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Programa Doutoral em Engenharia Química e Biológica

PRISCILLA BARBOSA SALES DE ALBUQUERQUE

AVALIAÇÃO DE ATIVIDADES BIOLÓGICAS DE MEMBRANA E GEL DA GALACTOMANANA EXTRAÍDA DAS SEMENTES DE *Cassia grandis* COM BIOMOLÉCULAS IMOBILIZADAS

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Orientadores: Profª; Dra. Maria das Graças Carneiro da Cunha (Departamento de Bioquímica, Centro de Biociências/UFPE) e Prof. Dr. José António Couto Teixeira (Departamento de Engenharia Biológica, Centro de Engenharia Biológica, UMINHO).

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Fevereiro, 2017

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RESUMO

A galactomanana extraída das sementes de *C. grandis* representa uma fonte alternativa para galactomananas principalmente devido à sua capacidade de atuar como líquido ou como gel. O presente trabalho teve por objetivo a caracterização de géis e membranas obtidas a partir da galactomanana extraída das sementes de *C. grandis*, com ou sem biomoléculas imobilizadas (lectina extraída das sementes de *Cratylia mollis* – Cramoll, lactoferrina, peptídeos bioativos, fitoesteróis e nanopartículas de lecitina com quercetina incorporada), através de análises reológicas, físico-químicas e mecânicas, além da avaliação das ações citotóxica e cicatrizante destes produtos. A galactomanana foi extraída por precipitação com etanol, enquanto a Cramoll foi obtida por precipitação salina seguida por cromatografia de afinidade em Sephadex G-75. As nanopartículas de lecitina contendo quercetina foram caracterizadas através da medição de diâmetro das partículas, grau de polidispersão, estabilidade e potencial zeta. Os géis de galactomanana (1,7 % p/v), sem e com Cramoll imobilizada por enclausuramento, tiveram sua estabilidade avaliada ao longo do tempo por reometria, pH, cor, avaliação microbiana e atividade hemaglutinante. O efeito da concentração de diferentes biomoléculas imobilizadas nas propriedades físico-químicas das membranas de galactomanana (0,8 % p/v) foi avaliado através de MEV, FTIR, propriedades mecânicas e térmicas, cor, conteúdo de água, solubilidade, permeabilidade ao vapor de água e ângulo de contato. As atividades citotóxica e cicatrizante das membranas com Cramoll imobilizada foram avaliadas em cultura de fibroblastos e em ratos machos, respectivamente. Os resultados revelaram que ambos os géis, sem e com Cramoll imobilizada, demonstraram transparência, estabilidade até 30 dias, não apresentaram contaminação microbiana e mantiveram o pH próximo da neutralidade, no entanto, as análises reológicas mostraram que o gel com Cramoll imobilizada perdeu substancialmente o seu módulo elástico após 60 dias. Todas as nanopartículas de lecitina contendo quercetina (25-150 µg/mL) apresentaram potencial zeta semelhante (-47,14). Nanopartículas de lecitina contendo até 50 µg/mL de quercetina apresentaram estabilidade e tamanho de partícula menores quando comparadas àquelas com 75 a 150 µg/mL de quercetina incorporada; estas apresentaram sinais de precipitação após 48 horas. Lactoferrina, peptídeos bioativos, fitoesteróis e nanopartículas de lecitina contendo quercetina, quando imobilizadas em membranas de galactomanana, levaram à produção de membranas com alta luminosidade e tendência ao amarelo, além de apresentarem maior rigidez, rugosidade e características físicas mais hidrofílicas que as membranas sem biomoléculas. A avaliação *in vivo* da atividade cicatrizante das membranas de galactomanana sem e com Cramoll imobilizada revelou uma efetiva regeneração tecidual em feridas tópicas produzidas em ratos machos, no entanto, as membranas com Cramoll imobilizada foram significativamente mais eficientes, promovendo a completa contração das feridas em apenas 11 dias. Em conclusão, os resultados obtidos sugerem que a galactomanana extraída das sementes de *C. grandis* é uma promissora matriz para a imobilização de biomoléculas, principalmente no que se refere à utilização de suas suspensões em membranas e géis úteis para as indústrias farmacêutica, cosmética e alimentícia.

Palavras-chave: Caracterização. Imobilização. Polissacarídeos.

ABSTRACT

The galactomannan extracted from the seeds of *C. grandis* represents an alternative source for galactomannans mainly due to its ability to act as a liquid or as a gel, suggesting that, due to its rheological properties, it can be used in different biotechnological applications, such as an effective support for immobilization of biomolecules. The present study aimed the characterization of gels and membranes prepared from the galactomannan extracted from *C. grandis* seeds with and without immobilized biomolecules (lectin extracted from the seeds of *Cratylia mollis* - Cramoll, lactoferrin, bioactive peptides, phytosterols and nanoparticles of lecithin with incorporated quercetin) through rheological, physicochemical and mechanical analysis, as well as the evaluation of cytotoxic and healing activities of the above mentioned matrices. The galactomannan was extracted by ethanol precipitation, while Cramoll was obtained by salt precipitation followed by affinity chromatography on Sephadex G-75. Lecithin nanoparticles containing quercetin were characterized by measurement the diameter of the particles, degree of polydispersity, stability and zeta potential. Galactomannan gels (1.7 %, w/v), with and without immobilized Cramoll, were evaluated along time by rheometry, pH, colour, microbial contamination and lectin hemagglutinating activity. MEV, FTIR, mechanical and thermal properties, colour, water content, solubility, water vapour permeability and contact angle were performed to evaluate the effect of the concentration of different on the physicochemical properties of galactomannan membranes (0.8 %, w/v). In addition, the cytotoxic and healing activities of membranes with immobilized Cramoll were evaluated in culture of fibroblasts and in male rats, respectively. The results showed that both gels, with and without immobilized lectin, demonstrated transparency, were stable up to 30 days, presented no microbial contamination as well as a pH close to neutral, however, rheological data showed that the gel with immobilized lectin loses its elastic modulus substantially after 60 days. All of the lecithin nanoparticles containing quercetin (25-150 µg/mL) showed similar zeta potential (-47.14). Lecithin nanoparticles containing up to 50 µg/mL of quercetin showed stability and particles size smaller when compared to those with 75 to 150 µg/mL of incorporated quercetin; the last one concentrations showed signs of precipitation after 48 hours of analysis. Lactoferrin, bioactive peptides, phytosterols and lecithin nanoparticles with incorporated quercetin, when immobilized on galactomannan membranes, lead to membranes with a strong whiteness tendency as well as a yellowish appearance, roughness on their surface and physical characteristics more hydrophilic than membranes without biomolecules (control). *In vivo* healing activity evaluation of the galactomannan membranes with and without immobilized Cramoll improved tissue regeneration on topical wounds of male rats; however, membranes with immobilized lectin were significantly better, promoting complete wounds´ contraction only in 11 days. In conclusion, the obtained results suggest the galactomannan extracted from *C. grandis* as a promising matrix for immobilization of biomolecules, mainly due to the use of their suspensions in membranes and gels useful for pharmaceutical, cosmetic and food industries.

Key-words: Characterization. Immobilization. Polysaccharides.

LISTA DE ILUSTRAÇÕES

Artigo III

Artigo IV

LISTA DE TABELAS

Artigo VI

LISTA DE ABREVIATURAS E SIGLAS

AH – Atividade Hemaglutinante
CA – Ângulo de contato
CAT – Catalase
ConA – Lectina isolada da *Canavalia ensiformis*
CK14 – Citoqueratina 14
Cramoll – Lectina extraída de sementes de *Cratylia mollis* Mart. (feijão Camaratu)
Cramoll 1,4 – Formulação contendo as isoformas 1 e 4 da Cramoll
DLS – Espalhamento dinâmico de luz
EB – Alongamento de ruptura
ERO – Espécies Reativas de Oxigênio
FTIR – Espectroscopia de infravermelho transformada de Fourier
GPX – Glutationa Peroxidase
GRAS – Geralmente Reconhecida como Segura
IFN-γ – Interferon γ
MC – Conteúdo de água
MEV – Microscopia eletrônica de varredura
MMPs – Metaloproteinases
Np – Nanopartículas
RL – Radicais Livres
SOD – Superóxido Dismutase
Sol – Solubilidade
TS – Resistência à tração e alongamento de ruptura
UV – Radiação Ultravioleta
WVP – Permeabilidade ao vapor de água
Y – Opacidade
Yw – Padrão de opacidade branco
Yb – Padrão de opacidade preto
Ym – Módulo de Young

SUMÁRIO

Fundamentação teórica

1. INTRODUÇÃO

Diversos produtos utilizados pela sociedade têm como fonte a flora e a fauna brasileira. Medicamentos, alimentos, fibras, óleos naturais e essenciais, cosméticos, produtos químicos e biocombustível são alguns exemplos dos produtos que podem ser fabricados a partir das inúmeras classes de compostos químicos extraídos das nossas espécies vegetais e animais. Os polissacarídeos representam uma das classes de maior importância, pois são polímeros naturais extraídos de plantas, algas, animais, fungos ou obtidos por via fermentativa, com uma ampla gama de aplicações, especialmente nas áreas alimentícia, biomédica, farmacêutica e cosmética (CUNHA; DE PAULA; FEITOSA, 2009).

Galactomananas são polissacarídeos extraídos com elevado rendimento a partir do endosperma de sementes de leguminosas, onde desempenham funções de reserva de energia e hidratação. Também apresentam propriedades particulares como massa molar elevada, solubilidade em água e caráter não-iônico (POLLARD et al., 2010), além da grande capacidade de moldar filmes/membranas (CERQUEIRA et al., 2009a; CERQUEIRA et al., 2009b; CERQUEIRA et al., 2011a; MARTINS et al., 2012) e atuar como agente gelificante (DA LOZZO et al., 2013; PINHEIRO et al., 2011).

Cassia grandis é uma planta de pequeno a médio porte, pertencente à família Leguminosae e à subfamília Caesalpinioideae, amplamente encontrada na região da mata do Brasil. O conteúdo de goma solúvel em água (32 – 34 %) encontrado nas sementes de *C. grandis* da Índia foi reportado por Joshi & Kapoor (2003) como a principal fonte de galactomanana. Albuquerque et al. (2014) caracterizaram a galactomanana extraída das sementes de *C. grandis* do estado de Pernambuco, Brasil, e observaram que concentrações menores que 1,5 % (p/v) exibem comportamento de solução polimérica, enquanto concentrações maiores associam-se ao comportamento de um gel fraco. A habilidade de apresentar-se tanto como solução quanto como gel sugere que as características físicas deste polissacarídeo podem ser úteis em uma gama de aplicações biotecnológicas.

Os filmes ou membranas para revestimentos são definidos como matrizes preparadas a partir de biomateriais e amplamente utilizadas desde a área farmacêutica até a alimentícia (BEVERLYA et al., 2008). No campo farmacêutico, por

exemplo, filmes preparados a base de Policaju (MONTEIRO et al., 2007) e quitosana (KATO et al., 2003) foram desenvolvidos como curativos para feridas, em virtude das suas características de libertação e de adesão. Além disso, eles têm sido aplicados no desenvolvimento de biossensores, membranas biológicas, experimentos imunológicos e fios de sutura por serem biodegradáveis e biocompatíveis (WATANABE et al., 2005).

Como ampla fonte alternativa na formação de filmes poliméricos, a galactomanana extraída das sementes de *C. grandis* apresenta propriedades físicas particulares para diferentes formulações filmogênicas, sugerindo sua promissora utilização como curativo tópico de feridas. Além do mais, por ser um polissacarídeo atóxico, hidrofílico, biocompatível, biodegradável e de acesso fácil, esta galactomanana possui características que tornam mais do que viável a sua utilização como suporte para imobilização de sistemas de liberação de drogas, os quais possam potencializar o efeito cicatrizante de feridas.

O gel é uma rede tridimensional obtida através de ligações das macromoléculas envoltas por um solvente, os quais suportam a própria massa e mantém a sua forma. A presença de ligações cruzadas não covalentes dificulta enormemente a descrição das propriedades físicas dessas redes, devido ao número e a posição destas ligações poderem variar com a temperatura e com o tempo. A reversibilidade dos géis físicos deve-se, portanto, à natureza das interações estabelecidas em sua estrutura (VALENGA, 2007).

Os géis geralmente apresentam propriedades viscosas e elásticas, com uma preponderância do modo elástico. As propriedades funcionais dos polissacarídeos solúveis em água são largamente utilizadas na indústria. Para a otimização desse emprego industrial, tornou-se necessário desenvolver métodos que permitissem prever a estrutura e a função destes polímeros através do conhecimento das conformações adotadas pelos mesmos. Entre os métodos fisico-químicos utilizados nessa avaliação estão, por exemplo, as técnicas reológicas, que descrevem as propriedades mecânicas dos vários materiais, sob várias condições de deformação, quando eles exibem a capacidade de escoar e/ou acumular deformações reversíveis (SCHRAMM, 2006).

Existem muitos estudos na literatura relacionados à sinergia que ocorre entre diferentes misturas de polissacarídeos. Géis mistos com dois ou mais biopolímeros

vêm sendo amplamente investigados, com ênfase para os que utilizam galactomananas com a finalidade de melhorar propriedades como coesão, aparência, estabilidade e durabilidade (MENEGUIN, 2012). Entre os exemplos incluem-se ágar e galactomanana (LUCYSZYN et al., 2006; PEREIRA-NETTO et al., 2012), galactomanana e xantana (SHOBHA; THARANATHAN, 2009), pectina e galactomanana (WU et al., 2009) e galactomananas não convencionais com xantana e carragenana (PINHEIRO et al., 2011).

Estudos envolvendo a utilização de galactomananas como suportes para incorporação de biomoléculas também já foram desenvolvidos, por exemplo, com a importante finalidade de avaliar a liberação controlada de suspensões contidas em estruturas nanoméricas (ALVAREZ-ROMÁN et al., 2001; COMBA; SETHI, 2009). Pertencente ao grupo de sistemas dispersos em escala nanomérica, nanocápsulas, nanopartículas, lipossomas, microesferas, microcápsulas e nanoesferas são formas farmacêuticas que têm por finalidade reduzir os efeitos colaterais de muitas substâncias e ao mesmo tempo aumentar a sua eficácia após administração por diversas vias, incluindo a cutânea (ANGELI, 2007).

Nesse sentido, o desenvolvimento tecnológico de novas formas farmacêuticas em escala nanomérica tem sido uma estratégia promissora para aumentar, de forma controlada, a penetração de fármacos através da pele. A utilização de matrizes da galactomanana extraída das sementes de *C. grandis* com estruturas nanoméricas incorporadas apresentaria, portanto, características vantajosas para a aplicação tópica, principalmente no que se refere ao contato íntimo estabelecido com o estrato córneo, o que possivelmente aumentaria a quantidade de agente encapsulado capaz de penetrar na pele.

No presente trabalho, a galactomanana extraída das sementes de *C. grandis* foi preparada em diferentes concentrações para formular membranas e géis potencialmente eficazes na imobilização de biomoléculas. As atividades citotóxica e cicatrizante foram avaliadas para as formulações puras e para as incorporadas com biomoléculas.

2. OBJETIVOS

2.1 GERAL

Caracterizar géis e membranas preparados a partir da galactomanana extraída das sementes de *C. grandis*, com biomoléculas imobilizadas ou não, através de análises reológicas, físico-químicas e mecânicas, e avaliar as ações citotóxica e cicatrizante dos produtos formados.

2.2 ESPECÍFICOS

- Extrair a galactomanana contida nas sementes de *C. grandis*;
- Produzir géis e membranas à base da galactomanana extraída das sementes de *C. grandis*;
- Purificar Cramoll 1,4 a partir das sementes de *Cratylia mollis* Mart. (feijão Camaratu);
- Incorporar Cramoll 1,4 em gel de galactomanana;
- Estudar o perfil reológico dos géis de galactomanana com e sem Cramoll 1,4 incorporada;
- Imobilizar por enclausuramento a Cramoll 1,4 em membranas de galactomanana;
- Avaliar o potencial citotóxico da membrana de galactomanana com Cramoll 1,4 incorporada;
- Avaliar a atividade cicatrizante da membrana de galactomanana com Cramoll 1,4;
- Acompanhar a evolução do processo de cicatrização do ponto de vista clínico, microbiológico e histopatológico;
- Imobilizar diferentes biomoléculas (lactoferrina, peptídeos bioativos e fitoesteróis) em membranas de galactomanana;
- Caracterizar membranas de galactomanana com biomoléculas imobilizadas de acordo com suas propriedades físico-químicas e mecânicas;
- Preparar nanopartículas de lecitina/quitosana com quercetina incorporada;

- Caracterizar as nanopartículas de lecitina/quitosana com quercetina incorporada quanto ao tamanho de partículas, carga de superfície e eficiência de encapsulamento;
- Imobilizar nanopartículas de lecitina/quitosana com quercetina incorporada em membrana de galactomanana;
- Caracterizar membranas de galactomanana com nanopartículas de lecitina/quitosana com quercetina incorporada de acordo com suas propriedades físico-químicas e mecânicas.

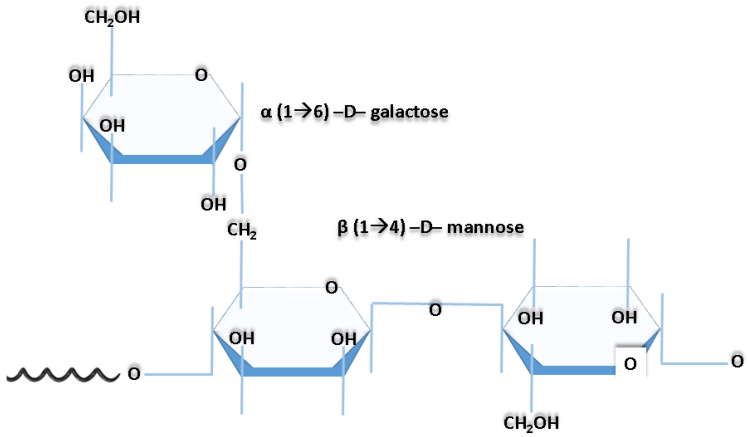
3. REVISÃO DE LITERATURA

3.1 GALACTOMANANAS

3.1.1 Considerações Gerais

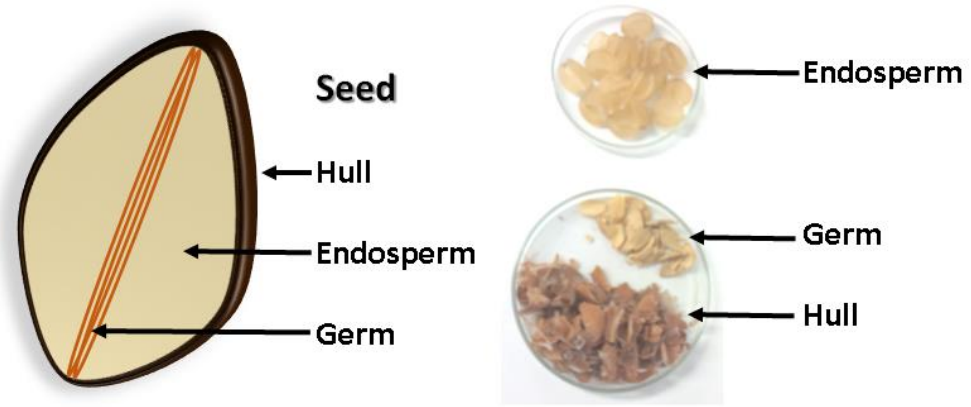
Galactomananas (Figura 1) são polissacarídeos formados por uma cadeia linear com unidades *β*-1,4-D-manopiranose substituídas por unidades ligantes *α*-1,6-D-galactopiranose, obtidos a partir de fontes microbianas, e principalmente de plantas, onde desempenham função de reserva e cuja principal fonte é o endosperma das sementes (Figura 2), com destaque para os membros da família Leguminosae (DEA; MORRINSON, 1975). Esta família possui espécies espalhadas por todo o mundo, especialmente nas regiões tropicais e subtropicais, que variam de árvores emergentes até ervas diminutas e efêmeras (LEWIS et al., 2005).

Figura 1 – Segmento representativo de uma cadeia de galactomanana de origem vegetal



FONTE: Albuquerque et al. (2016b). M: representação das unidades de manose da cadeia principal em ligação β (1→4). G: representação da substituição por unidade de galactose em α (1→6).

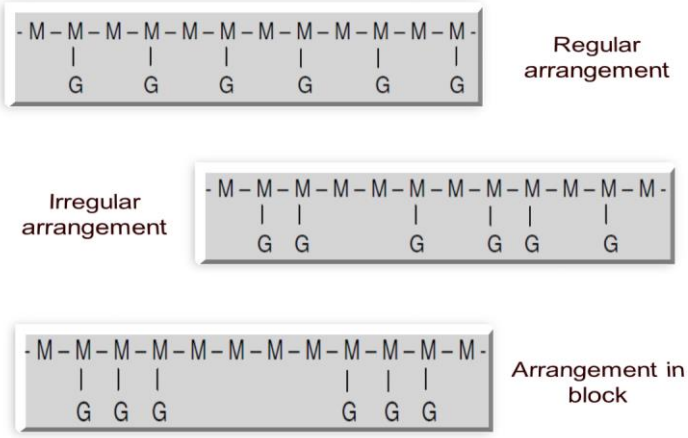
Figura 2 – Semente da família Leguminosae contendo casca, endosperma e gérmen



FONTE: Albuquerque et al. (2016a).

Os polissacarídeos de sementes são exemplos de classes de compostos naturais que têm contribuído na classificação da família Leguminosae, mas especial ênfase tem sido dada às galactomananas (HEGNAUER; GRAYER-BARKMEIJER, 1993). Segundo a classificação de Engler (1964), a família Leguminosae é dividida nas subfamílias Caesalpinioideae, Mimosoideae e Faboideae. A utilização de galactomananas como caráter taxonômico tem sido proposta por muitos autores, levando em consideração o rendimento em galactomanana extraída do endosperma das sementes desta família, assim como a proporção entre os resíduos de manose e galactose na molécula e os teores destes compostos nas sementes (ROSA et al., 2009). Outra importante utilidade das galactomananas leva em consideração que a família Leguminosae é a segunda dentro da classe das dicotiledôneas e a primeira em importância econômica. Dea e Morrison (1975) propuseram três possibilidades para a distribuição das substituições de galactose ao longo da cadeia principal de manana (Figura 3), ou seja, para a estrutura fina da molécula:

Figura 3 – Distribuição das substituições de galactose ao longo da cadeia principal de manana



FONTE: Albuquerque et al. (2016a). M: representação das unidades de manose da cadeia principal em ligação β (1→4). G: representação da substituição por unidade de galactose em α (1→6).

Além de proporcionar diferenças na densidade e na viscosidade das soluções, a proporção e distribuição das unidades de galactose tem papel fundamental na solubilidade das galactomananas, visto que, à medida que a razão manose/galactose diminui, a solubilidade aumenta. Caso não existam ramificações com o monossacarídeo galactose, tem-se uma manana pura ou verdadeira, ou seja, um polissacarídeo constituído de pelo menos 85 a 95% de unidades de manose, cuja distribuição das cadeias irá proporcionar interações intermoleculares do tipo ligação de hidrogênio entre as hidroxilas *cis* da manose, levando à formação de agregados completamente insolúveis em água (ASPINALL, 1959; GOYCOOLEA; MORRIS; GIDLEY, 1995; STEPHEN, 1983).

A presença de unidades de galactose produz cadeias ramificadas e realiza o impedimento estérico entre as ligações de hidrogênio intermoleculares, minimizando a formação de agregados. Por outro lado, galactomananas com poucas cadeias laterais (maior razão manose/galactose) podem interagir melhor com outros polissacarídeos devido às longas regiões não substituídas (SRIVASTAVA; KAPOOR, 2005).

3.1.2 Aplicação Biotecnológica

As três principais galactomananas utilizadas comercialmente em indústrias de alimentos e não-alimentos são goma guar (*Cyamopsis tetragonobus*, razão manose/galactose 1,6), goma tara (*Caesalpinea spinosa*, razão manose/galactose 3,3) e goma de feijão alfarroba (*Ceratonia siliqua,* razão manose/galactose 3,75)

(DAKIA et al., 2008; DEA; MORRISON, 1975; GIDLEY; REID, 2006). Outras galactomananas comercialmente conhecidas são a goma extraída de *Cassia tora*, com relação manose/galactose de aproximadamente 3,0 (SRIVASTAVA; KAPOOR, 2005) e a galactomanana de feno-grego *Trigonella foenum-graecum* (razão manose/galactose 1,1), comercializadas em menor escala, assim como aquela extraída das sementes de *Prosopis juliflora* (razão manose/galactose 4,2) (FIGUEIREDO, 1983).

Na indústria alimentícia, a goma guar e a alfarroba são as espécies mais utilizadas (DOYLE et al., 2006), enquanto a galactomanana de tara tem sido aceita como uma alternativa àquelas já utilizadas (AZERO; ANDRADE, 2002). As principais utilizações são como espessantes e substitutos de gordura, além de também melhorarem a textura e aparência dos alimentos e aumentarem a sua resistência a variações de temperatura. Em particular, as galactomananas podem ser usadas em produtos derivados do leite, sobremesas (especialmente sorvetes), geleias, produtos em pó, misturas para bolo e glacê, temperos, molhos, sopas e alimentos enlatados e congelados (REID; EDWARDS, 1995).

As galactomananas também podem entrar na composição de alimentos dietéticos, uma vez que não são digeridas pelo organismo. A adição da goma guar em refeições ricas em carboidratos reduz o aumento pós-prandial de glicose e de insulina no sangue. Além disso, melhorias no metabolismo de carboidratos e lipídeos em pacientes insulino-dependentes e insulino-independentes foram conseguidas usando preparações farmacêuticas de goma guar e alimentos adicionados da mesma goma. A ação fisiológica da goma guar parece depender principalmente da sua capacidade de hidratação rápida, conseguindo, assim, aumentar a viscosidade do bolo alimentar no estômago e no intestino delgado. A alta viscosidade no intestino delgado diminui a digestão e absorção de carboidratos, o que tende a reduzir a hiperglicemia pós prandial. Também existem estudos de que a goma guar é capaz de diminuir o LDL sanguíneo (BEHR et al., 1998; ELLIS et al., 1991).

Estudos relacionados às propriedades mecânicas e térmicas de filmes a base de galactomananas também vem sendo amplamente explorados para a aplicação biotecnológica deste polissacarídeo na indústria alimentícia, principalmente no que se refere ao desenvolvimento de revestimentos comestíveis (CERQUEIRA et al., 2011a), por exemplo, para frutas tropicais (CERQUEIRA et al., 2009b) e queijo ricota

(CERQUEIRA et al., 2009a).

Na indústria de cosméticos, as galactomananas são utilizadas como espessantes e estabilizantes em cremes e loções (STEPHEN, 1983). Géis mistos com dois ou mais biopolímeros também vêm sendo amplamente investigados, com ênfase para os que utilizam galactomananas com a finalidade de melhorar propriedades como coesão, aparência, estabilidade e durabilidade (MENEGUIN, 2012).

As galactomananas têm sido usadas como matriz na liberação controlada de drogas, a exemplo da formulação galactomanana/xantana, que já foi utilizada como veículo de liberação controlada de fármacos como diclofenato de sódio e teofilina. As galactomananas são adicionadas a comprimidos efervescentes, pois, como são agentes espessantes, formam uma suspensão estável, impedindo o assentamento das partículas, além de promover uma agradável sensação na boca (SCHIERMEIER; SCHMIDT, 2002; UGHINI et al., 2003; VENDRUSCOLO et al., 2005). Estudos envolvendo a utilização de galactomananas em géis para incorporação de biomoléculas também já foram desenvolvidos, por exemplo, com a importante finalidade de avaliar a liberação controlada de suspensões contidas em estruturas nanoméricas (ALVAREZ-ROMÁN et al., 2001; COMBA; SETHI, 2009).

Galactomananas, em combinação com pectinas, têm sido usadas como revestimento de cápsulas e comprimidos com a finalidade de liberação controlada de drogas no intestino grosso. Isoladamente, esses polissacarídeos não podem ser utilizados como carreadores de drogas ao cólon devido a sua solubilidade em água. Entretanto, quando o revestimento produzido pela combinação de galactomanana e pectina possui pH 7, ele torna-se elástico e insolúvel em fluidos gástricos e intestinais, e consegue atravessar o trato gastrointestinal superior, não ocorrendo liberação da droga no estômago e intestino delgado, havendo a liberação somente no intestino grosso (YANG; CHU; FIX, 2002).

As galactomananas também são adsorvidas pelas fibras de celulose e por isso são utilizadas na indústria de papel, pois melhoram as propriedades mecânicas do papel através da regulação do estado de floculação na suspensão de fibras celulósicas (NEWMAN; HEMMINGSON, 1998; LIMA; OLIVEIRA; BUCKERIDGE, 2003).

Diversas pesquisas já foram desenvolvidas a cerca da purificação, descrição das propriedades físicas, químicas e biológicas, e utilização de galactomananas

obtidas de distintas e variadas fontes. Galactomananas de diferentes espécies vegetais já foram caracterizadas, a exemplo da *Prosopis ruscifolia* (BUSCH et al., 2015), *Senna tora* (PAWAR; LALITHA, 2014), *C. grandis* (ALBUQUERQUE et al., 2014), *Dimorphandra gardneriana* Tul. (CUNHA et al., 2009), *Caesalpinia ferrea* var. *ferrea* (SOUZA et al., 2010a), *Caesalpinia pulcherrima*, *Gleditsia triacanthos* e *Adenanthera pavonina* (CERQUEIRA et al., 2011b).

O grau de substituição de unidades de galactose na cadeia central de manose é um caráter importante na interação das galactomananas com outros polímeros. Um dos primeiros relatos sobre as características e propriedades dos sistemas formados a partir de misturas binárias entre galactomananas e outros polissacarídeos foram observados por McCleary et al. (1981), que realizaram análises reológicas entre xantana e a galactomanana goma guar e demonstraram que essa interação diminui com o aumento do grau de substituição das galactomananas. Bresolin et al. (1997) também avaliaram o efeito sinérgico da xantana com galactomananas de *Mimosa scabrella* e *Schizolobium parahybum*, enquanto que as propriedades reológicas de gelificação foram estudadas por Amaral (1998). Mais recentemente, Grisel et al. (2015), avaliaram o efeito sinérgico das galactomananas goma guar e alfarroba com a xantana e confirmaram que o impacto de distribuição das unidades de galactose ao longo da cadeia principal de manose está ligado ao mecanismo de sinergia. Lucyszyn et al. (2006), por sua vez, aplicaram géis de misturas de galactomananas e ágar para cultivo de células vegetais. Outro gel binário amplamente estudado é constituído por k-carragenana e galactomanana. Gonçalves et al. (1997) observaram que a adição de galactomanana melhorou a qualidade do gel quando comparado ao obtido pela k-carragenana isolada. Em combinação com galactomanana, os géis tornaram-se menos quebradiços, mais resistentes e com menor tendência à sinerese. Pinheiro et al. (2011), por sua vez, quantificaram as interações sinérgicas entre as galactomananas extraídas de *Gleditsia triacanthos* e *Sophora japonica* com k-carragenana e xantana e compararam os resultados obtidos com as tradicionais galactomananas goma guar e alfarroba; os resultados demonstraram mais uma vez que o efeito sinérgico dos sistemas depende da razão entre manose e galactose, e, além disso, da estrutura fina da galactomanana.

Pode-se perceber que existe uma tendência mundial relacionada às pesquisas desenvolvidas sobre a purificação, a caracterização e a utilização de galactomananas,

indicando a grande necessidade de encontrar sementes que constituam novas fontes alternativas para extração deste polissacarídeo, especialmente para produção industrial. Em especial na América Latina, as possíveis fontes de extração de galactomananas são pouco conhecidas, apesar da rica biodiversidade da flora local (CUNHA; PAULA; FEITOSA, 2009).

O Brasil possui fontes ricas de espécies diversificadas para a extração de galactomananas a partir de sementes que poderiam alavancar a comercialização deste polissacarídeo, além de incluir a melhoria do valor agregado pela utilização de sementes sem capacidade germinativa. A *C. grandis*, árvore amplamente encontrada na região da mata do Estado de Pernambuco, Brasil, pode ser uma rica fonte fornecedora de sementes com elevado potencial de rendimento de extração de galactomanana, a qual tem destaque importante no âmbito do comércio e de promissoras pesquisas científicas.

3.1.3 Galactomananas de plantas do gênero Cassia

O gênero *Cassia* apresenta o maior número de representantes na subfamília Caesalpinioideae, a qual corresponde à cerca de 200 gêneros e mais de 2250 espécies (LEWIS et al., 2005). As espécies desta subfamília são plantas leguminosas arbustivas, arbóreas ou trepadeiras das matas, raramente são ervas. Apresentam folhas compostas e frutos leguminosos e estão muito bem representadas no Brasil (JOLY, 1998).

Diversos estudos desenvolvidos com espécies do gênero *Cassia* demonstraram importantes atividades biológicas para extratos obtidos de suas flores, folhas, vagens e sementes. Para a *C. fistula*, foram reportadas atividades larvicida (DURAIPANDIYAN; IGNACIMUTHU; PAULRAJ, 2011), antidiabética (DAISY et al., 2010), antifertilizante (CHAUHAN; AGARWAL, 2010), anticâncer (DURAIPANDIYAN et al., 2012), antioxidante e antibacteriana (SURESH et al., 2015). *C. tora* apresentou atividades antimicrobiana e antioxidante (SARAVANAKUMAR et al., 2015), enquanto atividade antidiurética foi observada para *C. occidentalis* (NTCHAPDA et al., 2015). Esta última também apresentou atividade antioxidante, assim como *C. obstusifolia* (VADIVEL; KUNYANGA; BIESALSKI, 2011) e *C. hirsuta* (VADIVEL; NANDETY; BIESALSKI, 2011).

As galactomananas obtidas por espécies de plantas do gênero *Cassia*

merecem importante destaque devido às diversas aplicações às quais se destinam, tanto de forma isolada ou associadas a demais produtos, inclusive outras galactomananas. Por exemplo, o enorme interesse médico a cerca destes polissacarídeos está relacionado ao seu bom valor terapêutico na medicina popular (LODHA et al., 2010).

Algumas possíveis fontes de galactomananas foram identificadas em *C. alata, C. angustifolia, C. didymobotrya, C. grandis, C. nodosa, C. occidentalis, C. saemaea, C. hirsuta e C. spectabilis*, cujas sementes, geralmente de tamanho médio e contendo mais de 40% de endosperma, são consideradas, portanto, preferíveis para a exploração comercial (SRIVASTAVA; KAPOOR, 2005).

3.1.4 *Cassia grandis*

Cassia grandis é a maior espécie brasileira do gênero *Cassia*. O nome do gênero *Cassia* é hebraico ou grego e *grandis* significa grande, visto que sua vagem pode atingir 60 cm de comprimento. Pertence à Divisão Magnoliophyta (Angiospermae), à Classe Magnoliopsida (Dicotiledonae), à Ordem Fabales e à Família Caesalpiniaceae (Leguminosae: Caesalpinioideae). Trata-se de uma árvore caducifólia com 10 a 15 m de altura e podendo atingir dimensões próximas a 30m de altura na idade adulta. Apresenta fruto (legume) lenhoso indeiscente, cilíndrico, irregular, geralmente com 11 a 60 cm de comprimento e 36 a 50 mm de diâmetro, de duas suturas longitudinais e nervuras salientes e grossas, que ligam as suturas. Quebrando o pericarpo de seus frutos, aparecem os septos circulares que separam as sementes, e uma massa preta, pegajosa e adocicada. O fruto maduro é marrom-escuro externamente e contém muitas sementes com aspecto oval e bastante duras, aplainada de um lado e carinada do outro, brilhante, castanho-amarelo-claro, com excisão no hilo de até 1 cm de comprimento (CARVALHO, 2003).

Figura 4 – Flores e Fruto com sementes de *Cassia grandis*

FONTE: Disponível em: <http://www.tramil.net/fototeca/imageDisplay.php?id_elem=112>. Acesso em: 12 ago. 2015.

Além do Brasil, *C. grandis* ocorre de forma natural no sul do México, Costa Rica, Honduras, Panamá, Porto Rico, Colômbia, Guiana, Guiana Francesa, Peru, Suriname e na Venezuela (CARVALHO, 2003). Outros países e localidades em que pode ser encontrada são Trinidad e Tobago, Havaí, Jamaica e Cuba (CORREA; BERNAL, 1990), assim como na Índia, onde foi introduzida como uma árvore ornamental em jardins e avenidas, devido a sua sombra (JOSHI; KAPOOR, 2003). Segundo Carvalho (2003), *C. grandis* ocorre nos seguintes estados brasileiros: Amazonas, Amapá, Bahia, Maranhão, Mato Grosso, Mato Grosso do Sul, Pará, Paraíba, Pernambuco, Rio de Janeiro, Roraima, Sergipe e Tocantins.

C. grandis pode ser empregada na alimentação humana e animal, bem como para fins medicinais, ornamentais, veterinários, entre outros. Os frutos da *C. grandis*, por exemplo, geralmente são deixados no pasto (CARVALHO, 2003) e usados na forragem (BODOWSKI, 1984). Em relação ao consumo humano, o fruto não é totalmente aceito devido à polpa amarga e adstringente com cheiro forte de sarro (VIEIRA, 1992), porém, ainda assim, apresenta ampla distribuição na forma silvestre e em áreas campestres, além da possibilidade de comércio da espécie (BODOWSKI, 1984; CORREA; BERNAL, 1990; MUTCHNICK; McCARTHY, 1997).

Segundo Santana et al. (2002), as sementes de *C. grandis* possuem um alto potencial de uso na elaboração de colares, brincos e cortinas, e Duarte (1979) sugere seu emprego na arborização urbana, em parques e estradas, indicando seu potencial agroflorestal principalmente recomendado para as zonas secas. Devido ao seu grande porte e ao peso e tamanho de seus frutos, no entanto, Brandão et al. (2002) sugerem que ela seja utilizada apenas em parques e grandes jardins.

A aplicação medicinal de *C. grandis* é sugerida contra o sorotipo 2 do vírus da dengue (HERNÁNDEZ-CASTRO; DIAZ-CASTILLO; MARTÍNEZ-GUTIERREZ, 2015), como promotor da diurese (LODHA et al., 2010), contra vermes e parasitas intestinais (LENTZ et al., 1998), doenças gástricas (BHAMARAPRAVATI et al., 2003), sanguíneas (PARRA; SADIÑAS, 2000), respiratórias (FREI et al., 1998) e de pele (CÁCERES et al*.,* 1991; SALINAS; GRIJALVA, 1994).

Joshi e Kapoor (2003) citam que as sementes contêm aproximadamente 50% de endosperma gomoso e possuem as características de se tornarem uma fonte em potencial de galactomanana, que é utilizada em larga escala para o consumo internacional. Algumas indústrias, tais como farmacêuticas, de minas e refinarias de petróleo, têxtil, de papel e cosmética utilizam essa matéria-prima por ser uma fonte barata, ecológica, não-tóxica e considerada GRAS (Geralmente Reconhecida como Segura).

Albuquerque et al. (2014) extraíram eficientemente a galactomanana contida nas sementes de *C. grandis* colhidas da zona da Mata do estado de Pernambuco, Brasil, obtendo um rendimento de extração de 36 ± 8 % e uma razão manose/galactose de 2,44. Uma extensiva caracterização reológica demonstrou que a galactomanana exibe um platô Newtoniano a baixas concentrações, seguido por um comportamento de solução polimérica e, após a faixa de transição próxima a 1,7 % (p/v) de concentração, o sistema adquire um estado de gel. Considerando tais resultados, a habilidade de apresentar-se tanto como solução quanto como gel permite uma ampla gama de aplicações das soluções aquosas da galactomanana.

3.2 LECTINAS

O termo lectina, proveniente do latim *legere* (pegar, escolher), foi primeiro empregado por Boyd e Shapleigh, em 1954, para descrever aglutininas grupo sanguíneo-específicas encontradas em sementes e outras partes de determinadas plantas. Atualmente, as lectinas representam uma classe de (glico)proteínas de origem não imune e amplamente distribuídas na natureza.

A característica fundamental das lectinas é a habilidade de ligar-se especificamente a carboidratos. O reconhecimento e a associação a essas biomoléculas ocorrem de forma reversível e com alta afinidade e especificidade (SILVA et al., 2011), permitindo o desempenho de atividades biológicas particulares

pelas lectinas. O mecanismo de ação deste reconhecimento acontece de acordo com o modelo chave-fechadura (KENNEDY et al., 1995), onde as lectinas fazem uso de uma série de diversas interações químicas fracas para produzir um reconhecimento altamente seletivo aos carboidratos ligados a uma superfície (SHARMA; SUROLIA, 1997).

As lectinas podem ser monovalentes (um sítio ligante glicídico por monômero), ou multivalentes (dois ou mais sítios de ligação para açúcar). Devido a tais propriedades ligantes, as lectinas são capazes de promover a aglutinação de células animais e de plantas e a precipitação de polissacarídeos, glicoproteínas, peptidoglicanos, ácido teicóico, glicofosfolipídios, etc (LIENER et al., 1986). Análises detalhadas demonstraram que as lectinas são um grupo grande e heterogêneo de proteínas que diferem fortemente quanto à especificidade aos carboidratos, estrutura molecular e atividade biológica (VAN DAMME et al., 1996), no entanto, é importante ressaltar que, além do sítio ligante para o carboidrato específico, algumas lectinas também possuem um segundo tipo de sítio ligante que interage, por exemplo, com bases nitrogenadas (SINGH.; TIWARY; KENNEDY, 1999).

Os monômeros mono ou multivalentes das lectinas estabelecem uma ponte ligante entre as células, formando uma rede que se aglutina. Esse fenômeno é uma das ferramentas auxiliares na avaliação da atividade das lectinas, uma vez que a aglutinação pode ser facilmente observada após um período de incubação adequado. Grande parte das lectinas se liga aos carboidratos presentes na superfície dos eritrócitos, permitindo, portanto, a determinação da presença da lectina pela formação de uma rede de aglutinação. Esta avaliação é denominada de Atividade Hemaglutinante (AH) (CORREIA; COELHO; PAIVA, 2008).

As atividades biológicas atribuídas às lectinas despertam o interesse destas moléculas pela investigação científica, ou seja, elas são utilizadas para explorar superfícies celulares, ligando-se à porção carboidrato de glicoproteínas ou glicofosfolipídios que se projetam da célula (UENO; LIM, 1991). Ainda por sua versatilidade, as lectinas têm sido estudadas quanto à atividade antitumoral (ARAÚJO et al., 2011; NOLTE et al., 2012), inseticida (ARAÚJO et al., 2012; ATALAH; SMAGGHE; VAN DAMME, 2014; OLIVEIRA et al., 2011) e antimicrobiana (COSTA et al., 2010; FERREIRA et al., 2011; QU et al., 2015); detecção e separação de glicoconjugados (LANZA et al., 2015); ação mitogênica (VIKRAM et al., 2010), entre

outras.

Em função da capacidade de interagir com carboidratos, as lectinas foram classificadas em específicas e não específicas. A especificidade da lectina é definida em termos do monossacarídeo ou oligossacarídeo que inibe as reações de precipitação ou aglutinação induzidas por elas (KOMPELLA; LEE, 2001). Por existirem plantas que possuem duas ou mais lectinas que diferem na especificidade, Sharon e Lis (1987) denominaram estas lectinas de isolectinas, que são definidas como um grupo de proteínas intimamente relacionadas, resultantes da expressão de diferentes genes, com estruturas semelhantes em uma mesma espécie, e que apresentam formas moleculares com mobilidade eletroforética diferente. O termo isoforma foi proposto para lectinas pertencentes à mesma espécie, cuja heterogeneidade de origem genética não foi bem definida (PAIVA; COELHO, 1992).

Apesar de muitas plantas possuírem uma lectina com especificidade para um único carboidrato, são conhecidas plantas que contêm lectinas com especificidade para açúcares diferentes. A Cramoll é uma delas: lectina extraída de sementes de *Cratylia mollis* Mart. (feijão Camaratu), uma planta nativa do Nordeste Brasileiro e pertencente à família Leguminosae. É fortemente inibida por metil α-D-manosídeo e, de acordo com a classe de lectinas ligantes de glicose/manose, similar às lectinas isoladas da *Canavalia ensiformis* (Concanavalina A ou ConA) e *Lens culinaris* (lectina de lentilha) (LIMA et al., 1997). Quatro formas múltiplas foram purificadas das sementes desta planta (Cramoll 1, Cramoll 2, Cramoll 3 e Cramoll 4), porém é possível desenvolver preparações que contenham múltiplos 1 e 4 associados, caracterizando a Cramoll 1,4 (CORREIA; COELHO, 1995).

Estudos desenvolvidos com a isoforma 1,4 da Cramoll demonstraram seu perfil imunomodulatório (SILVA et al., 2015), capacidade de produção de IFN-γ e de óxido nítrico (MELO et al., 2010a), atividade antitumoral (ANDRADE et al., 2004), atividade mitogênica em linfócitos humanos (MACIEL et al., 2004; MELO et al., 2010b) e atividade cicatrizante (MELO et al., 2011).

3.3 LACTOFERRINA

A lactoferrina (Lf), descrita pela primeira vez na literatura científica há aproximadamente 50 anos, é uma glicoproteína monomérica e globular com caráter básico e pertencente à família das transferrinas (GARCÍA-MONTOYA et al., 2012). A

Lf é produzida por células epiteliais mucosas em várias espécies de mamíferos, sendo encontrada nos humanos em secreções mucosas incluindo lágrimas, saliva, fluidos vaginais, sêmen, secreções nasais e brônquicas, bile, fluidos gastrintestinais, urina e mais frequentemente no leite e colostro; na verdade, Lf é a proteína mais abundante no leite após a caseína. Também pode ser encontrada em fluidos corporais, como plasma sanguíneo e líquido amniótico, e em quantidades consideráveis em grânulos de neutrófilos secundários, onde desempenha um papel fisiológico significativo (GONZÁ-LEZ-CHÁVEZ; ARÉVALO-GALLEGOS; RASCÓN-CRUZ, 2009).

Lf possui uma excelente afinidade de ligação ao ferro, sendo a única transferrina com capacidade de reter este metal em uma ampla faixa de pH, incluindo meios extremamente ácidos. Também exibe maior resistência à proteólise (AISEN; LEIBMAN, 1972). Além dessas diferenças, a carga positiva líquida da Lf e sua distribuição em vários tecidos fazem dela uma proteína multifuncional, isto é, esta proteína possui atividades biológicas importantes, como regulação da absorção de ferro no intestino, atividade anticancerígena, anti-inflamatória e moduladora imunológica, bem como propriedades antimicrobianas contra um grande número de microrganismos. Tais propriedades devem-se principalmente à sua capacidade de se ligar ao ferro e de interagir com compostos celulares e moleculares de hospedeiros e patógenos (GONZÁ-LEZ-CHÁVEZ; ARÉVALO-GALLEGOS; RASCÓN-CRUZ, 2009).

A atividade antimicrobiana da Lf tem sido amplamente reportada na literatura científica contra bactérias (Gram + e Gram-), fungos, leveduras, vírus e parasitas, além de também promover o crescimento de bactérias benéficas à flora humana, como *Lactobacillus* e Bifidobactéria. Geralmente, dois mecanismos de ação estão associados à atividade antimicrobiana da Lf: o primeiro é o sequestro de ferro em locais de infecção, o que priva o microrganismo deste nutriente, criando assim um efeito bacteriostático; o outro mecanismo é a interação direta da molécula de Lf com o agente infeccioso através da interação dos aminoácidos positivos na glicoproteína com moléculas aniônicas em algumas superfícies bacterianas, virais, fúngicas e parasitárias, causando a lise celular (SHERMAN, 2004; YAMAUCHI et al., 2006).

Considerando as atividades biológicas da Lf, além das atuais necessidades farmacêuticas e nutricionais da população, compreende-se o crescente aumento nas pesquisas científicas relacionadas à esta glicoproteína, principalmente no que se refere à sua produção, seja isolada do leite (RACHMAN; MAHESWARI; BACHROEM,

2015) ou obtida por engenharia genética (HWANG; KRUZEL; ACTOR, 2016), e das aplicações biotecnológicas testadas com Lf livre (SRIRAMOJU; KANWAR; KANWAR, 2015) ou imobilizada em suportes das mais variadas fontes (BOURBON et al., 2016; KIM et al., 2014).

3.4 PEPTÍDEOS BIOATIVOS

Peptídeos bioativos (BAPs) são fragmentos específicos de proteínas com aproximadamente 2 a 30 aminoácidos e baixo peso molecular. Podem ser obtidos através de fontes alimentares como ovo, leite, peixe, soja, carne ou sangue. Os BAPs são inativos dentro da sequência da proteína parental, porém têm um impacto positivo nos sistemas do corpo humano, com atividades semelhantes a drogas e/ou hormônios capazes de modular funções fisiológicas através da ligação a receptores específicos de células-alvo, levando à indução de respostas fisiológicas (DI BERNADINI et al., 2011; LAFARGA; HAYES, 2014).

BAPs são conhecidos por possuírem atividade antimicrobiana, antioxidante, antitrombótica, anti-hipertensiva, anticancerígena, reguladora da saciedade e imunomoduladora, podendo afetar os sistemas cardiovascular, imunológico, nervoso e digestivo (DI BERNARDINI et al., 2011; MARS; STAFLEU; DE GRAAF, 2012). Com relação aos efeitos descritos no sistema cardiovascular, os peptídeos bioativos são capazes de induzir uma atividade anti-hipertensiva através da inibição da enzima conversora de angiotensina, uma enzima chave na regulação da pressão arterial que converte a angiotensina I em angiotensina II, um potente vasoconstritor (NONGONIERMA; FITZGERALD, 2015a).

O número de estudos publicados na literatura sobre a utilização de BAPs em seres humanos tem aumentado nos últimos anos. Tal fato pode ser explicado pelo crescente interesse da comunidade científica, industrial e de saúde na aplicação de componentes naturais para o aperfeiçoamento da saúde, um fenômeno particularmente relevante no contexto em que as doenças metabólicas e suas complicações estão aumentando em todo o mundo. Os BAPs do leite ou de outras proteínas alimentares, isolados ou em combinação com fármacos (NONGONIERMA; FITZGERALD, 2015b), podem encontrar aplicação como alternativa natural às drogas por atuarem como agentes preventivos de várias doenças em humanos (LI-CHAN, 2015; SAADI et al., 2015). Isto pode ajudar, inclusive, a evitar os efeitos secundários

observados em muitos fármacos. As atividades anti-hipertensiva, opióide, saciante e reguladora de glucose no soro sanguíneo têm sido particularmente estudadas, no entanto, a avaliação bioatividade dos BAPs em humanos, seja com os peptídeos em forma livre ou imobilizados em alguma matriz, ainda é difícil de se estabelecer e requer mais estudos.

3.5 FITOESTERÓIS

Os esteróis vegetais, também chamados fitoesteróis, são relatados na literatura como mais de 250 esteróis distintos e compostos relacionados em vários materiais vegetais e marinhos; além disso, possuem estruturas químicas e funções biológicas semelhantes às do colesterol e, portanto, desempenham um papel importante na estrutura e função das membranas celulares (PIIRONEN et al., 2000).

Os fitoesteróis contêm grupamentos químicos adicionais à sua estrutura, como o metilo, o etilo, ou ligação dupla, e são conhecidos por terem propriedades hipocolesterolêmicas. Os análogos dos fitoesteróis são indicados para diminuir a absorção de colesterol e, assim, o nível de colesterol no soro em humanos, levando a benefícios cardiológicos para os usuários. Em geral, a dieta humana contém aproximadamente 200-300 mg/dia de fitoesteróis; quanto maior for o consumo de fitoesteróis a partir da dieta, menor é a absorção de colesterol e menor será seu nível sérico (CHEN et al., 2015).

Fitoesteróis são comumente adicionados a alimentos convencionais ou suplementos alimentares com o objetivo de reduzir o risco de doenças coronarianas em humanos (SRIGLEY; HAILE, 2015). A apresentação destes esteróis em formulações farmacológicas, tanto na forma livre quanto imobilizada em suportes poliméricos, representa uma inovação biotecnológica e requer mais investigações científicas.

3.6 FLAVONÓIDES

Os flavonóides compreendem um grupo de compostos fenólicos complexos que apresentam uma estrutura em comum, composta por dois anéis aromáticos e um grupo heterocíclico oxigenado (DIJK; DRIESSEN; RECOURT, 2000). As diferenças entre cada componente deste grupo estão relacionadas à variação no número e na posição dos grupamentos hidroxila da molécula, além de modificações nos núcleos e

grau de metilação e glicosilação que sofrem (DUGAS et al., 2000). São encontrados como componentes integrais da dieta humana e possuem propriedades antioxidantes (SAIT et al., 2015; SHARMA et al., 2015), antiinflamatórias (MASCARAQUE et al., 2014), antimicrobianas (AKHAVAN; JAHANGIRI; SHAFAGHAT, 2015; PERALTA et al., 2015), antitumorais (SEGUIN et al., 2013), entre outras.

Antioxidantes referem-se a substâncias que têm por característica diminuir ou bloquear as reações de oxidação induzidas por radicais livres (RL), os quais são caracterizados como moléculas instáveis liberadas em funções metabólicas de geração de energia. Por possuírem um elétron desemparelhado em seu orbital mais externo, estas espécies químicas são altamente reativas e dotadas de facilidade de produzir um campo magnético; atuam como agentes redutores, quando doam um elétron, ou como agentes oxidantes, ao ganharem um elétron em diferentes condições (RATNAM et al., 2006).

Os RL são formados em todas as organelas e compartimentos intracelulares pela ação de fagócitos contra agentes invasores, por regulação de fatores de crescimento envolvidos em respostas proliferativas ou, ainda, para desempenhar funções de sinalização celular (FINKEL; HOLBROOK, 2000; VALKO et al., 2007). A geração de RL é fundamental para a homeostase de células e tecidos, uma vez que cada organela e/ou compartimento possui alvos de dano oxidativo, além de abrigarem mecanismos próprios para eliminação do excesso destes radicais (SHIN et al., 2008).

Nosso organismo possui substâncias que têm por objetivo estabelecer um equilíbrio harmônico entre a presença das moléculas oxidantes, as antioxidantes e a pele. Quando existe um desequilíbrio entre as espécies reativas produzidas e as reservas antioxidantes, instala-se o estresse oxidativo (CHOW, 2002), que provoca danos importantes em nível celular: (1) RL atacam as membranas celulares; (2) danificam a mitocôndria, prejudicando a produção de energia; (3) interagem com as moléculas de proteína, danificando sistemas metabólicos cruciais, e (4) fragmentam o DNA, provocando mutações variadas e, muitas vezes, divisões celulares descontroladas.

Várias substâncias podem ser definidas como RL, porém o maior interesse é pelas espécies reativas de oxigênio (ERO). O termo ERO inclui não somente RL, mas também espécies não radicalares derivadas do oxigênio, a exemplo do peróxido de hidrogênio, que leva à formação da hidroxila (ADEGOKE et al., 1998). Embora a

geração endógena de ERO esteja atrelada às atividades metabólicas normais, vários estímulos ambientais como radiação ultravioleta (UV), agentes químicos tóxicos, radiação ionizante e hipertermia podem gerar altos níveis destas espécies, levando a um desequilíbrio na homeostase de células e tecidos (FINKEL; HOLBROOK, 2000). Quando o estresse é severo, a sobrevivência depende da habilidade das células adaptarem-se ou resistirem a ele e reparar ou remover as moléculas danosas e danificadas. Alternativamente, as células podem responder ao dano entrando em processo de apoptose (PORTUGAL et al., 2007).

Com o objetivo de minimizar os efeitos danosos das ERO, os organismos desenvolveram evolutivamente sistemas antioxidantes enzimáticos e não enzimáticos. No primeiro grupo, estão enzimas endógenas como a superóxido dismutase (SOD), a glutationa peroxidase (GPX) e a catalase (CAT), as quais protegem diretamente contra o radical O_2^- e o peróxido de hidrogênio, neutralizando-os ou convertendo-os em espécies menos reativas (LIPTON, 1999; RATNAM et al., 2006). Entre as defesas não-enzimáticas, diversos estudos reportam a ação antioxidante das vitaminas C e E (RENDÓN-RAMÍREZ et al., 2014; KARTHIKEYAN et al., 2014), glutationa (MAIN et al., 2012), ácido úrico (FABBRINI et al., 2014; ITAHANA et al., 2015), melatonina (AGUILERA et al., 2015; DING et al., 2014), carotenóides (GÓMEZ-ESTACA et al., 2015; ROSALIE et al., 2015) e a quercetina (RAVICHANDRAN; RAJENDRAN; DEVAPIRIAM, 2014; SOUZA et al., 2014; TAN et al., 2011).

A quercetina é um dos representantes de compostos polifenólicos mais estudados. Pertence à subfamília dos flavonóis e tem um excelente potencial antiradicalar, que está relacionado à capacidade de transferir elétrons aos RL, inibir a peroxidação lipídica, quelar íons metálicos, ativar enzimas antioxidantes, reduzir radicais α-tocoferóis, inibir oxidases, proteger o DNA e sequestrar peroxinitrito (HERTOG; HOLLMAN, 1993; OHSHIMA et al., 1998).

O uso tópico de formulações contendo quercetina sugere uma potencial aplicação biotecnológica deste antioxidante frente aos danos induzidos por ERO, a exemplo das espécies geradas pela radiação ultravioleta (UV). A insolubilidade da quercetina em água, no entanto, dificulta sua permeação na pele, limitando o seu poder de proteção ao tecido (TAN et al., 2011). O desenvolvimento de sistemas capazes de transportar quercetina através da pele, para exercer as atividades de

antioxidação e proteção contra o estresse oxidativo, é de grande importância na profilaxia e terapêutica de doenças de pele variadas.

3.7 IMOBILIZAÇÃO DE BIOMOLÉCULAS

Enzimas, anticorpos, proteínas, receptores celulares e drogas são exemplos de biomoléculas que já foram imobilizadas por meio químico ou físico em diferentes suportes biomateriais, para aplicações que vão das áreas terapêutica e diagnóstica, até métodos de separação e outros bioprocessos.

Entre as diferentes classes de biomateriais que podem ser utilizados como suportes, os polímeros têm interesse especial devido aos grupos reativos dispostos em sua superfície ou aos grupos que, sofrendo qualquer derivatização, podem ligar-se covalentemente a biomoléculas. Além disso, uma vantagem dos suportes poliméricos para biomoléculas está relacionada às diferentes formas de fabricação destes sistemas, incluindo filmes, membranas, tubos, fibras, partículas, cápsulas e estruturas porosas (HOFFMAN; HUBBEL, 2004).

Os vários métodos de imobilização empregados baseiam-se nas ligações físicas ou químicas entre a biomolécula e o suporte polimérico. Os mais utilizados são: adsorção física (interações hidrofóbicas, pontes de hidrogênio ou forças de van der Waals) e química (ligação covalente ou iônica), imobilização por confinamento em matriz e ligação cruzada. Importante observar que o termo imobilização refere-se tanto a uma localização transitória quanto a uma imobilização permanente da biomolécula dentro ou sobre o suporte polimérico utilizado. Ainda mais, se o suporte polimérico for biodegradável, a biomolécula imobilizada pode ser liberada pela degradação da matriz (HOFFMAN; HUBBEL, 2004).

Atualmente, diversas publicações científicas reportam os polissacarídeos como polímeros eficientes para imobilização de biomoléculas, por exemplo, filmes de quitosana de origem animal e microbiana, adicionados com o plasticizante glicerol, foram utilizados para a imobilização de bromelina por Zappino et al. (2015), os quais sugeriram os filmes de quitosana de baixo peso molecular como adequados para aplicação na indústria vinícola. Elchinger et al. (2015), por sua vez, imobilizaram proteases em filmes de quitosana e observaram um excelente efeito anti-biofilme, especialmente contra culturas de Staphylococcus. As áreas de bioengenharia e medicina também reportaram inovações, como a combinação de quitosana, gelatina

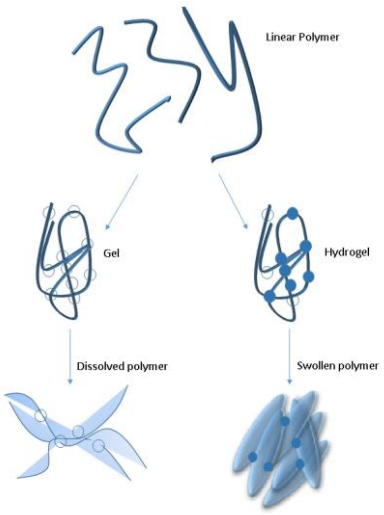
e alginato com nanotubos de carbono para imobilização de proteínas (DERKUS; EMREGUL; EMREGUL, 2015). A imobilização de biomoléculas em matrizes poliméricas também foi desenvolvida para a escala micro, a exemplo do trabalho que desenvolveu um biosensor de glicose por meio da imobilização de glicose oxidase em partículas de quitosana (ANUSHA et al., 2015), além das micropartículas de gel que foram produzidas a partir de lactoferrina e alginato (BOKKHIM et al., 2016).

Considerando os resultados acima mencionados, nota-se que na maioria das pesquisas desenvolvidas com polissacarídeos para imobilização de biomoléculas, as galactomananas ainda são pouco exploradas.

3.7.1 Géis como suportes de imobilização

Géis são sistemas semi-sólidos onde pequenas quantidades de sólido são dispersas em quantidades relativamente maiores de líquido, característica que garante uma natureza mais sólida do que líquida ao sistema (KLECH, 1990). Uma interpretação inadequada, mas bastante comum na ciência de polímeros, é o uso dos termos gel e hidrogel como sinônimos, pois mesmo que géis e hidrogéis sejam redes poliméricas quimicamente semelhantes (Figura 5), eles apresentam estruturas físicas particularmente distintas (GUPTA; VERMANI; GARG, 2002). Hidrogéis são caracterizados como uma rede de ligações cruzadas de polímeros hidrofílicos com capacidade de absorver grandes quantidades de água e inchar, embora mantenham a sua estrutura tridimensional (3D). Por vezes, os hidrogéis são também descritos como géis aquosos devido ao prefixo hidro, embora o termo hidrogel implica um material já inchado em água (GEHRKE; LEE, 1990).

Figura 5 – Polímero formando gel e hidrogel, mostrando diferentes comportamentos em meio aquoso. Os círculos fechados representam ligações covalentes, enquanto os círculos abertos representam ligações virtuais formadas por emaranhamento.



FONTE: Albuquerque et al. (2016a).

Embora alguns géis sejam suficientemente rígidos para manter sua estrutura sob baixas tensões, após exceder um determinado valor limite, a fluidez do gel desponta como uma característica atrelada à perca da estrutura polimérica. Os hidrogéis conseguem inchar em meio aquoso pelas mesmas razões que um polímero linear análogo consegue se dissolver em água para formar uma solução polimérica. Portanto, a característica central da formação de um hidrogel é sua inerente reticulação (capacidade de formar *cross-links*). Géis convencionais também podem desenvolver pequenos níveis de *cross-links*, como resultado do ganho de energia sob a influência de forças de tensão, mas este processo é reversível devido ao envolvimento de forças físicas fracas (GUPTA; VERMANI; GARG, 2002).

Moléculas biologicamente ativas como proteínas, peptídeos, sacarídeos, lipídeos, drogas, hormônios, receptores de superfície celular, conjugados, nucleotídeos e ácidos nucléicos podem ser química ou fisicamente imobilizadas em suportes poliméricos. Em relação a géis e hidrogéis, as biomoléculas podem ser imobilizadas na superfície exterior do gel ou dentro da rede polimérica do hidrogel (HOFFMAN; HUBBELL; 2004).

Aplicações que vão desde a indústria alimentícia até a farmacêutica têm utilizado géis desenvolvidos à base de polímeros como matrizes de liberação de compostos. A galactomanana das sementes de *Mimosa scabrella* Bentham, por exemplo, foi preparada em formulações com xantana e testada como matriz hidrofílica para liberação de teofilina (UGHINI et al., 2004) e diclofenaco de sódio (VENDRUSCOLO et al., 2005). Koop et al. (2009) utilizaram a galactomanana desta

mesma espécie como matriz para estabilização do ácido ascórbico. A goma do feijão alfarroba foi misturada com xantana e avaliada quanto à promoção da estabilidade de emulsões (MAKRI; DOXASTAKIS, 2006) e Rocha et al. (2012) utilizaram sílica e quitosana para imobilizar fungos dos gêneros *Aspergillus* e *Penicilium.*

A caracterização reológica da galactomanana extraída das sementes de *C. grandis* realizada por Albuquerque et al. (2014) demonstrou que este polissacarídeo é um fluido Newtoniano, podendo comportar-se tanto como líquido quanto como gel. Nesse sentido, o gel à base da galactomanana extraída das sementes de *C. grandis* possui características vantajosas para diversas aplicações biotecnológicas, incluindo sua atuação como efetivo suporte de imobilização para biomoléculas. O desenvolvimento tecnológico do gel de galactomanana como suporte de imobilização para lectinas desponta como uma estratégia promissora, especialmente no que se refere ao aumento da penetração e/ou disponibilidade da lectina no organismo.

3.7.2 Membranas como suportes de imobilização

Os polímeros, sejam naturais ou sintéticos, são moléculas cujas cadeias são longas e capazes de produzir matrizes contínuas que dão estrutura a filmes, membranas e revestimentos (MARTINS, 2003). A fim de evitar interpretações erradas, convêm fazer a distinção entre os termos mencionados: filmes ou membranas são películas formadas pela secagem (*casting*) de uma solução polimérica, a qual pode ser posteriormente aplicada em um produto; o revestimento, por sua vez, pode ser uma suspensão ou uma emulsão aplicada diretamente na superfície do produto e que, após secagem, leva à formação de um filme. Estes termos foram aperfeiçoados pela indústria alimentar para esclarecer a diferença entre revestimentos e filmes edíveis (PINHEIRO et al., 2010).

A preparação de filmes e revestimentos a partir de materiais biodegradáveis, a exemplo de polímeros naturais, tem despertado o interesse do meio científico nas últimas décadas, tendo em vista a necessidade de substituição dos polímeros sintéticos. De acordo com Yang e Paulson (2000), filmes e revestimentos desenvolvidos a partir de polissacarídeos constituem-se em excelentes barreiras ao oxigênio devido ao empacotamento das moléculas, formando uma rede estrutural ordenada através de ligações de hidrogênio, porém, existem algumas características higroscópicas que podem reduzir seu potencial para muitas aplicações.

Segundo Sobral (2000), as propriedades de filmes e revestimentos dependem do polímero usado, das condições de fabricação e das condições ambientais que são importantes fatores por causa da natureza higroscópica dos polímeros. A formulação destes produtos, na maioria das vezes, requer a utilização de plasticizantes, uma vez que filmes e revestimentos sem a adição do plasticizante apresentam uma estrutura frágil e dura devido às interações entre as moléculas do polímero (PINHEIRO et al., 2010).

Os plasticizantes são agentes de baixo peso molecular que, uma vez incorporados nos filmes e revestimentos, tornam-se capazes de se posicionar entre as moléculas do polímero. Eles interferem com as interações polímero-polímero e originam um aumento da flexibilidade e da capacidade de processamento (KROCHTA, 2002), além de melhorarem a resistência dos produtos à penetração de vapores e gases (SOTHORNVIT; KROCHTA, 2000). A água é um dos plasticizantes mais eficazes na composição de filmes e revestimentos, além da maioria dos demais plasticizantes também serem muito hidrofílicos e higroscópicos e poderem atrair moléculas de água. Devido a esta característica, a humidade relativa de armazenagem dos filmes e revestimentos torna-se uma das propriedades mais analisadas devido à influência da água na estrutura dos produtos formados (PINHEIRO et al., 2010).

Os surfactantes são substâncias anfipáticas devido às suas propriedades simultâneas de hidrofilicidade e hidrofobicidade e são geralmente adicionados para aumentar a estabilidade da emulsão na formulação de filmes e revestimentos. Os surfactantes podem ser incorporados para reduzir a tensão superficial da solução, melhorando a capacidade molhante dos produtos formados (KROCHTA, 2002).

Os polissacarídeos avaliados e/ou usados para formar filmes e revestimentos podem ser aplicados na área farmacêutica, em processos de encapsulação e liberação de princípios ativos; na indústria alimentícia, como filmes e revestimentos edíveis; na indústria de cosméticos; na área agrícola, como agente de liberação de pesticidas e nutrientes; entre outras aplicações. Os principais polissacarídeos reportados pela literatura científica sobre produção de filmes e revestimentos incluem: amido (ABREU et al., 2015; KIM et al., 2015; MENZEL et al., 2015), celulose (ASHOK et al., 2015; BEDANE et al., 2015; LEE; JEONG, 2015), alginatos (SOAZO et al., 2015; XIAO et al., 2015; ZHANG et al., 2015), carragenatos (FOUDA et al., 2015;

MEDEIROS et al., 2012; RHIM; WANG, 2014), quitosana (BIGUCCI et al., 2015; CHIEN; LIN; SU, 2013; GARCÍA et al., 2015) e gomas naturais, a exemplo do POLICAJU (CARNEIRO-DA-CUNHA et al., 2009; SOUZA et al., 2010b) e agar (SHANKAR; RHIM, 2015; SOUSA; GOLÇALVES, 2015).

Publicações científicas acerca da caracterização e aplicação de galactomananas como filmes e/ou revestimentos ainda são limitadas quando comparadas com aquelas já reportadas para outros polissacarídeos. Além deste fato, os principais trabalhos trouxeram novas perspectivas sobre as propriedades e a aplicação de filmes e revestimentos edíveis, como relatados a seguir.

Cerqueira et al. (2009b) estudaram a aplicação de revestimentos constituídos por galactomananas de diferentes fontes naturais (*Caesalpinia pulcherrima* e *Adenanthera pavonina*) em cinco frutas tropicais: acerola (*Malpighia emarginata*), cajá (*Spondias lutea*), manga (*Mangifera indica*), pitanga (*Eugenia uniflora*) e seriguela (*Spondias purpurea*). As propriedades de superfície dos cinco frutos foram determinadas para diferentes soluções aquosas das galactomananas acrescidas de glicerol. Lima et al. (2010) também utilizaram as galactomananas obtidas de *C. pulcherrima* e *A. pavonina* para revestir frutas, porém adicionaram colágeno e glicerol às soluções filmogênicas e avaliaram a aplicação dos revestimentos em mangas e maçãs. Cerqueira et al. (2009a), por sua vez, avaliaram a influência da temperatura de armazenamento na taxa de troca de gases em queijo revestido com galactomanana, enquanto Martins et al. (2012) estudaram as propriedades físico-químicas de filmes edíveis com diferentes concentrações da galactomanana do feijão alfarroba e k-carragenana. De maneira geral, revestimentos edíveis de galactomanana tendem a melhorar a aparência do alimento e podem ser utilizados como suportes de imobilização de conservantes naturais para reduzir o risco de contaminação microbiana, aumentando o tempo de prateleira dos alimentos revestidos com este polissacarídeo.

Importante salientar que a imobilização de compostos em filmes ou revestimentos de galactomanana deve ser avaliada quanto ao impacto na funcionalidade do produto final, uma vez que a molécula incorporada pode afetar propriedades funcionais básicas do polissacarídeo (CERQUEIRA et al., 2011a).

Martins et al. (2010) desenvolveram um filme da galactomanana extraída de *Gleditsia triacanthos* com nisina incorporada e obtiveram bons resultados na

prevenção de contaminação microbiana em queijos ricota. Cerqueira et al. (2010) usaram a mesma galactomanana de *G. triacanthos* para imobilizar extratos antioxidantes e implementar a ação antioxidante do produto final. Estes resultados demonstram que os trabalhos em que filmes de galactomanana são utilizados como suporte para imobilização de biomoléculas ainda são muito escassos, especialmente na área da saúde.

Valenga et al. (2012) realizaram a imobilização da lectina ConA (ligante glicose/manose) em filmes da galactomanana extraída das sementes de *Leucaena leucocephala*. Eles sugeriram que, como a espinha dorsal da galactomanana é composta por unidades β-d-manose em que algumas unidades de α-d-galactose estão ligadas na posição C-6, o reconhecimento de ConA pode acontecer através dos grupos OH na posição C-3 ou ainda C-6, caso esta última unidade de manose esteja livre. Levando em consideração que Cramoll 1,4 tem a sua especificidade correlacionada com a ConA, a interação galactomanana/Cramoll 1,4 tende a ocorrer através de ligações fracas, como pontes de hidrogênio e interações de Van der Waals, seguindo o padrão reportado por Valenga et al. (2012).

Apesar da gama de estudos sobre as aplicações biotecnológicas da Cramoll 1,4 na indústria farmacêutica, o uso terapêutico desta lectina no reparo de feridas ainda é pouco estudado. Devido às propriedades inerentes dos polissacarídeos e tendo em vista as características da Cramoll 1,4, a sua incorporação (imobilização) em filmes constituídos a partir da galactomanana da *C. grandis* possivelmente otimizará o efeito cicatrizante e promoverá a reabilitação de lesões cutâneas.

No que se refere ao aproveitamento da elevada área de superfície para a liberação de drogas, os sistemas nanoméricos têm maior eficácia no aumento da permeação da droga na pele do que muitos outros veículos (CHOKSI; POONAWALLA; WILKERSON, 2010). Sistemas nanoméricos e quercetina têm sido estudadas intensivamente (SOUZA et al., 2014), principalmente na área biológica (FANG et al., 2011; TAN et al., 2011), porém a pesquisa pela utilização da quercetina imobilizada em nanopartículas e apresentada em formulações à base da membrana da galactomanana extraída das sementes de *C. grandis* possivelmente representará uma inovação para os métodos de ação fotoprotetora e antioxidante, com consequente prevenção do envelhecimento cutâneo.

3.8 FERRAMENTAS PARA CARACTERIZAÇÃO FÍSICO-QUÍMICA DE BIOMOLÉCULAS

O consumo humano de polissacarídeos se estende por vários segmentos, principalmente devido às propriedades físicas que permitem o uso deste versátil material em diferentes aplicações. As galactomananas são polissacarídeos que formam soluções aquosas altamente viscosas; a variedade de propriedades físico-químicas torna este polímero um material bastante versátil, utilizado pelo homem de inúmeras formas nas indústrias alimentícia, farmacêutica, médica, cosmética, de papel, têxtil e mineração (SRIVASTAVA; KAPOOR, 2005; VIEIRA et al, 2007). Revelando a diversidade na sua aplicação, as galactomananas formam um grupo de polissacarídeos de especial interesse para a ciência aplicada.

A maioria das galactomananas utilizadas nas indústrias alimentar, farmacêutica e em cosméticos são gomas impuras, além da possibilidade de também serem utilizadas como matrizes para imobilização de outras biomoléculas, formando filmes, géis, hidrogéis e misturas binárias. A avaliação das características físico-químicas e reológicas das galactomananas, isoladas ou como matrizes de imobilização de biomoléculas, apresenta ampla aplicação no desenvolvimento de novos produtos, para determinar tanto a funcionalidade quanto a qualidade, e estabelecer, desta forma, as características do produto final.

Nesta seção serão descritas algumas das técnicas utilizadas neste trabalho para caracterização de biomoléculas.

3.8.1 Reologia aplicada à tecnologia

Reologia é a ciência que estuda a deformação e o fluxo dos materiais sob influência de tensões (BARNES et al., 1989). Dentro deste contexto, os materiais podem ser definidos como sólidos ou líquidos. Schramm (2006) define como sólidos ideais aqueles que se deformam elasticamente, ou seja, a energia necessária para a deformação é completamente recuperada quando a tensão é removida. Conforme o mesmo autor, fluidos ideais são aqueles que se deformam irreversivelmente, pois fluem sob ação de uma tensão. A energia requerida neste processo é dissipada em forma de calor e não é recuperada quando a tensão é retirada.

O tipo mais comum de deformação nos fluidos é por cisalhamento simples, que gera um escoamento caracterizado pelo movimento relativo das moléculas do fluido

devido à ação de uma força externa. Uma das propriedades medida na deformação de fluidos é a viscosidade, que assim como o módulo (para sólidos) é um fator determinante para o uso e aplicações dos materiais fluidos. Além de ser uma medida direta da qualidade do fluido em serviço, a viscosidade pode fornecer importantes informações sobre variações estruturais que ocorrem durante a aplicação de uma deformação ou tensão. A viscosidade, portanto, pode ser definida como a resistência ao movimento do fluir de um material (BARRA, 2015).

Desta maneira, na reologia de sólidos, a propriedade de maior interesse é a elasticidade, ao passo que em líquidos a viscosidade é a propriedade mais importante (TONELI; MURR; PARK, 2005). Entre os dois comportamentos extremos existem os materiais que se comportam ora como líquidos ora como sólidos, dependendo da tensão, da frequência ou da temperatura a que são expostos. Estes materiais são denominados de viscoelásticos (BARNES et al., 1989).

3.8.2 Espectroscopia de infravermelho transformada de Fourier

Uma das técnicas mais úteis para a identificação de biomoléculas é a espectroscopia de infravermelho transformada de Fourier (*Fourier Transformed Infrared Spectroscopy* - FTIR), que se baseia na análise de picos de absorção de determinados comprimentos de onda, expressos em cm^{-1} (GÓMEZ-ORDÓÑEZ e RUPÉREZ, 2011). Apresenta duas vantagens principais: requer pequenas quantidades de amostra (miligramas) e é um método não-agressivo com exatidão confiável (PEREIRA et al., 2003), no entanto, a técnica convencional necessita de procedimentos técnicos laboriosos para obter espectros com uma boa relação sinal/ruído (CHOPIN; WHALEN, 1993). Esta limitação foi superada com o desenvolvimento de técnicas de infravermelho interferométricas, associadas ao algoritmo transformado de Fourier, dando origem à espectroscopia de infravermelho transformada de Fourier (PEREIRA et al., 2009).

Na análise estrutural de polissacarídeos (MATHLOUTHI E KOENIG, 1987), cinco regiões podem ser distinguidas no espectro normal (entre 4000 e 650cm-1): (1) Região de estiramentos OH e CH, entre 3600 e 2800 cm-1; (2) Região de simetria local, entre 1500 e 1200 cm-1; (3) Região de estiramentos CO, entre 1200 e 950 cm-1; (4) Impressão digital ou região anomérica, entre 950 e 700 cm-1; (5) Região esquelética, abaixo de 700 cm-1. Dessa forma, informações sobre composição e

estrutura podem ser obtidos a partir do FTIR, para caracterização de biomoléculas.

3.8.3 Microscopia eletrônica de varredura

No estudo sobre a caracterização ou aplicação tecnológica das biomoléculas, é necessário observar a morfologia das amostras. A microscopia eletrônica é o método preferido para a visualização de detalhes estruturais com resolução em escala nanométrica. O grande poder de resolução de microscopia electrônica é resultado da aplicação de um feixe de elétrons com um comprimento de onda bem abaixo da gama nanométrica. A microscopia óptica, operando a comprimentos de onda (λ) no intervalo compreendido entre 400 e 800 nm, e abertura numérica (NA) ~ 1, com limite de difração d = λ / 2NA, dá uma resolução de aproximadamente 200 nm. Como o comprimento de onda de um feixe de elétrons é muito mais curto que o da luz, a resolução torna-se muito maior. Para um microscópio electrônico trabalhar com uma tensão de aceleração de 100 kV, o valor do comprimento de onda será de 0,0037 nm (SOUZA; CARNEIRO-DA-CUNHA, 2016). A microscopia eletrônica de varredura (MEV) é capaz de medir o tamanho, a distribuição de tamanho e a forma das biomoléculas; o princípio do referido microscópio é a utilização de um feixe de eletróns de pequeno diâmetro na investigação da superfície de uma amostra, ponto a ponto, em linhas sucessivas (KLANG et al., 2013). A interação entre a amostra e a sonda de elétrons produz vários tipos de emissões, que são captadas por diferentes detectores colocados em posições adequadas (SUGA et al., 2014)

3.8.4 Permeabilidade ao vapor de água, conteúdo de água, solubilidade e ângulo de contato

Permeabilidade ao vapor de água (WVP), conteúdo de água (MC), solubilidade (Sol) e ângulo de contato (CA) são metodologias geralmente utilizadas para caracterização física de estruturas compostas ou imobilizadas com biomoléculas. Cerqueira et al. (2010) relatam a WVP como a propriedade mais estudada na caracterização física de filmes e revestimentos devido à importância da água nas reações de deterioração. A capacidade de absorção de água é uma característica que pode afetar significativamente as propriedades físicas e de barreira exercidas por determinadas biomoléculas, por isso o conhecimento do conteúdo e da afinidade das biomoléculas pela água, respectivamente através dos métodos MC e Sol, é um

parâmetro-chave para a escolha em aplicações tecnológicas (KOWALCZYK et al., 2015). A determinação do CA é uma maneira simples de avaliar o grau de hidrofobicidade de biomoléculas dispostas em superfície, ou imobilizadas em alguma superfície, uma vez que os valores de CA aumentam com a diminuição da hidrofilicidade das amostras. Geralmente, se o CA for menor que 90º, a biomolécula é considerada hidrofílica (ALBUQUERQUE et al., 2017; MA et al., 2016).

3.8.5 Propriedades mecânicas

As propriedades mecânicas são características importantes a serem determinantes para biomoléculas com potencial aplicação industrial, devido ao manuseio a que estão sujeitos os produtos durante a sua distribuição e comercialização (OLIVAS; BARBOSA-CÁNOVAS, 2005). A força de tensão, o alongamento e o módulo elástico são as propriedades mecânicas mais comumente avaliadas em biomoléculas moldadas como filmes e revestimentos, ou ainda imobilizadas nestas matrizes. A força de tensão determina o esforço máximo desenvolvido no material em um teste elástico no momento de ruptura. O alongamento indica a capacidade de estiramento do material (SOUZA et al., 2010). Estas propriedades são muitas vezes relacionadas com as propriedades térmicas dos polímeros, como é caso da temperatura de transição vítrea, que é um dos parâmetros mais importantes na determinação das propriedades mecânicas de materiais no estado amorfo ou cristalino (PINHEIRO et al., 2010). Outro fator do qual dependem os valores da força de tensão e o alongamento dos materiais é a umidade relativa, cujo aumento provoca também o aumento da força de tensão e do alongamento devido ao acúmulo de água no material (OLIVAS; BARBOSA-CANOVAS, 2004). Do mesmo modo, a quantidade de plastificante utilizada afeta as propriedades mecânicas dos materiais (GENNADIOS et al., 1994)

3.8.6 Espalhamento dinâmico de luz e potencial zeta

Espalhamento dinâmico de luz (*Dynamic light scattering* - DLS) e potencial zeta são técnicas geralmente empregadas na caracterização de biomoléculas menores que 100nm, ou seja, nanomateriais. O prefixo “nano” está relacionado a uma escala de medida em que um nanômetro representa um bilionésimo do metro ou um milionésimo do milímetro. Estruturas nessa escala apresentam propriedades

funcionais únicas não encontradas na escala macro, especialmente sua elevada área de superfície, o que torna estes materiais muito mais reativos. Como resultado, os materiais com tamanho nano absorvem calor facilmente e a temperatura de fusão diminui em caso de sólidos (ASSIS et al., 2012). Este é apenas um exemplo de como os nanomateriais diferem dos demais materiais. Por definição, a nanotecnologia refere-se à tecnologia em que a matéria é manipulada à escala atômica e molecular para criar novos materiais e processos com características funcionais diferentes dos materiais comuns. Estes materiais não possuem necessariamente tamanho nanométrico, mas possuem em sua composição estruturas nanométricas que geraram novas propriedades e aplicações (SOUZA; CARNEIRO-DA-CUNHA, 2016).

O DLS é amplamente empregado na caracterização de nanomateriais, tais como partículas, emulsões e moléculas que foram dispersas ou dissolvidas em um líquido. O movimento browniano das partículas ou moléculas em suspensão faz com que a luz laser seja espalhada com intensidades diferentes. A análise dessas flutuações de intensidade resulta na velocidade do movimento browniano e assim, o tamanho de partícula usando a relação Stokes-Einstein (SABERI et al., 2013). É uma técnica especialmente utilizada para medir o tamanho e a distribuição de nanopartículas (CARO et al., 2016; SOUZA et al., 2014), nanoemulsões (SARI et al., 2015) e de soluções de polieletrólitos (CARNEIRO-DA-CUNHA et al 2010, MEDEIROS et al 2012; ZHANG et al 2014) usando a ideia de que pequenas partículas em suspensão se movem aleatoriamente e, portanto, chocam-se, promovendo o movimento browniano (MALVERN, 2004).

O potencial zeta é uma medida da magnitude da repulsão ou da atração eletrostática ou das cargas entre partículas, sendo um dos parâmetros fundamentais que, sabidamente, afetam a estabilidade dos materiais. Sua medição oferece uma visão detalhada sobre as causas da dispersão, agregação ou floculação, podendo ser aplicada para melhorar a formulação de dispersões, emulsões e suspensões formuladas à base de polissacarídeos, proteínas e lipídeos nanoestruturados (SOUZA; CARNEIRO-DA-CUNHA, 2016). O valor do potencial zeta pode ser relacionado à estabilidade de dispersões coloidais indicando o grau de repulsão entre partículas adjacentes e similarmente carregadas em uma dispersão. Sendo assim, um alto valor de potencial zeta indica que a solução ou dispersão resistirá à agregação; quando o potencial é baixo, a atração excede a repulsão e, portanto, a solução

floculará (MALVERN, 2004). Em geral, a linha que divide as suspensões em estáveis e instáveis é marcada pelo valor de +30 mV ou -30 mV, considerando-se estáveis os valores iguais ou superiores a estes (SOUZA; CARNEIRO-DA-CUNHA, 2016).

3.9 PROCESSO DE CICATRIZAÇÃO

A cicatrização é definida como um processo de reabilitação de feridas que conta com uma sequência de reações físicas, químicas e biológicas cuja finalidade é reconstituir a continuidade tecidual que foi interrompida. O evento é tão dinâmico que envolve componentes da parede celular, adesão e ativação plaquetária, inúmeros mediadores químicos, células inflamatórias (como leucócitos polimorfonucleares, macrófagos e linfócitos) e a fibronectina, os quais se comportam de forma harmoniosa a fim de garantir a restauração tissular (MANDELBAUM; DI SANTIS; MANDELBAUM, 2003; MODOLIN; BEVILACQUA, 1985).

A reabilitação de feridas é um processo universal e pode ser didaticamente dividido nas seguintes fases: hemostasia, fase inflamatória, fase proliferativa (ou de granulação) e fase de remodelação da matriz extracelular (SHIMIZU, 2005). Durante a evolução deste processo, as fases inflamatória, proliferativa e de maturação podem ser sequencialmente evidenciadas (KUMAR et al., 2005). Cada fase apresenta uma célula ou substância particular sem a qual o processo não evolui normalmente, no entanto, uma grande variedade de fatores pode interferir em qualquer uma das fases da cicatrização, sendo os principais fatores locais listados: sangramentos, tensão de oxigênio e contaminação microbiana (ARAÚJO et al., 1998; MENSAH et al., 2001).

Hemostasia refere-se à interrupção do sangramento de vasos sanguíneos lesados, através de interações reguladas com precisão entre os componentes da parede celular, adesão e ativação plaquetária e formação de uma trama de fibrina. Qualquer anormalidade decorrente do processo hemostático manifesta-se através de incapacidade de coagular normalmente (e, por conseguinte, de evitar a ocorrência de graus anormais de perdas sanguíneas) ou incapacidade de prevenir a coagulação excessiva (e, assim, de manter a integridade dos vasos sanguíneos) (RAVEL, 1997).

A coagulação do sangue envolve um sistema biológico de amplificação onde poucas substâncias de iniciação ativam, sequencialmente e por proteólise, uma cascata de proteínas circulantes precursoras (os fatores da coagulação), culminando na geração de trombina e, logo após, conversão de fibrinogênio em fibrina. A fibrina

infiltra os agregados de plaquetas nos locais de lesão vascular e transforma os tampões primários e instáveis de plaquetas em tampões hemostáticos firmes, definitivos e estáveis. As reações mediadas na superfície ocorrem com componentes específicos importantes: colágeno exposto, fosfolipídeo das plaquetas e fator tissular (HOFFBRAND; PETTIT; MOSS, 2004).

A fase inflamatória tem início logo após o dano tecidual e é caracterizada por vasodilatação local e sintomas como edema, calor, rubor e dor. O maior fluxo de sangue traz consigo proteínas séricas, fatores da coagulação e plaquetas, as quais, em sua forma ativada, garantem a eficiência do processo hemostático, liberam fatores de crescimento, fibrinogênio e fibronectina, e atraem células inflamatórias (KOOPMAN, 1995).

As células circulantes no foco inflamatório incluem neutrófilos, monócitos, eosinófilos, linfócitos, basófilos e plaquetas. Os neutrófilos são as primeiras células recrutadas, aparecendo aproximadamente 24 horas após a lesão, com funções de fagocitose e debridamento de tecidos necrosados, para minimizar a possibilidade de infecção do tecido agredido (PARK; BARBUL, 2004).

Os macrófagos migram ao local da ferida em cerca de 48 a 96 horas, tornando-se a população celular predominante antes da migração e proliferação dos fibroblastos. Sua ação antimicrobiana ocorre devido à geração de radicais reativos, como óxido nítrico e peróxido de oxigênio. Destacam-se por comandarem as demais células durante os eventos que determinam a cicatrização devido à capacidade de produzirem numerosas citocinas, como: Fator de Crescimento derivado de Plaqueta, Fator de Crescimento Transformante β, Fator de Crescimento Epidermal, Fator de Necrose Tumoral e Fator de Crescimento de Fibroblastos. A secreção de citocinas mencionadas e também de fatores de crescimento ativa e recruta outras células envolvidas no processo cicatricial (outros macrófagos e linfócitos), regula a quimiotaxia e a proliferação de fibroblastos, a síntese de colágeno, além da migração e replicação das células endoteliais, também envolvidas no processo de reparação tecidual (CHO et al., 2010; SCHIRATO et al., 2006).

A fase proliferativa consiste principalmente na formação de tecido de granulação, reepitelização e contração da ferida. O número de fibroblastos aumenta consideravelmente, a matriz extracelular de fibronectina formada por estas células ajuda a preencher a ferida e, por conseguinte, evitar a contaminação microbiana. Em

resposta aos fatores de crescimento e à baixa tensão de oxigênio no centro da ferida, os fibroblastos proliferam e aumentam a produção de colágeno (especialmente tipos I e III), elastina, proteoglicanos e glicosaminoglicanos (COTRAN; KUMAR, ROBBINS, 2000; STEED, 1997).

O tecido de granulação tem grande capacidade de contração, o que diminui a área de superfície da crosta formada e facilita e epitelização. Os fibroblastos são agentes especiais na contração, já que após a proliferação inicial, eles se diferenciam em miofibroblastos e estes, por sua vez, estendem e retraem pseudópodes ligados a fibras colágenas, alinhando as margens da lesão e promovendo a união das arestas da ferida (GOMATHI et al., 2003). A conversão de fibroblastos em miofibroblastos é realizada por fatores de crescimento expressando a α-actina do músculo liso, os quais tornam os miofibroblastos células musculares lisas eficientemente contráteis (MARTIN, 1997).

Por volta do terceiro ou quarto dia da cicatrização, o tecido de granulação é rico em vasos, para dar suporte à nova formação tecidual. A angiogênese é um processo complexo e envolve pelo menos cinco eventos celulares: dissolução local dos capilares endoteliais da membrana celular basal; migração direta de células endoteliais; proliferação das células endoteliais; formação de tubos capilares para embasamento do plexo venoso; e formação de nova membrana. O abastecimento da matriz extracelular por fibroblastos é gradualmente substituído por colágeno, cuja expressão alcança o máximo em 2 ou 3 semanas após a lesão tecidual, passando do tipo III, abundantemente produzido no início do reparo, para o colágeno I, tipo predominante antes da ferida. Uma vez que a matriz colagenosa abundante tenha sido depositada na ferida, os fibroblastos param de produzir colágeno e o tecido de granulação é substituído por uma cicatriz relativamente acelular. Quando a ferida já está preenchida pelo tecido de granulação, a angiogênese cessa e muitos dos vasos desintegram-se como resultado da apoptose (ILAN; MAHOOTI; MADRI, 1998).

O processo de contração da ferida envolve uma interação regulada e complexa de células, matriz celular e citocinas. Durante os primeiros dias, é a crosta que contrai, consistindo principalmente de fibrina seca, formada quando o exsudato escapa da mesma, coagulando e ressecando. A função da crosta, portanto, é selar a ferida, isolando-a do meio ambiente e evitando contaminação microbiana (DANTAS, 2000).

O processo de remodelação ocorre durante a fase final de reparo e pode

continuar pelos próximos 6 a 18 meses, onde a matriz extracelular é sintetizada, depositada, contraída e remodelada. Durante esta fase, o tipo e a aparência da cicatriz são funções da força de tensão, da pressão e do suprimento de oxigênio na área da ferida, além da idade do paciente. A transição do tecido de granulação à cicatriz é dependente da síntese e catabolismo de colágeno em velocidade baixa. A degradação do colágeno é controlada por um grupo de enzimas proteolíticas designadas por metaloproteinases (MMPs), que consistem em colagenases, gelatinases e estromolusinas, as quais são secretadas por macrófagos, células epidermais e fibroblastos (MIGNATTI et al., 1996; FAZIK; ZITE; GOSLEN, 2000).

O tratamento utilizado em lesões cutâneas pode desempenhar um papel integral no processo de cicatrização de feridas. Tão logo a fisiologia da cicatrização tenha se tornado melhor compreendida nos últimos anos, os reparos cutâneos sofreram mudanças drásticas no sentido de melhorar o processo que vai da hemostasia à remodelação. Pesquisas nesta área têm como base três aspectos principais: melhoria do processo de cicatrização por fatores que aceleram o processo e reduzem a cicatrização; desenvolvimento de substitutos de pele como equivalentes funcionais; e indução da cura através da regeneração em lugar da reparação tecidual (BALASUBRAMANI et al., 2001).

As pesquisas mais recentes a cerca do processo de cicatrização são desenvolvidas pela engenharia de tecidos com uma gama de substitutos de pele que podem ser utilizados na prática clínica. Não obstante a estas inovações, membranas para cicatrização de feridas também podem ser usadas como revestimentos temporários ou permanentes (MORGADO; AGUIAR–RICARDO; CORREIA, 2015). Polímeros obtidos de fontes naturais têm sido amplamente estudados para a confecção de curativos devido às características de biocompatibilidade, biodegradabilidade, atoxicidade, baixo custo e boa disponibilidade, além da similaridade com tecidos humanos e, portanto, potencial ação cicatrizante (BOATENG et al., 2015; MOGOSANUA; GRUMEZESCU, 2014).

Inúmeras pesquisas científicas reportam a avaliação da cicatrização de feridas tratadas com membranas polissacarídicas, como as preparadas a partir de quitina, quitosana e seus derivados (ITO; YOSHIDA; MURAKAMI, 2013; NASERI et al., 2014) e celulose e seus derivados (WEN et al., 2015) e alginato (MOMOHA et al., 2015).

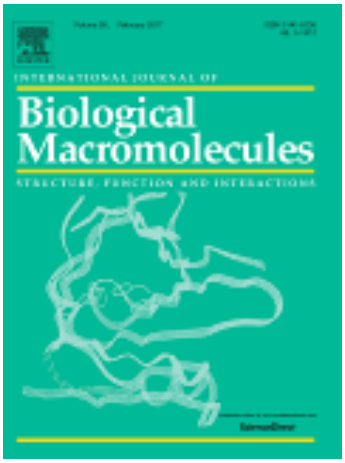
O mais importante no processo de cicatrização é manter a ferida oclusa e

úmida, portanto, a proposta de imobilizar Cramoll 1,4 em membranas da galactomanana extraída de *C. grandis* para avaliar a atividade cicatrizante em animais com feridas cutâneas desponta com vantagens no ramo científico devido ao fato das membranas funcionarem como tampões hemostáticos, mantendo a ferida fechada, úmida e suprida pela potencial atividade cicatrizante da lectina. Ainda com base na importância das propriedades de macrófagos e fibroblastos no processo de reparação tecidual, reveste-se de importância a avaliação da toxicidade das membranas de galactomanana, imobilizadas ou não com Cramoll 1,4, através da utilização de linhagens celulares deste tipo.

4 RESULTADOS

Artigo I

Investigating a galactomannan gel obtained from *Cassia grandis* seeds as immobilizing matrix for Cramoll lectin



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Investigating a galactomannan gel obtained from *Cassia grandis* seeds as immobilizing matrix for Cramoll lectin

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ABSTRACT

Characterization, with emphasis on the rheological properties, of *Cassia grandis* seeds galactomannan gel containing immobilized Cramoll 1–4 is presented. The gels, with and without immobilized Cramoll 1–4, were evaluated along time by rheometry, pH, color, microbial contamination and lectin hemagglutinating activity (HA). Rheological determinations confirmed the gels to be very stable up to 30 days with variations occurring after this period. Rheological data also showed that the gel/Cramoll 1–4 immobilizing matrix loses its elastic modulus substantially after 60 days. Both gels presented no microbial contamination as well as a pH close to neutral. Colorimetric parameters demonstrated the gels transparency with occasional yellowness. The opacity of the galactomannan gel did not change significantly along the study; the same did not occur for the gel with immobilized Cramoll 1–4 as a statistically significant reduction of its opacity was observed. In what concerns immobilized Cramoll 1–4 HA, up to 90% of its initial HA was maintained after 20 days, with a decrease to 60% after 60 days. These results combined with the thickening and stabilizing characteristics of the galactomannan gel make this gel a promising immobilizing matrix for Cramoll 1–4 that can be further exploited for clinical and cosmetic applications.

1. Introduction

Polysaccharides isolated from natural sources have been attracting much attention in the biochemistry and pharmacology fields [1] thanks to their bioactivity, biocompatibility, low cost and availability [2]. Galactomannans extracted from the endosperm of numerous plants (particularly Leguminosae) are polysaccharides widespread in nature. Their basic structure is composed by a central core of α(1 → 4)-linked D-mannopyranose to which β(1 → 6)-linked α-D-galactopyranosyl units are attached. High molecular weight, non-ionic character [3], and water solubility allow these systems to forming highly viscous and stable solutions at low concentrations [4].

Galactomannans are used in food, pharmaceutical, biomedical, cosmetic, textile and paper industries. Many studies have reported the potential use of galactomannans as thickeners [5], part of mixed systems such as hydrogels [6], emulsion stabilizers in the preparation of films [7] and edible films themselves with enhanced barrier as well as mechanical properties that extend and improve food shelf-life [8,9]. It is also important to highlight the application of galactomannans in the cosmetic industry [10] and as matrix for immobilization of certain biomolecules [11–14].

Galactomannan solutions exhibit non-Newtonian behavior and their rheological properties are also connected to their concentration [15], molar mass [5], and the mannose/galactose (M/G) ratio [16]. In order to guarantee the application efficacy of galactomannans in cosmetic and pharmaceutical fields, a full rheological characterization is usually necessary [2] as is the case of the Cross

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model [17] that has been used successfully to describe the shear-thinning properties of galactomannans [18,19].

Albuquerque et al. [20] have worked at an extensive rheological characterization of the galactomannan extracted from the seeds of *Cassia grandis*, a typical Brazilian tree, and observed that it presents fluid behavior, with Newtonian plateau followed by shear-thinning zones up to 1.5% (w/v) concentrations; above this value, the galactomannan behavior can be considered as that of a polymer solution of entangled strands. At 2.0% concentration, rheological data indicate that the system has reached a gel-like state which, in rheological terms, is one where the elastic modulus G′ is larger than the viscous modulus G″ and their dependence on oscillatory test frequency is low. The stress-strain study allowed a more accurate evaluation of this fluid–gel transition indicating that galactomannan concentrations higher than 1.7% (w/v) could be suitable for a range of applications including immobilizing matrix for certain biomolecules.

Lectins constitute a protein heterogeneous group of non-immune origin, containing two or more binding sites to mono or oligosaccharides [21]. The lectin extracted from *Cratylia mollis* seeds, also known as Cramoll, is a biomolecule with different molecular forms (Cramoll 1, Cramoll 2, Cramoll 3 and Cramoll 4) that has been well studied by structural analysis and employed in several biotechnological applications. In addition, its specificity is correlated with the so studied Concanavalin A lectin. Cramoll 1, Cramoll 2, and Cramoll 4 specifically bind to glucose or mannose, while Cramoll 3 is a galactose-specific glycoprotein [22,23]. Preparations containing isoforms 1 and 4 (Cramoll 1–4) were reported as presenting promising activities: antitumor [24], anti-parasitic [25,26], anti-inflammatory [27,28] and mitogenic [29,30]. Cramoll 1–4 is also able to bind, isolate, and characterize human plasma glycoproteins [31] and to act as a wound healing agent [32].

The technological development of novel forms for lectin immobilization has been a promising strategy to increase the penetration of drugs through the skin. The galactomannan gel obtained from the seeds of *C. grandis* has advantageous features for topical applications, especially due to the intimate contact made with the stratum corneum. Regarding these promising characteristics, developing an immobilizing matrix for Cramoll 1–4 based on the galactomannan gel would be a very important first step towards an improved drug delivery system for topical applications. By utilizing a series of tests (pH, colorimetry, rheometry, microbial contamination, and biological activity), the performance of the galactomannan gel at 1.7% (w/v) concentration, to work as a matrix for immobilizing Cramoll 1–4 was evaluated in this work. The above mentioned tests were conducted on specific days along a total experimental interval of 90 days, during which gel matrix samples were stored under controlled temperature and relative humidity conditions. Samples of the original gel, with no Cramoll 1–4, submitted to the same conditions of the immobilizing matrix system, were used for controlled comparison.

2. Materials and methods

The pods of *C. grandis* were collected at the rural zone of Pernambuco State, in the city of Angelim (Brazil), in July 2011. Ethanol 99.8%, acetone PA, sodium chloride and phenol were obtained from Vetec Fine Chemicals Ltd. (Brazil). All other chemicals were of analytical grade.

2.1. Extraction of the galactomannan from C. grandis seeds and gel preparation

The galactomannan from *C. grandis* seeds was obtained according to Albuquerque et al. [20]. Briefly, *C. grandis* pods were immersed in distilled water at 25 °C for 18 h and then separated in a half part, revealing the seeds trapped in a black and stick mass. The seeds were removed and placed dried until reaching a constant weight. The dry seeds were boiled in distilled water 1:5 (w/v) at 100 °C for 1 h for enzyme inactivation and maintained in water by 18 h at 25 °C to facilitate removal of the hull. After this period, the hull was removed and the residual was triturated in a blender with 0.1 M NaCl 5% (w/v) at 25 °C, filtered through a voil tissue and after using a screen printing cloth, and precipitated with 46% ethanol 1:3 (v/v) for 18 h. The white precipitate obtained was washed with 100% ethanol 1:3 (w/v) for 30 min and two times with acetone PA 1:3 (w/v) for 30 min, been filtered on screen printing cloth between each washing. The precipitate was dried until constant weight, pulverized and finally called galactomannan.

The galactomannan was dissolved in distilled water at 1.7% (w/v) under magnetic stirring (500 rpm) for 12 h at room temperature (25 °C). The obtained gel was stored at 4 °C and 80% relative humidity, been called pure galactomannan gel.

2.2. Extraction of the lectin from C. mollis seeds and immobilization on the galactomannan gel

Cramoll, containing its isoforms 1 and 4 (Cramoll 1–4) was obtained according to Correia and Coelho [22]. Briefly, the seeds were dried at 25 °C and crushed to obtain a flour, which was dissolved in 0.15 M NaCl 10% (w/v), under magnetic stirring (500 rpm) for 18 h at 4 °C. This saline extract was fractionated with ammonium sulphate (0–40% and 40–60%). The supernatant of the 40–60% fraction was dialysed and purified by affinity chromatography on Sephadex G-75 column.

Cramoll 1–4 was immobilized by entrapment on pure galactomannan gel as follows: 100 μg/ml of Cramoll 1–4 were added to the pure galactomannan gel under magnetic stirring (500 rpm) for 1 h at room temperature (25 °C). The gel with immobilized Cramoll 1–4, called gel/Cramoll 1–4 immobilizing matrix, was stored at 4 °C and 80% relative humidity.

The activity of Cramoll 1–4 free solution and Cramoll 1–4 immobilized on the gel matrix was evaluated by hemagglutinating activity (HA) assay according to the methodology described by Correia and Coelho [22], using a suspension [2.5% (v/v)] of rabbit erythrocytes treated with glutaraldehyde [33]. All HA results were expressed as log.

2.3. Stability of the pure galactomannan gel and the gel with immobilized Cramoll 1–4

The stability of the pure galactomannan gel and the gel/Cramoll 1–4 immobilizing matrix was evaluated by pH, colorimetry, and rheology for 90 days, been examined on the following days: 1, 5, 10, 20, 30, 60 and 90. All gel/Cramoll 1–4 immobilizing matrix samples and pure gel samples were prepared following systematic methods and controlled conditions where distinct samples, from the same initially prepared batch, were tested along different periods of storage.

The pH determination was conducted on a digital pH meter where two buffer solutions with pH 4.00 and 7.00 were employed for calibration. Three measurements of pH for each sample were performed for statistical analysis and their average was used. The color quantification was performed by a digital colorimeter (Konica Minolta, model CHROMA METER CR-410, Osaka, Japan) calibrated at illuminant C with a white standard. The parameters determined were L^* (L^* = 0 [black] and L^* = 100 [white]), a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness). These parameters are the ones recommended by the International Commission on Illumination. The opacity (Y) was calculated according to the Hunterlab color scale as the ratio between opacity of

each sample on the black standard (Yb) and opacity of each sample on the white standard (Yw). The results were expressed as a percentage: Y(%) = 100 (Yb/Yw). The analyses of mesophilic aerobes, yeasts and molds, lactic acid bacteria and psychrotrophs, to evaluate the microbiological contamination of the both gels, were carried according to the methodology described by the Compendium of Methods for the Microbiological Examination of Foods [34]. All measurements were performed in triplicate and the results expressed as mean ± standard deviation.

The mechanical stability studies were conducted on a stress controlled rheometer (Anton Paar MCR 301) equipped with a temperature controller. A circular parallel plate cell with internal diameter (i.d.) = 25 mm and a height (h) = 1 mm gap between plates was employed in the experiment. All rheology experiments were conducted at 25 °C. In order to guarantee reproducibility, for every experiment it was run a newly prepared sample, obtained from the same batch.

Stress-strain tests, rotational flow, and oscillatory viscoelastic linear-response measurements have been performed. The rheometer was operated in the oscillatory mode to the stress-strain tests: for a fixed frequency $\omega = 2$ rad/s, an externally applied shear stress varied from 1 to 300 Pa and the storage (G′) and loss (G″) moduli were recorded. That allows the sample mechanical responses to cover from the reversible linear response regime up to the nonlinear region, where the sample structure collapses and flow starts. Each experimental point was recorded after an exposure period of 5 s. The flow study was conducted in continuous steady state shear rotational mode by varying the shear-rate from 1 to 1000 s while acquiring 50 experimental points per run. The dynamic oscillatory mode study was conducted varying the applied torque/deformation angular frequency from 1 to 500 rad/s. The storage (G′) and loss (G″) modulus sample responses were recorded during frequency sweeps at 0.2% fixed strain amplitude, enough to guarantee linear reversible responses. Again, each experimental point was recorded after a 5 s exposure period.

2.4. Statistical analysis

The results were expressed as mean ± standard deviation, submitted to ANOVA (one-way) and Tukey's multiple comparison test. The values were considered statistically significant if compared to the significance level of $p \leq 0.05$. All statistical and graphical analyses were carried out with the Statistica 8.0 program (StatSoft Inc., 2008, Tulsa, OK, USA).

3. Results and discussion

The galactomannan extracted from the seeds of *C. grandis* showed extraction yield of 36 ± 8% and presented a mixture of 71.0% mannose and 29.0% galactose, identical to the reported by Albuquerque et al. [20]. No additional sugars were detected in the NMR spectra of this polysaccharide and the mannose/galactose ratio was 2.44:1. Cramoll 1–4 was efficiently extracted with the same chromatographic and electrophoretic profile reported by Correia and Coelho [22].

3.1. Stability investigation

3.1.1. pH value

The ability of galactomannans to form viscous solutions at relatively low concentration is their greatest advantage [17]. Furthermore aqueous solutions of galactomannan are basically neutral [35] and their viscosity is insensitive to pH variations (a broad range from 1 to 10.5); nevertheless some degradation may occur under highly acidic or alkaline conditions [7,35].

The pH stability assay resulted in values of 6.82 ± 0.16 for the pure galactomannan gel and 6.80 ± 0.39 for the gel/Cramoll 1–4 immobilizing matrix. The measured values were based on averages where no statistically significant variations ($p \geq 0.05$) over time were found. The viscosity and the pH stability of galactomannans depend on the initial preparation as well as the storage conditions. In our case, the close to neutral pH measured for both gels over time is considered optimum for most biotechnological applications.

3.1.2. Color

Color and appearance are important features for selection or acceptance of a product from food industry [36] to the cosmetics [37]. The galactomannan gel obtained from *C. grandis* seeds, with and without immobilized Cramoll 1–4, was characterized by instrumental colorimetry to ensure that color variations over storage time would not adversely affect the properties of product conservation.

The colorimetric L^*, a^* and b^* parameters of the pure galactomannan gel and the gel/Cramoll immobilizing matrix were expressed based on averages of the measured values, which did not showed statistically significant differences ($p > 0.05$) over time. The measured values for pure galactomannan gel were L^* (91.74 ± 1.32), a^* (−0.40 ± 0.14) and b^* (4.66 ± 0.40), similar to those obtained for gel/Cramoll 1–4 immobilizing matrix: L^* (88.73 ± 2.97), a^*(−0.36 ± 0.18) and b^* (5.00 ± 0.54). Both the pure galactomannan gel and the gel/Cramoll 1–4 immobilizing matrix had a strong whiteness tendency, represented by the L^* coordinate, a weak green color and special evidence for the yellowness appearance, respectively due to a^* and b^*. These steady values indicated minimal loss of color and thus confirmed the preservation of the gel initial state over time. This emerges as an advantage for the natural products (extracted from seeds) when compared to those obtained by fruit puree, which have the natural tendency to browning [38]. In addition, the pure galactomannan gel did not present significant color variations as those constituted, for example, by banana pulp, which tend to vary from deep yellow to red due to chemical reactions such as Maillard [38].

Opacity indicates the capacity of biopolymers for acting as barrier to light; and also can be reported as a way to relate their higher or lower miscibility [39]. The pure galactomannan gel has a three-dimensional network with a natural and stable organization, which might occur due to the high degree of substitution of galactose units in the main chain of mannose. Statistically, the opacity was not significantly different during the storage period for the pure galactomannan gel (10.49 ± 1.14). In turn, the gel/Cramoll 1–4 immobilizing matrix presented a statistically significant variation for the opacity value, with a decrease from 12.39 ± 0.64 on day 1 to 8.53 ± 0.38 on the 30th day. A small increase occurred on the 60th day (12.76 ± 1.66) and another reduction on the 90th day, at the final of the experimental time (9.83 ± 1.29). These results suggest that opacity depends on the structure level of the galactomannan as well as the interactions with other biomolecules; furthermore the opacity loss as a function of structure integrity is also corroborated by the rheological results reported in Section 3.1.3, where the apparent viscosity is reduced for a long storage period.

Our results are in agreement with Soares et al. [6], who developed a new hydrogel based upon optimized rheological characteristic of a mixture of κ-carrageenan and the galactomannan extracted from *C. grandis* seeds at the same concentration as that from our work. They also observed changes in opacity with the aging of the hydrogel, thus confirming the reorganization of the polysaccharide chains of the hydrogel that was reflected in a less opaque macroscopic sample.

3.1.3. Rheological study

Polysaccharide solutions are considered viscoelastic materials because they exhibit both solid (elastic) and viscous (liquid) char-

acteristics. The elastic and viscous nature of these materials is described respectively by the storage (G′) and loss (G″) linear moduli. Accordingly, it is possible to quantify the predominance of elastic or viscous character of a sample by submitting it to some external stress while monitoring the above mentioned responses [40–42]. G′ and G″ are key parameters for describing the mechanical stability of a system. For instance, how much stress the system can sustain before flowing or how its viscosity changes as the shear rate grows are fundamental for certain applications.

A rheological investigation was conducted aimed at evaluating the mechanical stability of the gel/Cramoll 1–4 immobilizing matrix composed by Cramoll 1–4 mixed into the pure galactomannan gel. The mechanical stability was evaluated by obtaining the viscoelastic properties of the immobilizing gel matrix via rheometry measurements, as a function of the sample storage period, divided into the following steps: (i) A stress-strain experiment where the matrix was submitted to a stress and where the linear moduli G′ and G″ were monitored. This measurement starts at a linear reversible regime (small strain) but eventually reaches a non-linear strain deformation regime where the sample structure fails with subsequent flowing; (ii) a flow curve where the gel/Cramoll 1–4 immobilizing matrix shear stress response was monitored as a function of a strain rate. This is a measurement where the matrix is submitted to a large destructive strain variation as a function of time; (iii) An oscillatory stress study where the gel/Cramoll 1–4 immobilizing matrix was submitted to a low deformation regime (0.2% strain) of oscillations and where the linear elastic (storage), G′, and viscous (loss), G″, moduli were monitored as a function of a variable oscillatory frequency. In this last experiment, specific structure length scales can be probed non-destructively. The pure galactomannan gel was submitted to the same set of experiments and was used as a control for monitoring the effect of the Cramoll 1–4 upon the immobilizing gel matrix mechanical properties.

3.1.3.1. Stress-strain test. The stress-strain test works as a preliminary investigation where one can separate the linear from non-linear structure response regime as well as identifying the elastic, G′, and loss, G″, moduli contributions. The Linear Viscoelastic Region (LVR) is considered as the one encompassing the area where the structure is deformed but not destroyed, represented by the constants G′ and G″, independent of stress or strain. This regime indicates the toughness—area under the curve—of the material. On the other hand, the values of G′ in this region indicate the stiffness of the material, or the degree of deformation for a given stress.

The results in Fig. 1A and B depict G′ and G″ moduli as a function of an applied shear stress for the pure galactomannan gel and the gel/Cramoll 1–4 immobilizing matrix, respectively. To help visualizing the data, we have depicted the 5 and 60 days data as representative of the entire storage period. Nevertheless, on Fig. 1C and D, the values of G′ and G″, extracted from the LVR from Fig. 1A and B respectively, are presented for the entire storage period recorded.

For the pure galactomannan gel, Fig. 1A and C, the results indicate that the gel suffers some mild stiffening—an increase on G′—during the storage period. G′ and G″ are almost identical with G″ > G′, at Fig. 1A, for the storage period of 5 days. We should mention that G′ ≫ G″ in a stress-strain test is a rheological signature for a strong elastic gel system. The similarity of G′ and G″ values is then indicative of a weak/soft gel behavior. This behavior is in agreement with Albuquerque et al. [20]. For the 60 days storage period, G′ > G″, reaching though a cross-over, point where G′ = G″ = 50 Pa, for a stress around 100 Pa. The latter will be considered the stress at which the gel structure fails completely and the system flows. Fig. 1C shows the data, G′ and G″ at the LVR, for the entire storage period. Initially, G′ is approximately equal to G″ but, as the storage period increases, a reduction of both moduli up to 20 days is observed. This reduction is followed by an increase of both moduli with a concomitant separation of their values. A gel stiffening as a function of storage period, for periods longer than 20 days, is indicated by the increase in the difference between G′ and G″. We should reinforce that the galactomannan gel behavior depends on concentration.

The moduli-stress curves for the gel/Cramoll 1–4 immobilizing matrix, Fig. 1B and D, indicate that the system suffers both G′ and G″ reduction as a function of the storage period. The data in Fig. 1B, for the storage period of 5 days, is essentially identical to that for the pure galactomannan gel for the same period. However, for the storage period of 60 days, there is a substantial decrease, almost one order of magnitude seen in Fig. 1B, for both moduli. Moreover, the gel/Cramoll 1–4 immobilizing matrix structure for the storage period of 60 days fails at a smaller stress value of approximately 20 Pa. It is important to note a modest maximum for G″ at this limit value. This maximum, known as weak strain/stress overshoot, indicates the presence of microstructures that required extra stress in order to align with the flow. The microstructure is not strong enough to cause a similar increase in the elastic modulus, common in shear thickening systems [43]. Fig. 1D shows the entire storage period data. One can notice G′ approximately equal to G″ for the entire interval with both moduli decreasing as a function of the storage period. The variation in moduli is more substantial than that observed for the pure galactomannan gel and, moreover, moves in the opposite direction. The gel/Cramoll 1–4 immobilizing matrix shows no variation in its moduli up to 20 days storage period. Nevertheless both moduli reduce substantially for longer periods, the system is said to soften as opposed to the stiffening that occurs in the pure galactomannan gel measurements. The latter is probably due to some aging of the pure gel structure along storage period.

A potential explanation for the decay on G′ and G″ moduli for our immobilizing gel matrix comes from Valenga et al. [13] study on the immobilization of the lectin extracted from *Canavalia ensiformis* seeds, also known as ConA, onto a monolayer made of a galactomannan extracted from *Leucaena leucocephala* seeds. They suggested that because the galactomannan backbone is β-D-mannose (1 → 4) linkage and some units at the C-6 position carries α-D-galactose units, the recognition of ConA might be for OH groups at the C-3 and C-6 position for the mannose units not substituted. Cramoll 1–4 has its specificity correlated with the ConA, then the interaction between the galactomannan extracted from *C. grandis* and Cramoll 1–4 might also occurs through weak monomeric bindings affinities such as hydrogen bonds and Van der Waals interaction, resulting in some depolymerisation in the central mannose core of the polysaccharide after a period of 60 days. The softening of the gel/Cramoll 1–4 structure as well as the appearance of a microstructure evidenced by the weak shear overshoot might be related with the biding processes described above. Moreover, this biding could also explain a potential degradation of Cramoll 1–4 for long storage periods as will be discussed in Section 3.2.

It is important to mention that, depending on the expected application of the immobilizing gel/Cramoll 1–4 immobilizing matrix, this substantial reduction may not be relevant.

3.1.3.2. Flow curves. The flow curves are experiments where the structure of the sample is tested against rotational flow shear rate and potential yield stresses are predicted. Since we are mostly interested in elastic immobilizing mechanical properties, stress response, where yield stresses can be obtained, instead of apparent viscosity, becomes more informative. Fig. 2 depicts shear stress response of the pure galactomannan gel (2A) and the gel/Cramoll 1–4 immobilizing matrix (2B) as a function of shear rate. The several plots present data for an interval of storage periods ranging from 5 to 90 days for the pure galactomannan gel (2A); and from 5 to 60 days for the gel/Cramoll 1–4 immobilizing matrix (2B).

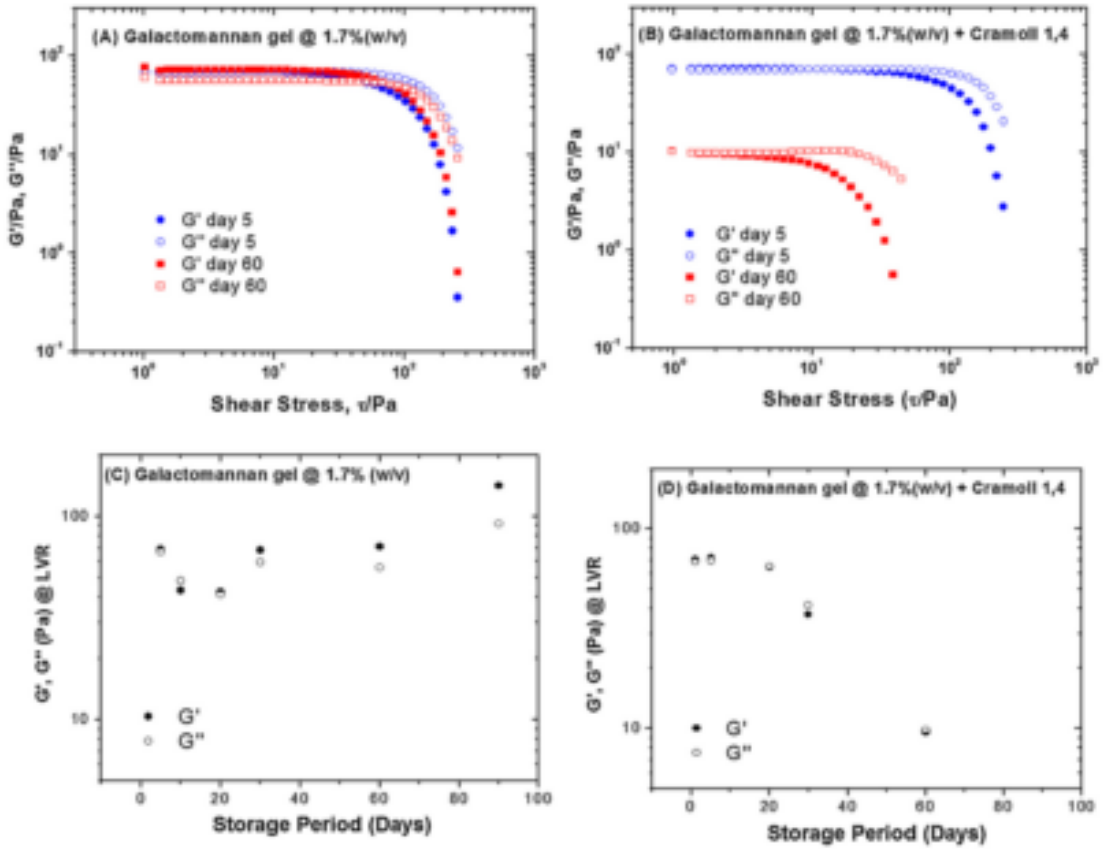

Fig. 1. Stress-strain experiment. The storage, G', and loss, G'', moduli are displayed as function of applied shear stress for the pure galactomannan gel at 1.7% (w/V) (A) and the gel/Cramoll 1–4 immobilizing matrix (B). The plots indicate storage periods of 5 and 60 days; (C) G' and G'' of the pure galactomannan gel obtained at the linear viscoelastic region (LVR) from (A) for several storage periods; (D) the same as (C) for the LVR obtained from (B).

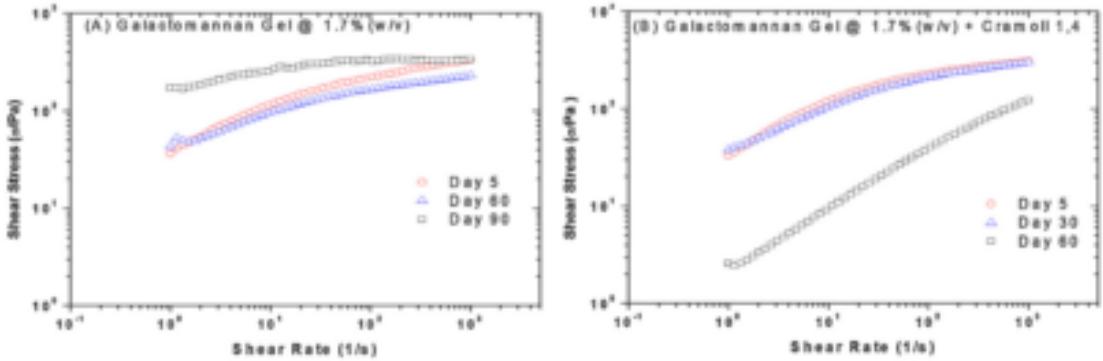

Fig. 2. Rotational flow experiments where the shear stress is measured as a function of the shear rate for the pure galactomannan gel at 1.7% (w/V) (A), and the gel/Cramoll 1–4 immobilizing matrix (B) for different storage periods.

The straight forward approach to describe shear stress σ as a function of shear rate $\dot{\gamma}$ is via the Herschel–Bulkley (HB) model [44]:

$$\sigma = \sigma_y k \dot{\gamma}^n, \quad for\, \sigma \geq \sigma_y \tag{1}$$

where σ_y denotes the yield stress, and the constant k is the consistency parameter associated with viscosity $\eta = k\dot{\gamma}^{n-1}$ The power n is a fitting parameter of the model describing how non-Newtonian ($n = 1$ for a Newtonian sample) the system is, with $0 < n < 1$ for shear-thinning, and $n > 1$ indicating shear-thickening. Fitting this model to the data on Fig. 2 one is able to obtain both n and the yield stress by extrapolating the experimental data towards zero shear rate. The $n = 0.12$ obtained indicate shear-thinning for the pure galactomannan gel identical to that obtained by Albuquerque et al.

[20]. Shear-thinning is also observed in the gel/Cramoll 1–4 immobilizing matrix but, as the storage period increases, a lower level of shear-thinning, and therefore lower n values are obtained. Overall, for storage periods from 5 to 30 days, both samples behave identically. However, for storage period of 60 days, the matrix exhibits a substantial decrease in the shear stress response, and therefore its apparent viscosity over the entire experimental shear rate interval. This reduction agrees with the stress-strain response already discussed. The extrapolated yield stress for the latter storage period is really small and can be neglected. The shear-thinning reduction, indicated by the value of n = 0.3, gives evidence to a reduction in the matrix structure length scale. One should consider at this point, from the rheology perspective, the gel/Cramoll 1–4 immobilizing matrix as a viscoelastic fluid.

The shear thinning behavior observed for the pure galactomannan gel (Fig. 2A) is similar to that reported for others galactomannans, such as those extracted from *L. leucocephala* seeds [45] and the most commercially used galactomannan, guar gum [46]. It is important to note though that this behavior is dependent on the molecular weight [5] the mannose/galactose ratio [19] and galactomannan concentration [18]. Moreover, the presence of Cramoll 1–4 appears not to be relevant regarding the degree of shear-thinning since both samples behave similarly within a given storage period from 5 to 30 days. Nevertheless, the pure galactomannan sample shows an increase in the stress response, as well as an increase in the yield stress, for the 90 days storage period. This is the same stiffening effect observed for the stress-strain measurements.

The gel/Cramoll 1–4 immobilizing matrix (2B), also kept under the same controlled storage conditions, presented similar stress response up to 30 days, decreasing its value significantly for 60 days storage period. The yield stress, obtained from the extrapolated theory fit, is negligible, again, for the latter storage period. This null yield stress agrees with the viscoelastic fluid signature observed by the stress-strain test.

3.1.3.3. Oscillatory results. Fig. 3 shows the results of G' and G'' moduli of the pure galactomannan gel (3A) and the gel/Cramoll 1–4 immobilizing matrix (3B) as a function of oscillatory frequency for a small amplitude oscillatory strain. The experiments were conducted keeping the samples at the LVR, where the gel structure is deformed reversibly and not destroyed, offering the opportunity to probe the microstructure of both systems as a function of the experimental storage period.

At the reversible small strain regime, the addition of Cramoll 1–4 on the pure galactomannan gel did not affect substantially, apart from a mild global reduction in both moduli, the rheological signature of a weak gel which consist of a small variation of G' and G'' as a function of frequency. The gel signature also agrees with visual observation of the samples standing against gravity. One can observe that both moduli continuously increase as a function of frequency for the storage interval depicted.

The cross-over between G' and G'' for both samples up to 30 days indicated a fluid behavior for small oscillation frequencies, dissipating most of the externally applied energy ($G'' > G'$), similar to the results reported by Albuquerque et al. [20] for the galactomannan extracted from *C. grandis* seeds at approximately 1.5% (w/v) and also to the oscillatory behavior observed for other galactomannans [45,47]. There was observed an inversion ($G'' < G'$) in the range between 3 and 9 Hz frequency, and the pure galactomannan gel acquired an elastic behavior (3A). The crossover disappears for the 60th day, so the two moduli evolved almost parallel with frequency increase; for the 90th day, the system had reached a gel state determined by an enhancement of G' and G'' values (data not shown).

The classical gel behavior (G' parallel to G'' as a function of frequency) was observed by Soares et al. [6] for the hydrogel composed by κ-carrageenan and the pure galactomannan gel. The pure galactomannan hydrogel was rheologicaly tested during 90 days period and a stiffening process was observed where both G' and G'' increase as a function of storage. An additional rheological study of the pure galactomannan gel as function of storage period may offer new opportunities for preparing solutions with smaller galactomannan concentrations that can exhibit a gel state after stiffening for a certain storage period.

3.2. Biological Activity of free Cramoll 1–4 and gel/Cramoll 1–4 immobilizing matrix

A range of Cramoll 1–4 concentrations was already evaluated: Maciel et al. [29] investigated Cramoll 1–4 in the mitogenic stimulation of human lymphocytes varying the lectin concentration from 0.78 to 100 μg/ml; Melo et al. [32] examined, *in vivo*, clinical and histopathological aspects of cutaneous wounds performed experimentally in healthy and immunocompromised mice using 100 μg/ml of Cramoll 1–4; Avelino et al. [48] described the development of a biosensor composed by 200 μg/ml Cramoll 1–4 immobilized by electrostatic interactions on hybrid Nanocomposite (gold nanoparticles and polyaniline) to distinguish abnormal glycoproteins of sera from patients infected with dengue serotypes I, II and III. Considering the broad range of Cramoll 1–4 concentrations in the development of their biological activities, a medium value of 100 μg/ml was chosen to be immobilized into the pure galactomannan gel.

The HA comparisons were made to a Cramoll 1–4 free solution which was benchmarked to 100% HA during the entire period. The HA of Cramoll 1–4 free solution was 3.01. Gel/Cramoll 1–4 immobilizing matrix presented HA of 2.71 when performed on days 1, 5 and 20, while for 30 and 60 days, its HA reduced to 1.81. HA was not significant after 90 days. These results demonstrate that the immobilized Cramoll 1–4 retained 90% of its initial HA during 20 days, decreasing to 60% up to 60 days.

The fundamental characteristic of lectins is its ability to bind specifically with carbohydrates, for instance, with glycoconjugates on cell surface. When that occurs, lectins deploy several biological effects. The HA allows for determining the lectin presence in a sample by forming a network agglutination between lectin binding sites and carbohydrates of the erythrocytes surface [49].The HA stability of the gel/Cramoll 1–4 immobilizing matrix ensures the maintenance of all lectin biological activities up to 60 days.

Cramoll 1–4 is classified as part of the mannose/glucose-specific binding group, as these are the monosaccharides to which Cramoll 1–4 exhibits the highest binding affinity. It is reasonable to predict that specific and non-specific hydrogen interactions between gel and lectin positively charged residues might occur, since the pure galactomannan gel has a central core of α(1 → 4)-linked D-mannopyranose to which β(1 → 6)-linked α-D-galactopyranosyl units are attached. That could be explained by potential conformational changes of Cramoll 1–4 for long storage periods.

The decrease in the opacity value for the gel/Cramoll 1–4 immobilizing matrix over time is, as already indicated in the flow curves, associated with the loss of gel structure stability. The stability loss seems to be corroborated by the loss of activity in HA during the same period, which might be associated with specific binding of the Cramoll 1–4 with the galactomannan backbone.

3.3. Microbial evaluation

A spoiled product may be described as one that has been rendered unfit for use. Microbial spoilage can be caused by bacteria, yeasts or fungi, which are all extremely versatile in their

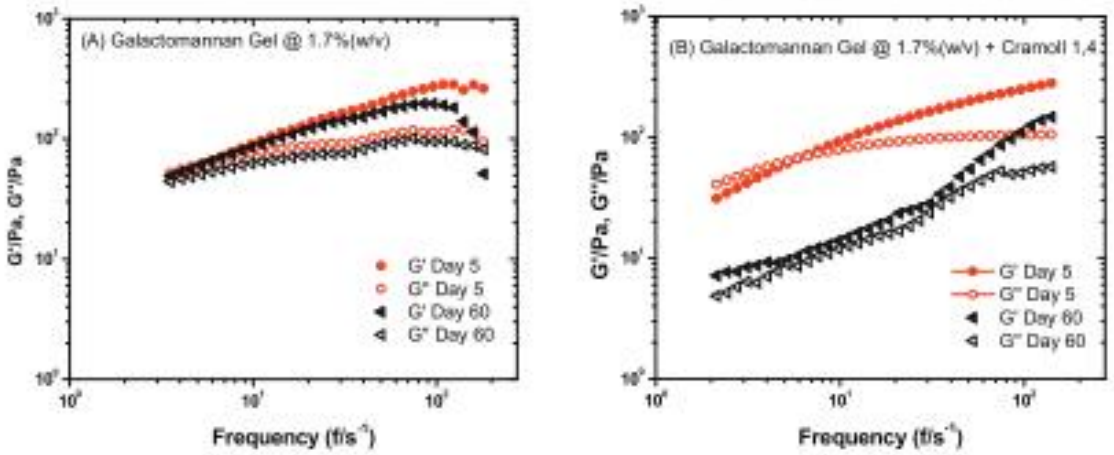

Fig. 3. The storage (G') and loss (G'') moduli as a function of an oscillatory stress frequency for different storage periods, for the pure galactomannan gel at 1.7% (w/v) (A) and the gel/Cramoll 1–4 immobilizing matrix (B).

metabolic activities. All classes of natural organic compounds, such as polysaccharides and proteins, are susceptible to degradation by the above mentioned microorganisms; synthetic compounds are also attacked, although often less readily [50]. To prevent the negative consequences ofmicrobial contamination, careful control measures are required during the storage process for industrial products, even food or cosmetics.

The pure galactomannan gel and the gel/Cramoll 1–4 immobilizing matrix presented no microbial counting for cultivable microorganisms along the total experimental interval. Our results are in accordance with the microbiological analysis of Soares et al. [6] for the hydrogel composed by κ-carrageenan and the pure galactomannan gel, since there was no observed microorganism growth in any of the experimentation days.

4. Conclusion

The gel/Cramoll 1–4 immobilizing matrix, as confirmed by the rheological measurements, proved to be stable up to 30 days with substantial variations occurring after this period. Moreover, throughout the entire storage period, no microbial contamination occurred, no changes in the neutral pH of the gels were observed and the colorimetric parameters remained constant. 90% of the HA was observed after 20 day of storage, decreasing to 60% up to 60 days. The rheological properties of the/Cramoll 1–4 immobilizing matrix and the maintenance of the HA of the immobilized Cramoll for a long period of time indicate the suitability for its use in topical applications, with potential use in pharmaceutical and cosmetics industries.

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References

[1] L. Yang, L. Zhang, Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources, Carbohydr. Polym. 76 (2009) 349–361.
[2] C.W. Vendruscolo, C. Ferrero, E.A.G. Pineda, J.L.M. Silveira, R.A. Freitas, M.B. Jiménez-Castellanos, T.M.B. Bresolin, Physicochemical and mechanical characterization of galactomannan from Mimosa scabrella: effect of drying method, Carbohydr. Polym. 76 (2009) 86–93.
[3] M.A. Pollard, R. Eder, P. Fischer, E.J. Windhab, Characterization of galactomannans isolated from legume endosperms of Caesalpinioideae and Faboideae subfamilies by multidetection aqueous SEC, Carbohydr. Polym. 79 (2010) 70–84.
[4] H. Neukom, Galactomannans: Properties and Applications, 22, Lebensmittel Wissenschaft und Technologie, London, 1989.
[5] A.I. Bourbon, A.C. Pinheiro, C. Ribeiro, C. Miranda, J.M. Maia, J.A. Teixeira, A.A. Vicente, Characterization of galactomannans extracted from seeds of Gleditsia triacanthos and Sophora japonica through shear and extensional rheology: comparison with guar gum and locust bean gum, Food Hydrocoll. 24 (2010) 184–192.
[6] P.A.G. Soares, J.R.P.C. Seixas, P.B.S. Albuquerque, G.R.C. Santos, P.A.S. Morelão, W. Barros Júnior, M.T.S. Correia, M.C. Carneiro-da-Cunha, Development and characterization of a new hydrogel based on galactomannan and κ-carrageenan, Carbohydr. Polym. 134 (2015) 673–679.
[7] M.A. Cerqueira, B.W.S. Souza, J.T. Martins, J.A. Teixeira, A.A. Vicente, Seed extracts of Gleditsia triacanthos: functional properties evaluation and incorporation into galactomannan films, Food Res. Int. 43 (2010) 2031–2038.
[8] M.A. Cerqueira, M.J. Sousa-Gallagher, L. Macedo, R. Rodríguez-Aguilera, B.W.S. Souza, J.A. Teixeira, A.A. Vicente, Use of galactomannan edible coating application and storage temperature for prolonging shelf-life of "Regional" cheese, J. Food Eng. 97 (2010) 87–94.
[9] J.T. Martins, M.A. Cerqueira, A.I. Bourbon, A.C. Pinheiro, B.W.S. Souza, A.A. Vicente, Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof, Food Hydrocoll. 29 (2012) 280–289.
[10] L. Gilbert, V. Loisel, G. Savary, M. Grisel, C. Picard, Stretching properties of xanthan cards, modified guar and celluloses in cosmetic emulsions, Carbohydr. Polym. 93 (2013) 644–650.
[11] R. Alvarez-Román, G. Barré, R.H. Guy, H. Fessi, Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection, Eur. J. Pharm. Biopharm. 52 (2001) 191–195.
[12] S. Comba, R. Sethi, Stabilization of highly concentrated suspensions of iron nanoparticles using shear-thinning gels of xanthan gum, Water Res. 43 (2009) 3717–3726.
[13] F. Valenga, D.F.S. Petri, N. Lucyszyn, T.A. Jó, M.R. Sierakowski, Galactomannan thin films as supports for the immobilization of Concanavalin A and/or dengue viruses, Int. J. Biol. Macromol. 50 (2012) 88–94.
[14] C.W. Vendruscolo, I.F. Andreazza, J.L.M.S. Ganter, C. Ferrero, T.M.B. Bresolin, Xanthan and galactomannan (from M. scabrella) matrix tablets for oral controlled delivery of theophylline, Int. J. Pharm. 296 (2005) 1–11.
[15] Y. Wu, W. Cui, N.A.M. Eskin, H.D. Goff, An investigation of four commercial galactomannans on their emulsion and rheological properties, Food Res. Int. 42 (2009) 1141–1146.

[16] P.B. Fernandes, M.P. Gonçalves, J.L. Doublier, A rheological characterization of Kappa-carrageenan/galactomannan mixed gels: a comparison of locust bean gum samples, Carbohydr. Polym. 16 (1991) 253–274.

[17] M.M. Cross, Rheology of non-Newtonian fluids: a new flow equation for pseudoplastic systems, J. Coll. Interface Sci. 20 (1965) 417.

[18] N. Garti, Z. Madar, A. Aserin, B. Sternheim, Fenugreek galactomannans as food emulsifiers, Food Sci. Technol. (Leb.) 30 (1997) 305–311.

[19] W. Sittikijyothin, D. Torres, M.P. Gonçalves, Modelling the rheological behaviour of galactomannan aqueous solutions, Carbohydr. Polym. 59 (2005) 339–350.

[20] P.B.S. Albuquerque, W. Barros Jr., G.R.C. Santos, M.T.S. Correia, P.A.S. Mourão, J.A. Teixeira, M.G. Carneiro-da-Cunha, Characterization and rheological study of the galactomannan extracted from seeds of Cassia grandis, Carbohydr. Polym. 104 (2014) 127–134.

[21] N. Sharon, H. Lis, The structural basis for carbohydrate recognition by lectins, in: A.M. Wu (Ed.), The Molecular Immunology of Complex Carbohydrates-2, Kluwer Academic/Plenum Publishers, Taiwan, 2001.

[22] M.T.S. Correia, L.C.B.B. Coelho, Purification of a glucose/mannose specific lectin isoform 1, from seeds of Cratylia mollis mart (Camaratu Bean), Appl. Biochem. Biotech. 55 (1995) 261–273.

[23] P.M.G. Paiva, L.C.B.B. Coelho, Purification and partial characterization of two lectin isoforms from Cratylia mollis Mart. (Camaratu Bean), Appl. Biochem. Biotechnol. 36 (1992) 113–118.

[24] C.A.S. Andrade, M.T.S. Correia, L.C.B.B. Coelho, S.C. Nascimento, N.S. Santos-Magalhães, Antitumor activity of Cratylia mollis lectin encapsulated into liposomes, Int. J. Pharm. 278 (2004) 435–445.

[25] M.P. Fernandes, N.M. Inada, M.R. Chiaratti, F.F.B. Araújo, F.V. Meirelles, M.T.S. Correia, L.C.B.B. Coelho, M.J.M. Alves, F.R. Gadelha, A.E. Vercesi, Mechanism of Trypanosoma cruzi death induced by Cratylia mollis seed lectin, J. Bioenerg. Biomembr. 42 (2010) 69–78.

[26] C.M.L. Melo, A.S.R. Lima, F.I.C. Beltrão, C.C.B. Cavalcanti, M.E. Melo-Júnior, S.M.L. Montenegro, L.C.B.B. Coelho, M.T.S. Correia, A.M.A. Carneiro-Leão, Potential effects of Cramoll 1 lectin on murine Schistosomiasis mansoni, Acta Trop. 118 (2011) 152–158.

[27] C.M.L. Melo, M.C.A.B. Castro, A.P. Oliveira, F.O.S. Gomes, V.R.A. Pereira, M.T.S. Correia, L.C.B.B. Coelho, P.M.G. Paiva, Immunomodulatory response of Cramoll 1–4 lectin on experimental lymphocytes, Phytother Res. 24 (2010) 1031–1038.

[28] C.M.L. Melo, B.A. Paim, K.G. Zecchin, J. Morari, M.R. Chiarrati, M.T.S. Correia, L.C.B.B. Coelho, P.M.G. Paiva, Cramoll 1–4 lectin increases BOS production, calcium levels and cytokine expression in treated spleen cells of rats, Mol. Cell. Biochem. 342 (2010) 165–169.

[29] E.V.M. Maciel, V.S. Araújo-Filho, M. Nakazawa, Y.M. Gomes, L.C.B.B. Coelho, M.T.S. Correia, Mitogenic activity of Cratylia mollis lectin on human lymphocytes, Biologia 59 (2004) 57–60.

[30] C.M.L. Melo, H. Melo, M.T.S. Correia, L.C.B.B. Coelho, M.B. Silva, V.R.A. Pereira, Mitogenic response and cytokine production induced by cramoll 1–4 lectin in splenocytes of inoculated mice, Scand. J. Immunol. 73 (2011) 112–121.

[31] V.L.M. Lima, M.T.S. Correia, Y.M.N. Cechinel, C.A.M. Sampaio, J.S. Owensand, L.C.B.B. Coelho, Immobilized Cratylia mollis lectin as a potential matrix to isolate plasma glycoproteins, including lecithin–cholesterol acyltransferase, Carbohydr. Polym. 33 (1997) 27–32.

[32] C.M.L. Melo, C.S. Porto, M.R. Melo-Júnior, C.M. Mendes, C.C.B. Cavalcanti, L.C.B.B. Coelho, A.L.F. Porto, A.M.A. Carneiro-Leão, M.T.S. Correia, Healing activity induced by Cramoll 1–4 lectin in healthy and immunocompromised mice, Int J. Pharm. 408 (2011) 113–119.

[33] D.H. Bing, J.G.M. Weyand, A.B. Stavitsky, Hemagglutination with aldehyde-fixed erythrocytes for assay of antigens and antibodies, Proc. Soc. Exp. Biol. Med. 124 (1967) 1166–1170.

[34] C. Vanderzant, D.F. Splittstoesser, Compendium of Methods for the Microbiological Examination of Foods, third ed., American Public Health Association (APHA), Washington, 1992.

[35] M. Srivastava, V.P. Kapoor, Seed galactomannans: an overview, Chem. Biodivers. 2 (2005) 295–317.

[36] T. Teniazo, I.F. Berbel, S. Cho, C.S. Favaro-Trindade, I.C.F. Moraes, S.C. Pinho, b-Carotene-loaded liposome dispersions stabilized with xanthan and guar gums: physico-chemical stability and feasibility of application in yogurt, LWT—Food Sci. Technol. 59 (2014) 1265–1273.

[37] J.H. Segura, F.B. Camargo Jr., E. Bagatin, M.B.G.M. Campos, Influence of thermal water and its oligoelements in the stability and efficacy of dermocosmetics formulations, Surg. Cosmt. Dermatol. 2 (2010) 11–17.

[38] M.R. Martelli, T.T. Barros, O.B.G. Assis, Puree films from bananas processed in the batching mode: mechanical properties and coloring variations, Polímeros 24 (2014) 137–142.

[39] B. Li, J.F. Kennedy, J.L. Peng, X. Yie, B.J. Xie, Preparation and performance evaluation of glucomannan–chitosan–nisin ternary antimicrobial blend film, Carbohydr. Polym. 65 (2006) 488–494.

[40] F. Ogher, F. Reicher, J.L.M.S. Ganter, Structural and rheological properties of polysaccharides from mango (Mangifera indica L.) pulp, Int. J. Biol. Macromol. 31 (2002) 9–17.

[41] G.M. Kavanagh, S.B. Ross-Murphy, Rheological characterization of polymer gels, Prog. Polym. Sci. 23 (1998) 533–562.

[42] G.E. Perissutti, T.M.B. Bresolin, J.L.M.S. Ganter, Interaction between the galactomannan from Mimosa scabrella and milk proteins, Food Hydrocoll. 16 (2002) 403–417.

[43] K. Hyun, S.H. Kim, K.H. Ahn, S.J. Lee, Large amplitude oscillatory shear as a way to classify the complex fluids, J. Non-Newtonian Fluid Mech. 107 (2002) 51–65.

[44] H.A. Barnes, Thixotropy—a review, J. Non-Newtonian Fluid Mech. 70 (1997) 1–33.

[45] L.M. Nwokocha, P.A. Williams, Rheological characterization of the galactomannan from Leucaena leucocephala seed, Carbohydr. Polym. 90 (2012) 833–838.

[46] D. Mudgil, S. Barak, B.S. Khatkar, Effect of enzymatic depolymerization on physicochemical and rheological properties of guar gum, Carbohydr. Polym. 90 (2012) 224–228.

[47] M.J. Perduca, M.J. Spotti, L.G. Santiago, M.A. Judis, A.C. Rubiolo, C.R. Carrara, Rheological characterization of the hydrocolloid from Gleditsia amorphoides seeds, LWT—Food Sci. Technol. 51 (2013) 143–147.

[48] K.Y.P.S. Avelino, C.A.S. Andrade, C.P. Melo, M.L. Nogueira, M.T.S. Correia, L.C.B.B. Coelho, Biosensor based on hybrid nanocomposite and Cramoll lectin for detection of dengue glycoproteins in real samples, Synth. Met. 194 (2014) 102–108.

[49] M.T.S. Correia, L.C.B.B. Coelho, P.M.G. Paiva, Lectins, carbohydrate recognition molecules: are they toxic? in: Y.H. Siddique (Ed.), Recent Trends in Toxicology, Transworld Research Network, India, 2008, pp. 47–59.

[50] R. Smart, D.F. Spooner, Microbiological spoilage in pharmaceuticals and cosmetics, J. Soc. Cosmetic Chem. 23 (1972) 721–737.

Artigo II

Biotechnological applications of galactomannan matrices: emphasis on immobilization of biomolecules

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Biotechnological Applications of Galactomannan Matrices: Emphasis on Immobilization of Biomolecules

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PBSA and LCBBC managed the literature searches and wrote the first draft of the manuscript. Authors MTSC, JAT and MGCC designed the study and managed the study performed. All authors read and approved the final manuscript.

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ABSTRACT

Polysaccharides are natural polymers extracted from plants, algae, animals, fungi or obtained via fermentation that can be applied on a wide range of uses, from food to biomedical industries. Galactomannans are polysaccharides mostly extracted from the endosperm of leguminous seeds and responsible to perform functions of energy reservation and hydration. They have singular properties that direct their potential use as films/coatings, gel agents, a part of mixed systems such

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as hydrogels, emulsion stabilizers, thickeners, and cosmetics. The characterization of galactomannans from conventional and nonconventional sources were reported as capable to produce the broad range of galactomannan matrices (films/membranes, coatings, gels and hydrogels). Matrices based on galactomannans, in addition, were explored as effective supports for immobilization of different functional compounds. The knowledge of the application of galactomannans as films and coatings is still limited compared with those already reported for other polysaccharides; moreover, the some publications brought new insights of the properties and characterization of edible films. The works in which galactomannan films are used as support for immobilization of biomolecules are still scarce, especially in health care. Due to their viscous and elastic properties, galactomannans have been widely investigated in mixed gels containing two or more biopolymers with the aim to improve cohesion, appearance, stability and durability of the gel. Studies involving the use of galactomannans in gels for immobilization of biomolecules have also been developed with the important purpose of evaluating the controlled release of suspensions contained in nanostructures. This review article aimed to approach the most recent literature dealing with galactomannan-based matrices and exposes the main strategies for the immobilization of biomolecules and their potential applications in industry.

Keywords: Galactomannan; film; gel; hydrogel; immobilization; matrices.

1. INTRODUCTION

Medicines, foods, fibers, natural and essential oils, cosmetics, chemical compounds and biofuels are examples of products that can be manufactured from a broad class of chemical substances from plant and animal species. Polysaccharides represent one of the most important classes, since they are natural polymers extracted from plants, algae, animals, fungi, or obtained by fermentation, with a wide range of applications, especially in food, biomedical, pharmaceutical and cosmetic fields [1].

Galactomannans are polysaccharides mostly extracted from the endosperm of leguminous seeds (Fig. 1) and responsible to perform functions of energy reservation and hydration. They have special properties such as high molar mass, water solubility and non-ionic character [2], which direct their potential use as films/coatings [3-8], as gel agents [9], as a part of mixed systems such as hydrogels [10,11], as emulsion stabilizers [12], thickeners [13], and cosmetic materials [14].

The characterization of galactomannans from conventional [3] and nonconventional [15-17] sources were reported as capable to produce the broad range of galactomannan matrices (films/membranes, coatings, gels and hydrogels). Matrices based on galactomannans, in addition, were explored as effective supports for immobilization of different functional compounds, such as peptides [4], antioxidants [8], lectins [18], and medicines [19].

Regarding the formulation of galactomannans as films/membranes and coatings, it is important to note the differences between the terms: Films or membranes are formed by drying of a polymeric

Fig. 1. Seed of Leguminosae family: representation of the constituents hull, endosperm and germ

solution, while coating is a suspension that can be directly applied to the product. Films based on galactomannans have been used in several applications, including pharmaceutical field and food industry. The most common polysaccharides used for production of edible films are cellulose, chitosan, agar and starch; galactomannans emerge as alternative materials that can be used for the production of edible films based on their edibility and biodegradability [5,20].

On the other hand, gel is considered a three-dimensional network obtained by the linkage of macromolecules wrapped in a solvent, which support their own weight and maintain its shape. The presence of non-covalent cross-links complicates the description of physical properties from such networks due to the influence of temperature and time in the number and position of these connections [21].

The gels generally exhibit viscous and elastic properties, with a predominance of the elastic mode. The functional properties of galactomannans are widely used in industry; then to optimize this industrial use, it is necessary to develop methods that would allow predicting the structure and the function of these polymers through the knowledge of their conformations.

Among the physicochemical methods used in this evaluation are the rheological techniques, which describe the mechanical properties of materials under distinct deformation conditions, since they exhibit the ability to flow and/or accumulate reversible deformations [22].

Galactomannans have been widely investigated in mixed gels containing two or more biopolymers with the aim to improve cohesion, appearance, stability and durability of the gel. The synergy between galactomannans and other polysaccharides, such as agar and galactomannan [23,24], galactomannan and xanthan [25], pectin and galactomannan [26], galactomannan and k-carrageenan [11], and unconventional galactomannans with xanthan and carrageenan [9] have been described.

This review article consider the most recent literature dealing with galactomannan matrices and emphasizes their potential use for the immobilization of distinct biomolecules. Moreover, their broad range of applications in industry was also exposed.

2. INTRODUCTION TO GALACTO-MANNANS

Galactomannans are polysaccharides composed by a linear chain of β-1,4-D-mannopyranose to which α-1,6-D-galactopyranose units are attached. They could be obtained from microbial sources, in particular yeasts and other fungi, and from plants. In what concerns the vast majority of galactomannans derived from plants, those polysaccharides display a reserve function and their main source is the endosperm of seeds, especially from members of the Leguminosae family [27]. The legume species are spread throughout the world, especially in tropical and subtropical regions, ranging from emergent trees to tiny and ephemeral herbs [28].

Polysaccharides from seeds are examples of natural compounds that have contributed to the Leguminosae family classification, but special emphasis has been given to galactomannans [29]. According to Engler classification [30], the Leguminosae family is divided into the subfamilies Caesalpinioideae, Mimosoideae and Faboideae. The use of galactomannans as a taxonomic character has been proposed by many authors due to the yield of extraction from the endosperm of the seeds as well as the ratio between the residues of mannose and galactose in the molecule and the contents of these compounds in the seeds [31].

Another utility of the galactomannans considers the Leguminosae family as the second most important into the Dicotyledoneae class and the first in economic importance. Three possibilities for the fine structure of the molecule, corresponding to the distribution of galactose along the mannose main chain, have already being proposed (Fig. 2).

To provide differences in density and viscosity of solutions, the proportion and distribution of galactose units has an essential role in solubility of galactomannans, its water solubility increasing with rising content of galactose (i.e. with decreasing M/G ratio). A polysaccharide chain composed of at least 85 to 95 % mannose units will provide intermolecular interactions like hydrogen bonds between cis hydroxyls of mannose, leading to formation of insoluble aggregates [32]. The presence of branched chains of galactose creates steric impediment between intermolecular hydrogen bonds, minimizing the formation of aggregates. Moreover, galactomannans with few side chains

(greater ratio mannose/galactose) may interact better with other polysaccharides due to the long unsubstituted regions [12]. For a better understanding of sources of galactomannans and their main properties, Table 1 summarizes those characteristics and the most important applicability of galactomannans from Leguminosae family.

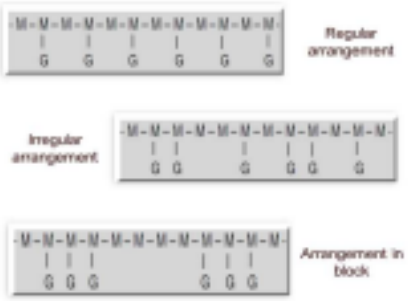

Fig. 2. Distribution of galactose units along the mannose main chain

M: representation of mannose units linked by β(1→4); G: representation of substitution by galactose units with linked α(1→6). Based upon Dea and Morrison [27]

3. BIOTECHNOLOGICAL APPLICATIONS OF GALACTOMANNANS

The three main commercially used galactomannans in food and non-food industries are guar gum (*Cyamopsis tetragonolobus*), tara gum (*Caesalpinea spinosa*) and locust bean gum (*Ceratonia siliqua*) [27,33]. Other galactomannans commercially known are the gum extracted from *Cassia tora* [12] and the galactomannan from fenugreek *Trigonella foenum-graecum*, marketed on a smaller scale as well as that extracted from the seeds of *Prosopis juliflora* (Please see Table 1).

In food industry, guar gum and locust bean gum are the most used species [34], while tara gum has been accepted as an alternative to those already used [35]. In general, galactomannans improve the texture and appearance of foods and increase its resistance to temperature changes, been especially applied as thickeners and fat substitutes; they also can be used in dairy products, desserts (especially ice cream), jellies, powders, cake mixes and frosting, spices, sauces, soups and canned and frozen foods [36].

Galactomannans enter into the composition of dietetic foods since they are not digested by the body. The addition of guar gum to meals that are rich in carbohydrates reduces the postprandial rise of glucose and insulin in the blood. Moreover, the use of pharmaceutical preparations of pure guar gum and the gum added to foods improved the metabolism of carbohydrates and lipids in insulin-dependent and insulin-independent patients. The physiological action of guar gum appears to depend mainly on their ability to rapid hydration, increasing the viscosity of the bolus in the stomach and small intestine. The high viscosity in the small intestine decreases both digestion and absorption of carbohydrates, which tends to reduce postprandial hyperglycemia. There are also studies demonstrating that guar gum is able to lower blood low density lipoproteins/ LDL [37]. Also as regards the food industry, studies related to the mechanical and thermal properties of films based on galactomannans have been widely exploited for biotechnological application especially as edible films [5], for example for tropical fruits [7] and ricotta cheese [38].

In what concerns non-food industries, galactomannans are used as thickeners and stabilizers in pharmaceutical formulations, such as creams and lotions for cosmetic fields [39]. In addition, these polysaccharides have been applied as matrix in the controlled release of drugs, such as the formulation composed by galactomannan and xanthan gum, which has been used as controlled release drug carrier of sodium diclofenate and theophylline. Galactomannans were also employed as thickeners in effervescent tablets and formed a stable suspension, thus preventing the settlement of the particles and promoting a pleasant feeling in the mouth [40,41].

Galactomannans were also applied for the controlled release of drugs in the large intestine. The polysaccharide have been used as coating capsules and tablets in combination with proteins. Individually, these polysaccharides cannot be applied as drug carriers to the colon due to their solubility in water. However, the coating based on galactomannan and pectin in pH 7 becomes elastic and insoluble in gastric and intestinal fluids, thus able to pass through the upper gastrointestinal tract and allow the release just in the intestine [42].

Further, galactomannans are adsorbed by cellulose fibers and used in paper industry to improve the mechanical properties of paper by regulating the flocculation state of cellulosic fiber suspension [43].

Table 1. Galactomannans from Leguminosae family and their main properties including M/G ratio and applications

Leguminosae family		M/G ratio	Applications	Reference
Subfamily	Species			
Caesalpinioideae	*Cassia absus*	3.0	Medicinal purposes.	[44,45,46]
	C. alata	3.3	-	[47]
	C. emarginata	2.70	-	[27]
	C. fistula	3.0	Possess antitumoral activity.	[48,49]
	C. grandis	2.44-3.15	-	[15,50]
	C. marylandica	3.76	-	[51]
	C. nodosa	3.5	-	[52]
	C. occidentalis	3.1	-	[51]
	C. tora	3.0	Disintegrant in the formulation of orodispersible tablets. Thickener or gelling agent.	[12,53,54]
	C. spectabilis	2.65	-	[55]
	C. spinosa	2.70-3.0	Possess protective colloidal properties and interfacial tension activity. Important agent of synergism with other polysaccharides.	[12,56]
	Caesalpinia pulcherrima	2.88	Used as coating for tropical fruits.	[7,57]
	Ceratonia siliqua	3.5-3.75	Binder, lubricator, and stabilizer. Provides heat-shock resistance in ice cream products. Speeds coagulation of cheeses. Used as beads for drug controlled release. Important agent of synergism with other polysaccharides.	[33,34,51,56]
	Caesalpinia cacalaco	2.5	-	[12]
	Delonix regia	4.28	-	[27]
	Gleditsia amorphoides	2.5	-	[58]
	G. triacanthos	2.82	Food industry purposes. Reduce intestinal absorption.	[8,13,17,59]

Leguminosae family		M/G ratio	Applications	Reference
Subfamily	Species			
			Possess immunomodulatory activity and anti-inflamatory and antioxidant effects	
	Gymnocladus dioica	2.71	-	[51]
	Parkinsonia aculeata	2.70	-	[51]
Mimosoideae	*Besmanthus illinoensis*	2.69	-	[12]
	Leucaena glauca	1.33	-	[12]
	Adenanthera pavonina	1.35	Used as coating for tropical fruits.	[7,57]
	Mimosa scabrella	1	Used as tablets for oral controlled drug delivery.	[19,48]
	Prosopis juliflora	1.1-1.6	Potential for use in the food industry.	35,60]
Faboideae	*Sophora japonica*	5.75	Food industry purposes.	[17,61]
	Trigonella foenum-graecum	0.95-1.1	Reduce surface tension.	[56,62,63]
			Possess anti-inflamatory and antioxidative effects.	
	Cyamopsis tetragonoloba	1.8	Thickener, stabilizer, emulsifier, and firming agent.	[51,56,64,65]
			Important agent of synergism with other polysaccharides.	
			Used as blends for food industry.	
			Part of mixed gels for topical drug delivery.	

Several studies have been developed about the purification, description of the physical, chemical and biological properties, and use of galactomannans obtained from different and varied sources. Galactomannans of distinct plant species have been well characterized, such as the *Prosopis ruscifolia* [16], *Senna tora* [66], *Cassia grandis* [15,50], *Dimorphandra gardneriana* Tul. [1], *Caesalpinia ferrea* var. *férrea* [67], *Caesalpinia pulcherrima*, *Gleditsia triacanthos* and *Adenanthera pavonina* [57].

The degree of substitution of the galactose units in the mannose backbone is an important character in the interaction of galactomannans with other polymers. One of the first reports on the characteristics and properties of systems formed from binary mixtures of galactomannans and other polysaccharides were performed by McCleary et al. [68], who described rheological analysis between xanthan and the guar gum galactomannan and demonstrated that this interaction decreases with increasing the degree of substitution of galactomannans. Bresolin et al. [69] also evaluated the synergistic effect of xanthan and the galactomannans from *Mimosa scabrella* and *Schizolobium parahybum*. Grisel et al. [70] evaluated the synergistic effect of guar gum galactomannan and locust bean gum with xanthan and confirmed that the impact distribution of galactose units along the main chain of mannose is attached to the synergy mechanism. Lucyszyn et al. [23] applied gel mixtures of galactomannans and agar for plant cell cultures.

Another widely studied gel comprises k-carrageenan and galactomannan. Gonçalves et al. [71] observed that the addition of galactomannan improved the gel quality when compared to the pure k-carrageenan gel. In combination with galactomannan, the mixture became less brittle, stronger, and less vulnerable to syneresis. Pinheiro et al. [9] quantified the synergistic interactions between the galactomannans extracted from *Sophora japonica* and *Gleditsia triacanthos* with k-carrageenan and xanthan and compared the results with the traditional guar gum galactomannan and locust bean gum. The results demonstrated once more that the synergistic effect of the system depends on the ratio of mannose and galactose; in addition, the fine structure of the galactomannan.

There is a worldwide trend related to researches on purification, characterization and application of galactomannans, indicating the need to find seeds, which are alternative sources for the extraction of this polysaccharide, especially for industrial production. The possible sources for galactomannan extraction in Latin America are still unfamiliar despite the rich biodiversity of the local flora [1].

Brazil has rich sources of diversified species for the extraction of galactomannans from seeds that could leverage the marketing of this polysaccharide. Considering the prices for commercial galactomannans including guar gum and locust bean gum (0,10 euros per gram, approximately) [72], it is important to note the potential of the Amazon as supplier of seeds with high potential for galactomannan extraction yield, which has important highlight in trade and promising scientific researches.

3.1 Immobilization of Biomolecules in Galactomannan Supports

Enzymes, antibodies, proteins, drugs and cellular receptors are examples of biomolecules already immobilized by chemical or physical means in different biomaterials for applications ranging from diagnostic and therapeutic areas to separation methods and other bioprocesses.

Among the different classes of biomaterials that can be used as carriers, polymers have special interest due to reactive groups on its surface or other derivative groups that may covalently bind to biomolecules. Moreover, an advantage of polymeric supports for biomolecules relates to distinct processes for manufacture these systems, including films, membranes, tubes, fibers, particles, gels, hydrogels, capsules and porous structures [73].

Several immobilization methods are based on physical or chemical linkages between the biomolecule and the polymeric support. The main used methods are physical adsorption (hydrophobic interactions, hydrogen bonds or van der Waals forces), chemical adsorption (covalent or ionic bonds), immobilization by containment matrix and crosslinks (Fig. 3). It is important to note that the term immobilization refers both for a transient location as a permanent immobilization of the biomolecule in or on the polymeric support. Moreover, if the polymeric carrier is biodegradable, the immobilized biomolecule can be released by degradation of the matrix [73].

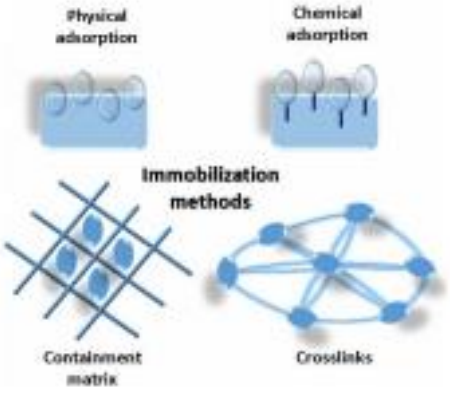

Fig. 3. Immobilizations based on physical or chemical linkages; containment matrix and crosslinks between the biomolecule and the polymeric support are represented

Currently, polysaccharides are reported as efficient polymers for biomolecule immobilization, for example, chitosan films from animal and microbial sources, added to glycerol, were used for the immobilization of bromelain [74]; the results suggested that the films with low molecular weight chitosan are suitable for application in wine industry. Proteases were immobilized on chitosan films and demonstrated an excellent anti-biofilm effect, especially against *Staphylococcus* cultures [75]. Bioengineering and medicine fields also reported innovations such as the combination of chitosan, gelatin and alginate with carbon nanotubes for protein immobilization [76]. Biomolecule immobilizations in polymeric matrices has also been developed for micro scale, for example the development of a glucose biosensor by immobilizing glucose oxidase in chitosan particles [77], in addition to the microparticles of gel produced from alginate and lactoferrin [78].

Considering the above-mentioned results, one can note that galactomannans are still little explored in most research carried out with polysaccharides for immobilization of biomolecules.

3.1.1 Galactomannan films/membranes as supports of immobilization

The polymers, whether natural or synthetic, are molecules whose chains are longer and able to produce continuous matrices vital for structuration of films, membranes and coatings. In order to avoid misinterpretations, it is important to distinguish the above-mentioned terms: Films or membranes are formed by drying (casting) of a polymeric solution, which can later be applied to a product; the coating can be a suspension or emulsion applied directly to the product surface and, after drying, leads to the formation of a film. These terms have been improved by the food industry to clarify the difference between coatings and edible films [79].

The preparation of films from biodegradable materials such as natural polymers has aroused the interest of the scientific community in recent decades, especially due to the importance given to the replacement of synthetic polymers. Films developed from polysaccharides (Fig. 4) are excellent barriers to oxygen due to packing of molecules, forming a structural network ordered through hydrogen bonds; however, there are hygroscopic characteristics that can reduce its potential for many applications [80].

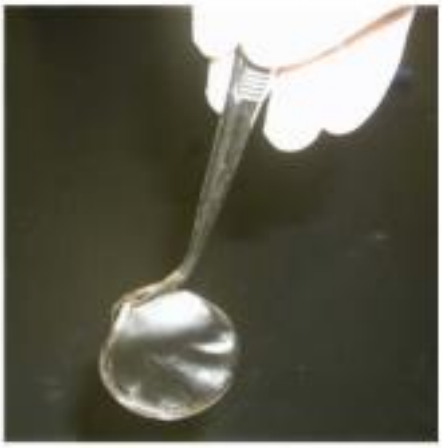

Fig. 4. Representation of films based on polysaccharides obtained from natural sources

The properties of films depend on the used polymer, the manufacturing conditions and environmental conditions, which are important factors due to the hygroscopic nature of the polymers [81]. The formulation of these products most often requires the use of plasticizers, since films without plasticizer addition exhibit a brittle and hard structure due to interactions among polymer molecules [79].

Plasticizers are low molecular weight agents that, once incorporated into films, are able to position themselves among the polymer molecules. They interfere with the polymer-polymer interactions and result in increased flexibility and processing capacity [82], as well as improve the product resistance to penetration of vapours and gases [40]. Water is a very effective plasticizer in the composition of films; other plasticizers are also hydrophilic and able to attract water molecules. Due to this feature, the relative humidity of films under storage becomes one of the main analysed properties especially due to the influence of water on the structure of the products [83].

Surfactants are considered amphipathic substances due to their hydrophilicity and hydrophobicity. They are usually added to enhance the emulsion stability of films. Surfactants could be incorporated to reduce surface tension of solutions, improving the wettability of the products [82].

The polysaccharides evaluated and/or employed to form films can be applied in the pharmaceutical field in encapsulation processes and release of active principles; as edible films in the food industry; in the cosmetic industry; in agriculture, such as pesticides and nutrients release agent; among other applications. The main polysaccharides reported on the production of films and coatings include: Starch [84,85], cellulose [86-88], alginate [89-91], carrageenan [92-94], chitosan [95-97] and natural gums, such as Policaju [98,99] and agar [100,101].

The knowledge of characterization and application of galactomannans as films is still limited compared with those already reported for other polysaccharides. Apart from this fact, the main publications brought new insights of the properties and uses of edible films, as reported below.

Cerqueira et al. [7] studied the application of coatings constituted by galactomannans from different natural sources (*Caesalpinia pulcherrima* and *Adenanthera pavonina*) in five tropical fruits: acerola (*Malpighia emarginata*), cajá (*Spondias lutea*), mango (*Mangifera indica*), pitanga (*Eugenia uniflora*) and seriguela (*Spondias purpurea*). The surface properties of the five fruits were determined for different aqueous solutions of galactomannans plus glycerol. Lima et al. [102] also used the galactomannans obtained from *C. pulcherrima* and *A. pavonina* to coat fruits, but added collagen and glycerol to the filmogenic solutions and evaluated the application of the coatings on mangoes and apples. The influence of storage temperature on the gas exchange rate of cheese coated with galactomannan was also evaluated [38] and the study of the physicochemical properties of edible films with different concentrations of locust bean gum and k-carrageenan was performed [3]. In general, edible films based on galactomannans tend to improve the appearance of the food and can be used as immobilizing media of nature preservatives in order to reduce microbial contamination, increasing the shelf life of foods coated with this polysaccharide.

It is important to highlight that mannose/galactose ratio, degree of substitution and degree of polymerization have been reported to directly affect edible films [103]. In addition, the immobilization of compounds in galactomannan films must be evaluated by the impact on the functionality of the final product, since the immobilized molecule can affect functional properties of the polysaccharide [5].

Martins et al. [4] developed a galactomannan film extracted from *Gleditsia triacanthos* with immobilized nisin and have been successful in preventing microbial contamination in cheese ricotta. Cerqueira et al. [8] used the same galactomannan from *G. triacanthos* to immobilize antioxidant extracts and implement the antioxidant activity of the final product. Valenga et al. [18] performed the immobilization of the lectin from *Canavalia ensiformis* seeds, ConA (glucose/ mannose binding), in galactomannan films obtained from the seeds of *Leucaena leucocephala*. They suggested that, as the backbone of the galactomannan is comprised of β-D-mannose units in which some α-D-galactose units are linked at the C-6 position, the recognition of ConA may occur through OH groups at C-3 or C-6 positions, if the latter mannose unit is free. These results demonstrate that the works in which galactomannan films are used as support for immobilization of biomolecules are still scarce, especially in health care.

3.1.2 Galactomannan gels and hydrogels as immobilizing supports

Gels are semi-solid systems in which small amounts of solid are dispersed in relatively larger quantities of liquid, a characteristic that provides a nature more solid than liquid to the system [104]. There is an inadequate interpretation in

polymer science under the use of the terms gel and hydrogel as synonyms. Even though gels and hydrogels are chemically similar polymeric networks (Fig. 5), they have distinct physical structures [105]. Hydrogels are characterized as crosslinked networks of hydrophilic polymers capable of absorbing large quantities of water and swell while retaining its three-dimensional structure. Sometimes hydrogels are also described as aqueous gels due to the hydro prefix, although the term hydrogel implies a material already swollen in water [106].

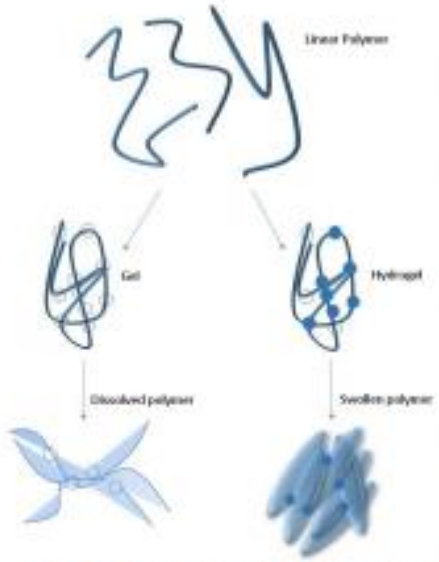

Fig. 5. Differential behavior of a polymer in aqueous solution

The closed circles represent covalent bonds and the open circles represent virtual entangled links

Although some gels are sufficiently rigid to maintain their structure under low stress, after exceeding a certain threshold value, the flow of the gel emerges as a characteristic linked to the loss in the polymer structure. Hydrogels can swell in aqueous medium for the same reason that a similar polymer can be dissolved in water to form a polymeric solution. Therefore, the central feature to form a hydrogel is its inherent crosslinking (ability to form cross-links). Conventional gels can also develop small levels of cross-links because of the energy gain under the influence of stress forces, but this process is reversible due to the involvement of weak physical forces [105].

Galactomannans themselves are nongelling agents, while some galactomannans are able to form gels with certain metal salts and others interact synergistically with different polysaccharides such as agar, xanthan, carrageenan, pectin and yellow mustard gum to form a three-dimensional gel network in appropriate conditions. Hydrated galactomannan molecules occupy a large hydrodynamic volume in aqueous solution and control the rheological behaviour of the entire solution [39]. The evaluation of this behaviour plays an important role in the characterization of galactomannan solutions, since these are often used to modify textural attributes [107]. This characterization can be performed through shear (steady and dynamic conditions) and extensional rheology [13].

Regarding the technical applications of galactomannan solutions, attention is drawn to the chemical behaviour of the different galactomannans. There are uses which benefit from the excellent viscosity formation of some galactomannans or their derivatives and there are utilizations which benefit from water absorption or from the formation of hydrogen bonds as well as gel formation [39].

Biologically active molecules such as proteins, peptides, saccharides, lipids, drugs, hormones, cell surface receptors, conjugates, nucleotides and nucleic acids can be immobilized based on physical or chemical linkages on polymeric supports. Biomolecules can be immobilized on the outer surface of the gel or within the hydrogel polymer network [73]. Applications ranging from the food to the pharmaceutical industry have used gels based on galactomannans as matrices for controlled release of compounds.

The galactomannan extracted from the seeds of *Mimosa scabrella*, for example, was prepared with xanthan and tested as hydrophilic matrix for controlled release of theophylline [40] and sodium diclofenac [41]. Koop et al. [108] utilized the galactomannan of the latter species as matrix for stabilizing ascorbic acid. The locust bean gum was mixed with xanthan and evaluated for promoting emulsion stability [109]. Rocha et al. [110] used silica and chitosan to immobilize *Aspergillus* and *Penicillium* fungal.

Studies involving the use of galactomannans in gels for immobilization of biomolecules have also been developed with the important purpose of evaluating the controlled release of suspensions contained in nanostructures [111,112]. Nanometric systems of polysaccharides have been studied intensively [113], especially in the biological field [114,115]. Nanocapsules, nanoparticles, liposomes, microspheres, microcapsules and nanospheres, belonging to the group of systems dispersed in nanometric scale, are pharmaceutical forms that intend to reduce side effects of many substances while increase its effectiveness after administration, even by different routes, including the cutaneous barrier. In this sense, the technological development of novel dosage forms in nanometric scale has been a promising strategy to increase the penetration of drugs through the skin in a controlled manner [116].

4. CONCLUSION

The purpose of this review was to approach the most recent scientific literature dealing with matrices based on galactomannans. Moreover, this review emphasis the main strategies for immobilization of biomolecules and their potential industrial applications. Solutions of galactomannans are considered viscoelastic materials since they exhibit both viscous (liquid) and solid (elastic) characteristics, which proposes its use as supports ranging from films/membranes to gels. This review article was motivated by the lack of adequate knowledge about the immobilization of biomolecules in galactomannan matrices and the impact on the functionality of the final product, since the immobilized biomolecule can affect functional properties of the polysaccharide.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Da Cunha PLR, De Paula RCM, JPA Feitosa, JPA. Polysaccharides from Brazilian biodiversity: An opportunity to change knowledge into economic value. Quim Nova. 2009;32(3):649-60. DOI:10.1590/S0100-40422009000300009
2. Pollard MA, Eder B, Fischer P, Windhab EJ. Characterization of galactomannans isolated from legume endosperms of Caesalpinioideae and Faboideae subfamilies by multidetection aqueous SEC. Carbohyd Polym. 2010;79:70-84. DOI:10.1016/j.carbpol.2009.07.028
3. Martins JT, Cerqueira MA, Bourbon AI, Pinheiro AC, Souza BWS, Vicente AA. Synergistic effects between k-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. Food Hydrocolloid. 2012;29:280-89. DOI:10.1016/j.foodhyd.2012.03.004
4. Martins JT, Cerqueira MA, Souza BWS, Avides MC, Vicente AA. Shelf life extension of ricotta cheese using coatings of galactomannans from nonconventional sources incorporating nisin against *Listeria monocytogenes*. J Agr Food Chem. 2010;58(3):1884-91. DOI:10.1021/jf902774z
5. Cerqueira MA, Bourbon AI, Pinheiro AC, Martins JT, Souza, BWS, Teixeira JA, et al. Galactomannans use in the development of edible films/coatings for food applications. Trends Food Sci Tech. 2011;22:662-71. DOI:10.1016/j.tifs.2011.07.002
6. Cerqueira MA, Lima AM, Souza BWS, Teixeira JÁ, Moreira RA, Vicente AA. Functional polysaccharides as edible coatings for cheese. J Agr Food Chem. 2009;57(4):1456-62. DOI:10.1016/j.foodres.2010.06.002
7. Cerqueira MA, Lima AM, Teixeira JA, Moreira RA, Vicente AA. Suitability of novel galactomannans as edible coatings for tropical fruits. J Food Eng. 2009;94,372-78. DOI:10.1016/j.jfoodeng.2009.04.003
8. Cerqueira MA, Souza BWS, Martins JT, Teixeira JA, Vicente AA. Seed extracts of *Gleditsia triacanthos*: Functional properties evaluation and incorporation into galactomannan films. Food Res Int. 2010;43(8):2031-38. DOI:10.1016/j.foodres.2010.06.002

9. Pinheiro AC, Bourbon AI, Rocha C, Ribeiro C, Maia JM, Gonçalves MP, et al. Rheological characterization of [kappa]-carrageenan/ galactomannan and xanthan/galactomannan gels: Comparison of galactomannans from non-traditional sources with conventional galactomannans. Carbohyd Polym. 2011;83(2):392-99. DOI:10.1016/j.carbpol.2010.07.058
10. Da-Lozzo EJ, Moledo RCA, Faraco CD, Ortolani-Machado CF, Bresolin TMB, Silveira JLM. Curcumin/xanthan–galactomannan hydrogels: Rheological analysis and biocompatibility. Carbohyd Polym. 2013;93:279–84. DOI:10.1016/j.carbpol.2012.02.036
11. Soares PAG, Seixas JRPC, Albuquerque PBS, Santos GRC, Mourão PAS, Barros Júnior W, et al. Development and characterization of a new hydrogel based on galactomannan and κ-carrageenan. Carbohyd Polym. 2015;134:673–79. DOI:10.1016/j.carbpol.2015.08.042.
12. Srivastava M, Kapoor VP. Seed galactomannans: An overview. Chem Biodivers. 2005;2(3):295–317. DOI:10.1002/cbdv.200590013
13. Bourbon AI, Pinheiro AC, Ribeiro C, Miranda C, Maia JM, Teixeira JA, et al. Characterization of galactomannans extracted from seeds of *Gleditsia triacanthos* and *Sophora japonica* through shear and extensional rheology: Comparison with guar gum and locust bean gum. Food Hydrocolloid. 2010;24(2-3):184–92. DOI:10.1016/j.foodhyd.2009.09.004
14. Gilbert L, Loisel V, Savary G, Grisel M, Picard C. Stretching properties of xanthan, carob, modified guar and celluloses in cosmetic emulsions. Carbohyd Polym. 2013;93:644–50. DOI:10.1016/j.carbpol.2012.12.028
15. Albuquerque PBS, Barros Júnior W, Santos GRC, Correia MTS, Mourão PAS, Teixeira JA, et al. Characterization and rheological study of the galactomannan extracted from seeds of *Cassia grandis*. Carbohyd Polym. 2014;104:127–34. DOI:10.1016/j.carbpol.2014.01.010
16. Busch VM, Kolender AA, Santagapita PR, Buera MP. Vinal gum, a galactomannan from *Prosopis ruscifolia* seeds: Physicochemical characterization. Food Hydrocolloid. 2015;51:495–502. DOI:10.1016/j.foodhyd.2015.04.035
17. Cerqueira MA, Pinheiro AC, Souza BWS, Lima AMP, Ribeiro C, Miranda C, et al. Extraction, purification and characterization of galactomannans from non-traditional sources. Carbohyd Polym. 2009;75(3):408–14. DOI:10.1016/j.carbpol.2008.07.036
18. Valenga F, Petri DFS, Lucyszyn N, Jó TA, Sierakowski MR. Galactomannan thin films as supports for the immobilization of Concanavalin A and/or dengue viruses. Int J Biol Macromol. 2012;50(1):88–94. DOI:10.1016/j.ijbiomac.2011.10.005
19. Vendruscolo CW, Andreazza IF, Ganter JLMS, Ferrero C, Bresolin TMB. Xanthan and galactomannan (from *M. scabrella*) matrix tablets for oral controlled delivery of theophylline. Int J Pharm. 2005;296(1-2):1–11. DOI:10.1016/j.ijpharm.2005.02.007
20. Antoniou J, Liu F, Majeed H, Zhong F. Characterization of Tara gum edible films incorporated with bulk chitosan and chitosan nanoparticles: A comparative study. Food Hydrocolloid. 2015;44:309–19. DOI:10.1016/j.foodhyd.2014.09.023
21. Ross-Murphy SB, Morris VJ, Morris ER. Molecular viscoelasticity of xanthan polysaccharide. Faraday Symp Chem S. 1983;18:115–29. DOI:10.1039/FS9831800115
22. Schramm G. Reologia e reometria: fundamentos teóricos e práticos. São Paulo: Artliber; 2006.
23. Lucyszyn N, Quoirin M, Ribas LLF, Koehler HR, Sierakowski MR. Micropropagation of "Durondeau" pear in modified-gelled medium. *In vitro* Cell Dev-pl. 2006;42(3):287–90. DOI:10.1079/IVP2006753
24. Pereira-Netto AB, Meneguin RG, Biz A, Silveira JLM. A galactomannan-driven enhancement of the in vitro multiplication rate for the marubakaido apple rootstock (*Malus prunifolia* (Willd.) Borkh) is not related to the degradation of the exogenous galactomannan. Appl Biochem Biotechnol. 2012;166:197–207. DOI:10.1007/s12010-011-9416-7
25. Shobha MS, Tharanathan RN. Rheological behaviour of pullulanase-treated guar galactomannan on co-gelation with xanthan. Food Hydrocolloid. 2009;23(3):749–54. DOI:10.1016/j.foodhyd.2008.04.006
26. Wu Y, Cui W, Eskin NAM, Goff HD. An investigation of four commercial

galactomannans on their emulsion and rheological properties. Food Res Int. 2009;42(8):1141–46.
DOI:10.1016/j.foodres.2009.05.015
27. Dea ICM, Morrison A. Chemistry and interactions of seed galactomannans. Adv Carbohyd Chem. 1975;31:241-312.
28. Lewis GP, Schrire BD, Mackinder B, Lock M. Legumes of the World. The Royal Botanic Gardens. Kew. 2005;577.
29. Hegnauer R, Grayer-Barkmeuer RJ. Relevance of seed polysaccharides and flavonoids for the classification of the Leguminosae: A chemotaxonomic approach. Phytochemistry. 1993;34(1):3-16.
DOI:10.1016/S0031-9422(00)90776-3
30. Engler, A. Syllabus der Pflanzenfamilien. Berlin: Gerbruder Borntraeger; 1964.
31. Rosa IG, Souza NS, Santana AA, Sousa H. Extração e caracterização fisico-química dos polissacarideos de *Leucaena leucocephala* (Lam.) de Wit. Pesquisa em Foco. 2009;17(1):22-30.
32. Dea ICM, Clark AH, McCleary BV. Effect of the molecular fine structure of galactomannans on their interaction properties — the role of unsubstituted sides. Food Hydrocolloid. 1986;1(2):129-140.
DOI:10.1016/S0268-005X(86)80015-7
33. Dakia PA, Blecker C, Robert C, Wathelet B, Paquot M. Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. Food Hydrocolloid.2008;22(5):807–18.
DOI:10.1016/j.foodhyd.2007.03.007
34. Doyle JP, Giannouli P, Martin EJ, Brooks M, Morris ER. Effect of sugars, galactose content and chainlength on freeze–thaw gelation of galactomannans. Carbohyd Polym. 2006;64(3):391–401.
DOI:10.1016/j.carbpol.2005.12.019
35. Azero EG, Andrade CT. Testing procedures for galactomannan purification. Polym Test. 2002;21(5):551–56.
DOI:10.1016/S0142-9418(01)00123-4
36. Reid JSG, Edwards ME. Galactomannans and other cell wall storage polysaccharides in seeds. In: Stephen AM, editor. Food Polysaccharides and Their Applications. New York: Marcel Dekker, Inc.; 1995.
37. Ellis PR, Dawoud FM, Morris ER. Blood glucose, plasma insulin and sensory responses to guar-containing wheat breads: Effects of molecular weight and particle size of guar gum. Br J Nutr. 1991;66(3):363–79.
DOI:10.1079/BJN19910041
38. Cerqueira MA, Sousa-Gallagher MJ, Macedo I, Rodriguez-Aguilera R, Souza BWS, Teixeira JA, et al. Use of galactomannan edible coating application and storage temperature for prolonging shelf-Life of "regional" cheese. J Food Eng. 2010;97(1):87–94.
DOI:10.1016/j.jfoodeng.2009.09.019
39. Prajapati VD, Jani GK, Moradiya NG, Randeria NP, Maheriya PM, Nagar BJ. Locust bean gum in the development of sustained release mucoadhesive macromolecules of aceclofenac. Carbohyd Polym. 2014;113:138–48.
DOI:10.1016/j.carbpol.2014.06.061
40. Ughini F, Andreazza IF, Ganter JLMS, Bresolin TMB. Evaluation of xanthan and highly substituted galactomannan from *M. scabrella* as a substained release matrix. Int J Pharm. 2004;271:197-205.
41. Vendruscolo CW, Andreazza IF, Ganter JLMS, Ferrero C, Bresolin TMB. Xanthan and galactomannan (from *M. scabrella*) matrix tablets for oral controlled delivery of theophylline. Int J Pharm. 2005;296:1–11.
42. Yang L, Chu JS, Fix JA. Colon-specific drug delivery: New approaches and in vitro/in vivo evaluation. Int J Pharm. 2002;235(1-2):1–15.
DOI:10.1016/S0378-5173(02)00004-2
43. Lima DU, Oliveira RC, Buckeridge MS. Seed storage hemicelluloses as wet-end additives in papermaking. Carbohyd Polym.2003;52:367–73.
DOI:10.1016/S0144-8617(03)00008-0
44. Kapoor VP, Mukherjee S. Isolation of five oligosaccharides from the galactomannan of *Cassia absus* seed. Phytochemistry. 1971;10(3):655–659.
DOI:10.1016/S0031-9422(00)94713-7
45. Pandya H, Kachwala Y, Sawant L, Pandita N. Pharmacognostical screening of seeds of *Cassia absus*. Pharmacogn J. 2010;2(11):419-426.
DOI:10.1016/S0975-3575(10)80025-2
46. Aftab K, Atta-Ur-Rahman, Ahmed SI, Usmanghani K. Traditional medicine *Cassia absus* L. (chaksu)-pharmacological evaluation. Phytomedicine. 1996;2(3):213–219.
DOI:10.1016/S0944-7113(96)80045-6
47. Gupta DS, Jann B, Bajpai KS, Sharma SC. Structure of a galactomannan from *Cassia*

alata seed. Carbohyd Res. 1987;162(2):271-276.
DOI:10.1016/0008-6215(87)80222-7

48. Petkowicz CLO, Reicher F, Mazeau K. Conformational analysis of galactomannans from oligomeric segments to polymeric chains. Carbohyd Polym. 1998;37(25).
DOI:10.1016/S0144-8617(98)00051-4

49. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich Ascites Carcinoma. J. Ethnopharmacol. 2000;72(1–2):151–156.
DOI:10.1016/S0378-8741(00)00227-0

50. Joshi H, Kapoor VP. *Cassia Grandis* Linn. F. seed galactomannan: structural and crystallographical studies. Carbohyd Res. 2003;338:1907–12.
DOI:10.1016/S0008-6215(03)00258-1

51. Prajapati VD, Jani GK, Moradiya NG, Randeria NP, Nagar BJ, Naikwadi NN, Variya BC. Galactomannan: A versatile biodegradable seed polysaccharide. Int J Biol Macromol. 2013;60:83-92.
DOI:10.1016/j.ijbiomac.2013.05.017

52. Kapoor VP. Rheological properties of seed galactomannan from *Cassia nodosa* buch.-hem. Carbohyd. Polym. 1994;25(2):79–84.
DOI:10.1016/0144-8617(94)90142-2.

53. Pawar H, Varkhade C, Jadhav P, Mehra K. Development and evaluation of orodispersible tablets using a natural polysaccharide isolated from *Cassia tora* seeds. Integr Med Int. 2014;3(2):91–98.
DOI:10.1016/j.imr.2014.03.002

54. Shang M, Zhang X, Dong Q, Yao J, Liu Q, Ding K. Isolation and structural characterization of the water-extractable polysaccharides from *Cassia obtusifolia* seeds. Carbohyd Polym. 2012;90:827-832.
DOI:10.1016/j.carbpol.2012.06.007

55. Kapoor VP, Taravel FR, Joseleau JP, Milas M, Chanzy H, Rinaudo M. *Cassia spectabilis* DC seed galactomannan: structural, crystallographical and rheological studies. Carbohydr Res. 1998;306(1-2):231-41.
DOI:10.1016/S0008-6215(97)00241-3

56. Prado BM, Kim S, Ozen BF, Mauer LJ. Differentiation of carbohydrate gums and mixtures using Fourier transform infrared spectroscopy and chemometrics. J Agric Food Chem. 2005;53:2823–2829.

57. Cerqueira MA, Souza BWS, Simões J, Teixeira JA, Domingues MRM, Coimbra MA, et al. Structural and thermal characterization of galactomannans from non-conventional sources. Carbohyd Polym. 2011;83(1):179–85.
DOI:10.1016/j.carbpol.2010.07.036

58. Perduca MJ, Spotti MJ, Santiago LG, Judis MA, Rubiolo AC, Carrara CR. Rheological characterization of the hydrocolloid from *Gleditsia amorphoides* seeds. LWT-Food Sci Technol. 2013;51(1):143-147.
DOI:10.1016/j.lwt.2012.09.007

59. Srichamroen A, Thomson ABR, Field CJ, Basu TP. In vitro intestinal glucose uptake is inhibited by galactomannan from Canadian fenugreek seed (*Trigonella foenum graecum* L) in genetically lean and obese rats. Nutr Res. 2009;29(1):49-54.

60. López-Franco YL, Cervantes-Montaño C, Martínez-Robinson KG, Lizardi-Mendoza J, Robles-Ozuna LE. Physicochemical characterization and functional properties of galactomannans from mesquite seeds (*Prosopis* spp.). Food Hydrocolloid. 2013;30:656-660.
DOI:10.1016/j.foodhyd.2012.08.012

61. Bourbon AI, Pinheiro AC, Ribeiro C, Miranda C, Maia JM, Teixeira JA, et al. Characterization of galactomannans extracted from seeds of *Gleditsia triacanthos* and *Sophora japonica* through shear and extensional rheology: Comparison with guar gum and locust bean gum. Food Hydrocolloid. 2010;24(2–3):184–192.
DOI:10.1016/j.foodhyd.2009.09.004

62. Brummer Y, Cui W, Wang Q. Extraction, purification, and physicochemical characterization of fenugreek gum. Food Hydrocolloid. 2003;17:229–236.
DOI:10.1016/S0268-005X(02)00054-1

63. Sindhu G, Ratheesh M, Shyni GL, Nambisan B, Helen A. Anti-inflammatory and antioxidative effects of mucilage of *Trigonella foenum graecum* (Fenugreek) on adjuvant induced arthritic rats. Int Immunopharmacol. 2012;12(1):205-211.
DOI:10.1016/j.intimp.2011.11.012

64. Daas P, Grolle K, Vliet T, Schols H, de Jongh H. Toward the recognition of structure–function relationships in galactomannans. J Agr Food Chem. 2002;50:4282–4289.
DOI:10.1021/jf011399t

65. Singh VK, Banerjee I, Agarwal T, Pramanik K, Bhattacharya MK, Pal K. Guar gum and sesame oil based novel bigels for

controlled drug delivery. Colloids Surf B Biointerfaces. 2014;123(1):582-592.
DOI:10.1016/j.colsurfb.2014.09.056

66. Pawar HA, Lalitha KG. Isolation, purification and characterization of galactomannans as an excipient from *Senna tora* seeds. Int J Biol Macromol. 2014;65:167–75.
DOI:10.1016/j.ijbiomac.2014.01.026

67. De Souza CF, Lucyszyn N, Ferraz FA, Sierakowski MR. *Caesalpinia ferrea* var. *ferrea* seeds as a new source of partially substituted galactomannan. Carbohyd Polym. 2010;82(3):641–47.
DOI:10.1016/j.carbpol.2010.05.031

68. McCleary, BV, Amado R, Waibel R, Neukom H. Effect of galactose content on the solution and interaction properties of guar and carob galactomannans. Carbohyd Res. 1981;92:269-285.
DOI:10.1016/S0008-6215(00)80398-5

69. Bresolin TMB, Sander PC, Reicher F, Sierakowski MR, Rinaudo M, Ganter JLMS. Viscometric studies on xanthan and galactomannan systems. Carbohyd Polym. 1997;33(97):131–38.
DOI:10.1016/S0144-8617(97)00051-9

70. Grisel M, Aguni Y, Renou F, Malhiac C. Impact of fine structure of galactomannans on their interactions with xanthan: Two co-existing mechanisms to explain the synergy. Food Hydrocolloid. 2015;51:449–58.
DOI:10.1016/j.foodhyd.2015.05.041

71. Gonçalves MP, Gomes C, Langdon MJ, Viebke C, Williams PA. Studies on k-carrageenan/locust bean gum mixtures in the presence of sodium chloride and sodium iodide. Biopolymers. 1997;41(6): 657-671,

72. Sigma-Aldrich. Biochemicals & Reagents. Available:http://www.sigmaaldrich.com/catalog/search?interface=All&term=%20guar%20gum&N=0+16184121&focus=product&lang=pt&region=PT
(Accessed 09 February 2016)

73. Hoffman AS, Hubbell JA. Surface immobilized biomolecules. In: Ratner BD, editor. Biomaterials Science: An introduction to materials in medicine. Part I: Materials Science and engineering. Chapter II: Classes of materials used in medicine. China: Academic Press; 2004.

74. Zappino M, Cacciotti I, Benucci I, Nanni F, Liburdi K, Valentini F, et al. Bromelain immobilization on microbial and animal source chitosan films, plasticized with glycerol, for application in wine-like medium: Microstructural, mechanical and catalytic characterisations. Food Hydrocolloid. 2015;45:41-47.
DOI:10.1016/j.foodhyd.2014.11.001

75. Elchinger PH, Delattre C, Faure S, Roy O, Badel S, Bernardi T, et al. Immobilization of proteases on chitosan for the development of films with anti-biofilm properties. Int J Biol Macromol. 2015;72:1063–68.
DOI:10.1016/j.ijbiomac.2014.09.061

76. Derkus B, Emregul KC, Emregul E. Evaluation of protein immobilization capacity on various carbon nanotube embedded hydrogel biomaterials. Mater Sci Eng C Mater Biol Appl. 2015;56:132–40.
DOI:10.1016/j.msec.2015.06.022

77. Anusha JR, Fleming AT, Kim HJ, Kim BC, Yu KH, Raj CJ. Effective immobilization of glucose oxidase on chitosan submicron particles from gladius of *Todarodes pacificus* for glucose sensing. Bioelectrochemistry. 2015;104:44–50.
DOI:10.1016/j.bioelechem.2015.02.004

78. Bokkhim H, Bansal N, Grøndahl L, Bhandari B. In-vitro digestion of different forms of bovine lactoferrin encapsulated in alginate micro-gel particles. Food Hydrocolloid. 2016;52:231–42.
DOI:10.1016/j.foodhyd.2015.07.007

79. Pinheiro AC, Cerqueira MA, Souza BWS, Martins JT, Teixeira JA, Vicente AA. Utilização de Revestimentos/filmes Edíveis Para Aplicações Alimentares. Boletim Da Biotecnologia. 2010;18–29.
Available:http://repositorium.sdum.uminho.pt/bitstream/1822/16725/1/3559.pdf
(Accessed 27 November 2015)

80. Yang L, Paulson AT. Mechanical and water vapour barrier properties of edible gellan films. Food Res Int. 2000;33(7):563–70.
DOI:10.1016/S0963-9969(00)00092-2

81. Do Amaral Sobral PJ. Thickness effects of myofibrillar protein based edible films on their functional properties. Pesqui Agropecu Bras. 2000;35(6):1251–59.
DOI:10.1590/S0100-204X2000000600022

82. Krochta JM. Proteins as raw materials for films and coatings: Definitions, current status, and opportunities. In: Gennadios A. Protein-based films and coatings. Boca Raton: CRC Press; 2002.

83. Sothornvit R, Krochta JM. Plasticizer effect on oxygen permeability of β-lactoglobulin

films. J Agr Food Chem. 2000;48:6298-6302.
DOI:10.1021/jf000836l
84. Abreu AS, Oliveira M, Sá A, Rodrigues RM, Cerqueira MA, Vicente AA, et al. Antimicrobial nanostructured starch based films for packaging. Carbohyd Polym. 2015;129:127–34.
DOI:10.1016/j.carbpol.2015.04.021
85. Kim SRB, Choi YG, Kim JY, Lim ST. Improvement of water solubility and humidity stability of tapioca starch film by incorporating various gums. LWT-Food Sci Technol. 2015;64(1):475–82.
DOI:10.1016/j.lwt.2015.05.009
86. Ashok B, Reddy KO, Madhukar K, Cai J, Zhang L, Rajulu AV. Properties of cellulose/thespesia lampas short fibers bio-composite films. Carbohyd Polym. 2015;127:110–15.
doi:10.1016/j.carbpol.2015.03.054
87. Bedane AH, Eić M, Farmahini-Farahani M, Xiao H. Water vapor transport properties of regenerated cellulose and nanofibrillated cellulose films. J Membrane Sc. 2015;493:46–57.
DOI:http://dx.doi.org/10.1016/j.memsci.2015.06.009
88. Lee TW, Jeong YG. Regenerated cellulose/multiwalled carbon nanotube composite films with efficient electric heating performance. Carbohyd Polym. 2015;133:456–63.
DOI:10.1016/j.carbpol.2015.06.053
89. Soazo M, Báez G, Barboza A, Busti PA, Rubiolo A, Verdini R, et al. Heat treatment of calcium alginate films obtained by ultrasonic atomizing: Physicochemical characterization. Food Hydrocolloid. 2015;51:193–99.
DOI:10.1016/j.foodhyd.2015.04.037
90. Xiao Q, Tong Q, Zhou Y, Deng F. Rheological properties of pullulan-sodium alginate based solutions during film formation. Carbohyd Polym. 2015;130:49–56.
DOI:10.1016/j.carbpol.2015.04.069
91. Zhang Y, Ma Q, Critzer F, Davidson PM, Zhong Q. Physical and antibacterial properties of alginate films containing cinnamon bark oil and soybean oil. LWT - Food Sc Technol. 2015;64(1):423–30.
DOI:10.1016/j.lwt.2015.05.008
92. Fouda MMG, El-Aassar MR, Fawal GF, Hafez EE, Masry SHD, Abdel-Megeed A. k-Carrageenan/ poly vinyl pyrollidone/ polyethylene glycol/silver nanoparticles film for biomedical application. Int J Biol Macromol. 2015;74:179-84.
DOI: 10.1016/j.ijbiomac.2014.11.040
93. Medeiros BGS, Pinheiro AC, Teixeira JA, Vicente AA, Carneiro-da-Cunha M.G. Polysaccharide/protein nanomultilayer coatings: Construction, characterization and evaluation of their effect on 'Rocha' pear (*Pyrus communis* L.) shelf-life. Food Bioprocess Tech. 2012;5(6):2435-45.
DOI: 10.4236/fns.2011.21001.
94. Rhim J, Wang L. Preparation and characterization of carrageenan-based nanocomposite films reinforced with clay mineral and silver nanoparticles. Appl Clay Sci. 2014;97–98:174-81.
DOI:10.1016/j.clay.2014.05.025
95. Bigucci F, Abruzzo A, Saladini B, Gallucci MC, Cerchiara T, Luppi B. Development and characterization of chitosan/ hyaluronan film for transdermal delivery of thiocolchicoside. Carbohyd Polym. 2015;130:32–40.
DOI:10.1016/j.carbpol.2015.04.067
96. Chien P, Lin H, Su M. Effects of Edible Micronized Chitosan Coating on Quality and Shelf Life of Sliced Papaya. FNS Food and Nutrition Sciences. 2013;4:9-13.
DOI: 10.4236/fns.2013.49A2002
97. García MA, Pérez L, Paz N, González J, Rapado M, Casariego A. Effect of molecular weight reduction by gamma irradiation on chitosan film properties. Mater Sci Eng C. 2015;55:174-80.
DOI:10.1016/j.jrras.2015.01.003
98. Carneiro-da-Cunha MG, Cerqueira MA, Souza BWS, Souza MP, Teixeira JA, Vicente AA. Physical properties of edible coatings and films made with a polysaccharide from *Anacardium occidentale* L. J Food Eng. 2009;95(3):379–85.
DOI:10.1016/j.jfoodeng.2009.05.020
99. Souza MP, Cerqueira MA, Souza BWS, Teixeira JA, Porto ALF, Vicente AA, et al. Polysaccharide from *Anacardium occidentale* L. tree gum (Policaju) as a coating for Tommy Atkins mangoes. Chem Pap. 2010;64(4):475–81.
DOI:10.2478/s11696-010-0017-7
100. Shankar S, Rhim J. Amino acid mediated synthesis of silver nanoparticles and preparation of antimicrobial agar/silver nanoparticles composite films. Carbohyd Polym. 2015;130:353-63.
DOI:10.1016/j.carbpol.2015.05.018

101. Sousa AMM, Gonçalves MP. Strategies to improve the mechanical strength and water resistance of agar films for food packaging applications. Carbohyd Polym. 2015;132: 196-204.
DOI:10.1016/j.carbpol.2015.06.022
102. Lima AM, Cerqueira MA, Souza BWS, Santos ECM, Teixeira JA, Moreira RA, et al. New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits – influence on fruits gas transfer rate. J Food Eng. 2010;97:101-09.
DOI:10.1016/j.jfoodeng.2009.09.021
103. Mikkonen KS, Rita H, Helén H, Talja RA, Hyvönen L, Tenkanen M. Effect of polysaccharide structure on mechanical and thermal properties of galactomannan-based films. Biomacromolecules. 2007;8:3198-3205.
DOI: 10.1021/bm700538c
104. Klech CM. Gels and jellies. In: Swarbrick J, Boylan JC, Editors. Encyclopedia of Pharmaceutical Technology. New York: Marcel Dekker; 1990.
105. Gupta P, Vermani K, Garg S. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today. 2002;7(10):569-79.
DOI:10.1016/S1359-6446(02)02255-9
106. Gehrke SH, Lee PI. Hydrogels for drug delivery systems. In: Tyle P, Editor. Specialized Drug Delivery Systems. New York: Marcel Dekker; 1990.
107. Marcotte M, Taherian AR, Trigui M, Ramaswamy HS. Evaluation of rheological characteristics of salt-enriched hydrocolloids in the context of ohmic heating. J Food Eng. 2001;48(2):157-67.
108. Koop HS, Praes CEDO, Reicher F, Petkowicz CLDO, Silveira JLM. Rheological behavior of gel of xanthan with seed galactomannan: Effect of hydroalcoholic-ascorbic acid. Mat Sci Eng C. 2009;29(2):559–63.
DOI:10.1016/j.msec.2008.10.004
109. Makri EA, Doxastakis GI. Study of emulsions stabilized with *Phaseolus vulgaris* or *Phaseolus coccineus* with the addition of Arabic gum, locust bean gum and xanthan gum. Food Hydrocolloid. 2006;20(8):1141–52.
DOI:10.1016/j.foodhyd.2005.12.008
110. Rocha LC, Souza AL, Rodrigues Filho UP, Campana Filho SP, Sette LD, Porto ALM. Immobilization of marine fungi on silica gel, silica xerogel and chitosan for biocatalytic reduction of ketones. J Mol Catal B-Enzym. 2012;84:160–65.
DOI:10.1016/j.molcatb.2012.05.025
111. Alvaréz-Román R, Barré G, Guy RH, Fessi H. Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection. Europ J Pharm Biopharm. 2001;52:191-95.
DOI:10.1016/S0939-6411(01)00188-6
112. Comba S, Sethi R. Stabilization of highly concentrated suspensions of iron nanoparticles using shear-thinning gels of xanthan gum. Water Res. 2009;43(15):3717-26.
DOI:10.1016/j.watres.2009.05.046
113. Souza MP, Vaz AFM, Correia MTS, Cerqueira MA, Vicente AA, Carneiro-da-Cunha MG. Quercetin-loaded lecithin/chitosan nanoparticles for functional food applications. Food Bioprocess Tech. 2014;7(4):1149–59.
DOI: 10.1007/s11947-013-1160-2
114. Fang R, Jing H, Chai Z, Zhao G, Stoll S, Ren F, et al. Design and characterization of protein-quercetin bioactive nanoparticles. J Nanobiotechnology. 2011;9(1):19.
DOI:10.1186/1477-3155-9-19
115. Tan Q, Liu W, Guo C, Zhai G. Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. Int J Nanomedicine. 2011;6:1621-30.
DOI: 10.2147/IJN.S22411
116. Choksi AN, Poonawalla T, Wilkerson MG. Nanoparticles: A closer look at their dermal effects. J Drugs Dermatol. 2010;9(5):475–81.

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Review

Approaches in biotechnological applications of natural polymers

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Abstract: Natural polymers, such as gums and mucilage, are biocompatible, cheap, easily available and non-toxic materials of native origin. These polymers are increasingly preferred over synthetic materials for industrial applications due to their intrinsic properties, as well as they are considered alternative sources of raw materials since they present characteristics of sustainability, biodegradability and biosafety. As definition, gums and mucilages are polysaccharides or complex carbohydrates consisting of one or more monosaccharides or their derivatives linked in bewildering variety of linkages and structures. Natural gums are considered polysaccharides naturally occurring in varieties of plant seeds and exudates, tree or shrub exudates, seaweed extracts, fungi, bacteria, and animal sources. Water-soluble gums, also known as hydrocolloids, are considered exudates and are pathological products; therefore, they do not form a part of cell wall. On the other hand, mucilages are part of cell and physiological products. It is important to highlight that gums represent the largest amounts of polymer materials derived from plants. Gums have enormously large and broad applications in both food and non-food industries, being commonly used as thickening, binding, emulsifying, suspending, stabilizing agents and matrices for drug release in pharmaceutical and cosmetic industries. In the food industry, their gelling properties and the ability to mold edible films and coatings are extensively studied. The use of gums depends on the intrinsic properties that they provide, often at costs below those of synthetic polymers. For upgrading the value of gums, they are being processed into various forms, including the most recent nanomaterials, for various biotechnological applications. Thus, the main natural polymers including galactomannans, cellulose,

chitin, agar, carrageenan, alginate, cashew gum, pectin and starch, in addition to the current researches about them are reviewed in this article.

Keywords: agarose; alginate; carrageenan; chitin; galactomannan; gum; hydrocolloids; mucilages; polysaccharides; starch

1. Introduction

Natural polymers are materials of natural origin with properties of biocompatibility, low cost, availability and lack of toxicity. These polymers, such as gums and mucilages, are increasingly preferred over synthetic materials due to their intrinsic properties; in addition to be considered alternative sources of raw materials for industrial applications.

As definition, gums are polysaccharides composed by multiple sugar units linked together to create large molecules with heterogeneous composition. Upon hydrolysis, they yield simple sugar units such as arabinose, galactose, glucose, mannose, xylose or uronic acids. The polysaccharide gums represent one of the most abundant industrial biomaterials and have been reported by several studies due to their sustainability, biodegradability and biosafety. Gums are abundant in nature and commonly found in many higher plants; in addition, they are frequently produced as a protection mechanism following plant injury [1].

Besides gums, the constituents of polysaccharides also include mucilages. Although their natural polymeric source, gums and mucilages have certain differences: gums readily dissolve in water, while mucilages form viscous masses; gums are considered pathological products, whereas mucilages are physiological products. In addition, their similarities are related to their broad range of physicochemical properties, which are widely used for applications including cosmetics, paper, pharmacy, textile, adhesive, inks, lithography, paint, explosive, and smoking products [2,3]. In order to avoid misinterpretations, it is important to distinguish the above-mentioned terms.

Mucilage is a polysaccharide mixture commonly found in various organs of many higher plant species. Due to its high variability in terms of chemical constituents, mucilage probably assumes a multitude of physiological functions in plants [4]. Mucilages found in rhizomes, roots and seed endosperms may act primarily as energy reserves [5], whereas foliar mucilages appear not to serve as storage carbohydrates. Generally, it has been assumed that foliar mucilages are merely secondary plant metabolites, but there was also reported that they may play a role in wound responses, frost tolerance, water transport, plant host-pathogen interactions, the ionic balance of plant cells, and as carbohydrate reserves. There is growing evidence that, due to the high concentration of hydroxyl groups in the polysaccharides, extracellular mucilages in particular have a high water-binding capacity and may play an important role in the drought resistance of certain plant species [2].

Natural gum is a term used to describe a group of naturally occurring polysaccharides. They have widespread industrial applications due to their ability either to form the gel, make the viscous solution or stabilize the emulsion systems [6]. Water-soluble gums are described as hydrocolloids; they are used for various applications, including dietary fibers [7], texture modifiers, stabilizers and/or emulsifiers [8,9], gelling agents [10], thickeners [11], coating agents [12,13] and packaging films [14]. Many natural gums form three dimensional interconnected molecular networks known as "gels". The strength of the gel depends on its structure and concentration, as well as on factors such as temperature,

pH, and ionic strength. The linear polysaccharides occupy greater volume than branched polymers of comparable molecular weight. Thus, at the same concentration, comparable linear polysaccharides exhibit greater viscosity. Therefore, it is difficult for the heterogeneous gum molecules to move freely without becoming entangled with each other (and any other large molecules also present). In addition, natural gums are often recognized by their swelling properties, which occurs due to the entrapment of large amounts of water between their chains and branches [1].

Cellulose, chitin, agar and starch are the most common polysaccharides used in industry. Regarding their broad range of applications, the preference for use of natural polymers is presumed over comparable synthetic materials due to their non-toxicity, low cost and availability. For instance, most of the natural gums are safe enough for oral consumption in the form of food additives or drug carriers [1]. In addition, synthesizing natural polymers as nanomaterials enhances the industrial applicability due to its larger surface, besides the intrinsic properties above mentioned. The present work considers the most recent literature dealing with natural polymers, including natural gums and mucilages, and their potential biotechnological applications. Moreover, we summarized information about their chemical structure, physicochemical and functional properties.

2. Classification of natural polymers and their biotechnological applications

Gums and mucilages are sourced from the endosperm of plant seeds, plant exudates, tree or shrub exudates, seaweed extracts, fungi, bacteria, and animal sources, where they perform a number of structural and metabolic functions; it is important to highlight that plant sources provide the largest amounts of gums. Natural gums are categorized based on their origins, behaviour and chemical structures. Gums submitted to a hydrolysis process, because of their polysaccharide nature, produce an indefinite number of monosaccharides. Depending on the type of the products obtained by hydrolysis, gums can be further classified into pentosans and hexosans. Chemically, they are pathological products consisting of calcium, potassium and magnesium salts of complex substances known as polyuronides, whose sugar units can be separated by hydrolysis using dilute mineral acids, followed by separation of liberated monosaccharides using different chromatographic techniques [3]. Different sources of gums and mucilages are listed in Table 1.

Plant-based gums are the polysaccharides originated from various parts of plant (e.g. plant cell walls, tree exudates, seeds, tuber/roots, seaweeds) [6]. Most of them belong to the Leguminosae family, which is divided into the subfamilies Caesalpinioideae, Mimosoideae and Faboideae [15]. The polysaccharides sourced from plants are examples of natural compounds that have contributed to the Leguminosae family classification, but special emphasis has been given to galactomannans [16].

The considerably growing interest in gums is due to their diverse structural properties and metabolic functions in food, pharmaceutical, cosmetic, textile and biomedical products [17], since they can be used as dietary fiber, texture modifiers, gelling agents, thickeners, emulsifiers, stabilizers, coating agents and packaging films [6,18]. In the recent years, the demand for plant-based gums in food, medicine and drug delivery systems have been considerably increased because they are the most notable ingredients in liquid and semisolid foods [19]. However, the market still desires new sources of plant-based gum to meet the demand for ingredients with more usefulness especially in food systems [6], hence they are the new generation of products sourced from sustainable materials that intend to comply the ecological and economic requirements.

Acting as soluble dietary fibres, gums play a crucial part in developing foods with high satiating capacity due to their viscosity. Most of the scientific researches mention that when gums are hydrated, they increase the viscosity of the solution medium and show a very wide range of rheological behaviour, generally pseudoplastic, depending on their concentration, chemical arrangement and structure [20].

Table 1. Classification of the different available gums and mucilages.

Source	Name	Type	Reference
Tree gum exudates	Arabic gum	Arabinogalactan	[21]
	Prosopis alba gum	Arabinogalactan	[22]
	Acacia tortuosa gum	Arabinogalactan	[23]
	Almond gum	Arabinogalactan	[24]
	Albizia stipulata gum	Arabinogalactan	[25]
	Acacia senegal gum	Arabinogalactan	[26]
	Cashew gum	Galactan with glucose, arabinose, rhamnose, mannose and glucuronic acid units	[27]
Extracts	Pectin	Galacturonic acid with rhamnose, arabinose, galactose, xylose, and glucose units	[28]
Seeds	*Cassia spectabilis* gum	Galactomannan	[29]
	Guar gum	Galactomannan	[30]
	Cassia nodosa gum	Galactomannan	[31]
	Cassia grandis gum	Galactomannan	[32]
	Fenugreek	Galactomannan	[33]
	Locust bean gum	Galactomannan	[34]
	Starch	Glucan	[35]
	Hymenaea courbaril gum	Xyloglucan	[36]
Red seaweeds	Agar	Galactan	[37]
	Carrageenans	Galactan	[38]
Brown seaweeds	Alginate	Mannuronic acid with guluronic acid units	[39]
Microbial	Xanthan gum	Glucan	[40]
	Cellulose	Glucan	[6]
Animal	Chitin	Glucosamine	[41]
	Hyaluronic acid	Glucosamine with glucuronic acid units	[42]
	Chondroitin sulphate	Galactosamine with glucuronic acid units	[43]

Studies dealing with gums as texture modifying agents are continuing to increase in the food and culinary industries, especially for gelling, thickening and emulsifying purposes. Some hydrocolloids are used as gelling agents, for example, to increase the satiety sensation in the stomach, brought about by different triggering factors. It is believed that, in order for gastric gelation to induce satiety, not only must a gel form in the stomach but the gel must also possess some strength [44]. Also as regards the food industry, the most common polysaccharides used for production of edible films are cellulose, chitosan, agar, starch, and no less important, galactomannans, since their mechanical and thermal properties have been widely exploited for biotechnological application.

Not only in food and culinary industry, but also in biomedical applications, the synergistic interactions that occur in systems containing different blends of polysaccharides have been used for a long time due to their ability to form strong gels even at low concentrations [45]. Still in view of the medical field, some of the current challenges in the conventional therapeutic systems include lack of proper drug delivery systems with efficient bioavailability or drug release capability, lack of efficient imaging and sensing technique, lack of targeted delivery systems, etc. As an attempt to circumvent these issues, the advent of nanotechnology allows the use nano-tools to overcome the shortcomings of the conventional methods [46], therefore, natural gums are being processed into various forms, including the most recent nanomaterials, for various biotechnological applications. The main natural polymers, including gums and mucilages, as well as the current researches about them are reviewed below.

3. Galactomannans

Galactomannans are neutral polysaccharides isolated from seeds. The main chain is composed of (1→4)-β-D-mannose (M) units with different degrees of substitution on O-6 with α-D-galactopyranosyl (G) units (Figure 1). Galactomannans are considered highly water soluble hydrocolloids providing highly viscous and stable aqueous solutions, in addition, its solubility depends on the composition (or M/G ratio) and on the distribution of galactose units along the mannan backbone chain: the larger the galactose content, the higher the solubility in water [47].

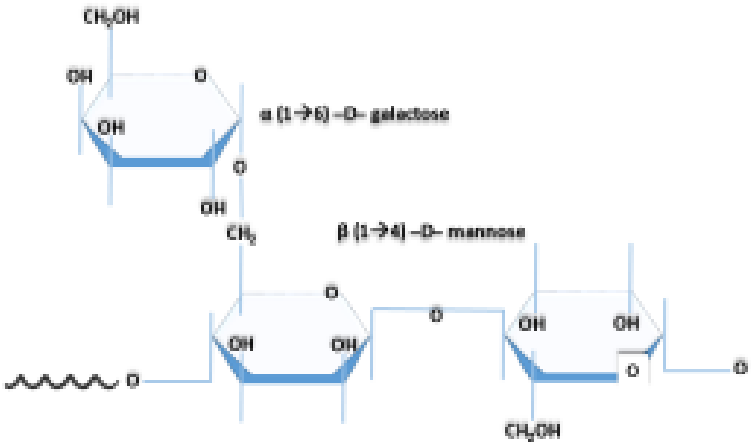

Figure 1. Representative segment of a galactomannan main chain from plant origin.

The M/G ratios varies among the species of the same subfamily. Caesalpinioideae presents M/G ratios ranging from 2.44 (*Cassia grandis*) [32] to 4.2 (*Delonix regia*) [48] and its main representatives are *Cassia tora* (M/G: 3.0) [49], *C. spinosa* (M/G: 2.7–3.0) [49,50] and *Ceratonia siliqua* (M/G: 3.5–3.75) [51]. Mimosoideae subfamily presents lower M/G ratios, usually varied from 1 (*Mimosa scabrella*) [52] to 2.6 (*Besmanthus illinoensis*) [49]. Faboideae subfamily presents a broad range of

M/G ratios for its representatives and includes the main commercially used galactomannan in food and non-food industries, the guar gum (*Cyamopsis tetragonolobus*, M/G: 1.8) [53].

Galactomannans are polysaccharide gums with several functions associated in terms of their physicochemical and biological properties, which in fact offer a wide variety of applications. Unlike other gums, galactomannans or their derivatives are less exploited on an industrial scale, despite milled endosperm powders of guar, locust bean (*Ceratonia siliqua*), and fenugreek (*Trigonella foenum-graecum*) represent the galactomannan seeds currently exploited on an industrial scale [54]. Among their applications, these gums accumulated moisture (desiccation tolerance) at the early stage of seed swelling and serve as a carbohydrate energy resource during seed germination. In addition, galactomannan gums may also play a protective role, since they impart certain strength to the endosperm surrounding the germ at the periphery [55].

Compared with other gums, galactomannans exhibited excellent retention of viscosity even at low concentrations. They can be used as mass-efficient aqueous thickeners, nutritional supplements [11,40], and component in a mixed gel [56,57]. In the food industry, galactomannans as guar and locust bean gum enjoy widespread use based on their ability to thicken and stabilize many food products [55]. More recently, some works reported the possibility of using galactomannans in the formation of films and coatings [14,59-61].

In some works, galactomannans have been used in binary mixtures with other polysaccharides such as xanthan gum [62-64], agar [65] and kappa carrageenan [58,66], to form gels with new properties. Among the oil and textile industries, galactomannan gums are the major ingredients in drilling mud and are used mainly also for their capacity to increase printing quality. The functional properties of these polysaccharides are of primary importance for controlling the release of drugs in the pharmaceutical industry [67,68].

4. Cellulose

Cellulose, the major structural constituent of the cell wall of plants, is the most abundant polysaccharide in nature. It has many advantages, such as superior thermal and mechanical properties, in addition to biocompatibility, biodegradability, and cost-effectiveness [69,70].

One of the most successful biomaterials for health is the bacterial cellulose, a promising biopolymer obtained through biosynthesis routes by some bacterial genera belonging to *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina* genera. Bacterial cellulose has been extensively studied due to its purity, superior physico-mechanical and biological properties, as well as its potential applications in numerous traditional industries, such as biomedical, construction, pulp and papermaking, in addition to textile industry [71-73].

The biosynthesis of cellulose is a process commonly associated to the living plant cell through photosynthesis. In the oceans, however, most cellulose is produced by unicellular plankton or algae using the same type of carbon dioxide fixation found in photosynthesis of land plants. In addition, several animals, and microbial can assemble cellulose, but these organisms are devoid of photosynthetic capacity and usually require glucose or some organic substrate synthesized by a photosynthetic organism to assemble the polymer [74]. According to Nobles et al. [75], even most cellulose widespread in nature is now being produced by plant cellulose synthase complexes; this

enzyme has bacterial origin, therefore its genes have been acquired by plants from cyanobacterial ancestors of their chloroplasts.

Despite the most recent literature dealing with the elucidation of the molecular mechanisms of cellulose biosynthesis in plants, many aspects of this process still remain obscure. Especially for bacterial cellulose biosynthesis, the model system associated with the microbial origin of cellulose has long been used as a simpler and genetically tractable model to study its biosynthesis in plants. Even after this model system became dispensable, studies of bacterial cellulose biosynthesis proved to be extremely important, since bacterial cellulose has advantageous characteristics over comparable with plant cellulose, including high crystallinity degree, elasticity, durability and higher water-absorbance [76-78].

In summary, there are four main different pathways to obtain cellulose: the first is the most popular and industrially important pathway for isolating cellulose from plants, which includes the chemical pulping, separation, and purification processes to remove lignin and other polysaccharides (hemicelluloses). The second pathway consists in the biosynthesis of cellulose by different types of micro-organisms (unicellular algae, fungi, and bacteria). The third pathway is the enzymatic *in vitro* synthesis starting from materials such as cellobiosyl fluoride. The last pathway is a chemical synthesis, that produces cellulose though a ring-opening polymerization of the benzylated and pivaloylated derivatives of glucose [73].

In what concerns the basic structure of cellulose, it is a simple polysaccharide with no branching or substituents in its homogeneous backbone (Figure 2). The morphological hierarchy of cellulose is composed by elementary fibrils, which pack into larger units called microfibrils, and these are in turn assembled into fibres. Within the cellulose fibrils, there are regions where the cellulose chains are arranged in a highly ordered structure (crystallites) and regions that are disordered (amorphous-like). The extraction of the crystalline regions results in nanocrystalline cellulose (NCC), while the interaction between inter- and intra-molecular networks can vary, giving rise to cellulose polymorphs or allomorphs [79,80].

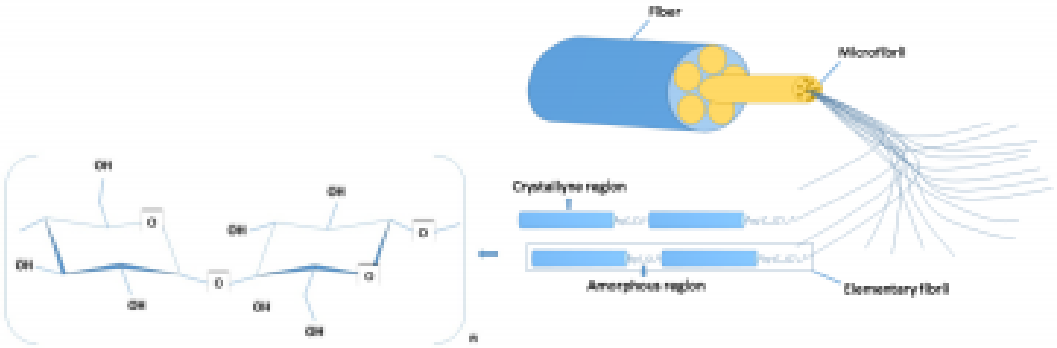

Figure 2. Representative image of the cellulose main chain and the hierarchical structure of this polysaccharide.

NCC is the base reinforcement unit that strengthen all subsequent structures in plant, trees, etc., and can be a useful material on which to base a new polymer composite industry. NCC has also properties such as low density, high specific strength and modulus, high surface area, unique optical properties, and modifiable surface properties due to the reactive hydroxyl groups [81]. Advantages in

the biotechnological application of NCC when compared to macrocellulose are related not only to their useful, unsurpassed, physical and chemical properties, or to their sustainability, biodegradability, renewability, abundance, high biocompatibility. Actually, the nanoscale dimension opens a wide range of possible properties to be discovered. According to Fujisawa et al. [82], NCC can be divided in three major groups: (1) cellulose nanocrystals (CNC), obtained from acid hydrolysis followed by mechanical agitation from the suspension of nanocrystals in water; (2) microfibrillated cellulose (MFC), prepared with mechanical disintegration method of the cellulosic pulp in water; and (3) nanofibrillated cellulose (NFC), prepared using a combination of chemical oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), followed by mechanical disintegration in water, or only by mechanical disintegration.

It is important to highlight that the hydroxyl groups are the most targeted reactive groups on the cellulose main chain; they can fully or partially react with chemical agents to obtain various derivatives with different degree of substitution. For upgrading the value of cellulose, the obtained derivatives have been developed and used in industries such as food, cosmetic, biomedical, and pharmaceutical. However, the application of cellulosic material is limited due to the difficulty in processing, for example, the high crystallinity degree and rigid intra/intermolecular hydrogen bonds which result in its insolubility in most solvents [35,83].

The above mentioned problems associated with the processing of cellulose could be observed for other natural polymers since they present increasingly application in the industrial technology. In addition, most polymers do not show biological activity unless some modifications are carried out, so it is important to mention that numerous attempts are being performed to minimize these certain drawbacks with chemical modifications in the polymeric structure.

The most important chemical transformation and functionalization of cellulose based on hydroxyl group include esterification, etherification, selective oxidation, graft copolymerization, and intermolecular crosslinking reaction [84]. The esterification process of cellulose was applied for different purposes, such as the preparation of superabsorbent hydrogels [85] and bacterial cellulose nanofibers [86]. The etherification process of cellulose was especially applied in the pharmaceutical industry, for example to obtain NFC as safe candidate for novel, bio based, and permanently antimicrobial material [87]. The oxidation of primary hydroxyl groups of cellulose is one of the most important methods of their functionalization, which yields valuable oxidized products such as polyuronic acids and aldehydes, to be further used in various fields as drugs, dyes, glues, thickeners, additives to foodstuffs, cosmetics and many other. Oxidized cellulose and regenerated cellulose are widely used as excellent hemostatic materials in various surgical operations and postsurgical adhesion prevention layer [88-90].

Regenerated cellulose has also been used as films; cellophane films, for example, have not been commonly used for food packaging, due to their high water vapour permeability (low barrier properties) compared to their synthetic counterparts. On the other hand, transparent cellulose films exhibit good oxygen and CO_2 barrier properties at dry conditions. They are made from purified wood or cotton pulp sheets and have important characteristics such as transparency, durability, flexibility, non-water solubility and non-permeability to air and grease. Other transparent cellulose films such as MFC and NFC film have emerged as a potential packaging material due to their strong mechanical properties [91,92].

Currently, there are two oxidation systems to selectively convert the primary hydroxyl groups in cellulose into carboxyl and/or aldehyde groups: (1) 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and its derivatives; (2) N-hydroxyphthalimide (NHPI), N-hydroxy-benzotriazole (HBT), and violuric

acid (VA) [93,94]. The amount of carboxylic groups formed by oxidation of cellulose was investigated for different and important purposes, such as the following examples. A comparatively investigation between TEMPO and NHPI to oxidize regenerated cellulose fibers [95], the potential of TEMPO-mediated oxidized NFC as bioremediator of metal ions from contaminated water [96]; the *in vitro* antibacterial evaluation of TEMPO-oxidized cellulose films functionalized with silver nanoparticles [97]; and the preparation of water stable films made of TEMPO/$NaClO_2$ oxidized NFC, covalently bonded with poly(vinyl alcohol) [98].

Another approach for cellulose modification via chemical redox initiation methods (ceric ammonium nitrate or ferrous ammonium sulphate–potassium persulfate) or irradiation methods (UV, microwave, and γ-rays) is the graft copolymerization, which provides a mean of altering the physical and chemical properties of cellulose and increasing its functionality [35,97-101].

Nowadays, the research on cellulose nanocomposites has grown exponentially. As definition, cellulose nanocomposites are structures composed of water-soluble or water-dispersive polymers mixed with cellulose nanomaterials, and manufactured using different processes able to affect properties such as dispersion, distribution and alignment of the reinforcing phase of the composite [102]. The most recent literature dealing with cellulose nanocomposites described the employment of the microwave-assisted method on the investigation of the influence of cellulose on nanocomposites of cellulose/Ag [103], cellulose Ag/Cl [104], cellulose/SrF_2 [105], cellulose/CaF_2 and cellulose/MgF_2 [106], and cellulose/F-substituted hydroxyapatite [107]. The pharmaceutical field has important studies, for example, all cellulose nanocomposite membranes with excellent performance were successfully fabricated as novel filtration system to remove nanoparticles and Hepatite C virus from aqueous medium [108]. Transparent porous nanodiamonds/cellulose nanocomposite membranes with controlled release of doxorubicin were tested as a candidate for wound dressing [109]; and cellulose-graft-polyacrylamide/nano-hydroxyapatite composites were reported as a promising scaffold for bone tissue engineering [110,111].

5. Polysaccharides from animal origin

Glycosaminoglycans (GAGs) are heteropolysaccharides composed by a repeating disaccharide unit without branched chains, being one of the two monosaccharides always an amino sugar (N-acetylgalactosamine or N-acetylglucosamine) and the other one an uronic acid. They are usually found on all animal cell surfaces and in the extracellular matrix, where are known to bind and regulate different proteins. In the last years, an increasing number of GAGs, chitin and chitosan applications have been reported by the scientific literature reporting their applicability in numerous contexts from food, cosmetic, textiles and clinical areas [43].

Chondroitin sulphate (CS) is an essential component of extracellular matrix of connective tissues in which plays a central role in various biological processes, including the elasticity of the articular cartilage, hemostasis and inflammation, regulation of cell development, cell adhesion, proliferation and differentiation [43]. Hyaluronic acid (HA) is classified as a viscous substance with multifunctional properties, especially those related to biological functions like cell proliferation, differentiation and tissue repair; the applicability of HA has been focused recently as drug delivery devices with studies suggesting a number of molecules might be used as gel preparations for drug transport. Since the application of the polysaccharide in the medicinal field has been increased, the interest in isolation of HA has been augmented to greater extents [112].

Although CS and HA has been isolated from terrestrial origin so far, the increasing interest on these polysaccharides significantly aroused the alternative search from marine sources since it is at the preliminary level. However, chitin is still the main waste product of the shellfish industry and, due of this importance, will be detailed revised below.

Chitin

Chitin is the second most abundant polymer after cellulose [89]. Its chemical structure (poly-β-(1→4)-*N*-acetyl-D-glucosamine) is widely synthesized in a number of living organisms, which depending on its source, chitin occurs mainly as two allomorphs, namely α (the most abundant) and β forms [47]. Sharing the classification of natural polymers from animal origin with CS and HA, chitin can be found in many organisms including fungi, yeasts, algae and squid pen; it is also usually found in lobsters, crab tendons, shrimp and crab shells as well as insect cuticles, being shells the preferred source of chitin due to their high availability as waste from the seafood processing industry [43,47].

Chitin occurs in nature as ordered crystalline microfibrils found as structural components in the cell wall of fungi and yeast or in the exoskeleton of arthropods. The main commercial sources of chitin are crab and shrimp shells, where chitin is associated with proteins, pigments and calcium carbonate. The physical properties of chitin in solution are still scarce in the literature due to the difficulties in dissolving the polymer; chitin is insoluble in all usual solvents, which represents a problem in view of the development of processing and of its uses [47].

Chitin can be partially degraded by acid to obtain a series of oligomers namely oligochitins. Instead, under alkaline conditions, a partial deacetylation of chitin results in the most important chitin derivative in terms of applications: chitosan (Figure 3). Oligochitins as well as those from chitosan are recognized for their bioactivity including antitumoral [113,114], antimicrobial [115], fungicidal [116] and immunotherapeutic [117] properties, eliciting chitinase [118], and regulating organism growth [111].

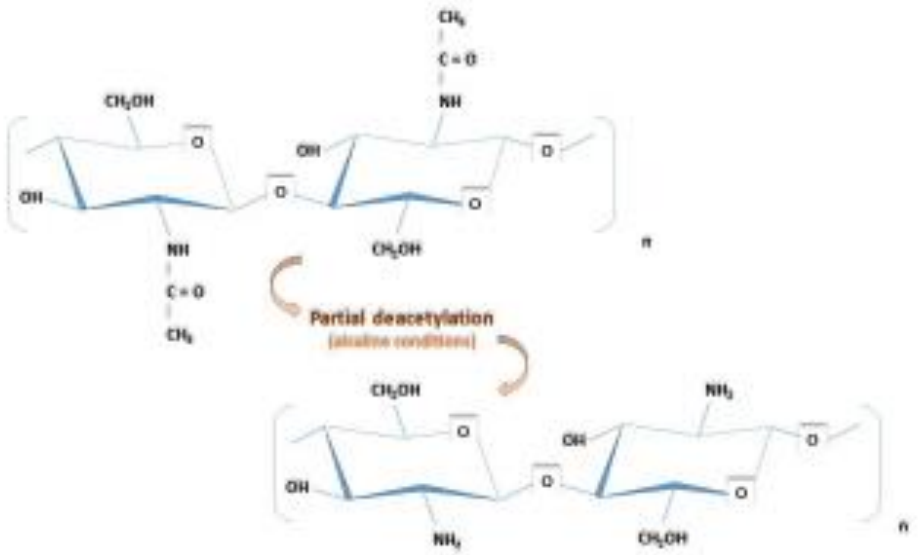

Figure 3. Representation of chitin and chitosan structures. "n" is the degree of polymerization.

Chitin is an amino polysaccharide having massive structural propensities to produce bioactive materials with innovative properties, functions and a range of biotechnological applications. In addition, the specific physicochemical, mechanical, biological and degradation properties offer efficient way to blend this biopolymer with synthetic ones [119].

Chitin has a low toxicity and it is inert in the gastrointestinal tract of mammals. Chitinases and lysozyme from egg white can degrade chitin; in the first case, the enzymes are widely distributed in nature, found in bacteria, fungi and plants as well as in the digestive tract of numerous animals. After the deacetylation process, it was shown that the rate of in vivo degradation is high for chitin, but decreases for chitosan [47,120].

The main applications of chitin are in medical and pharmaceutical fields, especially due to the presence of the acetamide group, which is similar to the amide bond of the protein of the living tissue and, therefore, is compatible with human or animal cells. Hence, chitin can be used in wound healing [121,122] and tissue engineering applications [123,124].

Still in view of wound dressing materials, Morgado et al. [125] remarked that chitosan had also been used for wound dressing production due to its intrinsic properties, including antimicrobial and hemostatic activities, biocompatibility, and biodegradability. Chen et al. [126] and Lih et al. [127] also highlighted the potential application of chitosan as wound dressing due to the recognition of its surface by platelets. The coagulation cascade starts in a few seconds with the protonated amine groups of chitosan attracting the negatively charged residues on red blood cell membranes, resulting on a strong agglutination, thrombin generation and fibrin mesh synthesis within the microenvironment created by this polysaccharide.

The marked insolubility of chitin in all usual solvents is the most different property compared to chitosan, which is the only commercially available water-soluble cationic polymer due to the positive charges on its amino groups. The water solubility of chitosan allows the interaction of this cationic biopolymer with anionic molecules such as glycosaminoglycans (GAG) and proteoglycans. Many cytokines/growth factors are linked to GAG, so the complex of chitosan-GAG may retain and concentrate those substances [128]. This unique property makes chitosan an appropriate material not only in the biomedical and pharmaceutical fields; instead, the antimicrobial and gas barrier properties of chitosan were successfully applied in the food industry, for example, in a nanomultilayer coating obtained by electrostatic layer-by-layer self-assembling technique; by combining the intrinsic properties of chitosan and the low oxygen permeability of pectin, layers were efficient in the reduction of gas flow and on the extension of the shelf-life of mangoes [129]. Chitosan-based films with quercetin incorporated also showed potential to be used as a solution for active food packaging [130]. The positive charge of chitosan was also used in the development of a high-efficient gene delivery formulation combined with polyethylenimine and DNA by Min et al. [131], who achieved a system less cytotoxic than those made of a single cationic carrier.

Many applications were described based on chitin and its blends for tissue engineering, for example, Wan and Tai [132] presented a revision of chitin as scaffolds and matrices for tissue engineering, stem cell propagation and differentiation. Polymer matrix–calcium based composites were used for hard tissue substitutes [133], in addition to drug delivery [134]. Chitin is also efficient to give porous forms providing scaffolds for complete tissue formation [135,136].

In what concerns drug delivery system, chitin and its derivative amorphous chitin, alone or with other polymers, have been widely used due to its biocompatible, biodegradable and non-toxic nature [137]. Further, more recently application takes into consideration the high surface area presented in nanometric

systems, especially because of the nano-size possess certain unique properties which enable them to be used in a number of biomedical applications. Prepared by cross-linking reaction using TPP, chitin nanoparticles were described as efficient networks for drugs with antitumoral [138,139] and antibacterial [137] purposes. Working with one derivative of chitin containing carboxyl groups (CMC), Dev et al. [140] have taken the approach of cross-linking the CMC as a way to control its biodegradability and to result in nanoparticles, providing a most favorable option for drug delivery applications in chemotherapy. Chitin nanoparticles can be also isolated from the purified chitin by repeated acid hydrolysis and applied for different purposes, such as the development of bio-nanocomposites with starch [141] and as a suitable adsorbent material for the removal of dyestuff from effluents [142].

Chitin can be used in the food industry because of its biological activities including antioxidant and antimicrobial effects. In addition to other properties that could improve food safety, quality, and shelf life [143], however the main publications dealing with protective barrier against food spoilage [144,145], edible film production [146-150], use as stabilizers and thickeners compounds [143], and prebiotics ingredients [151] related chitosan as the most efficient biomaterial in this field.

6. Seaweed polysaccharides

Algae are an important source of many polysaccharides from the point of view of applications, in addition to be the oldest known living organisms. Morphologically, they are very primitive and their evolution was very slow over time. Blue-green algae (Cyanophyta) appeared first, followed by red algae (Rhodophyta), green algae (Chlorophyta) and finally brown algae (Phaeophyta). Algae are in fact rich in non-essential and essential amino acids such as proline, glycine and lysine, besides many other molecules [39,47].

It was recognized that the three main gums, agar, alginate, and carrageenan, could be extracted from algae and used especially as thickeners or gelling polymers. Their physical properties are efficiently used in food, however new applications of algae are being developed, for example in the production of drugs and pharmaceuticals. Red seaweeds contain agar (mainly neutral), carrageenans and a few other polysaccharides included under the category of sulphated polysaccharides. Brown algae produce alginates, a carboxylic polymer, initially in different ionic forms and a few other polysaccharides, among them fucoidans [47].

6.1. Agar and carrageenan

Algae derived from Rhodophyta are an important source of polysaccharides, which are sulphated glycans together with a variety of *O*-methylated sugar residues, among them carrageenans and agars, polysaccharides commonly used in industry. The basic structure of these polymers is composed of a linear chain of β-D-(1→3) galactopyranose and α-D/L-(1→4) galactopyranose units (Figure 4) arranged in an alternating sequence [47].

Polysaccharides sourced from red algae have attracted an increasing interest due to their excellent physical properties, such as thickening, gelling, and stabilizing ability [152,153], and also due to their beneficial biological activities, such as anticoagulant [154], antithrombotic [38], antioxidant [155], antiviral [156,157], anti-inflammation [158], antitumour [159], and immunomodulatory activity [160]. It is important to highlight that their biological activities depend on the structural features, such as the sulphate content and distribution of sulphate groups on the main chain, molar mass, and stereo-chemistry.

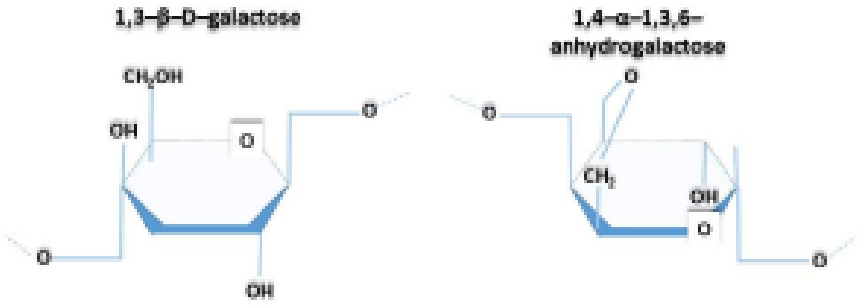

Figure 4. Chemical structure of repeat units of polysaccharides sourced from red algae.

6.1.1. Agar/agarose

Agar is a gelatinous substance composed of alternating D- and L- galactopyranose units. Its macromolecular structure consists in a mixture of agarose and agaropectin. Agarose is the neutral fraction of agar, and it is obtained by precipitation of the anionic agaropectins by quaternary ammonium salt and separation by centrifugation. Agaropectin is a heterogeneous mixture of smaller molecules, which have similar structures with agarose but contains many anionic groups such as sulphate, pyruvate and glycuronate. Due to the similar chemical and physical properties with agarose, agar has also been widely studied and applied in the biomedical field because of its good biocompatibility, biodegradability, nontoxicity, availability and lowcost [47,161].

Agarose adopts a single or double helical conformation in the solid state. Gelation occurs by aggregation of double helices at a temperature dependent on methoxyl and sulphate contents, which can modulate this process. The three-dimensional network based on association of double helices is thermoreversible, i.e., the crosslinks occur physically without chemical catalyst or any crosslinking agent, and it is stabilized by cooperative hydrogen bonds. Therefore, the low cost, special mechanical properties, biocompatibility and relatively bioinert nature make agarose a promising alternative as a gel matrix [47,162].

Since agar and agarose have good biocompatibility and excellent moisturizing capacity, many applications were described in the literature. Various forms of systems based on agar and agarose have been developed for the applications in pharmaceutical industries and medical research: Miguel et al. [163] produced a new in situ thermo responsive hydrogel composed by agarose and chitosan to be used as an injectable scaffold for tissue regeneration. They corroborated Varoni et al. [164], who described agarose hydrogels as mouldable materials, i.e., the hydrogel may be polymerized *in situ* reducing invasiveness of the surgery, besides it also be able to acquire the right shape at the wound site, without wrinkling or fluting and interacting with the damaged tissue. Agar and agarose fibers were fabricated by Bao et al. [161] and demonstrated to be good candidate materials for wound-dressing applications.

The scientific literature reported that the use of hydrogel as biomaterials for wound healing is also promising, however, they usually exhibit relatively poor mechanical properties due to the high swelling ratio, which limits their practical applications as wound dressings. Therefore, the most recent studies demonstrated that the incorporation of nanomaterials is probably the effective strategy to improve the mechanical property of hydrogels [165]. Wang et al. [166], for example, developed

nanocomposite hydrogels based on agarose and ZnO and concluded that the system might be an excellent candidate for wound dressings.

Agarose can be processed by extrusion from solution at high temperature, forming gel beads into ice-cold buffer. Another method is to emulsify agarose in a warm fluid which is immiscible with the aqueous polysaccharide solution; the emulsion is then cooled and produce microspheres [167]. Large-sized agarose beads can be prepared by some typical techniques, such as mechanical stirring and spraying methods. However, these methods suffer from poor control of droplet size and size distribution, low energy efficiency, and time consuming characteristics. Li et al. [168] improved the emulsification reproducibility and stable bead properties by developing a manufacturing protocol for uniform droplets and beads of controlled size with rotating membrane emulsification.

Agarose is also widely used as growth medium for microorganisms (bacteria and fungi) [169-171] and for biotechnological applications. Agarose was submitted to an enzymatic hydrolysis process with no acid treatment and converted in a potential hydrolysate for bioethanol production [172]. By casting and subsequent drying process, agar and agarose films can be formed depending on the application [173,174].

In the food industry, it is well known that agar films possess several properties adequate for food packaging applications, however, their high cost-production and quality variations caused by physiological and environmental factors affecting wild seaweeds make them less attractive for industries [175]. Despite having good mechanical and relatively good oxygen and water barriers properties, the most recent trends in food industry use nanomaterials as promising option to improve mechanical and barrier properties of biodegradable agar-based composite films [176-178].

In the domain of blends, agar was mixed with different materials, for example, poly(vinyl alcohol) and soy protein, respectively for specific food applications [179] and for scanning the variations in the matrix tensile strength [180]. Agar and agarose were also mixed with different polysaccharides to produce blends with potential biotechnological applications. Agarose was mixed with fenugreek galactomannan and formed a matrix efficiently used as biosensor to detect pesticides in food [181]. Multicomponent hydrogel films composed of agar, κ-carrageenan, konjac glucomannan powder, and nanoclay improved mechanical and water resistant properties of the blended hydrogel film [182]. Recently, agarose blends have been used in nanotechnological process, such as the rheological study of agarose hydrogels with tunicate cellulose nanowhiskers [183].

6.1.2. Carrageenan

Carrageenan is a word that seems to originate from the inhabitants of the country of Carrageen, on the south Irish coast where extracts from red algae for food and medicines were already used as early as 600 years ago. The major constituent of such algae is the so-called carrageenans, naturally occurring anionic sulphated linear polysaccharides with the linear backbone built up by β-D-galactose and 3,6-anhydro-α-D-galactose with variable density in the sulphated group [38,184].

There are several different carrageenans with slightly varied properties and chemical structures. The three most prevalent and of highest commercial interest are called iota, kappa, and lambda carrageenan, differentiated based on the amount and position of sulphate groups, and serving different properties [38].

Carrageenans have been extensively used in the food industry as thickening, gelling, stabilizing and protein-suspending agents due to its biocompatibility, biodegradability, high capacity of water retention and mechanical strength of its gels. According to the properties of carrageenan, it can split into two groups like, gelling (kappa and iota) and thickening agent (lambda); the 3,6-anhydro-α-D-galactose

is essential for the gelling properties of kappa and iota carrageenans. The molecular weight of carrageenans is critically important to its functionality in food, so, as stabilizers, carrageenans achieve its functionality due to its high molecular weight and binding capacity to proteins through charged sulphate and carboxyl groups [185].

The most recent applications of carrageenans are described in the following. Encapsulation of β-galactosidase in kappa carrageenan hydrogel beads was investigated for the application in food industry [186]. Multilayer coatings composed of kappa carrageenan and chitosan were produced, characterized in terms of their permeability's and surface properties [187], and used as model for the release behavior of Methylene blue; the industrial relevance of this type of nanolayered coatings consists in the development of bioactive compound release systems for application in food industry [188]. Multilayer films with a nanocomposition of kappa carrageenan, agar, clay and polylactide were prepared to modify the mechanical and barrier properties of the film for the food packaging application [189]. Still in view of the nanoscience, carrageenans were used not only as carriers of active antimicrobial nanoparticles, but also as polymer matrices for biodegradable films, for example, nanocomposite films with carrageenan, silver nanoparticles and clay mineral were prepared to test their combined effect on the antimicrobial activity and physicochemical film properties [190].

The biological activity of carrageenan as a natural occurring gum has been increasing widely for human applications and creates a strong position in the biomedical field [38], but it is important to highlight that the preliminary studies reported carrageenans administered in drinking water or diet could be a potential cause of intestinal inflammation and ulcers in animals [38,191]. Later, it was determined that these studies and others were conducted with a different material obtained by a degradation of the carrageenan that resulted in a sulfated polysaccharide with the same structural backbone to carrageenan, but with a lower molecular weight and different functional properties. Degraded carrageenan is referred to as poligeenan [185,192], polymer obtained by acid hydrolysis at high temperatures for several hours [185,193]. Poligeenan is also used in food applications, however, it was related to capable of causing gastrointestinal irritation in experimental animals and was considered to cause gastrointestinal cancer in animals. Carrageenans do not cause these effects and is safe at maximally administered oral doses [185]; they are related to inflammation just as employed in laboratories to create pathological models for exploration of the process and treatment of the inflammation [194-198].

Regarding the biological activities of carrageenans, its effect as food supplement was investigated on the immunity status and lipid profile in patients with cardiovascular disease. The results demonstrated that carrageenan moderately modulated all of the immunity system markers, caused statistically significant decreases in important biomarkers of chronic inflammation and significantly decreased cholesterol levels and low density lipoprotein cholesterol [199]. Besides the well-known biological activities related to inflammatory, immune [200], antioxidant [201-203], and antitumor responses [204,205], carrageenans are potent inhibitors of herpes and HPV viruses and there are indications that these polysaccharides may offer some protection against HIV infection [38,200].

Anticoagulant activity could be the most attractive property associated to carrageenans [154,160,206] and also to other sulphated polysaccharides, which were reported to possess similar or even stronger activity than those of heparin.

Carrageenans have been used by the pharmaceutical industry as excipient in different forms of presentation. Hydrogels of kappa carrageenan containing $CaCO_3$ and $NaHCO_3$ were tested as pore forming agents by Selvakumaran and Muhamad [207], and the system with $CaCO_3$ was considered an

efficient floating drug delivery system for amoxicillin trihydrate. Iota-carrageenan was investigated not only as a drug-loaded polymer but also as an ionic polymer to increase the solubility of the lappaconitine analgesic-alkaloid [208]. Hydrogels composed of kappa carrageenan and synthetic or natural polymers were reported, such as the blend constituted of kappa carrageenan and polythiophene for the electrically controlled drug release of acetylsalicylic acid [209]. Carrageenans were also used by the Interpenetrating Polymer Network (IPN) technique, which possess several advantages like high swelling capacity and tremendous mechanical strength on the targeted and controlled drug delivery. For example, IPN beads of kappa carrageenan and sodium carboxymethyl cellulose using $AlCl_3$ as a crosslinking agent were evaluated on the drug release behavior of ibuprofen [210].

6.2. Alginate

Alginate is the most important polysaccharide derived from brown algae (Phaephyta), and it can be also produced by soil bacteria (*Azotobacter vinelandii* and *Pseudomonas* species). This gum is composed of 1,4-linked *β*-D-mannuronic acid (M) with 4C1 ring conformation and *α*-L-guluronic acid (G) with 1C4 conformation, both in the pyranosic conformation and present in varying amounts. It was demonstrated that the physical properties in aqueous medium for these polymers depend not only on the M/G ratio, but also on the distribution of M and G units along the chain. In addition, the stiffness of the alginate chains as well as calcium complex formation could be attributed to the composition (M/G ratio) and distribution of M and G units in the chains [47].

The main property of alginates is their ability to retain water, in addition to gelling and stabilizing properties. Because of their linear structure, and high molecular weight, alginates form strong films and good fibres in the solid state. Gel formation is a very important characteristic for alginates; the higher content of G units form stable crosslinked junctions with divalent counterions (for example, Ca, Ba, and Sr, unless Mg), so the crosslinked network can be considered a gel (Figure 5). In addition, the low pH also forms acidic gels stabilized by hydrogen bonds [47].

As an anionic polymer, alginate forms electrostatic complex when mixed with a cationic polymer. The stability of the formed complex depends on the pH and salt concentration, but even in the best conditions, the complex is insoluble and thus allows the formation of fibres, films, and capsules. Many applications are proposed to these complexes, especially for the most investigated polyelectrolyte complex based on alginate and chitosan. For example, a nanomultilayer coating composed of sodium alginate and chitosan, obtained by electrostatic layer-by-layer self-assembling, onto aminolyzed/charged PET was characterized and presented highly functional properties and promising future for industrial applications [211]. This alginate-chitosan nanomultilayer coating was applied in fresh-cut mangoes and improved the microbiological and physicochemical quality, extending the quality and shelf life of fresh-cut mangoes up to 8 days [130]. The deposition of functionalized particles on multilayers based on chitosan-alginate was investigated with the aim to use the complex in opto-electronics, biosensors or medical applications [212]. Belscak-Cvitanovic [213] prepared chitosan-alginate beads to encapsulate and retard the release of caffeine in water. Wang et al. [214] developed chitosan-alginate coatings to deliver titanium substrates via electrodeposition and suggested the potential application of this composite coating in tissue engineering scaffolds field. One more example is the study of Seth et al. [215], who designed magnetic chitosan-alginate core–shell beads for oral administration of low permeable drug.

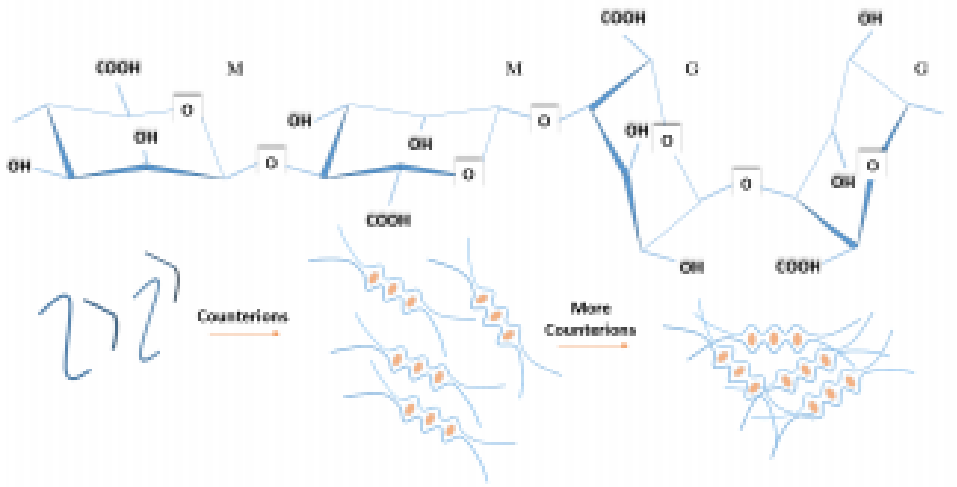

Figure 5. Chemical structure of repeated units of alginate: *β*-D-mannuronic acid (M) and *α*-L-guluronic acid (G), and the schematic crosslink between alginate and the counterions.

Several studies have shown that the chitosan-alginate complex acts as an effective way to improve de stability of encapsulated compounds [216,217]. It is important to highlight that, nowadays, the most extensively investigated delivery systems to encapsulate, protect and control the release of functional and unstable compounds in industries varying from food to pharmaceutics considers the development of capsules in micro and nano scales. Liu et al. [218] optimized a formula of chitosan-alginate deposited nanoliposomes and observed an improved *in vitro* digestion stability. Haidar et al. [219] developed core-shell hybrid nanoparticles based on the layer-by-layer assembly of alginate and chitosan on liposomes and demonstrated that the polymers shell could increase the membrane stability and sustain release of the model protein. Liu et al. [220] evaluated the environmental stress stability of microencapsules based on vitamin C liposomes decorated with chitosan-alginate membranes and observed the release kinetics of these formulations during *in vitro* gastrointestinal digestion. Chitosan-alginate complexes loaded with other nano compounds were tested with different purposes, including bactericidal activity [221], tissue engineering [222], and osteochondral [223] and food [224] applications.

Other polyelectrolytes may also be involved to form complexes with chitosan-alginate, such as collagen [225], gelatin [226], inulin [227], BSA protein [228], poly(L-lysine) [229], poly(L-lactide) [230], and hydroxyapatite [231].

Regarding the characteristic advantages of alginates, including biocompatibility, biodegradability, immunogenicity, and gel formation, alginates are well adapted for biomedical and pharmaceutical applications, such as tissue engineering, delivery vehicles for drugs and cell or enzyme entrapment. In addition, alginates have been successfully used in the food industry, for example, to prolong shelf life of the products and protect bioactive compounds encapsulated in the alginate matrix [232,233]; they can also be used as an antioxidant active interface for food preservation [234], and as a part of matrices designed to improve the oxidative stability during food storage [235].

Taking into account the above mentioned characteristics of alginates, nearly all of them are harvested in their wild state; cultivation is too expensive to provide alginates at a reasonable price for industrial applications. The quantity and quality of the alginates extracted depend on the algae species and on the season of harvest; furthermore, the higher content of alginate is still directed to the food industry.

7. Gum extracted from *Anarcadium occidentale*

Cashew gum (CG) is an exudate extracted from *Anacardium occidentale*, a popular tree belonging to the family Anacardiaceae. The cashew tree can grow up to 12 feet tall and is native to northeastern Brazil, however it can also be found in India, Mozambique, Tanzania, Kenya and among other countries. As a polysaccharide exudate, this gum is produced as a mechanism of plant defense against stress caused by physical injury or microbial attack. Its production can occur in all parts of the tree and its qualitative and quantitative depends on tree maturity and environmental conditions; initially, the gum is off white in colour but changes to reddish brown or yellowish brown on exposure. It is sparingly soluble in water but swells in contact with it giving a highly viscous solution [236-238].

The polyuronide CG is composed of a branched framework of D-galactose units. In addition, present are D-glucuronic acid, L-arabinose, L-rhamnose and, except in the case of a Venezuelan sample, D-glucose. D-xylose, D-mannose and 4-O-methyl-D-glucuronic acid have been found in some samples [239,240]. As a mixture of acid polysaccharides, CG contains various metal ions such as neutralized cations. The nature and content of these constituents depend on the composition of the soil upon which the trees grew. The major cations of *A. occidentale* are K^+, Na^+, Ca^{2+} and Mg^{2+}. Crude CG, containing these cations, tends to be naturally transformed into Na^+ salt, after purification or dialysis against NaCl 0.15 M, as previously described [237,239]. Figure 6 demonstrates the chemical composition of the galactose framework of CG and a graphic representation of the sugars and cations possibly associated to the polysaccharide.

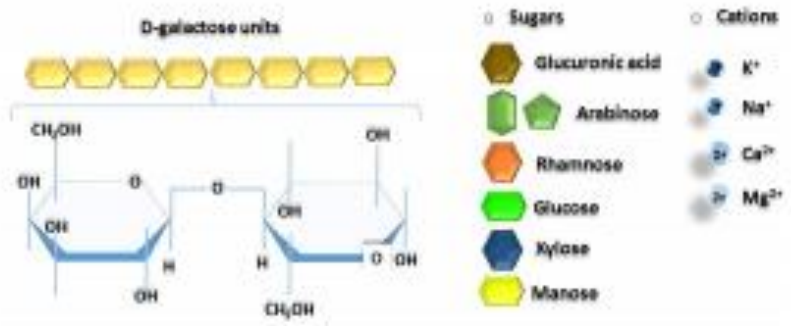

Figure 6. Representative image of the galactose main chain and possible sugars and counterions associated to the chemical structure of CG.

CG, as a versatile, naturally occurring biopolymer, is finding increasing applications in biotechnology industries, especially from pharmaceutical to food industry; however, there is a lack of understanding of its physicochemical properties thus limiting its applications. Chemical structure,

solubility and molecular weight of CG closely affect its solution properties as well as its interactions with other polysaccharides [239].

Modifications of gums have been tried through derivatisation of functional groups, grafting with polymers, cross-linking with ions, etc. The modification of CG can improve its technological and functional properties, since its application is related to a better understand on the physicochemical properties of the gum, in its original state or chemically modified, either isolated or blended with other polymer [241].

The unique properties of CG are due to hydroxyls, the majority, and to carboxyl reactive groups. This polysaccharide is easily chemically modified to reduce the water solubility or to develop pH sensitivity, introduce functional reactive groups, etc. Therefore, it can be utilised for a chosen application [237]. Thus, as the composition and properties of CG were disclosed, the chemical modification observed for blends composed of CG and other polymers were proposed to increase their reactivity. Following the use of CG blended with other polymers, the most recent literature reports the development of systems for drug release, like microbeads [242] and nanoparticles [243] of CG and alginate, and nanogels of CG and chitosan [244].

Several applications of blended CG were described, such as the production of films prepared with CG and polyvinyl alcohol (PVA) with the important purpose of wound therapeutics [245,246]. A layer-by-layer film containing CG intercalated with polyaniline unmodified or modified with phosphonic acid intercalated with CG was evaluated in determination of dopamine [247], and nanocomposite films of starch and CG were added to a nanoclay to increase the stability of cashew nut kernels [248]. Hydrogels based on CG (namely Policaju in this study) and chitosan were developed, characterized in their chemical structure, and indicated as a promising road to biomaterial fabrication and biomedical applications [249]. The larvicide activity of an essential oil loaded in beads based on chitosan and CG was evaluated by Paula et al. [250]; afterward, CG nanoparticles added with other essential oil was proposed to improve the controlled release of a larvicide system [251]. CG and carboxymethylcellulose based formulations have been evaluated as protective edible coatings on intact and cut red guavas [252].

The properties of CG have also been exploited as a binder, for example the binding efficacy of CG based on tablet formulation was investigated in comparison with standard binders such as acacia and polyvinyl pyrrolidone; the results suggested that CG can be used as an alternative binder to produce a tablet of better mechanical strength and dissolution profile of particular drug substance [253]. In addition, CG can be used as a substitute for liquid glue for paper, in the cosmetic industry, as an agglutinant for capsules and pills [237], and as edible coatings for application on mangoes [27] and apples [254].

Fabrication of systems utilizing CG for drug delivery have been an area of great interest, such as the transdermal release profile for diclofenac diethyl amine loaded with nanoparticles of acetylated CG [255]. Other example is the study about the controlled delivery of antimicrobial peptides immobilized in multilayer films of CG and indium tin oxide to combat and prevent anti-leishmanial activity [256]. Self-assembled nanoparticles from hydrophobized CG containing an anti-inflamatory drug was also characterized as a drug delivery device [257].

CG is one of few biopolymers that have been proposed to replace the similar but more popular polysaccharide gum arabic. As a biopolymer with amphiphilic compounds, CG was reported to have about 6% of polysaccharide-protein complex, suggesting that probably the same complex is present in the gum arabic. The similarity of both gums suggests the study of CG in the food and beverage industry as thickening and gelling agent, and as colloidal stabiliser [258].

8. Pectin

Pectin is a complex and heterogeneous polysaccharide located in the middle lamella and primary cell walls of plant tissues, and in fruits and vegetables. The chemical structure of pectin depends on the origin, location in the plant and extraction method, been composed of acidic polymers, known as homogalacturonan, rhamnogalacturonan and xylogalacturonan with several neutral sugars/polymers such as arabinans, galactans and arabinogalactans (attached as side chains). The available data support the highly schematic model of pectin shown in Figure 7. The backbone contains α-(1-4)-linked linear homogalacturonic units alternated with two types of highly branched rhamnogalacturonan regions. The first region is substituted with side chains of arabinose and galactose units, while the second has a highly conserved structure, consisting of the main chain branched with eleven different monosaccharides, including some rare sugars such as 2-O-methylxylose, 2-O-methylfucose, apiose, aceric acid, 2-keto-3-deoxy-d-manno-octulosonic acid, and 3-deoxy-d-lyxo-2-heptulosaric acid. Xylogalacturonan is similar to homogalacturonan except that it is substituted with single β-(1-3)-xylose units or such units substituted with a few additional β-(1-4)-xylose. In all natural pectins, some of the carboxyl groups exist in the methyl ester form [35,47,259].

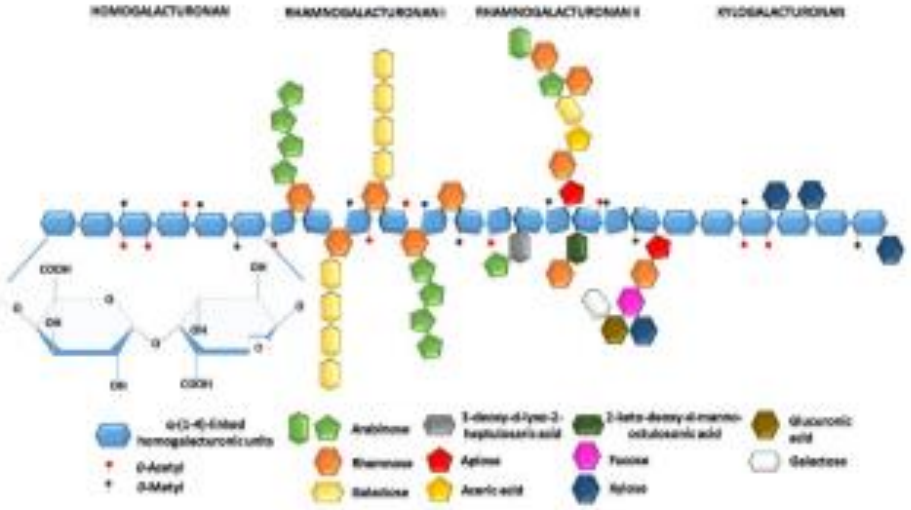

Figure 7. Schematic structure of pectin. The relative abundance of the different types of pectin varies, but homogalacturonan and rhamnogalacturonan I are considered the major components, while xylogalacturonan and rhamnogalacturonan II are minor components.

Pectin has a high molecular weight and can be converted into hydrogels, intended as flexible network of polymer chains that can swell but do not dissolve in water. Pectin solutions at high concentrations and low pH facilitate the formation of coil entanglements, resulting in formation of physical gels. Moreover, water-insoluble gels may be obtained with the use of divalent or trivalent cations. This simple gelling mechanism has raised interest for the preparation of hydrogels for biomedical applications, including drug delivery, gene delivery, tissue engineering and wound healing. It is also important to highlight that the monosaccharide content and the spatial disposition of

the crosslinking blocks in the pectin structure need to be carefully considered when designing pectin gels for specific biomedical applications [260].

Pectins exist with different degrees of esterification. They can be classified as high methoxyl pectin or low methoxyl pectin according to its degree, that yield some differences in their properties, for example, pectins with a high degree of esterification form thermoreversible gels in acidic conditions [47,261].

Pectins are widely used as technological adjuvants in the food industry, fully exploiting their structural diversity. Different structures lead to distinct gelling properties, emulsion and thickening activities, emulsion stabilities, and release effects in complex food matrices. In the pharmaceutical industry, pectin is commonly used as an excipient due to its non-toxicity, low production costs, and gelling activity properties [262].

Edible coatings based on pectin [263,264] and associated with other polysaccharides, including alginate [265] and chitosan [129], were developed in terms of evaluate the coating storage ability on foods and fruits. Pectin containing agricultural by-products are also used as potential sources of a new class of prebiotics known as pectic oligosaccharides [259,266,267]. The impact of protein content and combination mode between protein and pectin on emulsifying properties is one of the most properties studied by the scientific literature [268-270].

The increasing use of pectin in drug delivery is facilitated by the physicochemical properties of pectin. Muco-adhesiveness, ease of dissolution in basic environments, resistance to degradation by proteases and amylases of the upper gastrointestinal tract and the ability to form gels in acid environments, which allows this natural polysaccharide to target different drug delivery formulations. For example, films [271], beads [272], pellets and structures in micro and nanoscale [273-275], into completely different environments such as nasal, vaginal, ocular, gastric and, specially, large intestine [261,276]. The use of pectin-derived drug carries in colon cancer treatments has been extensively considered; pectin is intact in the upper gastrointestinal tract and degraded by specific colonic bacteria [277,278]. Modification of pectin via grafting with poly(N-vinylpyrrolidone) (PVP) has also been reported to form an effective hydrogel that can make effective colon-targeted drug delivery [279]. In terms of the gel-forming capacity, pectin lowers cholesterol by binding the cholesterol and bile acids in the gut, thus promoting their excretion [280-282].

In addition to food and pharmaceutical applications, recent studies reported that pectins showed immunomodulating activities [283-285]. The hydrogel of pectin has been explored in tissue engineering applications for bone cell culture and promoting the nucleation of minerals, and in wound healing applications for binding active drugs or growth factors and protecting against bacteria [260]. Still in view of the wound care, oxidized pectin and carboxymethyl chitosan membranes were developed by in situ cross-linking and demonstrated to be non-hemolytic and cytocompatible [286]. Pectin is a natural anti-glycation agent and has been used beneficially to treat healing damages. Wound dressings of oxidized pectin, gelatin and nonwoven cotton fabric were used as immobilizing supports for nanosilver and ciprofloxacin [287] and alow vera and curcumin [288] with the important propose to test their viability as effective materials for wound management.

9. Starch

Starch is a biopolymer synthesized in a granular form by green plants for energy storage over long periods. Starch granules consist of two major components, branched amylopectin and linear amylose;

in both cases, the basic structure is composed of α-D-glucopyranose residues, forming α-1,4-glucosidic bonds in linear structure of amylose and additional α-1,6-glycosydic branches in amylopectin molecules. Minor constituents such as lipids, proteins, and minerals are present in starch and the levels vary with the origin. The differences in structure of amylose and amylopectin have indeed significant variance in their properties. Amylose is much more prone to crystallization process, called retrogradation, and can produce tough gels and strong films, while amylopectin could be dispersed in water and retrogrades much slower, which results in soft gels and weak films [35,289].

The predominant model for starch structure is presented in multi-scale, consisting of granules, into which is found growth rings composed of amorphous and crystalline lamellae containing amylopectin and amylose chains [290], as can be observed in Figure 8.

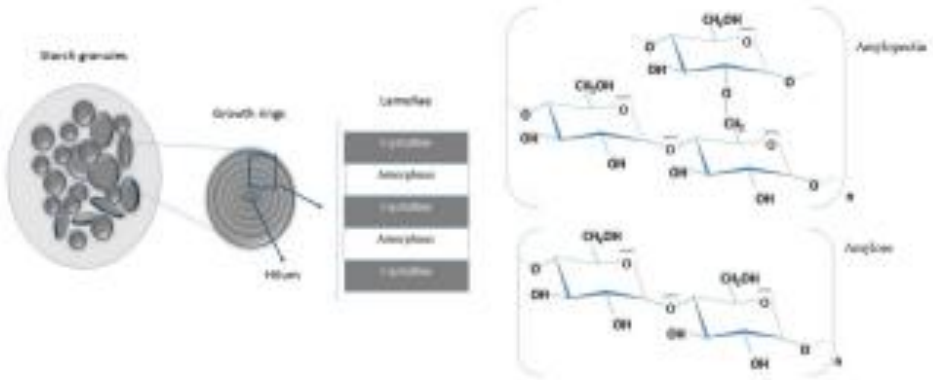

Figure 8. Multi-scale model for starch structure, containing granules, growth rings, amorphous and crystalline lamellae, hilum, and amylopectin and amylose molecular structures.

Starch is an excellent material for biotechnological applications due to its non-toxic, renewable and biodegradable characteristics; however, its direct applications are limited by its poor processability and intrinsic properties, such as thermal, mechanical, and biological properties. Thus, various chemical, physical, and enzymatic modifications or blending with other materials has supplied solutions to achieve properties that are more desirable. Similar to the cellulose (section 4), conventional chemical modifications of starch are performed based on the primary and secondary hydroxyl groups, including esterification, etherification, oxidation, and graft copolymerization [35].

Starch esters are generally prepared by reacting with fatty acids and fatty acid methyl ester in organic solvents [291,292]. Starch esters have been developed for pharmaceutical applications, e.g. used as superdisintegrant and matrix former in capsules and tablet formulations; and for medical application to maintain human colonic function and preventing colonic disease [35,293].

Quaternary ammonium cationic starches, the major commercial starch ethers, are commonly prepared by the reaction of an aqueous alkaline solution of 2,3-epoxypropyltrimethyl ammonium chloride or 3-chloro-2-hydroxypropyltrimethyl ammonium chloride. The quaternary ammonium-substituted cationic starches may form nanoparticles with anionic sodium tripolyphosphate. The nanoparticles could

entrap hydrophobic molecules, providing a great potential as nanosized carrier in health care and environmental sciences [35,294]. Hydroxyethyl starch is semisynthetic starch ether by reacting with ethylene oxide in alkaline medium, and it has been used as a plasma volume expander and cryoprotectant in medicine. A nanocarrier based on hydroxyethyl starch for active receptor-mediated targeting was synthesized [35,295]. The hydroxyethyl starch folic acid conjugate nanocarriers could be of high interest for the development of receptor mediated targeting using polymeric nanocapsules to deliver and accumulate their encapsulated molecules to the targeted area.

Selective oxidation of starch with N_2O_4 or a TEMPO/NaClO/NaBr system can exclusively yield carboxylates on the primary hydroxyl groups. Such oxidation approaches can also be applied to selectively oxidize starch derivatives, which bear another oxidation candidate primary hydroxyl group (e.g. hydroxyethyl starch) [35]. Recently, NaClO oxidation was applied in the aqueous re-dispersibility of starch nanocrystal powder and increased the oxidation degree from 0.41 to 0.581% (w/w), while the applied active chlorine increased from 1 to 4% (w/w) [296].

It is important to note that starches are chemically and/or physically modified to accentuate their positive characteristics, diminish their undesirable qualities (such as high viscosity, susceptibility to retrogradation, and lack of process tolerance), or improve new attributes (retention, film formation, digestibility, solubility, etc.). Acid hydrolysis has been used for a long time to modify starch and its properties because of its simplicity and controllability; recently, the main researches about acid hydrolysis has been applied for preparing starch nanocrystals (SNC). The preparation methods and applications of SNC have been extensively reported by the scientific literature [290,297-300]. In addition to SNC, starch crystallites, microcrystalline starch and hydrolysed starches all refer to the crystalline part of starch obtained by hydrolysis. They differ from each other in the extent of hydrolysis they have undergone (from the most to the least), and have to be distinguished from starch nanoparticles, of which they are just one kind, and which can be amorphous [290].

Because starch is environmentally friendly, nano-scale starch derivatives are suggested as one of the promising biomaterials for novel utilization in foods, cosmetics, medicines as well as various composites. For example, a comparative study was developed with waxy maize starch nano-derivatives extracted through acid hydrolysis and ultrasound treatment, respectively for nanocrystals and nanoparticles. Their application in nanocomposite films was suggested to improve their morphological, structural and thermal behaviour [301]. SNC and nanoparticles were also compared in a rheological characterization of suspensions containing its nano-derivatives under different ionic strength conditions [302]. SNC were also found to serve as an effective reinforcing agent for natural rubber [303], edible films from potato starch [304], and amaranth protein films [305].

10. Conclusions

The aim of this review was to approach the most recent scientific literature dealing with natural polymers, their chemical structure, physicochemical and functional properties, in addition to their biotechnological applications. From the fundamental point of view, it was remarkable to note the preference of industry applications for natural gums when comparable with synthetic materials due to their non-toxicity, low cost, availability, biodegradability and biosafety. The use of nano-tools to process natural polymers, their derivatives or blends, is gaining prominence in the industrial field due to the advantages in the use of nanomaterials sourced from natural origin, since the use of these materials are likely to cause less environmental impact.

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Conflict of interest

Authors have declared that no competing interests exist.

References

1. Rana V, Rai P, Tiwary AK, et al. (2011) Modified gums: Approaches and applications in drug delivery. *Carbohydr Polym* 83: 1031-1047.
2. Clifford SC, Arndt SK, Popp M, et al. (2002) Mucilages and polysaccharides in *Ziziphus* species (Rhamnaceae): Localization, composition and physiological roles during drought-stress. *J Exp Bot* 53: 131-138.
3. Prajapati VD, Jani GK, Moradiya NG, et al. (2013) Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydr Polym* 92: 1685-1699.
4. Ghanem ME, Hana RM, Classen B (2010) Mucilage and polysaccharides in the halophyte plant species Kosteletzkya virginica: Localization and composition in relation to salt stress. *J Plant Physiol* 167: 382-392.
5. Franz G (1979) Metabolism of reserve polysaccharides in tubers of *Orchis morio* L. *Planta Med* 36: 68-73.
6. Mirhosseini H, Amid BT (2012) A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. *Food Res Int* 46: 387-398.
7. Gupta S, Saurabh CK, Variyar PS, et al. (2015) Comparative analysis of dietary fiber activities of enzymatic and gamma depolymerized guar gum. *Food Hydrocoll* 48: 149-154.
8. Hosseini-Parvar SH, Osano JP, Matia-Merino L. (2016) Emulsifying properties of basil seed gum: Effect of pH and ionic strength. *Food Hydrocoll* 52: 838-847.
9. Buriti FCA, Freitas SC, Egito AS, et al. (2014) Effects of tropical fruit pulps and partially hydrolysed galactomannan from *Caesalpinia pulcherrima* seeds on the dietary fibre content, probiotic viability, texture and sensory features of goat dairy beverages. *LWT Food Sci Technol* 59: 196-203.
10. Pang Z, Deeth H, Prakash S, et al. (2016) Development of rheological and sensory properties of combinations of milk proteins and gelling polysaccharides as potential gelatin replacements in the manufacture of stirred acid milk gels and yogurt. *J Food Eng* 169: 27-37.
11. Cho HM, Yoo B (2015) Rheological characteristics of cold thickened beverages containing xanthan gum-based food thickeners used for dysphagia diets. *J Acad Nutr Diet* 115: 106-111.
12. Bouaziz F, Koubaa M, Neifar M, et al. (2016) Feasibility of using almond gum as coating agent to improve the quality of fried potato chips: Evaluation of sensorial properties. *LWT Food Sci Technol* 65: 800-807.

13. Yu L, Li J, Ding S, et al. (2016) Effect of guar gum with glycerol coating on the properties and oil absorption of fried potato chips. *Food Hydrocoll* 54: 211-219.
14. Ma Q, Hu D, Wang H, et al. (2016) Tara gum edible film incorporated with oleic acid. *Food Hydrocoll* 56: 127-133.
15. Engler A (1964) Syllabus der Pflanzenfamilien. Berlin: *Gerbruder Borntraeger*, 193.
16. Hegnauer R, Grayer-Barkmeuer RJ (1994) Relevance of seed polysaccharides and flavonoids for the classification of the Leguminosae: A chemotaxonomic approach. *Phytochemistry* 34: 3-16.
17. Nishinari K, Zhang H, Ikeda S (2000) Hydrocolloid gels of polysaccharides and proteins. *Curr Opin Colloid Interface Sci* 5: 195-201.
18. McClements DJ (2005) *Food emulsions: Principles, practices, and techniques* (2nd ed.). Boca Raton, FL: CRC Press.
19. Williams PA, Phillips GO (2000) Introduction to food hydrocolloids, In: Phillips GO, Williams PA, *Handbook of hydrocolloids*. New York: CRC Press, 1-19.
20. Fiszman S, Varela P (2013) The role of gums in satiety/satiation. A review. *Food Hydrocoll* 32: 147-154.
21. Deogade UM, Deshmukh VN, Sakarkar DM. (2012) Natural gums and mucilage's in NDDS: applications and recent approaches. *Int J PharmTech Res* 4: 799-814.
22. Vasile FC, Martinez MJ, Ruiz-Henestrosa VMP, et al. (2016) Physicochemical, interfacial and emulsifying properties of a non-conventional exudate gum (*Prosopis alba*) in comparison with gum Arabic. *Food Hydrocoll* 56: 245-253.
23. Martinez M, Beltrán O, Rincón F, et al. (2015) New structural features of *Acacia tortuosa* gum exudate. *Food Chem* 182: 105-110.
24. Rezaei A, Nasirpour A, Tavanai H (2016) Fractionation and some physicochemical properties of almond gum (*Amygdalus communis* L.) exudates. *Food Hydrocoll* 60: 461-469.
25. Thanzamia K, Malsawmtluangia C, Lalhlenmawia H, et al. (2015) Characterization and in vitro antioxidant activity of *Albizia stipulata* Boiv. gum exudates. *Int J Biol Macromol* 80: 231-239.
26. Gashua IB, Williams PA, Yadav MP, et al. (2015) Characterisation and molecular association of Nigerian and Sudanese Acacia gum exudates. *Food Hydrocoll* 51: 405-413.
27. Souza MP, Cerqueira MA, Souza BWS, et al. (2010) Polysaccharide from Anarcadium occidentale L. tree gum (Policaju) as a coating for Tommy atkins mangoes. *Chem Pap* 64: 475-481.
28. Lefsih K, Delattre C, Pierre G, et al. (2016) Extraction, characterization and gelling behavior enhancement of pectins from the cladodes of *Opuntia ficus* indica. *Int J Biol Macromol* 82: 645-652.
29. Kapoor VP, Taravel FR, Joseleau JP, et al. (1998) *Cassia spectabilis* DC seed galactomannan: structural, crystallographical and rheological studies. *Carbohydr Res* 306: 231-241.
30. McCleary BV, Amado R, Waibel R, et al. (1981) Effect of galactose content on the solution and interaction properties of guar and carob galactomannans. *Carbohyd Res* 92: 269-285.
31. Kapoor VP (1994) Rheological properties of seed galactomannan from *Cassia nodosa* buch.-hem. *Carbohyd. Polym* 25: 79-84.
32. Albuquerque PBS, Barros Júnior W, Santos GRC, et al. (2014) Characterization and rheological study of the galactomannan extracted from seeds of *Cassia grandis*. *Carbohyd Polym* 104: 127-134.

33. Brummer Y, Cui W, Wang Q (2003) Extraction, purification, and physicochemical characterization of fenugreek gum. *Food Hydrocoll* 17: 229-236.
34. Dakia PA, Blecker C, Robert C, et al. (2008) Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. *Food Hydrocoll* 22: 807-818.
35. Liu J, Willför S, Xu C (2014) A review of bioactive plant polysaccharides: Biological activities, functionalizatison, and biomedical applications. *Bioact Carbohydr Diet Fibre* 5: 31-61.
36. Arruda IRS, Albuquerque PBS, Santos GRC, et al. (2015) Structure and rheological properties of a xyloglucan extracted from *Hymenaea courbaril* var. *courbaril* seeds. *Int J Biol Macromol* 73: 31-38.
37. Yampakdee S, Benjakul S, Kingwascharapong P (2015) Physico-chemical and gel properties of agar from *Gracilaria tenuistipitata* from the lake of Songkhla, Thailand. *Food Hydrocoll* 51: 217-226.
38. Prajapati VD, Maheriya PM, Jani GK, et al. (2014) Carrageenan: A natural seaweed polysaccharide and its applications. *Carbohydr Polym* 105: 97-112.
39. Rinaudo M (2007) Seaweed polysaccharides, In: Kamerling JP, *Comprehensive Glycoscience*, vol. 4, Amsterdam: Elsevier, 2: 691-735.
40. Silva MF, Fornari RCG, Mazutti M, et al. (2009) Production and characterization of xantham gum by *Xanthomonas campestris* using cheese whey as sole carbon source. *J Food Eng* 90: 119-123.
41. Kumari S, Rath PK (2014) Extraction and Characterization of Chitin and Chitosan from (*Labeo rohit*) Fish Scales. *Procedia Mat Sci* 6: 482-489.
42. Sadhasivam G, Muthuvel A (2014) Isolation and characterization of hyaluronic acid from marine organisms. *Adv Food Nutr Res* 72: 61-77.
43. Vázquez JA, Rodriguez-Amado I, Montemayor MI, et al. (2013) Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review. *Mar Drugs* 11: 747-774.
44. Ström A, Boers HM, Koppert R, et al. (2009) Physico-chemical properties of hydrocolloids determine their appetite effects, In Williams PA, Phillips GO, *Gums and stabilisers for the food industry*. Cambridge: Royal Society of Chemistry, 341-355.
45. Rinaudo M, Moroni A (2009) Rheological behavior of binary and ternary mixtures of polysaccharides in aqueous medium. *Food Hydrocoll* 23: 1720-1728.
46. Priya MV, Sabitha M, Jayakumar R (2016) Colloidal chitin nanogels: A plethora of applications under one shell. *Carbohydr Polym* 136: 609-617.
47. Rinaudo M (2008) Main properties and current applications of some polysaccharides as biomaterials. *Polym Int* 57: 397-430.
48. Dea ICM, Morrison A (1975) Chemistry and interactions of seed galactomannans. *Adv Carbohydr Chem* 31: 241-312.
49. Srivastava M, Kapoor VP (2005) Seed galactomannans: An overview. *Chem Biodivers* 2: 295-317.
50. Prado BM, Kim S, Ozen BF, et al. (2005) Differentiation of carbohydrate gums and mixtures using Fourier transform infrared spectroscopy and chemometrics. *J Agric Food Chem* 53: 2823-2829.

51. Dakia PA, Blecker C, Robert C, et al. (2008) Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. *Food Hydrocoll* 22: 807-818.
52. Vendruscolo CW, Andreazza IF, Ganter JLMS, et al. Xanthan and galactomannan (from *M. scabrella*) matrix tablets for oral controlled delivery of theophylline. *Int J Pharm* 296:1-11.
53. Daas P, Grolle K, Vliet T, et al. (2002) Toward the recognition of structure–function relationships in galactomannans. *J Agric Food Chem* 50: 4282-4289.
54. Pollard MA, Eder B, Fischer P, et al. (2010) Characterization of galactomannans isolated from legume endosperms of Caesalpinioideae and Faboideae subfamilies by multidetection aqueous SEC. *Carbohydr Polym* 79: 70-84.
55. Jiang J-X, Jian H-l, Cristhian C (2011) Structural and thermal characterization of galactomannans from genus *Gleditsia* seeds as potential food gum substitutes. *J Sci Food Agric* 91: 732-737.
56. Reid JSG, Edwards ME (1995) Galactomannans and other cell wall storage polysaccharides in seeds, In Stephen AM, *Food Polysaccharides and Their Applications*, New York: Marcel Dekker, Inc.
57. Singh VK, Banerjee I, Agarwal T, et al. (2014) Guar gum and sesame oil based novel bigels for controlled drug delivery. *Colloids Surf B Biointerfaces* 123: 582-592.
58. Soares PAG, Seixas JRPC, Albuquerque JRPC, et al. (2015) Development and characterization of a new hydrogel based on galactomannan and k-carrageenan. *Carbohydr Polym* 134: 673-679.
59. Antoniou J, Liu F, Majeed H, et al. (2014) Physicochemical and thermomechanical characterization of tara gum edible films: Effect of polyols as plasticizers. *Carbohydr Polym* 111: 359-365.
60. Antoniou J, Liu F, Majeed H, et al. (2015) Characterization of tara gum edible films incorporated with bulk chitosan and chitosan nanoparticles: A comparative study. *Food Hydrocoll* 44: 309-319.
61. Rodrigues DC, Cunha AP, Brito ES, et al. (2016) Mesquite seed gum and palm fruit oil emulsion edible films: Influence of oil content and sonication. *Food Hydrocoll* 56: 227-235.
62. Sandolo C, Bulone D, Mangione MR, et al. (2010) Synergistic interaction of Locust Bean Gum and Xanthan investigated by rheology and light scattering. *Carbohyd Polym* 82: 733-741.
63. Grisel M, Aguni Y, Renou F, et al. (2015) Impact of fine structure of galactomannans on their interactions with xanthan: Two co-existing mechanisms to explain the synergy. *Food Hydrocoll* 51: 449-458.
64. Koop HS, Freitas RA, Souza MM, et al. (2015) Topical curcumin-loaded hydrogels obtained using galactomannan from Schizolobium parahybae and xanthan. *Carbohydr Polym* 116: 229-236.
65. Sousa AMM, Gonçalves MP (2015) The influence of locust bean gum on native and alkali-modified agar gels. *Food Hydrocoll* 44: 451-470.
66. Martins JT, Cerqueira MA, Bourbon AI, et al. (2012) Synergistic effects between k-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocoll* 29: 280-289.
67. Albuquerque PBS, Silva CS, Soares PAG, et al. (2016) Investigating a galactomannan gel obtained from *Cassia grandis* seeds as immobilizing matrix for Cramoll lectin. *Int J Biol Macromol* 86: 454-461.

68. Almeida RR, Magalhães HS, Souza JRR, et al. (2015) Exploring the potential of Dimorphandra gardneriana galactomannans as drug delivery systems. *Ind Crops Prod* 69: 284-289.
69. Klemm D, Heublein B, Fink HP (2005) Cellulose: fascinating biopolymer and sustainable raw material. *Angew Chem Int Ed* 44: 3358-3393.
70. Lee T-W, Jeong YG (2015) Regenerated cellulose/multiwalled carbon nanotube composite films with efficient electric heating performance. *Carbohydr Polym* 133: 456-463.
71. Ullah MW, Ul-Islam M, Khan S, et al. (2016) Structural and physico-mechanical characterization of bio-cellulose produced by a cell-free system. *Carbohydr Polym* 136: 908-916.
72. Dayal MS, Catchmark JM (2016) Mechanical and structural property analysis of bacterial cellulose composites. *Carbohydr Polym* 144: 447-453.
73. Chen P, Cho SY, Jin HJ (2010) Modification and applications of bacterial celluloses in polymer science. *Macromol Res* 18: 309-320.
74. Keshk SM (2014) Bacterial cellulose production and its industrial applications. *J Bioprocess Biotech* 4: 1-10.
75. Nobles DR, Romanovicz DK, Brown Jr RM (2001) Cellulose in Cyanobacteria. Origin of Vascular Plant Cellulose Synthase?. *Plant Physiol* 127: 529-542.
76. Castro C, Cordeiro N, Faria M, et al. (2015) In-situ glyoxalization during biosynthesis of bacterial cellulose. *Carbohydr Polym* 126: 32-39.
77. Kiziltas EE, Kiziltas A, Blumentritt M, et al. (2015))Biosynthesis of bacterial cellulose in the presence of different nanoparticles to create novel hybrid materials. *Carbohydr Polym* 129: 148-155.
78. Römling U, Galperin MY (2015) Bacterial cellulose biosynthesis: Diversity of operons, subunits, products, and functions. *Trends Microbiol* 23: 545-557.
79. Brinchi L, Cotana F, Fortunati E, et al. (2013) Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications. *Carbohydr Polym* 94: 154-169.
80. Moon RJ, Martini A, Nairn J, et al. (2011) Cellulose nanomaterials review: structure, properties and nanocomposites. *Chem Soc Rev* 40: 3941-3994.
81. Peng BL, Dhar N, Liu HL, et al. (2011) Chemistry and applications of nanocrystalline cellulose and its derivatives: A nanotechnology perspective. *Can J Chem Eng* 89: 1191-1206.
82. Fujisawa S, Okita Y, Fukuzumi H, et al. (2011) Preparation and characterization of TEMPO-oxidized cellulose nanofibril films with free carboxyl groups. *Carbohydr Polym* 84: 579-583.
83. Zhang Q, Lin D, Yao S (2015) Review on biomedical and bioengineering applications of cellulose sulfate. *Carbohydr Polym* 132: 311-322.
84. Pérez S, Samain D (2010) *Structure and engineering of celluloses*, In: Derek H, Advances in carbohydrate chemistry and biochemistry, Vol 64, New York: Academic Press, 25-116.
85. Kono H, Fujita S (2012) Biodegradable superabsorbent hydrogels derived from cellulose by esterification crosslinking with 1,2,3,4-butanetetracarboxylic dianhydride. *Carbohydr Polym* 87: 2582-2588.
86. Ramirez JAA, Suriano CJ, Cerrutti P, et al. (2014) Surface esterification of cellulose nanofibers by a simple organocatalytic methodology. *Carbohydr Polym* 114: 416-423.
87. Littunen K, De Castro JS, Samoylenko A, et al. (2016) Synthesis of cationized nanofibrillated cellulose and its antimicrobial properties. *Eur Polym J* 75: 116-124.

88. Genyk Y, Kato T, Pomposelli JJ, et al. (2016) Fibrin sealant patch (TachoSil) vs oxidized regenerated cellulose patch (Surgicel Original) for the secondary treatment of local bleeding in patients undergoing hepatic resection: a randomized controlled trial. *J Am Coll Surg* 222: 261-268.
89. Wu YD, He JM, Huang YD, et al. (2012) Oxidation of regenerated cellulose with nitrogen dioxide/carbon tetrachloride. *Fibers Polym* 13: 576-581.
90. Zacharias T, Ferreira N (2012) Carrier-bound fibrin sealant compared to oxidized cellulose application after liver resection. *HPB* 14: 839-347.
91. Bedane AH, Eić M, Farmahini-Farahani M, et al. (2015) Water vapor transport properties of regenerated cellulose and nanofibrillated cellulose films. *J Memb Sci* 493: 46-57.
92. Stevanic JS, Bergström EM, Gatenholm P, et al. (2012) Arabinoxylan/nanofibrillated cellulose composite films. *J Mater Sci* 47: 6724-6732.
93. Biliuta G, Fras L, Drobota M, et al. (2013) Comparison study of TEMPO and phthalimide-N-oxyl (PINO) radicals on oxidation efficiency toward cellulose. *Carbohydr Polym* 91: 502-507.
94. Liu P, Oksman K, Mathew AP (2016) Surface adsorption and self-assembly of Cu(II) ions on TEMPO-oxidized cellulose nanofibers in aqueous media. *J Colloid Interface Sci* 464:175-182.
95. Huang M, Chen F, Jiang Z, et al. (2013) Preparation of TEMPO-oxidized cellulose/amino acid/nanosilver biocomposite film and its antibacterial activity. *Int J Biol Macromol* 62:608-613.
96. Hakalahti M, Salminen A, Seppälä J, et al. (2015) Effect of interfibrillar PVA bridging on water stability and mechanical properties of TEMPO/NaClO2 oxidized cellulosic nanofibril films. *Carbohydr Polym* 126: 78-82.
97. Barshay M, Güven O, Kodama Y. (2015) Amine functionalization of cellulose surface grafted with glycidyl methacrylate by γ -initiated RAFT polymerization. *Radiat Phys Chem* 124: 140-144.
98. Carlmark A, Larsson E, Malmström E. (2012) Grafting of cellulose by ring-opening polymerisation - A review. *Eur Polym J* 48: 1646-1659.
99. Lizundia E, Fortunati E, Dominici F, et al. (2016) PLLA-grafted cellulose nanocrystals: Role of the CNC content and grafting on the PLA bionanocomposite film properties. *Carbohydr Polym* 142: 105-113.
100. Madrid JF, Abad LV (2015) Modification of microcrystalline cellulose by gamma radiation-induced grafting. *Radiat Phys Chem* 115: 143-147.
101. Kang H, Liu R, Huang Y (2015) Graft modification of cellulose : Methods , properties and applications. *Polymer* 70: A1-A16.
102. Oksman K, Aitomäki Y, Mathew AP, et al. (2016) Review of the recent developments in cellulose nanocomposite processing. *Compos Part A* 83: 2-18.
103. Fu L, Liu B, Meng L (2016) Comparative study of cellulose/Ag nanocomposites using four cellulose types. *Mater Lett* 171: 277-280.
104. Li S, Fu L, Ma M, et al. (2012) Simultaneous microwave-assisted synthesis, characterization, thermal stability, and antimicrobial activity of cellulose/AgCl nanocomposites. *Biomass Bioenergy* 47: 516-521.
105. Deng F, Dong Y, Liu S, et al. (2016) Revealing the influences of cellulose on cellulose/SrF_2 nanocomposites synthesized by microwave-assisted method. *Ind Crops Prod* 85: 258-265.
106. Deng F, Fu L-H, Ma M-G (2015) Microwave-assisted rapid synthesis and characterization of CaF_2 particles-filled cellulose nanocomposites in ionic liquid. *Carbohydr Polym* 121: 163-168.

107. Jia N, Li SM, Ma MG, et al. (2012) Rapid microwave-assisted fabrication of cellulose/F-substituted hydroxyapatite nanocomposites using green ionic liquids as additive. *Mater Lett* 68: 44-56.
108. Huang W, Wang Y, Chen C, et al. (2016) Fabrication of flexible self-standing all-cellulose nanofibrous composite membranes for virus removal. *Carbohydr Polym* 143: 9-17.
109. Luo X, Zhang H, Cao Z, et al. (2016) A simple route to develop transparent doxorubicin-loaded nanodiamonds/cellulose nanocomposite membranes as potential wound dressings. *Carbohydr Polym* 143: 231-238.
110. Kiyazar S, Aghazadeh J, Sadeghi A, et al. (2016) In vitro evaluation for apatite-forming ability of cellulose-based nanocomposite scaffolds for bone tissue engineering. *Int J Biol Macromol* 86: 434-442.
111. Blank CE, Hinman NW (2016) Cyanobacterial and algal growth on chitin as a source of nitrogen; ecological, evolutionary, and biotechnological implications. *Algal Res* 15: 152-163.
112. Giji S, Arumugam M (2014) Isolation and characterization of hyaluronic acid from marine organisms. *Adv Food Nutr Res* 72: 61-77.
113. Jiang Y, Meng X, Wu Z, et al. (2016) Modified chitosan thermosensitive hydrogel enables sustained and efficient anti-tumor therapy via intratumoral injection. *Carbohydr Polym* 144: 245-253.
114. Younes I, Hajji S, Frachet V, et al. (2014) Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. *Int J Biol Macromol* 69: 489-498.
115. Shankar S, Reddy JP, Rhim J-W, et al. (2015) Preparation, characterization, and antimicrobial activity of chitin nanofibrils reinforced carrageenan nanocomposite films. *Carbohydr Polym* 117: 468-475.
116. Ilnicka A, Walczyk M, Lukaszewicz JP (2015) The fungicidal properties of the carbon materials obtained from chitin and chitosan promoted by copper salts. *Mater Sci Eng C* 52: 31-36.
117. Hoseini MHM, Moradi M, Alimohammadian MH, et al. (2016) Immunotherapeutic effects of chitin in comparison with chitosan against Leishmania major infection. *Parasitol Int* 65: 99-104.
118. Munster JM, Sanders P, Kate GA, et al. (2015) Kinetic characterization of *Aspergillus niger* chitinase CfeI using a HPAEC-PAD method for native chitin oligosaccharides. *Carbohydr Res* 407: 73-78.
119. Usman A, Zia KM, Zuber M, et al. (2016) Chitin and chitosan based polyurethanes: A review of recent advances and prospective biomedical applications. *Int J Biol Macromol* 86: 630-645.
120. Tomihata K, Ikada Y (1997) In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. *Biomaterials* 18: 567-575.
121. Naseri N, Algan C, Jacobs V, et al. (2014) Electrospun chitosan-based nanocomposite mats reinforced with chitin nanocrystals for wound dressing. *Carbohydr Polym* 109: 7-15.
122. Xia G, Lang X, Kong M, et al. (2016) Surface fluid-swellable chitosan fiber as the wound dressing material. *Carbohydr Polym* 136: 860-866.
123. Busilacchi A, Gigante A, Mattioli-Belmonte M, et al. (2013) Chitosan stabilizes platelet growth factors and modulates stem cell differentiation toward tissue regeneration. *Carbohydr Polym* 98: 665-676.
124. Kanimozhi K, Basha SK, Kumari VS (2016) Processing and characterization of chitosan/PVA and methylcellulose porous scaffolds for tissue engineering. *Mater Sci Eng C* 61: 484-491.

125. Morgado PI, Aguiar-Ricardo A, Correia IJ (2015) Asymmetric membranes as ideal wound dressings: An overview on production methods, structure, properties and performance relationship. *J Membr Sci* 490: 139-151.
126. Chen K-Y, Liao W-J, Kuo S-M, et al. (2009) Asymmetric Chitosan Membrane Containing Collagen I Nanospheres for Skin Tissue Engineering. *Biomacromolecules* 10: 1642-1649.
127. Lih E, Lee JS, Park KM, et al. (2012) Rapidly curable chitosan–PEG hydrogels as tissue adhesives for hemostasis and wound healing. *Acta Biomater* 8: 3261-3269.
128. Ferraro V, Cruz IB, Jorge RF, et al. (2010) Valorisation of natural extracts from marine source focused on marine by-products: a review. *Food Res Int* 43: 2221-2233.
129. Medeiros BGDS, Pinheiro AC, Carneiro-da-cunha MG, et al. (2012) Development and characterization of a nanomultilayer coating of pectin and chitosan – Evaluation of its gas barrier properties and application on 'Tommy atkins' mangoes. *J Food Eng* 110: 457-464.
130. Souza MP, Vaz AFM, Silva HD, et al. (2015) Development and characterization of an active chitosan-based film containing quercetin. *Food Bioprocess Technol* 8: 2183-2219.
131. Min SH, Park KC, Yeom Y (2014) Chitosan-mediated non-viral gene delivery with improved serum stability and reduced cytotoxicity. *Biotechnol Bioprocess Eng* 19: 1077-1082.
132. Wan ACA, Tai BCU (2013) CHITIN - A promising biomaterial for tissue engineering and stem cell technologies. *Biotechnol Adv* 31: 1776-1785.
133. Pangon A, Saesoo S, Saengkrit N, et al. (2016) Hydroxyapatite-hybridized chitosan/chitin whisker bionanocomposite fibers for bone tissue engineering applications. *Carbohydr Polym* 144: 419-427.
134. Kumar PTS, Ramya C, Jayakumar R, et al. (2013) Drug delivery and tissue engineering applications of biocompatible pectin – chitin / nano $CaCO_3$ composite scaffolds. *Colloids Surf B Biointerfaces* 106:109-116.
135. Kanimozhi K, Basha SK, Kumari VS (2016) Processing and characterization of chitosan/PVA and methylcellulose porous scaffolds for tissue engineering. *Mater Sci Eng C* 61: 484-491.
136. Zhao L, Wu Y, Chen S, et al. (2015) Preparation and characterization of cross-linked carboxymethyl chitin porous membrane scaffold for biomedical applications. *Carbohydr Polym* 126:150-155.
137. Smitha KT, Nisha N, Maya S, et al. (2015) Delivery of rifampicin-chitin nanoparticles into the intracellular compartment of polymorphonuclear leukocytes. *Int J Biol Macromol* 74:36-43.
138. Geetha P, Sivaram AJ, Jayakumar R, et al. (2016) Integration of in silico modeling, prediction by binding energy and experimental approach to study the amorphous chitin nanocarriers for cancer drug delivery. *Carbohydr Polym* 142: 240-249.
139. Smitha KT, Anitha A, Furuike T, et al. (2013) In vitro evaluation of paclitaxel loaded amorphous chitin nanoparticles for colon cancer drug delivery. *Colloids Surf B Biointerfaces* 104: 245-253.
140. Dev A, Mohan JC, Sreeja V, et al. (2010) Novel carboxymethyl chitin nanoparticles for cancer drug delivery applications. *Carbohydr Polym* 79: 1073-1079.
141. Chang PR, Jian R, Yu J, et al. (2010) Starch-based composites reinforced with novel chitin nanoparticles. *Carbohydr Polym* 80: 420-425.
142. Dhananasekaran S, Palanivel R, Pappu S (2016) Adsorption of Methylene Blue, Bromophenol Blue, and Coomassie Brilliant Blue by α-chitin nanoparticles. *J Adv Res* 7: 113-124.
143. Hamed I, Özogul F, Regenstein JM (2016) Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. *Trends Food Sci Technol* 48: 40-50.

144. Chantarasataporn P, Tepkasikul P, Kingcha Y, et al. (2014) Water-based oligochitosan and nanowhisker chitosan as potential food preservatives for shelf-life extension of minced pork. *Food Chem* 159: 463-470.
145. Farajzadeh F, Motamedzadegan A, Shahidi S-A, et al. (2016) The Effect of Chitosan-Gelatin Coating on the Quality of Shrimp (Litopenaeus vannamei) under Refrigerated Condition. *Food Control* 67: 163-170.
146. Caro N, Medina E, Diaz-Dosque M, et al. (2016) Novel active packaging based on films of chitosan and chitosan/quinoa protein printed with chitosan-tripolyphosphate-thymol nanoparticles via thermal ink-jet printing. *Food Hydrocoll* 52: 520-532.
147. Carvalho RL, Cabral MF, Germano TA, et al. (2016) Chitosan coating with transcinnamaldehyde improves structural integrity and antioxidant metabolism of fresh-cut melon. *Postharvest Biol Technol* 113: 29-39.
148. Genskowsky E, Puente LA, Pèrez-Álvarez JA, et al. (2015) Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT Food Sci Technol* 64: 1057-1062.
149. Sun Q, Si F, Xiong L, et al. (2013) Effect of dry heating with ionic gums on physicochemical properties of starch. *Food Chem* 136: 1421-145.
150. Vieira JM, Flores-López ML, de Rodriguez DJ, et al. (2016) Effect of chitosan–*Aloe vera* coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biol Technol* 116: 88-97.
151. Hernández-Valdepeña MA, Pedraza-Chaverri J, Gracia-Mora I, et al. (2016) Suppression of the *tert*-butylhydroquinone toxicity by its grafting onto chitosan and further cross-linking to agavin toward a novel antioxidant and prebiotic material. *Food Chem* 199: 485-491.
152. Stenner R, Matubayasi N, Shimizu S (2016) Gelation of carrageenan: Effects of sugars and polyols. *Food Hydrocoll* 54: 284-292.
153. Tavassoli-Kafrani E, Shekarchizadeh H, Masoudpour-Behabadi M (2016) Development of edible films and coatings from alginates and carrageenans. *Carbohydr Polym* 137: 360-374.
154. Liang W, Mao X, Peng X, et al. (2014) Effects of sulfate group in red seaweed polysaccharides on anticoagulant activity and cytotoxicity. *Carbohydr Polym* 101: 776-785.
155. Sudharsan S, Subhapradha N, Seedevi P, et al. (2015) Antioxidant and anticoagulant activity of sulfated polysaccharide from *Gracilaria debilis* (Forsskal). *Int J Biol Macromol* 81:1031-1038.
156. Chiu YH, Chan YL, Tsai LW, et al. (2012) Prevention of human enterovirus 71 infection by kappa carrageenan. *Antiviral Res* 95: 128-134.
157. Cosenza VA, Navarro DA, Pujol CA, et al. (2015) Partial and total C-6 oxidation of gelling carrageenans. Modulation of the antiviral activity with the anionic character. *Carbohydr Polym* 128: 199-206.
158. El-Shitany NA, El-Bastawissy EA, El-Desoky K (2014) Ellagic acid protects against carrageenan-induced acute inflammation through inhibition of nuclear factor kappa B, inducible cyclooxygenase and proinflammatory cytokines and enhancement of interleukin-10 via an antioxidant mechanism. *Int Immunopharmacol* 19: 290-299.
159. Yao Z, Wu H, Zhang S, et al. (2014) Enzymatic preparation of kappa-carrageenan oligosaccharides and their anti-angiogenic activity. *Carbohydr Polym* 101: 359-367.
160. Yermak IM, Barabanova AO, Aminin DL, et al. (2012) Effects of structural peculiarities of carrageenans on their immunomodulatory and anticoagulant activities. *Carbohydr Polym* 87: 713-720.

161. Bao X, Hayashi K, Li Y, et al. (2010) Novel agarose and agar fibers: Fabrication and characterization. *Mater Lett* 64: 2435-2437.
162. Hu J, Zhu Y, Tong H, et al. (2016) A detailed study of homogeneous agarose / hydroxyapatite nanocomposites for load-bearing bone tissue. *Int J Biol Macromol* 82: 134-143.
163. Miguel SP, Ribeiro MP, Brancal H, et al. (2014) Thermoresponsive chitosan-agarose hydrogel for skin regeneration. *Carbohydr Polym* 111: 366-373.
164. Varoni E, Tschon M, Palazzo B, et al. (2012) Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response. *Connect Tissue Res* 53: 548-554.
165. Gonzalez JS, Ludueña LN, Ponce A, et al. (2014) Poly(vinyl alcohol)/cellulose nanowhiskers nanocomposite hydrogels for potential wound dressings. *Mater Sci Eng* C 34: 54-61.
166. Wang J, Hu H, Yang Z, et al. (2016) IPN hydrogel nanocomposites based on agarose and ZnO with antifouling and bactericidal properties. *Mater Sci Eng* C 61: 376-386.
167. Yi Y, Neufeld RJ, Poncelet D (2005) Immobilization of cells in Polysaccharide gels, In: Dumitriu S., Polysacharides. *Structural diversity and functional versatility*, 2d ed., New York: Marcel Dekker, 867-891.
168. Li XQ, Li Q, Gong FL, et al. (2015) Preparation of large-sized highly uniform agarose beads by novel rotating membrane emulsification. *J Membr Sci* 476: 30-39.
169. Droce A, Sørensen JL, Giese H, et al. (2013) Glass bead cultivation of fungi: Combining the best of liquid and agar media. *J Microbiol Methods* 94: 343-346.
170. Ganesan M, Reddy CRK, Jha B (2015) Impact of cultivation on growth rate and agar content of *Gelidiella acerosa* (Gelidiales, Rhodophyta). *Algal Res* 12: 398-404.
171. Griffitt KJ, Grimes DJ (2013) A novel agar formulation for isolation and direct enumeration of *Vibrio vulnificus* from oyster tissue. *J Microbiol Methods* 94: 98-102.
172. Seo BY, Park J, Huh IY, et al. (2016) Agarose hydrolysis by two-stage enzymatic process and bioethanol production from the hydrolysate. *Process Biochem* In press.
173. Giménez B, López de Lacey A, Pérez-Santin E, et al. (2013) Release of active compounds from agar and agar-gelatin films with green tea extract. *Food Hydrocoll* 30: 264-271.
174. Sousa AMM, Souza HKS, Latona N, et al. (2014) Choline chloride based ionic liquid analogues as tool for the fabrication of agar films with improved mechanical properties. *Carbohydr Polym* 111: 206-214.
175. Sousa AMM, Gonçalves MP (2015) The influence of locust bean gum on native and alkali-modified agar gels. *Food Hydrocoll* 44: 461-470.
176. Oun AA, Rhim J-W (2015) Effect of post-treatments and concentration of cotton linter cellulose nanocrystals on the properties of agar-based nanocomposite films. *Carbohydr Polym* 134: 20-29.
177. Shankar S, Rhim JW (2016) Preparation of nanocellulose from micro-crystalline cellulose: The effect on the performance and properties of agar-based composite films. *Carbohydr Polym* 135: 18-26.
178. Vejdan A, Ojagh SM, Adeli A, et al. (2016) Effect of TiO_2 nanoparticles on the physico-mechanical and ultraviolet light barrier properties of fish gelatin/agar bilayer film. LWT *Food Sci Technol* 71: 88-95.
179. Madera-Santana TJ, Freile-Pelegrin Y, Azamar-Barrios JA (2014) Physicochemical and morphological properties of plasticized poly(vinyl alcohol)-agar biodegradable films. *Int J Biol Macromol* 69:176-184.

180. Tian H, Xu G, Yang B, et al. (2011) Microstructure and mechanical properties of soy protein/agar blend films: Effect of composition and processing methods. *J Food Eng* 107: 21-26.
181. Kestwal RM, Bagal-Kestwal D, Chiang B-H (2015) Fenugreek hydrogel-agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection. *Anal Chim Acta* 886: 143-150.
182. Rhim JW, Wang LF (2013) Mechanical and water barrier properties of agar/k-carrageenan/konjac glucomannan ternary blend biohydrogel films. *Carbohydr Polym* 96: 71-81.
183. Le Goff KJ, Gaillard C, Helbert W, et al. (2015) Rheological study of reinforcement of agarose hydrogels by cellulose nanowhiskers. *Carbohydr Polym* 116: 117-123.
184. Campo VL, Kawano DF, Silva DB, et al. (2009) Carrageenans: Biological properties, chemical modifications and structural analysis - A review. *Carbohydr Polym* 77: 167-180.
185. Weiner ML (2016) Parameters and pitfalls to consider in the conduct of food additive research, Carrageenan as a case study. *Food Chem Toxicol* 87: 31-44.
186. Zhang Z, Zhang R, Chen L, et al. (2016) Encapsulation of lactase (b-galactosidase) into k-carrageenan-based hydrogel beads: Impact of environmental conditions on enzyme activity. *Food Chem* 200: 69-75.
187. Pinheiro AC, Bourbon AI, Medeiros BGDS, et al. (2012) Interactions between κ-carrageenan and chitosan in nanolayered coatings - Structural and transport properties. *Carbohydr Polym* 87: 1081-1090.
188. Pinheiro AC, Bourbon AI, Quintas MAC, et al. (2012) k-carrageenan/chitosan nanolayered coating for controlled release of a model bioactive compound. *Innov Food Sci Emerg* 16:227-232.
189. Rhim J (2013) Effect of PLA lamination on performance characteristics of agar/κ-carrageenan/clay bio-nanocomposite film. *Food Res Int* 51: 714-722.
190. Rhim J, Wang L (2014) Preparation and characterization of carrageenan-based nanocomposite films reinforced with clay mineral and silver nanoparticles. *Appl Clay Sci* 98: 174-181.
191. Benard C, Cultrone A, Michel C (2010) Degraded carrageenan causing colitis in rats induces TNF secretion and ICAM-1 upregulation in monocytes through NF-kB activation. *Plos One* 5: e8666.
192. Mckim JM, Wilga PC, Pregenzer JF, et al. (2015) The common food additive carrageenan is not a ligand for Toll-Like-Receptor 4 (TLR4) in an HEK293-TLR4 reporter cell-line model. *Food Chem Toxicol* 78: 153-158.
193. Weiner ML (2014) Food additive carrageenan: Part II: A critical review of carrageenan in vivo safety studies. *Crit Rev Toxicol* 44: 244-269.
194. De Souza RR, Bretanha LC, Dalmarco EM, et al. (2015) Modulatory effect of *Senecio brasiliensis* (Spreng) Less. in a murine model of inflammation induced by carrageenan into the pleural cavity. *J Ethnopharmacol* 168: 373-379.
195. Matsumoto K, Obara S, Kuroda Y, et al. (2015) Anti-inflammatory effects of linezolid on carrageenan-induced paw edema in rats. *J Infect Chemother* 21: 889-891.
196. Shalini V, Jayalekshmi A, Helen A (2015) Mechanism of anti-inflammatory effect of tricin, a flavonoid isolated from Njavara rice bran in LPS induced hPBMCs and carrageenan induced rats. *Mol Immunol* 66: 229-239.
197. Singh V, Tiwari S, Sharma AK, et al. (2007) Removal of lead from aqueous solutions using *Cassia grandis* seed gum-graft-poly(methylmethacrylate). *J Colloid Interface Sci* 316: 224-232.

198. Solanki HK, Shah DA, Maheriya PM, et al. (2015) Evaluation of anti-inflammatory activity of probiotic on carrageenan-induced paw edema in Wistar rats. *Int J Biol Macromol* 72: 1277-1282.
199. Sokolova EV, Bogdanovich LN, Ivanova TB, et al. (2014) Effect of carrageenan food supplement on patients with cardiovascular disease results in normalization of lipid profile and moderate modulation of immunity system markers. *Pharmanutrition* 2: 33-37.
200. Ngo DH, Kim SK (2013) Sulfated polysaccharides as bioactive agents from marine algae. *Int J Biol Macromol* 62: 70-75.
201. Abad LV, Relleve LS, Racadio CDT, et al. (2013) Antioxidant activity potential of gamma irradiated carrageenan. *Appl Radiat Isot* 79: 73-79.
202. Relleve L, Abad L (2015) Characterization and antioxidant properties of alcoholic extracts from gamma irradiated k-carrageenan. *Radiat Phys Chem* 112: 40-48.
203. Sun Y, Yang B, Wu Y, et al. (2015) Structural characterization and antioxidant activities of k-carrageenan oligosaccharides degraded by different methods. *Food Chem* 178: 311-318.
204. Niu T, Zhang D, Chen H, et al. (2015) Modulation of the binding of basic fibroblast growth factor and heparanase activity by purified λ-carrageenan oligosaccharides. *Carbohydr Polym* 125: 76-84.
205. Yao Z, Wu H, Zhang S (2014) Enzymatic preparation of k-carrageenan oligosaccharides and their anti-angiogenic activity. *Carbohydr Polym* 101: 359-367.
206. De Araújo CA, Noseda MD, Cipriani TR, et al. (2013) Selective sulfation of carrageenans and the influence of sulfate regiochemistry on anticoagulant properties. *Carbohydr Polym* 91: 483-491.
207. Selvakumaran S, Muhamad II (2015) Evaluation of kappa carrageenan as potential carrier for floating drug delivery system: Effect of cross linker. *Int J Pharm* 496: 323-331.
208. Sun W, Saldaña MDA, Zhao Y, et al. (2016) Hydrophobic lappaconitine loaded into iota-carrageenan by one step self-assembly. *Carbohydr Polym* 137: 231-238.
209. Pairatwachapun S, Paradee N, Sirivat A. (2016) Controlled release of acetylsalicylic acid from polythiophene/carrageenan hydrogel via electrical stimulation. *Carbohydr Polym* 137: 214-221.
210. Lohani A, Singh G, Sankar S, et al. (2016) Tailored-interpenetrating polymer network beads of k -carrageenan and sodium carboxymethyl cellulose for controlled drug delivery. *J Drug Deliv Sci Tec* 31: 53-64.
211. Carneiro-da-Cunha MG, Cerqueira MA, Souza BWS, et al. (2010) Physical and thermal properties of a chitosan/alginate nanolayered PET film. *Carbohydr Polym* 82: 153-159.
212. Dange-Delbaere C, Buron CC, Euvrard M, et al. (2016) Stability and cathodic electrophoretic deposition of polystyrene particles pre-coated with chitosan–alginate multilayer. *Colloids Surfaces A Physicochem Eng Asp* 493: 1-8.
213. Belscak-Cvitanovic A, Komes D, Karlović S, et al. (2015) Improving the controlled delivery formulations of caffeine in alginate hydrogel beads combined with pectin, carrageenan, chitosan and psyllium. *Food Chem* 167: 378-386.
214. Wang Z, Zhang X, Gu J, et al. (2014) Electrodeposition of alginate/chitosan layer-by-layer composite coatings on titanium substrates. *Carbohydr Polym* 103: 38-45.
215. Seth A, Lafargue D, Poirier C, et al. (2014) Performance of magnetic chitosan-alginate core-shell beads for increasing the bioavailability of a low permeable drug. *Eur J Pharm Biopharm* 88: 374-381.
216. Cheng HC, Chang CY, Hsieh FI, et al. (2011) Effects of tremella-alginate-liposome encapsulation on oral delivery of inactivated H5N3 vaccine. *J Microencapsul* 28: 55-61.

217. Shin GH, Chung SK, Kim JT, et al. (2013) Preparation of chitosan-coated nanoliposomes for improving the mucoadhesive property of curcumin using the ethanol injection method. *J Agric Food Chem* 61: 11119-11126.
218. Liu W, Liu J, Li T, et al. (2013) Improved physical and in vitro digestion stability of a polyelectrolyte delivery system based on layer-by-layer self-assembly alginate-chitosan-coated nanoliposomes. *J Agric Food Chem* 61: 4133-4144.
219. Haidar ZS, Hamdy RC, Tabrizian M (2008) Protein release kinetics for core-shell hybrid nanoparticles based on the layer-by-layer assembly of alginate and chitosan on liposomes. *Biomaterials* 29: 1207-1215.
220. Liu W, Liu W, Ye A, et al. (2016) Environmental stress stability of microencapsules based on liposomes decorated with chitosan and sodium alginate. *Food Chem* 196: 396-404.
221. Martins AF, Monteiro JP, Bonafé EG, et al. (2015) Bactericidal activity of hydrogel beads based on N,N,N-trimethyl chitosan/alginate complexes loaded with silver nanoparticles. *Chinese Chem Lett* 26: 1129-1132.
222. Jaikumar D, Sajesh KM, Soumya S, et al. (2015) Injectable alginate-O-carboxymethyl chitosan/nano fibrin composite hydrogels for adipose tissue engineering. *Int J Biol Macromol* 74: 318-326.
223. Algul D, Sipahi H, Aydin A, et al. (2015) Biocompatibility of biomimetic multilayered alginate-chitosan/β-TCP scaffold for osteochondral tissue. *Int J Biol Macromol* 79: 363-369.
224. Rivera MC, Pinheiro AC, Bourbon AI, et al. (2015) Hollow chitosan/alginate nanocapsules for bioactive compound delivery. *Int J Biol Macromol* 79: 95-102.
225. Wang J-Z, Zhu Y-X, Ma H-C, et al. (2016) Developing multi-cellular tumor spheroid model (MCTS) in the chitosan/collagen/alginate (CCA) fibrous scaffold for anticancer drug screening. *Mater Sci Eng C* 62: 215-225.
226. Trabelsi I, Ayadi D, Bejar W, et al. (2014) Effects of Lactobacillus plantarum immobilization in alginate coated with chitosan and gelatin on antibacterial activity. *Int J Biol Macromol* 64: 84-89.
227. Gandomi H, Abbaszadeh S, Misaghi A, et al. (2016) Effect of chitosan-alginate encapsulation with inulin on survival of *Lactobacillus rhamnosus* GG during apple juice storage and under simulated gastrointestinal conditions. *LWT Food Sci Technol* 69: 365-371.
228. Sáez MI, Barros AM, Vizcaino AJ, et al. (2015) Effect of alginate and chitosan encapsulation on the fate of BSA protein delivered orally to gilthead sea bream (*Sparus aurata*). *Anim Feed Sci Technol* 210: 114-124.
229. Ren Y, Xie H, Liu X, et al. (2016) Comparative investigation of the binding characteristics of poly-l-lysine and chitosan on alginate hydrogel. *Int J Biol Macromol* 84: 135-141.
230. Lin J-H, Chen C-K, Wen S-P, et al. (2015) Poly-l-lactide/sodium alginate/chitosan microsphere hybrid scaffolds made with braiding manufacture and adhesion technique: Solution to the incongruence between porosity and compressive strength. *Mater Sci Eng C* 52: 111-120.
231. Kim H-L, Jung G-Y, Yoon J-H, et al. (2015) Preparation and characterization of nano-sized hydroxyapatite/alginate/chitosan composite scaffolds for bone tissue engineering. *Mater Sci Eng C* 54: 20-25.
232. Garcia-Ceja A, Mani-López E, Palou E, et al. (2015) Viability during refrigerated storage in selected food products and during simulated gastrointestinal conditions of individual and combined lactobacilli encapsulated in alginate or alginate-chitosan. *LWT Food Sci Technol* 63: 482-489.

233. Strobel SA, Scher HB, Nitin N, et al. (2016) *In situ* cross-linking of alginate during spray-drying to microencapsulate lipids in powder. *Food Hydrocoll* 58: 141-149.
234. De'Nobili MD, Soria M, Martinefski MR, et al. (2016) Stability of L-(+)-ascorbic acid in alginate edible films loaded with citric acid for antioxidant food preservation. *J Food Eng* 175: 1-7.
235. Salvia-Trujillo L, Decker EA, McClements DJ (2016) Influence of an anionic polysaccharide on the physical and oxidative stability of omega-3 nanoemulsions: Antioxidant effects of alginate. *Food Hydrocoll* 52: 690-698.
236. Da Cunha PLR, De Paula RCM, Feitosa JPA (2009) Polysaccharides from brazilian biodiversity: an opportunity to change knowledge into economic value. *Quim Nova* 32: 649-660.
237. Kumar A (2012) Cashew Gum a versatile hydrophyllic polymer: a review. *Curr Drug Ther* 7: 2-12.
238. Kumar R, Patil MB, Patil SR, et al. (2009) Evaluation of *Anacardium occidentale* gum as gelling agent in aceclofenac gel. *Int J PharmTech Res* 1: 695-704.
239. Ribeiro AJ, de Souza FRL, Bezerra JMNA, et al. (2016) Gums' based delivery systems: review on cashew gum and its derivatives. *Carbohydr Polym* 147: 188-200.
240. Paula RCM, Heatley F, Budd PM (1998) Characterization of *Anacardium occidentale* exudate polysaccharide. *Polym Int* 45: 27-35.
241. Porto BC, Augusto PED, Terekhov A, et al. (2015) Effect of dynamic high pressure on technological properties of cashew tree gum (*Anacardium occidentale* L.). *Carbohydr Polym* 129: 187-193.
242. Das B, Dutta S, Nayak AK, et al. (2014) Zinc alginate-carboxymethyl cashew gum microbeads for prolonged drug release: development and optimization. *Int J Biol Macromol* 70: 506-515.
243. Oliveira EF, Paula HCB, Paula RCM. (2014) Alginate/cashew gum nanoparticles for essential oil encapsulation. *Colloids Surf B Biointerfaces* 113: 146-151.
244. Abreu FOMS, Oliveira EF, Paula HCB, et al. (2012) Chitosan/cashew gum nanogels for essential oil encapsulation. *Carbohydr Polym* 89: 1277-1282.
245. Moreira BR, Batista KA, Castro EG, et al. (2015) A bioactive film based on cashew gum polysaccharide for wound dressing applications. *Carbohydr Polym* 122: 69-76.
246. Silva FEF, Batista KA, Di-Medeiros MCB, et al. (2016) A stimuli-responsive and bioactive film based on blended polyvinyl alcohol and cashew gum polysaccharide. *Mater Sci Eng C* 58: 927-234.
247. Barros SBA, Leite CMDS, de Brito ACF, et al. (2012) Multilayer films electrodes consisted of Cashew Gum and polyaniline assembled by the Layer-by-Layer technique: electrochemical characterization and its use for dopamine determination. *Int J Anal Chem* 2012: 1-10.
248. Pinto AMB, Santos TM, Caceres CA, et al. (2015) Starch-cashew tree gum nanocomposite films and their application for coating cashew nuts. *LWT Food Sci Technol* 62: 549-554.
249. Soares PAG, Bourbon AI, Vicente AA, et al.(2014) Development and characterization of hydrogels based on natural polysaccharides: Policaju and chitosan. *Mater Sci Eng C* 42: 219-226.
250. Paula HCB, Sombra FM, Cavalcante RDF, et al. (2011) Preparation and characterization of chitosan/cashew gum beads loaded with Lippia sidoides essential oil. *Mater Sci Eng C* 31: 173-178.
251. Paula HCB, Rodrigues MLL, Ribeiro WLC, et al. (2012) Protective effect of Cashew Gum nanoparticles on natural larvicide from *Moringa oleifera* seeds. *J Appl Polym Sci* 124:1778-1784.

252. Forato LA, de Britto D, de Rizzo JS, et al. (2015) Effect of cashew gum-carboxymethylcellulose edible coatings in extending the shelf-life of fresh and cut guavas. *Food Packag Shelf Life* 5: 68-74.
253. Gowthamarajan K, Kumar GKP, Gaikwad NB, et al. (2011) Preliminary study of *Anacardium occidentale* gum as binder in formulation of paracetamol tablets. *Carbohydr Polym* 83: 506-511.
254. Carneiro-da-Cunha MG, Cerqueira MA, Souza BWS, et al. (2009) Physical properties of edible coatings and films made with a polysaccharide from *Anacardium occidentale* L. *J Food Eng* 95: 379-385.
255. Dias SFL, Nogueira SS, Dourado FF, et al. (2016) Acetylated cashew gum-based nanoparticles for transdermal delivery of diclofenac diethyl amine. *Carbohydr Polym* 143: 254-261.
256. Bittencourt CR, Farias EAO, Bezerra KC, et al. (2016) Immobilization of cationic antimicrobial peptides and natural cashew gum in nanosheet systems for the investigation of anti-leishmanial activity. *Mater Sci Eng C* 59: 549-555.
257. Pitombeira NAO, Veras Neto JG, Silva DA, et al. (2015) Self-assembled nanoparticles of acetylated cashew gum: Characterization and evaluation as potential drug carrier. *Carbohydr Polym* 117: 610-615.
258. Porto BC, Cristianini M (2014) Evaluation of cashew tree gum (*Anacardium occidentale* L.) emulsifying properties. *LWT Food Sci Technol* 59: 1325-1331.
259. Babbar N, Dejonghe W, Gatti M, et al. (2015) Pectic oligosaccharides from agricultural by-products: production, characterization and health benefits. *Crit Rev Biotechnol* 36: 594-606.
260. Munarin F, Tanzi MC, Petrini P (2012) Advances in biomedical applications of pectin gels. *Int J Biol Macromol* 51: 681-689.
261. Marras-Marquez T, Peña J, Veiga-Ochoa MD (2015) Robust and versatile pectin-based drug delivery systems. *Int J Pharm* 479: 265-276.
262. Müller-Maatsch J, Bencivenni M, Caligiani A, et al. (2016) Pectin content and composition from different food waste streams. *Food Chem* 201: 37-45.
263. Hua X, Wang K, Yang R, et al. (2015) Edible coatings from sun flower head pectin to reduce lipid uptake in fried potato chips. *LWT Food Sci Technol* 62: 1220-1225.
264. Krivorotova T, Cirkovas A, Maciulyte S, et al. (2016) Nisin-loaded pectin nanoparticles for food preservation. *Food Hydrocoll* 54: 49-56.
265. Guerreiro AC, Gago CML, Faleiro ML, et al. (2015) Raspberry fresh fruit quality as affected by pectin- and alginate-based edible coatings enriched with essential oils. *Sci Hortic* 194: 138-146.
266. Gullón B, Gullón P, Sanz Y, et al. (2011) Prebiotic potential of a refined product containing pectic oligosaccharides. *LWT Food Sci Technol* 44: 1687-1696.
267. Lama-Muñoz A, Rodriguez-Gutiérrez G, Rubio-Senent F, et al. (2012) Production, characterization and isolation of neutral and pectic oligosaccharides with low molecular weights from olive by-products thermally treated. *Food Hydrocoll* 28: 92-104.
268. Chen H, Qiu S, Gan J, et al. (2016) New insights into the functionality of protein to the emulsifying properties of sugar beet pectin. *Food Hydrocoll* 57: 262-270.
269. Schmidt US, Pietsch VL, Rentschler C, et al. (2016) Influence of the degree of esterification on the emulsifying performance of conjugates formed between whey protein isolate and citrus pectin. *Food Hydrocoll* 56: 1-8.

270. Tamnak S, Mirhosseini H, Ping C, et al. (2016) Physicochemical properties, rheological behavior and morphology of pectin-pea protein isolate mixtures and conjugates in aqueous system and oil in water emulsion. *Food Hydrocoll* 56: 405-416.
271. Meneguin AB, Cury BSF, Evangelista RC (2014) Films from resistant starch-pectin dispersions intended for colonic drug delivery. *Carbohydr Polym* 99: 140-149.
272. Jung J, Arnold RD, Wicker L (2013) Pectin and charge modified pectin hydrogel beads as a colon-targeted drug delivery carrier. *Colloids Surf B Biointerfaces* 104: 116-121.
273. Elisangela P, Sitta DLA, Fragal VH, et al. (2014) Covalent TiO_2/pectin microspheres with Fe_3O_4 nanoparticles for magnetic field-modulated drug delivery. *Int J Biol Macromol* 67: 43-52.
274. Zhang Y, Chen T, Yuan P, et al. (2015) Encapsulation of honokiol into self-assembled pectin nanoparticles for drug delivery to HepG2 cells. *Carbohydr Polym* 133: 31-38.
275. Zhou M, Wang T, Hu Q, et al. (2016) Low density lipoprotein/pectin complex nanogels as potential oral delivery vehicles for curcumin. *Food Hydrocoll* 57: 20-29.
276. Mashingaidze F, Choonara YE, Kumar P, et al. (2016) Poly (ethylene glycol) enclatherated pectin-mucin submicron matrices for intravaginal anti-HIV-1 drug delivery. *Int J Pharm* 503: 16-28.
277. Alvarez-Lorenzo C, Blanco-Fernandez B, Puga AM, et al. (2013) Crosslinked ionic polysaccharides for stimuli-sensitive drug delivery. *Adv Drug Deliv Rev* 65: 1148-1171.
278. Auriemma G, Mencherini T, Russo P, et al. (2013) Prilling for the development of multi-particulate colon drug delivery systems: Pectin vs. pectin-alginate beads. *Carbohydr Polym* 92: 367-373.
279. Fares MM, Assaf SM, Abul-Haija YM (2010). Pectin grafted poly(N-vinylpyrrolidone): Optimization and in vitro controllable theophylline drug release. *J Appl Polym Sci* 117: 1945-1954.
280. van der Gronde T, Hartog A, van Hees C, et al. (2016) Systematic review of the mechanisms and evidence behind the hypocholesterolaemic effects of HPMC, pectin and chitosan in animal trials. *Food Chem* 199: 746-759.
281. Zhu RG, Sun Y Di, Li TP, et al. (2015) Comparative effects of hawthorn (*Crataegus pinnatifida* Bunge) pectin and pectin hydrolyzates on the cholesterol homeostasis of hamsters fed high-cholesterol diets. *Chem Biol Interact* 238: 42-47.
282. Zhu R, Li T, Dong Y, et al. (2013) Pectin pentasaccharide from hawthorn (*Crataegus pinnatifida* Bunge. Var. major) ameliorates disorders of cholesterol metabolism in high-fat diet fed mice. *Food Res Int* 54: 261-268.
283. Austarheim I, Christensen BE, Hegna IK, et al. (2012) Chemical and biological characterization of pectin-like polysaccharides from the bark of the Malian medicinal tree Cola cordifolia. *Carbohydr Polym* 89: 259-268.
284. Le Normand M, Mélida H, Holmbom B, et al. (2014) Hot-water extracts from the inner bark of Norway spruce with immunomodulating activities. *Carbohydr Polym* 101: 699-704.
285. Zou YF, Zhang BZ, Inngjerdingen KT, et al. (2014) Polysaccharides with immunomodulating properties from the bark of *Parkia biglobosa*. *Carbohydr Polym* 101: 457-463.
286. Fan L, Sun Y, Xie W, et al. (2011) Oxidized pectin cross-linked carboxymethyl chitosan: a new class of hydrogels. *J Biomater Sci Polym Ed* 5063: 2119-2132.
287. Tummalapalli M, Berthet M, Verrier B, et al. (2016) Drug loaded composite oxidized pectin and gelatin networks for accelerated wound healing. *Int J Pharm* 505: 234-245.

288. Tummalapalli M, Berthet M, Verrier B, et al. (2016) Composite wound dressings of pectin and gelatin with aloe vera and curcumin as bioactive agents. *Int J Biol Macromol* 82: 104-113.
289. Pérez S, Bertoft E (2010) The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Staerke* 62: 389-420.
290. Le Corre D, Angellier-Coussy H (2014) Preparation and application of starch nanoparticles for nanocomposites: A review. *React Funct Polym* 85: 97-120.
291. Grote C, Heinze T (2005) Starch derivatives of high degree of functionalization 11: Studies on alternative acylation of starch with long-chain fatty acids homogeneously in N,N-dimethyl acetamide/LiCl. *Cellulose* 12: 435-444.
292. Xie W, Wang Y (2011) Synthesis of high fatty acid starch esters with 1-butyl-3-methylimidazolium chloride as a reaction medium. *Starch/Stärke* 63: 190-197.
293. Tupa M, Maldonado L, Vazquez A, et al. (2013). Simple organocatalytic route for the synthesis of starch esters. *Carbohydr Polym* 98: 349-357.
294. Rutkaite R, Bendoraitiene J, Klimaviciute R, et al. (2012) Cationic starch nanoparticles based on polyelectrolyte complexes. *Int J Biol Macromol* 50: 687-693.
295. Baier G, Baumann D, Siebert JM, et al. (2012) Suppressing unspecific cell uptake for targeted delivery using hydroxyethyl starch nanocapsules. *Biomacromolecules* 13: 2704-2715.
296. Wei B, Zhang B, Sun B (2016) Aqueous re-dispersibility of starch nanocrystal powder improved by sodium hypochlorite oxidation. *Food Hydrocoll* 52: 29-37.
297. Amini AM, Mohammad S, Razavi A (2016) A fast and efficient approach to prepare starch nanocrystals from normal corn starch. *Food Hydrocoll* 57: 132-138.
298. Kim HY, Park SS, Lim ST (2015) Preparation, characterization and utilization of starch nanoparticles. *Colloids Surf B Biointerfaces* 126: 607-620.
299. Lin N, Huang J, Chang PR, et al. (2011) Preparation, modification, and application of starch nanocrystals in nanomaterials: A review. *J Nanomater* 2011.
300. Lin N, Huang J, Dufresne A (2012) Preparation, properties and applications of polysaccharide nanocrystals in advanced functional nanomaterials: a review. *Nanoscale* 4: 3274-3294.
301. Angellier H, Molina-Boisseau S, Dufresne A (2005) Mechanical properties of waxy maize starch nanocrystal reinforced natural rubber. *Macromolecules* 38: 9161-9170.
302. Jiang S, Liu C, Han Z, et al. (2016) Evaluation of rheological behavior of starch nanocrystals by acid hydrolysis and starch nanoparticles by self-assembly: A comparative study. *Food Hydrocoll* 52: 914-922.
303. Rajisha KR, Maria HJ, Pothan LA, et al. (2014) Preparation and characterization of potato starch nanocrystal reinforced natural rubber nanocomposites. *Int J Biol Macromol* 67: 147-153.
304. Sessini V, Arrieta MP, Kenny JM, et al. (2016) Processing of edible films based on nanoreinforced gelatinized starch. *Polym Degrad Stabil* In Press.
305. Condés MC, Añón MC, Mauri AN, et al. (2015) Amaranth protein films reinforced with maize starch nanocrystals. *Food Hydrocoll* 47: 146-157.

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Artigo IV

Immobilization of bioactive compounds in *Cassia grandis* galactomannan-based films: influence on physicochemical properties

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Immobilization of bioactive compounds in *Cassia grandis* galactomannan-based films: Influence on physicochemical properties

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ABSTRACT

Galactomannan extracted from *Cassia grandis* seeds was used for the production of films containing different concentrations of the bioactive compounds lactoferrin (LF), bioactive peptides (BAPs), and phytosterols. SEM, FTIR, mechanical and thermal properties, colour, moisture content (MC), solubility, water vapour permeability (WVP), and contact angle (CA) were performed evaluating the effect of increasing concentrations of bioactive compounds on the films' physicochemical properties. The immobilization of bioactive compounds leads to films with roughness on their surface, as observed by SEM. The thermal events demonstrated that bioactive compounds avoided the establishment of more hydrogen bonds when compared to galactomannan control film; this behaviour was also confirmed by FTIR. All the studied films had a strong whiteness tendency as well as a yellowish appearance. The addition of Lf reduced MC and solubility values and leads to an increase of WVP and CA values, while the addition of BAPs and phytosterols did not changed the films' solubility. The mechanical properties were affected by the addition of bioactive compounds, which improved the stiffness of the films. Galactomannan-based films from *C. grandis* showed to be a promising structure for the immobilization of biomolecules, pointing at a significant number of possible applications in food and pharmaceutical industries.

1. Introduction

Polymers, whether natural or synthetic, are large molecules whose chains are able to produce continuous matrices that will influence the successful formation of films, membranes and coatings. Among natural polymers, polysaccharides have been used as an alternative material for the development of biodegradable and non-toxic films and membranes that can be used in different applications. In the pharmaceutical field, films based on alginate [1], chitosan [2], and POLICAJU [3] have been used as wound dressing due to their capacity of adhesion and controlled release of biomolecules; while galactomannan-based films have been applied as supports for the immobilization of Concanavalin A in immunological experiments, aiming the evaluation of interactions between polysaccharides, proteins and dengue virus [4]. In food industry, the use of polysaccharides for the production of films and coatings became an alternative for synthetic and wax materials, being cellulose derivatives, chitosan, agar and starch some of the most common used materials. They can act in the maintenance of safety and quality of foods, being used as carriers of biomolecules, not only for control of the microbial contaminations, but also to enhance the health benefits of food products [5].

Galactomannans are hydrophilic polysaccharides derived from leguminous seeds that possess a central core of (1 → 4)-linked D-mannopyranose units to which (1 → 6)-linked D-galactopyranose units are attached. They present as main advantages their emulsifying, thickening, and gelling capacity. In addition, they can be used as alternative sources for the production of edible films based on their edibility and biodegradability [6,7]. The galactomannan

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extracted from the seeds of *Cassia grandis*, a typical Brazilian tree, has been previously characterized by our lab [8]. Methylation analysis and NMR spectroscopy reported that the polysaccharide has a central core composed of 4-linked β-mannose units with branches of galactose, linked to the carbohydrate core through α(1–6) linkage. The rheological characterization indicated the galactomannan as an alternative source for conventional galactomannans due to its ability to present both liquid and gel behaviour suggesting that, due to its rheological properties, it can be useful in various biotechnological applications.

A great number of works reported the use of galactomannans from conventional [9] and non-conventional [10] sources to produce films. Other galactomannan-based films have also been explored as effective support for the immobilization of different functional compounds, such as peptides [11], antioxidants [12] and lectins [4]. The incorporation of bioactive compounds with functional properties in the galactomannan-based films can bring several advantages for pharmaceutical and food industries. By combining the excellent biological characteristics of the bioactive compounds and the physicochemical properties of the films, functional galactomannan-based films could bring several advantageous when compared with the synthetic alternatives and thus should be explored.

Lactoferrin (Lf) is a monomeric, globular, and basic 80 kDa iron-binding glycoprotein of the transferrin family. It is abundant in milk and in most biological fluids and holds important biological activities including anticancer, anti-inflammatory and immune modulator, as well as antimicrobial properties against a large number of microorganisms. These properties are mostly due to its ability to bind iron and to interact with cellular and molecular compounds of hosts and pathogens [13]. Bioactive peptides (BAPs) are specific fragments of proteins with activities similar to drugs and/or hormones, capable of modulating physiological functions by binding to specific target cell receptors, leading to induction of physiologic responses. Milk proteins are a good source of BAPs, which can positively affect various health biomarkers *in vitro* [14]. Regarding the described effects in the cardiovascular system, BAPs induce anti-hypertensive activity by inhibiting the angiotensin converting enzyme, a key enzyme in the regulation of blood pressure that converts angiotensin I into angiotensin II, a potent vasoconstrictor. Plant sterols, also called phytosterols, have been reported to include over than 250 distinct sterols and related compounds in various plant and marine materials; in addition, they have similar chemical structures and biological functions as cholesterol. Phytosterols contain an extra methyl group, ethyl group, or double bond, and they are known to have hypocholesterolemic properties. Phytosterols analogues are suggested to lower cholesterol absorption and thus the serum cholesterol level in humans, leading to cardiologic health benefits [15].

Several studies have reported the immobilization of Lf [16,17], BAPs [18], and phytosterols [19] in pharmaceutical solid dosage forms such as capsules, beads, particles and tablets, however the immobilization of those bioactive compounds in galactomannan-based films emerges as an effective way for biotechnological applications in pharmaceutical and food industries, since they can be an alternative, for example, for individuals with dermal wounds or with difficulty in swallowing those unit doses.

The objective of this work was to evaluate the effect of three different bioactive compounds (Lf, BAPs and phytosterols) on the properties of galactomannan-based films. The effect of increasing concentrations of bioactive compounds was evaluated through scanning electron microscopy (SEM), Fourier-transform infrared (FTIR) spectroscopy analyses and thermal properties. Colour, moisture content, solubility, water vapour permeability, water contact angle, and mechanical properties were also performed.

2. Materials and methods

2.1. Materials

The pods of *C. grandis* were collected at the rural zone of Pernambuco state, in the city of Angelim (Brazil), in July 2011. Ethanol (99.8%), acetone (PA) and sodium chloride were obtained from Vetec Fine Chemicals Ltda. (Brazil). Lf was obtained from DMV International (USA). The reported composition expressed as a dry weigh percentage was: 96% protein, 0.5% ash, 3.5% moisture and an iron content around 120 ppm. Glycerol 87% (Panreac, Spain), Lowpept® (hydrolysed milk protein, Innaves SA, Spain) and Lowcol® (phytosterols, Innaves SA, Spain) were obtained from commercial sources. Lowpept® (hydrolysed milk proteins, potassium chloride, cellulose microcrystalline as bulking agent, xantham gum as carrier and magnesium stearate and silicon dioxide as anti-caking agents) and Lowcol® (phytosterols, cellulose microcrystalline as bulking agent, xantham gum as carrier and magnesium stearate and silicon dioxide as anti-caking agents) were used as sources of BAPs and phytosterols, respectively. All other chemicals were of analytical grade.

2.2. Film preparation

The galactomannan contained in *C. grandis* seeds was obtained according to Albuquerque et al. (2014) [8], with an extraction yield of 36 ± 8% and a composition of 71.0% mannose and 29.0% galactose. Briefly, the purification process was performed by immersion of the pods of *C. grandis* in distilled water at 25 °C for 18 h; the pods were then separated in a half part, revealing the seeds which were removed and dried until reaching a constant weight. The dry seeds were boiled in distilled water 1:5 (w/v) at 100 °C for 1 h for enzyme inactivation and maintained in water by 18 h at 25 °C to facilitate removal of the hull. After that, the hull was removed and the residual was triturated in a blender with 0.1 M NaCl 5% (w/v) at 25 °C, filtered through a veil tissue and after using a screen printing cloth, and precipitated with 46% ethanol 1:3 (v/v) for 18 h. The white precipitate obtained was washed with 100% ethanol 1:3 (w/v) for 30 min and two times with acetone 1:3 (w/v) for 30 min, been filtered on screen printing cloth between each washing. The precipitate was dried until constant weight, milled and finally called galactomannan.

The film forming solutions were prepared in distilled water with 0.8% (w/v) of galactomannan and 0.2% (v/v) of glycerol, maintained under magnetic stirring (500 rpm) for 18 h, at room temperature (20 ± 2 °C), and finally called film A. Galactomannan and glycerol concentrations were chosen based on preliminary analyses (results not shown).

Lf (0.1% for B1 and 0.2% for B2), Lowpept® (0.1% for C1 and 0.2% for C2) and Lowcol® (0.1% D1 and 0.2% for D2) were added to the film forming solutions (w/v), and left under magnetic stirring (500 rpm) for 5 h, at room temperature (20 ± 2 °C). A constant amount (15 mL) of each of the obtained film forming solutions was cast onto a 90 mm diameter polystyrene Petri dish. The films were then dried in an oven at 33 °C for 9 h and maintained at 20 °C and 54% relative humidity (RH) until further characterization.

2.3. Film thickness

Film thickness was measured with a digital micrometer (No. 293–561, Mitutoyo, Japan). Five different randomly chosen points were performed on each film and the mean values were used in the calculations of water vapour permeability (WVP) and mechanical properties.

2.4. Scanning electron microscopy

The Scanning Electron Microscopy (SEM) surface scans of the films were performed on a scanning electron microscope (Nova NanoSEM 200, Netherlands) with an accelerating voltage of 10 kV under vacuum conditions. Each film was attached to a coverslip via a coated thin film of chromium and carbon. The coating also worked to prevent the accumulation of static electric charge on the surface during electron irradiation and to avoid scanning faults and other image artefacts. The samples were sputter-coated with colloidal gold particles and then left drying before scanning. The images were presented with 5000× of magnitude and 20 μm of amplitude.

2.5. Fourier-transform infrared (FTIR) spectroscopy

The films were characterized by Fourier Transform Infrared (FTIR) Spectroscopy on a Bruker FT-IR VERTEX 80/80 v (Boston, USA) in Attenuated Total Reflectance mode (ATR) with a platinum crystal accessory between 400 and 4000 cm^{-1}, using 16 scans at a resolution of 4 cm^{-1}. Before analysis, an open bean background spectrum was recorded as a blank. The galactomannan control film (A) was used as control and each spectrum recorded for the films with immobilized bioactive compounds (B1, B2, C1, C2, D1 and D2) was subtracted from the control spectrum. Data analysis was performed with GraphPad Prism 5.00.288 (GraphPad Software, Inc., San Diego, CA, USA).

2.6. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was completed with a PerkinElmer TGA 4000 (PerkinElmer, Massachusetts, EUA). Samples were placed in the balance system and heated from 20 °C to 450 °C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere.

2.7. Colour and opacity

The colour and opacity were determined using a digital colorimeter (Konica Minolta, model Chroma Meter CR-400, Osaka, Japan) calibrated at illuminant C with a white standard. The parameters determined were L^* (L^* = 0 [black] and L^* = 100 [white]), a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness). These parameters are the ones recommended by the International Commission on Illumination. Films opacity was calculated as the ratio between the opacity of each sample on the black standard (Y_b) and the opacity of each sample on the white standard (Y_w). Five measurements were determined at random Y_b and Y_w and an average of them was used for calculations. The experiment was done in triplicate and the results were expressed as percentage and determined through:

$$Y(\%) = (Y_b/Y_w)\,100 \quad (1)$$

2.8. Moisture content

The moisture content (MC) was expressed as the percentage of water removed from the initial mass sample. MC was determined gravimetrically by drying the films at 105 °C in an oven with forced air circulation for 24 h. The experiments were performed in triplicate.

2.9. Solubility

The measurement of solubility was determined according to Gontard, Duchez, Cuq, Guilberts (1994) [20]. Triplicates of each film were cut with a circular mould of 2 cm diameter, weighted and dried at 105 °C in an oven for 24 h. After reweighting, the films were placed in cups with 50 mL of distilled water, sealed over with parafilm and homogenized at 60 rpm for 24 h. The non-soluble part of each film was dried at 105 °C for 24 h and weighted again.

2.10. Water vapour permeability

The measurement of water vapour permeability (WVP) was determined gravimetrically based on ASTM E96-92 method [21,22]. The film was sealed on the top of a permeation cell containing distilled water (100% RH; 2337 Pa vapour pressure at 20 °C), placed in a desiccator at 20 °C and 0% RH (0 Pa water vapour pressure) containing silica. The cells were weighted at intervals of 2 h for 10 h. Steady-state and uniform water pressure conditions were assumed by maintaining the air circulation constant outside the test cell by using a miniature fan inside the desiccator[29]. The slope of weight loss versus time was obtained by linear regression. Three replicates were obtained for each sample.

2.11. Contact angle

Contact angle was measured in a face contact angle meter (OCA 20, Dataphysics, Germany). The samples of the films were taken with a 500 μL syringe (Hamilton, Switzerland), with a needle of 0.75 mm of diameter. The contact angle at the film surfaces was measured by the sessile drop method [23]. Measurements were made in less than 5 s. Thirty replicates of contact angle measurements were obtained at 19.8 (±0.3) °C.

2.12 Mechanical properties: Youngís modulus, tensile strength and elongation-at-break

Youngís modulus (YM), tensile strength (TS) and elongation-at-break (EB) were measured using a TA-HD plus Texture Analyzer (Serial RS232, Stable Micro Systems, Surrey, UK) following the guidelines of ASTM D 882-02 (2010). According to the ASTM standard, film strips with a length of 100 mm and a width of 20 mm were used and the average film thickness was measured as previously described in Section 2.3. The initial grip separation was set at 100 mm and the crosshead speed was set at 5 mm min^{-1}. YM was related as the slope of the elastic region in a plot of stress x strain. TS was expressed in MPa and was calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the specimen. EB was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (100 mm) and expressed as a percentage. At least six replicates were performed for each sample.

2.13. Statistical analyses

Statistical analyses were performed using Analysis of Variance (ANOVA) and linear regression analysis. The Tukey test (α=0.05) was used to determine any significance of differences between specific means (GraphPad Prism 5.00.288, GraphPad Software, Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

SEM is a technique that allows visualizing the surface morphology of the films, mainly the distribution of the immobilized bioactive compounds in the microstructure of the film matrix. Fig. 1 shows the surface morphology of the galactomannan-based films. Fig. 1A shows the image of the film without any bioactive compound (A), presenting a more uniform structure than the films

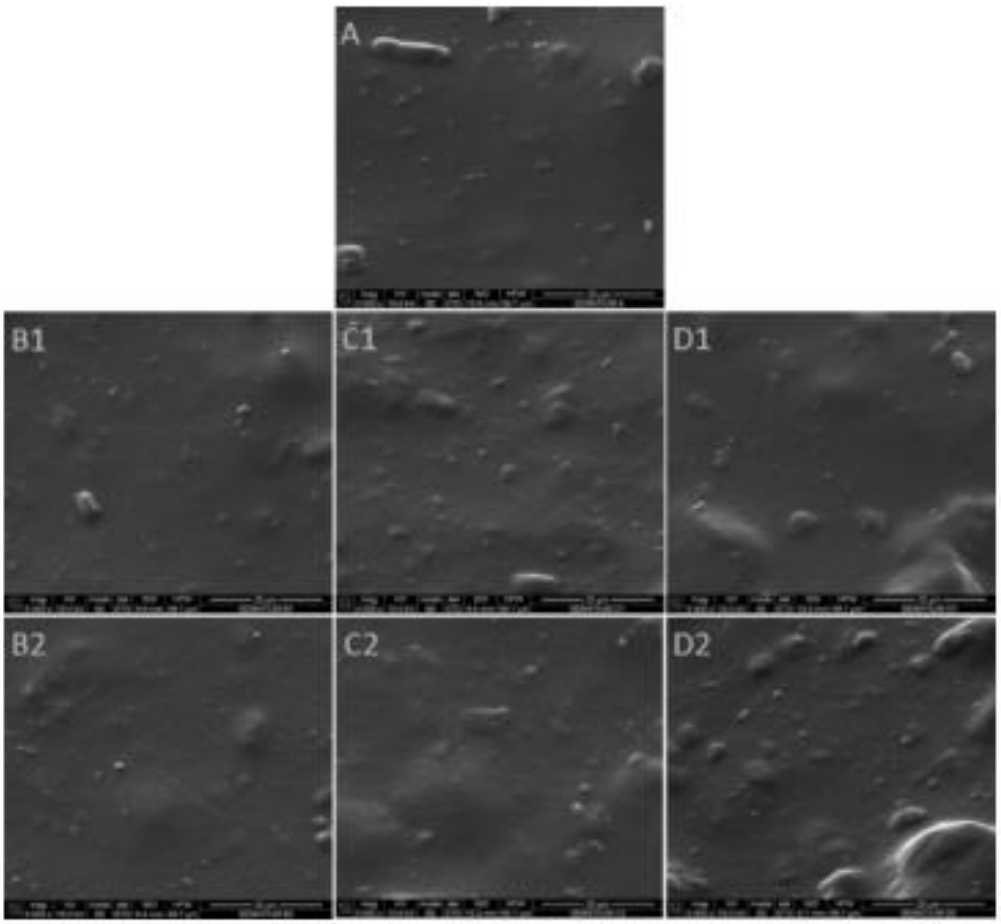

Fig. 1. SEM images showing the surface structure of (A) the control galactomannan film and after immobilization of (B1) Lf 0.1% (w/v), (B2) Lf 0.2% (w/v), (C1) Lowpept® 0.1% (w/v), (C2) Lowpept® 0.2% (w/v), (D1) Lowcol® 0.1% (w/v), and (D2) Lowcol® 0.2% (w/v) in the film-forming mixture.

with immobilized bioactive compounds (B-D). It is possible to observe by SEM some granules that maybe related to contaminating residues remaining from the galactomannan extraction. However, it is important to highlight that during galactomannan extraction NaCl was used to increase the solubility of the contaminating free proteins; also washing with ethanol allows to remove contaminants and a further wash with acetone that allows the removal of proteins [24]. In addition, galactomannan films with Lowpept® (C1 and C2) and Lowcol® (D1 and D2) showed more granules than B1 and B2 (immobilized with Lf) probably due to the presence of insoluble parts of these biomolecules. Is clear the presence of the immobilized bioactive compounds in the microstructure of the film matrix and their change according to the type of biomolecule.

For instance, working with the immobilization of bioactive compounds in galactomannan-based films, Cerqueira et al. (2010) [12] used different extracts and showed by SEM that the composite films present vesicles with spherical shapes, smooth surfaces and apparently free of visible cracks and pores. It is possible to observe in these results that Lf was homogenously distributed in the film matrix, while Lowpept® (Fig. 1C1 and C2) and Lowcol® (Fig. 1D1 and D2) presented a non-uniform pattern of distribution. In addition, according to the results presented in Table 1, film thickness was only affected ($p < 0.05$) by Lowcol® immobilization, maybe associated with voids formed during film preparation and which leads to films with certain roughness on their surface and thus higher values of thickness.

Table 1
Bioactive compounds concentration used in films formulation and the corresponding values of film thickness.

Films	Bioactive compound (w/v)	Thickness (mm)
A	–	0.031 ± 0.001^{a}
B1	Lf 0.1%	0.041 ± 0.008^{a}
B2	Lf 0.2%	0.044 ± 0.005^{a}
C1	Lowpept® 0.1%	0.038 ± 0.001^{a}
C2	Lowpept® 0.2%	0.044 ± 0.000^{a}
D1	Lowcol® 0.1%	0.069 ± 0.008^{b}
D2	Lowcol® 0.2%	0.069 ± 0.008^{b}

$^{a-b}$Different superscript letters in the same column indicate a statistically significant difference ($p < 0.05$).

3.2. FTIR

FTIR was used for the evaluation of possible chemical interactions between Lf, Lowpept®, Lowcol® and the film matrix and possible modifications in their structure (Fig. 2).

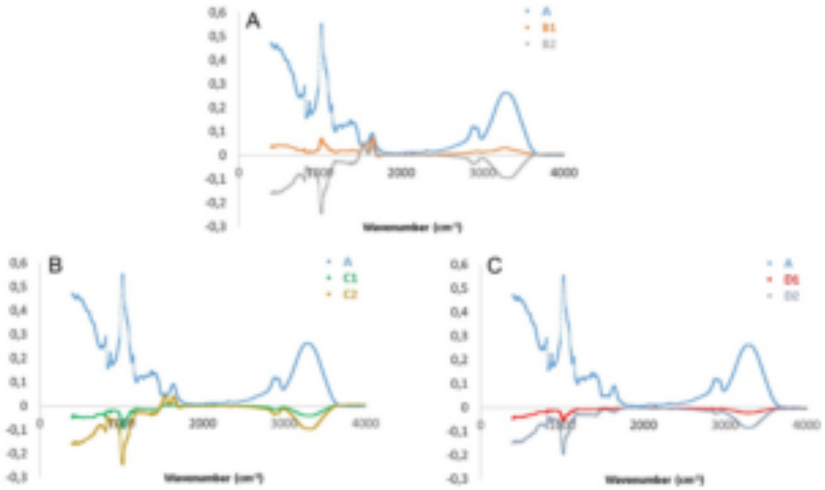

Fig. 2. FTIR spectra of the galactomannan control film (A) as blank and the subtracted spectra of the films after immobilization of (B1) Lf 0.1% (w/v), (B2) Lf 0.2% (w/v), (C1) Lowpept® 0.1% (w/v), (C2) Lowpept® 0.2% (w/v), (D1) Lowcol® 0.1% (w/v), and (D2) Lowcol® 0.2% (w/v) in the film-forming mixture.

The control film (A), composed only of galactomannan, displayed a FTIR spectrum similar to the result obtained by Albuquerque et al. (2014) [8], confirming that the control film is originated exclusively by the polysaccharide purified from *C. grandis* seeds; however, particular differences were observed for the films with immobilized bioactive compounds. The spectra recorded for these films were subtracted from the spectrum obtained for the galactomannan control film (please see Section 2.5). In general, all films presented a broad band between the regions 3100–3500 cm^{-1}, that represents O—H stretching vibration formed by hydroxyl groups of the galactomannan and water involved in hydrogen bonding. In addition, a broad band around 2800–3000 cm^{-1}, assigned to C—H stretching vibration, is also presented in all samples [25]. The incorporation of bioactive compounds leads to a great variation in the region related to hydroxyl groups, which can be related with the hydrophilicity character of the films. The addition of 0.2% (w/v) of Lf (B2), 0.1 and 0.2% (w/v) of Lowpept® (C1 and C2) and 0.1 and 0.2% (w/v) Lowcol® (D1 and D2) decreased the intensity of the bands, being representative of the lower availability of hydroxyl groups on these films and thus a less interaction with water. A different behaviour was observed for 0.1% (w/v) of Lf (B1), where the intensity of the O—H stretching vibration was similar to the galactomannan film (A) used as control.

The peak near to 1000 cm^{-1} corresponds to the stretching vibration of C—O in C—O—C bonds and appears in all studied films [26], while the anomeric region (950–700 cm^{-1}) and peaks near to 870 and 810 cm^{-1} are representative of the α-D-galactopyranose and β-D-mannopyranose units, respectively [27]. Despite the maintenance of these peaks for all films confirming the main structure of the galactomannan, it is possible to observe that the spectra of films with high concentrations of bioactive compounds (B2, C2 and D2) show an inflection of the peak (intensity decrease), which can be related to their influence in the galactomannan's film structure.

According to the literature [28], signals at 1650 and 1550 cm^{-1} are assigned to the axial deformation of the C=O bond (amide I) and the angular deformation of the N—H bond (amide II), proposed for identification of proteins. These peaks could be clearly observed in films with 0.1 and 0.2% (w/v) of Lf and 0.2% (w/v) of Lowpept®, respectively B1, B2 and C2, once the spectra of these films lead to an increase of the peak intensity in this range. The addition of Lowpept® at 0.1% (w/v, namely C1) and Lowcol® (0.1 and 0.2%, w/v, respectively for D1 and D2) displays no signals at 1650 and 1550 cm^{-1}, corresponding to a similar spectrum to the galactomannan control film (A).

3.3. Thermogravimetric analysis

The thermogravimetric analysis (TGA) for galactomannan films show at least three thermal events, being the first attributed to water evaporation, the second attributed to the presence of glycerol, and the third related to polysaccharide decomposition [29,30]. TGA experiments performed in this study are in accordance with those reported by the literature and show three mass loss events for the galactomannan films (Table 2). The first thermal event occurred near 65 °C and corresponds to dehydration, which may be attributed to the evaporation process, a characteristic phenomenon of a polysaccharide with a hydrophilic nature. The second, around 188 °C, is related to the weight loss associated with glycerol decomposition. For these results no statistically significant differences were observed. The third peak is associated with the dehydration, depolymerization and pyrolytic decomposition of the polysaccharide backbone [30,31]. The galactomannan control film (A) presented a peak of 294.19 ± 0.95 °C and a weight loss of 45.17 ± 2.33%, similar to the results obtained for other galactomannans [10,31,32]. In addition, B2, C1 and C2 were statistically different to the control film (A), i.e., the presence of Lf (0.2%, w/v) and Lowcol® (0.1 and 0.2%, w/v) avoided the establishment of more hydrogen bonds in the galactomannan film matrix, as confirmed by FTIR spectra analyses.

3.4. Colour and opacity

Colour and opacity are important features for the acceptance of products from pharmaceutical or food industry. Table 3 presents the colour parameters and opacity of the films. All studied films were bright with a strong whiteness tendency as presented by L^* coordinate values, and a yellowness appearance represented by b^* coordinate; for those parameters films did not present statistically significant differences ($p > 0.05$) among them. Results showed that the presence of Lf in higher concentrations (B2) lead to the increase ($p < 0.05$) of a^* values, i.e., the increasing concentrations of Lf lead

Table 2
Thermogravimetric behaviour of the galactomannan control film (A) and the films after immobilization of (B1) Lf 0.1% (w/v), (B2) Lf 0.2% (w/v), (C1) Lowpept® 0.1% (w/v), (C2) Lowpept® 0.2% (w/v), (D1) Lowcol® 0.1% (w/v), and (D2) Lowcol® 0.2% (w/v) in the film-forming mixture. The values of the peaks correspond to the values of derivative thermograms obtained by the TGA curve between 20 and 450 °C.

Sample	Peak 1 (°C)	ΔY 1 (%)	Peak 2 (°C)	ΔY 2 (%)	Peak 3 (°C)	ΔY 3 (%)
A	67.75 ± 6.94[a]	14.28 ± 4.08[a]	193.10 ± 11.50[a]	15.94 ± 0.78[b]	294.19 ± 0.99[b,c]	45.17 ± 2.33[b]
B1	67.18 ± 3.84[a]	12.81 ± 4.86[a]	189.87 ± 10.10[a]	14.54 ± 0.15[b,c]	289.90 ± 3.73[b,c]	46.90 ± 3.35[b]
B2	66.93 ± 2.15[a]	17.40 ± 0.64[a]	187.38 ± 3.00[a]	12.67 ± 1.15[c]	286.61 ± 1.53[c,d]	43.39 ± 1.20[b]
C1	63.45 ± 1.63[a]	16.09 ± 2.02[a]	188.74 ± 9.06[a]	12.21 ± 0.6[c]	282.33 ± 0.57[d]	46.28 ± 1.76[b]
C2	64.46 ± 3.10[a]	13.42 ± 4.08[a]	192.96 ± 2.88[a]	9.43 ± 0.76[b]	272.50 ± 0.55[e]	45.79 ± 3.10[b]
D1	63.80 ± 2.42[a]	16.99 ± 1.19[a]	183.63 ± 4.51[a]	13.11 ± 0.95[c]	295.34 ± 1.15[c]	48.89 ± 1.13[b]
D2	65.36 ± 4.38[a]	16.89 ± 2.71[a]	187.93 ± 1.72[a]	12.61 ± 1.36[c]	296.33 ± 0.33[c]	50.55 ± 2.87[a]

[a–e]Values with the same superscript letters within a column are not significantly different ($p < 0.05$).

Table 3
Colour parameters L^* (luminosity), a^* (−a^* = greenness and +a^* = redness), b^* (−b^* = blueness and +b^* = yellowness) and Y (opacity) for the galactomannan control film (A) and the films after immobilization of (B1) Lf 0.1% (w/v), (B2) Lf 0.2% (w/v), (C1) Lowpept® 0.1% (w/v), (C2) Lowpept® 0.2% (w/v), (D1) Lowcol® 0.1% (w/v), and (D2) Lowcol® 0.2% (w/v) in the film-forming mixture (values expressed as average ± standard deviation).

Film	L^*	a^*	b^*	Y (%)
A	95.59 ± 0.40[a]	0.917 ± 0.01[b,c]	7.017 ± 0.40[a]	11.83 ± 0.25[a]
B1	95.05 ± 0.42[a]	1.197 ± 0.13[a,b]	6.823 ± 0.78[a]	11.71 ±0.26[a]
B2	95.18 ± 0.09[a]	1.270 ± 0.01[a]	6.593 ± 0.04[a]	11.53 ± 0.15[a]
C1	95.47 ± 0.32[a]	0.903 ± 0.11[b,c]	7.607 ± 0.40[a]	11.84 ± 0.19[a]
C2	95.29 ± 0.24[a]	0.843 ± 0.19[c]	7.980 ± 1.08[a]	12.37 ± 0.16[a,b]
D1	95.30 ± 0.15[a]	1.040 ± 0.06[a,b,c]	7.257 ± 0.28[a]	13.43 ± 0.18[b]
D2	94.51 ± 0.90[a]	1.133 ± 0.11[a,b,c]	8.063 ± 0.91[a]	15.41 ± 1.40[c]

[a–c]Different superscript letters in the same column indicate a statistically significant difference ($p < 0.05$).

Table 4
Moisture content (MC), solubility (Sol), water vapour permeability (WVP) and contact angle (CA) values of the galactomannan control film (A) and the films after immobilization of (B1) Lf 0.1% (w/v), (B2) Lf 0.2% (w/v), (C1) Lowpept® 0.1% (w/v), (C2) Lowpept® 0.2% (w/v), (D1) Lowcol® 0.1% (w/v), and (D2) Lowcol® 0.2% (w/v) in the film-forming mixture.

Films	MC (%)	Solubility (%)	WVP x 10^{-7} ($g\,h^{-1}\,m^{-1}\,Pa^{-1}$)	CA (°)
A	29.33 ± 2.31[a,b]	74.00 ± 7.21[a]	5.60 ± 0.39[a]	68.72 ± 4.26[a]
B1	17.67 ± 5.51[b,c]	55.67 ± 7.02[b,c]	8.85 ± 1.37[a,b]	123.0 ± 4.27[b]
B2	15.67 ± 5.51[c]	51.67 ± 4.16[c]	9.39 ± 1.31[b]	122.8 ± 9.18[b]
C1	31.67 ± 4.04[a]	69.67 ± 7.77[a,b]	7.75 ± 0.75[a,b]	83.60 ± 3.46[c]
C2	26.33 ± 5.69[a,b,c]	63.67 ± 7.50[a,b,c]	8.72 ± 0.52[a,b]	93.05 ± 3.20[c]
D1	20.33 ± 3.79[a,b,c]	69.33 ± 4.93[a,b,c]	22.43 ± 0.21[c]	67.34 ± 7.07[a]
D2	18.00 ± 2.65[b,c]	56.00 ± 5.00[b,c]	21.29 ± 2.55[c]	62.06 ± 2.17[a]

[a–c]Values with the same superscript letters within a column are not significantly different ($p < 0.05$).

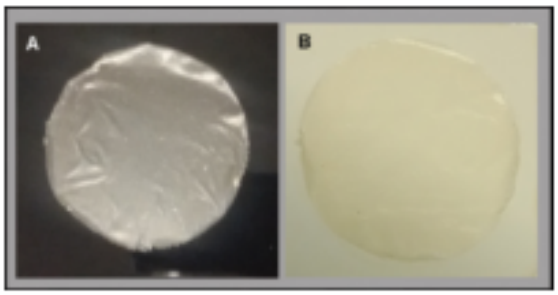

Fig. 3. Representative image of the galactomannan film with immobilized Lowpept® at 0.2% (w/v) in black (A) and white (B) background (D2).

to a redness appearance of the film, even considering their evident yellowness tendency.

All the films were slightly opaque (Table 3), however only galactomannan films with immobilized Lowcol® (D1 and D2) exhibited significantly higher ($p < 0.05$) values of opacity when compared to the control film (Fig. 3). This is in agreement with other published works [11,12,33], where it has been showed that the addition of bioactive compounds to the films could lead to an increase of opacity values.

3.5. *Moisture content (MC), solubility (Sol), water vapour permeability (WVP) and contact angle (CA)*

Moisture-binding abilities of films can affect significantly their physical and barrier properties, thus the knowledge of the content and affinity of film matrix to water is a key parameter when choosing a film for specific applications [34].

Table 4 shows the values of moisture content (MC), solubility, water vapour permeability (WVP), and contact angle (CA) of the films without any immobilized bioactive compounds (A) and of the films with immobilized Lf (B1 and B2), Lowpept® (C1 and C2), and Lowcol® (D1 and D2). MC results showed that galactomannan-based films with 0.2% (w/v) of Lf (B2) presented a significant ($p < 0.05$) reduction of MC values when compared to the control film (A). Bourbon et al. (2011) [5] reported that incorporating bioactive compounds in chitosan films led to an increase of MC, however this increase was not significant for films with Lf. Our results showed that the incorporation of Lf in its highest concentration decreased the MC, which can be explained by the fact that Lf presents both hydrophobic and hydrophilic parts in its conformation. At neutral pH, Lf has a net positive charge [35] with a hydrophobic domain [36], thus the higher concentration of Lf in the galactomannan-based film may have reduced the availability of hydroxyl groups to interact with water, resulting in a decrease of MC values, as confirmed by FTIR spectra analyses. Other explanation for the availability of hydroxyl groups on galactomannan composite films and a lower interaction with water was advanced by Antoniou et al. (2015) [6], who worked with tara gum-based films and showed the influence of the addition of compounds in the MC. They explained that the films with composites, nanoparticles in this case, presented a more compact structure, which allowed them to occupy more free volume in the polymer matrix, thus reducing the MC values.

Solubility results showed that the addition of Lf (B1 and B2) and Lowcol® in its highest concentration (D2) decreased significantly ($p < 0.05$) the solubility values when compared to the control film (A). Nevertheless, it is important to mention that when Lf, Lowpept®, and Lowcol® were added to galactomannan films, they maintained their structure even after 24 h immersion in water. On the other hand, the control film (A) became a cracked mass due to a potential dissolution pattern. The reduction of the solubility for the galactomannan-based films with immobilized Lf is in agreement with the decrease of MC values observed for B2, i.e., the film could be considered more hydrophobic than the control film (A).

It is known that the presence of a plasticizer like glycerol leads to a reduction of the hydrophobicity of polysaccharide-based films

[37,38], but also the proportion and distribution of galactose units along the mannan chain can have an essential role in water content of galactomannans. The water solubility tends to increase with increasing content of galactose for galactomannans with M/G ratio up to 3; in turn, several studies reported that higher values of M/G ratio lead to films with lower solubility [39,40]. The MC and solubility values obtained for the control film (A) are higher than the results obtained for the galactomannan studied by Antoniou, Liu, Majeed, Qazi, & Zhong (2014) [41], who evaluated how the properties of tara gum-based films were influenced by polyols, including glycerol at a similar concentration to the one used in our work. They obtained solubility values ranging between 14.37 and 32.40% according to the glycerol concentration used. Cerqueira, Souza, Teixeira, & Vicente (2012) [31] also reported that the addition of glycerol increased the solubility and MC of films of *Gleditsia triacanthos* galactomannan when compared with other plasticizers. The galactomannan extracted from the seeds of *C. grandis* used in this work has a mannose/galactose ratio (2.44:1) different from the galactomannans used in the above mentioned works. This molar ratio, in addition to the presence of glycerol may have influenced the galactomannan film structure, justifying the differences obtained for MC and solubility when compared to other galactomannan-based films; but also the extraction process (different in this case) can lead to great difference in the solubility of obtained galactomannans.

WVP is the most extensively studied property of edible films mainly because of the importance of the water in deteriorative reactions [12]. WVP values ranged from $5.60 \pm 0.39 \times 10^{-7}$ (A) to $22.43 \pm 0.21 \times 10^{-7}$ g h^{-1} m^{-1} Pa^{-1} (D1) (Table 2). The presence of phytosterols into galactomannan-based films (D1 and D2) increased significantly the WVP results, leading to values 3.9-fold higher than the control film (A). The same happened when Lf was added in a high concentration (B2), however in this case the increase was lower (1.7-fold). According to Antoniou, Liu, Majeed, Qazi, & Zhong (2014) [41], the high degree of hydrophilicity and the water attracted into the matrix contribute to create regions of higher water mobility, with greater inter-chain distances in the galactomannan film. Regarding the results for the film with immobilized Lowcol® in its highest concentration (D2), one can observe that it is more hydrophobic than the control film (A) in result of its reduced solubility, thus the increase in the WVP value can be associated to the irregular surface of the film, confirmed by SEM results (see Section 3.1). The presence of voids on the irregular surface of the film could function as site for water binding during moisture absorption, also allowing the water vapour to pass through the film. Finally, the increased WVP values reported in our results demonstrated that the contents of Lf in its highest concentration (B2), and phytosterols from Lowcol® (D1 and D2), in addition to the interaction with the galactomannan film, were the most significant factors affecting the WVP.

The determination of the contact-angle (CA) of films surface is a simple way to evaluate the degree of hydrophobicity of the films, since the CA values will increase for higher hydrophobic surfaces [42]. Generally, if surfaces have CA values less than 90°, they are considered hydrophilic. The CA of the control film (A) indicated that this film is essentially hydrophilic, which was quite similar to that reported for other galactomannan-based films [41,42]. Lf (B1 and B2) and Lowpept® (C1 and C2) immobilized in galactomannan films led to an increase ($p < 0.05$) of CA values of the control film (A), while no difference ($p > 0.05$) was observed for Lowcol® (D1 and D2). It is also important to note that the concentration effect did not affect each batch-film, i.e., there were no differences observed between B1 and B2, C1 and C2, and D1 and D2.

The results confirmed that the presence of Lf increased the hydrophobicity of the films at all concentrations (B1 and B2) with CA values almost 40° higher than the control films. The hydrophobic pattern observed for CA is also in agreement with the results obtained for MC and solubility of galactomannan-based films with immobilized Lf. Relatively to the addition of Lowpept®, the hydrophilic nature of the films (C1 and C2) remains unclear since their CA values were approximately 90°. A different behaviour was observed for the addition of Lowcol®: the results showed a higher water affinity for D1 and D2. CA provides information regarding the sorption of water molecules to the film structure [39], being related with the capacity for water binding during moisture absorption for films with immobilized Lowcol®, as confirmed by WVP results.

It is important to mention that, depending on the application of the films with immobilized bioactive compounds, the substantial differences in its physicochemical properties may be relevant for the biomolecules and the display of their activities, for instance, the relative hydrophobic pattern of the films with immobilized bioactive compounds allows its graduated release, once the compound guarantees its permanence into the film until the complete solubilisation of the matrix.

Table 5
Effect of concentration on Young's modulus (YM), tensile strength (TS) and elongation at break (EB) of the galactomannan control film (A) and the films after immobilization of (B1) Lf 0.1% (w/v), (B2) Lf 0.2% (w/v), (C1) Lowpept® 0.1% (w/v), (C2) Lowpept® 0.2% (w/v), (D1) Lowcol® 0.1% (w/v), and (D2) Lowcol® 0.2% (w/v) in the film-forming mixture.

Films	YM (MPa)	TS (MPa)	EB (%)
A	0.29 ± 0.14^{a}	4.73 ± 1.96^{a}	18.06 ± 6.44^{a}
B1	4.58 ± 1.12^{d}	$10.10 \pm 1.13^{b,c}$	3.76 ± 0.93^{b}
B2	$5.08 \pm 0.91^{c,d}$	$9.40 \pm 2.25^{b,c}$	3.37 ± 2.21^{b}
C1	3.83 ± 0.049^{c}	8.08 ± 1.05^{c}	3.64 ± 1.42^{b}
C2	$6.24 \pm 0.92^{b,c}$	11.37 ± 1.70^{b}	3.16 ± 0.87^{b}
D1	6.77 ± 1.10^{b}	$10.58 \pm 1.97^{b,c}$	2.36 ± 0.69^{b}
D2	4.51 ± 1.20^{d}	8.25 ± 1.40^{c}	3.29 ± 0.54^{b}

$^{a-d}$Values with the same superscript letters within a column are not significantly different ($p < 0.05$).

3.6. Mechanical properties

Table 5 shows the mechanical properties (Young's modulus – YM, tensile strength – TS and elongation at break – EB) of the galactomannan films without and with immobilized bioactive compounds. YM is a measure of the stiffness of a sample and a decrease in this value means a reduction of the film stiffness, i.e., the reduction of YM increases the deformability of the films [43]. Films with immobilized Lf (B1 and B2), Lowpept® (C1 and C2), and Lowcol® (D1 and D2) showed statistically significant ($p < 0.05$) difference in YM when compared to the control film (A), which means that the immobilized bioactive compounds improved the toughness of the films. Regarding the effect of the immobilized bioactive compounds concentration on the films, it is important to note that no significant ($p > 0.05$) impact occurred to the films with Lf, while for films with Lowpept® a concentration-dependent behaviour was observed, where films with higher concentrations of this biomolecule (C2) showed higher YM values than the films with 0.1% (C1). A distinct behaviour was observed for films with Lowcol®, where YM values decreased for higher concentrations of the bioactive compound.

TS indicates the maximum tensile stress that the film can sustain. It is a parameter related to the chemical structure of the film and depends strongly on film composition, being directly influenced by water, plasticizers, surfactants and bioactive compounds immobilized in the film matrix [5]. Galactomannan-based films with Lf, Lowpept®, and Lowcol® exhibited higher TS values compared to the control film (A), which means that the immobilization of bioactive compounds affected the structure of the films, improving the tensile stress to which the films may be subjected before deformation occurs. In addition, increasing the content of

Lowpept® leads to a significant increase of ($p < 0.05$) TS values of the films. The concentrations of the other bioactive compounds did not show effects on TS values.

EB is related with the film flexibility and the obtained results are presented in Table 5. Results show that films with immobilized bioactive compounds were less flexible ($p < 0.05$) than the control film (A). Nevertheless, the increase of the concentration of them did not affect EB. This is in agreement with other works, as the EB of galactomannan-based films decreased when biomolecules or other compounds were added to the matrix [7].

The M/G ratio of galactomannans significantly affects the mechanical properties of the films. For example, Mikkonen et al. (2007) [44] reported that films prepared from locust bean gum (M/G ratio of approximately 3.33) were stronger and more flexible than films prepared from guar gum (M/G ratio of approximately 1.67). Dos Santos et al. (2015) [39] produced films from five sources of galactomannans (*Adenanthera pavonina*, *Cyamopsis tetragonolobus*, *Caesalpinia pulcherrima*, *Ceratonia siliqua* and *Sophora japonica*, presenting mannose/galactose ratios of 1.3, 1.7, 2.9, 3.4 and 5.6, respectively) and reported that EB increases with the increase of the M/G ratio for values up to 3.0. For higher molar ratios, the EB values showed a reduction. TS values generally follow the inverse of those obtained for EB; they are high at low M/G ratios, rising again for high M/G ratios. The result for TS of the galactomannan control film (A) is in agreement with published works for other galactomannan-based films [45].

The galactomannan extracted from *C. grandis* seeds used in this study (M/G ratio of 2.44) produced more flexible control films (A) than the above mentioned galactomannans; in addition, the immobilization of Lf, Lowpept®, and Lowcol® affected the structure of the films, already reported for the physical characterization by MC, solubility, WVP and CA results, increasing the filmís stiffness (higher YM) and tensile strength (TS), confirming the reinforcing effect of the bioactive compounds into the film matrix.

4. Conclusion

Lactoferrin, Lowpept®, and Lowcol® can be added to galactomannan-based films maintaining their main structure and promoting significant variations on the physicochemical properties of the films. The incorporation of the bioactive compounds increased the films' stiffness and decreased their solubility, leading to a reinforcement of the films. It has also been shown how the structure, the surface and the hydrophilicity character of the films could be influenced by the presence of bioactive compounds through measurement of thermal properties (TGA), surface microstructure (SEM) and chemical structure (FTIR).

The immobilization of Lactoferrin (Lf), peptides (from Lowpept®) and phytosterols (from Lowcol®) in galactomannan-based films can be valuable for a great number of industrial applications. For example, in the pharmaceutical field, galactomannan-based films with immobilized Lf can be tested as a candidate for wound dressing by combining the physicochemical properties of the film and the excellent Lf biological activities. For biotechnological applications in food, the health benefits associated to BAPs and phytosterols could be achieved in galactomannan films with immobilized Lowpept® and Lowcol®, respectively, which can be used as alternative for individuals with difficulties for swallowing pharmaceutical solid dosage forms.

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References

[1] F.U. Momoh, J.S. Boateng, S.C.W. Richardson, B.Z. Chowdhry, J.C. Mitchell, Development and functional characterization of alginate dressing as potential protein delivery system for wound healing, Int. J. Biol. Macromol. 81 (2015) 137–150, http://dx.doi.org/10.1016/j.ijbiomac.2015.07.037.

[2] Y. Kato, H. Onishi, Y. Machida, Application of chitin and chitosan derivatives in the pharmaceutical field, Curr. Pharm. Biotechnol. 4 (2003) 303–309, http://dx.doi.org/10.2174/1389201033489748.

[3] F.M.F. Monteiro, G.M.M. Silva, J.B.R. Silva, C.S. Porto, L.B. Carvalho Júnior, J.L. Lima Filho, A.M.A. Carneiro-Leão, M.G. Carneiro-da-Cunha, A.L.F. Porto, Immobilization of trypsin on polysaccharide film from *Anacardium occidentale* L. and its application as cutaneous dressing, Process Biochem. 42 (2007) 884–888, http://dx.doi.org/10.1016/j.procbio.2007.01.006.

[4] F. Valenga, D.F.S. Petri, N. Lucyszyn, T.A. Ji, M.R. Sierakowski, Galactomannan thin films as supports for the immobilization of Concanavalin A and/or dengue viruses, Int. J. Biol. Macromol. 50 (2012) 88–94, http://dx.doi.org/10.1016/j.ijbiomac.2011.10.005.

[5] A.I. Bourbon, A.C. Pinheiro, M.A. Cerqueira, C.M.R. Rocha, M.C. Avides, M.A.C. Quintas, A.A. Vicente, Physico-chemical characterization of chitosan-based edible films incorporating bioactive compounds of different molecular weight, J. Food Eng. 106 (2011) 111–118, http://dx.doi.org/10.1016/j.jfoodeng.2011.03.024.

[6] J. Antoniou, F. Liu, H. Majeed, F. Zhong, Characterization of tara gum edible films incorporated with bulk chitosan and chitosan nanoparticles: a comparative study, Food Hydrocoll. 44 (2015) 309–319, http://dx.doi.org/10.1016/j.foodhyd.2014.09.023.

[7] M.A. Cerqueira, A.I. Bourbon, A.C. Pinheiro, J.T. Martins, B.W.S. Souza, J.A. Teixeira, A.A. Vicente, Galactomannans use in the development of edible films/coatings for food applications, Trends Food Sci. Technol. 22 (2011) 662–671, http://dx.doi.org/10.1016/j.tifs.2011.07.002.

[8] P.B.S. Albuquerque, W. Barros, G.R.C. Santos, M.T.S. Correia, P.A.S. Mourão, J.A. Teixeira, M.G. Carneiro-da-Cunha, Characterization and rheological study of the galactomannan extracted from seeds of Cassia grandis, Carbohydr. Polym. 104 (2014) 127–134, http://dx.doi.org/10.1016/j.carbpol.2014.01.010.

[9] J.T. Martins, M.A. Cerqueira, A.I. Bourbon, A.C. Pinheiro, B.W.S. Souza, A.A. Vicente, Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof, Food Hydrocoll. 29 (2012) 280–289, http://dx.doi.org/10.1016/j.foodhyd.2012.03.004.

[10] M.A. Cerqueira, B.W.S. Souza, J. Simões, J.A. Teixeira, M.R.M. Domingues, M.A. Coimbra, A.A. Vicente, Structural and thermal characterization of galactomannans from non-conventional sources, Carbohydr. Polym. 83 (2011) 179–185, http://dx.doi.org/10.1016/j.carbpol.2010.07.036.

[11] J.T. Martins, M.A. Cerqueira, B.W.S. Souza, M. do Carmo Avides, A.A. Vicente, Shelf life extension of ricotta cheese using coatings of galactomannans from nonconventional sources incorporating resin against *Listeria monocytogenes*, J. Agric. Food Chem. 58 (2010) 1884–1891, http://dx.doi.org/10.1021/jf902774z.

[12] M.A. Cerqueira, B.W.S. Souza, J.T. Martins, J.A. Teixeira, A.A. Vicente, Seed extracts of *Gleditsia triacanthos*: Functional properties evaluation and incorporation into galactomannan films, Food Res. Int. 43 (2010) 2031–2038, http://dx.doi.org/10.1016/j.foodres.2010.06.002.

[13] I.A. García-Montoya, T.S. Cendón, S. Arévalo-Gallegos, Q. Rascón-Cruz, Lactoferrin a multiple bioactive protein: an overview, Biochim. Biophys. Acta – Gen. Subj. 1820 (2012) 226–236, http://dx.doi.org/10.1016/j.bbagen.2011.06.018.

[14] A.B. Nongonierma, R.J. Fitzgerald, The scientific evidence for the role of milk protein-derived bioactive peptides in humans: a Review, J. Funct. Foods 17 (2015) 640–656, http://dx.doi.org/10.1016/j.jff.2015.06.021.

[15] V. Piironen, D.G. Lindsay, T. a. Miettinen, J. Toivo, A.M. Lampi, Plant sterols: biosynthesis, biological function and their importance to human nutrition, J. Sci. Food Agric. 80 (2000) 939–966, 10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C.

[16] H. Bekhit, N. Bansal, L. Grondahl, B. Bhandari, In-vitro digestion of different forms of bovine lactoferrin encapsulated in alginate micro-gel particles, Food Hydrocoll. 52 (2016) 231–242, http://dx.doi.org/10.1016/j.foodhyd.2015.07.007.

[17] H. Bekhit, N. Bansal, L. Grondahl, B. Bhandari, Characterization of alginate-lactoferrin beads prepared by extrusion gelation method, Food Hydrocoll. 53 (2016) 270–276, http://dx.doi.org/10.1016/j.foodhyd.2014.12.002.

[18] T. Lafarga, M. Hayes, Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients, Meat Sci. 98 (2014) 227–239, http://dx.doi.org/10.1016/j.meatsci.2014.05.036.

[19] C.T. Srigley, E.A. Haile, Quantification of plant sterols/stanols in foods and dietary supplements containing added phytosterols, J. Food Compos. Anal. 40 (2015) 163–176, http://dx.doi.org/10.1016/j.jfca.2015.01.008.

[20] N. Gontard, C. Duchez, B. Cuq, S. Guilbert, Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties, Int. J. Food Sci. Tech. 29 (1994) 39–50, http://dx.doi.org/10.1111/j.1365-2621.1994.tb02043.x.

[21] T.H. McHugh, R.J. Avena-Bustillos, J.M. Krochta, Hydrophilic edible film: modified procedure for water vapor permeability and explanation of thickness effects, J. Food Sci. 58 (1993) 899–903, http://dx.doi.org/10.1111/j.1365-2621.1993.tb09387.x.

[22] V. Guillard, B. Broyart, C. Bonazzi, S. Guilbert, N. Gontard, Preventing moisture transfer in a composite food using edible films: experimental and mathematical study, J. Food Sci. 68 (2003) 2267–2277, http://dx.doi.org/10.1111/j.1365-2621.2003.tb05758.x.

[23] A.W. Newman, D.Y. Kwok, Contact angle measurement and contact angle interpretation, Adv. Colloid Interfac. 81 (1999) 167–249, http://dx.doi.org/10.1016/S0001-8686(98)00087-6.

[24] I.R.S. Arruda, P.B.S. Albuquerque, G.R.C. Santos, A.G. Silva, P.A.S. Mourão, M.T.S. Correia, A.A. Vicente, M.G. Carneiro-da-Cunha, Structure and rheological properties of a xyloglucan extracted from Hymenaea courbaril var. courbaril seeds, Int. J. Biol. Macromol. 73 (2015) 31–38, http://dx.doi.org/10.1016/j.ijbiomac.2014.11.001.

[25] S.N. Yuen, S.M. Choi, D.L. Phillips, C.Y. Ma, Raman and FTIR spectroscopy study of carboxymethylated non-starch polysaccharides, Food Chem. 114 (2009) 1091–1098, http://dx.doi.org/10.1016/j.foodchem.2008.10.053.

[26] F.M. Pelissari, M.V.E. Grossmann, F. Yamashita, E.A.G. Pineda, Anti- microbial, mechanical, and barrier properties of cassava starchechitosan films incorporated with oregano essential oil, J. Agr. Food Chem. 57 (2009) 7499–7504, http://dx.doi.org/10.1021/jf9002363.

[27] S.N. Yuen, S.M. Choi, D.L. Phillips, C.Y. Ma, Raman and FTIR spectroscopy study of carboxymethylated non-starch polysaccharides, Food Chem. 114 (2009) 1091–1098, http://dx.doi.org/10.1016/j.foodchem.2008.10.053.

[28] S.D. Figueiró, J.C. Góes, R.A. Moreira, A.S.B. Sombra, On the physicochemical and dielectric properties of glutaraldehyde crosslinked galactomannan-collagen films, Carbohydr. Polym. 56 (2004) 313–320, http://dx.doi.org/10.1016/j.carbpol.2004.01.011.

[29] C.G. Methé, M.A. Rao, Thermal behavior of gum arabic in comparison with cashew gum, Thermochim. Acta. 357–358 (2000) 9–13, http://dx.doi.org/10.1016/S0040-6031(00)00358-0.

[30] M.J. Zohuriaan, F. Shokrolahi, Thermal studies on natural and modified gums, Polym. Test. 23 (2004) 575–579, http://dx.doi.org/10.1016/j.polymertesting.2003.11.001.

[31] M.A. Cerqueira, B.W.S. Souza, J.A. Teixeira, A.A. Vicente, Effect of glycerol and corn oil on physicochemical properties of polysaccharide films –a comparative study, Food Hydrocolloids 27 (2012) 175–184, http://dx.doi.org/10.1016/j.foodhyd.2011.07.007.

[32] C.W. Vendruscolo, C. Ferrero, E.A.G. Pineda, J.L.M. Silveira, R.A. Freitas, M.R. Jiménez-Castellanos, T.M.B. Bresolin, Physicochemical and mechanical characterization of galactomannan from Mimosa scabrella: effect of drying method, Carbohydr. Polym. 76 (2009) 86–93, http://dx.doi.org/10.1016/j.carbpol.2008.09.028.

[33] M.A. Cerqueira, Â.M. Lima, B.W.S. Souza, J.A. Teixeira, R.A. Moreira, A.A. Vicente, Functional polysaccharides as edible coatings for cheese, J. Agric. Food Chem. 57 (2009) 1456–1462, http://dx.doi.org/10.1021/jf8027264.

[34] D. Kowalczyk, M. Kordowska-Wiater, J. Nowak, B. Baraniak, Characterization of films based on chitosan lactate and its blends with oxidized starch and gelatin, Int. J. Biol. Macromol. 77 (2015) 350–359, http://dx.doi.org/10.1016/j.ijbiomac.2015.03.032.

[35] A. Teo, K.K.T. Goh, J. Wen, I. Oey, S. Ko, H.-S. Kwak, S.J. Lee, Physicochemical properties of whey protein, lactoferrin and Tween 20 stabilised nanoemulsions: effect of temperature, pH and salt, Food Chem. 197 (2016) 297–306, http://dx.doi.org/10.1016/j.foodchem.2015.10.086.

[36] M. Sinha, S. Kaushik, P. Kaur, S. Sharma, T.P. Singh, Antimicrobial lactoferrin peptides: the hidden players in the protective function of a multifunctional protein, Int. J. Pept. (2013), http://dx.doi.org/10.1155/2013/390230.

[37] R. Ahmadi, A. Kalbasi-Ashtari, A. Oromiehie, M. Yarmand, F. Jahandideh, Development and characterization of a novel biodegradable edible film obtained from psyllium seed (Plantago ovata Forsk), J. Food Eng. 109 (2012) 745–751, http://dx.doi.org/10.1016/j.jfoodeng.2011.11.010.

[38] M. Ghasemlou, F. Khodaiyan, A. Oromiehie, Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran, Carbohydr. Polym. 84 (2011) 477–483, http://dx.doi.org/10.1016/j.carbpol.2010.12.010.

[39] V.R.F. Dos Santos, B.W.S. Souza, J.A. Teixeira, A.A. Vicente, M.A. Cerqueira, Relationship between galactomannan structure and physicochemical properties of films produced thereof, J. Food Sci. Technol. 52 (2015) 8292–8299, http://dx.doi.org/10.1007/s13197-015-1961-6.

[40] M. Jouki, N. Khazaei, M. Ghasemlou, M. Hadinezhad, Effect of glycerol concentration on edible film production from cress seed carbohydrate gum, Carbohydr. Polym. 96 (2013) 39–46, http://dx.doi.org/10.1016/j.carbpol.2013.03.077.

[41] J. Antoniou, F. Liu, H. Majeed, H.J. Qazi, F. Zhong, Physicochemical and thermomechanical characterization of tara gum edible films: effect of polyols as plasticizers, Carbohydr. Polym. 111 (2014) 359–365, http://dx.doi.org/10.1016/j.carbpol.2014.04.005.

[42] Q. Ma, D. Hu, H. Wang, L. Wang, Tara gum edible fi lm incorporated with oleic acid, Food Hydrocolloids 56 (2016) 127–133, http://dx.doi.org/10.1016/j.foodhyd.2015.11.033.

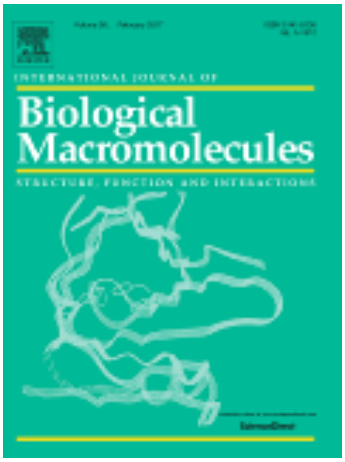
[43] C.K. Saurabh, S. Gupta, J. Bahadur, S. Mazumder, P.S. Variyar, A. Sharma, Radiation dose dependent change in physiochemical, mechanical and barrier properties of guar gum based films, Carbohydr. Polym. 98 (2013) 1610–1617, http://dx.doi.org/10.1016/j.carbpol.2013.07.041.

[44] K.S. Mikkonen, H. Rita, H. Helén, R.A. Talja, L. Hyvönen, M. Tenkanen, Effect of polysaccharide structure on mechanical and thermal properties of galactomannan-based films, Biomacromolecules 8 (2007) 3198–3205, http://dx.doi.org/10.1021/bm700538c.

[45] M.A. Cerqueira, Â.M. Lima, J.A. Teixeira, R.A. Moreira, A.A. Vicente, Suitability of novel galactomannans as edible coatings for tropical fruits, J. Food Eng. 94 (2009) 372–378, http://dx.doi.org/10.1016/j.jfoodeng.2009.04.003.

Artigo V

Healing activity evaluation of the galactomannan film obtained from *Cassia grandis* seeds with immobilized *Cratylia mollis* seed lectin



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Healing activity evaluation of the galactomannan film obtained from *Cassia grandis* seeds with immobilized C*ratylia mollis* seed lectin

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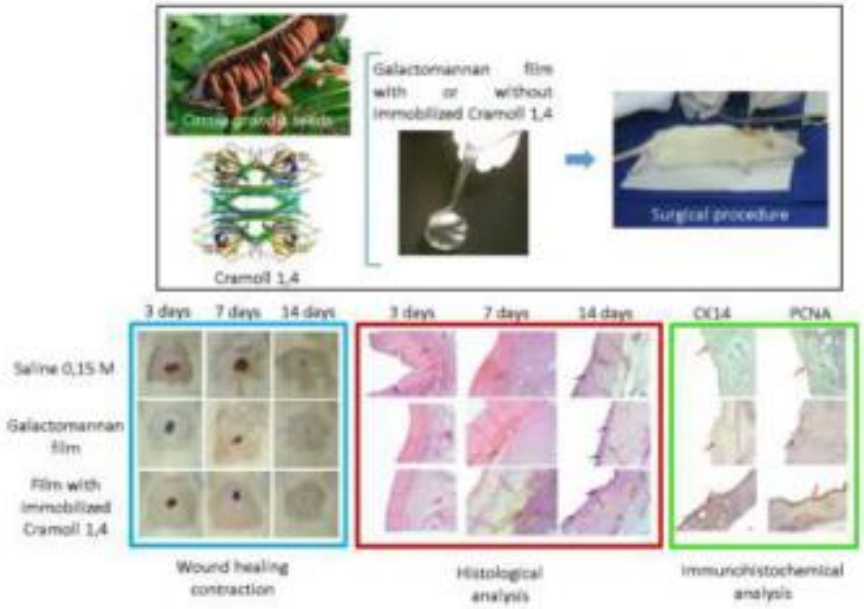
Graphical Abstract



HIGHLIGHTS

- Cramoll 1,4 was efficiently immobilized in films based on the galactomannan extracted from *C. grandis* seeds;
- Cramoll 1,4 maintained 90.94% of the initial lectin hemagglutinating activity after the immobilization process;
- Galactomannan film and the film with immobilized Cramoll 1.4 showed no cytotoxicity;
- Galactomannan film with immobilized Cramoll 1,4 completely closed the wounds with 11 days of experiment;
- Galactomannan film and the film with immobilized Cramoll 1.4 showed to be a promising candidate as topical wound curatives;

1. **Abstract**

Galactomannan films from *Cassia grandis* seeds, associated or not with Cramoll 1,4, were used on topical wounds of rats for the evaluation of the healing process during 14 days. All of the films were evaluated by cytotoxic assay, FTIR and lectin hemagglutinating activity (HA). Forty-five male rats were submitted to aseptic dermal wounds (Ø=0.8 cm) and divided in groups (n=15): control, test 1, and test 2, treated respectively with saline, galactomannan film and film with immobilized Cramoll 1,4. Macroscopic evaluations were performed by clinical observations and area measurements, and microscopic analysis by histological criteria. Epithelial cell proliferation and differentiation was immunohistochemically assessed using CK14 and PCNA. The presence of CO peaks in the FTIR spectrum confirmed the immobilization of Cramoll 1,4 in the film, while the residual HA confirmed the stability of the lectin after immobilization with 90.94% of the initial HA. The films presented non-cytotoxicity and cell viability exceeding 80%. All of the animals presented re-epithelization around 10 days, furthermore test 2 group showed a diffuse response at the stromal tissue and the basal layer associated with wounds completely closed with 11 days of experiment. The results suggest a promising use of the films as topical wound curatives.

Keywords: natural polymers; topical wound curatives; wound healing.

1. Introduction

The wound healing is defined as a rehabilitation process with an ordered sequence of physical, chemical and biological reactions. This dynamic event comprises overlapped phases including coagulation, inflammation, proliferation and remodelling, which involves cell wall components, adhesion and activation of platelets, chemical mediators, inflammatory cells (such as polymorphonuclear leukocytes, macrophages and lymphocytes) and fibronectin; all of them acting in consonance in order to reconstruct the tissues´ integrity which was previously interrupted by an injury. The process initiates by extravasation of blood constituents, platelet aggregation and migration of inflammatory cells to the site of the wound; the transition from the inflammatory to the proliferative phase is characterized by migration and proliferation of fibroblasts, keratinocytes and endothelial cells, followed by angiogenesis, leading to re-epithelialisation and granulation tissue formation. Finally, the recovery of the normal tissue occurs by reduction in cellularity, vascularization, and collagen deposition, consisting in the remodelling phase [1,2].

Natural polymers are biodegradable products with potential interesting for biomedical engineering due to two major advantages over non-biodegradable polymers: they are gradually absorbed by the human body, and some of them are able to regenerate tissues through the interaction of their biodegradation with immunologic cells [3]. In what concerns the development of different dressings for damaged tissues, natural polymers have been well studied due to their inherent characteristics, including biodegradability, biocompatibility, absence of toxicity, and some structural similarities with human tissues, as well as their implication in the wound repair [4].

The most used wound dressings are biopolymers such as chitin, chitosan and derivatives [5,6], hyaluronic acid [7], cellulose and derivatives [8], alginate [9], collagen [10], fibrin [11] or silk fibroin [12]. In general terms and according to their applications, wound dressings can be classified in different types; the film-type wound dressings are normally adhesive, with a whiteness tendency, durable, conformable, easy to manipulate, cheap, semi-permeable to oxygen and water vapour, and often impermeable to liquid and to bacterial contamination [13]. Polysaccharides are largely used as film-type wound dressings due to their ability to mimic the structure and composition of the skin. When in contact with wound exudate, these systems efficiently work on the absorption of wound fluids, thus creating a moist environment [14].

Galactomannans are polysaccharides widespread in nature. They are mostly obtained from leguminous plants and their purification process generally yields heterogeneous polymers with (1→4)-linked D-mannopyranose main chains to which (1→6)-linked α-D-galactopyranosyl units are attached [15].

Scientific publications about the characterization and application of galactomannan films are still scarce when compared to other polysaccharides. In addition, the main works reported films´ physicochemical properties rather than their biotechnological applications [16,17].

Albuquerque et al. (2014) [18] extracted the galactomannan from *Cassia grandis* seeds, a native plant in Brazil northeast. An extensive experimental rheological characterization showed that the galactomannan presents flow behaviour, exhibiting shear-thinning zones at low concentrations, and a gel-like state above the transition liquid-solid point. The ability to provide both liquid and solid features suggests the use of low concentrations of galactomannan as films with potential application in pharmaceutical and biomedical industries.

Recently, Albuquerque et al. (2017) [19] used the galactomannan extracted from *C. grandis* seeds at 0.8 % (w/v) for the production of films containing different concentrations of lactoferrin, bioactive peptides, and phytosterols. The galactomannan film behaved as a promising structure for the immobilization of biomolecules foreseeing a great number of possible applications in food and pharmaceutical industries. Other galactomannans have been successfully used as matrix for immobilization of different biomolecules, such as nanoparticles [16], lipids [17], peptides [20], antioxidants [21] and lectins [22]. Therefore, galactomannan-based films with an immobilized biomolecule that improves the wound healing process may represent a good candidate for the treatment of wounds and should be exploited.

The lectin extracted from *Cratylia mollis* seeds, also known as Cramoll, is a biomolecule with different molecular forms that has been well studied by structural analysis [23,24] and employed in several biotechnological applications [25]. Preparations containing isoforms 1 and 4 (Cramoll 1,4) have been reported as potent healing agent in different experimental models [26,27]. In fact, Cramoll present properties that could enhance the wound healing process, e. g. immunomodulatory [28], antitumor [29], anti-inflammatory [30], and proliferative potential even in oxidative stress situation [31].

The objective of this work was the development of a potential wound dressing based on the galactomannan extracted from *C. grandis* seeds, with enhanced wound healing activity by Cramoll 1,4 immobilization. NaCl 0,15 M and galactomannan films with or without immobilized Cramoll 1,4 were used for evaluate the healing activity in male rats. Further, the epithelial cell proliferation and differentiation process was immunohistochemically assessed using cytokeratin 14- (CK14) and proliferation cell nuclear antigen (PCNA)-specific antibodies.

2. Material and methods

The pods of *C. grandis* were collected in the city of Angelim, while *C. mollis* seeds were collected in Ibimirim, both of cities in the State of Pernambuco (Brazil). Ethanol 99.8%, acetone PA, sodium chloride and phenol were obtained from Vetec Fine Chemicals Ltda. (Brazil). All other chemicals were of analytical grade.

2.1 Extraction of the galactomannan from *C. grandis* seeds and preparation of the filmogenic solution

The galactomannan from *C. grandis* seeds was obtained according to Albuquerque et al. (2014) [18]. Briefly, the pods of *C. grandis* were immersed in distilled water at room temperature (25 ºC) for 18 h and then separated in a half part, revealing the seeds, which were removed and dried until reaching a constant weight. The dry seeds were boiled in distilled water 1:5 (w/v) at 100 ºC for 1 h and conserved in water at 25 ºC, for 18 h, to facilitate removal of the hull. The residual without hull was triturated in a blender with 0.1 M NaCl 5% (w/v) at 25 ºC, filtered through a veil tissue and after using a screen printing cloth, and precipitated with 46% ethanol 1:3 (v/v) for 18 h. The white precipitate obtained was washed with 100% ethanol 1:3 (w/v) for 30 min and two times with acetone 1:3 (w/v) for 30 min, filtered on screen printing cloth between each washing and finally dried until constant weight. The dry precipitate was milled and called galactomannan.

Pilot experiment indicated that low concentrations of galactomannan [< 1.0 % (w/v)] were able to produce films, however filmogenic solutions containing polysaccharide concentrations below 0.8 % (w/v) produced brittle films. Thus, the filmogenic solution was prepared in distilled water with galactomannan at 0.8 % (w/v) and glycerol at 0.2 % (v/v), under magnetic stirring (500 rpm), for 12 h, at 25 °C, to achieve thinner, more flexible films.

2.2 Extraction of the lectin from *Cratylia mollis* seeds and immobilization on the filmogenic solution

The lectin extracted from *C. mollis* seeds, known as Cramoll, containing its isoforms 1 and 4 (Cramoll 1,4), was obtained according to Correia and Coelho (1995) [23]. Briefly, the seeds were dried at 25 ºC and crushed to obtain a flour, which was dissolved in 0.15 M NaCl 10 % (w/v), under magnetic stirring (500 rpm), for 18 h, at 4 ºC. Thus, the obtained saline extract was fractionated with ammonium sulphate (0–40% and 40–60%). The precipitate obtained from the 40–60% fraction was dialysed and purified by affinity chromatography on Sephadex G-75 column.

Cramoll 1,4 was immobilized by entrapment as follows: 0 - 0.5 mg/mL of Cramoll 1,4 were added to the filmogenic solution under magnetic stirring (500 rpm), for 30 min, at 25 ºC, and the pH was adjusted to 5.8. The final solution was cast onto a 90 mm diameter Petri dish and kept at 30 ºC until drying.

2.3 Cytotoxicity assay

The cytotoxic activity was determined by the bromide method [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT). Volumes of filmogenic solutions (200 µL) containing galactomannan 0.8% (w/v), added to glycerol 0.2 % (v/v) and different Cramoll 1,4 concentrations (0.1 - 0.5 mg/mL), were prepared under sterile conditions; then, the solutions were placed in 96-well plate and maintained at 25 °C in order to obtain the films. The dried films received a suspension of Vero cells with 10^5 cells/mL (monkey kidney fibroblasts) in RPMI medium, and the plate was incubated for 24 to 48 h at 37 °C. Subsequently, 5 mg/mL of the MTT stock solution was added to each well to stop the incubation time. After 3 h, the RPMI medium and the MTT excess were aspirated and 200µL of dimethylsulfoxide (DMSO) were added to each well to dissolve the formazan crystals, whose quantity was measured at 595 nm absorbance [32]. All experiments were repeated three times with three replications per experiment.

2.4 Biological activity

The biological activity of the lectin and its stability after the immobilization in the galactomannan film were evaluated via hemagglutinating activity (HA) according to the methodology described by Correia and Coelho (1995) [23], using a suspension of rabbit erythrocytes at 2.5 % (v/v) treated with glutaraldehyde. The HA was tested for a Cramoll 1,4 (0.5 mg/mL) free solution and the Cramoll 1,4 immobilized in the galactomannan filmogenic solution, being expressed as $\log_{10}$ in the results.

2.5 Immobilization of Cramoll 1,4 in the galactomannan film

The immobilization of Cramoll 1,4 (0.5 mg/mL) in the galactomannan film was confirmed by determining the composition of chemical bonds through Fourier Transformed Infrared (FTIR) spectroscopy on a VERTEX 70 (Bruker Optics, USA) spectrometer in Attenuated Total Reflectance mode (ATR). The spectra were scanned between 4000 and 500 cm^{-1} using 16 scans at a resolution of 4 cm^{-1}.

2.6 Surgical procedure and treatment groups

All experimental procedures involving animals were approved by the Animal Ethical Committee of the Universidade Federal de Pernambuco (protocol nº 23076.027752/2012-81). The rats were carried out in

the LIKA/UFPE vivarium and kept in individual cages in a macro environment controlled with *ad libitum* supply of water and food, complying with the standards established by the Brazilian College of Animal Experimentation.

Forty-five male Wistar rats (90 – 120 day-old, weighting 250 – 300 g) were divided into experimental groups (n = 15) and anesthetized by intramuscular injection with xylazine hydrochloride 2 % (w/v) and ketamine hydrochloride 10% (w/v) at 1:1 ratio. After the anaesthesia, each animal was shaved on the dorsal thoracic region and the clean skin was marked for the production of cutaneous wound. Aseptic dermal wound (Ø = 0.8 cm) was made by skin incision and divulsion of epidermal layer under antisepsis with povidone-iodine 1% (w/v) and sterile solution of NaCl 0.15 M. The lesions were treated according to the group to which the animal belonged: control group, treated with NaCl 0.15 M; test 1 group, treated with galactomannan film; test 2 group, treated with the film with immobilized Cramoll 1,4 (0.5 mg/mL).

2.7 Healing activity evaluation

The healing activity was assessed according to the methodology described by Monteiro et al. (2007) [33]. After surgical procedures, the animals were clinical evaluated daily for observation of the following parameters: edema, hyperemia, exudate, primary and secondary crusts, detachment and re-epithelialization. On specific days (3, 7 and 14), a visual proof of the wound healing pattern was recorded by taking digital photograph from a constant distance at the indicated time point. The time taken for full re-epithelialization of the wound biopsies was noted, the rate of contraction and surface area was measured by the standard planimetric method, by tracing the wound on transparent graph sheet. The percentage of wound contraction was calculated using the following formula:

$$\%\ \text{wound contraction} = \frac{[\text{wound area day } 0 - \text{wound area day (n)}]}{\text{wound area day } 0} \times 100$$

where n = number of days (3^{rd}, 7^{th}, 14^{th} day). Then, five animals from each group were sacrificed following the surgical procedure, using lethal doses of sodium thiopental (200 mg.Kg-1). Skin fragments were collected with a wide margin (± 1 cm) from the original lesion and stored in 10 % (v/v) formalin, according to Aragão-Neto et al. (2016) [34].

2.8 Histological analysis

The skin lesions induced in rats undergoing different treatments were located, sectioned, and set in 10 % (v/v) buffered formalin. After setting, the samples were washed with water, immersed in 70 % (v/v)

ethyl alcohol for 3-4 days and embedded in paraffin. Five-µm thick paraffin sections were taken and stained with hematoxylin & eosin (HE). Histological analysis of the skin sections was carried out using a light microscopy system Zeiss (Axio Scope A1) with Axion Vision software (40 x Magnification).

2.9 Immunohistochemical analysis

Serial sections were immunohistochemically processed by the method described by Hosoya et al. (2008) [35], with some modifications. The sections were subjected to autoclaving, while immersed in citric acid buffer (pH 6.0), for 15 min at 121 °C for antigen retrieval. After that, they stained for 20 min at room temperature (25 ºC) and were subsequently treated with 0.3 % H_2O_2 in a solution of 0.1 M phosphate buffered saline (PBS, pH 7.4), for 15 min at room temperature in order to inactivate endogenous peroxidase. Then, the sections were pre-treated with goat serum for 30 min at room temperature and incubated with primary antibodies for 18 h, overnight, at 4 °C. Mouse monoclonal antibodies against rat Cytokeratin-14 (CK14, 1:200) and rat Proliferative Nuclear Cell Antigen (PCNA, 1:400) were employed. The sections were rinsed in PBS and consecutively reacted with biotinylated goat antibody against mouse IgG (secondary antibody). They were then allowed to react with horseradish peroxidase (HRP)-conjugated streptavidin. The immune complexes were visualized using 3,3'-diaminobenzidine tetrahydrochloride (Liquid DAB Substrate kit). The immunostained sections were counter-stained with Mayer's hematoxylin (Lab Vision, CA, USA), dehydrated with xylene and mounted with Entellan® (Merk). Immunohistolochemical analysis was carried out using a light microscopy system Zeiss (Axio Scope A1) with Axion Vision software.

2.10 Statistical analysis

Statistical analysis were performed using two-way method of analysis of variance (two-way ANOVA). The statistical significance was set at 5 % ($p < 0.05$) and the software used for data entry and processing was the Graphpad Prism for Windows, version 5.0 from Graphpad Software, Inc.

3. Results and discussion

3.1 Cytotoxicity evaluation

The immune system is challenged constantly by adverse factors able to cause diseases to which the medicine still has limited tools for treatment and prevention. In this context, products from natural sources are generating renewed interest in scientific research for the development of new treatments [36].

Macrophages are immunologic cells with essential role in maintaining homeostasis regardless the variations in external conditions. When an injury occurs, macrophages become the predominant cell population before migration and proliferation of fibroblasts; they also attract additional cells related to the healing process and regulate the proliferation and chemotaxis of fibroblasts, the collagen synthesis and the migration and replication of endothelial cells [36]. Regarding the importance of the properties of immunologic cells in the wound repair, the usage of fibroblast cell lines to evaluate the toxicity of products suitable for biotechnological applications is a considerable factor in the biological characterization of potential wound dressings.

Cell viability of the galactomannan film and the film with immobilized Cramoll 1,4 in different concentrations were calculated as a percentage of viable cells that were treated and compared to the untreated cells 100% viable (positive control) for 24 and 48 h. None of the films showed significant cytotoxicity when compared to respective untreated cells (Fig. 1). These results corroborate previously reported works showing that Cramoll did not induce apoptosis or toxic effects in a range of normal cells, for instance immune and proliferative cells [28,31,38] and Vero cells [31]. Moreover, these findings encouraged us to test this new formulation (the film with immobilized Cramoll 1,4) in a wound healing model.

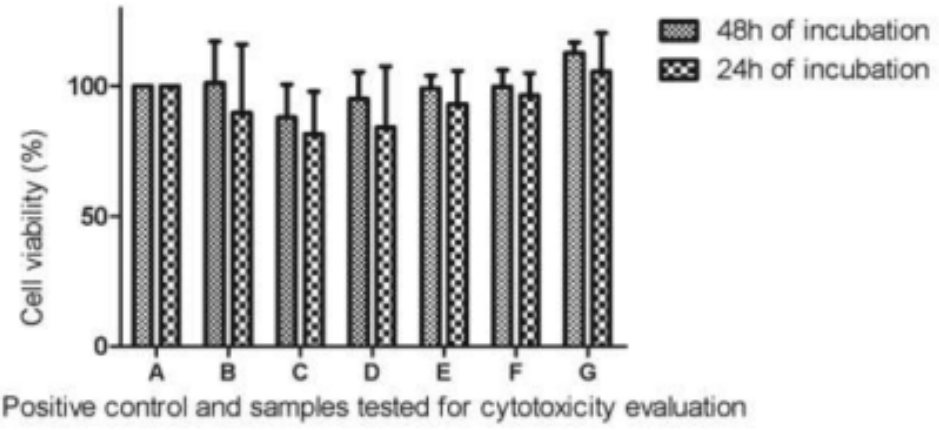


Fig.1. Cell viability for A: positive control; B: galactomannan film; C: galactomannan film + Cramoll 0.1 mg/mL; D: galactomannan film + Cramoll 0.2 mg/mL; E: galactomannan film + Cramoll 0.3 mg/mL; F: galactomannan film + Cramoll 0.4 mg/mL; G: galactomannan film + Cramoll 0.5 mg/mL.

The galactomannan film was non-cytotoxic and presented cell viability of 89.80 ± 26.27 % and 101.2 ± 16.07 %, respectively after 24 and 48 h of incubation. The film with immobilized Cramoll 1,4 in different

concentrations (0.1 to 0.5 mg/mL) presented similar behaviour with cell viability continually increasing with the increase in the concentration of immobilized lectin. Our results suggest that the immunomodulatory and mitogenic properties of Cramoll 1,4 allowed fibroblast cells to exercise their activity in healing process. In addition, the cytotoxicity evaluation was performed to simulate *in vivo* processes that usually occur when any substance promotes wound healing and tissue regeneration. With this result, the samples were test for other assays, as one can confirm in the following results.

3.2 Stability and successfully Immobilization of Cramoll 1,4 into galactomannan film

Classified as part of the mannose/glucose-specific binding group, Cramoll 1,4 is a lectin that binds specifically these monosaccharides and, when that occurs, deploy several biological effects [25]. HA is a test for determining the lectin presence in a sample by forming a network agglutination between lectin binding sites and carbohydrates of the erythrocytes surface [39].

Cramoll 1,4 at 0.5 mg/mL was chosen to be used in the following analyses due to its immunomodulatory and mitogenic pattern, as well as the percentage of viable cells already presented on the cytotoxic evaluation. The HA was made to a Cramoll 1,4 free solution (0.5 mg/mL) which was benchmarked to 100 % HA. The HA for the lectin free solution and the galactomannan film with immobilized Cramoll 1,4 (at 0.5 mg/mL) were 3.31 and 3.01, respectively. The residual HA confirmed the stability of Cramoll 1,4 after immobilization because the lectin retained 90.94 % of its initial HA. The galactomannan film without immobilized Cramoll 1,4 did not show any HA.

FTIR was used in order to evaluate possible chemical interactions between Cramoll 1,4 and the galactomannan film, in addition to modifications in their structure (Fig. 2). The FTIR spectrum for the galactomannan film is observed in the black line, which is similar to other galactomannan spectra [18]; this result confirms the nature of the polysaccharide, i.e., the galactomannan extracted from *C. grandis* seeds. Particular differences could be observed for the film with immobilized Cramoll 1,4 (observed in the red line of Fig. 2). According to the literature [40], characteristics amide I band corresponds to C-O stretching near to 1640 cm^{-1} and amide II band corresponds to C–N stretching and N–H bending near to 1540 cm^{-1} proposing the identification of proteins. The shifting observed for the peaks 1634.47 and 1535.43 cm^{-1} can be related to the results reported for identification of proteins by FTIR, thus confirming the presence of the lectin after the immobilization process.

Cramoll 1,4 was proved to be a stable biomolecule after the immobilization process in different supports. For example, Albuquerque et al. (2016) [41] evaluated Cramoll 1,4 contained in *C. grandis* seeds galactomannan gel [1.7 % (w/v)] by rheometry, pH, colour, microbial contamination and lectin hemagglutinating activity along time. They suggested that this gel is a promising immobilizing matrix for Cramoll 1,4 and can be further exploited for clinical and cosmetic applications. Silva et al. (2016) [42] developed a biosensor for metastatic disease diagnosis and demonstrated that Cramoll 1,4 was able to distinguish the degree of staging prostate cancer, providing the diagnostic differentiation of benign and malign hyperplasia. Avelino et al. (2014) [43] described the development of a biosensor composed by Cramoll 1,4 immobilized by electrostatic interactions on hybrid nanocomposite (gold nanoparticles and polyaniline) to distinguish abnormal glycoproteins of sera from patients infected with dengue serotypes I, II and III. Considering the broad range of Cramoll 1,4 biological activities and possible chemical interactions, it was important to guarantee the presence and stability of the lectin after the immobilization process. In our case, the successful immobilization of Cramoll 1,4 on the film matrix is an important feature to ensure the release of the lectin biological activities.

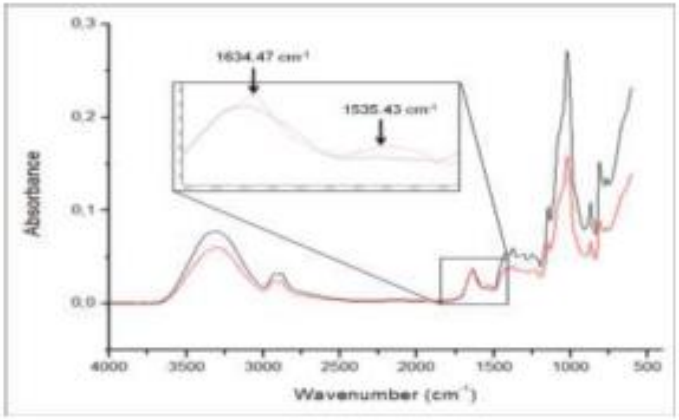


Fig.2. FTIR spectrum of the galactomannan film (black line) with immobilized Cramoll 1,4 (red line).

3.3 Rate of wound contracture and macroscopic evaluation

Percentages of wound contraction for control and tested groups are shown in Fig. 3A. The comparison between the wound area on the surgery day and on the sacrifice day was expressed as percentage of retraction and analysed statistically using two-way ANOVA. The results showed that the tested groups were significantly different ($p<0.05$) from control on days 3 and 7. Furthermore, control group showed the slow rate of wound contraction for the entire experimental interval. There was a significant

increase (p<0.05) in the percentage of wound contraction in the test 2 group on the 7th day, however all of the groups showed same wound contraction and re-epithelialization feature at 14 days.

Regarding the clinical observations (Fig. 3B), from the first to the third day after surgery, all of the studied groups presented crust with edema and hyperemia. Further, control group presented exudate throughout the first two days of the healing process, which was not observed in animals of the tested groups. The presence of exudate indicates an intense inflammatory response, which means more pain and confirms the difficulty to eat and drink of these animals. The galactomannan film and the film with immobilized Cramoll 1,4 worked as hemostatic tampons, protecting the wound from fluid loss and microbial contamination [44]. Comparing the tested groups, test 2 group showed less intense edema and hyperemia and an accelerated crust detachment. This fact could be related to the anti-inflammatory and wound healing activities of Cramoll 1,4. These characteristics result in significant reduction of pain and edema, as well as a better circulation to the injured site.

It is possible to observe in Fig. 3B that wound closing in tested groups happened faster than control wounds after the same time points. According to Ranjbar-Mohammadi et al. (2016) [45], the quicker wound closure facilitates the biological event of healing by joining the wound edges. Due to the inherent characteristics of a polysaccharide, seems that the galactomannan contributed to the healing effects and promoted a faster wound healing. About 10 days, all animals presented re-epithelization, however it is important to point that Cramoll 1,4 improved significantly the wound healing when compared to the control group, and the wounds of test 2 group were completely closed with 11 days of the experiment. Finally, the scar tissue formed after the surgical procedure was much reduced in test 1 and test 2 groups when compared to the control, and its coloration was paler and more similar to mature surrounding tissue.

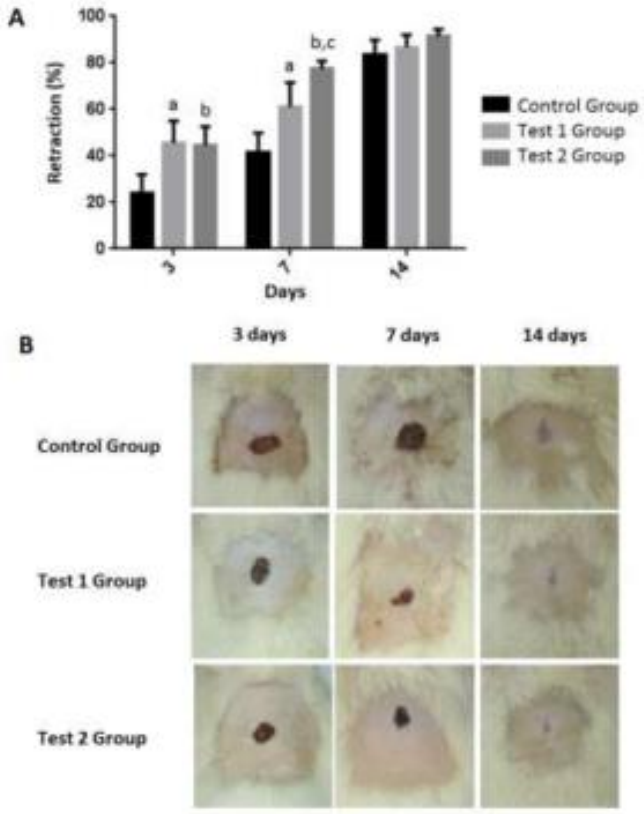


Fig.3 (A) – Percentage of retraction of wound area for control (treated with saline 0.15 M), test 1(treated with galactomannan film) and test 2 (treated with the film with immobilized Cramoll 1,4): The bars are expressed as mean ± standard error (n = 5). [a]Represent significant difference between test 1 and control; [b]Represent significant difference between test 2 and control; [c]Represent significant difference between test groups ($p<0.05$). (B) – Images representing the wound healing for control, test 1 and test 2 groups.

3.4 Histological observations

Cellular observation of wound tissues was possible by HE staining for control and the tested groups. The formation of epithelium, connective tissue, inflammatory response, fibroblast proliferation and collagen deposition could be seen in Fig. 4. On day 3, HE stained on tissue sections of control group showed inflammatory infiltrate, few fibroblasts and a dysmorphic protein layer directly in contact with the wound area; there was no evidence of collagen and epithelium extract for this group. The tested groups showed less inflammatory response and a slight deposition of collagen, confirming the quick healing effects of galactomannan and Cramoll 1,4 on full wound compared with control in the same time. It is important to highlight that the inflammation stage in wound healing process occurs shortly after the injury, so the immune

system components act by removing damaged tissue and bacteria from the wound. Galactomannan and Cramoll 1,4 had absorb the protein layer on the wounds of the tested groups, thus enhancing the healing process and confirming the behavior also observed in macroscopic evaluation (see Fig. 3B).

On the 7th day, HE stained histopathological sections of all of the groups showed moderate inflammatory infiltrate; however, some particular differences could be observed for the tested groups. The connective tissue is fibrous in nature with less inflammatory components such as lymphocytes and blood vessels, which clearly indicates that galactomannan and Cramoll 1,4 improved a faster healing by preventing the prolonged inflammatory phase. It is possible to observe the presence of collagen for all of the groups, but collagen with much fibrils and moderate epithelial layer was formed in wounds that were treated with the film with immobilized Cramoll 1,4 (test 2 group).

On day 14 after surgery, the wounds of the tested groups showed higher degree of healing compared with the control, as confirmed by macroscopic evaluation (see Fig. 3). The inflammatory components already observed for all of the groups in the 3rd and the 7th days were substituted by a connective tissue with collagen fibers, but the tested groups showed complete epithelialization with focal acanthosis, indicating a good and complete healing, while the control group presented rare epithelialization.

Our results agree to which has been reported for other works about wound healing experiments employing polysaccharides in association with different therapies, biomolecules or cells. In general, they reported that their components accelerated the transition from the inflammation and tissue granulation phases of the wound healing process and enhanced mature scar formation and extracellular matrix remodeling, leading to a faster wound contracture and closure. For example, Ranjbar-Mohammadi et al. (2016) [45] worked with a scaffold of gum tragacanth, curcumin and cells, and observed after 15 days a wound closure with well-formed granulation tissue. Aragão-Neto et al. (2016) [34] evaluated the effect of a hydrogel based on cashew tree (*Anacardium occidentale* L.) gum and chitosan associated or not with low level laser therapy and concluded that the hydrogel contributed for a most effective wound healing and modulation of the inflammatory process until 14 days. The healing mechanism of the polysaccharide extracted from *Caesalpinia ferrea* stem barks was investigated by Pereira et al. (2016) [2], who observed the complete cutaneous healing acquired by topical application (during 21 days, twice a day) at day 10. Tabandeh et al. (2014) [46] evaluated the skin wound repair of rats using a gel containing polysaccharides of *Aloe vera* and observed wound closure in 15 days. In this paper, the fact that the films were only applied on the day of the surgery, in addition to the presence of collagen in the tested groups already in the 3rd day

could be considered as a great advantage for this matrix. It is also important to highlight that the wound repair observed in microscopic evaluation, confirmed by the contraction observed in macroscopic results, was an important characteristic for validation of the galactomannan as a healing agent.

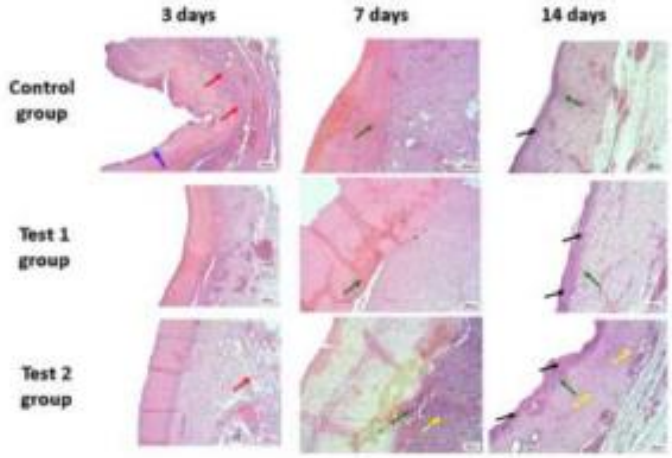


Fig.4. Cellular observations of control (treated with saline 0.15 M), test 1 (treated with galactomannan film) and test 2 (treated with the film with immobilized Cramoll 1,4) at 3rd, 7th and 14th day of treatment. Dysmorphic protein layer, inflammatory response, collagen fibers, connective tissue and epithelium were indicated by blue, red, yellow, green and black arrows, respectively. Scale bars: 100μm.

3.5 Immunohistochemical analyses for CK14 and PCNA

The immunohistochemical analyses for CK14 and PCNA in the wound tissues were performed for all of the groups using DAB and hematoxylin staining. PCNA is able for detecting the proliferating cells, while CK14 is for epithelial differentiation. Cytokeratins are major intermediate filaments in all types of epithelia and are the most fundamental markers of epithelial differentiation. CK14 is a marker for undifferentiated keratinocytes and disappears at the onset of differentiation; it is normally expressed in several layers of the epidermis (basal and suprabasal layers), being expressed differently in the epidermal tissue during the re-epithelialization process [35,47]. On the 3rd and 7th days, no reactivity was observed for CK14 in all of the groups. On the 14th day, the results for immunohistochemical analysis of this antibody showed that there is a mark representative of the epithelium, i.e., CK14 was contained in the basal layer of the epithelium for all of the groups; however, this reaction revealed a more stratified tissue in the wounds of the tested groups. In addition, the regenerated epithelium of the test 2 group reacted strongly with antibodies for CK14 throughout the entire basal layer, thus the reaction was more immunopositive when compared to the test 1 group (Fig.

5). CK14 increased with the increase of cellular proliferation and tissue differentiation. This result agrees with those observed in microscopic and macroscopic evaluations until 14 days, and with Seltmann et al. (2013) [48], who reported that high levels of CK14 are known to lead to proliferation in vivo and found close relation with skin tensegrity. The same observations were reported in oral wounds during the re-epithelization process of mucosa [35].

To explore the cellular mechanisms for the acceleration of wound healing by galactomannan film and the film with immobilized Cramoll 1,4, PCNA was used as a marker of cell proliferation by immunohistochemical analysis. As already observed for CK14 results, there was no reactivity for PCNA on days 3 and 7 for all of the groups. On the 14th day, we observed a similar behavior of the above mentioned immunohistochemical result: the control group showed a focal response in both stromal tissue and epithelial layer, while the tested groups showed a diffuse response. It is important to highlight that test 2 group had a stronger reaction when compared to test 1 (Fig. 5). The behavior observed for test 2 group corroborates the results already observed in the cytotoxic assay, i.e., that Cramoll 1,4 immobilized in galactomannan-based films could display efficiently its biological activities, including mitogenic pattern, immunomodulation, and wound healing agent.

Up to 14 days of experiment, all of the groups still showed a focal response at the basal layer of the re-epithelialized tissue, but intense diffuse response was observed at the stromal tissue and the basal layer of the re-epithelialized wound of test 2 group, which suggests that the healing process is almost finalized. Hence, the galactomannan film can be effectively used as wound dressing, especially as an alternative for synthetic ones; additionally, the wound repair can be stimulated and accelerated by the incorporation of Cramoll 1,4 into the matrix.

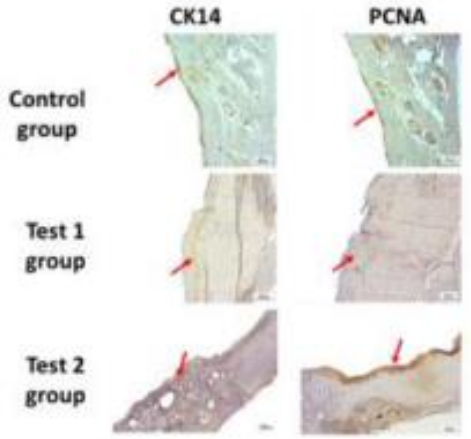


Fig.5. Immunohistochemical observations of CK14 and PCNA in control (treated with saline 0.15 M), test 1 (treated with galactomannan film) and test 2 (treated with the film with immobilized Cramoll 1,4) at 14th day of treatment. DAB and hematoxylin contrast. Scale bars: 100μm.

4. Conclusions

In this work, galactomannan films with or without immobilized Cramoll 1,4, proved to be an effective alternative to replace synthetic wound dressings in healing of wounds made on rat models. The lectin was successfully immobilized in the galactomannan film, producing films with 90.94 % of the initial HA of Cramoll 1,4 and cell viability exceeding 80 %. In what concerns the wounds of the tested groups, there were observed improved granulation, epithelium formation and collagen regeneration. These results could be associated with the innate properties of the galactomannan, which can introduce faster signalling pathway, resembling natural extracellular matrix and attracting fibroblasts to the derma layer. Moreover, the immunomodulatory and mitogenic characteristics of Cramoll 1,4 accelerated wound healing process, as confirmed by cytotoxicity evaluation and immunohistochemical analyses.

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References

[1] S.H. Mandelbaum, É.P. Di Santis, M.H.S. Mandelbaum, Cicatrização: conceitos atuais e recursos auxiliares-Parte I Cicatrization: current concepts and auxiliary resources-Part I, An. Bras. Dermatologia. 78 (2003) 393–410. doi:10.1590/S0365-05962003000400002.

[2] L.P. Pereira, M.R.L. Mota, L.A.C. Brizeno, F.C. Nogueira, E.G.M. Ferreira, M.G. Pereira, A.M.S. Assreuy, Modulator effect of a polysaccharide-rich extract from *Caesalpinia ferrea* stem barks in rat cutaneous wound healing: Role of TNF-α, IL-1β, NO, TGF- β. J Ethnopharmacol. 187 (2016) 213–223. doi:10.1016/j.jep.2016.04.043.

[3] M. Cho, C. Yang, S.M. Kim, S.G. You, Molecular characterization and biological activities of watersoluble sulfated polysaccharides from *Enteromorpha prolifera*, Food Sci. Biotechnol. 19 (2010) 525–533. doi:10.1007/s10068-010-0073-3.

[4] J. Boateng, R. Burgos-Amador, O. Okeke, H. Pawar, Composite alginate and gelatin based bio-polymeric wafers containing silver sulfadiazine for wound healing., Int. J. Biol. Macromol. 79 (2015) 63–71. doi:10.1016/j.ijbiomac.2015.04.048.

[5] T. Ito, C. Yoshida, Y. Murakami, Design of novel sheet-shaped chitosan hydrogel for wound healing: A hybrid biomaterial consisting of both PEG-grafted chitosan and crosslinkable polymeric micelles acting as drug containers, Mater. Sci. Eng. C. 33 (2013) 3697–3703. doi:10.1016/j.msec.2013.04.056.

[6] N. Naseri, C. Algan, V. Jacobs, M. John, K. Oksman, A.P. Mathew, Electrospun chitosan-based nanocomposite mats reinforced with chitin nanocrystals for wound dressing, Carbohydr. Polym. 109 (2014) 7–15. doi:10.1016/j.carbpol.2014.03.031.

[7] F. Reyes-Ortega, A. Cifuentes, G. Rodríguez, M.R. Aguilar, Á. González-Gómez, R. Solis, N. García-Honduvilla, J. Buján, J. García-Sanmartín, A. Martínez, J.S. Román, Bioactive bilayered dressing for compromised epidermal tissue regeneration with sequential activity of complementary agents., Acta Biomater. 23 (2015) 103–115. doi:10.1016/j.actbio.2015.05.012.

[8] X. Wen, Y. Zheng, J. Wu, L. Yue, C. Wang, J. Luan, Z. Wu, K. Wang, In vitro and in vivo investigation of bacterial cellulose dressing containing uniform silver sulfadiazine nanoparticles for burn wound healing, Prog. Nat. Sci. Mater. Int. 25 (2015) 197–203. doi:10.1016/j.pnsc.2015.05.004.

[9] F.U. Momoh, J.S. Boateng, S.C.W. Richardson, B.Z. Chowdhry, J.C. Mitchell, Development and functional characterization of alginate dressing as potential protein delivery system for wound healing, Int. J. Biol. Macromol. 81 (2015) 137–150. doi:10.1016/j.ijbiomac.2015.07.037.

[10] L.I.F. Moura, A.M.A. Dias, E. Suesca, S. Casadiegos, E.C. Leal, M.R. Fontanilla, L. Carvalho, H.C. de Sousa, E. Carvalho, Neurotensin-loaded collagen dressings reduce inflammation and improve wound healing in diabetic mice, Biochim. Biophys. Acta - Mol. Basis Dis. 1842 (2014) 32–43. doi:10.1016/j.bbadis.2013.10.009.

[11] G. Gainza, W.S. Chu, R.H. Guy, J.L. Pedraz, R.M. Hernandez, B. Delgado-Charro, M. Igartua, Development and in vitro evaluation of lipid nanoparticle-based dressings for topical treatment of chronic wounds, Int. J. Pharm. 490 (2015) 404–411. doi:10.1016/j.ijpharm.2015.05.075.

[12] S. Çalamak, C. Erdoğdu, M. Özalp, K. Ulubayram, Silk fibroin based antibacterial bionanotextiles as wound dressing materials, Mater. Sci. Eng. C. 43 (2014) 11–20. doi:10.1016/j.msec.2014.07.001.

[13] L.I.F. Moura, A.M.A. Dias, E. Carvalho, H.C. De Sousa, Recent advances on the development of wound dressings for diabetic foot ulcer treatment - A review, Acta Biomater. 9 (2013) 7093–7114. doi:10.1016/j.actbio.2013.03.033.

[14] P.I. Morgado, A. Aguiar-Ricardo, I.J. Correia, Asymmetric membranes as ideal wound dressings: An overview on production methods, structure, properties and performance relationship, J. Memb. Sci. 490 (2015) 139–151. doi:10.1016/j.memsci.2015.04.064.

[15] M. Srivastava, V.P. Kapoor, Seed galactomannans: An overview, Chem. Biodivers. 2 (2005) 295–317. doi:10.1002/cbdv.200590013.

[16] J. Antoniou, F. Liu, H. Majeed, F. Zhong, Characterization of tara gum edible films incorporated with bulk chitosan and chitosan nanoparticles: A comparative study, Food Hydrocoll. 44 (2015) 309–319. doi:10.1016/j.foodhyd.2014.09.023.

[17] Q. Ma, D. Hu, H. Wang, L. Wang, Food Hydrocolloids Tara gum edible fi lm incorporated with oleic acid, 56 (2016) 127–133.

[18] P.B.S. Albuquerque, W. Barros, G.R.C. Santos, M.T.S. Correia, P.A.S. Mourão, J.A. Teixeira, M.G. Carneiro-da-Cunha, Characterization and rheological study of the galactomannan extracted from seeds of *Cassia grandis*, Carbohydr. Polym. 104 (2014) 127–134. doi:10.1016/j.carbpol.2014.01.010.

[19] P.B.S. Albuquerque, M.A. Cerqueira, A.A. Vicente, J.A. Teixeira, M.G. Carneiro-da-cunha, International Journal of Biological Macromolecules Immobilization of bioactive compounds in *Cassia grandis* galactomannan-based films : Influence on physicochemical properties, Int. J. Biol. Macromol. 96 (2017) 727–735. doi:10.1016/j.ijbiomac.2016.12.081.

[20] J.T. Martins, M.A. Cerqueira, B.W.S. Souza, M. do Carmo Avides, A.A. Vicente, Shelf Life Extension of Ricotta Cheese Using Coatings of Galactomannans from Nonconventional Sources Incorporating Nisin against *Listeria monocytogenes*, J. Agric. Food Chem. 58 (2010) 1884–1891. doi:10.1021/jf902774z.

[21] M.A. Cerqueira, B.W.S. Souza, J.T. Martins, J.A. Teixeira, A.A. Vicente, Seed extracts of Gleditsia triacanthos: Functional properties evaluation and incorporation into galactomannan films, Food Res. Int. 43 (2010) 2031–2038. doi:10.1016/j.foodres.2010.06.002.

[22] F. Valenga, D.F.S. Petri, N. Lucyszyn, T.A. Jó, M.R. Sierakowski, Galactomannan thin films as supports for the immobilization of Concanavalin A and/or dengue viruses, Int. J. Biol. Macromol. 50 (2012) 88–94. doi:10.1016/j.ijbiomac.2011.10.005.

[23] M.T.S. Correia, L.C.B.B. Coelho, Purification of a glucose/mannose specific lectin, isoform 1, from seeds of *Cratylia mollis* mart. (Camaratu Bean), Appl. Biochem. Biotechnol. 55 (1995) 261–273. doi:10.1007/BF02786865.

[24] P.M.G. Paiva, L.C.B.B. Coelho, Purification and partial characterization of two lectin isoforms from *Cratylia mollis* mart. (camaratu bean), Appl. Biochem. Biotechnol. 36 (1992) 113–118. doi:10.1007/BF02929691.

[25] L.C.N. Silva, C.M.B. Filho, R.A. De Paula, L.C.B.B. Coelho, M. V Silva, T. Maria, S. Correia, *Cratylia mollis* Lectin : A Versatile Tool for Biomedical Studies, (2014) 44–54. doi:10.2174/1573407210011407250000701.

[26] C.M.L. De Melo, C.S. Porto, M.R. Melo, C.M.I. Mendes, C.C.B. Cavalcanti, L.C.B.B. Coelho, A.L.F. Porto, A.M. Dos Anjos Carneiro Leão, M.T. Dos Santos Correia, Healing activity induced by Cramoll 1,4 lectin in healthy and immunocompromised mice, Int. J. Pharm. 408 (2011) 113–119. doi:10.1016/j.ijpharm.2011.02.011.

[27] D. Dos Santos Tavares Pereira, M.H. Madruga Lima-Ribeiro, R. Santos-Oliveira, C. De Lima Bezerra Cavalcanti, N. Teles De Pontes-Filho, L. Cassandra Breitenbach Barroso Coelho, A.M. Dos Anjos Carneiro-Leão, M.T. Dos Santos Correia, Topical application effect of the isolectin hydrogel (Cramoll 1,4) on second-degree burns: Experimental model, J. Biomed. Biotechnol. 2012 (2012). doi:10.1155/2012/184538.

[28] L.C.N. da Silva, N.M.P. Alves, M.C.A.B. de Castro, V.R.A. Pereira, N.V.N. da Paz, L.C.B.B. Coelho, R.C.B.Q. de Figueiredo, M.T. dos S. Correia, Immunomodulatory effects of pCramoll and rCramoll on peritoneal exudate cells (PECs) infected and non-infected with *Staphylococcus aureus*, Int. J. Biol. Macromol. 72 (2015) 848–854. doi:10.1016/j.ijbiomac.2014.09.045.

[29] C.R.A. da Cunha, L.C.N. da Silva, F.J.F. Almeida, M.S. Ferraz, N. Varejão, M.F. de S. Cartaxo, R. de C.M. de Miranda, F.C.A. de Aguiar, N.P. da S. Santos, L.C.B.B. Coelho, N.S. Santos-Magalhães, M.T. dos S. Correia, Encapsulation into stealth liposomes enhances the antitumor action of recombinant *Cratylia mollis* lectin expressed in Escherichia coli, Front. Microbiol. 7 (2016) 1–11. doi:10.3389/fmicb.2016.01355.

[30] C.M.L. De Melo, B.A. Paim, K.G. Zecchin, J. Morari, M.R. Chiaratti, M.T.S. Correia, L.C.B. Barroso Coelho, P.M.G. Paiva, Cramoll 1,4 lectin increases ROS production, calcium levels, and cytokine expression in treated spleen cells of rats, Mol. Cell. Biochem. 342 (2010) 163–169. doi:10.1007/s11010-010-0480-z.

[31] L.C.N. Da Silva, N.M.P. Alves, M.C.A.B. De Castro, T.M.M. Higino, C.R.A. Da Cunha, V.R.A. Pereira, N.V.N. Da Paz, L.C.B.B. Coelho, M.T.D.S. Correia, R.C.B.Q. De Figueiredo, PCramoll and rCramoll as New Preventive Agents against the Oxidative Dysfunction Induced by Hydrogen Peroxide, Oxid. Med. Cell. Longev. 2015 (2015). doi:10.1155/2015/520872.

[32] M.C. Alley, D.A. Scudiero, A. Monks, M. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R. Shoemaker, M.R. Boyd, Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay, Cancer Res. 48 (1988) 584–588.

[33] F.M.F. Monteiro, G.M. de Medeiros e Silva, J.B.R. da Silva, C.S. Porto, L.B. de Carvalho, J.L. de Lima Filho, A.M.D.A. Carneiro-Leão, M.D.G. Carneiro-da-Cunha, A.L.F. Porto, Immobilization of trypsin on polysaccharide film from Anacardium occidentale L. and its application as cutaneous dressing, Process Biochem. 42 (2007) 884–888. doi:10.1016/j.procbio.2007.01.006.

[34] A.C. Aragão-Neto, P.A.G. Soares, M.H.M. Lima-Ribeiro, E.J.A. Carvalho, M.T.S. Correia, M.G. Carneiro-da-Cunha, Combined therapy using low level laser and chitosan-policaju hydrogel for wound healing, Int. J. Biol. Macromol. 95 (2016) 268–272. doi: 10.1016/j.ijbiomac.2016.11.019.

[35] A. Hosoya, J.M. Lee, S.W. Cho, J.Y. Kim, N. Shinozaki, T. Shibahara, M. Shimono, H.S. Jung, Morphological evidence of basal keratinocyte migration during the re-epithelialization process, Histochem. Cell Biol. 130 (2008) 1165–1175. doi:10.1007/s00418-008-0499-3.

[36] M. Sengupta, G.D. Sharma, B. Chakraborty, Hepatoprotective and immunomodulatory properties of aqueous extract of Curcuma longa in carbon tetra chloride intoxicated Swiss albino mice, Asian Pac. J. Trop. Biomed. 1 (2011) 193–199. doi:10.1016/S2221-1691(11)60026-9.

[37] G.V. Schirato, F.M.F. Monteiro, F. de O. Silva, J.L. de Lima Filho, A.M. dos A.C. Leão, A.L.F. Porto, O polissacarídeo do *Anacardium occidentale* L. na fase inflamatória do processo cicatricial de lesões cutâneas, Ciência Rural. 36 (2006) 149–154. doi:10.1590/S0103-84782006000100022.

[38] C.M.L. De Melo, M.C.A.B. De Castro, A.P. De Oliveira, F.O.S. Gomes, V.R.A. Pereira, M.T.S. Correia, L.C.B.B. Coelho, P.M.G. Paiva, Immunomodulatory response of Cramoll 1,4 lectin on experimental lymphocytes, Phyther. Res. 24 (2010) 1631–1636. doi:10.1002/ptr.3156.

[39] P.M.G. Correia, M.T.S., Coelho, L.C.B.B., Paiva, Lectins, carbohydrate recognition molecules: Are they toxic?, Recent Trends Toxicol. 37 (2008) 47–59.

[40] S.D. Figueiró, J.C. Góes, R. a. Moreira, a. S.B. Sombra, On the physico-chemical and dielectric properties of glutaraldehyde crosslinked galactomannan-collagen films, Carbohydr. Polym. 56 (2004) 313–320. doi:10.1016/j.carbpol.2004.01.011.

[41] P.B.S. Albuquerque, C.S. Silva, P.A.G. Soares, W. Barros, M.T.S. Correia, L.C.B.B. Coelho, J.A. Teixeira, M.G. Carneiro-da-Cunha, Investigating a galactomannan gel obtained from *Cassia grandis* seeds as immobilizing matrix for Cramoll lectin, Int. J. Biol. Macromol. 86 (2016) 454–461. doi:10.1016/j.ijbiomac.2016.01.107.

[42] P.M.S. Silva, A.L.R. Lima, B.V.M. Silva, L.C.B.B. Coelho, R.F. Dutra, M.T.S. Correia, *Cratylia mollis* lectin nanoelectrode for differential diagnostic of prostate cancer and benign prostatic hyperplasia based on label-free detection, Biosens. Bioelectron. 85 (2016) 171–177. doi:10.1016/j.bios.2016.05.004.

[43] K.Y.P.S. Avelino, C.A.S. Andrade, C.P. De Melo, M.L. Nogueira, M.T.S. Correia, L.C.B.B. Coelho, M.D.L. Oliveira, Biosensor based on hybrid nanocomposite and CramoLL lectin for detection of dengue glycoproteins in real samples, Synth. Met. 194 (2014) 102–108. doi:10.1016/j.synthmet.2014.05.001.

[44] G.D. Mogoşanu, A.M. Grumezescu, Natural and synthetic polymers for wounds and burns dressing, Int. J. Pharm. 463 (2014) 127–136. doi:10.1016/j.ijpharm.2013.12.015.

[45] M. Ranjbar-Mohammadi, S. Rabbani, S.H. Bahrami, M.T. Joghataei, F. Moayer, Antibacterial performance and in vivo diabetic wound healing of curcumin loaded gum tragacanth/poly(ε-caprolactone) electrospun nanofibers. Mater. Sci. Eng. C Mater. Biol. Appl. 69 (2016) 1183–1191. doi:10.1016/j.msec.2016.08.032.

[46] M.R. Tabandeh, A. Oryan, A. Mohammadalipour, Polysaccharides of Aloe vera induce MMP-3 and TIMP-2 gene expression during the skin wound repair of rat, Int. J. Biol. Macromol. 65 (2014) 424–430. doi:10.1016/j.ijbiomac.2014.01.055.

[47] J. Mendoza-Garcia, A. Sebastian, T. Alonso-Rasgado, A. Bayat, Optimization of an ex vivo wound healing model in the adult human skin: Functional evaluation using photodynamic therapy, Wound Repair Regen. 23 (2015) 685–702. doi:10.1111/wrr.12325.

[48] K. Seltmann, A.W. Fritsch, J. a Käs, T.M. Magin, Keratins significantly contribute to cell stiffness and impact invasive behavior., Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 18507–12. doi:10.1073/pnas.1310493110.

Artigo VI

Development of lecithin nanoparticles containing quercetin for immobilization in galactomannan-based films

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Development of lecithin nanoparticles containing quercetin for immobilization in galactomannan-based films

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Abstract

The immobilization of lecithin nanoparticles containing quercetin in galactomannan-based films emerges as a promising strategy to increase the absorption of quercetin through the skin. This work aims evaluating the effect of different concentrations of lecithin nanoparticles containing quercetin on the properties of galactomannan-based films obtained from *Cassia grandis* seeds. Nanoparticles suspension was obtained by injection of lecithin in distilled water. Quercetin was incorporated at different concentrations. Lecithin nanoparticles (LNp) and quercetin-loaded lecithin nanoparticles (QT-LNp) were analysed by dynamic light scattering and zeta potential. After the characterization, QT-LNp (75µg/mL of quercetin) was immobilized at different concentrations in galactomannan films and submitted to analyses trough colour parameters, solubility, moisture content (MC) and water vapour permeability (WVP). LNp was statistically smaller when compared with those with quercetin above 50 µg/mL. All LNps presented low polydispersity index even considering their significant differences. LNp with and without quercetin presented similar values for zeta potential. All of the studied films had a strong whiteness tendency, a yellowness appearance and low opacity values. The incorporation of QT-LNp in galactomannan-based films did not lead to statistical differences for solubility and MC, while for WVP the results were significantly different. Galactomannan-based films from *C. grandis* showed to be a promising structure for the immobilization of lecithin nanoparticles containing quercetin.

Key-words: encapsulation; quercetin-loaded nanoparticles; polysaccharide.

1. Introduction

Natural or synthetic polymers are molecules whose chains are longer and able to produce continuous matrices vital for structuration of films, membranes and coatings. The preparation of films from biodegradable materials has aroused the interest of the scientific community in recent decades, especially due to the importance given to the replacement of synthetic polymers. Films developed from polysaccharides act as excellent barriers to oxygen due to packing of molecules, forming a structural network ordered through hydrogen bonds; however, there are hygroscopic characteristics that can reduce its potential for many applications (Albuquerque, Coelho, Correia, Teixeira, & Carneiro-da-Cunha, 2016; Yang & Paulson, 2000).

Galactomannans are polysaccharides derived from leguminous seeds composed by a central core of (1 → 4)-linked D-mannopyranose units to which (1 → 6)-linked D-galactopyranose units are attached. They are an essential hydrophilic material with a variety of properties, such as nontoxic, biodegradable, inexpensive, and readily available. Albuquerque et al. (2014) extracted the galactomannan from the seeds of *Cassia grandis*, a typical Brazilian tree, and performed an extensive rheological characterization, demonstrating that this polysaccharide presents a fluid behaviour; specifically, the concentration of 1.7% (w/v) represents the fluid-gel transition of the galactomannan. More recently, Albuquerque, Cerqueira, Vicente, Teixeira, & Carneiro-da-cunha, (2017) used the same galactomannan for the production of films containing different concentrations of bioactive compounds and concluded that the galactomannan matrix showed to be a promising structure for the immobilization of biomolecules, pointing at a significant number of possible applications in food and pharmaceutical industries.

Biologically active molecules such as proteins, peptides, saccharides, lipids,

drugs, hormones, cell surface receptors, conjugates, nucleotides and nucleic acids can be immobilized based on physical or chemical linkages on polymeric supports. Applications ranging from the food to the pharmaceutical industry have used films based on galactomannans as matrices for controlled release of compounds (Albuquerque et al., 2016; Bassi & Kaur, 2015; Jian, Zhu, Zhang, Sun, & Jiang, 2012; Rossi et al., 2016), however, in what concern our knowledge, no work have described the immobilization of quercetin in galactomannan matrices.

Quercetin, 3,3´,4´,5´-7-pentahydroxy flavone is one of the most abundant flavonoid in plants. In spite of this wide spectrum of pharmacological properties, the use of quercetin in pharmaceutical field is limited due to its low aqueous solubility and instability in physiological medium. These properties of quercetin result in poor bioavailability, poor permeability, and instability, which may difficult its absorption, for example, through the skin. One-way to circumvent these problems is to entrap/adsorb the quercetin into biodegradable polymeric systems (Kumari, Yadav, Pakade, Singh, & Yadav, 2010).

Lecithin is a mixture of phospholipids of vegetable origin comprised mainly of phosphatidylcholine and phosphatidyl-ethanolamine, and it is considered a safe and biocompatible excipient, already used in many pharmaceutical formulations (Şenyiğit et al. 2010). The demand for encapsulation systems continues to grow as the industries needs to preserve the benefits of active compounds and deliver them at specific conditions. The development of polymeric systems at nano-scale is increasingly being studied for their attractive properties, e.g. ability to encapsulate different bioactive compounds, large surface area, and response to environmental changes (Bourbon, Cerqueira, & Vicente, 2016). In this sense, the technological development of a system composed of lecithin and quercetin in nanometric scale emerges as a promising

strategy to increase the penetration of quercetin through the skin in a controlled manner. In addition, the immobilization of the system in galactomannan-based films may enhance its permeability across the stratum corneum due to the bioadhesion of the polysaccharide.

During this work, quercetin was incorporated in lecithin nanoparticles and characterized by particle size and zeta potential measurements, and encapsulation efficiency and loading. Then, the effect of different concentrations of the system was evaluated on the properties of galactomannan films from *C. grandis* seeds. Colour, moisture content, solubility, water vapour permeability, and water contact angle were performed on the films aiming the development of a matrix valuable for a great number of industrial applications.

2. Materials and Methods

2.1 Materials

Soya lecithin (Lipoid S45) was kindly provided by Lipoid (Ludwigshafen, Germany), 99 % ethanol and quercetin from Sigma Alderich. The pods of *C. grandis* were collected at the rural zone of Pernambuco state, in the city of Angelim (Brazil), in July 2011. Ethanol (99.8%), acetone (PA) and sodium chloride were obtained from Vetec Fine Chemicals Ltda. (Brazil). The solvents used were of analytical grade and all other reagents were purchased from Sigma (Steinheim, Germany).

2.2 Nanoparticles preparation

Nanoparticles suspension was obtained by injection, drop by drop, during 2 min, of 2 mL of ethanolic solution of Lipoid S45 (25 mg/mL) in 23 mL of distilled water under magnetic stirring (600 rpm). The suspension was leaved in magnetic stirring for 8 min

and then submitted to a vigorous shaking on a Vortex mixer for 2 min at room temperature, being called LNp. The incorporation of quercetin at different concentrations (0 - 150 µg/mL) was performed by prior dissolution in the ethanolic solution of lipoid S45 and subsequent addition into distilled water as described above. Finally, quercetin-loaded lecithin nanoparticles were namely QT-LNp.

2.3 Physicochemical characterization of the nanoparticles

The hydrodynamic diameter (size-average) and polydispersity index (PDI) of the nanoparticles were determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, UK). The samples were analysed in a folded capillary cell. Detection of the scattered light was carried out at 173° (NIBS = non-invasive backscatter detection) and temperature of 25 °C. The zeta potential was measured by laser Doppler micro-electrophoresis using a Zetasizer ZS Nano (Zetasizer Nano ZS, Malvern Instruments, UK). The samples were filtered with 0.45 µm syringe filter (Minisart® High Flow, Sartorius, Gloucestershire, UK) to eliminate eventual particles above 450 nm and compared to the samples without filtering. At least five replicates were performed for each sample and the results are given as mean ± standard deviation of the values obtained.

2.4 Quercetin encapsulation efficiency and loading

The quercetin encapsulation efficiency and loading of the Np were conducted according in a ultracentrifuge at 180000 g over 30 min and 4ºC (Microultracentrifuge Sorvall MTX 150, Thermo Fisher Scientific, Germany). A sample of QT-LNp suspension was measured with a microplate reader (GENios, TECAN, Switzerland) at 373 nm, obtaining the total content of quercetin. The same volume of QT-LNp after

ultrafiltration was accurately taken and determined under the same condition, and the total amount of free drug was obtained. The content of quercetin loaded in LNp was calculated by subtracting the total amount of quercetin and the amount of free quercetin in QT-LNp suspension. The encapsulation efficiency of quercetin (EE) and quercetin-loading (L) were calculated by the following equations:

$$EE\ (\%) = \frac{Ws}{Wtotal}\ x\ 100$$

$$L\ (\%) = \frac{Ws}{WLNp}\ x\ 100$$

where W_s was the amount of quercetin loaded in LNp; W_{total} was the total amount of quercetin in the LNp suspension; and $W_{LNp\ was}$ the vehicle weight.

2.5 Film preparation

The galactomannan contained in *C. grandis* seeds was obtained according to Albuquerque et al., (2014). Briefly, the purification process was performed by immersion of the pods of *C. grandis* in distilled water at 25 ºC for 18 h; the pods were then separated in a half part, revealing the seeds which were removed and dried until reaching a constant weight. The dry seeds were boiled in distilled water 1:5 (w/v) at 100 ºC for 1 h for enzyme inactivation and maintained in water by 18 h at 25 ºC to facilitate removal of the hull. After that, the hull was removed and the residual was triturated in a blender with 0.1 M NaCl 5% (w/v) at 25 ºC, filtered through a veil tissue and after using a screen printing cloth, and precipitated with 46% ethanol 1:3 (v/v) for 18 h. The white precipitate obtained was washed with 100% ethanol 1:3 (w/v) for 30

min and two times with acetone 1:3 (w/v) for 30 min, been filtered on screen printing cloth between each washing. The precipitate was dried until constant weight, milled and finally called galactomannan.

The film forming solutions were prepared in accordance to the methodology described by Albuquerque et al. (2017). Briefly, the filmogenic solution was prepared with 0.8 % (w/v) of galactomannan added of 0.2 % (v/v) of glycerol at 500 rpm, 20 ± 2 °C, for 12h. QT-LNp (75µg of quercetin/mL suspension) was immobilized at different concentrations [0 – 0.5% (v/v)] in the film forming solutions and left under magnetic stirring at 500 rpm for 2h. A constant amount of 15 mL of each of the obtained film forming solutions was cast onto 90 mm diameter polystyrene Petri dishes and dried in an oven at 33 ºC for 9 h. Finally, the films were maintained at 20 ºC and 54% relative humidity (RH) until further characterization.

2.6 Film thickness

Film thickness was measured with a digital micrometer (No. 293–561, Mitutoyo, Japan). Five different randomly chosen points were performed on each film and the mean values were used in the calculations of water vapour permeability (WVP) and mechanical properties.

2.7 Colour and opacity

Colour and opacity were determined using a digital colorimeter (Konica Minolta, model Chroma Meter CR-400, Osaka, Japan) calibrated at illuminant C with a white standard. The parameters determined were L* (L* = 0 [black] and L* = 100 [white]), a* (-a* = greenness and + a* = redness) and b* (-b* = blueness and + b* = yellowness). These parameters are the ones recommended by the International Commission on

Illumination. Films opacity was calculated as the ratio between the opacity of each sample on the black standard (Yb) and the opacity of each sample on the white standard (Yw). Five measurements were determined at random Yb and Yw and an average of them was used for calculations. The experiment was done in triplicate and the results were expressed as percentage and determined through:

$$Y\ (\%) = (Yb/Yw) \cdot 100 \qquad \text{Equation 1}$$

2.8 Moisture content

The moisture content (MC) was expressed as the percentage of water removed from the initial mass sample. MC was determined gravimetrically by drying the films at 105 °C in an oven with forced air circulation for 24h. The experiments were performed in triplicate.

2.9 Solubility

The measurement of solubility was determined according to Gontard, Duchez, Cuq, Guilberts (1994). Triplicates of each film were cut with a circular mould of 2 cm diameter, weighted and dried at 105 °C in an oven for 24 h. After reweighting, the films were placed in cups with 50 mL of distilled water, sealed over with parafilm and homogenized at 60 rpm for 24 h. The non-soluble part of each film was dried at 105 ºC for 24 h and weighted again.

2.10 Water vapour permeability

Water vapour permeability (WVP) was determined gravimetrically based on ASTM E96-92 method. The film was sealed on the top of a permeation cell containing distilled water (100% RH; 2337 Pa vapour pressure at 20 ºC), placed in a desiccator

at 20 ºC and 0% RH (0 Pa water vapour pressure) containing silica. The cells were weighted at intervals of 2 h for 10 h. Steady-state and uniform water pressure conditions were assumed by maintaining the air circulation constant outside the test cell by using a miniature fan inside the desiccator. The slope of weight loss versus time was obtained by linear regression. Three replicates were obtained for each sample.

2.11 Contact angle

The degree of hydrophilicity of the nanoparticles was measured by the sessile drop method in a face contact angle meter (OCA 20, Dataphysics, Germany). The methodology was based on Mahaling & Katti (2016) with some modifications. 1 mL of the nanoparticle suspensions was placed on a glass slide to form a particle thin film and allowed to dry at room temperature in a desiccator. The samples were taken with a 500 µL syringe (Hamilton, Switzerland), with a needle of 0.75 mm of diameter. Measurements were made in less than 5 s. Thirty replicates of contact angle measurements were obtained at 24.0 (± 0.2) ºC and ~65% humidity, then calculated using the inbuilt software of the instrument. Contact angle for galactomannan-based films with different concentrations [0 – 0.5% (v/v)] of QT-LNp (75µg of quercetin/mL suspension) were evaluated as described above, in addition to a new measurement at 30 s to evaluate the pattern of hydrophobicity of the films.

2.12 Statistical analyses

Statistical analyses were performed using Analysis of Variance (ANOVA) and linear regression analysis. The Tukey test (α=0.05) was used to determine any significance of differences between specific means (GraphPad Prism 5.00.288, GraphPad Software, Inc., San Diego, CA, USA).

3. Results and discussion

3.1 Physicochemical characterization of the nanoparticles

The average size and polydispersity index analyses with fundamental importance for nanoparticle´s characterization, since they directly influence parameters such as loading, release, and stability of the compound inside nanoparticles. It is known that the smaller the particle is, the greater is the exposed surface area, which leads to a faster release of encapsulated drugs. Smaller particles also have an increased risk of aggregation during a period of time, being important to reach the production of particles with a low PDI, this achieving maximum stability by a better control (and lesser dispersion) of particles' size. In this sense, it is important to mention that the reproducibility of parameters such as stability and release is directly connected to a low PDI (≤ 0.4), since a high PDI means that there is no uniformity in the size distribution of the sample. (Souza et al., 2014).

Quercetin concentrations in LNp, their average size and polydispersity index are presented in Table 1. Regarding the results of Z average, it is possible to note that LNp was statistically smaller (p<0.05) when compared with those of concentrations above 50 µg/mL of quercetin, while for QT-LNp 25 no difference was observed. In addition, increasing quercetin concentrations above 100 µg/mL lead to higher values of average size. The results related to polydispersity index showed that all of the nanoparticles presented low PDI (≤ 0.29) even considering their significant differences. LNp containing 0, 25, 50, and 75 µg/mL of quercetin were homogeneous, however formulations with 100, 125, and 150 µg/mL showed signs of instability (precipitation): 100 and 125 µg/mL after 72 h, and 150 µg/mL after 48 h (Fig. 1).

Table 1 - Size-average and PDI for LNp and QT-LNp with different concentrations of quercetin (µg/mL).

Sample	Z average	PDI
LNp	94,26 ± 0,8234^{c}	0.2679 ± 0,003985^{b,d}
QT-LNp 25	93,85 ± 4,343^{c}	0.2784 ± 0,01159^{c,d}
QT-LNp 50	100,2 ± 0,5670^{d}	0.2355 ± 0,009969^{a}
QT-LNp 75	100,5 ± 4,569^{d}	0.2754 ± 0,01242^{c,d}
QT-LNp 100	104,1 ± 4,214^{d}	0.2828 ± 0,007239^{c}
QT-LNp 125	114,0 ± 1,045^{b}	0.2693 ± 0,006865^{d}
QT-LNp 150	131,8 ± 3,469^{a}	0.2571 ± 0,005259^{b}

$^{a-d}$Different superscript letters in the same column indicate a statistically significant difference (p< 0.05).

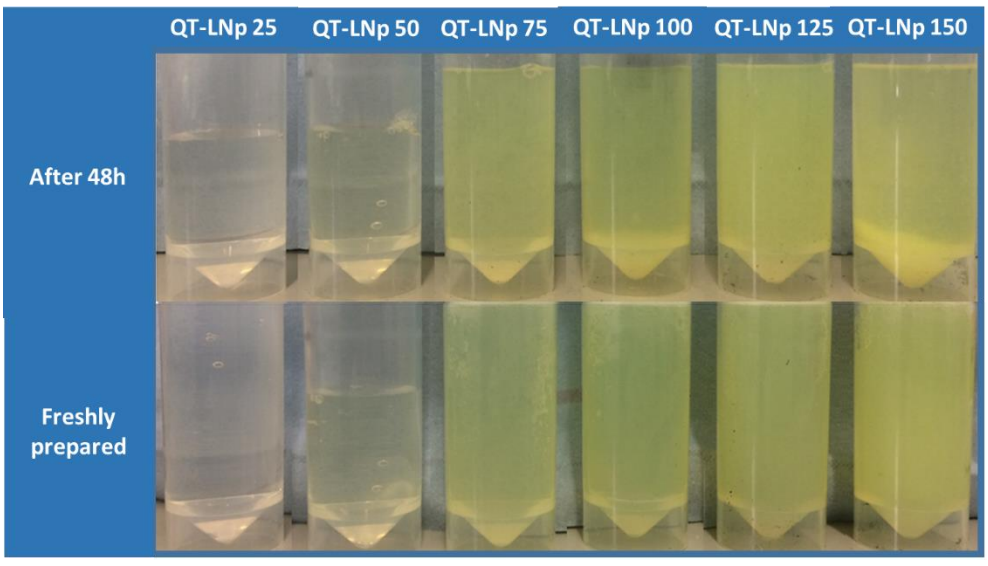

Fig.1. Profile of precipitation over time for QT-LNp with different concentrations of quercetin (µg/mL).

Zeta potential is related to the surface charge that can influence the stability of

suspended particles through electrostatic repulsion among them. All LNp with and without quercetin presented zeta potential values around -45 mV (Fig. 2), with no statistical differences (p<0.05) among them. The negatively charge could be associated to the phosphate groups of lecithin.

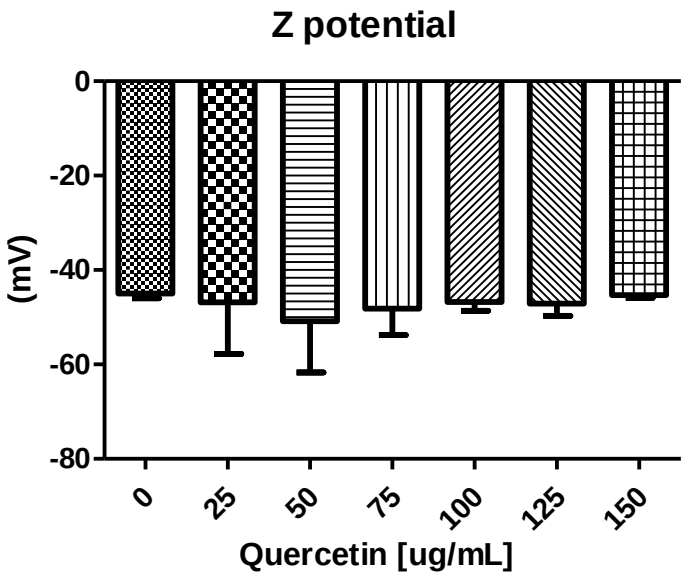

Fig. 2. Z potential for LNp and QT-LNp with different concentrations of quercetin (µg/mL).

3.2 Quercetin encapsulation efficiency and loading

Encapsulation efficiency and the loading of the compound depends largely on its solubility in the matrix (Kumari et al., 2010). The encapsulation efficiency of quercetin into lecithin nanoparticles (Fig. 3A) presented better values for concentrations above 75 µg/mL. Fig. 3B shows that increasing the quercetin concentration in the formulation resulted in improved quercetin loading, however, as already mentioned in section 3.1, formulations containing more than 75 µg/mL of quercetin showed signs of instability; therefore, nanoparticles with 75 µg/mL of

quercetin were chosen to be used in further analyses.

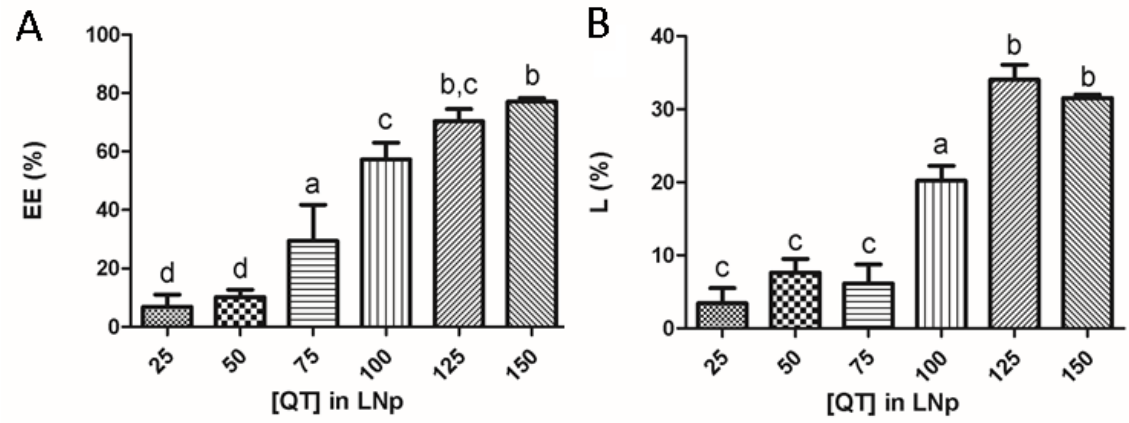

Fig.3. Influence of quercetin concentration in the encapsulation efficiency (A) and loading (B) of the nanoparticles. All determinations were performed in triplicate and the results expressed as mean ± standard deviation.

3.3 Colour and opacity

After the nanoparticles characterization, QT-LNp 75 (with 75 µg of quercetin/mL suspension) was chosen to be used in films characterization due to its stable pattern. Table 2 shows the films with QT-LNp 75 at different concentrations [0 – 0.5% (v/v)] and their thickness values.

Table 2 – QT-LNp 75 concentrations used in films formulation and the corresponding values of film thickness.

Films	QT-LNp 75 (v/v)	Thickness (mm)
A	-	0,046 ± 0,004[a]
B	0.1 %	0,051 ± 0,001[a]
C	0.2 %	0,058 ± 0,002[a]
D	0.3 %	0,056 ± 0,008[a]

E	0.4 %	0,051 ± 0,002[a]
F	0.5 %	0,054 ± 0,008[a]

[a-b]Different superscript letters in the same column indicate a statistically significant difference (p<0.05).

Colour and opacity are important features for the acceptance of products from pharmaceutical or food industry. So, the measurement of the colour of the products potentially valuable for the industry is an important point at the physical characterization. Table 3 presents the colour parameters and opacity of the films. All of them had a strong whiteness tendency, represented by L* coordinate values. The presence of QT-LNp 75 at 0.5% (v/v) decreased (p<0.05) the redness appearance of the films, even considering their evident yellowness tendency. All the studied films presented low opacity values with no significant differences between them.

Table 3 – Color parameters L (luminosity), a* (-a* = greenness and +a* = redness), b* (-b* = blueness and +b* = yellowness) and Y (opacity) for (A) the galactomannan-based film without QT-LNp 75 and (B-F) with different concentrations of QT-LNp 75 (values expressed as average ± standard deviation).

Films	L	a*	b*	Y
A	95.62 ± 0.40[a]	0.35 ± 0.06[b,c]	7.93 ± 0.61[a]	12.30 ± 0.32[a]
B	95.66 ± 0.22[a]	0.35 ± 0.01[b,c]	7.46 ± 0.09[a]	12.59 ± 0.26[a]
C	95.77 ± 0.22[a]	0.30 ± 0.01[a,b,c]	7.24 ± 0.33[a]	12.49 ± 0.24[a]
D	95.01 ± 1.09[a]	0.39 ± 0.04[b]	8.17 ± 2.08[a]	12.11 ± 0.36[a]
E	96.05 ± 0.31[a]	0.27 ± 0.03[a,c]	7.46 ± 0.99[a]	12.27 ± 0.29[a]
F	95.84 ± 0.42[a]	0.25 ± 0.04[a]	7.25 ± 0.36[a]	12.62 ± 0.14[a]

[a-c]Different superscript letters in the same column indicate a statistically significant difference (p<0.05).

3.4 Moisture content, solubility, water vapour permeability, and contact angle

Moisture-binding abilities of films can affect significantly their physical and barrier properties, thus the knowledge of the content and affinity of film matrix to water is a key parameter when choosing a film for specific applications. Table 4 shows the values of moisture content (MC), solubility, and water vapour permeability (WVP) of the films without QT-LNp 75 immobilized (A) and those with different concentrations of QT-LNp 75 (B-F). The incorporation of QT-LNp 75 in galactomannan-based films did not lead to statistical differences (p>0.05) for solubility and moisture content, while for WVP the results were significantly different.

Table 4 – Values of solubility (Sol), moisture content (MC), and water vapour permeability (WVP) of the films.

Films	Sol (%)	MC (%)	WVP x 10^{-7} (g h-1 m-1 Pa-1
A	77 ± 8[a]	17 ± 1[a]	7.27 ± 0.80[a]
B	68 ± 10[a]	19 ± 2[a]	8.07 ± 0.63[a,c]
C	77 ± 4[a]	22 ± 3[a]	10.44 ± 0.48[b,c]
D	75 ± 2[a]	27 ± 5[a]	11.41 ± 1.05[b]
E	54 ± 22[a]	28 ± 9[a]	9.72 ± 0.57[a,b,c]
F	73 ± 13[a]	23 ± 2[a]	9.94 ± 1.46[b,c]

[a-c]Different superscript letters in the same column indicate a statistically significant difference (p<0.05).

The determination of the contact angle (CA) is a simple way to evaluate materials´ degree of hydrophobicity, even layered on a surface or immobilized on a ready-surface. Commonly, if surfaces have CA values less than 90º, they are considered hydrophilic (Albuquerque et al., 2017; Ma, Hu, & Wang, 2016). Fig. 4 shows CA values for LNp and QT-LNp in different concentrations of quercetin, demonstrating the higher hydrophilicity of the nanoparticles. It is possible to observe particular differences by increasing quercetin concentration above 75 µg/mL, which lead to higher values of CA.

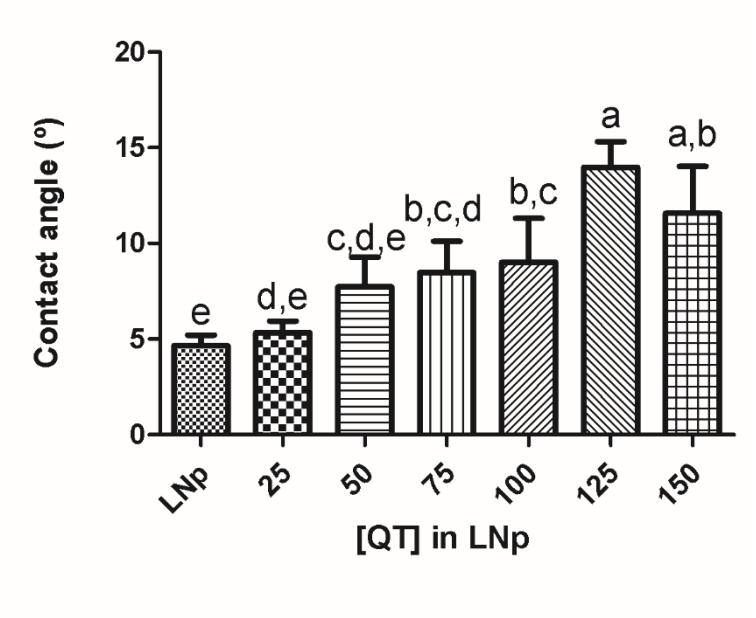

Fig.4. Contact angle values for LNp and QT-LNp (after incorporation of quercetin between 0 and 150 µg/mL).

After LNp and QT-LNp in deferent concentrations were characterized by CA, this work proceeded with the immobilization of QT-LNp 75 on the galactomannan film forming solution. The (A) galactomannan film without QT-LNp 75 and the ones (B-F) with immobilized QT-LNp 75 were measured also at 5 s and additionally 30 s, to

understand the pattern of hydrophobicity of the samples over time. The results presented in Table 5 demonstrates that all of the films were essentially hydrophobic with no statistical differences between them and when analyzed over time.

Table 5 – Values of contact angle (CA) measured at 5 s and 30 s for (A) the galactomannan-based film without QT-LNp 75 and (B-F) with different concentrations of QT-LNp 75 (values expressed as average ± standard deviation).

Films	CA at 5 s	CA at 30 s
A	113.5 ± 9,57[a]	111.5 ± 2.61[a]
B	114,2 ± 5.93[a]	119.1 ± 3.47[a]
C	108,3 ± 4.36[a]	112.2 ± 3.04[a]
D	108,2 + 3.98[a]	114.2 ± 6.02[a]
E	112,8 ± 7.03[a]	115.6 ± 6.22[a]
F	113,8 ± 4.81[a]	117.1 ± 3.78[a]

[a-c]Different superscript letters in the columns indicate a statistically significant difference ($p<0.05$).

4. Conclusion

Quercetin has been successfully encapsulated in lecithin nanoparticles using a simple technique. In addition, galactomannan-based films from *C. grandis* showed to be a promising structure for the immobilization of lecithin nanoparticles containing quercetin and a permeability enhancer of quercetin across the skin.

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References

Albuquerque, P. B. S., Barros, W., Santos, G. R. C., Correia, M. T. S., Mourão, P. A. S., Teixeira, J. A., & Carneiro-da-Cunha, M. G. (2014). Characterization and rheological study of the galactomannan extracted from seeds of Cassia grandis. *Carbohydrate Polymers*, *104*, 127–134. http://doi.org/10.1016/j.carbpol.2014.01.010

Albuquerque, P. B. S., Cerqueira, M. A., Vicente, A. A., Teixeira, J. A., & Carneiro-da-cunha, M. G. (2017). International Journal of Biological Macromolecules Immobilization of bioactive compounds in Cassia grandis galactomannan-based films : Influence on physicochemical properties. *International Journal of Biological Macromolecules*, *96*, 727–735. http://doi.org/10.1016/j.ijbiomac.2016.12.081

Albuquerque, P. B. S., Silva, C. S., Soares, P. A. G., Jr., W. B., Correia, M. T. S., Coelho, L. C. B. B., … Carneiro-da-Cunha, M. G. (2016). Investigating a galactomannan gel obtained from Cassia grandis seeds as immobilizing matrix for Cramoll lectin. *International Journal of Biological Macromolecules*.

http://doi.org/10.1016/j.ijbiomac.2016.01.107

Albuquerque, P., Coelho, L., Correia, M., Teixeira, J., & Carneiro-da-Cunha, M. (2016). Biotechnological Applications of Galactomannan Matrices: Emphasis on Immobilization of Biomolecules. *Advances in Research*, *6*(5), 1–17. http://doi.org/10.9734/AIR/2016/23435

Bassi, P., & Kaur, G. (2015). Fenugreek gum derivatives with improved bioadhesion and controlled drug release: In vitro and in vivo characterization. *Journal of Drug Delivery Science and Technology*, *29*, 42–54. http://doi.org/10.1016/j.jddst.2015.06.006

Bourbon, A. I., Cerqueira, M. A., & Vicente, A. A. (2016). Encapsulation and controlled release of bioactive compounds in lactoferrin-glycomacropeptide nanohydrogels: Curcumin and caffeine as model compounds. *Journal of Food Engineering*, *180*, 110–119. http://doi.org/10.1016/j.jfoodeng.2016.02.016

Jian, H., Zhu, L., Zhang, W., Sun, D., & Jiang, J. (2012). Galactomannan (from Gleditsia sinensis Lam.) and xanthan gum matrix tablets for controlled delivery of theophylline: In vitro drug release and swelling behavior. *Carbohydrate Polymers*, *87*(3), 2176–2182. http://doi.org/10.1016/j.carbpol.2011.10.043

Kumari, A., Yadav, S. K., Pakade, Y. B., Singh, B., & Yadav, S. C. (2010). Development of biodegradable nanoparticles for delivery of quercetin. *Colloids and Surfaces B: Biointerfaces*, *80*(2), 184–192. http://doi.org/10.1016/j.colsurfb.2010.06.002

Ma, Q., Hu, D., & Wang, L. (2016). Preparation and physical properties of tara gum film reinforced with cellulose nanocrystals. *International Journal of Biological Macromolecules*. http://doi.org/10.1016/j.ijbiomac.2016.01.104

Mahaling, B., & Katti, D. S. (2016). Understanding the in fl uence of surface properties of nanoparticles and penetration enhancers for improving bioavailability in eye

tissues in vivo. *International Journal of Pharmaceutics*, *501*(1–2), 1–9. http://doi.org/10.1016/j.ijpharm.2016.01.053

Rossi, B., Campia, P., Merlini, L., Brasca, M., Pastori, N., Farris, S., … Galante, Y. M. (2016). An aerogel obtained from chemo-enzymatically oxidized fenugreek galactomannans as a versatile delivery system. *Carbohydrate Polymers*, *144*, 353–361. http://doi.org/10.1016/j.carbpol.2016.02.007

Souza, M. P., Vaz, A. F. M., Correia, M. T. S., Cerqueira, M. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2014). Quercetin-Loaded Lecithin/Chitosan Nanoparticles for Functional Food Applications. *Food and Bioprocess Technology*, *7*(4), 1149–1159. http://doi.org/10.1007/s11947-013-1160-2

Yang, L., & Paulson, A. . (2000). Mechanical and water vapour barrier properties of edible gellan films. *Food Research International*, *33*(7), 563–570. http://doi.org/10.1016/S0963-9969(00)00092-2

Figure captions

Fig.1. Profile of precipitation over time for QT-LNp with different concentrations of quercetin (µg/mL).

Fig. 2. Z potential for LNp and QT-LNp with different concentrations of quercetin (µg/mL).

Fig.3. Influence of quercetin concentration in the encapsulation efficiency (A) and loading (B) of the nanoparticles. All determinations were performed in triplicate and the results expressed as mean ± standard deviation.

Fig.4. Contact angle values for LNp and QT-LNp (after incorporation of quercetin between 0 and 150 µg/mL).

5 CONCLUSÕES

Os resultados obtidos permitem concluir que:

- Os géis de galactomanana, sem ou com Cramoll 1,4 imobilizada, apresentaram estabilidade ao pH, cor, parâmetros reológicos e ausência de contaminação microbiana ao longo de 30 dias;
- A atividade hemaglutinante da Cramoll 1,4 imobilizada no gel de galactomanana foi mantida a 90% da sua atividade inicial durante um período de 20 dias, diminuindo para 60% após 60 dias;
- Os filmes da galactomanana extraída das sementes de *C. grandis* com Cramoll 1,4 imobilizada não apresentaram citotoxicidade e mantiveram 90,94% da atividade hemaglutinante inicial da lectina;
- Os filmes de galactomanana sem e com Cramol 1,4 imobilizada, quando utilizados como curativos tópicos, aceleraram o processo cicatricial de feridas, destacando-se aqueles contendo Cramoll 1,4. Provavelmente as propriedades inatas da galactomanana podem ter induzido uma via de sinalização mais rápida, imitando a matriz extracelular natural e atraindo fibroblastos para a camada da derme;
- A imobilização de compostos bioativos tais como lactoferrina (Lf), peptídeos bioativos (BAPs) e fitoesteróis em filmes da galactomanana extraída das sementes de *C. grandis* resultaram em filmes com superfícies mais rugosas e afetaram suas propriedades mecânicas, o que melhorou a rigidez dos filmes;
- Quercetina foi eficientemente imobilizada em nanopartículas de lecitina através de uma técnica simples e rápida;
- A incorporação de nanopartículas de lecitina contendo quercetina em filmes à base de galactomanana não afetou os parâmetros solubilidade e teor de umidade, contudo afetou a permeabilidade aos vapores de água;

E finalmente, a galactomanana extraída das sementes de *C. grandis* é uma promissora matriz em forma de gel e/ou membrana para a imobilização de biomoléculas, para utilização nas indústrias farmacêutica, cosmética e alimentícia.

REFERÊNCIAS

ABREU, A.S.; OLIVEIRA, M.; SÁ, A.; RODRIGUES, R.M.; CERQUEIRA, M.A.; VICENTE, A.A.; MACHADO, A.V. Antimicrobial nanostructured starch based films for packaging. **Carbohydrate Polymers**. 129, 127-134, 2015.

ADEGOKE, G.O.; KUMAR, M.V.; KRISHNA, A.G.G.; VARADAJ, M.C.; SAMBAIAH, K.; LOKESH, B.R. Antioxidants and lipid oxidation in foods – a critical appraisal. **Journal of Food Science and Technology**. 35(4), 283-298, 1998.

AGUILERA, Y.; HERRERA, T.; BENÍTEZ, V.; ARRIBAS, S.M.; PABLO, A.L.L.; ESTEBAN, R.M.; MARTÍN-CABREJAS, M.A. Estimation of scavenging capacity of melatonin and other antioxidants: Contribution and evaluation in germinated seeds. **Food Chemistry**. 170, 203-211, 2015.

AISEN, P.; LEIBMAN, A. Lactoferrin and transferrin: a comparative study. **Biochimica et Biophysica Acta**. 257, 314–23, 1972.

AKHAVAN, M.; JAHANGIRI, S.; SHAFAGHAT, A. Studies on the antioxidant and antimicrobial activity and flavonoid derivatives from the fruit of *Trigonosciadium brachytaenium* (Boiss.) Alava. **Industrial Crops and Products**. 63, 114-118, 2015.

ALBUQUERQUE, P.B.S.; BARROS JÚNIOR, W.; SANTOS, G.R.C.; CORREIA, M.T.S.; MOURAO, P.A.S.; TEIXEIRA, J.A.; CARNEIRO-DA-CUNHA, M.G. Characterization and rheological study of the galactomannan extracted from seeds of *Cassia grandis.* **Carbohydrate Polymers.** 104, 127–134, 2014.

ALBUQUERQUE, P.B.S.; CERQUEIRA, M.A.; VICENTE, A.A.; TEIXEIRA, J.A.; CARNEIRO-DA-CUNHA, M.G. Immobilization of bioactive compounds in Cassia grandis galactomannan-based films: Influence on physicochemical properties. **International Journal of Biological Macromolecules.** 96: 727-735, 2017.

ALBUQUERQUE, P.B.S.; COELHO, L.C.B.B.; CORREIA, M.T.S.; TEIXEIRA, J.A.; CARNEIRO-DA-CUNHA, M.G. Biotechnological applications of galactomannan matrices: emphasis on immobilization of biomolecules. **Advances in research**, 6(5), 1-17, 2016a.

ALBUQUERQUE, P.B.S.; COELHO, L.C.B.B.; TEIXEIRA, J.A.; CARNEIRO-DA-CUNHA, M.G. Approaches in biotechnological applications of natural polymers.

AIMS Molecular Science, 3(3): 386-425, 2016b.

ALVARÉZ-ROMÁN, R.; BARRÉ, G.; GUY, R.H.; FESSI, H. Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection. **European Journal Pharmaceutics and Biopharmaceutics,** 52, 191-195, 2001.

AMARAL, J. **Estudo reológico da galactomanana de sementes de *Cassia fastuosa*.** 1998. 123 f. Dissertação (Mestrado em Ciências - Bioquímica). Universidade Federal do Paraná. Curitiba, 1998.

ANDRADE, C.A.S.; CORREIA, M.T.S.; COELHO, L.C.B.B.; NASCIMENTO, S.C.; SANTOS-MAGALHÃES, N.S. Antitumor activity of *Cratylia mollis* lectin encapsulated into liposomes. **International Journal of Pharmaceutics**. 278, 435–445, 2004.

ANGELI, V.W. Desenvolvimento e caracterização de formulações fotoprotetoras contendo nanocápsulas. 2007. 110p. Tese (Doutorado em Ciências Farmacêuticas). Universidade Federal do Rio Grande do Sul, Porto Alegre, 2007.

ANUSHA, J.R.; FLEMING, A.T.; KIM, H.J.; KIM, B.C.; YU, K.H.; RAJ, C.J. Effective immobilization of glucose oxidase on chitosan submicron particles from gladius of Todarodes pacificus for glucose sensing. **Bioelectrochemistry**. 104, 44-50, 2015.

ARAÚJO, C.F.R.; SOUZA FILHO, Z.A.; GRECA, F.H.; GUERREIRO, M.M.C.P.M.; LEITE, L.A.; MANSUR, A.E.C.; KANTOR, D.C.; NASSIF, A.E. Efeitos do Agarol ® e do Trigliceril ® sobre a cicatrização de pele, estudo experimental em ratos. **Acta Cirúrgica Brasileira**. 13(4), 232-237, 1998.

ARAÚJO, R.M.S.; FERREIRA, R.S.; NAPOLEÃO, T.H.; CARNEIRO-DA-CUNHA, M.G.; COELHO, L.C.B.B.; CORREIA, M.T.S.; OLIVA, M.L.V.; PAIVA, P.M.G. *Crataeva tapia* bark lectin is an affinity adsorbent and insecticidal agent. **Plant Science**. 183, 20-26, 2012.

ARAÚJO, R.M.S.; VAZ, A.F.M.; AGUIAR, J.S.; COELHO, L.C.B.B.; PAIVA, P.M.G.; MELO, A.M.M.; SILVA, T.G.; CORREIA, M.T.S. Lectin from *Crataeva tapia* bark exerts antitumor, anti-inflammatory and analgesic activities. **Natural Products and Bioprospecting**. 1, 97–100, 2011.

ASHOK, B.; REDDY, K.O.; MADHUKAR, K.; CAI, J.; ZHANG, L.; RAJULU, A.V. Properties of cellulose/*Thespesia lampas* short fibers bio-composite films. **Carbohydrate Polymers**. 127, 110-115, 2015.

ASPINALL, G.O. Structural chemistry of the hemicelluloses. **Advances in Carbohydrate Chemistry**, 14, 429–468, 1959.

ASSIS, L.M.; ZAVAREZE, E.R.; PRENTICE-HERNÁNDEZ, C.; SOUZA SOARES, L.A. Characteristics of nanoparticles and their potential applications in foods. **Brazilian Journal of Food Technology**. 15, 99-109, 2012.

ATALAH, B.; SMAGGHE, G.; VAN DAMME, E.J.M. Orysata, a jacalin-related lectin from rice, could protect plants against biting-chewing and piercing-sucking insects. **Plant Science**. 221–222, 21-28, 2014.

AZERO, E. G.; ANDRADE, C. T. Testing produces for galactomannan purification. **Polymer testing**, 21, 551-556, 2002.

AZULAY, R.D.; AZULAY, D.R. **Dermatologia**. Rio de Janeiro: Guanabara Koogan. 4ª ed., p. 10-11, 2006.

BARNES, H. A.; HUTTON, J. F.; WALTERS, K. **An Introduction to Rheology**. 1st ed. Amsterdam: Elsevier, 1989.

BARRA, G. **Fundamentos da Reologia de Materiais Poliméricos.** Apostila de processos 4. EMC 5744. Disponível em: <http://emc5744.barra.prof.ufsc.br/Reologia%20parte%201.pdf>. Acesso em 08 de agosto de 2015.

BEDANE, A.H.; EIĆ, M.; FARMAHINI-FARAHANI, M.; XIAO, H. Water vapor transport properties of regenerated cellulose and nanofibrillated cellulose films. **Journal of Membrane Science**. 493, 46-57, 2015.

BEHR, S.H.; MUSTAD, V.A.; WALTER, D.; BLOCH, T.D. Cholesterol-lowering effect of food bars containing guar gum in healthy women and men. **Journal of the American Dietetic Association**. Poster Section: Medical Nutrition Therapy, 1998.

BEVERLYA, R.L.; JANES, M.E.; PRINYAWIWATKULA, W.; NO, H.K. Edibles chitosan films on read-to-eat roast beef for the control of *Listeria monocytogenes.* **Food Microbiology**, 25, 534–537, 2008.

BHAMARAPRAVATI, S.; PENDLAND, S.L.; MAHADY, G.B. Extracts of spice and food plants from Thai traditional medicine inhibit the growth of the human carcinogen *Helicobacter pylori*. **In Vivo**, 17(6), 541-544, 2003.

BIGUCCI, F.; ABRUZZO, A.; SALADINI, B.; GALLUCCI, M.A.; CERCHIARA, T.; LUPPI, B. Development and characterization of chitosan/hyaluronan film for

transdermal delivery of thiocolchicoside. **Carbohydrate Polymers**. 130, 32-40, 2015.

BOATENG, J.; BURGOS-AMADOR, R.; OKEKE, O.; PAWAR, H.; Composite alginate and gelatin based bio-polymeric wafers containing silver sulfadiazine for wound healing. **International Journal of Biological Macromolecules**. 79, 63–71, 2015.

BOKKHIM, H.; BANSAL, N.; GRØNDAHL, L.; BHANDARI, B. *In-vitro* digestion of different forms of bovine lactoferrin encapsulated in alginate micro-gel particles. **Food Hydrocolloids**. 52, 231-242, 2016.

BOURBON, A.I.; PINHEIRO, A.C.; CERQUEIRA, M.A.; VICENTE, A.A. Influence of chitosan coating on protein-based nanohydrogels properties and in vitro gastric digestibility. Food Hydrocolloids. 60, 109-118, 2016.

BOYD, W.C.; SHAPLEIGH, E. Specific precipitating activity of plant agglutinins (lectins). **Science**. 119(3091), 419, 1954.

BRANDÃO, M.; LACA-BUENDIA, J.P.; MACEDO, J.F. **Árvores nativas e exóticas do estado de Minas Gerais**. Belo Horizonte: EPAMIG, 2002. 528p.

BRESOLIN, T. M. B.; SANDER, P.C.; REICHER, F.; SIERAKOWSKI, M. R.; RINAUDO, M.; GANTER, J. L. M. S. Viscosimetric studies on xanthan and galactomannan systems. **Carbohydrate Polymers**, 33, 131-138, 1997.

BUDOWSKI, G.; KASS, D.C.L.; RUSSO, R.O. Leguminous trees for shade. **Pesquisa Agropecuária Brasileira**, 19, 205-222, 1984.

BUSCH, V.M.; KOLENDER, A.A.; SANTAGAPITA, P.R.; BUERA, M.P. *Vinal* gum, a galactomannan from *Prosopis ruscifolia* seeds: Physicochemical characterization. **Food Hydrocolloids**. 51, 495-502, 2015.

CÁCERES, A.; LÓPEZ, B.; JUÁREZ, X.; AGUILA J. del.; GARCÍA, S. Plants used in Guatemala for the treatment of dermatophytic infections. 2. Evaluation of antifungal activity of seven American plants. **Journal of Ethnopharmacology**, 40, 207-213, 1993.

CARNEIRO-DA-CUNHA, M.G.; CERQUEIRA, M.A.; SOUZA B.W.S.; CARVALHO, S.; QUINTAS, M.A.C.; TEIXEIRA, J.A.; VICENTE, A.A. Physical and thermal properties of a chitosan/alginate nanolayered PET film. **Carbohydrate Polymers**. 82, 153–159, 2010.

CARNEIRO-DA-CUNHA, M.G.; CERQUEIRA, M.A.; SOUZA, B.W.S.; SOUZA, M.P.;

TEIXEIRA, J.A.; VICENTE, A.A. Physical properties of edible coatings and films made with a polysaccharide from *Anacardium occidentale* L. **Journal of Food Engineering**. 95, 379–385, 2009.

CARO, N.; MEDINA, E.; DÍAZ-DOSQUE, M.; LÓPEZ, L.; ABUGOCH, L.; TAPIA, C. Novel active packaging based on films of chitosan and chitosan/quinoa protein printed with chitosan-tripolyphosphate-thymol nanoparticles via thermal ink-jet printing. **Food Hydrocolloids**. 52, 520-532, 2016.

CARVALHO, P.E.R. **Espécies arbóreas brasileiras**. Brasília: Embrapa Informação Tecnológica, Colombo, PR: Embrapa Florestas, 2003. 1. 1039p.

CERQUEIRA, M.A.; BOURBON, A.I.; PINHEIRO, A.C.; MARTINS, J.T.; SOUZA, B.W.S.; TEIXEIRA, J.A.; VICENTE, A.A.. Galactomannans use in the development of edible films/coatings for food applications. **Trends in Food Science & Technology**, 22, 662-671, 2011a.

CERQUEIRA, M.A.; LIMA, A.M.; SOUZA, B.W.S.; TEIXEIRA, J.A.; MOREIRA, R.A.; VICENTE, A.A. Functional polysaccharides as edible coatings for cheese. **Journal of Agricultural and Food Chemistry**, 57(4), 1456-1462, 2009a.

CERQUEIRA, M.A.; LIMA, A.M.; TEIXEIRA, J.A.; MOREIRA, R.A.; VICENTE, A.A. Suitability of novel galactomannans as edible coatings for tropical fruits. **Journal of Food Engineering,** 94, 372-378, 2009b.

CERQUEIRA, M.A.; SOUZA, B.W.S.; MARTINS, J.T.; TEIXEIRA, J.A.; VICENTE, A.A. Seed extracts of *Gleditsia triacanthos*: functional properties evaluation and incorporation into galactomannan films. **Food Research International**. 43(8), 2031-2038, 2010.

CERQUEIRA, M.A.; SOUZA, B.W.S.; SIMÕES, J.; TEIXEIRA, J.A.; ROSÁRIO, M.; DOMINGUES, M.; COIMBRA. M.A.; VICENTE, A.A. Structural and thermal characterization of galactomannans from non-conventional sources. **Carbohydrate Polymers,** 83, 179–185, 2011b.

CHAUHAN, A.; AGARWAL, M. Evaluating the antifertility potential of an aqueous extract from *Cassia fistula* seeds in male rats. **Fertility and Sterility**, 93(5), 1706-1710, 2010.

CHEN, Y.-Z.; KAO, S.-Y.; JIAN, H.-C.; YU, Y.-M.; LI, J.-Y.; WANG, W.-H.; TSAI, C.-W. Determination of cholesterol and four phytosterols in foods without derivatization by gas chromatography-tandem mass spectrometry. Journal of food and drug

analysis. 23, 636-644, 2015.

CHIEN, P.; LIN, H.; SU, M. Effects of Edible Micronized Chitosan Coating on Quality and Shelf Life of Sliced Papaya. **Food and Nutrition Sciences**. 4, 9-13, 2013.

CHO, M.L.; YANG, C.; KIM, S.M.; YOU, S.G. Molecular characterization and biological activities of water soluble sulfated polysaccharides from *Enteromorpha prolifera.* **Food Science and Biotechnology**. 19(2): 525-533, 2010.

CHOKSI, A.N.; POONAWALLA, T.; WILKERSON, M.G. **Journal of Drugs in Dermatology**. 9(5), 475–481, 2010.

CHOPIN, T.,; WHALEN, E. A new and rapid method for carrageenan identification by FT IR diffuse reflectance spectroscopy directly on dried, ground algal material. **Carbohydrate Research**, 246, 51-59, 1993.

CHOW, C.K. Antioxidant nutrients and environmental health: Introduction. **Toxicology**. 180(1), 1-3, 2002.

COMBA, S; SETHI, R. Stabilization of highly concentrated suspersions of iron nanoparticles using shear-thinning gels of xanthan gum. **Water Research**, 43(15), 3717-3726, 2009.

CORREA, J.E.; BERNAL, H.Y. **Especies vegetales promisorias de lós paises del convenio Andrés Bello**. Bogotá: Guadalupe, 1990. 485p.

CORREIA, M.T.S.; COELHO, L.C.B.B. Purification of a Glucose/Mannose Specific Lectin, Isoform 1, from Seeds of *Cratylia mollis* Mart. (Camaratu Bean). **Applied Biochemistry and Biotechnology.** 55, 261-273, 1995.

CORREIA, M.T.S.; COELHO, L.C.B.B.; PAIVA, P.M.G. Lectins, carbohydrate recognition molecules: Are they toxic?, In: SIDDIQUE, Y.H. **Recent Trends in Toxicology vol. 37**, Kerala, India: Transworld Research Network, 2008, Vol. 37, p. 47-59.

COSTA, R.M.P.B.; VAZ, A.F.M.; OLIVA, M.L.V.; COELHO, L.C.B.B.; CORREIA, M.T.S.; CARNEIRO-DA-CUNHA, M.G. A new mistletoe *Phthirusa pyrifolia* leaf lectin with antimicrobial properties. **Process Biochemistry**. 45, 526–533, 2010.

COTRAN, R.S.; KUMAR, V.; ROBBINS, S.L. Neoplasia. In: COTRAN, R.S.; KUMAR, V.; ROBBINS, S.L. **Pathologic basis of disease**. 6 ed. Philadelphia: Saunders, 2000.

CUNHA, P.L.R.; DE PAULA, R.C.M.; FEITOSA, J.P.A.; Polissacarídeos da biodiversidade brasileira: uma oportunidade de transformar conhecimento em

valor econômico. **Quimica Nova***.* 32(3), 649-660, 2009.

CUNHA, P.L.R.; VIEIRA, I.G.P.; ARRIAGA., A.M.C.; DE PAULA, R.C.M.; FEITOSA; J.P.A. Isolation and characterization of galactomannan from *Dimorphandra gardneriana* Tul. seeds as a potential guar gum substitute. **Food Hydrocolloids,** 23(2), 880-885, 2009.

DAISY, P.; BALASUBRAMANIAN, K.; RAJALAKSHMI, M.; ELIZA, J.; SELVARAJ, J.Insulin mimetic impact of Catechin isolated from *Cassia fistula* on the glucose oxidation and molecular mechanisms of glucose uptake on Streptozotocin-induced diabetic Wistar rats Original Research Article. **Phytomedicine,** 17(1), 28-36, 2010.

DAKIA, P.A.; BLECKER, C.; ROBERTA, C.; WATHELETA, B.; PAQUOTA, M. Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. **Food Hydrocolloids**, 22, 807–818, 2008.

DA-LOZZO, E.J.; MOLEDO, R.C.A.; FARACO, C.D.; ORTOLANI-MACHADO, C.F.; BRESOLIN, T.M.B.; SILVEIRA, J.L.M. (2013). Curcumin/xanthan–galactomannan hydrogels: Rheological analysis and biocompatibility. **Carbohydrate Polymers.** 93, 279– 284, 2013.

DANTAS, C.J.S. Reparação Tecidual. In: **Mecanismos Celulares da Inflamação**. Rio de Janeiro: Medsi, 2000, cap. 17, p. 197-225.

DE POLO, K.F. **A short textbook of cosmetology**. Augsburg: H. Ziolkowsky GmbH. p. 19-42, 1998.

DEA, I.C.M.; MORRISON, A. Chemistry and interactions of seed galactomannans. **Advances in Carbohydrate Chemistry**, 31, 241-312. 1975.

DERKUS, B.; EMREGUL, K.C.; EMREGUL, E. Evaluation of protein immobilization capacity on various carbono nanotube embedded hydrogel biomaterials. **Materials Science & Engineering. C, Materials for biological applications**. 56, 132-140, 2015.

DI BERNARDINI, R.; HARNEDY, P.; BOLTON, D.; KERRY, J.; O'NEILL, E.; MULLEN, A.M.; HAYES, M. Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. **Food Chemistry**. 124(4), 1296–1307, 2011.

DIJK, C.; DRIESSEN, A.J.M.; RECOURT, K. The uncoupling efficiency and affinity of

flavonoids for vesicles. **Biochemical Pharmacology**. 60, 1593-1600, 2000.

DING, K.; WANG, H.; XU, J.; LI, T.; ZHANG, L.; DING, Y.; ZHU, L.; HE, J.; ZHOU, M. Melatonin stimulates antioxidant enzymes and reduces oxidative stress in experimental traumatic brain injury: the Nrf2–ARE signaling pathway as a potential mechanism. **Free Radical Biology and Medicine**. 73, 1-11, 2014.

DOYLE, J.P.; GIANNOULI, P.; MARTIN, E.J.; BROOKS, M., MORRIS, E.R. Effect of sugars, galactose content and chainlenght on freeze-thaw gelation of galactomannans. **Carbohydrate Polymers**, 64, 391-401, 2006.

DUARTE, A.P. Contribuição ao conhecimento da germinação de algumas essências florestais. **Rodriguésia**, 30, 56, 1979.

DUDKIEWICZ, A., TIEDE, K., LOESCHNER, K., et al. Characterization of nanomaterials in food by electron microscopy. **Trends Analyt Chem**. 30: 28-43, 2011.

DUGAS, A.J.; CASTAÑEDA-COSTA, J.; BONIN, G.C.; PRICE, K.L.; FISCHER, N.H.; WINSTON, G.W. Evaluation of the total peroxyl radical-scavenging capacity of flavonoids: structure-activity relationships. **Journal of Natural Products**. 63, 327-331, 2000.

DURAIPANDIYAN, V.; BASKAR, A.; IGNACIMUTHU, S.; MUTHUKUMAR, C.; AL-HARBI, N.A. Anticancer activity of Rhein isolated from *Cassia fistula* L. flower. **Asian Pacific Journal of Tropical Disease**, 2(1), 2012.

DURAIPANDIYAN, V.; IGNACIMUTHU, S.; PAULRAJ, M.G. Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. **Saudi Journal of Biological Sciences**, 18(2), 129-133, 2011.

EBAH. **Anatomo-fisiologia da pele**. Disponível em: <http://www.ebah.com.br/content/ABAAABqeEAC/02-anatomo-fisiologia-pele>. Acesso em: 11 de agosto de 2015.

ELCHINGER, P.; DELATTRE, C.; FAURE, S.; ROY, O.; BADEL, S.; BERNARDI, T.; TAILLEFUMIER, C.; MICHAUD, P. Immobilization of proteases on chitosan for the development of films with anti-biofilm properties**. International Journal of Biological Macromolecules**. 72, 1063-1068, 2015.

ELLIS, P.R.; DAWOUD, F.M.; MORRIS, E.R.; Blood glucose, plasma insulin and sensory responses to guar containing what breads: effects of molecular weight and particle size of guar gum. **British Journal of Nutrition,** 66, 363-379, 1991.

ENGLER, A. **Syllabus der Pflanzenfamilien.** Berlin: Gerbruder Borntraeger, 1964, 193p.

FABBRINI, E.; SERAFINI, M.; BARIC, I.C.; HAZEN, I.L.; KLEIN, S. Effect of Plasma Uric Acid on Antioxidant Capacity, Oxidative Stress, and Insulin Sensitivity in Obese Subjects. **Diabetes**. 63(3), 976-981, 2014.

FANG, R.; JING, H.; CHAI, Z.; ZHAO, G.; STOLL, S.; REN, F.; LIU, F.; LENG, X. Design and characterization of protein-quercetin bioactive nanoparticles. **Journal of Nanobiotechnology**, 9:19, 1-14, 2011.

FAZIK, M.K.; ZITE, J.A.; GOSLEN, J.B. Cicatrização das Feridas. In: COLEMAN, W.P.; HANKE, W. **Cirurgia Cosmética. Princípios e Técnicas**. 2 ed. Rio de Janeiro: Revinter, cap.3, 2000, p. 18-38.

FERREIRA, R.S.; NAPOLEÃO, T.H.; SANTOS, A.F.S.; SÁ, R.A.; CARNEIRO-DA-CUNHA, M.G.; MORAIS, M.M.C.; SILVA-LUCCA, R.A.; OLIVA, M.L.V.; COELHO, L.C.B.B.; PAIVA, P.M.G. Coagulant and antibacterial activities of the water-soluble seed lectina from *Moringa oleifera*. **Letters in Applied Microbiology**. 53(2), 186-192, 2011.

FIGUEIREDO, A. A. Extração, identificação e características do polissacarídeo da semente de algaroba (*Prosopis juliflora* DC). **Ciência e Tecnologia de Alimentos**, 3(1), 82–89, 1983.

FINKEL, T.; HOLBROOK, N.J. Oxidants, oxidative stress and the biology of ageing. **Nature**. 408(6809), 239-247, 2000.

FOUDA, M.M.G.; EL-AASSAR, M.R.; FAWAL, G.F.; HAFEZ, E.E.; MASRY, S.H.D.; ABDEL-MEGEED, A. k-Carrageenan/poly vinyl pyrollidone/polyethylene glycol/silver nanoparticles film for biomedical application. **International Journal of Biological Macromolecules**. 74, 179-184, 2015.

FREI, B.; BALTISBERGER, M.; STICHER, O.; HEINRICH, M. Medical ethnobotany of the Zapotecs of the Isthmus-Sierra (Oaxaca, Mexico): Documentation and assessment of indigenous uses. **Journal of Ethnopharmacology**, 62, 149-165, 1998.

GANTER, J.L.M.S. **Galactomanana de sementes de *Mimosa scabrella* (Bracatinga)**. Tese (Mestrado em Bioquímica) - Departamento de Bioquímica, Universidade Federal do Paraná. Curitiba, 1988.

GARCÍA, M.A.; PÉREZ, L.; PAZ, N.; GONZÁLEZ, J.; RAPADO, M.; CASARIEGO, A.

Effect of molecular weight reduction by gamma irradiation on chitosan film properties. **Materials Science and Engineering: C**. 55, 174-180, 2015.

GARCÍA-MONTOYA, I.A.; CENDÓN, T.S.; ARÉVALO-GALLEGOS. S.; RASCÓN-CRUZ, Q. Lactoferrin a multiple bioactive protein: An overview. **Biochimica et Biophysica Acta**. 1820, 226–236, 2012.

GEHRKE, S.H.; LEE, P.I. (1990) Hydrogels for drug delivery systems. In: TYLE, P. **Specialized Drug Delivery Systems**, Marcel Dekker, 1990, p. 333-392.

GENNADIOS, A.; WELLER, C. L.; GOODING, C. H. Measurement errors in water vapor permeability of highly permeable, hydrophilic edible films. **Journal of Food Engineering**. 21: 395–409, 1994.

GENSKOWSKY, E.; PUENTE, L.A.; PÉREZ-ÁLVAREZ, J.A.; FERNANDEZ-LOPEZ, J.; MUÑOZ, L.A.; VIUDA-MARTOS, M.. Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). **LWT - Food Science and Technology**. 64(2), 1057-1062, 2015.

GIDLEY, M.J.; REID, J.S.G. Galactomannans and other cell wall storage polysaccharides in seeds. **Food polysaccharides and their applications**. 2 ed. Boca Raton: CRC Press, 2006.

GOMATHI, K.; GOPINATH, D.; AHMED, M.R.; JAYAKUMAR, R. Quercetin incorporated collagen matrices for dermal wound healing processes in rat. **Biomaterials**. 24(16), 2767-2772, 2003.

GÓMEZ-ESTACA, J.; BRAVO, L.; GÓMEZ-GUILLÉN, M.C.; ALEMÁN, A.; MONTERO, P. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. **Food Chemistry**. 112, 18-25, 2009.

GÓMEZ-ESTACA, J.; CALVO, M.M.; SÁNCHEZ-FAURE, A.; MONTERO, P.; GÓMEZ-GUILLÉN, M.C. Development, properties, and stability of antioxidant shrimp muscle protein films incorporating carotenoid-containing extracts from food by-products. **LWT - Food Science and Technology**. 64(1), 189-196, 2015.

GÓMEZ-ORDÓÑEZ, EV.; RUPÉREZ, P. FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. **Food Hydrocolloids**, 25, 1514-1520, 2011.

GONÇALVES, M.P.; GOMES, C.; LANGDON, M.J.; VIEBKE, C.; WILLIAMS, P.A.

Studies on k-carrageenan/locust bean gum mixtures in the presence of sodium chloride and sodium iodide. **Biopolymers**, 41(6), 657-671, 1997.

GONZÁLEZ-CHÁVEZ, S.A.; ARÉVALO-GALLEGOS, S.; RASCÓN-CRUZ, Q. Lactoferrin: structure, function and applications. **International Journal of Antimicrobial Agents**. 33, 301.e1–301.e8, 2009.

GOYCOOLEA, F.M.; MORRIS, E.R.; GIDLEY, M.J. Viscosity of galactomannans at alkaline and neutral pH: evidence of hyperentanglement in solution. **Carbohydrate Polymers**, 27, 69–71, 1995.

GRISEL, M.; AGUNI, Y.; RENOU, F.; MALHIAC, C. Impact of fine structure of galactomannans on their interactions with xanthan: Two co-existing mechanisms to explain the synergy. **Food Hydrocolloids**. 51, 449-458, 2015.

GUPTA, P.; VERMANI, K.; GARG, S. Hydrogels: from controlled release to pH-responsive drug delivery. **Drug Discovery Today**. 7(10), 569-579, 2002.

HADDAD, M.C.L.; BRUSCHI, L.C.; MARTINS, E.A.P. Influência do açúcar no processo de cicatrização de incisões cirúrgicas infectadas. **Revista latino-americana de Enfermagem.** 8(1), 57-65, 2000.

HAM, S.A.; YOO, T.; HWANG, J.S.; KANG, E.S.; PAEK, K.S.; PARK, C.; KIM, J.; DO, J.T.; SEO, H.G. Peroxisome proliferator-activated receptor δ modulates MMP-2 secretion and elastin expression in human dermal fibroblasts exposed to ultraviolet B radiation. **Journal of Dermatological Science**. 76(1), 44-50, 2014.

HEGNAUER, R.; GRAYER-BARKMEUER, R.J. Relevance of seed polysaccharides and flavonoids for the classification of the Leguminosae: a chemotaxonomic approach. **Phytochemistry**, 34(1), 3-16, 1993.

HERNÁNDEZ-CASTRO, C.; DIAZ-CASTILLO, F.; MARTÍNEZ-GUTIERREZ, M. Ethanol extracts of *Cassia grandis* and *Tabernaemontana cymosa* inhibit the *in vitro* replication of dengue virus serotype 2**. Asian Pacific Journal of Tropical Disease**. 5(2), 98-106, 2015.

HERTOG, M.G.L.; HOLLMAN, P.C.H.; Content of potentially anticarcinogenic flavonoids in tea, wine and fruit juice. **Journal Agriculture Food Chemistry.** 41, 1241-1246, 1993.

HOFFBRAND, A.V.; PETTIT, J.E.; MOSS, P.A.H. Fundamentos em hematologia. Porto Alegre: Artmed, 2004.

HOFFMAN, A.S.; HUBBELL, J.A. Surface immobilized biomolecules. In: RATNER,

B.D. **Biomaterials Science: An introduction to materials in medicine**. Part I: Materials Science and engineering. Chapter II: Classes of materials used in medicine. China: Academic Press, 2004, p. 225-233.

HWANG, S.-A.; KRUZEL, M.L.; ACTOR, J.K. Recombinant human lactoferrin modulates human PBMC derived macrophage responses to BCG and LPS. Tuberculosis. 101, 53-62, 2016.

ILAN, N.; MAHOOTI, S.; MADRI, J.A. Distinct signal transduction pathways are utilized during the tube formation and survival phases of *in vitro* angiogenesis. **Journal of Cell Science.** 11, 3621-3631, 1998.

ITAHANA, Y.; HAN, R.; BARBIER, S.; LEI, Z.; ROZEN, S.; ITAHANA, K. The uric acid transporter SLC2A9 is a direct target gene of the tumor suppressor p53 contributing to antioxidant defense. **Oncogene**. 34, 1799-1810, 2015.

ITO, T.; YOSHIDA, C.; MURAKAMI, Y., Design of novel sheet-shaped chitosan hydrogel for wound healing: A hybrid biomaterial consisting of both PEG-grafted chitosan and crosslinkable polymeric micelles acting as drug containers. **Materials Science and Engineering: C**. 33(7), 3697-3703, 2013.

JOLY, A.B. **Botânica: Introdução à Taxonomia Vegetal.** São Paulo: Companhia Editorial nacional, 1998, 777p.

KARTHIKEYAN, S.; SRIDHAR, M.; RAMAJAYAM, G.; LAVANYA, R.; ARUNAKARAN, J.; SRINIVASA, N. Polychlorinated biphenyl (PCBs)-induced oxidative stress plays a role on vertebral antioxidant system: Ameliorative role ofvitamin C and E in male Wistar rats. **Biomedicine & Preventive Nutrition**. 4(3), 411-416, 2014.

KATO, Y.; ONISHI, H.; MACHIDA, Y. Application of chitin and chitosan derivatives in the pharmaceutical field. **Current Pharmaceutical Biotechnology.** 4(5), 303–9, 2003.

KENNEDY, J.F., PAIVA, P.M.G., CORREIA, M.T.S., CAVALCANTI, M.S.M.; COELHO, L.C.B.B. Lectins, versatile proteins of recognition: a review. **Carbohydrate Polymers.** 26, 219–30, 1995.

KLANG, V.; VALENTA, C.; MATSKO, N. B. Electron microscopy of pharmaceutical systems. **Micron**. 44: 45–74, 2013.

KIM, S.E.; YUN, Y.-P.; LEE, J.Y.; PARK, K.; SUH, D.H. Osteoblast activity of MG-63 cells is enhanced by growth on a lactoferrin-immobilized titanium substrate.

Colloids and Surfaces B: Biointerfaces. 123, 191-198, 2014.

KIM, S.R.B.; CHOI, Y.; KIM, J.; LIM, S. Improvement of water solubility and humidity stability of tapioca starch film by incorporating various gums. **LWT - Food Science and Technology**.64(1), 475-482, 2015.

KLECH, C.M. Gels and jellies. In: SWARBRICK, J.; BOYLAN, J.C. **Encyclopedia of Pharmaceutical Technology**, Marcel Dekker, 1990, p. 415–439.

KOHEN, R. Skin antioxidants: their role in aging and in oxidative stress – new approaches for their evaluation. **Biomedicine and Pharmacotherapy**. 53, 181-92, 1999.

KOMPELLA, U.B.; LEE, V.H.L. Delivery systems for penetration enhancement of peptide and protein drugs: design considerations. **Advanced Drug Delivery Reviews**. 46, 211–245, 2001.

KOOP, H.S.; PRAES, C.E.D.O.; REICHER, F.; PETKOWICZ, C.L.D.O.; SILVEIRA, J.L.M. Rheological behavior of gel of xanthan with seed galactomannan: Effect of hydroalcoholic-ascorbic acid. **Materials Science and Engineering C**. 29(2), 559–563, 2009.

KOOPMANN, C. Cutaneous wound healing. Otolaringologic Clinics of North America. 28(5), 835-845, 1995.

KOWALCZYK, D., KORDOWSKA-WIATER, M., NOWAK, J., BARANIAK, B. Characterization of films based on chitosan lactate and its blends with oxidized starch and gelatin, **International Journal of Biological Macromolecules**. 77: 350–359, 2015.

KROCHTA, J.M. Proteins as raw materials for films and coatings: definitions, current status, and opportunities. In: GENNADIOS, A. **Protein-based films and coatings**, Boca Raton: CRC press, 2002, p. 367.

KUMAR, V.; ABBAS, A.K.; FAUSTO, N.;ROBBINS, S.L. **Patologia – Bases patológicas das doenças.** Rio de Janeiro: Elsevier, 2005, 1592 p.

LAFARGA, T.; HAYES, M. Bioactive peptides frommeatmuscle and by-products: generation, functionality and application as functional ingredientes. Meat Science. 98, 227-239, 2014.

LAN, C.E.; WU C.; YU, H. Solar-simulated radiation and heat treatment induced metalloproteinase-1 expression in cultured dermal fibroblasts via distinct pathways: Implications on reduction of sun-associated aging. **Journal of**

Dermatological Science. 72, 290–295, 2013.

LANZA, V.; D'AGATA, R.; IACONO, G.; BELLIA, F.; SPOTO, G.; VECCHIO, G. Cyclam glycoconjugates as lectin ligands and protective agents of metal-induced amyloid aggregation. **Journal of Inorganic Biochemistry** (In Press, Corrected Proof, Available online 19 June 2015).

LAURI, A.; POMPILIO, G.; CAPOGROSSI, M.C. The mitochondrial genome in aging and senescence. **Ageing Research Reviews**. 18, 1-15, 2014.

LEE, M.; JEONG, N.; JANG, B. Antioxidative activity and antiaging effect of carrot glycoprotein. **Journal of Industrial and Engineering Chemistry**. 25, 216-221, 2015.

LEE, T.; JEONG, Y.G. Regenerated cellulose/multiwalled carbon nanotube composite films with efficient electric heating performance. **Carbohydrate Polymers**. 133, 456-463, 2015.

LENTZ, D.L.; CLARK, A.M.; HUFFORD, C.D.; MEURER-GRIMES, B.; PASSREITER, C.M.; CORDERO, J.; IBRAHIMI, O.; OKUNADE, A.L. Antimicrobial properties of Honduran medicinal plants. **Journal of Ethnopharmacology**, 63, 253-256, 1998.

LEWIS, G.P.; SCHRIRE, B.D.; MACKINDER, B.; LOCK, M. Legumes of the World. **The Royal Botanic Gardens.** Kew, p. 577, 2005.

LEWIS, K.G.; BERCOVITCH, L.; DILL, L.S.W.; ROBINSON-BOSTOM, L. Acquired disorders of elastic tissue: part I. Increased elastic tissue and solar elastotic syndromes. **Journal of the American Academy Dermatology**. 51, 1-21. 2004.

LI-CHAN, E.C. Bioactive peptides and protein hydrolysates: Research trends and challenges for application as nutraceuticals and functional food ingredients. Current Opinion in Food Science. 1, 28–37, 2015.

LI, M.; VIERKÖTTER, A.; SCHIKOWSKI, T.; HÜLS, A.; DING, A.; MATSUI, M.S.; DENG, B.; MA, C.; REN, A.; ZHANG, H.; TAN, J.; YANG, Y.; JIN, L.; KRUTMANN, J.; LI, Z.; WANG, S. Epidemiological evidence that indoor air pollution from cooking with solid fuels accelerates skin aging in Chinese women. **Journal of Dermatological Science**. 79(2), 148-154, 2015.

LIENER, I.E.; SHARON, N.; GOLDSTEIN, I.J. Preface in: LIENER, I.E.; SHARON, N.; GOLDSTEIN, I.J. (Eds.). **The lectins: Properties, functions and applications in biology and medicine**, Orlando: Academic Press, 1986. p. xi-vv.

LIMA, A.M.; CERQUEIRA, M.A.; SOUZA, B.W.S.; SANTOS, E.C.M.; TEIXEIRA, J.A.; MOREIRA, R.A.; VICENTE, A.A. New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits – Influence on fruits gas transfer rate. **Journal of Food Engineering**. 97(1), 101-109, 2010.

LIMA, D.U.; OLIVEIRA, R.C.; BUCKERIDGE, M.S. Seed storage hemicelluloses as wet-end additives in paper making. **Carbohydrate Polymers**, 52, 367-373, 2003.

LIMA, V. L. M., CORREIA, M. T. S., CECHINEL, Y. M. N., SAMPAIO, C. A. M., OWEN, J. S. AND COELHO, L. C. B. B. Immobilized *Cratylia mollis* lectin as a potential matrix to isolate plasma glycoproteins, including lecithin–cholesterol acyltransferase. **Carbohydrate Polymers**. 33, 27–32, 1997.

LIN, J.-Y.; SELIM, M.A.; SHEA, C.R.; GRICHNIK, J.M.; OMAR, M.M.; MONTEIRO-RIVIERE, N.A.; PINNELL, S.R. UV photoprotection by combination topical antioxidants vitamin C and vitamin E. **Journal of the American Academy of Dermatology**. 48(6), 866-874, 2003.

LIPTON, P. Ischemic cell death in brain neurons. **Physiological Reviews**. 79(4), 1431-1568, 1999.

LIV, N., LAZIĆ, I., KRUIT, P. AND HOOGENBOOM, J. P. Scanning electron microscopy of individual nanoparticle bio-markers in liquid. **Ultramicroscopy**. 143: 93–9, 2014.

LODHA, S.R.; JOSHI, S.V.; VYAS, B.A.; UPADHYE, M.C.; KIRVE, M.S.; SALUNKE, S.S.; KADU, S.K.; ROGYE, M.V. Assessment of the antidiabetic potential of *Cassia grandis* using an *in vivo* model. **Journal of Advanced Pharmaceutical Technology & Research**, 1(3), 330-333, 2010.

LUCYSZYN, N.; QUORIN, M.; RIBAS, L. F.; KOEHLER, H. S.; REICHER, F., SIERAKOWSKI, M-R. Micropropagation of *Durondeau* pear in modified – gelled medium. **In Vitro Cellular & Developmental Biology – Plant**, 42, 287-290. 2006.

MA, Q., HU, D., WANG, H., WANG, L. Tara gum edible fi lm incorporated with oleic acid. **Food Hydrocolloids.** 56: 127–133, 2016.

MACIEL, E.V.M.; ARAÚJO-FILHO, V.S.; NAKAZAWA, M.; GOMES, Y.M.; COELHO, L.C.B.B.; CORREIA, M.T.S. Mitogenic activity of *Cratylia mollis* lectin on human lymphocytes. **Biology**, 32, 57–60, 2004.

MAIN, P.A.; ANGLEY, M.T.; O'DOHERTY, C.E.; THOMAS, P.; FENECH, M. The potential role of the antioxidant and detoxification properties of glutathione in autism spectrum disorders: a systematic review and meta-analysis. **Nutrition & Metabolism**. 9(35), 1-37, 2012.

MAKRI, E.A.; DOXASTAKIS, G.I. Study of emulsions stabilized with Phaseolus vulgaris or *Phaseolus coccineus* with the addition of Arabic gum, locust bean gum and xanthan gum. **Food Hydrocolloids**. 20(8), 1141–1152, 2006.

MALVERN, ZETA SIZER NANO. **Series User Manual Mano,** 317, 270p, 2004.

MANDELBAUM, S.H.; DI SANTIS, E.P.; MANDELBAUM, M.H.S. Cicatrização: conceitos atuais e recursos auxiliares – Parte I. **Anais Brasileiros de Dermatologia**. 78(4), 393-410, 2003.

MANDELBAUM, S.H.; DI SANTIS, E.P.; MANDELBAUM, M.H.S. Cicatrização: conceitos atuais e recursos auxiliares – Parte II. **Anais Brasileiros de Dermatologia**. 78(5), 525-542, 2003.

MARS, M.; STAFLEU, A.; DE GRAAF, C. Use of satiety peptides in assessing the satiatingcapacityof foods. Physiology & Behavior. 105(2), 483–488, 2012.

MARTIN, P. Wound healing – aiming for perfect skin regeneration. **Science**. 276, 74-81, 1997.

MARTINS, J. Amigo da pressa. **Ciência Hoje**. 33(196), 42-43, 2003.

MARTINS, J.T.; CERQUEIRA, M.A.; BOURBON, A.I.; PINHEIRO, A.C.; SOUZA B.W.S.; VICENTE, A.A. Synergistic effects between k-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. **Food Hydrocolloids**, 29, 280-289, 2012.

MARTINS, J.T.; CERQUEIRA, M.A.; SOUZA, B.W.S.; AVIDES, M.C.; VICENTE, A.A. Shelf life extension of ricotta cheese using coatings of galactomannans from nonconventional sources incorporating nisin against Listeria monocytogenes. **Journal of Agricultural and Food Chemistry**. 58(3), 1884-1891, 2010.

MASCARAQUE, C.; ARANDA, C.; OCÓN, B.; MONTE, M.J.; SUÁREZ, M.D.; ZARZUELO, A.; MARÍN, J.J.G.; MARTÍNEZ-AUGUSTIN, O.; MEDINA, F.S. Rutin has intestinal antiinflammatory effects in the CD4+ CD62L+ T cell transfer model of colitis. **Pharmacological Research**. 90, 48-57, 2014.

MATHLOUTHI, M.; KOENIG, J.L. Vibrational spectra of carbohydrates. **Advances in Carbohydrate Chemistry and Biochemistry**, 44, 82-89, 1987.

MAUCH, C.; HERRON, G.S.; BAUER, E.A. Regulation of connective tissue turnover by matrix metalloproteinases. In: FREINKEL, R.K.; WOODLEY, D. **The Biology of the Skin**. New York: The Parthenon Publishing Group, 2001, p. 353-64.

McCLEARY, B.V.; AMADO, R.; WAIBEL, R.; NEUKOM, H. Effect of galactose content on the solution and interaction properties of guar and carob galactomannans. **Carbohydrate Research**, 92, 269-285, 1981.

MEDEIROS, B.G.S.; PINHEIRO, A.C.; TEIXEIRA, J.A.; VICENTE, A.A.; CARNEIRO-DA-CUNHA, M.G. Polysaccharide/Protein Nanomultilayer Coatings: Construction, Characterization and Evaluation of Their Effect on 'Rocha' Pear (Pyrus communis L.) Shelf-Life. Food and Bioprocess Technology. 5(6), 2435-2445, 2012.

MELO, C.M.L.; LIMA, A.L.R.; BELTRÃO, E.I.C.; CAVALCANTI, C.C.B.; MELO-JÚNIOR, M.R.; MONTENEGRO, S.M.L.; COELHO, L.C.B.B.; CORREIA, M.T.S.; CARNEIRO-LEÃO, A.M.A. Potential effects of Cramolll 1,4 lectin on murine *Schistosomiasis mansoni*. **Acta Tropica**. 118, 152–158, 2011.

MELO, C.M.L.; MELO, H.; CORREIA, M.T.S.; COELHO, L.C.B.B.; SILVA, M.B.; PEREIRA, V.R.A. Mitogenic response and cytokine production induced by cramoll 1,4 lectin in splenocytes of inoculated mice. **Scandinavian Journal of Immunology.** 73, 112–121, 2010b.

MELO, C.M.L.; PAIM, B.A.; ZECCHIN, K.G.; MORARI, J.; CHIARRATI, M.R.; CORREIA, M.T.S.; COELHO, L.C.B.B.; PAIVA, P.M.G. Cramoll 1,4 lectin increases ROS production, calcium levels and cytokine expression in treated spleen cells of rats. **Molecular and Cellular Biochemistry**. 342, 163-169, 2010a.

MENEGUIN, R.G. **Géis de ágar /galactomanana de *schizolobium parahybae* ((vell.) blake) (guapuruvu): análises reológicas e por microscopia eletrônica de varredura.** 2012. 101p. Dissertação (Mestrado em Ciências – Bioquímica). Universidade Federal do Paraná, Curitiba, 2012.

MENSAH, J.M.; IACOMINI, M.; JONES, C.; GORIN, P.A.J.; Similarity of monosaccharide, oligosaccharide and polysaccharide structures in gum exudate of *Anacardium occidentale*. **Phytochemistry**. 47, 715-721, 1998.

MENZEL, C.; ANDERSSON, M.; ANDERSSON, R.; VÁZQUEZ-GUTIÉRREZ, J.L.; DANIEL, G.; LANGTON, M.; GÄLLSTEDT, M.; KOCH, K. Improved material

properties of solution-cast starch films: Effect of varying amylopectin structure and amylose content ofstarch from genetically modified potatoes. **Carbohydrate Polymers**. 130, 388-397, 2015.

MIGNATTI, P.; RIFKIN, D.B.; WELGUS, H.G.; PARKS, W.C. Proteinases and tissue remodelling. In: CLARK, R.A.F. **The molecular and cellular biology of wound repair**. New York: Plenum, 1996, p. 171-194.

MODOLIN, M.; BEVILACQUA, R.G.. Cicatrização das feridas: síntese das aquisições recentes. **Revista Brasileira de Clínica e Terapêutica**. 14(6), 55-64, 1985.

MOGOSANUA, G.D.; GRUMEZESCU, A.M. Natural and synthetic polymers for wounds and burns dressing. **International Journal of Pharmaceutics**. 463, 127–136, 2014.

MOMOHA, F.U.; BOATENGA, J.S.; RICHARDSON, S.C.W.; CHOWDHRY, B.Z.; MITCHELL, J.C.; 2015. Development and functional characterization of alginate dressing as potential protein delivery system for wound healing. **International Journal of Biological Macromolecules**. 81, 137–150, 2015.

MONTEIRO, F.M.F.; SILVA, G.M.M.; SILVA, J.B.R.; PORTO, C.S.; CARVALHO JÚNIOR, L.B.; LIMA FILHO, J.L.; CARNEIRO-LEÃO, A.M.A.; CARNEIRO-DA-CUNHA, M.G.; PORTO, A.L.F. Immobilization of trypsin on polysaccharide film from *Anacardium occidentale* L. and its application as cutaneous dressing. **Process Biochemistry**. 42, 884–888, 2007.

MORGADO, P.I.; AGUIAR-RICARDO, A.; CORREIA, I.J. Asymmetric membranes as ideal wound dressings: An overview on production methods, structure, properties and performance relationship. **Journal of Membrane Science**. 490, 139–151, 2015.

MUTCHNICK, P.A.; McCARTHY, B.C. An ethnobotanical analysis of the three species commom to the subtropical moist forests of the Petén, Guatemala. **Economic Botany**, 51(2), 158-183, 1997.

NASERI, N.; ALGAN, C.; JACOBS, V.; JOHN, M.; OKSMAN, K.; MATHEW, A.P. Electrospun chitosan-based nanocomposite mats reinforced with chitin nanocrystals for wound dressing. **Carbohydrate Polymers**. 109, 7-15, 2014.

NEWMAN, R.H.; HEMMINGSON, J.A. Interactions between locust bean gum and cellulose characterized by 13C n.m.r. spectroscopy. **Carbohydrate Polymers**, 36, 167-172, 1998.

NICOLAY, J.F.; PAILLET, C. Poluição e envelhecimento: nova classe de antioxidantes para a Pele. **Cosmetics & Toiletries**. 14(2), 58-63. 2002.

NOLTE, S.; DAMASIO, D.C.; BARÉA, A.C.; GOMES, J.; MAGALHÃES, A.; ZISCHLER, L.F.C.M.; STUELP-CAMPELO, P.M.; ELÍFIO-ESPOSITO, S.L.; ROQUE-BARREIRA, M.C.; REIS, C.A.; MORENO-AMARAL, A.N. BJcuL, a lectin purified from *Bothrops jararacussu* venom, induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly**. Toxicon**. 59(1), 81-85, 2012.

NONGONIERMA, A.B.; FITZGERALD, R.J. The scientific evidence for the role of milk protein-derived bioactive peptides in humans: a Review. Journal of Functional Foods. 17, 640–656, 2015a.

NONGONIERMA, A.B.; FITZGERALD, R.J. Utilisation of the isobole methodology to study dietary peptide–drug and peptide–peptide interactive effects on dipeptidyl peptidase IV (DPP-IV) inhibition. Food & Function. 6, 312–319, 2015b.

NOUVEAU-RICHARD, S.; YANG, Z.; MAC-MARY, S.; LI, L.; BASTIEN, P.; TARDY, I.; BOUILLON, C.; HUMBERT, P.; DE LACHARRIÈRE, O. Skin ageing: a comparison between Chinese and European populations. A pilot study. **Journal of Dermatological Science**. 40, 187-193, 2005.

NTCHAPDA, F.; BARAMA, J.; AZAMBOU, D.R.K.; ETET, P.F.S.; DIMO, T. Diuretic and antioxidante activities of the aqueous extract of leaves of *Cassia occidentalis* (Linn.) in rats. **Asian Pacific Journal of Tropical Medicine**. **In Press, Accepted Manuscript**, Available online 6 August 2015.

OHSHIMA, H.; YOSHIE, Y.; AURIOL, S.; GILIBERT, I. Antioxidant and pro-oxidant actions of flavonoids: effects on DNA damage induced by nitric oxide, peroxinitrite and nitroxyl anion. **Free Radical Biology & Medicina**. 25(9), 1057-1065, 1998.

OLIVAS, G.I.; BARBOSA-CÁNOVAS, G.V. Edible coatings for fresh-cut fruits. **Critical reviews in food science and nutrition**. 45: 657-670, 2005.

OLIVEIRA, C.F.R.; LUZ, L.A.; PAIVA, P.M.G.; COELHO, L.C.B.B.; MARANGONI, S.; MACEDO, M.L.R. Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with potential for the control ofinsects**. Process Biochemistry**, 46 (2), 498-504, 2011.

ONO, L.; WOLLINGER, W.; ROCCO, I.M.; COIMBRA, T.L.M.; GORIN, P.A.J.; SIERAKOWSKI, M.R. In vitro and in vivo antiviral properties of sulfated

galactomannans against yellow fever virus (BeH111 strain) and dengue 1 virus (Hawaii strain). **Antiviral Research**, 60, 201-208, 2003.

PAIVA, P. M. G.; COELHO, L. C. B. B. Purification and partial characterization of two lectin isoforms from *Cratylia mollis* Mart. (Camaratu bean). **Applied Biochemistry and Biotechnology**. 36, 113–17, 1992.

PARK, J.E.; BARBUL, A. Understanding the role of immune regulation in wound healing. **The American Journal of Surgery**. 187, 11S-16S, 2004.

PARRA, A.L.; SARDIÑAS, M.I.G. Toxicidad aguda oral de 3 formas farmacéuticas a partir de *Cassia grandis* L. Centro de Investigación y Desarrollo de Medicamentos (CIDEM), Departamento de Investigaciones Biológicas. **Revista Cubana de Plantas Medicinais**, 5(2), 68-70, 2000.

PAWAR, H.A.; LALITHA, K.G. Isolation, purification and characterization of galactomannans as an excipient from Senna tora seeds. **International Journal of Biological Macromolecules**. 65, 167-175, 2014.

PERALTA, M.A.; SILVA, M.A.; ORTEGA, M.G.; CABRERA, J.L.; PARAJE, M.G. Antifungal activity of a prenylated flavonoid from *Dalea elegans* against *Candida albicans* biofilms. **Phytomedicine**, In Press, Uncorrected Proof, Available online 26 July 2015.

PEREIRA, L.; AMADO, A.M.; CRITCHLEY, A.T.; VAN DE VELDE, F.; RIBEIRO-CLARO, P.J.A. Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). **Food Hydrocolloids**, 23(7), 1903-1909, 2009.

PEREIRA, L.; SOUSA, A.; COELHO, H.; AMADO, A.M.; RIBEIRO-CLARO, P.J.A. Use of FTIR, FT-Raman and 13C-NMR spectroscopy for identification of some seaweed phycocolloids. **Biomolecular Engineering**, 20(4 e 6), 223-228, 2003.

PEREIRA-NETTO, A. B.; MENEGUIN, R. G.; BIZ, A.; SILVEIRA, J. L. M. A galactomannan-driven enhancement of the in vitro multiplication rate for the maubakaido apple rootstock (*Malus prunifolia* (Willd.) Borkh) is not related to the polysaccharide degradation. **Applied Biochemistry Biotechnology**, 166(1), 197-207, 2012.

PIIRONEN, V.; LINDSAY, D.G.; MIETTINEN, T.A.; TOIVO, J.; LAMPI, A.-M. Plant sterols: biosynthesis, biological function and their importance to human nutrition. **Journal of the science of Food and Agriculture**. 80, 939-966, 2000.

PINHEIRO, A.C.; BOURBON, A.I.; ROCHA, C;, RIBEIRO, C.; MAIA, J.M.; GONÇALVES, M.P., et al. Rheological characterization of [kappa]-carrageenan/galactomannan and xanthan/galactomannan gels: comparison of galactomannans from non-traditional sources with conventional galactomannans. **Carbohydrate Polymers**, 83(2), 392-399, 2011.

PINHEIRO, A. C.; CERQUEIRA, M. A.; SOUZA, B. W. S.; MARTINS, J. T.; TEIXEIRA, J. A.; VICENTE, A. A. Utilização de revestimentos/filmes edíveis para aplicações alimentares. **Boletim de Biotecnologia**. 18-28, 2010.

POLLARD, M.A.; EDER, B.; FISCHER, P.; WINDHAB, E.J. Characterization of galactomannans isolated from legume endosperms of Caesalpinioideae and Faboideae subfamilies by multidetection aqueous SEC. **Carbohydrate Polymers.** 79, 70-84, 2010.

POOL, H.; QUINTANAR, D.; FIGUEROA, J.D.; MANO, C.M.; BECHARA, J.E.H.; GODINEZ, L.A.; MENDOZA, S. Antioxidant Effects of Quercetin and Catechin Encapsulated into PLGA Nanoparticles. **Journal of Nanomaterials**. 2012, 1-12, 2012.

PORTUGAL, M.; BARAK, V.; GINSBURG, I.; KOHEN, R. Interplay among oxidants, antioxidants, and cytokines in skin disorders: Present status and future considerations. **Biomedicine and Pharmacotherapy**. 61, 412-422, 2007.

QU, M.; TONG, C.; KONG, L.; YAN, X.; CHERNIKOV, O.V.; LUKYANOV, P.A.; JIN, Q.; LI, W. Purification of a secreted lectin from *Andrias davidianus* skin and its antibacterial activity. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**. 167, 140-146, 2015.

RACHMAN, A.B.; MAHESWARI, R.R.A.; BACHROEM, M.S. Composition and isolation of lactoferrin from colostrum and milk of various goat breeds. Procedia Food Science, 3, 200-210, 2015.

RATNAM, D.V.; ANKOLA, D.D.; BHARDWAJ, V.; SAHANA, D.K.; KUMAR, M.N.V.R. Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. **Journal of Controlled Release**. 113, 189-207, 2006.

RAVEL, R. **Laboratório Clínico: Aplicações Clínicas dos Dados Laboratoriais**. 6. ed. Rio de Janeiro: Guanabara Koogan, 1997. p. 90-91.

RAVICHANDRAN, R.; RAJENDRAN, M.; DEVAPIRIAM, D. Antioxidant study of quercetin and their metal complex and determination of stability constant by

spectrophotometry method. **Food Chemistry.** 146, 472–478, 2014.

RAWLINGS, A.V.; MATTS, P.J.; ANDERSON, C.D.; ROBERTS, M.S. Skin biology, xerosis, barrier repair and measurement. **Drug Discovery Today**. 5(2), 127-136, 2008.

RECHIA-VARGAS, C. G. Polysaccharides from the of *Senna multijuga*. **International Journal of Biological Macromolecules**, 17(6), 409-412, 1995.

REID, J.S.G.; EDWARDS, M.E. Galactomannans and other cell wall storage polysaccharidesin seeds. In: STEPHEN, A.M. **Food Polysaccharides and Their Applications**. New York: Marcel Dekker, Inc., 1995. p. 155-186.

RENDÓN-RAMÍREZ, A.; MALDONADO-VEGA, M.; QUINTANAR-ESCORZA, M.; HERNÁNDEZ, G.; ARÉVALO-RIVAS, B.; ZENTELLA-DEHESA, A.; CALDERÓN-SALINAS, J. Effect of vitamin E and C supplementation on oxidative damage and total antioxidant capacity in lead-exposed workers. **Environmental Toxicology and Pharmacology**. 37(1), 45-54, 2014.

RHIM, J.; WANG, L. Preparation and characterization of carrageenan-based nanocomposite films reinforced with clay mineral and silver nanoparticles**.** **Applied Clay Science**. 97–98, 174-181, 2014.

RIGO, L.A.; SILVA, C.R.; OLIVEIRA, S.M.; CABREIRA, T.N.; SILVA, C.B.; FERREIRA, J.; BECK, R.C.R. Nanoencapsulation of rice bran oil increases its protective effects against UVB radiation-induced skin injury in mice. **European Journal of Pharmaceutics and Biopharmaceutics**. 93, 11-17, 2015.

ROCHA, L.C.; SOUZA, A.L.; RODRIGUES FILHO, U.P.; CAMPANA FILHOA, S.P.; SETTE, L.D.; PORTO, A.L.M. Immobilization of marine fungi on silica gel, silica xerogel and chitosan for biocatalytic reduction of ketones. **Journal of Molecular Catalysis B: Enzymatic**. 84, 160–165, 2012.

ROSA, I.G.; SOUZA, N.S.; SANTANA, A.A.; LIMA, H.S. Extração e caracterização fisico-química dos polissacarideos de *Leucaena leucocephala* (Lam.) DE Wit. **Pesquisa em Foco**, 17(1), 22-30, 2009.

ROSALIE, R.; JOAS, J.; DEYTIEUX-BELLEAU, C.; VULCAIN, E.; PAYET, B.; DUFOSSÉ, L.; LÉCHAUDEL, M. Antioxidant and enzymatic responses to oxidative stress induced by pre-harvest water supply reduction and ripening on mango (*Mangifera indica* L. cv. ‘Cogshall’) in relation to carotenoid content. **Journal of Plant Physiology**. 184, 68-78, 2015.

SAADI, S.; SAARI, N.; ANWAR, F.; HAMID, A.A.; GHAZALI, H.M. Recent advances in food biopeptides: Production, biological functionalities and therapeutic applications. Biotechnology Advances. 33, 80–116, 2015.

SABERI, A. H., FANG, Y., & MCCLEMENTS, D. J. Fabrication of vitamin E-enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. **Journal of Colloid and Interface Science**. 391: 95-102, 2013.

SAIT, S.; HAMRI-ZEGHICHI, S.; BOULEKBACHE-MAKHLOUF, L.; MADANI, K.; RIGOU, P.; BRIGHENTI, V.; PRENCIPE, F.P.; BENVENUTI, S.; PELLATI, F. HPLC-UV/DAD and ESI-MS^n analysis of flavonoids and antioxidant activity of an Algerian medicinal plant: *Paronychia argentea* Lam. **Journal of Pharmaceutical and Biomedical Analysis**. 111, 231-240, 2015.

SALINAS, B.E.; GRIJALVA, A. Diagnóstico de Nicarágua. In: Ocampo, R.A. (ed.). **Domesticación de plantas medicinales em Centroamérica**. Turrialba: CATIE/OEA, 1994. 135p.

SANTANA, R.; MONTAGNINI, F.; LOUMAN, B.; VILLALOBOS, R.; GOMEZ, M. Secondary forest products from Southern Nicarágua with potential for their use in Masaya crafts. **Revista Florestal Centroamericana**, 38, 85-90, 2002.

SARAVANAKUMAR, A.; GANESH, M.; JAYAPRAKASH, J.; JANG, H.T. Biosynthesis of silver nanoparticles using *Cassia tora* leaf extract and its antioxidant and antibacterial activities. **Journal of Industrial and Engineering Chemistry**. 28, 277-281, 2015.

SARI, T.P.; MANN, B.; KUMAR, R.; SINGH, R.R.B.; SHARMA, R.; BHARDWAJ, M.; ATHIRA, S. Preparation and characterization of nanoemulsion encapsulating curcumin. **Food Hydrocolloids**. 43, 540-546, 2015.

SASAKI, T.; KISHI, S. Molecular and chemical genetic approaches to developmental origins of aging and disease in zebrafish. **Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease**. 1832(9), 1362-1370, 2013.

SCHAEFER, H.; REDELMEIER, T.E. **Skin barrier - principles of percutaneous Absorption**. Basel-Suíça: Editora Karger. p. 13-15, 1996.

SCHIERMEIER, S.; SCHMIDT, P.C. Fast dispersible ibuprofen tablets. **European Journal of Pharmaceutical Sciences**, 15, 295-305, 2002.

SCHIRATO, G.V.; MONTEIRO, F.M.F.; SILVA, F.O.; LIMA FILHO, J.L.; CARNEIRO-LEÃO, A.M.A.; PORTO, A.L.F. The polysaccharide from *Anacardium occidentale*

L. in the inflammatory phase of the cutaneous wound healing. **Ciência Rural**. 36(1), 149-154, 2006.

SCHRAMM, G. **Reologia e Reometria:** Fundamentos Teóricos e Práticos. São Paulo: Artliber, 2006.

SCHRAMM, G. **Reologia e Reometria:** Fundamentos Teóricos e Práticos. São Paulo: Artliber, 2006.

SCHULZ, J.; HOHENBERGE, H.; PFLÜCKER, F.; GÄRTER, E.; WILL, T.; PFEIFFER, S.; WEPF, R.; WENDEL, V.; GERS-BARLAG, H.; WITTERN, P.; Distribution of sunscreens on skin. **Advanced Drug Delivery Reviews**. 54(1), 157-163, 2002.

SEGUIN, J.; BRULLÉ, L.; BOYER, R.; LU, Y.M.; ROMANO, Y.M.; TOUIL, Y.S.; SCHERMAN, D.; BESSODES, M.; MIGNET, N.; CHABOT, G.G. Liposomal encapsulation of the natural flavonoid fisetin improves bioavailability and antitumor efficacy. **International Journal of Pharmaceutics**. 444 (1–2), 146-154, 2013.

SEO, J.Y.; CHUNG, J.H. Thermal aging: a new concept of skin aging. **Journal of Dermatological Science**. 2, 13-22, 2006.

SHAHA, H.; MAHAJAN, S.R. Screening of topical gel containing lycopene and dexamethasone against UV radiation induced photoaging in mice. **Biomedicine & Aging Pathology**. 4, 303–308, 2014.

SHANKAR, S.; RHIM, J. Amino acid mediated synthesis of silver nanoparticles and preparation of antimicrobial agar/silver nanoparticles composite films. **Carbohydrate Polymers**. 130, 353-363, 2015.

SHARMA, K.; KO, E.Y.; ASSEFA, A.D.; HA, S.; NILE, S.H.; LEE, E.T.; PARK, S.W. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. **Journal of Food and Drug Analysis**. 23(2), 243-252, 2015.

SHARMA, V.; SUROLIA, A. Analysis of carbohydrate recognition by legume lectins: size of the combining site loops and their primary specificity. **Journal of Molecular Biology**. 267, 433–45, 1997.

SHARON, N.; LIS, H. A century of lectin research (1888 – 1988). **Trends in Biochemistry Sciences**. 12, 488–491, 1987.

SHERMAN, M.P.; BENNETT, S.H.; HWANG, F.F.; Yu, C. Neonatal small bowel epithelia: enhancing anti-bacterial defense with lactoferrin and Lactobacillus GG.

BioMetals. 17, 285–289, 2004.

SHIMIZU, T. Role of macrophage migration inhibitory factor (MIF) in the skin. **Biomaterials**, 24, 4353-4364, 2005.

SHIN, M.H.; MOON, Y.J.; SEO, J.; LEE, Y.; KIM, K.H.; CHUNG, J.H. Reactive oxygen species produced by NADPH oxidase, xanthine oxidase, and mitochondrial electron transport system mediate heat shock-induced MMP-1 and MMP-9 expression. **Free Radicals Biology and Medicine**. 44, 635-645, 2008.

SHOBHA, M. S.; THARANATHAN, R. N. Rheological behaviour of pullulanase treated guar galactomannan on co-gelation with xanthan. **Food Hydrocolloid.** 23, 749-754, 2009.

SIERAKOWSKI et al. Specifc modifications of galactomannans. **Carbohydrate Polymers**, 42, 51-57, 2000.

SILVA, A.R. **Efeito de extratos vegetais com potencial antioxidante em fibroblastos submetidos à radiação UV**. 2009, 102 f. Dissertação (Mestrado em Ciências Farmacêuticas). Universidade Federal do Paraná. Curitiba, 2009.

SILVA, L.C.N.; ALVES, N.M.P.; CASTRO, M.C.A.B.; PEREIRA, V.R.A.; PAZ, N.V.N.; COELHO, L.C.B.B.; FIGUEIREDO, R.C.B.Q.; CORREIA, M.T.S. Immunomodulatory effects of pCramoll and rCramoll on peritoneal exudate cells (PECs) infected and non-infected with*Staphylococcus aureus*. **International Journal of Biological Macromolecules**. 72, 848-854, 2015.

SILVA, M.C.C.; SANTANA, L.A.; SILVA-LUCCA, R.A.; LIMA, A.L.R.; FERREIRA, J.G.; PAIVA, P.M.G.; COELHO, L.C.B.B.; OLIVA, M.L.V.; ZINGALI, R.B.; CORREIA, M.T.S. Immobilized *Cratylia mollis* lectin: An affinity matrix to purify a soybean (Glycine max) seed protein with in vitro platelet antiaggregation and anticoagulant activities. **Process Biochemistry**. 46, 74–80, 2011.

SINGH, R.S.; TIWARY, A.K.; KENNEDY, J.F. Lectins: Sources, Activities and Applications. **Critical Reviews in Biotechnology**. 19, 145-178, 1999.

SOAZO, M.; BÁEZ, G.; BARBOZA, A.; BUSTI, P.A.; RUBIOLO, A.; VERDINI, R.; DELORENZI. N.J. Heat treatment of calcium alginate films obtained by ultrasonic atomizing: Physicochemical characterization. **Food Hydrocolloids**. 51, 193-199, 2015.

SOBRAL, P.J.A. Influência da espessura de biofilmes feitos à base de proteínas miofibrilares sobre suas propriedades funcionais. **Pesquisa Agropecuária**

Brasileira. 35(6), 1251-1259, 2000.

SOTHORNVIT, R.; KROCHTA, J.M. Plasticizer effect on oxygen permeability of β-lactoglobulin films. **Journal of Agricultural and Food Chemistry**. 48, 6298-6302, 2000.

SOUSA, A.M.M.; GONÇALVES, M.P. Strategies to improve the mechanical strength and water resistance of agar films for food packaging applications**.** **Carbohydrate Polymers**. 132, 196-204, 2015.

SOUZA, C.F.; LUCYSZYN, N.; FERRAZ, F.A.; SIERAKOWSKI, M.R. *Caesalpinia ferrea* var. *ferrea* seeds as a new source of partially substituted galactomannan. **Carbohydrate Polymers**, 82, 641–647, 2010a.

SOUZA, M.P.; CERQUEIRA, M.A.; SOUZA, B.W.S.; TEIXEIRA, J.T.; PORTO, A.L.F.; VICENTE, A.; CARNEIRO-DA-CUNHA, M.G. Polysaccharide from *Anacardium occidentale* L. tree gum (Policaju) as a coating for Tommy Atkins mangoes. **Chemical Papers**. 64(4), 475–481, 2010b.

SOUZA, M.P.; CARNEIRO-DA-CUNHA, M.G. Nanotechnology in edible packaging. In: CERQUEIRA, M.A.P.R.; PEREIRA, R.N.C.; RAMOS, O.L.S.; TEIXEIRA, J.A.C.; VICENTE, A.A. **Edible Food Packaging: materials and processing technologies**. CRC Press, 2016, p. 288-311.

SOUZA, M.P.; VAZ, A.F.M.; CORREIA, M.T.S.; CERQUEIRA, M.; VICENTE, A.; CARNEIRO-DA-CUNHA, M.G. Quercetin-Loaded Lecithin/Chitosan Nanoparticles for Functional Food Applications. Food and Bioprocess Technology. 7(4), 1149-1159, 2014.

SOUZA, V.M. **Ativos Dermatológicos**. São Paulo: Tecnopress. p. 23-25, 2003.

SRIGLEY, C.T.; HAILE, E.A. Quantification of plant sterols/stanols in foods and dietary supplements containing added phytosterols. Journal of Food Composition and Analysis. 40, 163–176, 2015.

SRIRAMOJU, B.; KANWAR, R.K.; KANWAR, J.R. Lactoferrin induced neuronal differentiation: A boon for brain tumours. **International Journal of Developmental Neuroscience**. 41, 28-36, 2015.

SRIVASTAVA, M.; KAPOOR, V.P. Seed galactomannans: An overview. **Chemistry & Biodiversity**, 2, 295–317. 2005.

STEED, D.L.; Papel dos fatores de crescimento na cicatrização das feridas. In: BARBUL, A. **Clínica Cirúrgica da América do Norte**. Rio de Janeiro: Interlivros,

v.3, 1997, p. 571-582.

STEPHEN, A.M. Other plant polysaccharides. In: ASPINALL, G.O. (Ed.), **The polysaccharides**, London: Academic Press, 1983. p. 97-180.

SUGA, M.; ASAHINA, S.; SAKUDA Y.; et al. Recent progress in scanning electron microscopy for the characterization of fine structural details of nano materials. **Progress in Solid State Chemistry**. 42: 1-21, 2014.

SURESH, D.; NETHRAVATHI, P.C.; UDAYABHANU, RAJANAIKA, H.; NAGABHUSHANA, H.; SHARMA, S.C. Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using *Cassia fistula* plant extract and their photodegradative, antioxidant and antibacterial activities. **Materials Science in Semiconductor Processing**. 31, 446-454, 2015.

TAN, Q.; LIU, W.; GUO, C.; ZHAI, G. Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. **International Journal of Nanomedicine**. 6, 1621-1630, 2011.

TAVARES, C.; MONTEIRO, S. R.; MORENO, N.; LOPES DA SILVA, J.A. Does the branching degree of galactomannans influence their effect on whey protein gelation? **Colloids and Surfaces A: Physicochemcal and Engineering Aspects**, 270, 213-219, 2005.

THIELE, J.J.; EKANAYAKE-MUDIYANSELAGE, S. Vitamin E in human skin: organ-specific physiology and considerations for its use in dermatology. **Molecular Aspects of Medicine**. 28, 646-667, 2007.

TONELI, J.T.C.L.; MURR, F.E.X.; PARK, K.J. Estudo da reologia de polissacarídeos utilizados na indústria de alimentos. **Revista Brasileira de Produtos Agroindustriais**, 7, 181-204, 2005.

TRAMIL. *Cassia grandis*. Disponível em: <http://www.tramil.net/fototeca/imageDisplay.php?id_elem=112>. Acesso em: 12 ago. 2015.

UENO, K.; LIM, D.J. Heterogeneity of glycoconjugates in the secretory cells of the chinchilla middle ear and eustachian tubal epithelia: a lectin-gold cytochemical study. **Journal of Histochemistry & Cytochemistry**. 39, 71-80, 1991.

UGHINI, F.; ANDREAZZA, I.F.; GANTER, J.L.M.S.; BRESOLIN, T.M.B. Evaluation of xanthan and highly substituted galactomannan from *M. scabrella* as a substained release matrix. **International Journal of Pharmaceutics**, 271, 197-205, 2004.

VADIVEL, V.; NANDETY, A.; BIESALSKI, H.K. Antioxidant Potential and Health Relevant Functionality of Traditionally Processed *Cassia hirsuta* L. Seeds: An Indian Underutilized Food Legume. **Plant Foods for Human Nutrition**, 66, 245–253, 2011.

VALENGA, F. **Estudos de interação entre galactomanana e alginato e possíveis aplicações.** 2007. 74 f. Dissertação (Mestrado em Ciências – Bioquímica). Universidade Federal do Paraná. Curitiba, 2007.

VALENGA, F.; PETRI, D.F.S.; LUCYSZYN, N.; JÓ, T.A.; SIERAKOWSKI, M.R. Galactomannan thin films as supports for the immobilization of Concanavalin A and/or dengue viroses. **International Journal of Biological Macromolecules**. 50, 88– 94, 2012.

VALKO, M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M.T.D.; MAZUR, M.; TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. **The International Journal of Biochemistry & Cell Biology**. 39, 44-84. 2007.

VAN DAMME, E.J.M.; BARRE, A.; ROUGE, P.; VAN LEUVEN, F.; PEUMANS, W.J. The NeuAc(a-2,6)–Gal/GalNac – binding lectin from elderberry (*Sambucus nigra*) bark, a type–2 ribosome-inactivating protein with an unusual specificity and structure. **European Journal of Biochemistry**. 235, 128–37, 1996.

VENDRUSCOLO, C.W.; ANDREAZZA, I.F.; GANTER, J.L.M.S.; FERRERO, C.; BRESOLIN, T.M.B. Xanthan and galactomannan (from M. scabrella) matrix tablets for oral controlled delivery of theophylline. **International Journal of Pharmaceutics**, 296, 1–11, 2005.

VIEIRA, I. G. P.; MENDES, F. N. P.; GALLÃO, M. I.; BRITO, E. S. NMR study of galactomannas from the seeds of mesquite tree (*Prosopis juliflora* (Sw) DC). **Food Chemistry**, 101, 70-73, 2007.

VIEIRA, L.S. **Fitoterapia da Amazônia**: manual de plantas medicinais (A Farmácia de Deus). 2ª ed. São Paulo: Agronômica Ceres, 1992. 347p.

VIKRAM, D.; KSHITIJA, D.; JATINDER, S.; KUMAR, S.A.; KUMAR, A.S.; SINGH, K.S. Purification and characterization of an Anti-proliferative and mitogenic plant lectin from tubers of *Arisaema speciosum*. **Pharmacognosy Journal**. 2(9), 266-277, 2010.

WATANABE, J.; IWAMOTO, S.; ICHIKAWA, S. Entrapment of some compounds into

biocompatible nano-sized particles and their releasing properties. **Colloids and Surfaces. B, Biointerfaces.** 42, 141–146, 2005.

WEN, X.; ZHENG, Y.; WU, J.; YUE, L.; WANG, C.; LUAN, J.; WU, Z.; WANG, Z. *In vitro* and *in vivo* investigation of bacterial cellulose dressing containing uniform silver sulfadiazine nanoparticles for burn wound healing. **Progress in Natural Science: Materials International**. 25(3), 197-203, 2015.

WILKINSON, J.B.; MOORE, R.J. **Harry`s Cosmetology**. New York: Chemical Publishing, p. 5-20, 1982.

WU, T.H.; YEN, F.L.; LIN, L.T.; TSAI, T.R.; LIN, C.C.; CHAMD, T.M. Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. **International Journal of Pharmaceutics**, 346, 160–168, 2008.

WULF, H.C.; SANDBY-MOLLER, J.; KOBAYASI, T.; GNIADECKI, R. Skin aging and natural photoprotection. **Micron**. 35, 185-191, 2004.

XIAO, Q.; TONG, Q.; ZHOU, Y.; DENG, F. Rheological properties of pullulan-sodium alginate based solutions during film formation. **Carbohydrate Polymers**. 130, 49-56, 2015.

YADAV, T.; MISHRA, S.; DAS, S.; AGGARWAL, S.; RANI, V. Anticedants and natural prevention of environmental toxicants induced accelerated aging of skin. **Environmental Toxicology and Pharmacology**. 39(1), 384-391, 2015.

YAMAUCHI, K.; WAKABAYASHI, H.; SHIN, K.; TAKASE, M. Bovine lactoferrin: benefits and mechanism of action against infections. Biochemistry and Cell Biology. 84, 291–296, 2006.

YANG, L.; CHU, J.S.; FIX, J.A. Colon-specific drug delivery:new approaches and in vitro/in vivo evaluation. **International Journal of Pharmaceutics**, 235, 1-15, 2002.

YANG, L.; PAULSON, A.T. Mechanical and water vapour barrier properties of edible gellan films. **Food. Res. Int**. 33, 563-570, 2000.

YENILMEZ, E.; BASARAN, E.; YAZAN, Y. Release characteristics of vitamin E incorporated chitosan microspheres and in vitro–in vivo evaluation for topical application. **Carbohydrate Polymers**. 84, 807–811, 2011.

ZAPPINO, M.; CACCIOTTI, I.; BENUCCI, I.; NANNI, F.; LIBURDI, K.; VALENTINI, F.; ESTI, M. Bromelain immobilization on microbial and animal source chitosan films, plasticized with glycerol, for application in wine-like medium:

Microstructural, mechanical and catalytic characterisations. **Food Hydrocolloids**. 45, 41-47, 2015.

ZHANG, Y.; MA, Q.; CRITZER, F.; DAVIDSON, P.M.; ZHONG, Q. Physical and antibacterial properties of alginate films containing cinnamon bark oil and soybean oil. **LWT - Food Science and Technology**. 64(1), 423-430, 2015.

ZHANG, Y.; NIU, Y.; LUO, Y.; Ge, M.; YANG, T.; YU, L.L.; WANG, Q. Fabrication, characterization and antimicrobial activities of thymolloaded zein nanoparticles stabilized by sodium caseinate–chitosan hydrochloride double layers. **Food Chemistry**. 142, 269–75, 2014.

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Structure and rheological properties of a xyloglucan extracted from *Hymenaea courbaril* var. *courbaril* seeds

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ABSTRACT

Hymenaea courbaril var *courbaril* seed xyloglucan was efficiently extracted with 0.1 M NaCl, followed by ethanol precipitation (yield = 72 ± 5% w/w). Its amorphous structure was identified by the pattern of X-ray diffraction. The monosaccharide composition was determined by GC/MS analysis of the alditol acetates and showed the occurrence of glucose:xylose:galactose:arabinose (40:34:20:6). One-(1D) and two-dimensional-(2D) NMR spectra confirmed a central backbone composed by 4-linked β-glucose units partially branched at position 6 with non-reducing terminal units of α-xylose or β-galactose-(1→2)-α-xylose disaccharides. The xyloglucan solution was evaluated by dynamic light scattering and presents a polydisperse and practically neutral profile, and at 0.5 and 1.0% (w/v) the solutions behave as a viscoelastic fluid. The polysaccharide did not show significant antibacterial or hemolytic activities. Overall our results indicate that xyloglucan from *H. courbaril* is a promising polysaccharide for food and pharmaceutical industries.

1. Introduction

Polysaccharides are polymers widespread in nature and synthesized in high amounts by plants and microorganisms. They are a source of biopolymers generally non-toxic, biodegradable, and biocompatible, sometimes exhibiting properties of biorecognition [1]. The polysaccharides have a wide range of chemical structure and physiochemical properties that cannot be easily reproduced synthetically. Thus, the possibility to obtain these compounds from natural sources renders numerous polysaccharides, which often present a lower cost than synthetic polymers [2].

Xyloglucans are branched polysaccharides usually found in the primary cell walls and in the higher plants seeds with structural and storage functions, respectively. They have a 4-linked β-D-glucan backbone, substituted at position O-6 by branches of α-D-xylose or of β-D-galactose-1→2-α-D-xylose disaccharide [3–5]. Xyloglucans can be extracted from seeds of different species such as *Copaifera langsdorffii*, *Hymenaea courbaril* and *Tamarindus indica* that are combined with the same structural groups, but the proportion and arrangement of these groups give a fine structure that varies according to the species and even within the same species [6–8].

Xyloglucans can be used in food, medical and pharmaceutical industries, even to biotechnological processes [9], due to its characteristics, such as Newtonian flow behavior in the wide range of shear rate, water-holding ability and their resistance against heat, salt and pH regimes [10].

H. courbaril var. *courbaril* belongs to Caesalpiniaceae family that occurs abundantly throughout Brazilian forests [11]. It is undemanding regarding fertility and soil moisture, besides it is important for the recovery of deforested areas and afforestation of large parks and gardens [12]. Each tree produces an average of 10 kg of seeds/year that are a source of xyloglucan [11–13].

This work aims to extract and purify xyloglucan from seeds of *H. courbaril*. Furthermore, we intend to determine the structure of the polysaccharide and its rheological properties, complement by evaluation of their antimicrobial and hemolytic activities, in order to determine the potential biotechnological applications of the polysaccharide.

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2. Materials and methods

2.1. Materials

The pods of *H. courbaril* were collected in September 2010 at the Parque Nacional do Catimbau, Buíque-Pernambuco, Brazil. Botanical identification was made by depositing testimony specimens in the Herbarium of the Instituto Agronômico de Pernambuco (IPA 84893), Brazil. All the chemicals were of analytical grade.

2.2. Extraction and purification of xyloglucan

The xyloglucan from the seeds of *H. courbaril* was obtained by the method of Albuquerque et al. (2014) [14]. The seeds were manually removed from dry pods, washed and boiled in distilled water at 100 °C for 30 min for enzyme inactivation and softening of the hulls. After this period, the hull was removed and seeds were dried at 60 °C to constant weight (m_i). The dry seeds were triturated with 0.1 mol L^{-1} NaCl [15% (w/v)] at 25 °C in an industrial blender (Model TR-02, Metalúrgica Siemsen Ltda, Santa Catarina, Brazil) and this crude extract obtained was subsequently centrifuged (Thermo Fisher Scientific Sorvall RC6, USA) for 20 min at 1500 × g. The supernatant was filtered through a voil tissue, followed by a new filtration using screen printing cloth (90 thread type), and precipitated with 46% ethanol [1:3 (v/v)] for 16 h. The precipitate was filtered on screen printing cloth (110 thread type), washed with 100% ethanol [1:3 (w/v)] for 30 min and twice with acetone PA [1:3 (w/v)] for 30 min, filtered on screen printing cloth (110 thread type) between each washing and finally dried at 60 °C until constant weight (m_f). The dry precipitate was milled and stored in amber glass bottles.

The yield of the extraction in mass was determined as the ratio of the final mass of the precipitate powder (m_f) by the initial mass of the dry seeds (m_i) and expressed as % (w/w). The total content of carbohydrate and protein in the precipitated powder was determined by the phenol sulfuric acid method [15] and by Bradford method [16] respectively.

2.3. Structural characterization

2.3.1. Gel filtration chromatography

Xyloglucan (1 mg) was applied to a Superose 6 column (30 × 1 cm) from Pharmacia, linked to a HPLC (high performance liquid chromatography) system, equilibrated with 0.5 mol L^{-1} ammonium acetate (pH 5.). The column was eluted with the same solution at a flow rate of 0.5 mL min^{-1}. The eluent was monitored simultaneously by absorbance at 280 nm and by differential refractive index. The column was calibrated with dextrans with average Mr of 500 and 50 kDa.

2.3.2. X-ray patterns

X-ray diffraction pattern of the xyloglucan was obtained using a Bruker D8 Advance (Germany) diffractometer (30 kV, 30 mA) equipped with CuKα radiation at wavelength of 0.154 nm. The measurements were carried out for an angular interval varying from 5–60° (2θ range), scanning rate of 5° min^{-1}, step of 0.02° and 2 s per step. The total diffracted area and the area under the crystallinity peaks were evaluated by integration after correcting the data for absorption in order to determine the polysaccharide crystallinity.

2.3.3. Monosaccharide composition

After acid hydrolysis of the xyloglucan (5 mol L^{-1} trifluoroacetic acid for 2 h at 100 °C), the alditol acetate derivatives were analyzed by gas-liquid chromatography/mass spectrometry (GCMS-QP2010 Shimadzu, Japan) [17].

2.3.4. Methylation analysis

Xyloglucan (5 mg) was subjected to two rounds of methylation [18]. The methylated polysaccharide was hydrolyzed with 5 mol L^{-1} trifluoroacetic acid for 2 h at 100 °C, reduced with borohydride, and the alditols were acetylated with acetic anhydride:pyridine (1:1 v/v). The alditol acetates of the methylated sugars were dissolved in chloroform and analyzed in a gas chromatography/mass spectrometry unit (GCMS-QP2010 Shimadzu, Japan) with a Restek column RTX-5MS. The sample was analyzed using a split ratio of 30. The carrier gas was helium and a temperature gradient of 110 °C to 250 °C, with variation of 2 °C min^{-1}. The temperatures of the injector, ion source and interface were 260 °C, 200 °C and 230 °C, respectively.

Different periods of time for hydrolysis of the methylated polysaccharide were tested (from 1 up to 4 h) in order to assure total hydrolysis of the constitutive units but without significant lost of xylose residues. Two hours were chosen as the appropriated time for the hydrolysis.

2.3.5. Nuclear magnetic resonance spectroscopy

One (1D) and two-dimensional (2D) spectra of the xyloglucan were recorded using a Bruker DRX 400 MHz apparatus with a triple resonance probe, as described previously [11,19]. Approximately 5 mg of each sample was dissolved in 0.5 mL of 99.9% deuterium oxide (Cambridge Isotope Laboratory, Cambridge, MA, USA). All spectra were recorded at 50 °C with HOD (deuterated water exhibiting a peak due to exchange with residual H_2O) suppression by presaturation. For 1D ^{1}H NMR spectra, 32 scans were recorded, using an inter-scan delay equal to 1 s. For 2D ^{1}H/^{1}H TOCSY (total correlated spectroscopy) and ^{1}H/^{13}C HSQC (heteronuclear single quantum coherence) experiments, spectra were recorded using states TPPI (time proportion phase incrementation) for quadrature detection in the indirect dimension. TOCSY spectra were run with 4046 × 400 points with a spinlock field of 10 kHz and a mixing time of 80 ms. Two-dimensional ^{1}H/^{13}C Multiplicity-Edited HSQC spectra were recorded at 50 °C with HOD suppression by presaturation, with 256 scans. The increment number setup was set to 64, and states-TPPI were used for quadrature detection in the indirect dimension and run with 1024 × 256 points with globally optimized alternating phase rectangular pulses (GARP) for decoupling. Chemical shifts were displayed relative to external trimethyl-silylpropionic acid at 0 ppm for ^{1}H and relative to methanol for ^{13}C.

2.4. ζ-potential and dynamic light scattering (DLS)

DLS and ζ-potential (ζ) measurements were carried out with the xyloglucan solution 0.5% (w/v) in water at pH 5.8 adjusted with 0.1 M NaOH. The measurements were carried out on a ZetaSizer Nano ZS90 (Malvern Instruments, U.K.). The DLS cumulants analysis (30 scans) provides the characterization of a sample through the mean apparent *Z*-average hydrodynamic diameter (Rh) for the particle or molecule size (nm). The width parameter, known as the polydispersity index (PDI), was determined from the intensity of scattered light (fixed angle of 90°) at 25 °C. The *Z*-average diameter is the mean hydrodynamic diameter (molecule size), determined from the intensity of scattered light. This measuring was carried out in triplicate with samples analyzed in a period of 144 h, with intervals at 48 h for each analysis. The ζ-potential values were calculated using the Smoluchowski equation [20]. Each sample was analyzed in a folded capillary cell. The results are given as average ± standard deviation.

2.5. Rheological measurements

The rheological properties of the xyloglucan aqueous solutions at 0.5 and 1.0% (w/v) were conducted in a stress controlled rheometer (Anton Paar MCR 301, Austria) equipped with two concentric cylinder geometry cell with outer diameter (o.d.) = 28 mm and internal diameter (i.d.) = 24 mm, with temperature controlled at 25 °C. Measurements were performed and 50 points were acquired.

2.5.1. Rotational flow

Continuous shear tests were carried out over a shear rate ($\dot{\gamma}$) range of 1–1000 s^{-1} to measure the apparent viscosity (η). The points were acquired while simultaneously collecting viscosity data.

2.5.2. Oscillatory flow

Dynamic oscillatory mode measurements were conducted varying the applied torque/deformation angular frequency (ω) from 1 to 500 rad s^{-1}. Storage (G') and loss (G'') moduli sample responses were recorded during the frequency sweep at a rate of one acquisition every 5 s. The strain deformation amplitude (γ) was fixed at 0.2%.

2.5.3. Stress-strain

The rheometer was operated in oscillatory mode. For a fixed frequency $\omega \equiv 2\pi$ – rad s^{-1} an externally applied shear stress (τ) was varied from 1 to 300 Pa and the (G') and (G'') moduli sample responses were recorded. One acquisition was carried out every 5 s.

2.6. Biological activities

2.6.1. Antibacterial activity

Gram-negative strains [*Escherichia coli* (UFPEDA 224), *Klebsiella pneumoniae* (UFPEDA 396), *Salmonella enteritidis* (UFPEDA 414), *Pseudomonas aeruginosa* (UFPEDA 416), *Proteus vulgaris* (UFPEDA 740)] and gram-positive strains [*Staphylococcus aureus* (UFPEDA 02), *Bacillus subtilis* (UFPEDA 86), *Micrococcus luteus* (UFPEDA 100), *Enterococcus faecalis* (UFPEDA 138)] were provided by Culture Collection of Microorganisms of Department of Antibiotics, Universidade Federal de Pernambuco (UFPEDA). Bacteria strains were grown in shaker flasks (250 mL) containing Nutrient Broth and incubated overnight in an orbital shaker at 100 rpm and 37 °C. The biomass concentration was determined measuring the suspension turbidity at 600 nm and then converted to colony forming units (10^5–10^6 CFU mL^{-1}) using appropriate calibration curves (turbidity equivalent to 0.5 in the McFarland scale). Xyloglucan antibacterial activity was investigated by the disc diffusion method [21], using 20 μL of xyloglucan solution (0.5% w/v) and positive controls containing neomicin and clindamicin (both with 10 μg/disc). All tests were carried out in triplicate.

2.6.2. Hemolytic activity for toxicity evaluation

The hemolytic activity was determined according to a literature method [22]. The red blood cells were diluted in saline solution to obtain a 1% suspension (v/v) and 1.1 mL was mixed with 0.4 mL of xyloglucan solution (0.25–2.0 mg mL^{-1} in saline). The minimum and maximum hemolytic controls were suspensions of red blood cells containing saline (hemolysis 0%) and Triton X-100 2.0 mg mL^{-1} (hemolysis 100%), respectively. All experiments were carried out in triplicate and the results expressed as average ± standard deviation. The hemolytic activity was expressed in relation to Triton X-100 and calculated by the following equation: Hemolytic activity (%) = $(As - Ab)/(Ac - Ab) \times 100$, where *Ac* is the absorbance of the control, *As* is the absorbance in the presence of the xyloglucan solution and *Ab* is the absorbance of Triton X-100 solution.

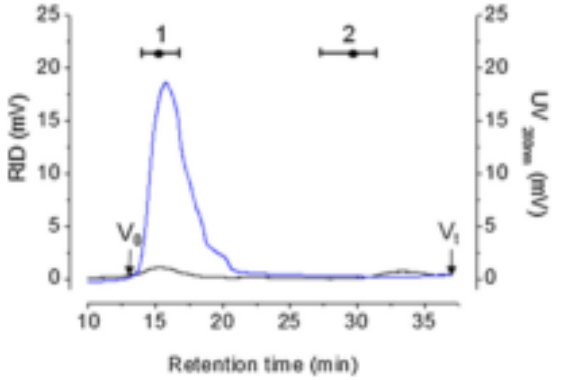

Fig. 1. Gel filtration chromatography (numbers 1 and 2 indicate the elution volumes of dextrans with 500 and 50 kDa, respectively).

2.7. Statistical analyses

Statistical analyses were carried out using Analysis of Variance (ANOVA). Comparisons between samples were analyzed using Student's *t*-test. Statistical significance was established at $p < 0.05$ (Graph Pad Prism, version 6, 2012, USA).

3. Results and discussion

3.1. Extraction and yield

Most methods for extraction of xyloglucan from seeds described in the literature consist of two basic stages, where the triturated seeds pass by aqueous extraction, followed by purification using alcoholic precipitation [8,23,24].

In the present work extraction with NaCl was used because it increases the solubility of the contaminating free proteins (*salting in* effect). The crude extract obtained was subsequently centrifuged to remove the residue, in agreement with the literature [5]. Washing with ethanol removed contaminant, while washing with acetone permitted further removal of proteins. These washes with ethanol and acetone also promote dehydration of the polysaccharide, thus contributing to preparation of a complete dried sample. The extraction yield was 72 ± 5% (w/w) related to dry weight of the seeds, being 81 ± 7% of this mass of polysaccharide content and by Bradford method [16] no protein was detected.

We believe that our methodology allowed us to achieve a high yield which is far above those of previous works, such as 15.5% [23] and 38% [5]. Different extraction times for xyloglucan were compared and demonstrated a variation of yield from 5.4 to 40.8% [25]. However, these authors did not mention clearly if your yield are related to moist or dry weight of the seeds.

3.2. Gel filtration chromatography

Gel filtration chromatography reveals that xyloglucan is a polydisperse polysaccharide with average molecular mass ≥500 kDa (Fig. 1). Clearly no degradation occurred during extraction and purification of the polysaccharide. The absence of fractions with the absorbance at 280 nm confirms that the xyloglucan does not contain contaminant proteins.

3.3. Patterns of X-ray diffraction

X-ray diffraction profile (Fig. 2) shows characteristics of amorphous structures with no sharp peaks. X-ray diffractogram

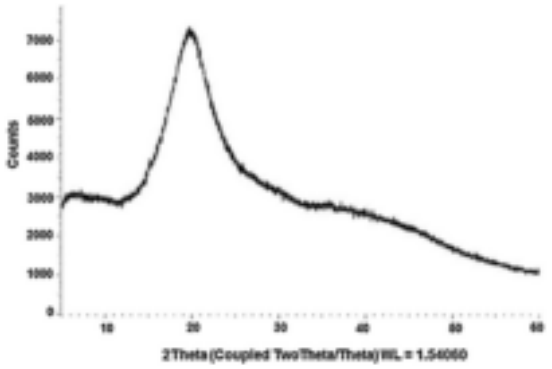

Fig. 2. Pattern of X-ray diffraction of the xyloglucan obtained from *H. courbaril* seeds.

presented a typical peak at approximately $2\theta = 20^{\circ}$, similar to those found for a xyloglucan from Gokyo Food and Chemical Co. Ltd. [26] and a galactoxyloglucan extracted from Tamarind seed [27].

It is known that crystalline structures generate sharp narrow diffraction peaks while amorphous components generate a broad one. The interpretation of broad amorphous peaks of several amorphous structures in X-ray scattering profile is difficult and hence the ratio between these intensities is used to calculate the index of crystallinity in the material [28]. The crystallinity index, related with the alignment of molecules in a particle structure [29], was calculated from X-ray diffraction profile for the xyloglucan, giving a value of 0.37. Furthermore, the crystallinity depends on how the material is dried or prepared, and that the drying process can lead to a better organization of the chain, thus increasing the crystallinity of the material [30].

3.4. Monosaccharide composition

The monosaccharide composition of the xyloglucan was determined based on gas-liquid chromatography/mass spectrometry analysis of the alditol acetates formed after acid hydrolysis, yielding (in %w/w): glucose (40%), xylose (34%), galactose (20%) and arabinose (6%).

Glucose, xylose and galactose were detected with a molar ratio of ~4:3:2 respectively. Minor amount of araninose was also detected (~6%). No other sugar was detected up to a limit of <2% as% of dry weight. The branching pattern of xyloglucan from different plant species was evaluated and demonstrated that some xyloglucans have an additional arabinose unit linked to the galactose residues at the non-reducing end of the side chain [31]. Kay & Petkowicz [25] suggest that the presence of arabinose is due to co-purified arabinan, which corroborates our result. They also confirmed the absence of arabinose residues in shorter extraction processes, while others workers [3,11] described arabinose traces in xyloglucans from seeds.

3.5. Methylation analysis

The substitution of hydroxyl free radical by methyl is accomplished in methylation analysis, where different *O*-methyl derivatives are obtained after hydrolysis and acetylation. The methylation of this polysaccharide, followed by acid hydrolysis and acetylation of the alditols, yields the derivatives units shown in Table 1.

The structure proposed for the polysaccharide was based on our experimental data (methylation and NMR analysis) and also on literature information [5,11,32] as summarized in Fig. 3.

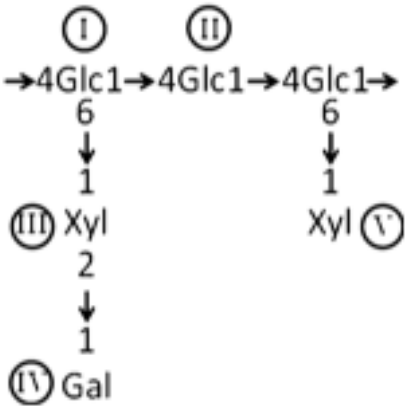

Fig. 3. Proposed structure for the xyloglucan under analysis.

The types of units found in the polysaccharide are shown in Fig. 3 and confirmed in Table 1. Clearly the preponderance of 2,3,6-tri-*O*-methyl and 2,3-di-*O*-methyl derivatives from glucose indicates that the preponderant structure is composed of 4-linked (unit II) and 4,6-substituted units (unit **I**) of this sugar. The presence of these two derivatives was also observed in a very similar proportion in the xyloglucan from *Guibourtia hymenifolia* (Moric.) J. Leonard [9] and in the xyloglucan from *H. courbaril* seeds [13], confirming 4-linked glucose residues as typical of xyloglucan cellulosic backbones.

Galactose occurs as 2,3,4,6-tetra-*O*-methyl derivative, indicating that this sugar is at non-reducing terminal (unit **IV**). Two third of the xylose units occur as 2,3,4-tri-*O*-methyl derivative, indicating non-reducing terminal branches (unit **V**), as galactose. One third of xylose residues occur as 3,4-di-*O*-methyl derivatives, meaning that the structure presents 2-linked xylosil units (unit **III**). Of course, the alditol acetate derivatives from 2,3-di-*O*- and 3,4-di-*O*-methyl xyloses are the same compound. We attributed our results to the 2,3-di-*O*-methyl xylose based on literature data for similar compounds [5,11,32] and also on our own NMR data (see next section).

The detection of 2,3-di-*O*-methyl glucose in approximately the same proportion as the sum of tetra-methyl derivative from galactose + tri-methyl derivatives from xylose, agrees with the structure proposed for the polysaccharide, contain a central core of 4-linked glucose units (unit **II**), partially branched at position 6 (unit **I**). Two third of the xylose units (unit **V**) and the totality of the galactose units (unit **IV**) occur as non-reducing terminal. One third of the xylose units (unit **III**) are 2-linked intermediate residues on the branches.

The overall recoveries of methylated alditol acetates were in good agreement with the monosaccharide composition obtained for the native polysaccharide after hydrolysis, reduction and acetylation (Section 3.4). Arabinose, which is present in small amount in the polysaccharide, was not detected even when we employed different periods of hydrolysis (from 1 up to 4 h).

3.6. Nuclear magnetic resonance spectroscopy

The structure of the xyloglucan from *H. courbaril* was investigated using one-dimensional (1D) and two-dimensional (2D) NMR spectra. The 1D ^{1}H-NMR spectrum (Fig. 4A) showed three signals in the anomeric region: one at 5.17 ppm, ascribed to substituted α-xylose units (Xyl′) (unit III in Fig. 3), another at 4.98 ppm to terminal non-reducing α-xylose (Xyl) (unit V in Fig. 3), and finally the signal at 4.58 ppm, which is an overlapped of β-glucose + β-galactose anomeric protons (units I, II + IV in Fig. 3). No signals assigned to

Table 1
Retention times and the proportions of the methylated derivatives obtained from the xyloglucan of *H. courbaril* seeds after acid hydrolysis for 2 h.

Peak number	Retention time (t_R - min)	Relative retention time	Proportion (% of total)	Methyl derivative[a]	Units[b]
1	24.33	1.00	22	2,3,4-tri-Me-Xyl	V
2	29.46	1.21	11	3,4-di-Me-Xyl	III
3	32.57	1.33	15	2,3,4,6-tetra-Me-Gal	IV
4	36.69	1.50	19	2,3,6-tri-Me-Glc	II
5	41.90	1.72	33	2,3-di-Me-Glc	I

[a] The type of methyl derivative was identified by the typical fragmentation spectrum.
[b] See Fig. 3.

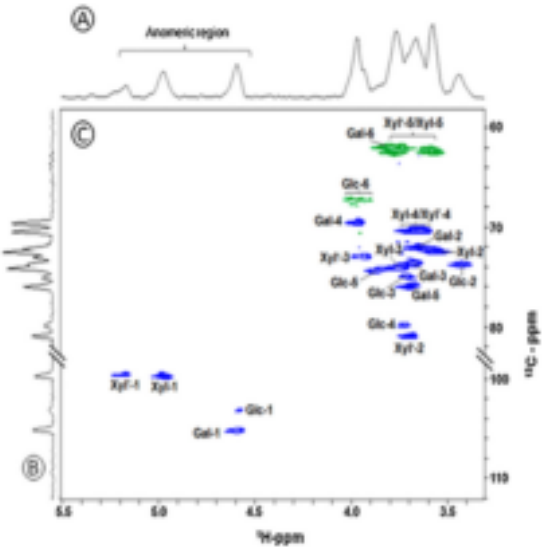

Fig. 4. One-dimensional ^{1}H-NMR (A), ^{13}C-NMR (B) and two-dimensional ^{13}C-^{1}H HSQC (C) spectra of the xyloglucan from *H. courbaril* seeds. In Panel C, signals of CH carbon/proton are in blue (phase signals) and those from CH_2 in green (antiphase). Panel B displays only the phase signals of the ^{13}C-NMR spectrum, shown in blue on Panel C.

contaminant proteins were observed in the 1D ^{1}H-NMR spectrum, such as signals from aromatic amino acids at 6.0–7.0 ppm.

The edited ^{13}C-^{1}H HSQC spectrum (Fig. 4C) allowed us to distinguish the anomeric signals from β-galactose (105.13 and 4.59 ^{13}C/^{1}H ppm) (unit IV) and β-glucose (103.02 and 4.58 ^{13}C/^{1}H ppm) (units I and II), which were coincident in the proton spectra. Signals from CH occur in phase (positive peaks in blue) while those from CH_2 occur as anti-phase (negative peaks in green). This spectrum allowed us to identify signals from C6/H6 (67.30/3.90–4.04 ppm), ascribed to β-glucose and β-galactose units, and those from C5/H5 (62.4/3.58–3.76 ppm) of α-xylose. Signals from C4 (79.75 ppm) and some of C6 (67.30 ppm) from β-glucose, as well as signals from C2 of some α-xylose units (80.81 ppm, Xyl′), were shifted downfield compared with non-glycosylated units (compared chemical shifts from units III vs V, in Table 2 and also literature data [32]. No signals ascribed to non-reducing β-arabinofuranosyl units were observed at 109/5.3 ppm. This type of residue was reported in other plant polysaccharide [25]. They may occur only in minor amounts in our xyloglucan preparation and not detected on the NMR spectrum.

The ^{1}H-^{1}H COSY and ^{1}H-^{1}H TOCSY spectra (Fig. 5) allowed us to identify the chemical shifts by scalar coupling of the spin systems. The COSY spectrum (Fig. 5A) confirmed the overlapping of the anomeric signals from β-galactose and β-glucose units at 4.58 ppm. The TOCSY spectrum (Fig. 5B) allowed us to identify the four spin systems and to determine their proton chemical shifts (Table 2). However some signals from β-glucose and β-galactose are coincident. Furthermore the chemical shifts of H2 and H3 from β-galactose units are very close as well as the chemical shifts of H3 and H4 from β-glucose.

The backbone formed by 4-linked β-D-glucan is common to all xyloglucans, but considerable differences are observed in the branches of the polysaccharide, such as arrangements in number, position and type of residues. This variation is a consequence of interspecific variations [3] and also of the extraction methods [25].

In conclusion, the ^{1}H and ^{13}C chemical shifts shown in Table 2 were similar to those reported for a plant polysaccharide composed of a central backbone, containing 4-linked β-glucose units (unit II), partially branched at position 6 (unit I) [32]. The polysaccharide also is highly branched, containing β-galactose and α-xylose as non-reducing terminal units (unit IV and V respectively). Furthermore, residues of 2-linked α-xylose are also found as intermediated residues in the branches (unit III).

3.7. ζ-potential and dynamic light scattering (DLS)

The information obtained by ζ-potential and Dynamic Light Scattering (DLS) are crucial to indicate the occurrence of stable, functional nanostructures [33]. PDI, obtained by DLS, is a measure of the size distribution width. When polydispersity equals zero, the sample is monodisperse. Values of PDI close to or above 0.5 represent heterogeneous solutions in relation to the particle size and are characteristic of samples outside the standards [34]. The term "particle" represents the molecule of polysaccharide, which stay disperses into diluted solution. This term has been used in the literature for different polysaccharides [35]. For a xyloglucan solution 0.5% (w/v) at pH 5.8, *Z*-average and PDI were 296.00 ± 12.40 nm and 0.32 ± 0.02, respectively, indicating a reasonably polydisperse solution. *Z*-averages values of 170 nm [24] and 80 nm [25] were reported for xyloglucan from *H. courbaril* seeds at 1 mg mL^{-1}, which are lower concentrations than ours (0.5% w/v), as well as the different method for extraction.

ζ-potential of xyloglucan was evaluated in order to determine the charges of the polysaccharide; a ζ-potential value of −11.3 ± 0.27 mV was found, thus showing that xyloglucan is a practically neutral polysaccharide. The polysaccharides may be constituted either by polycations or by polyanions, depending on their functional group, and may also be neutral, which is the case of different types of polysaccharides with a higher content of mannose and galactose units, with ζ – potential values between −13.7 and −2.1 mV [33]. In this work the obtained xyloglucan shows a structure composed by glucose (40%), xylose (34%), galactose (20%) and arabinose (6%), typical of a neutral polysaccharide.

Table 2
^{1}H and ^{13}C chemical shifts of the units found in the xyloglucan from *H. courbaril* seeds.

Structure/Chemical shifts	Units[a]	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
β-D-Glcp	I/II	103.02/4.58	73.65/3.43	74.93/3.70	79.75/3.73	74.14/3.84	67.30/3.90–4.04
β-D-Galp	IV	105.13/4.59	72/3.66	73.5/3.68	69.4/3.96	75.8/3.7	61.84/3.79–3.88
α-D-Xylp	III	99.69/4.97	72.3/3.57	73.93/3.75	70.2/3.65	62.4/3.58–3.76	
α-D-Xylp'	V	99.5/5.17	80.81/3.7	72.8/3.96	70.2/3.65	62.4/3.58–3.76	

[a] See Fig. 3.

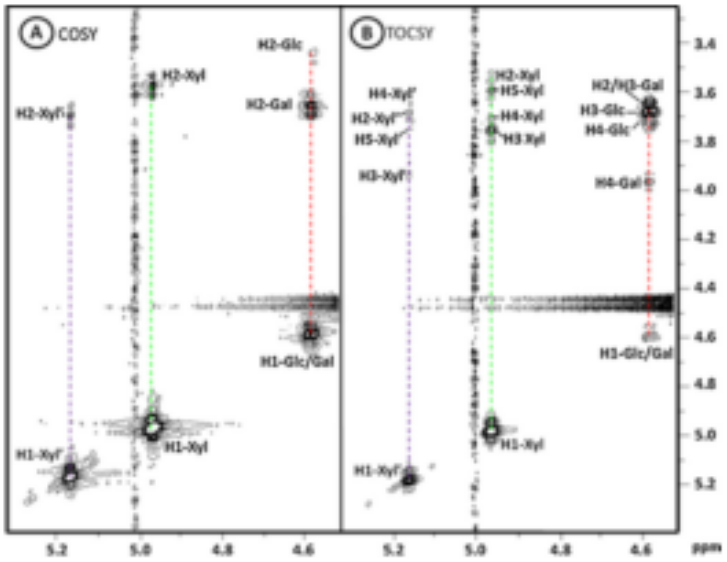

Fig. 5. ^{1}H-^{1}H COSY (A) and ^{1}H-^{1}H TOCSY (B) spectra of the xyloglucan from *H. courbaril* seeds. Xyl' and Xyl represent glycosylated (Xyl') and non-glycosylated (Xyl) xylose spin systems traced in pink and green, respectively). The coincident spin system of glucose and galactose is traced in red.

ζ-potential values are also related to the stability of solutions. As a general rule absolute values of ζ-potential above 60 mV indicate an excellent stability, from 60 to 30 mV are physically stable, from 30 to 5 mV are at the limit of stability and below 5 mV not stable and there is a strong likelihood to form aggregates [36]. Therefore, the result obtained with the xyloglucan (ζ-potential value = −11.3 ± 0.27 mV) indicates the polysaccharide solutions are stable. This solution was clear and transparent without the presence of aggregates demonstrating the total dissolution and stability of the system.

Polysaccharide concentration is the factor that mostly affects *Z*-average and ζ-potential values [33]. Anionic xyloglucans obtained from Gokyo Food and Chemical Co. Ltd (Fukusima, Japan) showed ζ-potential values of −21.7 ± 1.67 [26], but the authors worked with more concentrated solution of xyloglucan (1.0% w/v) and at a lower pH (4.5) than in our work, which may explain the different results they reported.

3.8. Rheological analyses

The rheological behavior of the xyloglucan at concentrations of 0.5 and 1.0% (w/v) was investigated by rotational, non-destructive oscillatory and stress-strain studies in order to evaluate its performance and potential as a biotechnological product.

Most part of the rheological characterization of xyloglucans from different sources investigates flow properties of this polysaccharide by mixing with other components [37–39] or chemical modification [23,24,40].

The rotational flow studies were performed on the xyloglucan with the apparent viscosity as a function of shear rate (Fig. 6A). For both concentrations, the system behaves as a Newtonian fluid for most part of the shear-rate interval in agreement with data from the literature [41–43]. These authors show xyloglucan aqueous solutions exhibiting typical Newtonian behavior at low concentrations (≤0.5% w/w) and shear-thinning behavior for concentrations usually higher than 1% (w/v).

In addition to the properties of stability against heat, pH and shear, xyloglucan cannot form gel simply by changes in its concentration. Although there is an increase in its viscosity, xyloglucan gels are formed only in the presence of alcohol or high levels of sugar [10,42,44].

Furthermore, the properties of xyloglucans in water, such as solubility, viscosity and gelling ability, are closely related to their chemical structures. This type of behavior is expected for most polymer solutions and was already observed for other polysaccharides composed essentially of glucose, xylose and galactose [23,40].

The oscillatory studies measured G' and G'' as a function of the oscillatory angular frequency (Fig. 6B) are similar to the behavior observed for others native xyloglucan solutions [23,41,45,46]. For the two concentrations tested, G'' was higher than G' for low frequencies, indicating that the system has a liquid behavior who dissipates most of the energy externally applied. G and G'' continuously increase as a function of oscillating frequency, so it is possible to observe a cross-over between the moduli; above the cross-over, an elastic behavior sets in $G' > G''$, typical for a concentrated polymer solution [47].

The ability to provide both behaviors between liquid and solid states suggests the use of our native xyloglucan with 0.5 and 1.0% (w/v) concentrations as films/coatings widely useful in many industries as a biodegradable material.

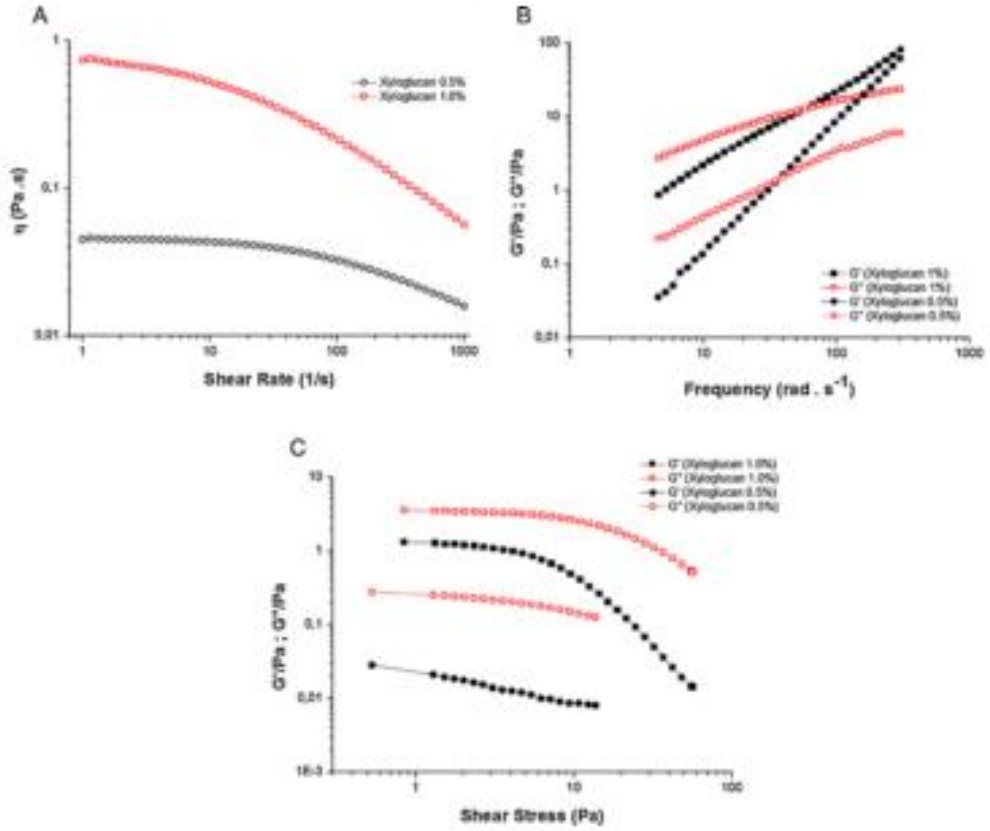

Fig. 6. Rheological analysis of the solutions obtained from the xyloglucan of the *H. courbaril* seeds at 0.5 (ball symbols) and 1.0% (square symbols) [w/v]. (A) rotational flow studies of apparent viscosity as a function of shear-rate, (B) oscillatory studies measuring G' (filled symbols) and G'' (open symbols) as a function of frequency and (C) stress-strain experiment measuring G' and G'' as a function of shear stress.

Fig. 6C shows a stress-strain experiment where G' and G'' are measured as a function of shear-stress and increase as a function of concentration with $G'' > G'$ for the entire externally applied stress interval. Similar behavior has been observed for the oscillatory measurements (see Fig. 6B) confirming that the system is essentially a Newtonian liquid for moderate applied stresses and cannot sustain elastic linear deformations. Both at 0.5 and 1.0% concentrations it behaves as a viscoelastic liquid where the G'' contribution has a constant value (Newtonian behavior) for a finite stress interval whereas the G' contribution decreases as a function of stress with $G'' > G'$ for the entire stress interval.

3.9. Biological activities: antibacterial and hemolytic

Antibacterial activity of xyloglucans was not tested previously. We now tested this possible effect but did not observe any action of the xyloglucan as inhibitor of bacterial growth. Perhaps the monosaccharides from xyloglucan could serve as carbon source for microbial growth, differently from that observed e.g. for chitosan [48], that exhibits antimicrobial activity.

The hemolytic activity of xyloglucan was performed to rule out a possible mechanism of toxicity and to check the safety of the polysaccharide, thus indicating if it is suitability for pharmaceutical preparations [49]. In hemolytic tests the compounds are considered toxic when the hemolysis contents equal or surpass 50%, the so-called HC_{50} [50].

Our results showed that solutions Triton X-100 at concentration raging from 0.25 to 2.00 mg mL^{-1} presented HC_{50} from 50.70 ± 0.04 to 100%, based on the highest hemolytic effect observed. Significant hemolysis was not detected at these concentrations of xyloglucan, with HC_{50} of 5.40 ± 0.32 to 6.10 ± 0.08%, thus leading to the conclusion that the polysaccharide is devoid of hemolytic activity and therefore shows potential for application in the health industry without apparent toxicity.

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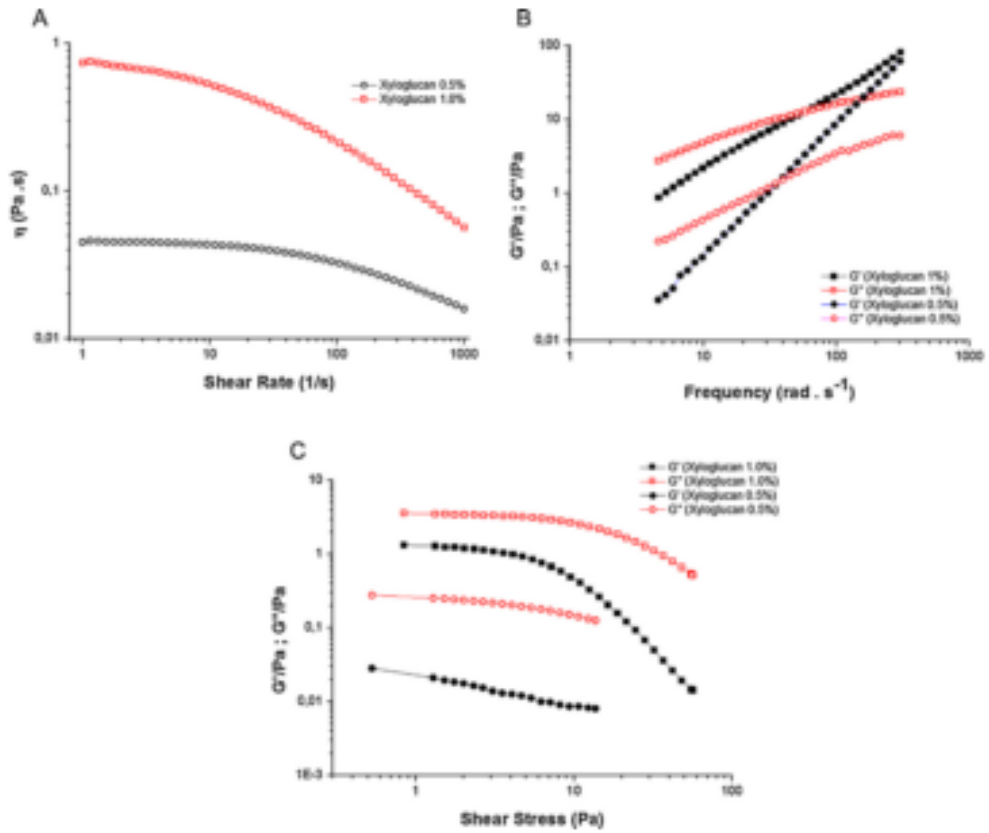

Fig. 6. Rheological analysis of the solutions obtained from the xyloglucan of the *H. courbaril* seeds at 0.5 (ball symbols) and 1.0% (square symbols) [w/V]. (A) rotational flow studies of apparent viscosity as a function of shear-rate, (B) oscillatory studies measuring G' (filled symbols) and G'' (open symbols) as a function of frequency and (C) stress-strain experiment measuring G' and G'' as a function of shear stress.

Fig. 6C shows a stress-strain experiment where G' and G'' are measured as a function of shear-stress and increase as a function of concentration with $G'' > G'$ for the entire externally applied stress interval. Similar behavior has been observed for the oscillatory measurements (see Fig. 6B) confirming that the system is essentially a Newtonian liquid for moderate applied stresses and cannot sustain elastic linear deformations. Both at 0.5 and 1.0% concentrations it behaves as a viscoelastic liquid where the G'' contribution has a constant value (Newtonian behavior) for a finite stress interval whereas the G' contribution decreases as a function of stress with $G'' > G'$ for the entire stress interval.

3.9. *Biological activities: antibacterial and hemolytic*

Antibacterial activity of xyloglucans was not tested previously. We now tested this possible effect but did not observe any action of the xyloglucan as inhibitor of bacterial growth. Perhaps the monosaccharides from xyloglucan could serve as carbon source for microbial growth, differently from that observed e.g. for chitosan [48], that exhibits antimicrobial activity.

The hemolytic activity of xyloglucan was performed to rule out a possible mechanism of toxicity and to check the safety of the polysaccharide, thus indicating if it is suitability for pharmaceutical preparations [49]. In hemolytic tests the compounds are considered toxic when the hemolysis contents equal or surpass 50%, the so-called HC_{50} [50].

Our results showed that solutions Triton X-100 at concentration raging from 0.25 to 2.00 mg mL^{-1} presented HC_{50} from 50.70 ± 0.04 to 100%, based on the highest hemolytic effect observed. Significant hemolysis was not detected at these concentrations of xyloglucan, with HC_{50} of 5.40 ± 0.32 to 6.10 ± 0.08%, thus leading to the conclusion that the polysaccharide is devoid of hemolytic activity and therefore shows potential for application in the health industry without apparent toxicity.

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References

[1] I. Mioedder, C. Travelet, A. Durand-Terrasson, S. Halila, F. Dubreuil, R. Borsali, Carbohydr. Polym. 94 (2013) 934–939.
[2] T. Coviello, P. Matricardi, C. Marianecci, F. Alhaique, J. Control. Release 119 (2007) 5–24.
[3] R.A. Freitas, S. Martin, G.L. Santos, F. Valenga, M.S. Buckeridged, F. Reicher, M.-R. Sierakowski, Carbohydr. Polym. 60 (2005) 507–514.
[4] T.A. Jó, D.F.S. Petri, L.M. Beltramini, N. Lucyszyn, M.R. Sierakowski, Carbohydr. Polym. 82 (2010) 355–362.
[5] N. Lucyszyn, A.F. Lubambo, K.F. Matos, I. Marvila, C.F. Souza, M.-R. Sierakowski, Mater. Sci. Eng. C. 29 (2009) 552–558.
[6] M. Buckeridge, D.C. Rocha, J.S.G. Reid, S.M.C. Dietrich, Physiol. Plant. 86 (1992) 145–151.
[7] M.S. Buckeridge, H.J. Crombie, C.J.M. Mendes, J.S.G. Reid, M.J. Gidley, C.C.J. Vieira, Carbohydr. Res. 303 (1997) 233–237.
[8] M.M.T. Rosário, M.M. Kanzawsu-Marcolino, A.E. Amaral, G.R. Noleto, C.L.O. Petkowicz, Chem. Biol. Interact. 189 (2011) 127–133.
[9] N. Lucyszyn, A.F. Lubambo, L. Ono, T.A. Jó, C.F. Souza, M.R. Sierakowski, Food Hydrocolloids 25 (2011) 1242–1250.
[10] K. Nishinari, M. Takemasa, R. Zhang, R. Takahashi, in: J.P. Kamerling (Ed.), Storage Plant Polysaccharides: Xyloglucans, Galactomannans, Glucomannans, Elsevier, New York, 2007, pp. 613–652.
[11] A.P. Busato, C.G. Vargas-Rechia, F. Reicher, Phytochemistry 58 (2001) 525–531.
[12] J.L.S. Carvalho Filho, M.F.A. Arrigoni-Blank, A.F. Blank, M.S.A. Rangel, Cerne 9 (2003) 109–118.
[13] N.N. Lima, F. Reicher, J.B.C. Corrêa, J.L.M.S. Ganter, M.R. Sierakowski, Cien. Cult. 45 (1993) 22–26.
[14] P.B.S. Albuquerque, W. Barros Jr., G.R.C. Santos, M.T.S. Correia, P.A.S. Mourão, J.A. Teixeira, M.G. Carneiro-da-Cunha, Carbohydr. Polym. 104 (2014) 127–134.
[15] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Anal. Chem. 28 (1956) 350–356.
[16] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[17] H.W. Kircher, Anal. Chem. 32 (1960) 1103–1106.
[18] I. Ciucanu, F. Kerek, Carbohydr. Res. 131 (1984) 209–217.
[19] A.M.F. Tovar, N.V.M. Capillé, G.R.C. Santos, B.C. Vairo, S-N.M.C.G. Oliveira, R.J.C. Fonseca, P.A.S. Mourão, Thromb. Haemostasis. 107 (2012) 903–915.
[20] R.J. Hunter, Zeta Potential in Colloid Science: Principles and Applications, 2nd ed., Academic Press, New York, 1981.
[21] M.D. Cole, Biochem. Syst. Ecol. 22 (1994) 837–858.
[22] J.F.V. Silva, L.C.N. Silva, I.R.S. Arruda, A.G. Silva, A.J. Macedo, J.M. Araújo, M.T.S. Correia, M.V. Silva, J. Med. Plants Res. 7 (2013) 2067–2072.
[23] A.P. Busato, F. Reicher, R. Domingues, J.L.M. Silveira, Mater. Sci. Eng. C. 29 (2009) 410–414.
[24] R.A. Freitas, A.P. Busato, D.A. Mitchell, J.L.M. Silveira, Carbohydr. Polym. 83 (2011) 1636–1642.
[25] K.C. Kai, C.L.O. Petkowicz, Int. J. Biol. Macromol. 46 (2010) 223–228.
[26] H.S. Mahajan, V.K. Tyagi, R.R. Patil, S.B. Dunange, Carbohydr. Polym. 91 (2013) 618–625.
[27] H. Kaur, S. Yadav, M. Ahuja, N. Dilbaghi, Carbohydr. Polym. 90 (2012) 1543–1549.
[28] A. Mishra, K. Kavita, B. Jha, Carbohydr. Polym 83 (2011) 852–857.
[29] D. Mudgil, S. Barak, B.S. Khatkar, Carbohydr. Polym 90 (2012) 224–228.
[30] C.W. Vendruscolo, C. Ferrero, E.A.G. Pineda, J.L.M. Silveira, R.A. Freitas, M.R. Jiménez-Castellanos, T.M.B. Bresolin, Carbohydr. Polym. 76 (2009) 86–93.
[31] M. Hoffman, Z. Jia, M.J. Peña, M. Cash, A. Harper, A.R. Blackburn, A.D. Darvill, W.S. York, Carbohydr. Res. 340 (2005) 1826–1840.
[32] W.S. York, R. Hawkins, Glycobiology 10 (2000) 193–201.
[33] M.G. Carneiro-da-Cunha, M.A. Cerqueira, B.W.S. Souza, J.A. Teixeira, A.A. Vicente, Carbohydr. Polym. 85 (2011) 522–528.
[34] P. Atkins, J. De Paula, Atkins' Physical Chemistry, 9th ed, W. H. Freeman and Company, New York, 2010.
[35] S. Dumitriu, Polysaccharides: Structrural Diversity Functional Versatility, 2nd ed, Marcel Dekker, New York, 2005.
[36] R.H. Müller, Zetapotential und Partikelladung in der Laborpraxis, 1st ed., Wissenschaftliche, Verlagsgesellschaft Stuttgart, 1996.
[37] N. Hiran, V. Tantishaiyakul, W. Pichayakorn, Int. J. Pharm. 388 (2010) 196–201.
[38] R. Pongsawatmanit, T. Temsiripong, T. Suwonsichon, Food Res. Int. 40 (2007) 239–248.
[39] R.P. Vianna-Filho, C.L. Petkowicz, J.L. Silveira, Carbohydr. Polym. 93 (2013) 266–272.
[40] K. Yamatoya, M. Shirakawa, Curr. Trends Polym. Sci. 8 (2003) 27–72.
[41] K. Khounvilay, W. Sittikijyothin, Food Hydrocolloids 26 (2012) 334–338.
[42] K. Nishinari, K. Yamatoya, M. Shirakawa, Xyloglucan, in: G.O. Phillips, P.A. Williams (Eds.), Handbook of Hydrocolloids, CRC, Press, Boca Raton, 2000, pp. 247–267.
[43] Y. Ren, D.R. Picout, P.R. Ellis, S.B. Ross-Murphy, J.S.G. Reid, Carbohydr. Res. 340 (2005) 997–1005.
[44] K. Nishinari, H. Zhang, S. Ikeda, S. Curr. Opin. Colloid Interface Sci. 5 (2000) 195–201.
[45] C. Ribeiro, G.G.C. Arizaga, F. Wypych, M.-R. Sierakowski, Int. J. Pharm. 167 (2009) 204–210.
[46] Q. Wang, P.R. Ellis, S.B. Ross-Murphy, W. Burchardh, Carbohydr. Polym. 33 (1997) 115–124.
[47] R. Lapasin, S. Pricl, Rheology of Industrial Polysaccharides: Theory and Applications, 1st ed., Blackie Academic & Professional, London, 1995.
[48] M. Kong, X.G. Chen, K. Xing, H.J. Park, Int. J. Food Microbiol. 144 (2010) 51–63.
[49] T. Kalaivani, C. Rajasekaran, K. Suthindhiran, L.J. Mathew, Evid. Based Complement. Altern. Med. 2011 (2011) 1–8.
[50] M. Ahn, R.N. Murugan, B. Jacob, J.K. Hyun, C. Cheong, E. Hwang, H.-N. Park, J.-H. Seo, G. Srinivasrao, K.S. Lee, S.Y. Shin, J.K. Bang, Eur. J. Med. Chem. 68 (2013) 10–18.

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Development and characterization of a new hydrogel based on galactomannan and κ-carrageenan

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ABSTRACT

A new hydrogel based on two natural polysaccharides was prepared in aqueous medium with 1.7% (w/v) galactomannan (from *Cassia grandis* seeds) and different concentrations of κ-carrageenan (0.3, 0.4 and 0.5% w/v), $CaCl_2$ (0.0, 0.1 and 0.2 M) and pH (5.0, 5.5 and 6.0), using a full factorial design based on rheological parameters. The best formulation was obtained with 1.7% (w/v) galactomannan and 0.5% (w/v) κ-carrageenan, containing 0.2 M $CaCl_2$ at pH 5.0. Nuclear magnetic resonance and scanning electron microscopy where used in order to characterize the hydrogel formulation. A shelf life study was carried out with this formulation along 90 days-period of storage at 4 °C, evaluating pH, color, microbial contamination and rheology. This hydrogel showed no significant changes in pH, no microbial contamination and became more translucent along the aging. Analyses by nuclear magnetic resonance and rheology showed a larger organization of the polysaccharides in the hydrogel matrix. The results demonstrated that this hydrogel was stable with possible applications in medical and cosmetic fields.

1. Introduction

Hydrogels are macromolecular networks formed from natural or synthetic hydrophilic polymers that can absorb and retain a significant amount of water without dissolving (Lefnaoui & Moulai-Mostefa, 2014; Ahmed, 2013). The hydrogel resistance to dissolution arises from cross-links between network chains, while their ability to absorb water arises from hydrophilic functional groups distributed along the polymeric backbone (Ahmed, 2013). Due to the combination of solid-like properties from the macromolecular network and liquid-like properties from the absorbed water, hydrogels exhibit viscoelasticity resembling that of biological tissue. In addition to these rheological properties, their high biocompatibility have attracted attention in drug delivery applications (Da-Lozzo et al., 2013), pharmaceuticals (Laurén et al., 2014), biomedical applications (Kenawy, Kamoun, Mohy Eldin, & El-Meligy, 2014), tissue engineering and regenerative medicine (Huang, He, & Wang, 2013), diagnostics (Feyzkhanova et al., 2014), wound dressing (De Cicco et al., 2014), separation of biomolecules or cells (Ahmad et al., 2014), barrier materials to regulate biological adhesions (Zhang et al., 2011), and biosensors (Liu et al., 2015).

When forming hydrogels, polysaccharides of natural origin have improved properties in comparison with synthetic polymers (Manjanna, Pramod Kumar, & Shivakumar, 2010). Among natural polymers, there are the gelling polymer agents as carrageenans, and also thickening polymers, such as galactomannans, that, although not producing network real cross-links, possess gel properties above some specific concentration. Kappa-carrageenan (κC) is obtained by alkaline extraction (or by modification of other carrageenans) from red seaweed (Rhodophyceae) (Prajapati, Maheriya, Jani, & Solanki, 2014). Without chemical modifications, it has the ability to form a strong three-dimensional network in presence of

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counterions (K^+ and Ca^{+2}) when compared with other types of carrageenans (lambda-carrageenan). κC is composed of alternating residues of 3-linked β-D-galactopyranose 4-sulfate and 4-linked 3,6-anhydro-α-D-galactopyranose residues (Prajapati et al., 2014; Pinheiro et al., 2011). Due to its high hydrophilicity, mechanical strength, biocompatibility and biodegradability, this polymer has been mostly used in food industry as gelling, stabilizing and thickener agent (Dima, Cotârlet, Alexe, & Dima, 2014). Nevertheless it has also been used to reduce or to eliminate toxicity in biomedical applications (Muhamad, Fen, Hui, & Mustapha, 2011).

Galactomannans are widespread polysaccharides commonly extracted from the endosperm of numerous seed plants (particularly the *Leguminosae*) which develop energy-reserve and hydration functions (Prajapati et al., 2013). The galactomannan extracted from *Cassia grandis* seeds have a core constituted by 4-linked β-D-mannopyranose with a repetitive single branch of α-D-galactopyranosyl units linked to position 6 of the central chain (Albuquerque et al., 2014). Due to its high molecular weight, water solubility, non-ionic character, biocompatibility and gel properties in higher concentrations, this polysaccharide can be used in food, pharmaceutical, biomedical, cosmetic, textile and paper industries, especially as an emulsion stabilizer (Albuquerque et al., 2014; Pinheiro et al., 2011).

Various hydrogels formulated from natural polymers have been used in industry. However, it has become clear that hydrogels consisting of single polymer arrangements cannot meet entirely the demands in terms of both properties and performance (Ahmed, 2013). In order to improve gel properties, new hydrogels based on two or more polymers have been developed in the last years (Jang et al., 2014; Diao, Li, Xiao, Duan, & Xu, 2014; Soares et al., 2014; Dash, Foston, & Ragauskas, 2013; Martins et al., 2012). Sometimes, chemical modification in involved polymers, chemical crosslinks and/or physical interactions is necessary. Such mixed systems include polymer blends, copolymer, interpenetrating or semi-interpenetrating polymer networks, with desirable properties (Liu et al., 2014; Lv et al., 2014; Vudjung et al., 2014; Bhattacharyya & Ray, 2014).

Along the years, the use of rheology for characterization of viscoelastic properties in the production and application of polymeric materials such as gels and hydrogels has been essential (Albuquerque et al., 2014; Jang et al., 2014; Dash et al., 2013; Sandolo, Matricardi, Alhaique, & Coviello, 2007). The hydrogels behavior during flow and applied stress are important quality control parameters responsible for maintaining the products superiority and increase its shelf life. In this work we have developed a new hydrogel based upon optimized rheological characteristic of a mixture of κ-carrageenan and galactomannan from *C. grandis* seeds. This optimization was achieved via a full factorial design 2^3 by which changing pH and both κC and cation concentrations, the hydrogels formed were rheologicaly tested. In addition to a primary characterization of the new hydrogel, shelf life studies were also carried out to guarantee its features during storage, making it viable in biomaterial industry applications.

2. Material and methods

2.1. Material

The pods of *C. grandis* were collected at the rural zone of Pernambuco State, in the city of Angelim (Brazil), in July 2011. Ethanol 99.8%, acetone PA, NaCl and phenol were obtained from Vetec Fine Chemicals Ltda. (Brazil). κ-Carrageenan was purchased from Sigma Aldrich (U.S.A.). All other chemicals were of analytical grade.

2.2. Extraction of the galactomannan from C. grandis seeds

The galactomannan from *C. grandis* seeds was extracted and purified according to the method described by Albuquerque et al. (2014). Briefly, dried seeds were boiled in distilled water 1:5 (w/v) at 100 °C for 1 h and maintained for 18 h at 25 °C to facilitate removal of the hull. The endosperm plus germ, were triturated in a blender with 0.1 M NaCl 5% (w/v) at 25 °C, filtered and precipitated with 46% ethanol 1:3 (v/v) for 24 h. The precipitate was filtered again, followed by a wash with 100% ethanol 1:3 (v/v) and twice with acetone P.A. 1:3 (v/v), with filtrations between each washing. The precipitated galactomannan obtained was dried in an oven at 100 °C and finally pulverized and kept in a dry place until further use.

2.3. Hydrogel preparation

Stock solutions of κC in different concentrations [0.3, 0.4 and 0.5% (w/v)] and galactomannan (1.7% w/v) were prepared. A known volume of κC was added into a beaker in addition to a known concentration of $CaCl_2$. The solution was maintained under magnetic stirring (850 rpm), at 50 °C during 20 min. The addition of $CaCl_2$ [1.0% (v/v) of the final volume of desired mixture] was necessary to ignite a crosslinking process. In order to evaluate the latter process, hydrogel samples with three $CaCl_2$ concentrations were actually utilized: 0 M, 0.1 M, and 0.2 M. After that, a well-known volume of galactomannan solution, was added into the beaker containing the κC + $CaCl_2$ solution at 1 ml/min flow rate, using a needle syringe (27G). The mixture was left stirring under 850 rpm at 50 °C, during additional 30 min. Several samples were prepared from the mixture adjusting their pH to 5.0, 5.5 or 6.0 using a 1 M HCl solution. These final samples, now named hydrogels, were distributed in a cell culture plate (6-well plate) and stored at 4 °C.

2.3.1. Experimental design and statistical analysis

The best hydrogel mixture was selected by monitoring the influence of the following independently controlled parameters: κ-carrageenan (κC) and $CaCl_2$ (Ci) concentrations, pH onto the measured complex viscosity (η^*), viscous–elastic moduli ratio ($G''/G' = \tan\delta$), and the phase transition temperature (T_t). The study was conducted employing a 2^3 full factorial design (see Supplementary material), in addition to a central point in quadruplicate to allow for the estimation of pure experimental error. The results were statistically validated by an analysis of variance (ANOVA) at a significance level of $p < 0.05$. All statistical and graphical analyses were carried out with the Statistica 8.0 program (StatSoft Inc., 2008, Tulsa, OK, USA).

2.4. Hydrogel characterization

2.4.1. Nuclear magnetic resonance spectroscopy

One dimensional (1D) ^{1}H NMR spectra of the polysaccharides and hydrogels were recorded using a Bruker DRX 600 MHz apparatus with a triple resonance probe, as described previously by Tovar et al. (2012). Approximately 5 mg of each sample was dissolved in 0.5 ml of 99.9% deuterium oxide (Cambridge Isotope Laboratory, Cambridge, MA, USA). The spectra were recorded at different temperatures (25 °C to 55 °C) with HOD (deuterated water exhibiting a peak due to exchange with residual H_2O) suppression by presaturation. For NMR spectra, 32 scans were recorded using an inter-scan delay equal to 1 s and the chemical shifts were displayed relative to external trimethyl-silylpropionic acid at 0 ppm for ^{1}H. The range of temperature was made in order to observe the temperature influence on the hydrogel matrix.

2.4.2. Rheological measurements

All rheological measurements were carried out, in triplicate for each sample, according to Pinheiro et al. (2011) using a rheometer MCR301 (Anton-Paar, Austria), in a parallel plate geometry (diameter: 25 mm) cell with a gap between plates of 1 mm. In order to determine the appropriate strain for linear viscoelastic regime, preliminary strain sweeps were conducted at different frequencies (0.1, 1.0 and 10.0 Hz) for variable strains ranging from 0.1 to 10.0%. Frequency sweeps were carried out within a 0.1–100 Hz range, maintaining a linear viscoelastic regime (0.2% strain deformation). Moreover, the hydrogel was tested for temperature stability (see Supplementary material) at oscillatory mode (frequency = 1.0 Hz) for a range of temperature from 0 to 70 °C, at a rate of 5 °C/min increase. Unless explicitly mentioned, all rheological measurements were conducted at 25 °C.

2.4.3. Stability and shelf life studies

Triplicates of the most stable hydrogel (Following a full factorial test criterion—see Supplementary material) were storaged at 4 °C in sterile petri dishes (35 × 10 mm) and during 90 days-period, a shelf life study were carried out by pH, colorimetric, rheological measurements and microbiological analysis. After 1, 5, 10, 20, 30, 60 and 90 days, the hydrogel samples were taken randomly. To evaluate the microbiological contamination of the hydrogel, analyses of mesophilic aerobes, yeasts and molds, lactic acid bacteria and psychrotrophs were carried according to the methodology described by the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant & Splittstoesser, 1992). The hydrogels opacity was determined using a Minolta color meter (CR 400; Minolta, Japan) and according to the Hunterlab color scale. Following this method, the degree of opacity (*Y*) was calculated as the ratio between the opacity of each sample on a black standard (*Yb*) and the opacity of each sample on a white standard (*Yw*). An average of random three *Yb* and *Yw* measurements was used for calculations. The results were expressed as a percentage: *Y* (%) = 100 (*Yb*/*Yw*). The rheological measurements were performed as described before.

2.4.4. Scanning electron microscopy (SEM)

The SEM surface scans of the hydrogel were conducted on a scanning electron microscope EVO LS15 (ZEISS, Germany) with an accelerating voltage of 10 kV under vacuum conditions. The hydrogel samples were prepared 24 h before measurements. For each sample internal matrix structure analysis, a cross-section of the hydrogel was lyophilized utilizing the cryofracture method. The lyophilized sample was attached to a coverslip via a coated thin film of chromium and carbon. The coating also worked to prevent the accumulation of static electric charge on the surface during electron irradiation and to avoid scanning faults and other image artifacts. The samples were sprayed with colloidal gold particles and then left drying at room temperature (25 °C) before scanning.

3. Results and discussion

3.1. Hydrogel characterization

In order to obtain an optimized hydrogel by mixing the galactomannan from *C. grandis* seeds and κ-carrageenan, a full factorial design 2^3 was performed (see Supplementary material). According to the results, it was possible to obtain a stable hydrogel composed by 1.7% (w/v) of galactomannan and 0.5% (w/v) of κ-carrageenan, with 0.2 M of $CaCl_2$ at pH 5.0 which was capable of supporting higher temperature (40.6 °C).

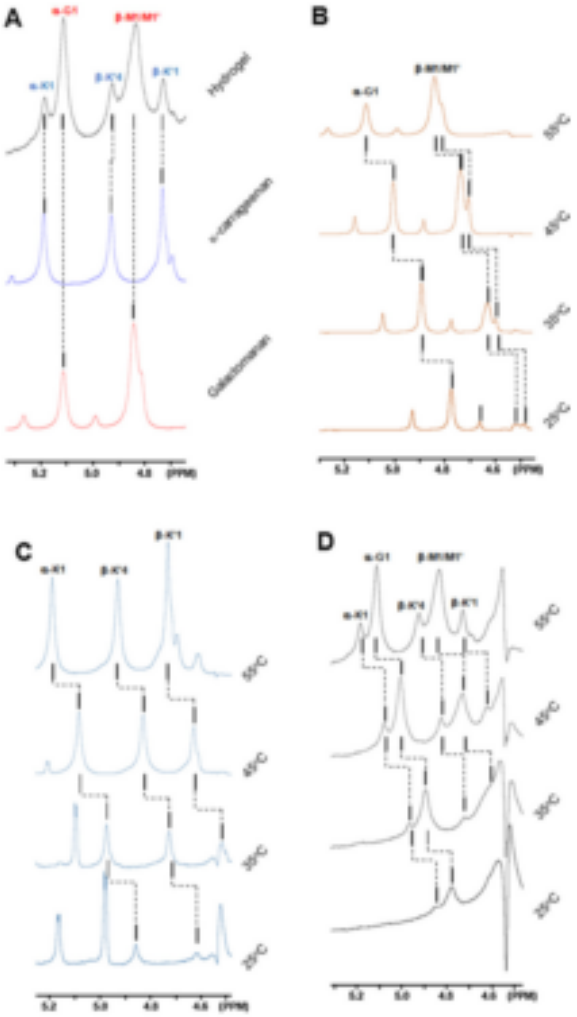

Fig. 1. One-dimensional ^{1}H NMR spectra (4.6–5.3 ppm region) of the purified galactomannan, κ-carrageenan and of hydrogel obtained by the mixture of these two polysaccharides at 45 °C (A). The impact of temperature variation was observed by the broken horizontal lines on the ^{1}H chemical shifts observed in the spectra of purified galactomannan (B), κ-carrageenan (C) and the hydrogel (D).

3.1.1. Nuclear magnetic resonance (NMR) spectroscopy

We characterized the hydrogel formed by κ-carrageenan and galactomannan using 1D ^{1}H NMR spectroscopy. The ^{1}H chemical shifts of these two polysaccharides are similar irrespective the spectra were run using purified polysaccharide solutions or hydrogel. Fig. 1 exemplifies this observation for the 4.6–5.3 ppm region of the spectra run at 45 °C. In this region, we can easily identify the anomeric protons of the α-galactopyranosyl (α-G1) and β-mannopyranosyl (β-M1) units at 5.11 and 4.84 ppm, respectively, of the galactomannan (bottom spectrum in Fig. 1A). It is also possible to visualize the anomeric protons of the 3,6-anhydro-α-galactose (α-K1) and of the β-galactopyranosyl unit (β-K1) at 5.19 and 4.72 ppm, respectively, of the κ-carrageenan (middle spectrum at 45 °C in Fig. 1A). We can also identify H4 of the 4-sulfated β-galactose (β-K4) due to the characteristic downfield shift of the 4-sulfation site. These results are in accordance with the

Table 1
Color parameters L^* and Y for the galactomannan)κ-carrageenan hydrogel along 90 days-period.

Days	L^*	Y (%)
1st	69.96 ± 0.91[a]	14.42 ± 0.81[a]
5th	72.72 ± 1.97[a]	13.40 ± 0.78[a]
10th	86.66 ± 5.93[b]	10.41 ± 0.36[b]
15th	90.03 ± 2.27[b]	9.89 ± 0.29[c]
30th	88.35 ± 2.55[b]	9.90 ± 0.27[c]
60th	87.91 ± 4.45[b]	12.76 ± 0.78[b]
90th	89.98 ± 1.91[b]	11.10 ± 0.49[b]

L^* (Light); Y (%) (Opacity); Values expressed as an average ± standard deviation. [a–c] Different superscript letters in the same column indicate a statistically significant difference ($p < 0.05$.).

solution NMR analysis of galactomannans and carrageenans previously reported in literature (Albuquerque et al., 2014; Cheng et al., 2013; Yao, Wu, Zhang, & Du, 2014; Farias et al., 2008; Bosco, Segre, Miertus, Cesa'ro, & Paoletti, 2005).

These characteristic ^{1}H NMR signals of the galactomannan and κ-carrageenan show no significant shifts in the ^{1}H NMR spectrum of the hydrogel (top spectrum in Fig. 1A), except for a very modest ~0.02 ppm upfield shift of β-K4. Possibly this is a consequence of interaction of 4-sulfate groups with calcium ions in the hydrogel formulation. These results indicate that κ-carrageenan and galactomannan form a physical type hydrogel, containing semi-interpenetrating polymer network (SEMI-IPN). Clearly, these polysaccharides did not form a chemical type hydrogel, which would result in shifts of their ^{1}H-signals.

We also investigated the impact of temperature on the ^{1}H spectra of the purified galactomannan (Fig. 1B) and κ-carrageenan (Fig. 1C) and of hydrogel (Fig. 1D). The ^{1}H signals show marked downfield shifts as the temperature increases from 25 °C to 55 °C (shifts indicated by horizontal broken lines in the Panels). However, more significant, the shifts are similar on the spectra run with the purified polysaccharides solutions (Fig. 1B and C) or with the hydrogel (Fig. 1D). Some ^{1}H signals are broad or even not identified at 25 °C, especially those of the β-anomers, mostly because of the interference of HOD signal at this temperature and of the water suppression by presaturation. This is particularly relevant for the ^{1}H spectra of the hydrogel at 25 °C and 35 °C (bottom panels in Fig. 1D), which suggest strong intermolecular interaction and self-organization of the polysaccharides in the gel state. The ^{1}H signals were recovered as the temperature increases, indicating disruption of the self-organization due to the increased dynamic of the polysaccharides solutions. This is a typical phenomenon of viscous solutions obtained with macromolecules (Shapiro, 2011).

3.1.2. Stability and shelf life evaluation of the hydrogel

The pH is an important parameter for evaluation of stability and biocompatibility of hydrogel. The hydrogel formed using galactomannan and κ-carrageenan showed an average pH = 5.70, without significant variations ($p \leq 0.05$). According to Segura, Camargo Junior, Bagatin, and Campos (2010), this pH range is considered suitable for various applications, especially in cosmetics, food and pharmaceutical industries. According to the microbiological analysis, there was no observed microorganism growth in any of the experimentation days (data not shown). The colorimetric results for the L^* and Y parameters are shown in Table 1. L^* denotes the light content into the sample. The opacity (Y%) is another important characteristic that indicates the capacity of biomaterials to act as a barrier against light, and it is also a way to relate the major or minor degree of miscibility of polymers (Li & Shimizu, 2006).

The experimental results have shown a significant increase in luminosity of approximately 24% along the course of the measurements followed by a reduction of opacity of 29%. On the first days it is most likely that the hydrogel is on a globular/double helix stage which scatters light more efficiently. With the aging of the hydrogel, as it keeps reacting with the free calcium ions, these helices start aggregating and organizing in junction zones in a way that reduces scattering and turns it more transparent. This comportment was also observed by the changes in opacity, which exhibited a reduction until the 30th day, with a small increase at the final of the experimental time (60th and 90th days). This confirmed the reorganization of the polysaccharide chains of the hydrogel that is reflected in a less opaque macroscopic sample, it is, more transparent. A similar observation was reported by Silva, Pereira, Carvalho, and Ferrua (2007), who showed that starch gels suffer structural changes along its formation with decreasing opacity.

3.1.3. Rheometry

The hydrogel rheological studies are depicted on Fig. 2 Fig. 2A shows an oscillatory experiment where the elastic (storage), G', and dissipation (loss), G'', moduli are monitored as a function of the oscillatory frequency. Regarding hydrogels, once the crosslinks between the different chains of the polymers are established, the network thus obtained shows a solid viscoelastic behavior and, sometimes, a pure elastic behavior (Hennink & van Nostrum, 2002; Cuggino et al., 2008). This can be visualized in Fig. 2A, which shows $G' \gg G''$ as a function of the oscillatory frequency. For longer periods of shelf storage, the gel matrix is reinforced and, in addition to $G' \gg G''$, the values of individual moduli do not vary substantially with frequency. These characteristics are a pristine signature of gel rheological behavior. Visually the hydrogel is self-standing (see Supplementary material). For short shelf storage periods, although $G' > G''$ and the gel behavior still preserved, the data indicate that the long-range network structure is not fully developed. For instance, at higher frequencies, both storage and loss moduli increase indicating the presence of a small length scale structure. In Fig. 2A the elastic modulus G' increases too steeply in contrast to what is expected for continuous gels. Instead its behavior resembles that of a packing of emulsion droplets at the jamming transition. It is important to note the classical gel behavior (parallel G' to G'' as a function of frequency) occurs above the 15 days storage period. In addition, the gel formed can be considered a strong gel when compared to the pure galactomanan gel (Albuquerque et al., 2014). Therefore, during the shelf storage (4 °C storage temperature) period the gel long-range structure is consolidated. These results were similar to those obtained by Pinheiro et al. (2011), who reported long-range gellification for mixtures of κ-carrageenan and galactomannan extracted from *Gleditsia triacanthos* seeds (60/40, respectively) and *Sophora japonica* (60/40, respectively).

Macroscopic hydrogels can support only a limited amount of stress and break when stress or strain exceeds some yielding limit. The data show the two main characteristics of the formed gel: a linear elastic region where G' is constant regardless of the stress applied; and a yield stress where the gel structure is destroyed and the system starts flowing (Fig. 2B). Although G' and G'' lose meaning at nonlinear regions, we will consider the point at the cross over, when $G' = G''$, as the stress necessary to yield. Conversely G'' does not show a linear region. Instead there is a weak overshoot at the crossover point. This overshoot does not exist in pure galactomannan samples from *C. grandis* (Albuquerque et al., 2014) and is most likely due to the existence of long chains in the κ-carrageenan as also found in xanthan gum (Hyum, Kim, Ahn, & Lee, 2002). During a stress test, in order to keep oscillating frequency fixed, as the stress (strain) increases the shear rate has to increase. Slow structure relaxation depends on the applied shear rate and gets faster as the shear rate increases. That explains the increase in dissipation G'' as the stress increases. However there is a peak at the point where the shear rate reaches the oscillating frequency causing dissipation to become inefficient. Overall, the increase in loss, G'', or storage, G',

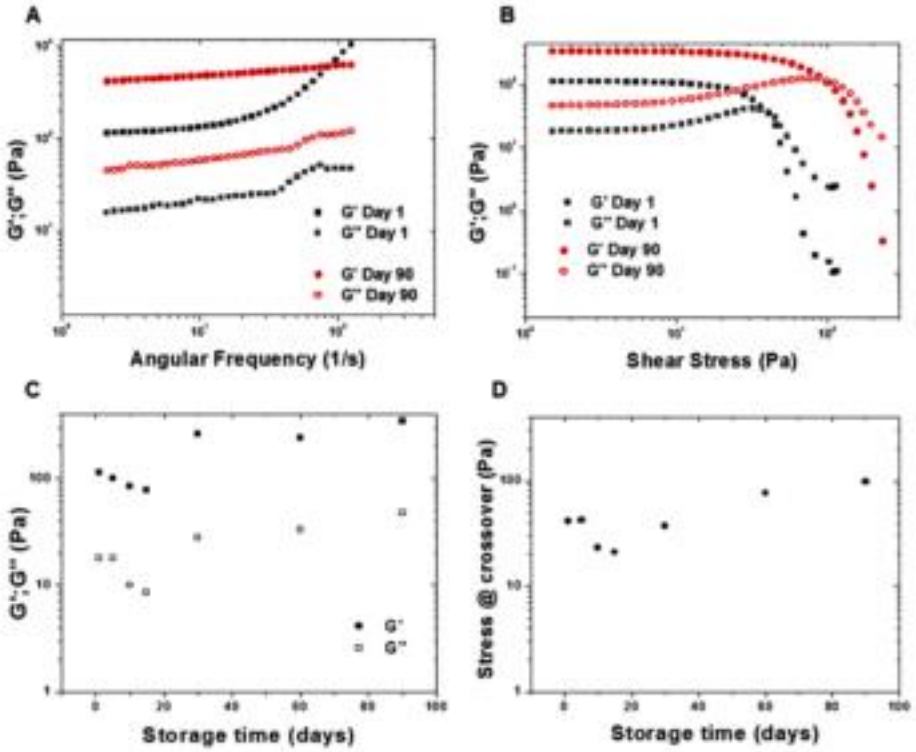

Fig. 2. Rheological measurements of the best hydrogel formulation obtained from the full factorial design 2^3. A—Elastic (G') and viscous (G'') moduli as a function of the angular frequency, dynamic oscillatory flow. B—Elastic (G') and viscous (G'') moduli as a function of the shear stress, dynamic oscillatory flow. C—Elastic (G') and viscous (G'') moduli as a function of the storage time (90 days). D—Stress at crossover as a function of the storage time (90 days).

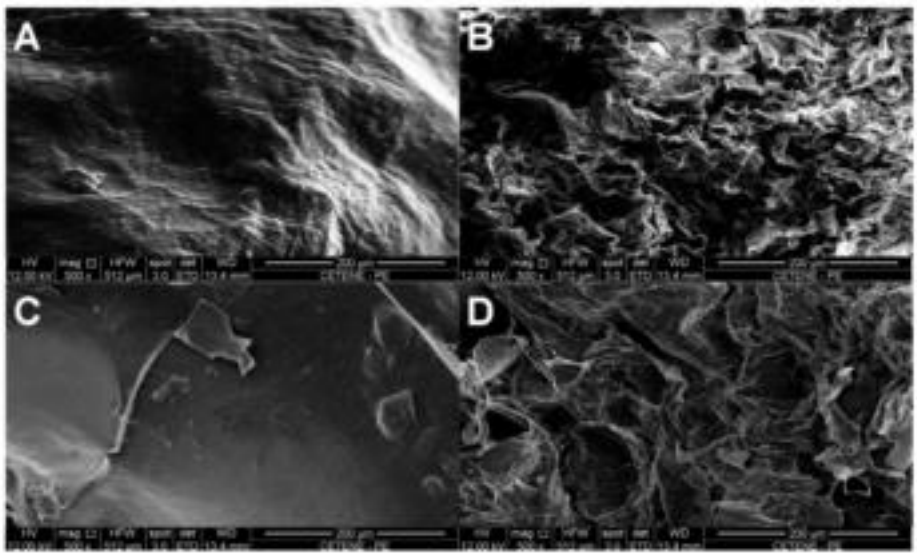

Fig. 3. SEM micrographs of the surface and matrix of the galactomannan/κ-carrageenan hydrogel at the first day (A and B, respectively) and after 90 days-period of storage at 4 °C (C and D, respectively).

moduli as a function of the storage time (Fig. 2C) corroborates what was previously discussed about an increase in the organization of the polysaccharide chains in the hydrogel matrix.

The elastic modulus for day 1 is very similar to the G' for pure galactomanan. It is worth noting that the galactomanan/κ-carrageenan combination is stiffer – higher G' – but less tougher, i.e. the area G' x stress is smaller than that of the pure galactomannan (Albuquerque et al., 2014). The accommodation of the interstitial entangled galactomannan network reduces the elastic modulus G' – there is a lower energy required to deform the network – at the same time that it increases the dissipation via the loss modulus locally. Finally, as the shelf storage period increases, there was an increase in the stress where the crossover between elastic and viscous moduli occurred (Fig. 2D). These results corroborate those presented in Fig. 2A, indicating that the hydrogel matrix was getting more organized and stiffer along the shelf storage period.

3.2. Scanning electron microscopy (SEM)

Studies of scanning electron microscopy were carried out to characterize the microstructure morphology of the freeze-dried galactomannan/κ-carrageenan hydrogel. Fig. 3 shows the SEM images of the surface and matrix of the hydrogel at the 1st day after preparation and after 90 days of storage at 4 °C. In the 1st day, the surface image indicates a rough and wavy morphology (Fig. 3A). In contrast, the matrix shows a 3D network, composed of 2D folded sheets, allowing for a reasonable interconnection between macropores (Fig. 3B). The roughness on the surface of the hydrogel is attributed to the presence of galactomannan (Lombardi & Mercê, 2003). It can also be observed an interconnection between pores that could be assigned to the crosslinking network formation between the sulfate group from κ-carrageenan chains and Ca^{+2} ions in the hydrogel formulation. Pore connectivity determines the easy flow of drugs and indicates if the hydrogel is appropriate for tissue engineering applications (Varghese, Chellappa, & Fathima, 2014).

After 90 days of storage at 4 °C, both surface and matrix of the hydrogel changed (Fig. 3C and D). The surface of hydrogel became less rough and the pores in the matrix became larger than the first day. This could be attributed to the organization of the polysaccharide chains in thin helices that starts to aggregate, thus reducing the fibers, and increasing the pores of the hydrogel matrix. Besides the fact that the porosity created by cryofracture method does not represent the hydrated porosity since the lyophilization process creates artifacts, the SEM analysis of the hydrogel remains interesting since these artifacts are linked to the real structures and the density of the hydrogels. The same observation was reported for thermosensitive chitosan hydrogels (Assaad, Maire, & Lerouge, 2015). The differences observed along the 90 days-period suggests that the hydrogel morphology can be modified by the aging time and depending on the targeted application, a smaller or larger porosity may be more favorable, given that porosity influences the drug release and cell invasion in tissue regeneration applications.

4. Conclusion

According to the results obtained of a full factorial design 2^3 based on rheological parameters, it was possible to obtain a stable hydrogel composed by 1.7% (w/v) of galactomannan (from *C. grandis* seeds) and 0.5% (w/v) of κ-carrageenan, with 0.2 M of $CaCl_2$ at pH 5.0. This hydrogel showed a temperature resistance until 42 °C without losing its shape. Furthermore, there were no significant changes in pH and no microbial contamination. Nevertheless it was observed a decrease in opacity and an increase in the transparency along of the 90 days-period of storage at 4 °C. The nuclear magnetic resonance has indicated the absence of covalent bonds which classify the investigated hydrogel as a physical gel, and confirmed that the temperature plays an important role in the maintenance of this physical gel matrix stability. Furthermore, the thermo-rheological tests confirmed a higher organization of the polysaccharides in the hydrogel matrix over the storage period with the elastic modulus always higher than the viscous modulus. The SEM analysis of the hydrogel matrix showed interconnected macropore architecture with a rough surface. These pore matrix interconnections may lead to an easy flow for biomolecules as well as scaffold matrices for tissue engineering. These results showed that this hydrogel has good and stable physical properties with potential application in medical and cosmetic industries.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.carbpol.2015.08.042.

References

Ahmad, H., Nurunnabi, M., Rahman, M. M., Kumar, K., Tauer, K., Minami, H., et al. (2014). Magnetically doped multi stimuli-responsive hydrogel microspheres with IPN structure and application in dye removal. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 459*, 39–47.

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications. *Journal of Advanced Research, 6*, 105–121.

Albuquerque, P. B. S., Barros, W., Jr., Santos, G. B. C., Correia, M. T. S., Moreira, P. A. S., Teixeira, J. A., et al. (2014). Characterization and rheological study of the galactomannan extracted from seeds of *Cassia grandis*. *Carbohydrate Polymers, 104*, 127–134.

Assaad, E., Maire, M., & Lerouge, S. (2015). Injectable thermosensitive chitosan hydrogels with controlledgelation kinetics and enhanced mechanical resistance. *Carbohydrate Polymers, 130*, 87–96.

Bhattacharyya, R., & Ray, S. K. (2014). Enhanced adsorption of synthetic dyes from aqueous solution by a semi-interpenetrating network hydrogel based on starch. *Journal of Industrial and Engineering Chemistry, 20*, 3714–3725.

Bosco, M., Segre, A., Miertus, S., Cesaro, A., & Paoletti, S. (2005). The disordered conformation of κ-carrageenan in solution as determined by NMR experiments and molecular modeling. *Carbohydrate Research, 340*, 943–958.

Cheng, Y., He, C., Ding, J., Xiao, C., Zhuang, X., & Chen, X. (2013). Thermosensitive hydrogels based on polypeptides for localized and sustained delivery of anticancer drugs. *Biomaterials, 34*, 10338–10347.

Cuggino, J. C., Igarzabal, C. A., Rueda, J. C., Quinzani, L. M., Komber, H., & Strumia, M. C. (2008). Synthesis and characterization of new hydrogels through copolymerization of N-acryloyl-tris-(hydroxymethyl) aminomethane and different crosslinking agents. *European Polymer Journal, 44*, 3548–3555.

Da-Lozzo, E. J., Moledo, R. C. A., Faraco, C. D. F., Ortolani-Machado, C. F., Bresolin, T. M. B., & Silveira, J. L. M. (2013). Curcumin/xanthan–galactomannan hydrogels: Rheological analysis and biocompatibility. *Carbohydrate Polymers, 93*, 279–284.

Dash, R., Foston, M., & Ragauskas, A. J. (2013). Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers. *Carbohydrate Polymers, 91*, 638–645.

De Cicco, F., Reverchon, E., Adami, R., Aurienmma, G., Russo, P., Calabrese, E. C., et al. (2014). In situ forming antibacterial dextran blend hydrogel for wound dressing: SAA technology vs. spray drying. *Carbohydrate Polymers, 101*, 1216–1224.

Diao, M., Li, Q., Xiao, H., Duan, N., & Xu, J. (2014). Synthesis and adsorption properties of superabsorbent hydrogel and peanut hull composite. *Journal of Environmental Chemical Engineering, 2*, 1558–1567.

Dima, C., Cotârlet, M., Alexe, P., & Dima, S. (2014). Reprint of "microencapsulation of essential oil of pimento [Pimenta dioica (L) Merr.] by

chitosan/k-carrageenan complex coacervation method". *Innovative Food Science and Emerging Technologies, 25*, 97–105.
Farias, E. H. C., Pomin, V. H., Valente, A. P., Nader, H., Rocha, H. A. O., & Mourão, P. A. S. (2008). A preponderantly 4-sulfated, 3-linked galactan from the green alga *Codium isthmocladum*. *Glycobiology (Oxford), 18*, 250–259.
Feyzikhanova, G. U., Filippova, M. A., Talibov, V. O., Dementieva, E. I., Maslennikov, V. V., Reznikov, Yu. P., et al. (2014). Development of hydrogel biochip for in vitro allergy diagnostics. *Journal of Immunological Methods, 406*, 51–57.
Hennink, W. E., & van Nostrum, C. F. (2002). Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews, 54*, 13–36.
Huang, Y., He, K., & Wang, X. (2013). Rapid prototyping of a hybrid hierarchical polyurethane–cell/hydrogel construct for regenerative medicine. *Materials Science and Engineering C, 33*, 3220–3229.
Hyun, K., Kim, S. H., Ahn, K. H., & Lee, S. J. (2002). Large amplitude oscillatory shear as a way to classify the complex fluids. *Journal of Non-Newtonian Fluid Mechanics, 107*, 51–65.
Jang, J., Seol, Y.-J., Kim, H. J., Kundu, J., Kim, S. W., & Cho, D.-W. (2014). Effects of alginate hydrogel cross-linking density on mechanical and biological behaviors for tissue engineering. *Journal of the Mechanical Behavior of Biomedical Materials, 37*, 69–77.
Kenawy, R., Kamoun, E. A., Moby Eldin, M. S., & El-Meligy, M. A. (2014). Physically crosslinked poly(vinyl alcohol)–hydroxyethyl starch blend hydrogel membranes: Synthesis and characterization for biomedical applications. *Arabian Journal of Chemistry, 7*, 372–380.
Laurén, P., Lou, Y. R., Raki, M., Urtti, A., Bergström, K., & Yliperttula, M. (2014). Technetium-99 m-labeled nanofibrillar cellulose hydrogel for in vivo drug release. *European Journal of Pharmaceutical Sciences, 65*, 79–88.
Lefnaoui, S., & Moulai-Mostefa, N. (2014). Investigation and optimization of formulation factors of a hydrogel network based on kappa carrageenan–pregelatinized starch blend using an experimental design. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 458*, 117–125.
Li, Y., & Shimizu, H. (2006). Morphological investigations on the nanostructured poly(vinylidene fluoride)/polyamide 11 blends by high-shear processing. *European Polymer Journal, 42*, 3202–3211.
Liu, L., Zhai, J., Zhu, C., Gao, Y., Wang, Y., Han, Y., et al. (2015). One-pot synthesis of 3-dimensional reduced graphene oxide-based hydrogel as support for microbe immobilization and BOD biosensor preparation. *Biosensors and Bioelectronics, 63*, 483–489.
Liu, Y., Yu, S., Wu, H., Li, Y., Wang, S., Tian, Z., et al. (2014). High permeability hydrogel membranes of chitosan/poly ether–block–amide blends for CO_2 separation. *Journal of Membrane Science, 469*, 198–208.
Lombardi, S. C., & Mercê, A. L. (2003). Properties of complexes of galactomannan of *Leucaena leucocephala* and Al^{3+}, Cu^{2+} and Pb^{2+}. *Bioresource Technology, 89*, 63–73.
Lv, Z., Chang, L., Long, K., Liu, J., Xiang, Y., Liu, J., et al. (2014). Thermosensitive in situ hydrogel based on the hybrid of hyaluronic acid and modified PCL/PEG triblock copolymer. *Carbohydrate Polymers, 108*, 26–33.
Manjanna, K. M., Pramod Kumar, T. M., & Shivakumar, B. (2010). Natural polysaccharide hydrogels as novel excipients for modified drug delivery systems: A review. *International Journal of ChemTech Research, 2*, 508–525.
Martins, J. T., Cerqueira, M. A., Bourbon, A. I., Pinheiro, A. C., Souza, B. W. S., & Vicente, A. A. (2012). Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids, 29*, 280–289.
Muhamad, I. I., Fen, L. S., Hui, H. Ng., & Mustapha, N. A. (2011). Genipin-cross-linked kappa carrageenan/carboxymethyl cellulose beads and effects on beta-carotene release. *Carbohydrate Polymers, 83*, 1207–1212.
Pinheiro, A. C., Bourbon, A. I., Rocha, C., Ribeiro, C., Maia, J. M., Gonçalves, M. P., et al. (2011). Rheological characterization of κ-carrageenan/galactomannan and xanthan/galactomannan gels: Comparison of galactomannans from non-traditional sources with conventional galactomannans. *Carbohydrate Polymers, 83*, 392–399.
Prajapati, V. D., Jani, G. K., Moradiya, N. G., Randeria, N. P., Nagar, B. J., Naikwadi, N. N., et al. (2013). Galactomannan: A versatile biodegradable seed polysaccharide. *International Journal of Biological Macromolecules, 60*, 83–92.
Prajapati, V. D., Maheriya, P. M., Jani, G. K., & Solanki, H. K. (2014). Carrageenan: A natural seaweed polysaccharide and its applications. *Carbohydrate Polymers, 105*, 97–112.
Sandolo, C., Matricardi, P., Alhaique, F., & Coviello, T. (2007). Dynamo-mechanical and rheological characterization of guar gum hydrogels. *European Polymer Journal, 43*, 3355–3367.
Segura, J. H., Camargo Junior, F. B., Bagatin, E., & Campos, P. M. B. G. M. (2010). Influência da água termal e de seus oligoelementos na estabilidade e eficácia de formulações dermocosméticas. *Surgical & Cosmetic Dermatology, 2*, 7–11.
Shapiro, Y. E. (2011). Structure and dynamics of hydrogels and organogels: An NMR spectroscopy approach. *Progress in Polymer Science, 36*, 1184–1253.
Silva, W. A., Pereira, J., Carvalho, C. W. P., & Ferrua, F. Q. (2007). Determinação da cor, imagem superficial topográfica e ângulo de contato de biofilmes de diferentes fontes de amido. *Ciência e Agrotecnologia, 31*, 154–163.
Soares, P. A. G., Bourbon, A. I., Vicente, A. A., Andrade, C. A. S., Barros, W., Jr., Correia, M. T. S., et al. (2014). Development and characterization of hydrogels based on natural polysaccharides: Policaju and chitosan. *Materials Science and Engineering C, 42*, 210–216.
Tovar, A. M. F., Capillé, N. V. M., Santos, G. R. C., Vairo, B. C., Oliveira, S-N. M. C. G., Fonseca, R. J. C., et al. (2012). Heparin from bovine intestinal mucosa: Gly-cam with multiple sulfation patterns and anticoagulant effects. *Thrombosis and Haemostasis, 107*, 903–915.
Vanderzant, C., & Splittstoesser, D. F. (1992). *Compendium of methods for the microbiological examination of foods* (3rd ed., pp. 1219). Washington: American Public Health Association (APHA).
Varghese, J. S., Chellappa, N., & Fathima, N. N. (2014). Gelatin–carrageenan hydrogels: Role of pore size distribution on drug delivery process. *Colloids and Surfaces B: Biointerfaces, 113*, 346–351.
Vudjung, C., Chaiwaan, U., Pangan, U., Chaipugdee, N., Boonyod, S., Saenjawitee, O., et al. (2014). Effect of natural rubber contents on biodegradation and water absorption of Interpenetrating Polymer Network (IPN) hydrogel from natural rubber and cassava starch. *Energy Procedia, 56*, 255–263.
Yao, Z., Wu, H., Zhang, S., & Du, Y. (2014). Enzymatic preparation of κ-carrageenan oligosaccharides and their anti-angiogenic activity. *Carbohydrate Polymers, 101*, 359–367.
Zhang, Z., Ni, J., Chen, L., Yu, L., Xu, J., & Ding, J. (2011). Biodegradable and thermoreversible PCLA-PEG-PCLA hydrogel as a barrier for prevention of post-operative adhesion. *Biomaterials, 32*, 4725–4736.