UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE CIÊNCIAS
DEPARTAMENTO DE BIOQUÍMICA E BIOLOGIA MOLECULAR
CURSO DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

DESENVOLVIMENTO DE FILMES COMESTÍVEIS À BASE DE GALACTOMANANAS VEGETAIS PARA CONSERVAÇÃO DE FRUTOS

ÁLVARO MARCOS PEREIRA LIMA

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Tese submetida à Coordenação do Curso de Pós-Graduação em Bioquímica, da Universidade Federal do Ceará, como requisito parcial para obtenção do grau de Doutor em Bioquímica.

Orientador: Dr. Renato de Azevedo Moreira
Co-orientador: Dr. António Augusto Martins de Oliveira Soares Vicente

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Álvaro Marcos Pereira Lima

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Prof. Dr. Renato de Azevedo Moreira
Depto. de Bioquímica e Biologia Molecular
Universidade Federal do Ceará (UFC)
Orientador

Profa. Dra. Kátia Flávia Fernandes Silva
Depto. de Ciências Fisiológicas
Universidade Federal de Goiás(UFG)

Profa. Dra. Ana Cristina de Oliveira Monteiro Moreira
Centro de Ciências da Saúde
Universidade de Fortaleza (UNIFOR)

Profa. Dra. Norma Maria Barros Benevides
Depto. de Bioquímica e Biologia Molecular
Universidade Federal do Ceará (UFC)

Profa. Dra. Maria Raquel Alcântara de Miranda
Depto. de Bioquímica e Biologia Molecular
Universidade Federal do Ceará (UFC)

A Adman e Letícia com todo meu amor

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AP	*Adenanthera pavonina*
CP	*Caesalpinia pulcherrima*
TA	Temperatura ambiente
UR	Umidade relativa
PEG	Polietilenoglicol
PCO_2	Permeabilidade ao dióxido de Carbono
PO_2	Permeabilidade ao Oxigênio
P H_2O	Permabilidade ao vapor de água
TS	Tração
E	Elasticidade até ruptura
Ws	Coeficiente de espalhamento
Wa	Coeficiente de adesão
Wc	Coeficiente de coesão
θ	Ângulo de contato

Resumo

Grandes perdas (25 a 80%) na qualidade de frutos frescos, ocorrem entre a colheita e o consumo. Um fator limitante na cadeia de distribuição deste tipo de alimento é o seu curto tempo de prateleira. Este fato, gera um grande desperdício econômico em países desenvolvidos, e em países tropicais assume proporções devastadoras. O emprego de películas de revestimento à base de galactomananas naturais, atóxicas e comestíveis pode trazer benefícios econômicos no aumento do tempo de prateleira das frutas mais perecíveis e ambientais por ser um material biodegradável. O objetivo deste trabalho foi de preparar diferentes revestimentos comestíveis, a partir de galactomananas de sementes de leguminosas (*Adenanthera pavonina* e *Caesalpinia pulcherrima*) visando o seu emprego no aumento de tempo de prateleira de frutos. Tendo ainda como parte complementar a avaliação da capacidade dessas galactomananas em formarem blendas com colágeno, e analisar as características dos filmes formados a partir dessas blendas. Soluções filmogênicas de galactomanana e glicerol foram preparadas variando a concentração de galactomanana de 0,5% a 1,5%; e concentrações de glicerol que variaram entre 1% e 2%. Os filmes foram avaliados com relação a sua capacidade molhante (*Ws*), sendo selecionados 8 filmes de *A. pavonina* que apresentaram melhores valores de *Ws* para cada fruto estudado e 4 filmes de *C. pulcherrima*. Estes filmes foram submetidos a testes de permeabilidade ao vapor de água, permeabilidade ao O_2 e permeabilidade ao CO_2. Para os dois primeiros as concentrações mais altas de galactomanana reduziram a permeabilidade, bem como o uso de concentrações mais baixas de glicerol. Para a permeabilidade ao dióxido de carbono ocorre o inverso: concentrações mais elevadas de glicerol reduzem PCO_2. Os filmes apresentaram boas características mecânicas, como uma considerável elasticidade e resistência à tração, sendo compatíveis com filmes de outros biopolímeros previamente estudados. Tendo por base os critérios - valores mais altos de capacidade molhante, valores mais baixos de permeabilidade ao vapor de água, valores mais baixos de permeabilidade ao oxigênio e valores mais baixos de permeabilidade ao

dióxido de carbono – as seguintes formulações de filmes mostraram-se mais adequadas para cada fruto: acerola – 0,5% de galactomanana de *A. pavonina* e 1% de glicerol; cajá – 1% de galactomanana de *A. pavonina* e 1% de glicerol; manga e pitanga – 1,5% de galactomanana de *A. pavonina* e 1% de glicerol e seriguëla 0,5%de galactomanana de *C. pulcherrima* e 1,5% de glicerol. Blendas de galactomanana colágeno e glicerol foram ainda estudadas com relação as mesmas propriedades, tendo por objetivo a melhoria de características de barreira ou mecânicas destes filmes. Foram selecionadas em termos de *Ws* a melhor blenda no revestimento de manga (0,5% de galactomanana de *A. Pavonina*, 1,5% de colágeno e 1,5% de glicerol) e a melhor blenda no revestimento de maçã (0,5% de galactomanana de *C.pulcherrima,* 1,5% de colágeno e sem glicerol). A blenda de galactomanana de *C. pulcherrima* – colágeno mostrou-se menos permeável ao vapor de água e mais permeável ao O_2 e CO_2. Mangas foram revestidas com a blenda de *A. Pavonina* – colágeno – glicerol e a taxa de transferência gasosa foi comparada com mangas sem revestimento. Foi observado um consumo 28% menor de O_2 e uma produção 11% menor de CO_2. O mesmo procedimento foi repetido para maçãs (desta vez utilizando a blenda de *C. pulcherrima*). O CO_2 produzido e o O_2 consumido foram aproximadamente 50% menores em maçãs revestidas quando comparadas a maçãs sem o revestimento.

Abstract

Great losses (25 to 80%) in the quality of fresh fruits occur between their harvest and consumption. A limiting factor in the distribution chain of these foods is their short shelf life. This fact creates a lot of economic waste in developed countries, and in tropical countries assume devastating proportions. The use of film-based coating natural galactomannans, nontoxic and eatable can bring economic benefits in increasing the shelf life of perishable fruits and more environmental because it is a biodegradable material. The objective of this work was to produce different edible coatings from galactomannans otained in *leguminosae* seeds (*Adenanthera pavonina* and *Caesalpinia pulcherrima*) looking for his use in order to increase shelf life of fruits. Having also as a complementary part to evaluate the ability of these galactomannans in forming blends with collagen, and analyze the characteristics of the films formed from these blends. Filmogenic solution of galactomannan and glycerol were prepared by varying the concentration of galactomannan from 0.5% to 1.5% and glycerol concentrations ranging from 1% to 2%. The films were evaluated with respect to their wettability (Ws), eight films were selected from *A. pavonina* which showed higher values of Ws for each fruit and studied four *C. pulcherrima* films. These films were tested for permeability to water vapor, O_2 and CO_2 . For the first two, higher concentrations of galactomannan reduced permeability, as well as the use of lower concentrations of glycerol. For the permeability to carbon dioxide is the opposite: higher concentrations of glycerol reduces PCO_2. The films showed good mechanical properties such as elasticity and considerable tensile strength, compatible with other films of polymers previously studied. Based on the criteria - the highest values of the wettability, lower values of permeability to water vapor, the lower values of oxygen permeability and lower values of permeability to carbon dioxide - the following film formulations were more appropriate for each fruit: acerola - 0.5% *A. pavonina* galactomannan and 1% glycerol; caja - 1% of *A. pavonina* galactomannan and 1% glycerol; mango and pitanga - 1.5% of *A. pavonina* galactomannan and 1% glycerol and seriguela- 0.5% *C. pulcherrima* galactomannan and 1.5% glycerol.

Galactomannan-collagen-glycerol blends were also studied regarding the same properties, with the objective of improving mechanical and barrier characteristics of these films. Were selected in terms of Ws the best blend to coat mango (0.5% of *A. pavonina* galactomannan, 1.5% collagen and 1.5% glycerol) and best blend to coat apple (0.5% of *C.pulcherrima* galactomannan, 1.5% collagen and without glycerol). The blend of galactomannan of *C. pulcherrima* - collagen was less permeable to water vapor and more permeable to O_2 and CO_2. Mangoes were coated with the *A.Pavonina* - collagen - glycerol blend and the rate of gas transfer was compared with uncoated mangoes. Was observed a 28% lower consumption of O_2 and a 11% lower production of CO_2. The same procedure was repeated for apples (this time using the blend of *C. pulcherrima*).The CO_2 produced and O_2 consumed was approximately 50% lower in coated apples to apples comparison without the coating.

1 – INTRODUÇÃO

Grandes perdas (25 a 80%) na qualidade de frutos frescos, ocorrem entre a colheita e o consumo. Um fator limitante na cadeia de distribuição deste tipo de alimento é o seu curto tempo de prateleira. Este fato, gera um grande desperdício econômico em países desenvolvidos, e em países tropicais assume proporções devastadoras (WILLS *et al.*, 1981).

Os produtores de frutas estão se adaptando às mudanças nos padrões de consumo e exigência crescentes dos consumidores quanto à qualidade das frutas frescas disponíveis no mercado. A necessidade do mercado de atender à demanda dos centros de consumo faz com que seja necessário, cada vez mais, prolongar a vida útil das frutas sem que estas percam seus atributos de proteção à saúde. Por isso há um interesse em pesquisas que levem ao desenvolvimento de novos materiais que preservem os frutos por mais tempo, sem que haja a adição de antimicrobianos (CHIEN, SHEU; YANG, 2007). Uma dessas técnicas de conservação é o uso de embalagens que modificam a atmosfera ao redor do produto, associado ao armazenamento sob refrigeração.

Os principais fatores responsáveis por prolongar o tempo de prateleira de frutas e verduras são: cuidados durante a colheita (não causar injúrias no fruto); colher o produto nas condições ideais de maturação e as boas condições sanitárias durante todas as etapas do processo (MOLEYAR; NARASIMHAM, 1994; LEE *et al.* 1996). Quando essas práticas são adotadas, a implementação de condições adequadas de armazenamento (controle de temperatura, controle de umidade relativa, modificação de atmosfera através do uso de gases, controle de luz e condições de stress mecânico e físico) pode ser efetiva, maximizando a "vida de prateleira" desse produto. Os usos de ambiente refrigerado e atmosfera controlada no armazenamento, levam a uma diminuição nas taxas de respiração desses frutos, retardando sua senescência e preservando sua qualidade por maior tempo (SALUNKHE; BOLIN; REDDY, 1991). Contudo, esses métodos possuem um alto custo de implementação, possuem um custo considerável de manutenção, e tornam-se impraticáveis no uso de pequenas quantidades de produto. A

aplicação de filmes comestíveis em frutos recém colhidos oferece uma alternativa menos dispendiosa, e igualmente eficaz na conservação desse tipo de alimento. A atmosfera modificada criada por um filme comestível pode proteger o fruto desde o momento da sua colheita, durante o transporte até o local de venda, e até mesmo na casa do consumidor (DIAB *et al.*, 2001; DURANGO; SOARES; ANDRADE, 2006; RIBEIRO *et al.*, 2007).

Atualmente, em torno de 150 milhões de toneladas de plástico são produzidas no mundo por ano, e essa produção e consumo continuam aumentando. A maioria das embalagens que se utiliza nos dias atuais é baseada em produtos derivados de petróleo o que leva a um aumento no consumo do mesmo, aumento nos níveis de poluição, e pelo fato de não serem biodegradáveis esses plásticos permanecem por séculos na natureza até que esta possa eliminá-los. Uma das estratégias utilizadas para solucionar as dificuldades com recursos fósseis é a reciclagem desses materiais. Contudo a reciclagem consome quantidades consideráveis de energia térmica, além de haver sempre perdas durante o processo (OKADA, 2002; PARRA, *et al.,* 2004). A produção de filmes a partir de materiais poliméricos biodegradáveis, naturais e abundantes (como é o caso das galactomananas) traz um grande benefício ambiental quando comparado aos plásticos sintéticos usados atualmente (CUTTER, 2006; CHILLO *et al.*, 2008).

O emprego de películas de revestimento à base de galactomananas naturais, atóxicas e comestíveis pode trazer benefícios ambientais com o menor consumo de combustíveis fósseis e por ser um material biodegradável, traz benefícios econômicos com a diminuição de perdas de alimentos, por um aumento no tempo de prateleira dos frutos mais perecíveis.

2 – FUNDAMENTAÇÃO TEÓRICA

Um dos principais problemas, no transporte, armazenamento e comercialização de frutas e hortaliças, está na senescência dos mesmos. A origem de toda essa problemática dá-se no momento da colheita do fruto, onde o mesmo é desligado da planta-mãe. Ele passa a usar a respiração como seu principal processo fisiológico, já que torna-se independente da absorção da água e minerais efetuada pelas raízes; da translocação dos nutrientes pelo sistema vascular e da atividade fotossintética da planta-mãe. O fruto passa a depender exclusivamente das reservas de substratos acumulados durante seu crescimento (CHITARRA; CHITARRA, 1990).

A respiração consiste na decomposição oxidativa de substâncias complexas presentes nas células, como açúcares e ácidos orgânicos, a moléculas simples como o CO_2 e H_2O, com a concomitante produção de energia nas formas de calor e ATP (KLUGE *et al.*, 2002). Sob condições normais, a maioria das plantas, incluindo seus frutos, respira aerobicamente, contudo na ausência de O_2 podem fazer respiração anaeróbica, em um processo conhecido por fermentação. Em frutos que sofrem fermentação, ocorre um acréscimo na produção de determinados álcoois, aldeídos e cetonas, que interferem no seu paladar e aroma, tornando-os depreciados (KADER, 1995).

Quando o fruto é colhido, há uma interrupção no balanço gasoso, ocorrendo um alto influxo do oxigênio com a perda proporcional de gás carbônico. Nesta nova condição as células internas não são renovadas e a respiração aumenta, provocando uma perda metabólica e levando o fruto a um gradual amadurecimento e eventual senescência.

A taxa de respiração depende tanto de fatores internos quanto de fatores externos. Dentre os fatores internos estão a espécie, o cultivar e o estágio de desenvolvimento. Entre os fatores externos estão a composição atmosférica (concentrações de O_2, CO_2 e etileno), a temperatura e os estresses (KLUGE *et al.*, 2002).

A modificação da atmosfera do ambiente de armazenamento de frutos, com a redução no conteúdo de O_2 e aumento de CO_2, resulta em diminuição da

taxa respiratória e o conseqüente prolongamento da vida útil desses produtos. As técnicas mais utilizadas na modificação da atmosfera de armazenamento de frutos são: o envolvimento com filmes plásticos, acondicionamento em embalagens e a cobertura por películas (SASS, 1993).

As películas funcionam como barreiras semipermeáveis capazes de assegurar a qualidade do alimento, além de serem biodegradáveis, oferecendo embalagens alternativas sem causar danos ambientais. A atmosfera modificada, criada pelo revestimento, gera um aprisionamento físico de CO_2, dentro do fruto e uma parcial ocupação dos poros, reduzindo desta forma a troca gasosa, ou seja, reduzindo a taxa respiratória. Se a permeação de oxigênio (O_2) para o seu interior é reduzida, ocorre um prolongamento do tempo de maturação (ODILIO; LEONI, 2003).

Os revestimentos comestíveis em frutos frescos surgem como uma alternativa ao armazenamento por atmosfera modificada (PARK, 1999). O emprego de revestimento e coberturas comestíveis, produzidos a partir de polissacarídeos de origem vegetal é visto como uma alternativa para aumentar a vida de prateleira desses alimentos, protegendo-os dos efeitos da umidade e do oxigênio, retardando a sua deterioração, sem introduzir materiais que alterem as características organolépticas do produto ou prejudiquem a saúde.

A utilização de filmes e revestimentos comestíveis para prolongar a vida útil dos alimentos não é um procedimento recente. O revestimento de laranjas e limões frescos com ceras, visando retardar a perda de água, foi usado na China nos séculos XII e XIII (HARDENBURG, 1967). Na Inglaterra do século XVI, era comum utilizar-se o revestimento de alimentos com materiais gordurosos, para diminuir a velocidade de desidratação destes produtos (LABUZA; CONTRERAS-MEDELLIN, 1981). Em meados de 1930 foi disponibilizado comercialmente um revestimento para frutos cítricos a base de parafina. No ano de 1950 a cera de carnaúba foi introduzida para esse fim, mas devido à aparência fosca resultante de sua aplicação, passou a ser misturada com polietileno e parafina (KAPLAN, 1986). Nos anos 60, ceras e vernizes processados a partir de gomas solúveis em água se tornaram populares no revestimento de frutos em geral.

As coberturas denominadas comestíveis são mais recentes, datando do início dos anos 80, quando passou a ocorrer um crescente interesse por esse tipo de produto, devido à uma expansão no mercado de produtos minimamente processados. Essa expansão no mercado desses produtos, deveu-se inicialmente ao consumo dem restaurantes e hotéis. Mas a conveniência da vida moderna, acabou levando esse tipo de produto, cada vez mais, ao interior dos lares (ODÍLIO, 2003).

2.1 Revestimentos Comestíveis

Revestimento comestível é definido como uma fina camada de material comestível, depositado em um alimento como cobertura, que vem sendo utilizado para incrementar a vida de pós-colheita dos vegetais. Sua finalidade é inibir ou reduzir a migração de umidade, oxigênio, dióxido de carbono, aromas, dentre outros constituintes, pois atuam como barreiras semi-permeáveis. Além disso, podem ser acrescidos de antioxidantes, antimicrobianos e flavorizantes, tendo ainda uma importância na melhoria da integridade mecânica e características de manuseio do alimento (KROCHTA; MULDER-JOHNSTON, 1997; LEE *et al*, 2003).

Por estarem em contato com o alimento, é desejável que películas e revestimentos comestíveis apresentem propriedades organolépticas neutras (transparente, inodoro, insípido), de modo a não alterar as características sensoriais dos alimentos (GONTARD, 1991). Revestimentos à base de hidrocolóides apresentam geralmente características organolépticas mais neutras do que os formados a partir de lipídeos ou derivados, os quais apresentam maior opacidade e sabor residual (GONTARD; GUILBERT, 1994).

Os revestimentos e películas comestíveis são elaborados tendo como base macromoléculas biológicas (biopolímeros) capazes de formar uma matriz contínua, homogênea e coesa (KESTER; FENNEMA, 1986). Conforme Cuq (1996), revestimentos são os que formam a matriz diretamente sobre os alimentos, diferentemente das películas, que são matrizes pré-formadas separadamente do alimento.

Estes revestimentos podem consistir de três materiais biológicos:

- Polissacarídeos (gomas, celulose, amido, alginatos, carragenanas, pectinas)
- Proteínas (colágeno, gelatina, zeína, proteína de soja, glúten e proteína do soro de leite)
- Lipídeos (Acetilglicerídeos e Ceras)

A escolha e constituição dos materiais empregados para formar a película comestível dependem de parâmetros como custo, disponibilidade, propriedades mecânicas (resistência e flexibilidade), propriedades ópticas (cor e opacidade), espessura, permeabilidade ao vapor de água e gases como oxigênio e dióxido de carbono, solubilidade em água e propriedades sensoriais. Essas propriedades dependem do biopolímero utilizado (conformação, peso molecular, distribuição de cargas, polaridade), das condições de fabricação (pH, concentração de proteínas, tratamento térmico da solução, tipo e teor de aditivos, como os plastificantes) e das condições ambientais (temperatura e umidade relativa), que são importantes devido à natureza higroscópica dos biopolímeros e do plastificante usado (CUQ , 1996).

O uso de plastificantes, geralmente polióis, que reduzem as interações intermoleculares entre as cadeias adjacentes do biopolímero, resultando no aumento da mobilidade dessas cadeias e, conseqüentemente, em materiais flexíveis é essencial na elaboração de películas comestíveis (GONTARD *et al.*, 1993; CUQ *et al.*, 1997). A diminuição da força e o aumento da deformação na ruptura com o aumento do plastificante são componentes típicos desses tipos de filmes (PARRIS *et al.*, 1995). A presença de plastificantes diminui a intensidade das interações proteína-proteína aumentando a mobilidade das cadeias polipeptídicas e tornando os filmes menos resistentes e mais deformáveis (CUQ, 1996).

Os revestimentos podem ser feitos de base simples ou compostas, utilizando materiais diferentes, consistindo de simples camada, dupla camada ou multicamadas (GUILBERT *et al.*, 1997). Krochta e Mulder-Johnston (1997)

definem películas e revestimentos de dupla camada como sendo formados como uma camada de película com propriedades de barreira a oxigênio e aromas e uma segunda camada, hidrofóbica, protegendo o produto da perda de umidade.

A produção de revestimentos comestíveis pode se fazer por um dos seguintes mecanismos (KESTER; FENNEMA, 1986):

- Coacervação simples: quando um hidrocolóide, disperso em água, é precipitado ou sofre uma mudança de fase após a evaporação do solvente (secagem), ou depois da adição de um não-eletrólito hidrossolúvel no qual o hidrocolóide é insolúvel (ex.etanol), ou após um ajuste de pH ou adição de um eletrólito que induza o *salting-out* ou a formação de ligações cruzadas.
- Coacervação complexa: quando duas soluções de hidrocolóides com cargas opostas são misturadas, causando a interação e precipitação do complexo polimérico.
- Gelificação ou coagulação térmica: quando o aquecimento da macromolécula, que provoca a desnaturação, é seguido por gelificação (ex. proteínas) ou precipitação, ou quando o arrefecimento de uma dispersão de hidrocolóide provoca a gelificação (ex. gelatina ou agar).

Alguns polissacarídeos, tais como alginato, pectina, carragenana, amido e derivados da celulose têm sido estudados, por seu potencial uso como revestimentos comestíveis. No entanto, espera-se uma mínima propriedade de barreira contra umidade nestes filmes, devido à sua natureza hidrofílica. Contudo, alguns filmes de polissacarídeos podem retardar a perda de umidade de alguns alimentos, quando aplicados na forma de gel, que age como agente sacrificante, ou seja, a umidade do gel evapora antes da desidratação do alimento revestido (KESTER; FENNEMA, 1986). Observa-se, às vezes, relação inversa entre a permeabilidade ao vapor de água e a permeabilidade ao oxigênio, então certos

filmes de polissacarídeos podem prover proteção efetiva dos alimentos quanto às alterações do oxigênio.

Os revestimentos e as películas de polissacarídeos têm um aspecto não gorduroso, têm um baixo teor calórico e podem ser utilizados para aumentar o tempo de prateleira de frutos, verduras, mariscos ou carnes evitando a desidratação, o ranço oxidativo e o escurecimento da superfície. Estes revestimentos permitem a criação de atmosferas modificadas, uma vantagem sobre o plástico que além de ser dispendioso é de difícil aplicação e prejudicial ao meio ambiente (BALDWIN *et al.*, 1995; PETERSEN *et al.*, 1999; DANG *et al.*, 2008).

Existem vários estudos que evidenciam os efeitos benéficos da utilização dos revestimentos na agricultura (NISPEROS-CARRIEDO, 1994), entre os quais se destacam:

1. Maior retenção de aroma, ácidos, açúcares, textura e cor;
2. Aumento da estabilidade durante o transporte e armazenamento;
3. Melhor aspecto visual;
4. Redução da contaminação microbiológica.

Alguns polissacarídeos solúveis em água, com a capacidade de formar revestimentos gelatinosos, têm sido aplicados em alimentos sujeitos a um processo de fritura, com o objetivo de reduzir a absorção de óleo (FISZMAN; SALVADOR, 2003).

2.2 Galactomananas

As galactomananas são polissacarídeos de reserva presentes em endospermas de sementes, principalmente de leguminosas. A sua função na planta também está relacionada com a retenção e regulação de água durante a germinação além da defesa contra predadores (SCHERBURKIN; ANULOV, 1999).

A maioria dos estudos desenvolvidos com galactomananas visa principalmente o interesse comercial que envolve estes polissacarídeos, com diferentes aplicações na indústria. Estas aplicações são decorrentes das

propriedades reológicas das soluções aquosas, formando soluções de alta viscosidade em baixas concentrações. As galactomananas são amplamente utilizadas como agentes espessantes, estabilizantes, gelificantes, encapsuladores, em uma variedade de aplicações industriais. E por não serem tóxicas, é vasto seu uso em indústrias têxteis, farmacêuticas, biomédicas, cosméticas e de alimentos (SRIVASTAVA; KAPOOR, 2005; VIEIRA *et al.*, 2007).

Estes polissacarídeos são constituídos, de cadeias lineares de D-manose unidas por ligações glicosídicas β-(1-4), com substituições de galactose ligadas às unidades de D-manose da cadeia linear através de ligações glicosídicas α-(1-6), como pode ser visto na Figura 1 (KÖK *et al.,* 1999).

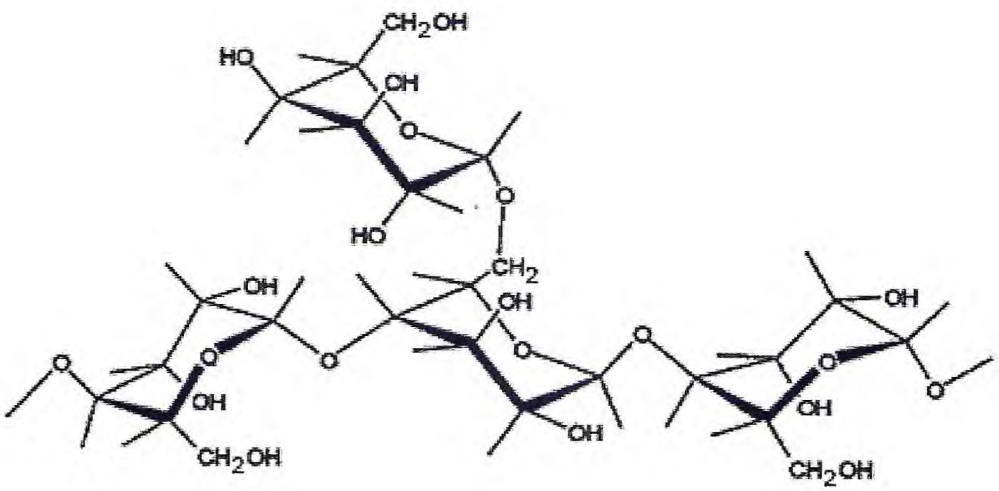


Figura 1 – Segmento de uma galactomanana com proporção manose/ galactose 3:1.

As propriedades físico-químicas e conformacionais das galactomananas estão relacionadas com a relação M/G e a distribuição de galactoses ao longo da cadeia principal. A solubilidade em água é afetada pelo grau de substituição de galactose na cadeia principal. A galactomana de alfarroba (*Ceratonia siliqua*), com razão M/G 3,5 apresenta baixa solubilidade à temperatura ambiente quando comparada a goma de Guar (*Cyamopsis tetragonolobus* L.), cuja relação M/G é cerca de 1,8 (DEA; MORRISON, 1975).

Apesar da relação manose/galactose das galactomananas fornecerem informações importantes, muitas vezes espécies com a mesma relação manose/galactose podem apresentar propriedades distintas. Este fato pode ser explicado quando se observa a estrutura fina de cada um desses polissacarídeos. A distribuição de galactose lateral ao longo da cadeia principal de manose pode se apresentar, aleatória, alternada ou em blocos.

Esta distribuição e a relação manose/galactose são fatores que afetam as propriedades das galactomananas, além de solubilidade (PETKOWICZ *et al.,* 1998), a susceptibilidade à degradação enzimática e interação molecular (DEA; MORRISON, 1975). O teor de galactose no polissacarídeo produz, ainda, um efeito pronunciado em suas propriedades reológicas. As galactomananas, em água, formam soluções altamente viscosas, e as propriedades do polímero em solução, são controladas pelas características moleculares tais como peso molecular e estrutura química (ANDRADE *et al.,* 1999).

A investigação de novas fontes de galactomananas constitui um assunto de grande relevância, tanto do ponto de vista acadêmico como industrial. Países tropicais como o Brasil apresentam grande potencial como produtores de recursos renováveis, que ainda não foram suficientemente explorados. O Brasil possui vastas regiões apropriadas ao cultivo de leguminosas. As leguminosas constituem uma das principais fontes de galactomananas, como têm comprovado as pesquisas que as identificaram em inúmeras espécies desta família. Algumas famílias como Annonaceae, Convolvulaceae, Loganiaceae e Palmae, também podem conter galactomanana (DEY, 1978). Embora muitas galactomananas tenham sido isoladas nos últimos anos, somente as galactomananas de guar e de alfarroba são utilizadas comercialmente. A goma de alfarroba é a mais conhecida e uma das mais antigas gomas extraídas de sementes.

As galactomananas podem também ser encontradas em fontes bacterianas e em fungos, assim como a D-manose e a D-galactose são encontradas em outros polissacarídeos de plantas, como glucomananas, mananas e galactanas (MATOS, 2000).

A obtenção de galactomananas que possam substituir em parte ou totalmente as gomas tradicionais pode trazer muitos benefícios econômicos e sociais, levando-se em conta que estes polímeros não possuem nenhum valor agregado no momento.

Segundo Azero e Andrade (2002), a demanda no Brasil por hidrocolóides e por polímeros biocompatíveis é crescente e, apesar das condições favoráveis à sua produção, as empresas nacionais dependem da importação desses produtos. Mikkonen *et al.*(2007) mostraram o uso de galactomananas como fontes alternativas na formação de filmes, avaliaram ainda os efeitos de diferentes razões M/G nas propriedades térmicas e mecânicas desses filmes.

Em estudo recente Cerqueira *et al.* (2009) apresentaram a metodologia de extração de galactomananas de 4 espécies distintas de leguminosas de fontes não-tradicionais na indústria (*Adenanthera pavonina, Caesalpinia pulcherrima, Gleditsia triacanthos e Sophora japonica*), com os respectivos rendimentos desses processos extrativos. Foram ainda avaliados parâmetros físicos e químicos desses polissacarídeos, bem como sua composição monossacarídica.

As galactomananas também podem ser importantes ferramentas biotecnológicas, como é o caso da extraída de *Adenanthera pavonina* que foi empregada como matriz de afinidade para isolamento de lectinas galactose-ligantes de sementes de *Artocarpus incisa* (MONTEIRO,1998), *Vaiterea macrocarpa, Abrus precatorius*, *Abrus pulchellus*. Sua estrutura é típica das galactomananas de leguminosas, uma cadeia linear de D-manose com ligações glicosídicas do tipo β-(1-4), e ramificações de galactose ligadas a unidades de D-manose da cadeia principal por ligações glicosídicas α-(1-6). A proporção M/G para *Adenanthera pavonina* é de 1,35:1 (CERQUEIRA, 2009).

Outras galactomananas, isoladas de sementes de *Delonix regia, Schizolobium parahybae* (MATOS, 2000) e *Parkisonia aculeata* (GARROS-ROSA, 2000), também se mostraram capazes de se ligar a lectinas α-D-galactose ligantes e servirem de matrizes para isolamento das mesmas.

2.3 Blendas ou misturas de componentes filmogênicos

A produção de blendas de materiais poliméricos tem atingido valores crescentes, por ser uma estratégia versátil e economicamente viável na obtenção de materiais com um amplo espectro de características desejáveis (THAKORE *et al.*, 2001).

Filmes biodegradáveis de proteínas e polissacarídeos apresentam propriedades mecânicas e visuais satisfatórias, bem como se mostram potenciais alternativas como substituintes de revestimentos de polímeros sintéticos nas indústrias de alimento e farmacêuticas. Pavlath e Robertson (1999), descreveram uma melhoria nas propriedades físico-químicas desse tipo de filme (após algumas modificações químicas), tornando-os comparáveis a filmes comerciais não-biodegradáveis.

Polissacarídeos originam filmes com boas propriedades mecânicas e revestimentos que são eficientes barreiras para compostos de baixa polaridade, apesar de não serem boas barreiras contra a passagem de água (AZEREDO *et al.*, 2000). Proteínas (queratina, caseína, colágeno, albumina e outras) são capazes de formar filmes com melhores propriedades de barreira ao vapor de água quando comparados aos filmes de polissacarídeos (GENNADIOS, 2002).

A natureza polar das proteínas confere aos filmes protéicos a característica de serem excelentes barreiras ao oxigênio e ao dióxido de carbono (apolares), contudo dentre as principais limitações existentes no uso de proteínas para produção de filmes, sem dúvida alguma, preponderam a baixa força mecânica e a pobreza de resistência à passagem de vapor de água comparados aos filmes de polímeros de origem sintética. Essa última característica é atribuída a hidrofobicidade oriunda das proteínas e às quantidades significativas de plastificantes como a glicerina e o sorbitol usadas para aumentar a flexibilidade. Entretanto, para filmes de colágeno (LIEBERMAN; GILBERT, 1973), glúten de trigo e zeína (AYDT *et al.*, 1991; GENNADIOS *et al.*, 1993; PARK; CHINNAN, 1995) boas propriedades como barreira ao oxigênio em ambientes com baixa umidade relativa foram relatados.

Uma das alternativas para diminuir essas limitações é o uso de componentes hidrofóbicos, tais como lipídeos, nas soluções formadoras de filmes (HERNÁNDEZ-MUÑOZ *et al.*, 2004). Outra maneira para melhorar a funcionalidade de filmes produzidos com proteínas seria a modificação da rede do polímero através de ligação cruzada das cadeias poliméricas. Isso pode ser viabilizado por meio de tratamento químico, físico ou enzimático devido à presença de grupos reativos nas cadeias laterais dos aminoácidos constituintes das proteínas. Por exemplo, Park *et al.* (2000), com proteína de soja e Orliac *et al.* (2002) com proteínas de girassol, todos, modificaram as redes do polímero filmogênico através do uso de aldeídos antes da preparação dos filmes com a intenção de melhorar as propriedades funcionais daqueles. Mahmoud e Savello (1993) com proteína do soro do leite, Lim *et al.* (1998,1999) com proteína de clara de ovo e gelatina e Larré *et al.* (2000) com glúten desaminado, utilizaram transglutaminase a fim de, enzimaticamente, introduzir ligações cruzadas nos polímeros. Irradiação UV foi utilizada como tratamento físico em proteínas de glúten de trigo, zeína de milho, albumina de ovo e caseinato sódico (RHIM *et al.,* 1999) com o intuito de também introduzir ligações cruzadas nos polímeros formadores de filmes.

Os compostos formadores de filmes podem interagir fisicamente e/ou quimicamente, resultando em filmes ou coberturas com propriedades melhoradas quando combinados (TANADA-PALMU ; GROSSO, 2005). A essas combinações ou misturas de componentes formadores de filmes denominamos blendas. O efeito ocasionado no filme, produzido pela formação da blenda chamamos de sinergismo, o que não necessariamente reflete a soma das propriedades individuais de cada componente da mistura. Por exemplo, Arvanitoyannis *et al.* (1998) estudaram as propriedades térmicas e funcionais de filmes comestíveis feitos de blendas de gelatina e alguns tipos de amido em função de vários tipos de plastificantes. Bertan *et al.* (2005) analisaram as propriedades microestruturais e físicas de filmes baseados em blendas de gelatina, ácidos graxos e triacetina usada como plastificante. Nesses estudos, com base nas propriedades

analisadas, os materiais testados apresentaram características de filmes comestíveis.

A formação de complexos proteína-polissacarídeo tem sido relatada por aumentar a funcionalidade de proteínas adsorvidas na interface de fluidos (DICKINSON, 2003). As interações proteínas-polissacarídeos são sensíveis a detalhes estruturais das proteínas e dos polissacarídeos, bem como aos valores de pH do meio (TOLSTOGUZOV, 1997; DICKINSON, 2003;).

Soluções aquosas de proteínas e polissacarídeos podem apresentar um dos fenômenos: coacervação complexa, miscibilidade e segregação. A coacervação complexa ocorre abaixo do ponto isoelétrico das proteínas como um resultado de uma rede formada por interações de biopolímeros carregados por cargas opostas. Isso leva a uma separação em duas fases: uma delas rica em biopolímeros complexados e a outra desprovida desses complexos (BAEZA *et al.*, 2004). Acima do ponto isoelétrico da proteína, ocorre uma incompatibilidade termodinâmica entre a proteína e o polissacarídeo, ocasionada por uma repulsão eletrostática e também por uma diferença de afinidade pelo solvente (TOLSTOGUZOV, 1997).

A miscibilidade de blendas de polímeros é atribuída às interações entre esses componentes poliméricos, que em geral produzem uma energia livre negativa na mistura, apesar de seu alto peso molecular. As interações mais comuns neste tipo de mistura são as pontes de hidrogênio, as interações iônicas e as ligações do tipo dipólo-dipólo. A maioria dos polímeros são imiscíveis, contudo a mistura entre polímeros tem aumentado a miscibilidade, e levado a um crescente interesse nos estudos de misturas de colágeno com polímeros naturais e sintéticos (SIONKOWSKA, 2004).

Sionkowska *et al.* (2004) analisando blendas de colágeno e quitosana através da técnica de difração de raios - X constataram que a estrutura em hélice tripla é perdida com o aumento do conteúdo de quitosana. Por outro lado, Figueiró *et al.* (2004) verificaram que num filme produzido com uma blenda de colágeno e o polissacarídeo galactomanana, a integridade estrutural da tripla hélice do colágeno foi preservada e que a goma foi retida nas fibras do polímero. A retenção dessas

propriedades dos dois polímeros no referido estudo permitirá o desenvolvimento de uma série de possíveis aplicações biomédicas, mas sobretudo, nas indústrias cosmética e alimentícia.

2.4 Propriedades Mecânicas e Térmicas de Filmes

Filmes biodegradáveis de polissacarídeos e proteínas apresentam propriedades mecânicas satisfatórias e boa aparência, sendo alternativas ecológicas e com grande potencial na substituição de embalagens sintéticas na indústria alimentícia e farmacêutica. Em 1999, Pavlath e Robertson, descreveram melhorias nas propriedades físico-químicas (através de modificações químicas) desse tipo de filme, tornando-os comparáveis em muitas características com os filmes não-biodegradáveis comumente utilizados.

Muitos pesquisadores têm estudado propriedades mecânicas e térmicas de filmes comestíveis, principalmente em função do uso de plastificantes. Em 1998, Arvanitoyannis *et al.* relataram a diminuição da temperatura de transição vítrea (*Tg*) e da resistência à ruptura (*Ts*) com o aumento na concentração de água ou polióis em blendas de gelatina e amido solúvel. Chiellini *et al.* (2001) observaram a ação de agentes reticulantes, como o glutaraldeído, na taxa de degradação e nas propriedades térmicas de blendas de gelatina e PVA (álcool polivinílico), através do uso de DSC (calorimetria diferencial de varredura) e DMTA (análise dinâmica térmica e mecânica). Os resultados apontaram para uma rápida e completa biodegradabilidade desses filmes. Sobral, Monterrey e Habitante (2002) determinaram a Tg de filmes de proteína miofibrilar de tilápia do Nilo, água e glicerol (por DSC), e relataram uma separação de fases entre a proteína e o plastificante, onde a Tg do plastificante é deslocada por influência da água (PARRA *et al.*, 2004).

Em 2008, Kristo, Koutsoumanis e Biliaderis, mostraram que a incorporação de alguns agentes antimicrobianos em filmes de caseinato alteram as propriedades termo-mecânicas e de barreira ao vapor de água desses filmes. Lactato de sódio e sorbato de sódio agiram como plastificantes aumentando o

equilíbrio no conteúdo de água, na permeabilidade ao vapor de água e na elasticidade desses filmes.

2.5 Propriedades de Barreira de Filmes Comestíveis

O principal interesse no estudo de filmes e revestimentos comestíveis baseia-se principalmente no seu potencial para fornecer melhorias em uma combinação de fatores como: umidade, oxigênio, sabor, aroma, cor, ou barreira lipídica para um alimento ou medicamento, com um conseqüente aumento da qualidade e vida de prateleira. Assim, a permeabilidade de filmes comestíveis a estas substâncias é objeto de grande interesse.

Permeabilidade é uma propriedade que descreve a medida em que uma substância que permeia se dissolve e, em seguida, a taxa com a qual o permeante difunde através de um filme, com uma força motora relacionada com a diferença de concentração do permeante entre os dois lados dos filmes . Permeabilidade é, assim, definida como a concentração ou diferença de pressão parcial entre as fases adjacentes aos dois lados do filme (KROCHTA, 2002).

O caráter polar das proteínas determina as propriedades de barreira dos filmes protéicos. Filmes protéicos têm alta permeabilidade a substâncias polares, como o vapor de água e baixa permeabilidade a substâncias apolares, tais como oxigênio, aromas e óleos. Plastificantes, incluindo a água, geralmente aumentam a permeabilidade dos filmes protéicos, tanto para substâncias polares como apolares; esse aumento de permeabilidade também é influenciado por um aumento na umidade relativa. Revestimentos polissacarídicos têm sido utilizados para estender a vida de prateleira de frutas e verduras, reduzindo a respiração e trocas gasosas devido à permeabilidade seletiva ao O_2 e CO_2.

Revestimentos e filmes hidrofílicos, como os de natureza protéica e polissacarídica, em geral são boas barreiras à passagem de gases como O_2 e CO_2, mas não são capazes de interferir muito na passagem do vapor de água (GARCÍA; MARTINÓ; ZARITZKY, 1998). Contudo uma certa quantidade de oxigênio e dióxido de carbono são necessários para a respiração dos tecidos

vivos, desta forma, o ideal é que se tenha um filme semipermeável, capaz de minimizar essas trocas gasosas, mas sem interrompê-las por completo, evitando que ocorra respiração anaeróbica (AYRANCI ; TUNC, 2004).

Filmes produzidos a partir de diferentes materiais, apresentam diferentes valores de permeabilidade ao oxigênio e ao dióxido de carbono, como pode ser visto nas tabelas 1 e 2.

A transferência de massa de vapor de água através de filmes comestíveis tem sido amplamente estudada, não só devido à importância do controle de umidade para manter a qualidade dos alimentos, mas também devido à facilidade com que pode ser medida com precisão, utilizando equipamentos simples (AYRANCI ;TUNC, 2004). Os resultados de permeabilidade ao vapor de água de filmes podem ser úteis na compreensão de mecanismos de transferência de massa, bem como nas interações entre soluto e polímero nos filmes comestíveis. A permeabilidade ao vapor de água de diferentes filmes comestíveis pode ser observada na Tabela 3. De acordo com a termodinâmica dos processos irreversíveis, a diferença de potencial químico da água é a força motriz da transferência de água através do filme. Quando o processo ocorre à temperatura e pressão constantes, a diferença de potencial químico da água resultante é proporcional à diferença de concentração de vapor de água entre as duas interfaces (MORILLON *et al.*,2000).

Tabela 1 – Comparação da permeabilidade ao oxigênio em filmes de diferentes polímeros comestíveis e filmes convencionais sintéticos.

Tipo de filme	Condições do Teste	Permeabilidade ao o_2 (cm^3 x µm/ m^2 x d x kPa)
Filmes de Celulose		
Metilcelulose	24° C,50%UR	97
Hidroxipropil-metilcelulose	24° C,50% UR	272
Metilcelulose	25° C,52% UR	90
Filmes de Amido		
Amido de milho	25° C,<100% UR	<65
Amido de milho modificado (hidroxipropil)	25° C,<78% UR	~0
Filmes Protéicos		
Colágeno	TA,0% UR	<0.04 – 0.5
Colágeno	TA,63% UR	23.3
Colágeno	TA,93% UR	890
Zeína: PEG + Glicerol (2.6:1)	25° C,0% UR	38.7 – 90.3
Glúten: Glicerol (2.5:1)	25° C,0% UR	6.1
Proteína de soja: Glicerol (2.4:1)	25° C,0% UR	6.1
Proteína do soro do leite:Glicerol (2.3:1)	23° C,50% UR	76.1
Proteína do soro do leite:Glicerol (2.3:1)	23° C,50% UR	4.3
Sintéticos		
Polietileno de baixa densidade	23° C,50% UR	1870
Polietileno de alta densidade	23° C,50% UR	427
Poliéster	23° C,50% UR	15.6
EVOH (70% VOH)	23° C,0% UR	0.1
EVOH (70% VOH)	23° C,95% UR	12
PVDC	23° C,50% UR	0.4 – 5.1

* TA – temperatura ambiente ** UR – umidade relativa *** Extraído de Miller & Krochta,1997

Tabela 2 - Comparação da permeabilidade ao dióxido de carbono em alguns filmes comestíveis com e sem adição de plastificante.

TIPO DE FILME	PERMEABILIDADE AO CO_2 ($cm^3 \times m^{-1} \times s^{-1}Pa^{-1}$)	REFERÊNCIAS
Amido de milho	$29.21x10^{-10}$	García *et al.* (2000)
Amido de milho + glicerol	$5.69x10^{-10}$	García *et al.* (2000)
Amylomaize**	$28.05x10^{-10}$	García *et al.* (2000)
Amylomaize**+glicerol	$3.85x10^{-10}$	García *et al.* (2000)

* Extraído de Bifani *et al.*, 2007
** Amylomaize é um tipo de amido de milho com um alto teor de amilose (>50%)

Tabela 3 – Comparação da permeabilidade ao vapor de água em alguns filmes comestíveis

TIPO DE FILME	WVP x 10^{11} ($g \times m^{-1} \times s^{-1}Pa^{-1}$)	REFERÊNCIAS
Amido de inhame	$0.99–1.88 \times 10^{-10}$	Mali *et al.* (2002)
Proteína de soja	38.3×10^{-10}	Anker *et al.* (2002)
Amido de Milho	3.68×10^{-10}	García *et al.* (2000)
Amido de Milho + glicerol	2.57×10^{-10}	García *et al.* (2000)
Amylomaize**	2.62×10^{-10}	García *et al.* (2000)
Amylomaize**+glicerol	2.14×10^{-10}	García *et al.* (2000)
Metilcelulose	$8.4–12.1 \times 10^{-10}$	Park *et al.* (1993)
Metilcelulose	$0.49–0.60 \times 10^{-10}$	Turhan and Sahbaz (2004)
Metilcelulose +PEG	0.747×10^{-10}	Turhan and Sahbaz (2004)
Hidroxipropilcelulose	$5.2–6.6 \times 10^{-10}$	Park *et al.* (1993)
Amido de batata	$1.36–2.17 \times 10^{-10}$	Petersson and Stading (2005)
Gelatina bovina	$4.7–10.6 \times 10^{-10}$	Sobral *et al.* (2001)
Gelatina suína	$5–8.9 \times 10^{-10}$	Sobral *et al.* (2001)
Gelana	1.58×10^{-10}	Yang and Paulson (2000)
β-caseína	$1.82–4.79x\ 10^{-10}$	Mauer *et al.* (2000)

* Adaptado de Bifani *et al.*, 2007
** Amylomaize é um tipo de amido de milho com um alto teor de amilose (>50%)

Os aromas e os sabores em geral são produzidos por compostos orgânicos voláteis. O transporte desses aromas e sabores através de materias plásticos de revestimento, não tem sido tão profundamente estudado como o transporte de outros gases, devido a complexidade destes compostos nos alimentos e a sua interação com vários polímeros. A presença de compostos orgânicos co-permeantes pode influenciar sensivelmente a permeabilidade desses aromas e sabores, quando os valores são comparados aos mesmos compostos puros (HERNANDEZ ; GIACIN, 1998). Em geral, os compostos responsáveis pelo aroma e sabor apresentam os mesmos princípios de permeabilidade em materiais plásticos como descrito anteriormente para outros gases como oxigênio e dióxido de carbono, contudo a determinção da taxa de transmissão desses gases é um pouco mais complexa pois a solubilidade desses compostos orgânicos é diretamente influenciada pela pressão (pressão-dependente) e os coeficientes de difusão sofrem influência direta da concentração (TUNG *et al.*, 2001).

As características exigidas para filmes e revestimentos comestíveis dependem, principalmente, da forma de deterioração que o produto alimentar (onde serão aplicados) pode sofrer. Portanto, baixa permeabilidade ao oxigênio é necessária para produtos que sejam sensíveis à oxidação, como é o caso das gorduras poliinsaturadas. Para frutas e verduras é importante que haja uma seletividade à transferência de massa de alguns gases como o oxigênio, o dióxido de carbono e o etileno. É importante que haja uma diminuição na transferência desses gases, sem que ocorra uma interrupção total dessas trocas gasosas (EL GHAOUTH *et al.*, 1991).

A expessura dos filmes é um fator de suma importância e que pode ser controlado. Se um filme é muito espesso, efeitos deletérios podem ser observado ocasionados por níveis de O_2 abaixo dos valores mínimos necessários, bem como por um aumento nos níveis de CO_2. Essa condição leva à fermentação anaeróbica, que gera produtos de aromas e sabores desegradáveis. Para prevenir esse tipo de condição se fazem necessários a caracterização e o estudo de propriedades importantes aos filmes comestíveis como: capacidade molhante, porpriedades de permebilidades a gases, a composição da atmosfera interna dos

frutos revestidos, e o efeito gerado por esses revestimentos na qualidade dos frutos revestidos (PARK, 2002).

2.6 Espécies Vegetais

2.6.1Sementes

2.6.1.1 *Adenanthera pavonina*

O gênero *Adenanthera* pertence à família Fabaeae (Leguminosae), subfamília *Mimosoideae*, tribo *Mimoseae*. Compreende mais de 10 espécies tropicais da Ásia, Austrália e ilhas do Pacifico (HEGNAUER, R.; HEGNAUER, M., 1996). Na Ásia, é conhecida como olho de dragão. Não existe qualquer registro oficial de quando essa planta foi introduzida no Brasil, mas ela encontrou um clima favorável para desenvolver-se e proliferar-se.

Apresenta crescimento rápido (alcançando de 15 a 20 metros de altura) e serve como bom dossel para plantas herbáceas, arbustivas e trepadeiras que não toleram altas intensidades luminosas. O cerne vermelho de *A. pavonina* é usado como um substituto da madeira do sândalo vermelho (*Pterocarpus sandalinus* L.), sendo assim, importante fonte fornecedora de madeira de boa qualidade para construções (BASU; CHAKRAVERTY, 1986). É utilizada em reflorestamentos, como planta ornamental e como forrageira na Tailândia, o cozimento das sementes e da madeira permite o seu uso no tratamento de infecções pulmonares, podendo também o produto ser aplicado externamente no tratamento da oftalmia crônica (BABURAJ ; GUNASEKARAN, 1993).

Estudos de caracterização da goma de sementes de *A. pavonina* são escassos e nenhum estudo estrutural foi relatado ainda. Tavares (1998) relatou o uso da galactomanana como matriz de afinidade para purificar lectinas galactose ligantes. Embora esta planta mostre amplo potencial biotecnológico, seu potencial ainda é subexplorado, sobretudo, como fonte de polissacarídeos hemicelulósicos.

Figura 2 – Árvore, folhas e frutos de *Adenanthera pavonina*.

2.6.1.2 Caesalpinia pulcherrima

Caesalpinia pulcherrima é a espécie mais abundante do gênero *Caesalpinia.* É conhecida popularmente pelo nome de flamboyanzinho, flor-de-pavão, flamboyant-mirim, poinciana. É um arbusto que atinge em média 3 m de altura, e é nativo da América Central. As folhas são paripenadas, com 20 a 40 cm de comprimento. Suas flores possuem pétalas que podem ser vermelhas, laranjas ou amarelas. O fruto é uma vagem com 6 a 12 cm de comprimento.

É bastante usada como uma planta ornamental, sendo bem comum em jardins de regiões tropicais. Os índios já utilizam a bastante tempo o sumo das folhas de *Caesalpinia pulcherrima* como antitérmico. O extrato das flores é utilizado no tratamento de ferimentos. As sementes são utilizadas como antitussígeno e broncodilatador (COUNTER, 2006).

Em 2005 Rao *et al.* mostraram que flavonóides extraídos das partes aéreas de *Caesalpinia pulcherrima* apresentavam atividade antiinflamatória, atuando na inibição da produção do óxido nítrico, bem como na supressão de TNF-α e IL-12. Essas duas citocinas são secretadas na fase inicial do processo inflamatório agudo e crônico, e estão intimamente relacionados com algumas

patologias como asma, artrite reumatóide e até mesmo o choque séptico. Chiang *et al.*(2003) demonstraram que flavonóides de *C. Pulcherrima* também apresentavam atividade antiviral contra herpes vírus e adenovírus, sendo mais potente sua ação contra os adenovírus ADV-8 e ADV-3.

Em 2006 Sudhakar *et al.* mostraram que extratos de sementes de *C. puclherrima* apresentavam atividade antimicrobiana contra *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* e *Staphylococcus aureus*. Apresentavam ainda atividade antifúngica contra *Cândida albicans*, *Aspergillus niger*, *Rhizopus oligosporus.*

Figura 3 – Árvore, flores e frutos de *Caesalpinia pulcherrima.*

2.6.2 Frutos

2.6.2.1 Acerola

A acerola (*Malpighia glabra*) é um fruto da família *Malpighiaceae* e também é conhecido popularmente por cereja-das-antilhas, cereja-de-barbados ou acerola, como é mais conhecida atualmente no Brasil, apresentando uma certa semelhança com a cereja européia. O fruto apresenta cor vermelha forte quando

maduro, variando entre os tons avermelhados e alaranjados (Figura 4), com um perfume semelhante ao da maçã, com sabor ácido, polpa macia e cheia de suco (GOMES *et al.*, 2002). A acerola é uma planta nativa das Antilhas, América Central e do norte da América do Sul. Por ser uma planta rústica e resistente, a acerola se propaga com facilidade em toda parte do mundo. O fruto nasce na aceroleira que é um arbusto de até três metros de altura, seu tronco se ramifica desde a base, e sua copa é bastante densa com pequenas folhas verde-escuras e brilhantes. Suas flores, de cor rósea-esbranquiçada, são dispostas em cachos, têm floração durante todo o ano, e após três ou quatro semanas se dá sua frutificação. Por ser uma planta muito rústica e resistente, ela se espalhou facilmente por várias áreas tropicais, subtropicais e até semi-áridas. A acerola, quando madura, tem uma variação de cor que vai do vermelho ao vinho, passando pelo alaranjado. Esta coloração é resultado da presença de antocianinas, especialmente pelargonidina e malvidina. Sua superfície é lisa ou divida em três gomos e possui três sementes no seu interior (COUTINHO, 1995).

O interesse pela acerola e os estudos sobre suas potencialidades econômicas, no entanto, só foram despertadas a partir dos anos 40, quando descobriu-se na porção comestível da fruta altos teores de ácido ascórbico (até 5 000 miligramas por 100 gramas de polpa.) Sendo assim, o consumo de acerola é indicado para o combate de várias doenças humanas, como a gripe e afecções pulmonares, controle de hemorragias nasais e gengivais, auxilia no tratamento de doenças do fígado, além de evitar a perda de apetite e dores musculares. Possui ainda vitaminas A, B_1 (tiamina), B_2 (riboflavina), B_3 (niacina), cálcio, fósforo, ferro (CHAVES *et al.*, 2004).

No Brasil, o cultivo de acerolas teve um forte crescimento nos últimos vinte anos, sendo hoje uma importante cultura principalmente para a economia da Região Nordeste, assim como um impulso para a agroindústria de polpa de fruta congelada. Existem mais de 42 variedades de acerola que são cultivadas no Brasil dentre as quais as principais são:Apodi (BRS 235), Cabocla, Cereja (BRS 236),

Frutacor (BRS 238), Okinawa, Olivier, Roxinha (BRS 237), Rubra e Sertaneja (http://www.wikipedia.org).

Figura 4 – Frutos de *Malpighia glabra*.

2.6.2.2 Cajá

O cajá é o fruto da cajazeira (*Spondias mombin*). É também chamada de ambaló, ambaró, cajá-mirim, cajazinha, tapareba, taperebá, taperibá, ou tapiriba. A cajazeira (*Spondias mombin L.*) é uma espécie frutífera da família *Anacardiaceae*, com altura de 20-25m, dotado de copa baixa e densa muita característica. Tronco curto e muito ramificado, revestido por casca rugosa, de 40-60cm de diâmetro. É originária da região tropical do continente americano. Na região sudeste da Bahia, a cajazeira é encontrada como árvore usada para sombreamento permanente do cacaueiro e também como produtora de frutos que servem como importante fonte de renda adicional para o produtor. Os frutos da cajazeira são muito apreciados pelo excelente sabor de sua polpa. Além disso, apresentam boas características agroindustriais como rendimento de polpa de 56% em média e suas características químicas, como Brix de 13°. A polpa de cajá está entre as mais comercializadas na região. A cajazeira desenvolve-se bem em climas úmidos, sub-úmidos e quentes, com precipitação anual entre 1.100 a 2.000 mm. Os solos recomendados para o plantio são os profundos e bem drenados, para

que as raízes tenham um desenvolvimento satisfatório. Os terrenos com declividade acima de 20% não são recomendáveis (http://www.wikipedia.org).

Dentre os seus principais constituintes químicos destacam-se as vitaminas A e C, alguns minerais como cálcio, ferro e fósforo, bem como alguns carboidratos e taninos.

Popularmente tem diversos usos como planta medicinal, sendo usado no tratamento de hematomas, hemorróida, diarréias. Um dos usos do cajá que tem adquirido maior respaldo científico, é o uso de sua atividade anti-viral. Em 1991, Pesquisadores da Universidade de Antuérpia na Bélgica, isolaram das folhas e talos desta espécie, dois elagitaninos (CORTHOUT *et al.,* 1991) , Geranina (MATOS, 1994) e Galoilgeranina (HASLAM, 1996), que demonstraram atividade pronunciada contra os vírus *Herpes simplex* tipo 1 e Coxsackie B2 , confirmando os estudos realizados por Fukuchi *et al.* em 1989 que relataram a atividade anti-herpética da Geranina e Taninos encontrados nas folhas de *Spondias Mombin.* A atividade desta substância foi determinada ser da ordem de 50 mcg/ml. A Universidade Federal do Ceará, lançou um medicamento a base de um extrato alcoólico obtido das folhas da cajazeira que vem sendo usado devido às suas propriedades anti-viróticas, apresentando resultados muito significativos no combate ao vírus da herpes tipo I e II.

Figura 5 – Árvore, folha e fruto de *Spondias mombim.*

2.6.2.3 Maçã

Maçã é o nome dado ao fruto da macieira, árvore da família *Rosaceae*, pertencente ao gênero *Malus*. As variedades mais comuns são *M. domestica* e *M. sieversii* e respectivos híbridos. A maçã, do ponto de vista científico, não é realmente um fruto, e sim um pseudofruto.

A maçã foi uma importante fonte alimentícia em todos os climas frios e, provavelmente, a macieira é a árvore cultivada há mais tempo. É a espécie de fruta, à exceção dos cítricos, que pode ser conservada durante mais tempo, conservando boa parte de seu valor nutritivo. As maçãs de inverno, colhidas no final do outono e guardadas em câmaras ou armazéns acima do ponto de congelamento, têm sido um alimento destacado durante milênios na Ásia, Europa e nos Estados Unidos (desde 1800). Mais de 7500 cultivares diferentes existem, e de acordo com o clima e a região escolhida, existem cultivares mais adaptados.

Em 2007 pelo menos 64 milhões de toneladas de maçãs foram colhidas em todo o mundo, gerando um valor econômico estimado de 10 bilhões de dólares. A China é o maior produtor mundial contribuindo com aproximadamente 42% de toda a produção global, sendo seguida por países como Estados Unidos, Irã, Turquia, Rússia e Itália. O Brasil produziu aproximadamente 1,1 milhão de toneladas no ano de 2007, em uma área cultivada de 37.562 hectares (FAO, 2007).

O consumo regular de maçã tem alegativas funcionais ajudando na prevenção e manutenção da taxa de colesterol em níveis aceitáveis, prevenção de problemas cardíacos e ajudando na perda de peso (FULGONI *et al.*, 2008). Esse efeito é devido ao alto teor de pectina, encontrada na casca. A maçã contém as vitaminas: B_1, B_2, B_3 e B_6, além de sais minerais, como fósforo e potássio. É rica

em quercetina, substância que ajuda a evitar a formação dos coágulos sanguíneos capazes de provocar acidentes vasculares.

In vitro alguns compostos fenólicos isolados da maçã (querecetina,epicatequina e procianidina), apresentaram atividade antioxidante e são capazes de ajudar na prevenção do câncer (LEE, 2003). O suco de maçã concentrado mostrou-se capaz de aumentar os teores de acetilcolina em ratos (CHAN *et al.*, 2006).

Figura 6 – Fruto e árvore de *Malus domestica.*

2.6.2.4 Manga

A mangueira pertence à família *Anarcadiaceae*, sendo uma árvore de grande porte e em forma de domo. Um elemento que pode identificá-la com facilidade é a presença de apenas um estame e as flores amarela-esverdeadas que desenvolvem-se a partir de uma panícula em forma de cone. Mangifera indica pode atingir 45 m de altura geralmente com uma circunferência de 3,6 m ou mais. A casca é rugosa, cinza escura e fibrosa. A folhas acumulam-se na ponta dos galhos e têm de 10 - 30 cm de comprimento por 2 - 10 cm de largura, oblongas ou lanceoladas, acuminadas, de cor verde-escura brilhante, rosedas quando novas,

com uma resina aromática quando amassada, pecíolo com 2,5 - 6 cm de comprimento, inchado na base, folhas novas pendem verticalmente para baixo, enquanto a cor é rosa. Inflorescência cônica, as flores tem 0,4 cm de diâmetro, amarelo-esverdeadas, aromáticas, masculinas e bisexuais na mesma panícula, cálice com 4 ou 5 sépalas, corola carnosa com 4 ou 5 pétalas, amarelo-claras, maiores que as sépalas, possui 4 ou 5 estaminóides e apenas um estame fértil e muito maior que os outros. Fruto em drupa com 5 - 20 cm de comprimento, carnoso, amarelo quando maduro, fibroso (Figura 7). Muitas variedades de frutos podem ser encontradas em função do local onde a planta se encontra. Na região nativa, as flores surgem de janeiro a março, e frutos maduros de abril a julho. Apesar de ser originária do Sul da Ásia, esta espécie se adaptou muito bem ao clima tropical, sendo amplamente cultivada nestas regiões.

A manga é um dos mais apreciados frutos de origem tropical, sendo atualmente cultivada em todos os países da faixa tropical e equatorial do mundo. Em 1998, esta foi a fruta que mais contribuiu para a pauta das exportações brasileiras de frutas frescas (SOUZA *et al.*, 2002). Em 2007, o Brasil foi responsável pela produção de 1 milhão e 546 mil toneladas em uma área de aproximadamente 90 mil hectares, ocupando o segundo lugar como principal país exportador em quantidades (FAO, 2007). A manga encontra excelentes condições para o seu desenvolvimento e produção, sendo cultivada em quase todos os estados brasileiros. A Região Nordeste destaca-se no cenário nacional como grande produtora de manga tipo exportação, onde estão localizados os principais pólos de irrigação da zona semi-árida como a região do vale do rio São Francisco e o pólo agrícola de Mossoró-Açu no Rio Grande do Norte (PIMENTEL *et al.*, 2000). Devido às condições climáticas benéficas, os mangicultores podem planejar suas colheitas para qualquer período do ano, possibilitando aos mesmos, colocarem o produto no mercado em épocas de melhores preços (SOUZA *et al.*, 2002).

Para que o Brasil atenda às exigências dos mercados nacional e estrangeiro, se faz necessário o aprimoramento e desenvolvimento de novas tecnologias em conservação e processamento pós-colheita, reduzindo as perdas

de qualidade dos frutos durante, principalmente, o transporte marítimo. Portanto, todos os cuidados tomados com esses frutos desde o momento da colheita, armazenamento, transporte e comercialização se justificam quando se obtém um produto de melhor qualidade que pode atingir um preço mais alto no mercado.

Como todo fruto de origem tropical, a manga apresenta obstáculos em relação a sua vida útil pós-colheita como uma elevada taxa respiratória. Quanto maior a taxa respiratória de um fruto, mais rápido será seu metabolismo e, portanto, mais rápido será seu amadurecimento e senescência (TUCKER, 1993). Mesmo depois de colhidos, os frutos continuam a realizar processos metabólicos como respiração e transpiração, com a diferença que as perdas de constituintes então observadas não são mais repostas pela planta-mãe. Isso requer reservas suficientes que permitam continuar o seu desenvolvimento depois da colheita.

A manga depois de colhida apresenta uma vida de armazenamento muito curta que varia entre 10 e 12 dias dependendo das condições de armazenamento (AVILÁN; ALVAREZ, 1990). Para uma manga ganhar importância comercial, esta deve apresentar um conjunto de características como boa palatabilidade, pouca fibra e razoáveis teores de açúcares e acidez (DONADIO, 1989), e para que estas características sejam obtidas, condições adequadas devem ser utilizadas durante o armazenamento.

Figura 7 – Fruto e árvore de *Mangifera indica* (cultivar Tommy Atkins).

2.6.2.5 Pitanga

A pitanga (*Eugenia uniflora* Berg.) é o fruto da pitangueira , a qual pertence a família *Myrtaceae.* O fruto é uma drupa globosa (Figura 8), carnosa, vermelha (a mais comum), amarela ou preta, e bastante saborosa, rica em cálcio, a pitanga é nativa da Mata Atlântica brasileira, onde é encontrada na floresta estacional semidecidual do planalto, desde Minas Gerais até o Rio Grande do Sul. Ocorre também nas restingas. Também pode ser encontrada na ilha da Madeira, Portugal, onde foi introduzida.

A palavra "pitanga" vem do tupi-guarani, e significa "vermelho". A planta é cultivada tradicionalmente em quintais domésticos. Dá-se bem em terrenos arenosos junto às praias e os frutos são ótimos atrativos para pássaros. É também usada como árvore ornamental em várias cidades brasileiras. Nos sistemas agroflorestais multiestrato e em reflorestamentos heterogêneos, é planta importante na recuperação de áreas degradadas. A pitangueira é uma árvore de porte médio(no máximo 12 m) de desenvolvimento moderado, e medianamente rústica. A copa globosa é dotada de folhagem perene. Apresenta formato cônico, suas folhas são de cor verde intensa e em geral apresentam aproximadamente 4 cm de comprimento. O fruto é rico em vitamina C, e seu aroma e sabor agradáveis fazem com que seja bastante utilizado na fabricação de doces e geléias (http://www.wikipedia.org).

Figura 8– Árvore e fruto de *Eugenia uniflora.*

2.6.2.6 Seriguela

Seriguela, cirigüela ou ciruela (*Spondias purpurea*) é o nome de uma árvore das anacardiáceas e também do seu fruto. É uma árvore de porte médio, podendo atingir até 7 metros. Originária das Américas (Central e do Sul), é bastante comum na Região Nordeste do Brasil. A seriguela encontra-se no sul do México, América Central, Antilhas, Nigéria, Filipinas e Brasil (Cerrado e Caatinga), em altitudes diversas. Tem ocorrido exemplares desta árvore em cidades do estado do Rio de Janeiro, Mato Grosso do Sul, Minas Gerais e São Paulo, sendo encontrada inclusive no paisagismo. Há diversos exemplares da fruta vermelha em chácaras do Distrito Federal. A região sul do Ceará é hoje a maior produtora de seriguela no Brasil.

Sua frutificação se dá nos meses de outubro e novembro, sendo colhida entre os meses de dezembro e janeiro. Está adaptada a solos fracos e com baixa pluviosidade. Sendo lavoura permanente, e de uso pouco difundido, não há apreciação econômica senão para produção sazonal em pequenas plantações.A

seriguela dificilmente se propaga por sementes, sendo multiplicada por estacas de 30 a 50 cm de comprimento e de 7 a 12 centímetros de diâmetro.

O fruto é uma drupa elipsodial de cor laranja-avermelhada e até amarelada quando maduro, com 2,5 centímetros a 5 centímetros de comprimento e 15 a 20 gramas de peso (Figura 9). A camada de polpa é fina, com cerca de 3 milímetros, com um caroço do tamanho de uma azeitona grande. É parecido com o cajá, mas ao contrário deste é bastante doce.O seu consumo é feito de diversas formas, desde *in natura* até na confecção de sucos, sorvetes e doces. É rico em carboidratos, cálcio, fósforo e ferro, a seriguela possui ainda outras vitaminas como A, B e C. É eficaz contra anemia e a diminuição dos glóbulos brancos (MILLER; SCHALL, 2005).

Figura 9 - Frutos de *Spondias purpurea*.

3 - OBJETIVOS

3.1 - Objetivo Geral

Produzir revestimentos comestíveis, a partir de galactomananas de sementes de leguminosas visando o seu emprego no aumento de tempo de prateleira de frutos tropicais.

Avaliar a capacidade dessas galactomananas em formarem blendas com colágeno, e analisar as características dos filmes formados a partir dessas blendas.

3.2 – Estratégia Experimental

1. Isolamento de galactomananas de sementes presentes no Estado do Ceará;

2. Preparação de revestimentos comestíveis, a partir de galactomananas de sementes e de blendas com colágeno;

3. Estudo das propriedades de superfície dos frutos utilizados para a aplicação dos revestimentos;

4. Otimização das misturas filmogênicas, baseada em sua capacidade de espalhamento sobre a superfície dos frutos;

5. Avaliação das propriedades mecânicas e de barreira dos revestimentos desenvolvidos;

6. Avaliação da influência do revestimento na respiração dos frutos

4 - MATERIAIS E MÉTODOS

4.1 Solventes e Reagentes

Os reagentes, utilizados, foram de grau analítico. Para obtenção da preparação final das soluções filmogênicas, foram ainda utilizados etanol (96 %) e glicerina comerciais.

4.2 Material vegetal

4.2.1 Sementes

Sementes de *Caesalpinia pulcherrima* foram coletadas no campo experimental do Laboratório de Lectinas e Glicoconjugados (LABLEC) do Departamento de Bioquímica e Biologia Molecular, da Universidade Federal do Ceará. Sementes de *Adenanthera pavonina* foram coletadas no Campus do Pici, da Universidade Federal do Ceará.

Após coletadas, as sementes foram retiradas das vagens (*C. pulcherrima*), limpas, devidamente selecionadas, lavadas e, depois de secas, guardadas em ambiente fresco e seco, para utilizações posteriores.

4.2.2 Frutos

Como modelos experimentais, foram utilizados frutos de manga, maçã, cajá, acerola, pitanga e seriguela. Os frutos foram adquiridos em supermercados nas cidade de Fortaleza e Braga (Portugal), sendo selecionados aqueles que apresentavam condições homogêneas com relação a maturação e ausência de contaminações microbiológicas.

Os frutos, menos perecíveis eram mantidos refrigerados a 10° C, até o período de utilização. Enquanto os mais perecíveis, eram adquiridos somente para utilização imediata.

4.2.3 Extração de polissacarídeos das sementes

Sementes quiescentes foram submetidas à fervura em etanol (70° C) por um período de 20 minutos, com o objetivo de inativar enzimas capazes de clivar o polissacarídeo, o etanol foi decantado após o resfriamento e foi adicionada água em seu lugar em uma razão 1:5 (m:v, sementes/água). As sementes foram deixadas intumescer por 12 h a 25 °C e então separadas manualmente em endosperma, tegumento e cotilédones.

Os endospermas obtidos das sementes de *Adenanthera pavonina* e de *Caesalpinia pulcherrima* foram homogeneizados em água destilada (utilizando um mixer) até obtenção de uma solução viscosa. O material foi peneirado (40 mesh) e a solução homogênea precipitada com álcool etílico 96° GL na relação de solução/álcool (1/2, v/v) e ao resíduo acrescido água destilada para uma nova extração.

O precipitado (galactomanana) foi liofilizado e guardado sob a forma de pó seco, até uso posterior.

A galactomanana pode, ainda, ser ressolubilizada em água e sua concentração em açúcar determinada pelo método do fenol sulfúrico (DUBOIS *et al.*, 1956) e estocada a 7 °C, até uso posterior.

4.2.4 Obtenção de Colágeno Solúvel

O colágeno solúvel foi obtido por tratamento alcalino de serosa de intestino bovino (FIGUEIRÓ *et al.*,1994), conforme pode ser visualizado na figura 10.

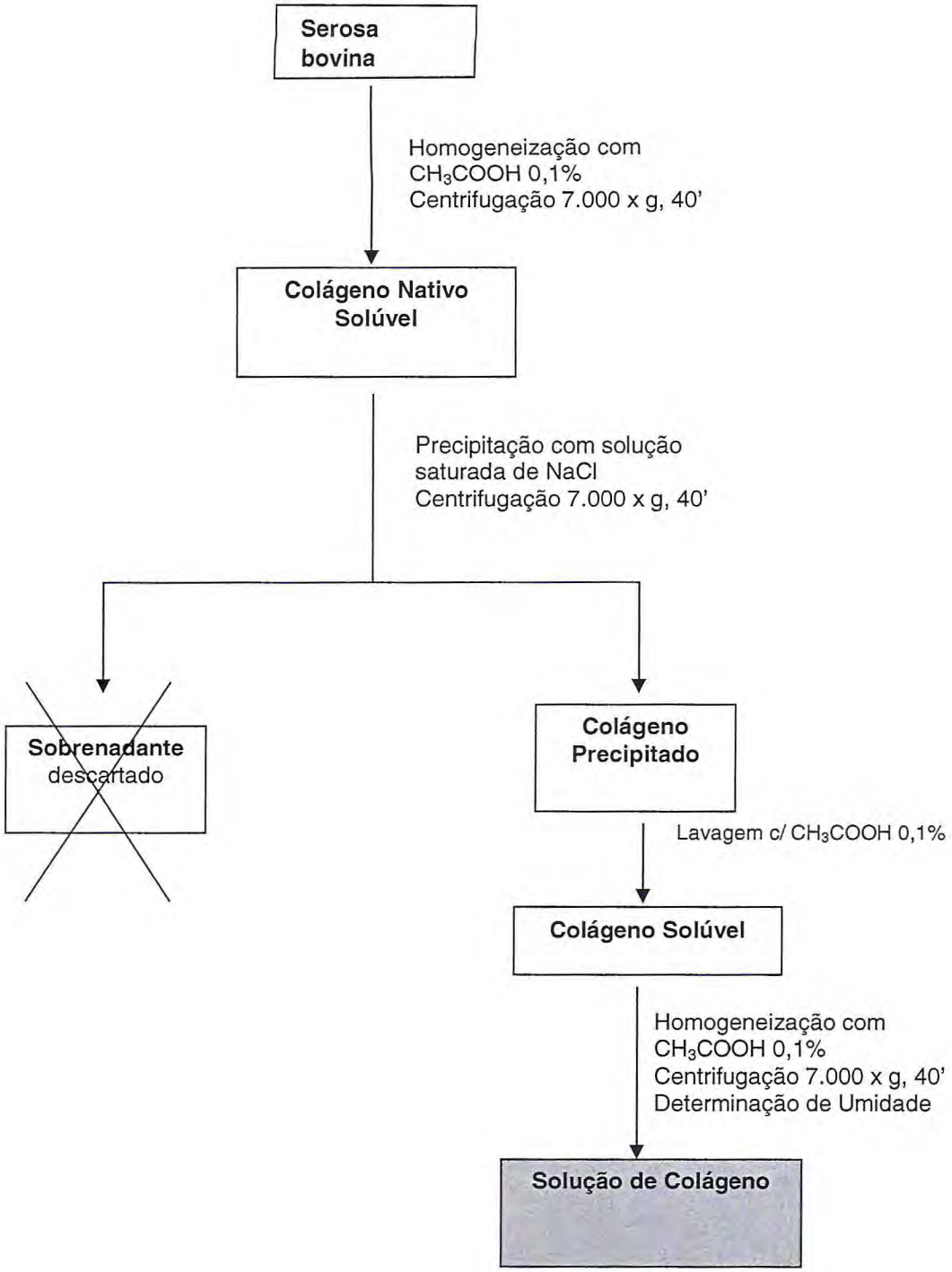


Figura 10 - Fluxograma da obtenção de colágeno solúvel a partir de serosa bovina

4.2.5. Preparação das Soluções Filmogênicas

As soluções filmogênicas de *A. pavonina* e *C. pulcherrima* foram preparadas dissolvendo a galactomanana liofilizada em água, com posterior adição do plastificante (glicerol) e, em parte dos experimentos uma solução de colágeno para elaboração de blendas galactomanana-colágeno. Cada solução foi homogeneizada por 5 minutos à temperatura ambiente (21 °C) e deixadas estabilizar por mais 10 minutos na mesma temperatura.

As tabelas 4 e 5 descrevem as concentrações utilizadas ao longo dos experimentos.

Tabela 4 - Diferentes concentrações utilizadas de galactomanana e glicerol na preparação de soluções filmogênicas de *A. pavonina* ou *C. pulcherrima*

Amostra	[Galactomanana]	[Glicerol]
Am.1	0.5%	1.0%
Am.2	1.0%	1.0%
Am.3	1.5%	1.0%
Am.4	0.5%	1.5%
Am.5	1.0%	1.5%
Am.6	1.5%	1.5%
Am.7	0.5%	2.0%
Am.8	1.0%	2.0%
Am.9	1.5%	2.0%

Tabela 5 - Concentrações utilizadas de galactomanana adicionada de colágeno e glicerol, na preparação de blendas de soluções filmogênicas de *A. pavonina* ou *C. pulcherrima*.

Amostra	[Galactomanana]	[Colágeno]	[Glicerol]
Am.1	0.5%	1.5%	0%
Am.2	1%	1%	0%
Am.3	1.5%	0.5%	0%
Am.4	0.5%	1.5%	0.5%
Am.5	1%	1%	0.5%
Am.6	1.5%	0.5%	0.5%
Am.7	0.5%	1.5%	1.0%
Am.8	1%	1%	1.0%
Am.9	1.5%	0.5%	1.0%
Am.10	0.5%	1.5%	1.5%
Am.11	1%	1%	1.5%
Am.12	1.5%	0.5%	1.5%

4.2.6. Capacidade Molhante e Tensão Superficial Crítica

Para se obter a capacidade molhante dos revestimentos foi necessário determinar o ângulo de contato do revestimento nas superfícies de frutos de acerola, cajá, manga ,maçã, pitanga e seriguela.

Foram testadas as soluções de Colágeno-Galactomanana de *Adenanthera pavonina* e Colágeno-Galactomanana de *Caesalpinia pulcherrima* nas proporções: 1,5%Col-0,5%Gal, 1,0%Col-1,0%Gal e 0,5%Col-1,5%Gal, variando-se as concentrações de glicerol em 0; 0,5; 1,0 e 1,5%. Foram feitos ainda ensaios utilizando somente galactomanana-glicerol, onde as concentrações de galactomanana variaram entre 0,5%, 1% e 1,5% e as concentrações de glicerol variaram entre 1%, 1,5% e 2%, conforme descrito anteriormente.

As soluções foram retiradas com uma seringa de 500 μL (Hamilton, Suíça). As análises das várias soluções estudadas foram realizadas utilizando o método da gota pendente disponível no medidor de ângulo de contacto OCA 20

(Dataphysics, Alemanha), selecionando a aproximação de Laplace-Young como método de cálculo.

Os frutos foram lavados com água destilada e só então foi retirada uma secção retangular que se fixou de forma adequada a um pedaço de vidro retangular. Foram feitas 20 medidas para cada amostra na obtenção do ângulo de contato a (20 ± 1) °C.

A tensão superficial crítica (γ_c), da superfície dos frutos estudados, foi determinada por extrapolação do gráfico de Zisman (ZISMAN, 1964), e utilizando como padrões: formamida, bromonaftaleno e água.

4.2.7. Preparação dos filmes

Na preparação dos filmes, foram utilizadas soluções filmogênicas conforme descrito nas tabelas 4 e 5. As soluções foram distribuídas em placas de petri (9 cm de diâmetro) utilizando o volume constante de 28 mL por cada placa. Os filmes foram secos em estufa a 35 °C por um período de 16 horas, e após a efetiva formação do filme, eles eram conservados a 20 °C e 50% de umidade relativa, até sua posterior utilização.

4.2.8. Espessura dos Filmes

A espessura dos filmes foi medida com um micrômetro digital (Mitutoyo, Japão). Cinco medidas de espessura foram obtidas a partir de diferentes pontos (selecionados aleatoriamente) do filme, e a média dessas medidas foi considerada. Esses valores de espessura foram considerados nos experimentos de permeabilidades a gases e propriedades mecânicas.

4.2.9. Permeabilidade ao vapor de água

A permeabilidade ao vapor de água dos filmes foi determinada gravimetricamente tendo como base o método ASTM E-96-92 (MC HUGH *et al.*, 1993; GUILLARD *et al.*,2003). O filme foi depositado na parte superior de uma célula de permeação contendo água destilada, e essa célula por sua vez foi inserida em um dessecador a 20 °C e com 0% de umidade relativa, contendo sílica. As células foram pesadas a cada 2 horas durante um período total de 10 horas (Figura 11). Foi feita uma curva da perda de massa x tempo, e a equação dessa curva foi obtida por regressão linear.

Figura 11 – Modelo adotado nos experimentos de Permeabilidade ao Vapor de Água. A – Célula de Permeação ; B – Pesagem da Célula de Permeação com o filme de galactomanana.

4.2.10. Permeabilidade ao Oxigênio

A permeabilidade ao Oxigênio foi determinada segundo o método ASTM D 3985–02 (2002). Os filmes foram selados entre 2 compartimentos, tendo cada um deles 2 canais. Na porção inferior era passado um fluxo controlado e constante de O_2, já no compartimento superior era passado um fluxo contínuo controlado de N_2. O oxigênio que permeava através do filme, atingia a porção

superior da câmara. O nitrogênio agia como um carreador para o O_2 e o fluxo de gás que saia dessa secção era conduzido a um sensor de oxigênio.

4.2.11. Permeabilidade ao Dióxido de Carbono

A permeabilidade ao dióxido de carbono foi determinada segundo o método ASTM D 3985–02 (2002). Os filmes foram selados entre 2 compartimentos, tendo cada um deles 2 canais. Na porção inferior era passado um fluxo controlado e constante de CO_2, já no compartimento superior era passado um fluxo contínuo controlado de N_2. O dióxido de carbono que permeava através do filme, atingia a porção superior da câmara. O nitrogênio agia como um carreador para o CO_2. Para medir o dióxido de carbono, era coletada uma alíquota de 1 mL do gás-teste, que era injetada em um cromatógrafo a gás (Chrompack CP 9001, Middelburg) a 110° C, com uma coluna Porapak Q80/100 2 m x 1/8" x 2 mm, utilizando detector do tipo FID. Como padrão para a cromatografia foi utilizada uma mistura gasosa contendo: 10 % CO_2, 20 % O_2 e 70 % N_2.

4.2.12. Propriedades Mecânicas dos Filmes

Os valores de *TS* (tração) e *E* (elasticidade até ruptura) foram mensurados utilizando uma máquina de testes universais da Instron (Modelo 4500), seguindo o método ASTM D 882-91 (1991). A distância inicial de separação entre as duas garras do aparelho era de 30 mm e a velocidade de deslocamento de 5 mm/min.

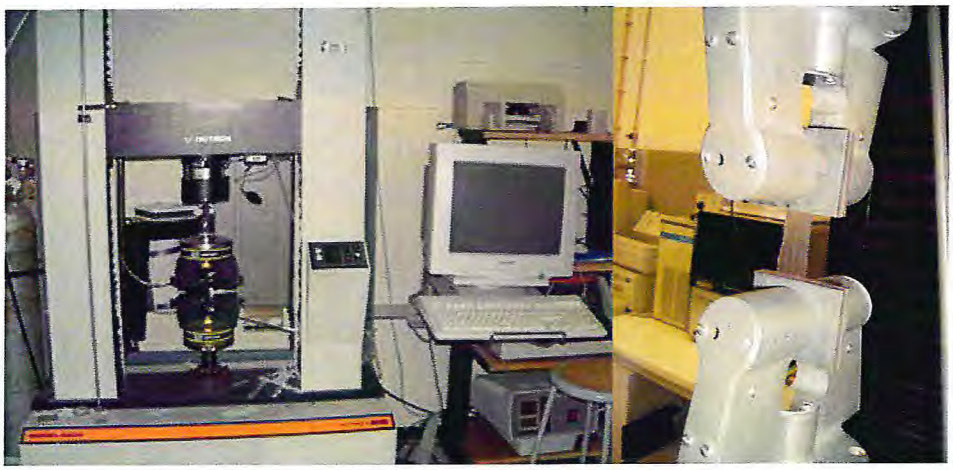


Figura 12 – Imagem da máquina de testes universais Instron, com ampliação do filme de galactomanana posicionado entre as garras durante o teste.

4.2.13. Cor e Opacidade dos Filmes

A opacidade dos filmes foi determinada utilizando um colorímetro Minolta (Cr 400; Minolta, Japan). A opacidade foi calculada utilizando o método de Hunther, que avalia a relação entre a opacidade de cada amostra frente um padrão negro (*Yb*) e a opacidade de cada amostra frente um padrão branco (*Yw*).

4.2.14. Taxa Respiratória dos frutos

Para quantificar o CO_2 liberado e O_2 consumido dos frutos inteiros (controle – sem revestimento e teste - envolvidos com películas de galactomanana) estes foram acondicionados em frascos de 600 mL (hermeticamente fechados). Foram utilizadas 3 repetições para cada tratamento com aproximadamente 120 g de frutos por frasco. A atmosfera no interior desses recipientes era retirada com seringas de 1 mL e as amostras injetadas em cromatógrafo a gás (Chrompack CP 9001) a 110° Celsius com coluna mol.sieve 5ª 80/100 mesh 1 m x 1,8" x 2 mm para separar o oxigênio e coluna Porapack Q 80/100 mesh 1 m x 1,8" x 2mm SS para separar o dióxido de carbono. Um detector por ionização de chama foi utilizado, bem como gás Hélio a um fluxo de

23 mL por minuto como gás carreador. Os valores obtidos foram comparados com uma mistura padrão contendo 10% de dióxido de carbono, 20% de Oxigênio e 70% de Nitrogênio.

Figura 13 – Imagem de frutos de *Mangifera indica* durante avaliação da influência dos filmes na taxa respiratória dos frutos.

4.2.15. Análises Estatísticas

As análises estatísticas foram feitas utilizando análise de variância (ANOVA), teste de Tukey ($p<0,05$) e regressão linear (Sigma Stat, USA).

5 - RESULTADOS E DISCUSSÕES

5.1 Revestimentos de Galactomanana – Glicerol

5.1.1 Tensão superficial e Tensão superficial crítica da superfície dos frutos

De acordo com Zisman (1964), sistemas que apresentam tensão superficial inferiores a 100 $mN \cdot m^{-1}$ (superfícies de baixa energia), o ângulo de contato formado por uma gota do líquido em uma superfície sólida será uma função linear da tensão superficial do líquido - γ_{LV} (onde a fase *V* é o ar saturado com o vapor do líquido *L*). O método de Zisman é aplicável somente para superfícies de baixa energia, portanto fazia-se necessária a determinação da energia nas superfícies dos frutos que seriam revestidos.

Para um líquido puro, se as interações polares (γ^p) e dispersivas (γ^d) são conhecidas, e se θ é o ângulo de contato entre este líquido e um sólido, as interações podem ser descritas em termos de trabalhos reversíveis de adesão, *Wa*, como:

$$W_a = W_a^d + W_a^p \Leftrightarrow W_a = 2 \cdot \left(\sqrt{\gamma_S^d \cdot \gamma_L^d} + \sqrt{\gamma_S^p \cdot \gamma_L^p} \right) \quad (1)$$

Onde γ_S^p e γ_S^d são as contribuições polares e dispersivas da superfície do sólido estudado. Rearranjando a equação (1) temos:

$$\frac{1 + \cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^p} \cdot \sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_S^d} \quad (2)$$

A determinação do ângulo de contato de pelo menos três compostos puros: bromonaftaleno (Merck, Alemanha), formamida (Merck, Alemanha) e água ultra pura, na superfície do fruto combinado com cada valor de componente

dispersivo e polar, permitem o cálculo da variável independete $\left(\sqrt{\frac{\gamma_L^p}{\gamma_L^d}}\right)$ e a variável dependente $\left(\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}}\right)$ da equação 2.

A tensão superficial, o componente dispersivo e o componente polar foram respectivamente: 72,10; 19,90 e 52,20 $mN{\cdot}m^{-1}$ para água; 44,40; 44,40 e 0,00 $mN{\cdot}m^{-1}$ para o bromonaftaleno e 56,90; 23,50 e 33,40 $mN{\cdot}m^{-1}$ para a formamida (BUSSCHER *et al.,* 1984).

A estimativa da tensão superficial crítica (γ_C) foi realizada por extrapolação do método de Zisman (Zisman, 1964). Este método vêm sendo há muito tempo utilizado para caracterizar a capacidade molhante de superfícies de baixa energia. Para obtenção do gráfico de Zisman faz-se necessário o estudo do Cos do ângulo de contato de um líquido puro na superfície do sólido que se pretende estudar bem como a tensão superficial desse líquido em estudo. A interseção das curvas obtidas com o Cos θ = 1 é conhecida como tensão superficial crítica (γ_C). A tensão superficial crítica é um ponto imaginário do valor da tensão da interface sólido-vapor (γ_{sv}) e é frequentemente utilizada para descrever a capacidade molhante de uma superfície. Ela representa o valor da tensão da interface líquido-vapor (γ_{lv}) de um líquido, acima do qual o espalhamento deste líquido na superfície de um sólido é completo. A tensão superficial crítica é definida como:

$$\gamma_C = \lim \gamma_{LV} \quad \text{as } \theta \rightarrow 0 \tag{3}$$

Após a determinação das medidas de tensão superficial crítica, tensão superficial, componentes polares e dispersivos dos frutos, o que se observou é que a tensão superficial entre os frutos é bastante semelhante, contudo existem diferenças entre os componentes polares e dispersivos (Tabela 6). Todos os frutos apresentaram um componente dispersivo com valores mais altos que o componente polar, indicando a habilidade da superfície dos frutos em formarem interações hidrofóbicas. Este fato já havia sido observado por Ribeiro *et al.* (2007)

para morangos, onde os valores do componente dispersivo foram superiores aos do componente polar. Uma superfície com este tipo de característica interage com líquidos primariamente por forças de dispersão, que por sua vez podem influenciar no espalhamento efetivo do revestimento aplicado sobre a superfície do fruto em estudo: a compatibilidade da polaridade (polar ou apolar) da superfície e do revestimento pode desempenhar um papel importante na capacidade molhante de uma superfície por um líquido e pode condicionar a composição deste último.

Neste trabalho, os frutos que apresentaram valores mais altos de interações polares foram a acerola (4,35) e a seriguela (4,59), enquanto a manga (1,47) apresentou os valores mais baixos do componente polar, tendo assim uma menor habilidade na formação de interações polares. Estas diferenças, apesar de serem estatisticamente significativas, não são suficientes para impor um comportamento muito diferente da superfície frente à solução de revestimento. Esta hipótese é confirmada com os resultados obtidos na tabela 7, onde não se percebe diferenças claras entre os valores de *Ws* obtidos para os frutos e revestimentos avaliados neste trabalho.

A tabela 6 mostra que os valores de tensão superficial crítica obtidos para cada frutos variaram entre 9,39 $mN \cdot m^{-1}$ e 23,92 $mN \cdot m^{-1}$. A acerola apresentou os valores mais baixos de tensão superficial crítica, por sua vez o cajá apresentou os valores mais altos. Os valores obtidos neste trabalho estão próximos dos valores de tensão superficial crítica da maçã (18,70 $mN \cdot m^{-1}$) e da laranja (20 $mN \cdot m^{-1}$) obtidos por Choi *et al.* (2002), excetuando-se apenas a acerola e a pitanga que apresentaram valores mais baixos de tensão superficial crítica. Segundo Dann (1970) os valores de tensão superficial crítica devem ser inferiores aos valores de tensão superficial fato que foi confirmado para todos os frutos deste estudo. Para todos os casos podemos concluir que os frutos analisados apresentam superfícies de baixa energia (inferiores a 100 $mN \cdot m^{-1}$) indicando que o métodos de Zisman pode ser aplicado no estudo destes frutos.

Tabela 6 – Valores de tensão superficial crítica, tensão superficial, componente polar e componente dispersivo dos frutos analisados.

Fruta	Tensão Superficial Crítica ($mN \cdot m^{-1}$)	Tensão Superficial ($mN \cdot m^{-1}$)	Componente Polar ($mN \cdot m^{-1}$)	Componente Dispersivo ($mN \cdot m^{-1}$)
Acerola	9,39 ± 0,07 [a]	27,94 ± 0,03 [a]	4,35 ± 0,01 [a]	23,59 ± 0,02 [a]
Cajá	23,92 ± 0,10 [b]	30,15 ± 0,02 [b]	2,29 ± 0,01 [b]	27,86 ± 0,01 [b]
Manga	22,68 ± 0,09 [c]	29,04 ± 0,02 [c]	1,47 ± 0,01 [c]	27,57 ± 0,01 [c]
Pitanga	13,42 ± 0,09 [d]	26,95 ± 0,02 [d]	3,07 ± 0,01 [d]	23,88 ± 0,01 [d]
Seriguela	19,62 ± 0,09 [e]	31,48 ± 0,05 [e]	4,59 ± 0,03 [e]	26,89 ± 0,02 [e]

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

5.1.2 Capacidade Molhante

A capacidade molhante foi estudada por determinação dos valores coeficiente de espalhamento (*Ws*) e dos trabalhos de adesão (*Wa*) e coesão (*Wc*). As forças adesivas promovem o espalhamento do líquido na superfície de um sólido enquanto as forças coesivas promovem a sua contração, impedindo a formação de espaços vazios ao longo da superfície coberta. A boa capacidade de revestir um sólido depende basicamente do balanço entre estas 3 forças. A tensão superficial da solução de revestimento foi mensurada utilizando o método da gota suspensa com aproximação de Laplace-Young (SONG; SPRINGER, 1996).

O ângulo de contato (θ) da gota de um líquido na superfície de um sólido é definido pelo equilíbrio mecânico da gota sobre a ação dessas três tensões interfaciais: sólido-vapor (γ_{sv}), sólido-líquido (γ_{sl}) e líquido-vapor (γ_{lv}), como pode ser visto na figura 14.

Figura 14 - Definição do ângulo de contato θ entre uma gota de um líquido e uma superfície plana e horizontal, mostrando as forças que atuam no sistema.

O valor do coeficiente de espalhamento (*Ws*) pode ser obtido pela equação 4 e seus valores sempre são negativos ou zero:

$$Ws = Wa - Wc = \gamma_{sv} - \gamma_{lv} - \gamma_{sl} \quad (4)$$

Onde *Wa* e *Wc* são os trabalhos de adesão e coesão, definidos nas equações 5 e 6, respectivamente

$$Wa = \gamma_{lv} + \gamma_{sv} - \gamma_{sl} \quad (5)$$

$$Wc = 2\,\gamma_{lv} \quad (6)$$

Como mencionado previamente as determinações de capacidade molhante foram feitas com diferentes concentrações de galactomanana, variando ainda a concentração de plastificante – glicerol (Tabela 4). A capacidade molhante foi estudada através da determinação dos valores do coeficiente de espalhamento (*Ws*). Os coeficientes de espalhamento (*Ws*), das soluções de galactomanana (*A. pavonina* e *C. pulcherrima*) e glicerol aplicadas em cada fruto, foram analisados e estão demonstrados nas Tabelas 7 e 8. Para cada fruto, o melhor (mais alto) valor de *Ws* para a respectiva solução de galactomanana foi determinado (Teste de Tukey, $p<0,05$) e estão destacadas em cinza nas tabelas. Quando não foram observadas diferenças estatisticamente significativas entre as soluções de galactomananas, estas foram consideradas igualmente boas em termos de capacidade molhante e a sua diferenciação foi feita baseada em outros critérios (como a permeabilidade a gases, as quais serão demonstradas posteriormente).

Os resultados obtidos mostraram que os valores de *Ws*, são muito dependentes tanto da fonte como da concentração de galactomanana, e também variam de acordo com o fruto testado. As soluções com concentrações mais baixas de galactomanana de *Adenanthera pavonina* apresentaram melhores (mais altos) valores de *Ws* ($p<0,05$) para os frutos com valores maiores do componente polar (acerola e seriguela). Para galactomanana de *Caesalpinia pulcherrima* os melhores valores de *Ws* para manga (valores de componente polar menores) foram encontrados em uma concentração de 1,5% de galactomanana. Estes resultados mostram que há uma relação direta com a polaridade das soluções aquosas, onde um aumento na concentração de galactomanana reduz a polaridade das soluções tornando-as mais adequadas no revestimento de superfícies apolares (como ocorre com a superfície da manga).

A tabela 7 apresenta os valores de *Ws* obtidos utilizando galactomanana de *Adenanthera pavonina* (AP). No caso das frutas testadas a acerola apresentou melhores valores de *Ws* quando revestida com soluções contendo 0,5% de galactomanana e 1% de glicerol; a seriguela apresentou os melhores valores de *Ws* com a mesma concentração de galactomanana e 1,5% de glicerol. Para manga, pitanga e cajá soluções com 0,5%, 1% e 1,5% de galactomanana não apresentaram diferenças estatisticamente significativas, apresentando bons valores de *Ws* para as concentrações diferentes de galactomanana e glicerol testadas.

Quando a galactomanana de *Caesalpinia pulcherrima* (CP) foi utilizada, os valores de *Ws* (Tabela 8) apresentaram diferenças estatisticamente significativas para cada fruto; sendo selecionadas as formulações que apresentaram melhores valores de *Ws* para cada fruto especificamente. Para manga os melhores valores de *Ws* foram obtidos com a concentração de 1,5% de galactomanana de *C. pulcherrima*, para os demais frutos os melhores valores de *Ws* foram obtidos com soluções contendo 0,5% de galactomanana de *C. pulcherrima.*

Os melhores revestimentos de *A. pavonina* e *C. pulcherrima* em termos de capacidade molhante (representado pelo coeficiente de espalhamento – *Ws*) foram subseqüentemente analisados para permeabilidade ao vapor de água, permeabilidade ao oxigênio e ao dióxido de carbono.

Tabela 7 – Coeficiente de Espalhamento *(Ws)* obtido para soluções de galactomanana de *Adenanthera pavonina* e glicerol nos frutos analisados.

Galactomanana % (m/v)	Glicerol % (v/v)	Acerola	Cajá	Manga	Pitanga	Seriguela
0,5	1,0	-29,92 ± 2,10 ^{a}	-36,50 ± 3,05 ^{a}	-30,97 ± 2,17 ^{ade}	-28,17 ± 7,27 ^{a}	-29,15 ± 2,78 ^{a}
0,5	1,5	-36,35 ± 3,95 ^{b}	-35,32 ± 3,74 ^{a}	-31,40 ± 2,97 ^{ade}	-31,71 ± 6,11 ^{a}	-23,72 ± 2,01 ^{b}
0,5	2,0	-42,38 ± 3,58 ^{c}	-27,84 ± 2,89 ^{b}	-37,91 ± 3,80 ^{b}	-39,13 ± 7,15 ^{bcd}	-28,95 ± 3,74 ^{a}
1,0	1,0	-42,11 ± 3,03 ^{c}	-30,80 ± 2,96 ^{b}	-34,37 ± 2,43 ^{abc}	-38,53 ± 3,91 ^{bcd}	-31,11 ± 2,79 ^{ad}
1,0	1,5	-46,71 ± 2,91 ^{d}	-36,96 ± 4,37 ^{a}	-30,93 ± 3,62 ^{ade}	-38,18 ± 3,78 ^{bcd}	-37,46 ± 2,65 ^{c}
1,0	2,0	-47,09 ± 4,54 ^{d}	-32,00 ± 3,44 ^{c}	-35,39 ± 1,75 ^{bc}	-44,22 ± 6,98 ^{c}	-36,86 ± 3,15 ^{c}
1,5	1,0	-41,57 ± 5,04 ^{c}	-32,85 ± 2,67 ^{c}	-29,18 ± 3,57 ^{e}	-26,45 ± 4,58 ^{a}	-32,35 ± 2,96 ^{d}
1,5	1,5	-41,06± 3,04 ^{c}	-31,24 ± 2,91 ^{bc}	-33,71 ± 2,95 ^{acd}	-32,75 ± 3,27 ^{ca}	-32,54 ± 3,70 ^{d}
1,5	2,0	-42,68 ± 1,41 ^{c}	-31,54 ± 3,21 ^{bc}	-30,38 ± 2,39 ^{ade}	-35,15 ± 2,68 ^{cd}	-37,90 ± 2,26 ^{c}

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

Tabela 8 – Coeficiente de Espalhamento *(Ws)* obtido para soluções de galactomanana de *Caesalpinia pulcherrima* e glicerol nos frutos analisados.

Galactomanana %(m/v)	Glicerol % (v/v)	Acerola	Cajá	Manga	Pitanga	Seriguela
0,5	1,0	-42,68 ± 6,50 ^{a}	-27,69 ± 3,73 ^{a}	-51,29 ± 4,14 ^{a}	-40,76 ± 4,61 ^{a}	-40,57 ± 3,10 ^{a}
0,5	1,5	-39,83 ± 5,95 ^{a}	-31,85 ± 2,37 ^{b}	-65,48 ± 4,57 ^{b}	-35,54 ± 5,39 ^{b}	-36,33 ± 3,39 ^{b}
0,5	2,0	-32,59 ± 4,65 ^{b}	-38,86 ± 5,14 ^{ce}	-49,73 ± 6,09 ^{a}	-39,70 ± 3,71 ^{a}	-40,66 ± 2,82 ^{a}
1,0	1,0	-44,70 ± 4,42 ^{a}	-45,34 ± 4,46 ^{d}	-68,82 ± 6,38 ^{c}	-47,91 ± 6,25 ^{c}	-45,27 ± 3,17 ^{c}
1,0	1,5	-43,47 ± 3,37 ^{a}	-45,08 ± 3,54 ^{d}	-77,83 ± 5,87 ^{d}	-50,01 ± 4,73 ^{c}	-51,16 ± 3,82 ^{d}
1,0	2,0	-41,36 ± 3,32 ^{a}	-47,48 ± 4,31 ^{d}	-66,24 ± 7,72 ^{ec}	-57,02 ± 2,86 ^{d}	-49,87 ± 3,51 ^{d}
1,5	1,0	-42,38 ± 6,32 ^{a}	-40,06 ± 6,04 ^{ce}	-64,36 ± 7,84 ^{ec}	-41,88 ± 4,30 ^{a}	-41,44 ± 4,72 ^{a}
1,5	1,5	-40,60 ± 3,77 ^{a}	-37,55 ± 2,59 ^{c}	-62,81 ± 4,26 ^{e}	-43,01 ± 5,46 ^{a}	-42,29 ± 3,37 ^{a}
1,5	2,0	-58,65 ± 5,65 ^{c}	-43,58 ± 3,72 ^{ced}	-45,20 ± 4,49 ^{f}	-58,83 ± 5,31 ^{d}	-47,81 ± 3,59 ^{d}

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

5.1.3 Permeabilidade ao vapor de água ($P\ H_2O$)

A permeabilidade ao vapor de água é a propriedade mais exaustivamente estudada dos filmes comestíveis, isto se deve principalmente ao fato da água estar envolvida em boa parte das reações degradativas. A tabela 9 e a figura 15 mostram que os valores de permeabilidade ao vapor de água mudam com o uso de diferentes concentrações de glicerol nas soluções de galactomanana. Quando há um aumento na concentração de glicerol ocorre também um aumento na permeabilidade ao vapor de água do filme em estudo. Estas diferenças mostraram-se estatisticamente significativas quando a concentração de plastificante (glicerol) foi aumentada de 1% para 2%.

Gontard *et al.* (1993) explicaram que o efeito do glicerol pode ser atribuído as propriedades hidrofílicas deste composto, as quais favorecem a adsorção de moléculas de água. O glicerol, através da sua ação como plastificante, consegue modificar a rede polimérica criando regiões móveis com grandes espaços intercadeias, promovendo a formação de agregados de moléculas de água por competição com as moléculas de água presentes no sítio ativo da matriz polimérica e a formação de microcavidades na estrutura da rede polimérica (DIAB *et al.*, 2001).

O aumento na permeabilidade ao vapor de água em filmes comestíveis relacionado com o aumento da concentração de plastificante tem sido descrito por outros autores: filmes de amido (MALI *et al.,* 2006), filmes de pululana (DIAB *et al.*, 2001), filmes de glúten de trigo (GONTARD *et al.*, 1993; CHERIAN *et al.*, 1995) e blendas de caseinato de sódio/amido (ARVANITOYANNIS, BILIADERIS, 1998).

Quando a concentração de galactomanana de *A. pavonina* ou *C. pulcherrima* é aumentada, e a concentração de glicerol é a mesma, o que se observa é um decréscimo na permeabilidade ao vapor de água, possivelmente devido a formação de uma rede polimérica mais coesa e mais forte, onde as moléculas de polissacarídeo se encontram mais próximas, deixando um menor espaço livre para que haja migração de moléculas de água. Estas mudanças mostraram-se estatisticamente significativas quando o aumento na concentração de galactomanana foi de 0,5% para 1,5% (tabela 9 e Figura 15).

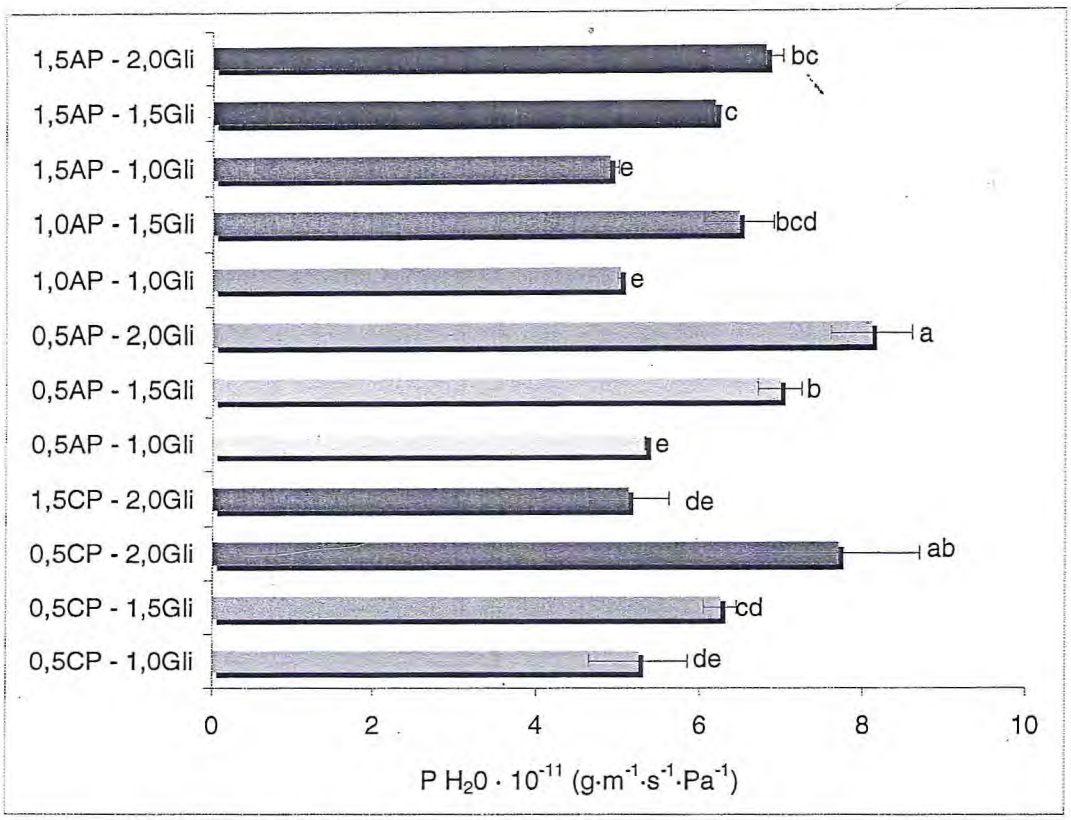
As formulações que apresentaram menor permeabilidade ao vapor de água foram: 0,5% de galactomanana de *C.pulcherrima* – 1% de glicerol; 1,5% de galactomanana de *C.pulcherrima* – 2% de glicerol; 0,5% de galactomanana de *A.pavonina* – 1% de glicerol; 1% de galactomanana de *A.pavonina* – 1% de glicerol e 1,5% de galactomanana de *A.pavonina* – 1% de glicerol.

Os valores de permeabilidade ao vapor de água obtidos para os filmes de galactomanana estão em concordância com valores obtidos para outros filmes de galactomanana e polissacarídeos previamente estudados. Aydinli e Tutas (2000) obtiveram valores de permeabilidade ao vapor de água variando entre 3,2 e 1,8 (10^{-11}. g.m $^{-1}$. s^{-1}. Pa^{-1}) para filmes de goma de "locust bean" (≈1% m/v) com polietilenoglicol (≈0,4% e 1,7% v/v). Garcia *et al.* (2006) obtiveram para filmes de amido de milho (5%) com glicerol (1,4%) valores de permeabilidade ao vapor de água de 8,7 (10^{-11}. g.m $^{-1}$. s^{-1}. Pa^{-1}).

Tabela 9 – Permeabilidade ao vapor de água dos filmes de galactomanana de *A. pavonina* (AP) e *C. pulcherima* (CP).

Filmes	$P\ H_2O \cdot 10^{-11}$ ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	Espessura $\cdot 10^{-4}$ (m)
0,5 CP - 1,0 Gli	5,25 ± 0,60 ^{de}	0,028 ± 0,004 ^{a}
0,5 CP - 1,5 Gli	6,25 ± 0,20 ^{cd}	0,030 ± 0,002 ^{ab}
0,5 CP - 2,0 Gli	7,70 ± 1,00 ^{ab}	0,032 ± 0,003 ^{ab}
1,5 CP - 2,0 Gli	5,12 ± 0,50 ^{de}	0,035 ± 0,005 ^{abc}
0,5 AP - 1,0 Gli	5,33 ± 0,01 ^{e}	0,030 ± 0,001 ^{a}
0,5 AP - 1,5 Gli	6,98 ± 0,27 ^{b}	0,031 ± 0,003 ^{ab}
0,5 AP - 2,0 Gli	8,10 ± 0,50 ^{a}	0,032 ± 0,003 ^{ab}
1,0 AP - 1,0 Gli	5,02 ± 0,03 ^{e}	0,035 ± 0,002 ^{bc}
1,0 AP - 1,5 Gli	6,47 ± 0,43 ^{bcd}	0,034 ± 0,002 ^{b}
1,5 AP - 1,0 Gli	4,89 ± 0,11 ^{e}	0,036 ± 0,001 ^{c}
1,5 AP - 1,5 Gli	6,18 ± 0,08 ^{c}	0,038 ± 0,003 ^{c}
1,5 AP - 2,0 Gli	6,81 ± 0,21 ^{bc}	0,037 ± 0,001 ^{c}

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).



*Letras diferentes indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

Figura 15 - Permeabilidade ao vapor de água para diferentes filmes de galactomanana de *A. pavonina* e *C.pulcherrima* (n=10, 95% de intervalo de confiança).

5.1.4 Permeabilidade ao Oxigênio (*P O_2*)

O oxigênio desempenha um papel fundamental nos processos oxidativos de alimentos, os quais são responsáveis por modificações no odor, cor, sabor e deterioração destes alimentos. Portanto, filmes que sejam capazes de fornecer uma barreira ao oxigênio podem ajudar na melhoria da qualidade deste alimento e aumentando seu tempo de prateleira. A tabela 10 e a figura 16 apresentam as permeabilidades ao oxigênio dos filmes de *A. pavonina* e *C. pulcherrima* que foram selecionados para serem testados como revestimentos para frutos.

Alguns autores já demonstraram anteriormente que um aumento na concentração de galactomanana contribui para um decréscimo na permeabilidade, enquanto é também de senso comum que concentrações mais elevadas de glicerol aumentam a permeabilidade do revestimento ao oxigênio (CANER et al., 1998; KESTER; FENNEMA, 1986). Como descrito anteriormente, comportamento semelhante também é observado quando se analisa a permeabilidade ao vapor de água.

Em geral, as amostram com concentrações mais elevadas de plastificante apresentaram valores mais elevados de permeabilidade ao oxigênio que as amostras que continham proporções mais baixas de glicerol. Estes resultados podem ser explicados pela natureza apolar do oxigênio que não interage com as propriedades polares da molécula de glicerol, aumentando a permeabilidade do filme ao oxigênio. Resultados semelhantes foram obtidos por Caner *et al.* (1998) e Kester e Fennema (1986) onde foi proposto que o plastificante reduz as forças de atração intermolecularares entre as cadeias do polímero, facilitando a penetração das moléculas de gás.

Com os dados obtidos no presente trabalho o que podemos observar é que o efeito da fonte de galactomanana sobre a permeabilidade ao oxigênio mostrou-se mais influente que o efeito provocado pela variação da concentração de glicerol. Podemos observar que filmes com concentrações semelhantes de galactomanana e glicerol, apresentaram diferenças estatisticamente significativas para a *P O_2* quando a fonte de galactomanana era diferente (*A. pavonina* ou *C.*

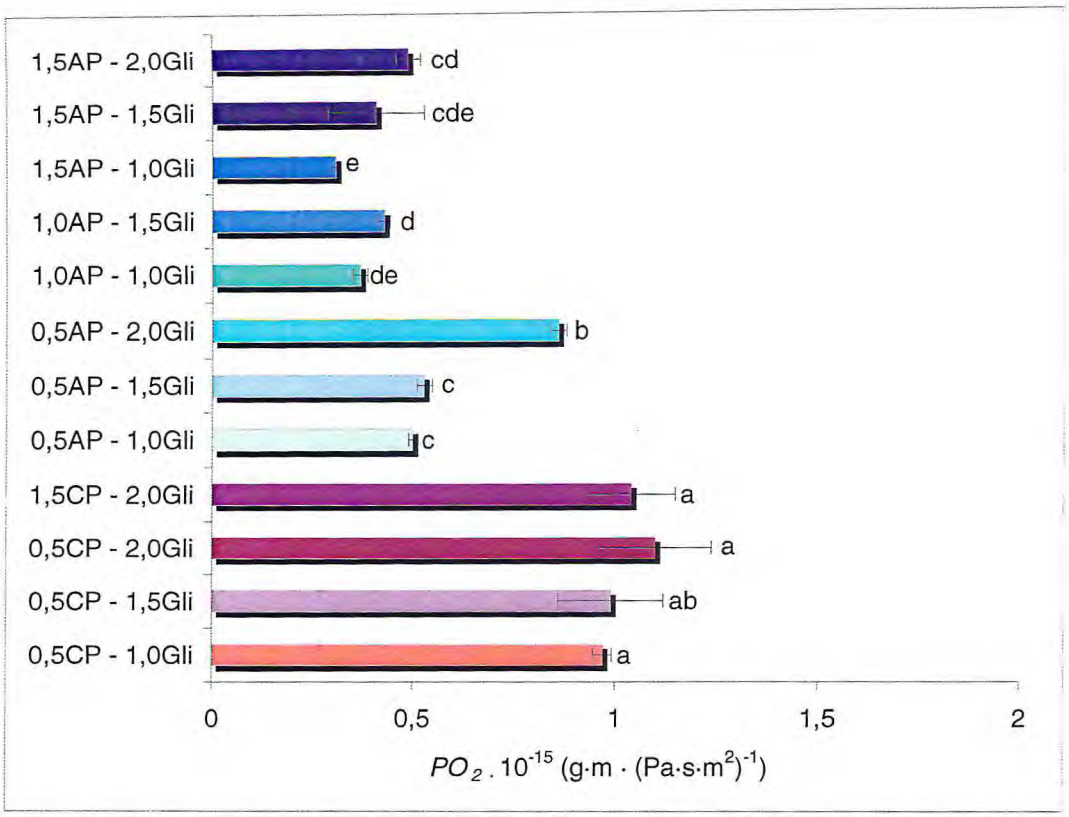
pulcherima). Estas diferenças podem ser explicadas pelo fato de que galactomananas de fontes diferentes apresentam uma constituição diferente na sua cadeia polimérica. A galactomanana de *A. pavonina* apresenta uma razão manose/galactose de 1,35 enquanto a galactomanana de *C. pulcherrima* é menos substituída apresentando uma razão manose/galactose de 2,88 (CERQUEIRA *et al.*,2009).

Os valores mais baixos de permeabilidade ao oxigênio foram obtidos para estas três formulações: 1% de galactomanana de *A. pavonina* – 1% de glicerol; 1,5% de galactomanana de *A. pavonina* – 1% de glicerol; 1,5% de galactomanana de *A. pavonina* – 1,5% de glicerol.

Tabela 10 – Permeabilidade ao oxigênio (*P* O_2) dos filmes de galactomanana de *A. pavonina* (AP) e *C. pulcherima* (CP).

Filmes	*P* O_2 $\cdot 10^{-15}$ (g·m · (Pa·s·$m^{2)-1}$)	Espessura $\cdot 10^{-4}$ (m)
0,5 CP - 1,0 Gli	0,97 ± 0,02 ^{a}	0,028 ± 0,004 ^{a}
0,5 CP - 1,5 Gli	0,99 ± 0,13 ^{ab}	0,030 ± 0,002 ^{ab}
0,5 CP - 2,0 Gli	1,10 ± 0,14 ^{a}	0,032 ± 0,003 ^{ab}
1,5 CP - 2,0 Gli	1,04 ± 0,11 ^{a}	0,035 ± 0,005 ^{abc}
0,5 AP - 1,0 Gli	0,50 ± 0,01 ^{c}	0,030 ± 0,001 ^{a}
0,5 AP - 1,5 Gli	0,53 ± 0,02 ^{c}	0,031 ± 0,003 ^{ab}
0,5 AP - 2,0 Gli	0,86 ± 0,02 ^{b}	0,032 ± 0,003 ^{ab}
1,0 AP - 1,0 Gli	0,37 ± 0,02 ^{de}	0,035 ± 0,002 ^{bc}
1,0 AP - 1,5 Gli	0,43 ± 0,02 ^{d}	0,034 ± 0,002 ^{b}
1,5 AP - 1,0 Gli	0,31 ± 0,01 ^{e}	0,036 ± 0,001 ^{c}
1,5 AP - 1,5 Gli	0,41 ± 0,12 ^{cde}	0,038 ± 0,003 ^{c}
1,5 AP - 2,0 Gli	0,49 ± 0,03 ^{cd}	0,037 ± 0,001 ^{c}

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).



*Letras diferentes indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

Figura 16 - Permeabilidade ao oxigênio para diferentes filmes de galactomanana de *A. pavonina* e *C.pulcherrima* (n=10, 95% de intervalo de confiança).

5.1.5 Permeabilidade ao Dióxido de Carbono (*P CO_2*)

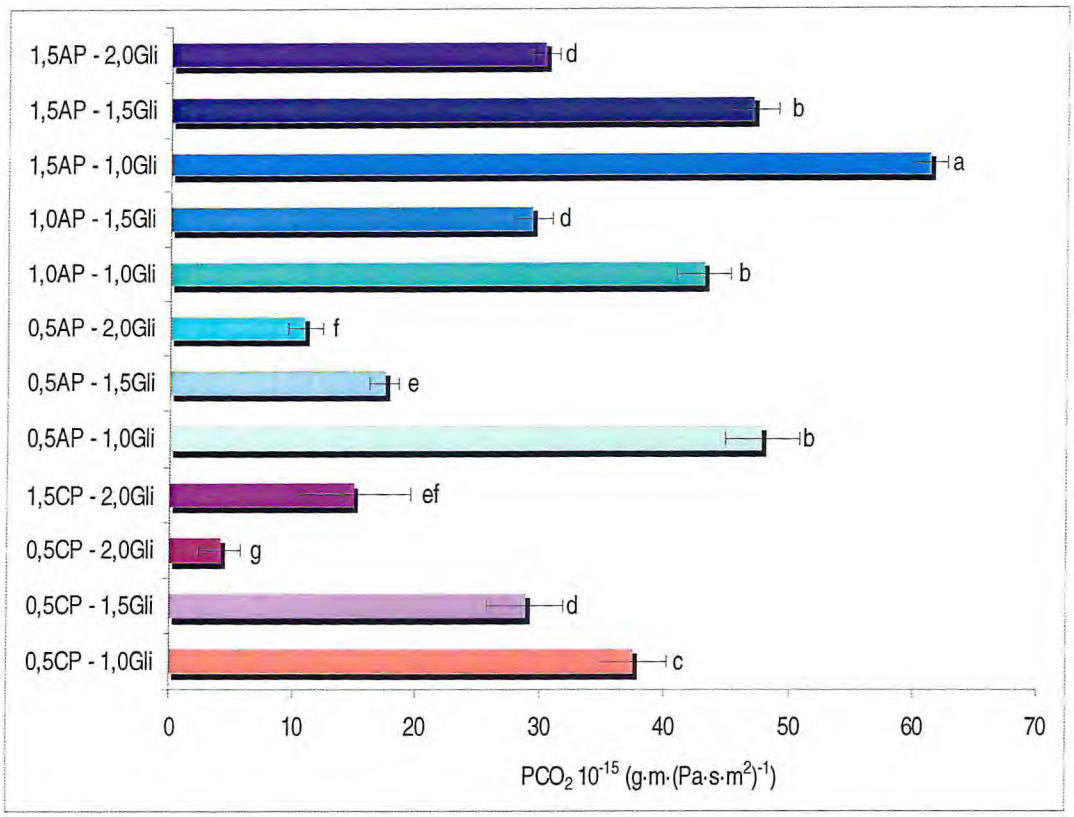
Em 1993 Ke *et al.* demonstraram que atmosferas controladas com autos teores de CO_2 e baixas concentrações de O_2 tinham efeitos benéficos na pós-colheita de morangos. Também foi observado que concentrações elevadas de dióxido de carbono exerciam influência na cor do morango e no metabolismo das antocianinas (GIL *et al,*1997). Concentrações elevadas de CO_2 retardam o apodrecimento de frutos além de manter sua firmeza por um maior período (HOLCROFT; KADER, 1999).

A tabela 11 e a figura 17 mostram os valores de permeabilidade ao dióxido de carbono para os filmes testados de galactomanana de *A.pavonina* e *C.pulcherrima.* Os resultados obtidos indicaram que soluções com concentrações mais elevadas de plastificante produziram filmes com valores mais baixos de permeabilidade ao CO_2. O Efeito do glicerol na permeabilidade ao dióxido de carbono, foi aquele que se sobressaiu em relação as outras variáveis avaliadas no experimento. O efeito do glicerol na permeabilidade ao CO_2 é exatamente contrário ao que é observado com relação à permeabilidade ao vapor de água e a permeabilidade ao O_2, onde filmes que apresentam valores de permeabilidade ao O_2 mais baixos são os que apresentam valores mais altos de permeabilidade ao CO_2. Quando a concentração de glicerol é aumentada à permeabilidade ao vapor de água e ao O_2 aumentam e há um decréscimo na permeabilidade ao CO_2 provavelmente como um resultado das características polares da molécula de glicerol e de sua capacidade de formar pontes de hidrogênio. Os valores mais altos de permeabilidade ao CO_2 foram obtidos para a formulação de 1,5% de galactomanana de *A. pavonina* – 1% de glicerol.

Tabela 11 – Permeabilidade ao dióxido de carbono (*P* CO_2) dos filmes de galactomanana de *A. pavonina* (AP) e *C. pulcherima* (CP).

Filmes	*P* CO_2 $\cdot 10^{-15}$ (g·m · (Pa·s·$m^{2)-1}$)	Espessura $\cdot 10^{-4}$ (m)
0,5 CP - 1,0 Gli	37,57 ± 2,75 ^{c}	0,028 ± 0,004 ^{a}
0,5 CP - 1,5 Gli	28,81 ± 3,08 ^{d}	0,030 ± 0,002 ^{ab}
0,5 CP - 2,0 Gli	4,10 ± 1,67 ^{g}	0,032 ± 0,003 ^{ab}
1,5 CP - 2,0 Gli	14,95 ± 4,57 ^{ef}	0,035 ± 0,005 ^{abc}
0,5 AP - 1,0 Gli	47,85 ± 3,00 ^{b}	0,030 ± 0,001 ^{a}
0,5 AP - 1,5 Gli	17,40 ± 1,21 ^{e}	0,031 ± 0,003 ^{ab}
0,5 AP - 2,0 Gli	10,94 ± 1,44 ^{f}	0,032 ± 0,003 ^{ab}
1,0 AP - 1,0 Gli	43,13 ± 2,21 ^{b}	0,035 ± 0,002 ^{bc}
1,0 AP - 1,5 Gli	29,29 ± 1,51 ^{d}	0,034 ± 0,002 ^{b}
1,5 AP - 1,0 Gli	61,19 ± 1,44 ^{a}	0,036 ± 0,001 ^{c}
1,5 AP - 1,5 Gli	47,08 ± 1,94 ^{b}	0,038 ± 0,003 ^{c}
1,5 AP - 2,0 Gli	30,26 ± 1,14 ^{d}	0,037 ± 0,001 ^{c}

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).



*Letras diferentes indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

Figura 17 - Permeabilidade ao dióxido de carbono para diferentes filmes de galactomanana de *A. pavonina* e *C.pulcherrima* (n=10, 95% de intervalo de confiança).

Tendo por base os critérios - valores mais altos de capacidade molhante, valores mais baixos de permeabilidade ao vapor de água, valores mais baixos de permeabilidade ao oxigênio e valores mais baixos de permeabilidade ao dióxido de carbono – as seguintes formulações de filmes mostraram-se mais adequadas para cada fruto: acerola – 0,5% de galactomanana de *A. pavonina* e 1% de glicerol; cajá – 1% de galactomanana de *A. pavonina* e 1% de glicerol; manga e pitanga – 1,5% de galactomanana de *A. pavonina* e 1% de glicerol e seriguela 0,5%de galactomanana de *C. pulcherrima* e 1,5% de glicerol (Tabela 12).

Tabela 12 – Valores de permeabilidade ao vapor de água, permeabilidade ao dióxido de carbono (*P* CO_2), Permeabilidade ao oxigênio (*P* O_2) e espessura dos filmes de galactomanana de *A. pavonina* (AP) e *C. pulcherrima* (CP).

Filmes	$PH_2O \cdot 10^{-11}$ ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	$P\,O_2 \cdot 10^{-15}$ ($g \cdot m \cdot (Pa \cdot s \cdot m^2)^{-1}$)	$P\,CO_2 \cdot 10^{-15}$ ($g \cdot m \cdot (Pa \cdot s \cdot m^2)^{-1}$)	Espessura $\cdot 10^{-4}$ (m)
0,5 CP - 1,0 Gli	5,25 ± 0,60 ^{de}	0,97 ± 0,02 ^{a}	37,57 ± 2,75 ^{c}	0,028 ± 0,004 ^{a}
0,5 CP - 1,5 Gli	6,25 ± 0,20 ^{cd}	0,99 ± 0,13 ^{ab}	28,81 ± 3,08 ^{d}	0,030 ± 0,002 ^{ab}
0,5 CP - 2,0 Gli	7,70 ± 1,00 ^{ab}	1,10 ± 0,14 ^{a}	4,10 ± 1,67 ^{g}	0,032 ± 0,003 ^{ab}
1,5 CP - 2,0 Gli	5,12 ± 0,50 ^{de}	1,04 ± 0,11 ^{a}	14,95 ± 4,57 ^{ef}	0,035 ± 0,005 ^{abc}
0,5 AP - 1,0 Gli	5,33 ± 0,01 ^{e}	0,50 ± 0,01 ^{c}	47,85 ± 3,00 ^{b}	0,030 ± 0,001 ^{a}
0,5 AP - 1,5 Gli	6,98 ± 0,27 ^{b}	0,53 ± 0,02 ^{c}	17,40 ± 1,21 ^{e}	0,031 ± 0,003 ^{ab}
0,5 AP - 2,0 Gli	8,10 ± 0,50 ^{a}	0,86 ± 0,02 ^{b}	10,94 ± 1,44 ^{f}	0,032 ± 0,003 ^{ab}
1,0 AP - 1,0 Gli	5,02 ± 0,03 ^{e}	0,37 ± 0,02 ^{de}	43,13 ± 2,21 ^{b}	0,035 ± 0,002 ^{bc}
1,0 AP - 1,5 Gli	6,47 ± 0,43 ^{bcd}	0,43 ± 0,02 ^{d}	29,29 ± 1,51 ^{d}	0,034 ± 0,002 ^{b}
1,5 AP - 1,0 Gli	4,89 ± 0,11 ^{e}	0,31 ± 0,01 ^{e}	61,19 ± 1,44 ^{a}	0,036 ± 0,001 ^{c}
1,5 AP - 1,5 Gli	6,18 ± 0,08 ^{c}	0,41 ± 0,12 ^{cde}	47,08 ± 1,94 ^{b}	0,038 ± 0,003 ^{c}
1,5 AP - 2,0 Gli	6,81 ± 0,21 ^{bc}	0,49 ± 0,03 ^{cd}	30,26 ± 1,14 ^{d}	0,037 ± 0,001 ^{c}

*Valores indicam a média ± desvio padrão (n=10, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

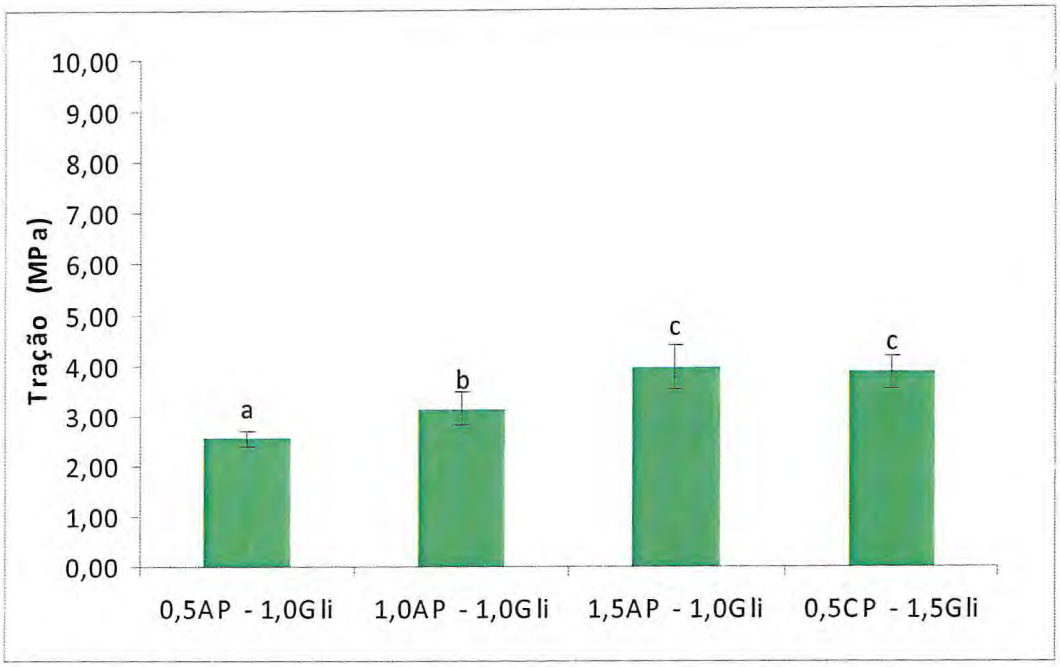
5.1.6 Propriedades Mecânicas

Os valores da *TS* (tração) e *E* (elasticidade até ruptura) foram mensurados para os filmes selecionados para cada fruto (correspondendo as células destacadas em cinza na (Tabela 12). A Figura 18 mostra que os valores de *TS* aumentam com um acréscimo nas concentrações de galactomanana dos filmes de *A. pavonina.* Quando comparamos os valores de *TS* entre os filmes de *A. pavonina e C. pulcherrima*, conforme demonstrado na Figura 18, observamos que filmes de *C. pulcherrima* com uma concentração de 0,5% de galactomanana e 1,5% de glicerol não apresentam diferenças estatisticamente significativas ($p<0,05$) quando comparados com filmes de *A. pavonina* com concentrações de 1,5% de galactomanana e 1% de glicerol. Este fato pode ser explicado devido a um menor grau de substituição da cadeia principal de manana da galactomanana de *C. pulcherrima* (razão manose/galactose 2,88) se sobrepondo a galactomanana de *A. pavonina* (razão manose/galactose 1,35). Por ser menos ramificada a galactomanana de *Caesalpinia* é mais propensa a formar pontes intercadeias capazes de prover uma maior estabilidade ao filme a ser formado. Por isso valores de *TS* semelhantes são observados mesmo quando a concentração de galactomanana de *Caesalpinia* é inferior a concentração da galactomanana de *A. pavonina.* Em 2007 Mikkonen *et al.* obtiveram resultados semelhantes quando compararam o *TS* de filmes da goma de "locust bean" (razão manose/galactose 3,5) e filmes de goma de guar (razão manose/galactose 1,5).

Com relação aos valores de *E* (elasticidade até ruptura) o que se pôde observar é que os filmes de *C. pulcherrima* apresentaram valores mais altos de *E* (Figura 19), o que pode ser explicado por um maior conteúdo de glicerol, mas também pela maior flexibilidade de estruturas menos substituídas (galactomanana de *C. pulcherrima*) quando comparada com estruturas mais substituídas (galactomanana de *A. pavonina*). Contudo com os dados obtidos neste trabalho ainda não podemos concluir qual destes dois fatores exerce uma maior influência

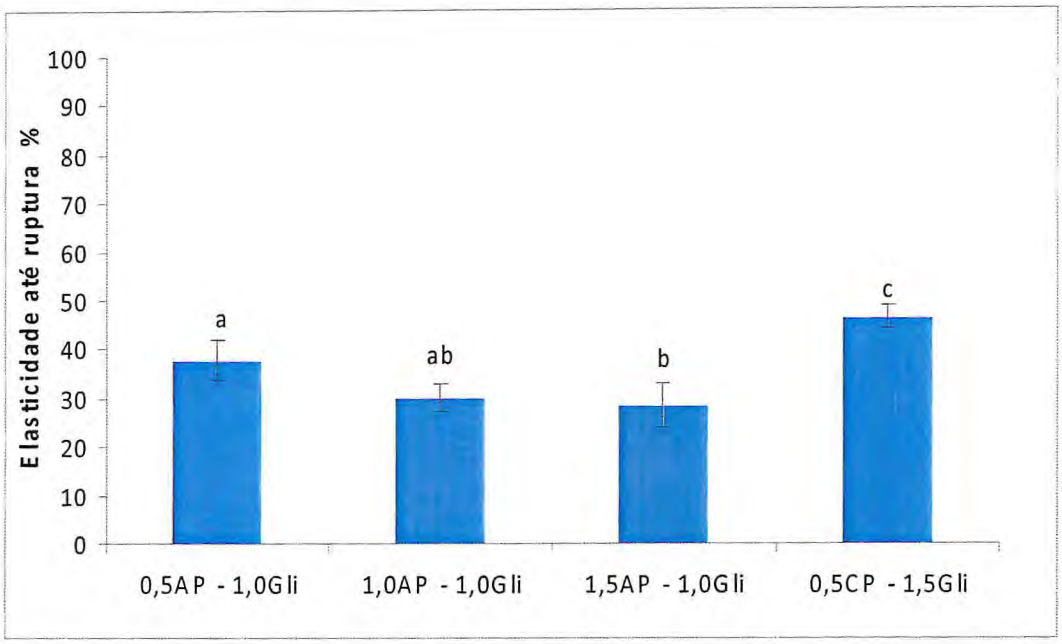
nos valores de *E.* Resultados semelhantes foram relatados por Mikkonen *et al.*(2007), onde mostraram que filmes de goma de "locust bean" são mais felxíveis que filmes de goma de guar.

Os valores obtidos estão de acordo com os valores observados em outros estudos que abordaram filmes de polissacarídeos. Srinivasa *et al.* (2007) mostraram que filmes de quitosana e glicerol apresentavam valores de 14,14 MPa e 34% de *TS* e *E* respectivamente. Em 2006, Garcia *et al.* mostraram que filmes com diferentes proporções de amido de milho, quitosana e glicerol apresentavam valores de *TS* variando entre 7,1 MPa a 60,7 MPa e valores de *E* variando entre 3% a 22,5%, onde o filme apresentando amido de milho e glicerol tinha valores bem próximos aos valores obtidos para as galactomanana de *A. pavonina* e *C. pulcherrima.*



*Valores indicam a média ± desvio padrão (n=3, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

Figura 18 – Valores de *TS* (tração) para os filmes selecionados de *A. pavonina* (0,5% galactomanana - 1% glicerol; 1% galactomanana - 1% glicerol; 1,5% galactomanana – 1% glicerol) e *C. pulcherrima* (0,5% galactomanana – 1,5% glicerol)



*Valores indicam a média ± desvio padrão (n=3, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

Figura 19 – Valores de *E* (elasticidade até ruptura) para os filmes selecionados de *A. pavonina* (0,5% galactomanana - 1% glicerol; 1% galactomanana - 1% glicerol; 1,5% galactomanana – 1% glicerol) e *C. pulcherrima* (0,5% galactomanana – 1,5% glicerol).

5.2 Revestimentos de blendas de Galactomanana – Colágeno – Glicerol

Com o intuito de desenvolver filmes com diferentes características no que concerne as propriedades de capacidade molhante, permeabilidades, e propriedades mecânicas, foram estudadas soluções filmogênicas formadas a partir de blendas de galactomanana, colágeno e glicerol.

5.2.1 Tensão superficial e Tensão superficial crítica da superfície dos frutos

No estudo de blendas de galactomanana – colágeno - glicerol, foram utilizados como modelo experimental para serem revestidos frutos de manga e maçã. A tensão superficial dos frutos foi avaliada em seus 2 componentes: componente polar e componente dispersivo. Os valores estimados do componente polar e do componente dispersivo da tensão superficial foram de 1,71 e 24,77 mN . m^{-1} para manga e 0,68 e 27,13 mN . m^{-1} para maçã. As tensões superficiais da maçã e da manga são a soma destes dois componentes (26,48 e 27,81 mN . m^{-1}, respectivamente). Os dois frutos possuem superfícies de baixa energia (inferior a 100 mN . m^{-1}), sendo aplicável o método de Zisman, no cálculo da tensão superficial crítica.

Os valores obtidos neste trabalho estão de acordo com os valores obtidos para outros frutos e hortaliças em trabalhos anteriores (Tabela13):

Tabela 13 – Tensão superficial crítica de alguns frutos e hortaliças.

Fruto	Tensão Superficial Crítica ($mN \cdot m^{-1}$)	Temperatura (°C)	Referência
Manga	19,5	20	Este trabalho
Maçã	25,4	20	Este trabalho
Morango	18,8	19	Ribeiro et al., 2007
Alho	18,3	23	Hershko and Nussinovitch, 1998
Laranja	23,0	25	Hagenmaier and Baker, 1993
Grapefruit	23,0	25	Hagenmaier and Baker, 1993
Tomate	17,4	20	Casariego et al. 2008
Cenoura	24,1	20	Casariego et al. 2008

5.2.2 Capacidade Molhante

A otimização de soluções de revestimento pode ser feita considerando 3 parâmetros: a capacidade molhante, os coeficientes de adesão e de coesão. O controle dos coeficientes de adesão e coesão é muito importante porque o primeiro promove o espalhamento da solução sobre a superfície a ser revestida e o segundo promove a sua contração, impedindo a formação de lacunas (RIBEIRO *et al.,* 2007). As análises de capacidade molhante foram feitas com diferentes concentrações de galactomanana (0,5%; 1% e 1,5%), com diferentes concentrações de colágeno (0,5%; 1% e 1,5%) e variando a concentração de glicerol (0;0,5%; 1% e 1,5%), como demonstrado na tabela 5. Neste estudo a capacidade molhante foi analisada pela determinação dos valores do coeficiente de espalhamento (*Ws*).

A Figura 20 mostra a variação do coeficiente de espalhamento dos revestimentos em manga *versus* concentração de glicerol, para diferentes proporções de galactomanana de *C.pulcherrima* – colágeno.

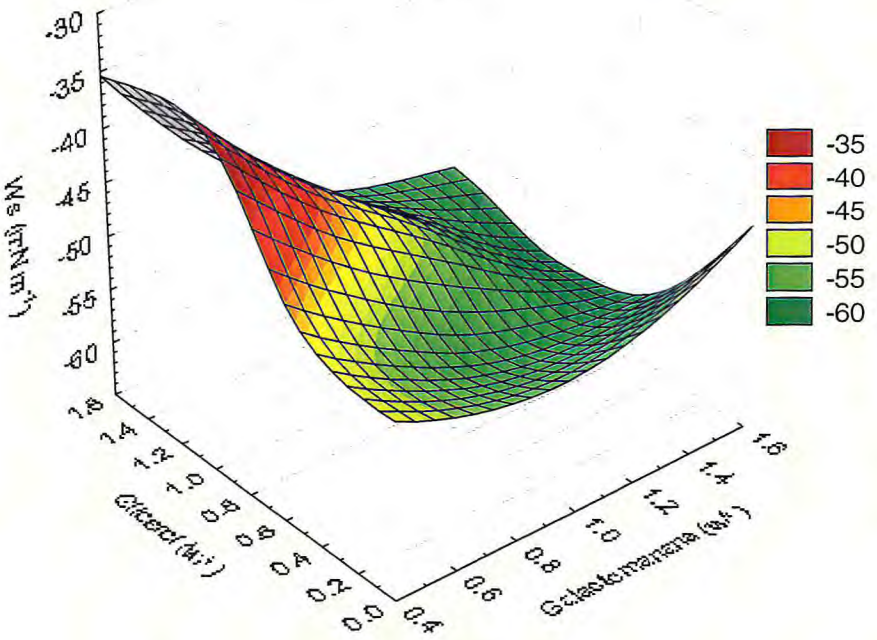


Figura 20 - Valores do coeficiente de espalhamento (*Ws*) para diferentes concentrações de galactomanana de *C. pulcherrima* – colágeno e glicerol em superfícies de manga.

O mesmo experimento foi repetido em manga usando desta vez a galactomanana de *A. pavonina* no lugar da galactomanana de *C. pulcherrima*. Os resultados estão demonstrados na figura 21.

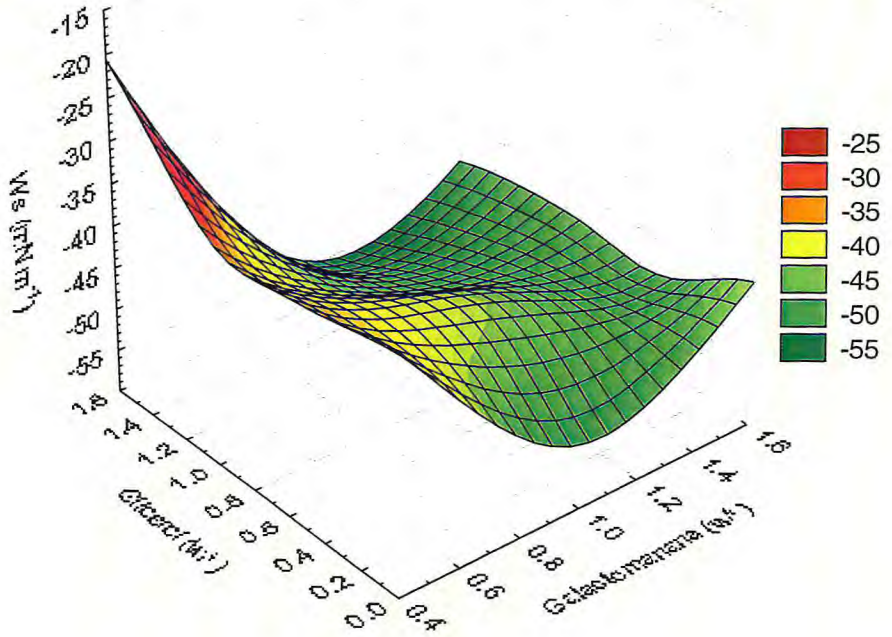


Figura 21 - Valores do coeficiente de espalhamento (*Ws*) para diferentes concentrações de galactomanana de *A.pavonina* – colágeno e glicerol em superfícies de manga.

A figura 22 representa a variação do coeficiente de espalhamento de revestimentos em maçã *versus* concentração de glicerol, para diferentes proporções de galactomanana de *C.pulcherrima* – colágeno.

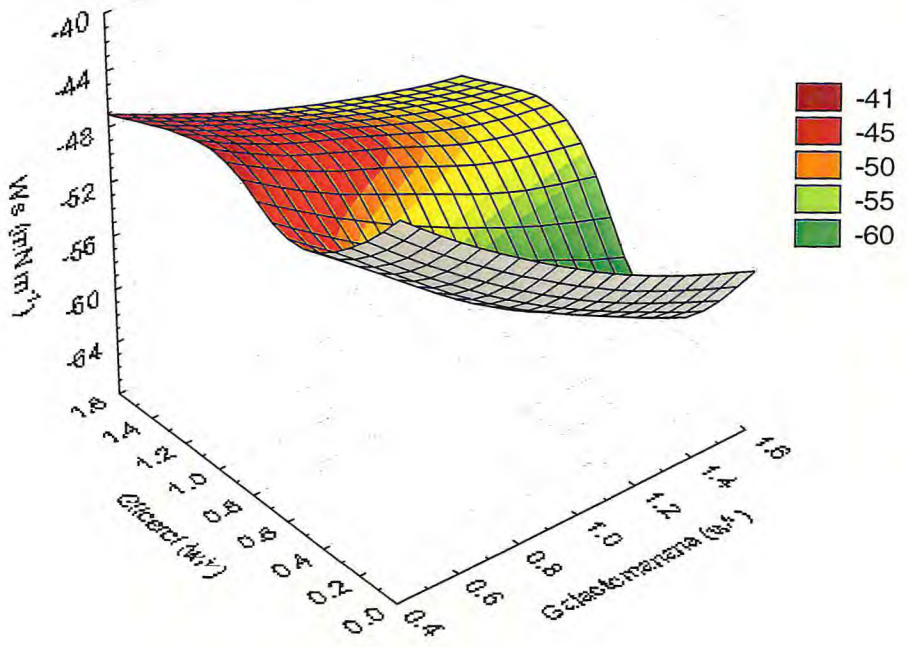


Figura 22 - Valores do coeficiente de espalhamento (*Ws*) para diferentes concentrações de galactomanana de *C. pulcherrima* – colágeno e glicerol em superfícies de maçã.

A figura 23 representa a variação do *coeficiente de espalhamento* de revestimentos em maçã *versus* concentração de glicerol, para diferentes proporções de galactomanana de *A.pavonina* – colágeno.

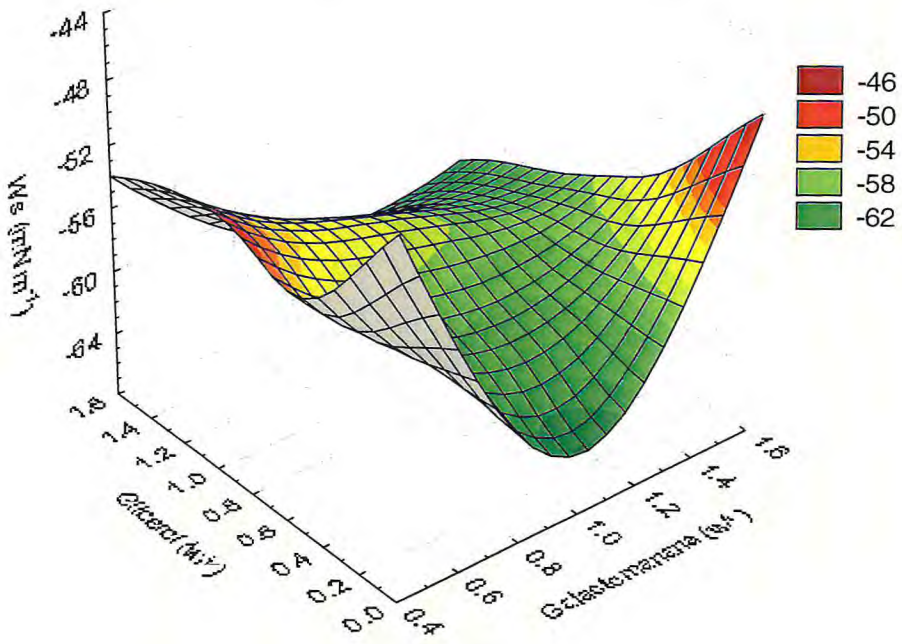


Figura 23 - Valores do coeficiente de espalhamento (*Ws*) para diferentes concentrações de galactomanana de *A. pavonina* – colágeno e glicerol em superfícies de maçã.

Como pode ser observado nas figuras 22 e 23 os melhores valores de coeficiente de espalhamento das soluções filmogências em maçã foram obtidas na ausência de glicerol. Este fato provavelmente está relacionado a particularidade da superfície da maçã apresentar um alto componente dispersivo, denotando a predominância de forças apolares. Como o glicerol é uma substância polar, esta fato explica o porque de filmes desprovidos de glicerol apresentarem melhores valores de *Ws.*

Os melhores valores de coeficiente de espalhamento, em manga, foram obtidos com blendas de 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol (-29,07 mN . m^{-1}). Os melhores valores de coeficiente de espalhamento, em maçã, foram obtidos com blendas de 0,5% de galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol (-42,79 mN . m^{-1}).

Estas melhores soluções em termos de capacidade molhante (representado aqui pelo coeficiente de espalhamento – *Ws*) foram analisadas com relação a permeabilidade ao vapor de água, permeabilidade ao oxigênio, permeabilidade ao dióxido de carbono e propriedades mecânicas.

5.2.3 Permeabilidade ao vapor de água, oxigênio e dióxido de carbono

As figuras 24, 25 e 26 mostram as diferenças de permeabilidade ao oxigênio (PO_2), permeabilidade ao dióxido de carbono (PCO_2) e permeabilidade ao vapor de água ($P\ H_2O$), respectivamente, para os filmes selecionados. O filme contendo 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol é menos permeável ao oxigênio que o filme contendo 0,5% de galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol. Isto pode ser explicado pelo fato da presença de plastificante diminuir a presença de regiões quebradiças e poros, aumentando a dispersão e diminuindo a permeabilidade a gases (GARCIA; MARTINO; ZARITZKY; 2000). Resultados semelhantes foram obtidos para a permeabilidade ao dióxido de carbono. O filme de galactomanana

de *A. pavonina* é aproximadamente 18 vezes menos permeável ao dióxido de carbono que o filme de galactomanana de *C. pulcherrima.*

Com relação a permeabilidade ao vapor de água o inverso ocorre quando comparado as permeabilidades ao O_2 e CO_2. O revestimento com 0,5% de galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol é aproximadamente 60% menos permeável a água que o filme contendo 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol. O plastificante diminui as atrações intermoleculares entre as cadeias poliméricas, facilitando a penetração das moléculas de vapor de água (KESTER; FENNEMA, 1986). O glicerol é uma molécula hidrofílica (polar) e o seu aumento provoca um aumento na massa transferida de vapor de água.

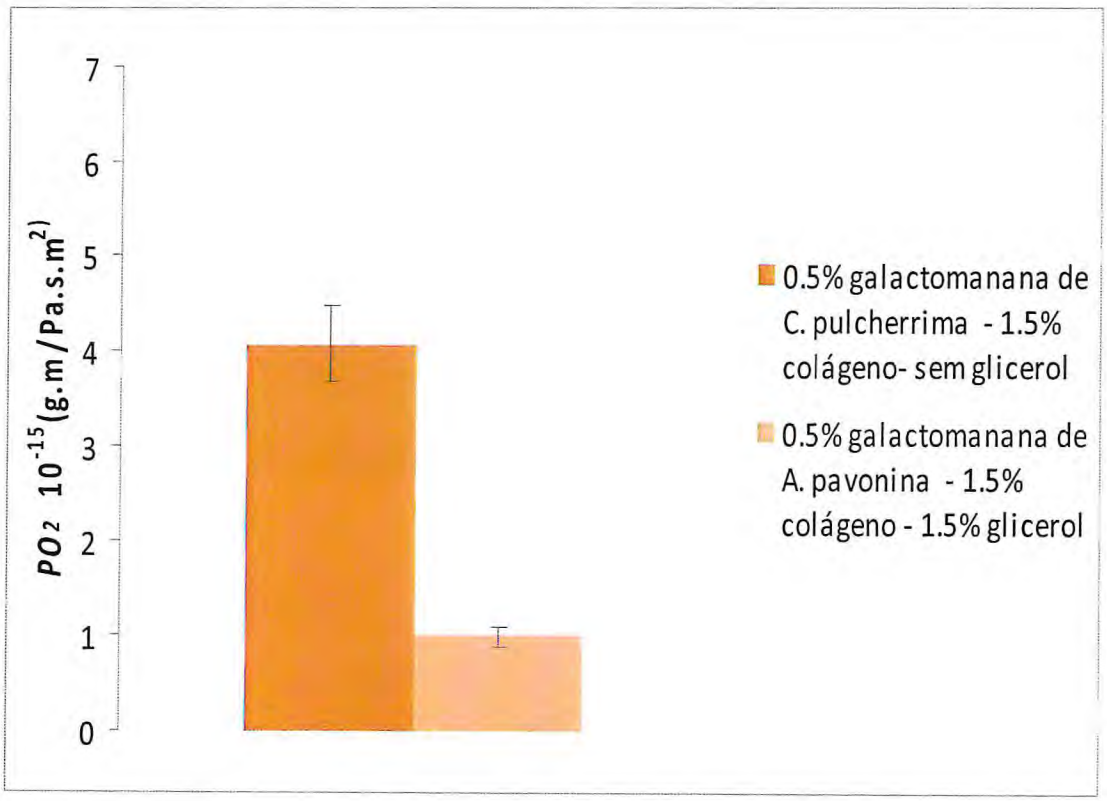


Figura 24 – Permeabilidade ao Oxigênio dos filmes com melhores valores de *Ws* para Maçã e Manga respectivamente (n= 3; 95% de intervalo de confiança).

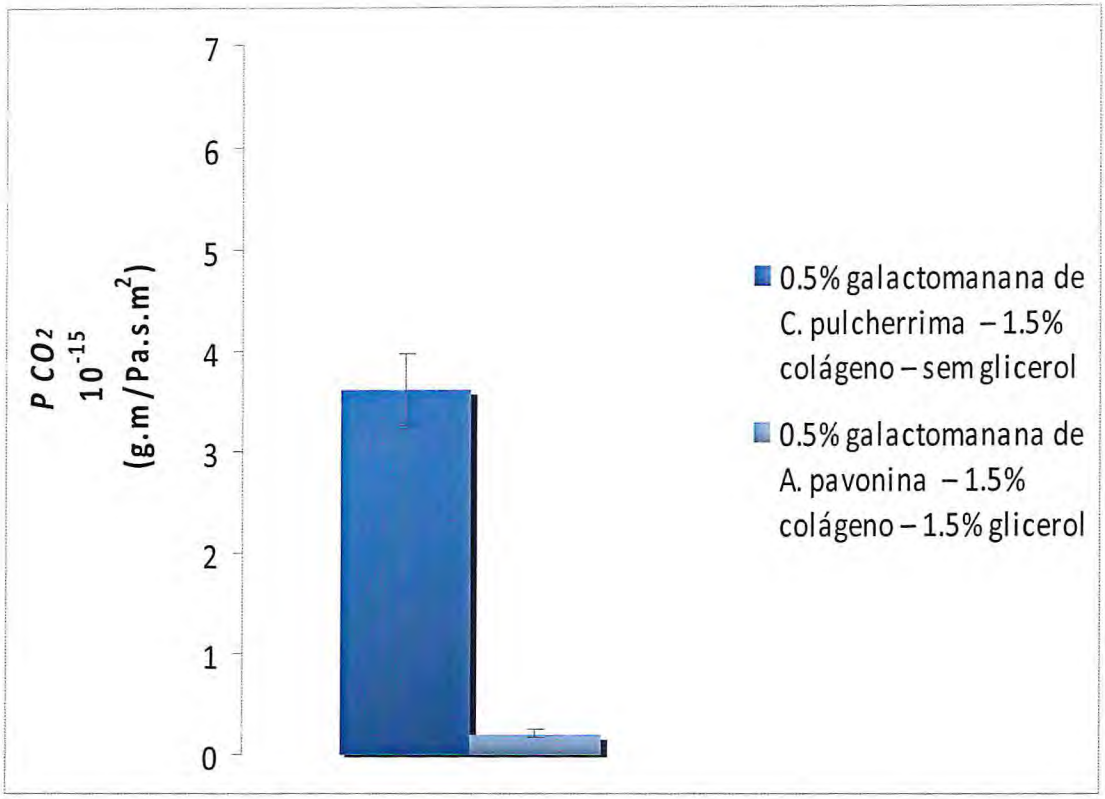


Figura 25 – Permeabilidade ao dióxido de carbono dos filmes com melhores valores de Ws para Maçã e Manga respectivamente (n= 3; 95% de intervalo de confiança)

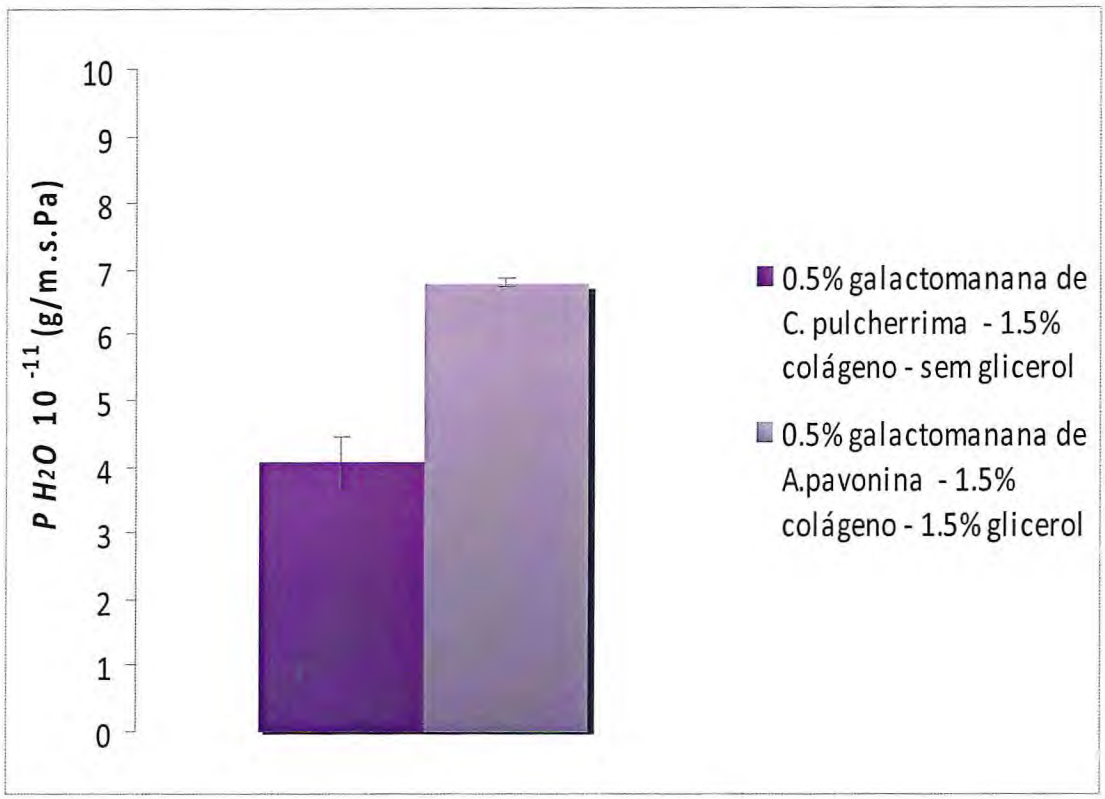


Figura 26 – Permeabilidade ao vapor de água dos filmes com melhores valores de Ws para Maçã e Manga respectivamente (n= 3; 95% de intervalo de confiança).

Os valores de permeabilidade estão de acordo com outros filmes feitos a partir de polissacarídeos e proteínas, apresentando valores mais baixos que outros filmes reportados (Tabela 14). Somente os valores de permeabilidade ao dióxido de carbono mostraram-se maiores quando comparados com filmes de zeína e gérmen de trigo.

Tabela 14 – Valores de permeabilidade de filmes comestíveis.

Filme comestível	$P\ H_2O \cdot 10^{-11}$ ($g \cdot m^{-1}\ s^{-1}\ Pa^{-1}$)	$P\ O_2 \cdot 10^{-12}$ ($cm^3 \cdot m \cdot (Pa \cdot s \cdot m^2)^{-1}$)	$P\ CO_2 \cdot 10^{-12}$ ($cm^3 \cdot m \cdot (Pa \cdot s \cdot m^2)^{-1}$)	Referência
Galactomanana – colágeno - glicerol	4,06 - 6,79	0,76 - 2,86	0,10 -1,83	Este trabalho
Goma de Locust bean (*Ceratonia siliqua* L.)	1,75 - 3,22	-	-	AYDINLI;TUTAS (2000)
Gérmen de trigo	61,6	14,04	1,08	PARK, 1999
Zeína	11,6	25,26	1,35	PARK, 1999
Amido	21,22	-	-	GARCIA; PINOTTI; ZARITZKY, 2006
Arabinoxilanas	11,8 - 17,7	-	-	PÉROVAL *et al.*, 2002
Quitosana	26,7	-	-	PRANOTO; RAKSHIT; SALOKHE, 2005
Amido	-	104,1 -131,9	-	RIBEIRO *et al.*, (2007)
Carragenana	-	41,9 - 44,4	-	RIBEIRO *et al.*, (2007)
Quitosana	-	0,09 -37,37	-	CANER; VERGANO; WILES (1998)
Proteína do soro do leite	-	26,6	-	GOUNGA; XU; WANG (2007)
Quitosana	-	-	20,1	SATHIVEL *et al.*, (2007)

5.2.4 Cor e Opacidade

Para mensurar a cor foram medidos os valores de *L*, a* e b**. Os valores destes parâmetros estão demonstrados na tabela 15. Comparando os dois filmes é possível observar que o filme contendo 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol possui valores mais altos de *L** (94,52) e *b** (5,84). Isso é um indicativo que o filme tem uma leve tendência a cor amarela por um maior valor de *b**. Por outro lado o filme contendo 0,5% de galactomanana de *C. pulcherrima* e 1,5% de colágeno apresentou valores mais baixos de L* (91,85) e valores mais altos de *a** (5,23) quando comparado ao filme de galactomanana de *A. Pavonina.* Isto é um indicativo de que o filme de *Caesalpinia* é um pouco mais escuro e com a coloração tendendo um pouco mais ao vermelho.

O filme de galactomanana de *A. Pavonina* é menos opaco que o filme de *C. pulcherrima.* Contudo ambos os filmes tem valores de opacidade mais elevados que filmes feitos exclusivamente de galactomanana e glicerol. Esta característica é típica de filmes de origem protéica.

Tabela 15 – Medidas de parâmetros de cor e opacidade para as blendas com melhores valores de *Ws* para maçã e para manga.

Filme	*L** (preto-branco)	*a** (verde-vermelho)	*b** (azul – amarelo)	Opacidade (%)
0.5% galactomanana de *C. pulcherrima* – 1.5% colágeno – sem glicerol	91,848± 0,38[a]	5,23 ± 0,01[a]	4,85 ± 0,12[a]	13,67 ± 0,001
0.5% galactomanana de *A. pavonina* –colágeno – 1.5% glicerol	94,52 ± 0,66[b]	4,61 ± 0,04[b]	5,84 ± 0,03[b]	11,34 ± 0,009

*Valores indicam a média ± desvio padrão (n=3, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

5.2.5 Propriedades mecânicas

O filme contendo 0,5% de galactomanana de *C. pulcherrima* e 1,5% de colágeno possui valores mais altos de *TS* (tração) e valores mais baixos de *E* (elasticidades até ruptura) quando comparado ao filme de *A. pavonina*, colágeno e glicerol (tabela 16). Estes resultados já eram esperados pois a presença do glicerol provoca uma redução na tração do filmes e um acréscimo na sua elasticidade. Quando comparado com outros filmes, as blendas de galactomanana - colágeno são mais resistentes que filmes de amido e filmes de galactomanana (PARRA *et al.*, 2004).

Tabela 16 – Tração (*TS*) e Elasticidade até ruptura (*E*) de filmes de blendas de galactomanana e colágeno.

Blenda	Tração (MPa)	Elasticidade até ruptura (%)
0.5% galactomanana de *C. pulcherrima* – 1.5% colágeno – sem glicerol	$117,56 \pm 25,58^{a}$	$18,74 \pm 0,01^{a}$
0.5% galactomanana de *A. pavonina* – colágeno – 1.5% glicerol	$8,34 \pm 2,72^{b}$	$47,17 \pm 0,03^{b}$

*Valores indicam a média ± desvio padrão (n=5, 95% de intervalo de confiança). Letras diferentes na mesma coluna indicam uma diferença estatisticamente significativa (teste de Tukey $p < 0,05$).

5.2.6 Taxas de transferência de O_2 e CO_2 (Respiração dos frutos)

Maçãs foram revestidas utilizando uma solução com 0,5% de galactomanana de *C.pulcherrima*, 1,5% de colágeno e sem glicerol. As taxas de transferência de O_2 e CO_2 foram comparadas com maçãs que não receberam qualquer tipo de revestimento. Os gases foram medidos por 120 horas (figuras 27 e 28) e a taxa de transferência de gases foi calculada e os resultados estão demonstrados na figura 29. As maçãs que foram revestidas apresentaram uma menor troca gasosa. A produção de CO_2 e o consumo de O_2 é aproximadamente 50% menor em maçãs com o revestimento, quando comparadas a maçãs que não foram revestidas. A taxa de produção de CO_2 é maior que o consumo de O_2.

Mangas revestidas com uma solução de 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol foram comparadas com mangas sem revestimento no que concerne às taxas de transferência de O_2 e CO_2. Os gases foram mensurados por um período de 60 horas (figuras 30 e 31) e a taxa de transferência de gases foi calculada e os resultados estão demonstrados na figura 32. Assim como ocorreu com as maçãs, as mangas revestidas apresentaram uma menor troca gasosa. Seu consumo de O_2 foi 28% menor que em mangas sem revestimento, e a produção de CO_2 foi 11% menor que em mangas não revestidas.

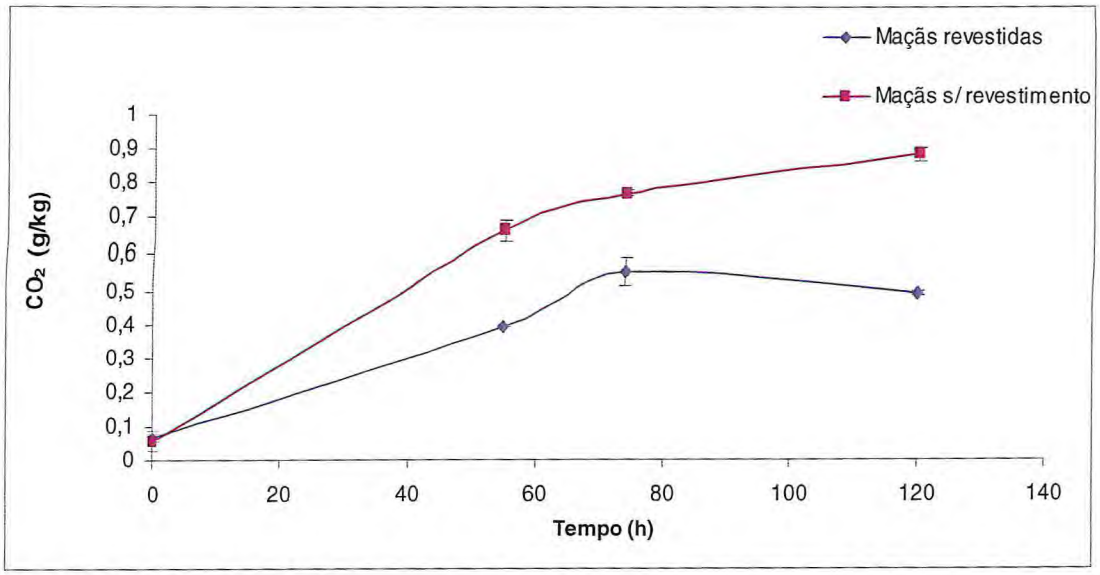


Figura 27 – Curva de produção de CO_2 ao longo do tempo em maçãs revestidas com solução filmogênica (0,5% de galactomanana de *C. pulcherrima* e 1,5% de Colágeno) e sem revestimento.

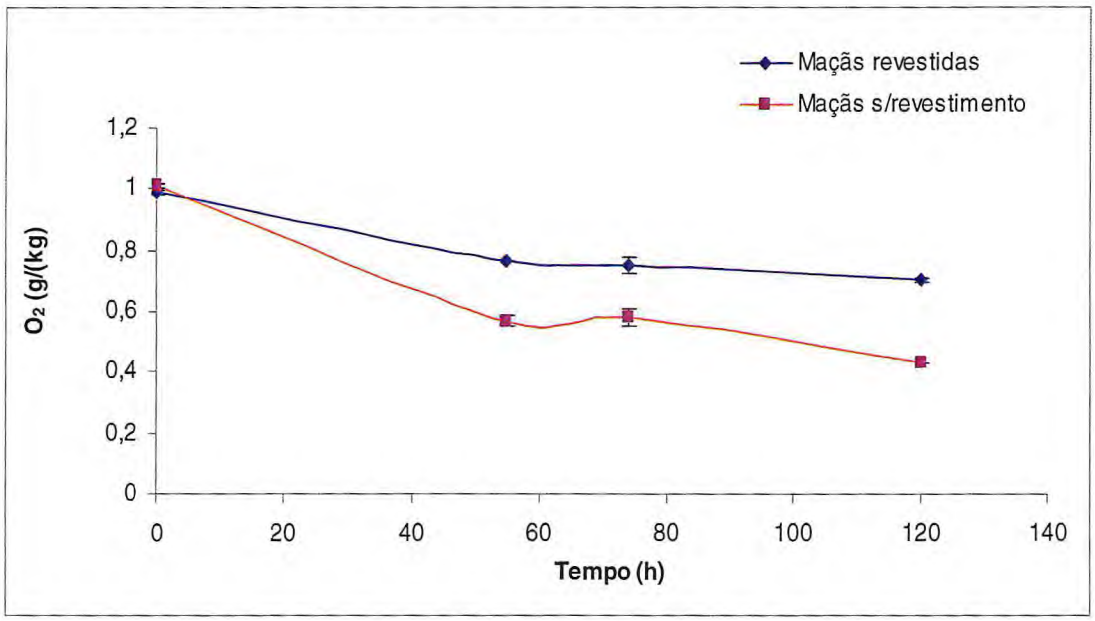


Figura 28 – Curva de consumo de O_2 ao longo do tempo em maçãs revestidas com solução filmogênica (0,5% de galactomanana de *C. pulcherrima* e 1,5% de Colágeno) e sem revestimento.

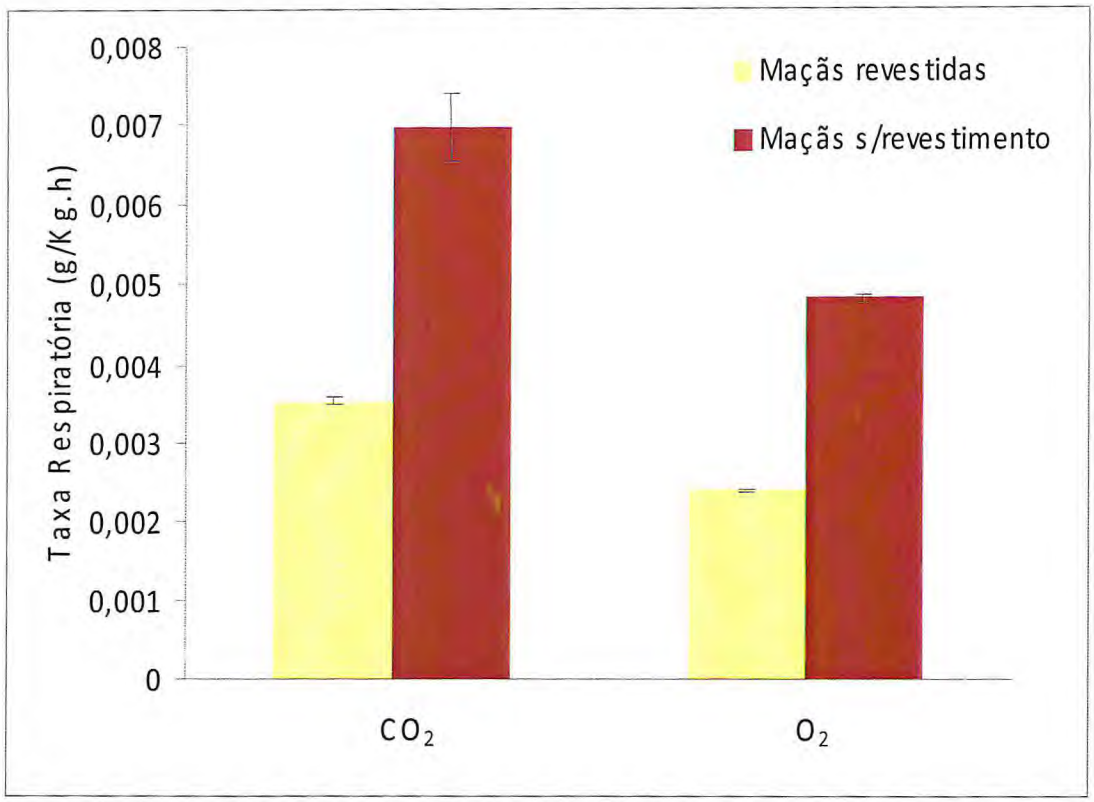


Figura 29 – Taxas de transferências de O_2 e CO_2 em maçãs revestidas e sem revestimento.

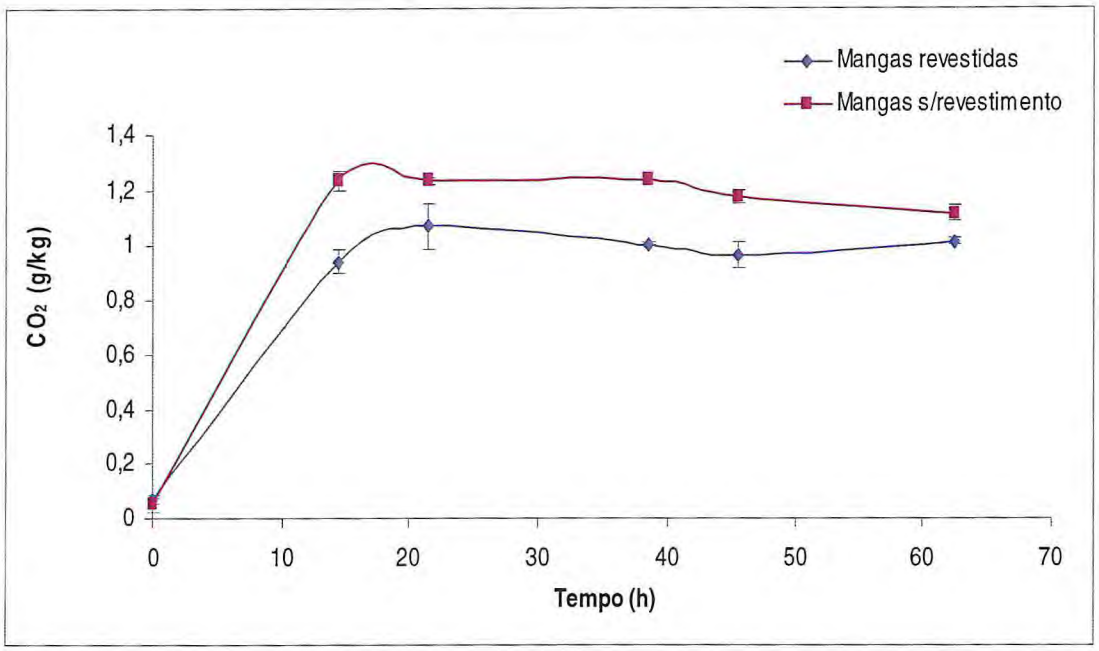


Figura 30 – Curva de produção de CO_2 ao longo do tempo em mangas revestidas com solução filmogênica (0,5% de galactomanana de *A. pavonina,* 1,5% de Colágeno e 1,5% de glicerol) e sem revestimento.

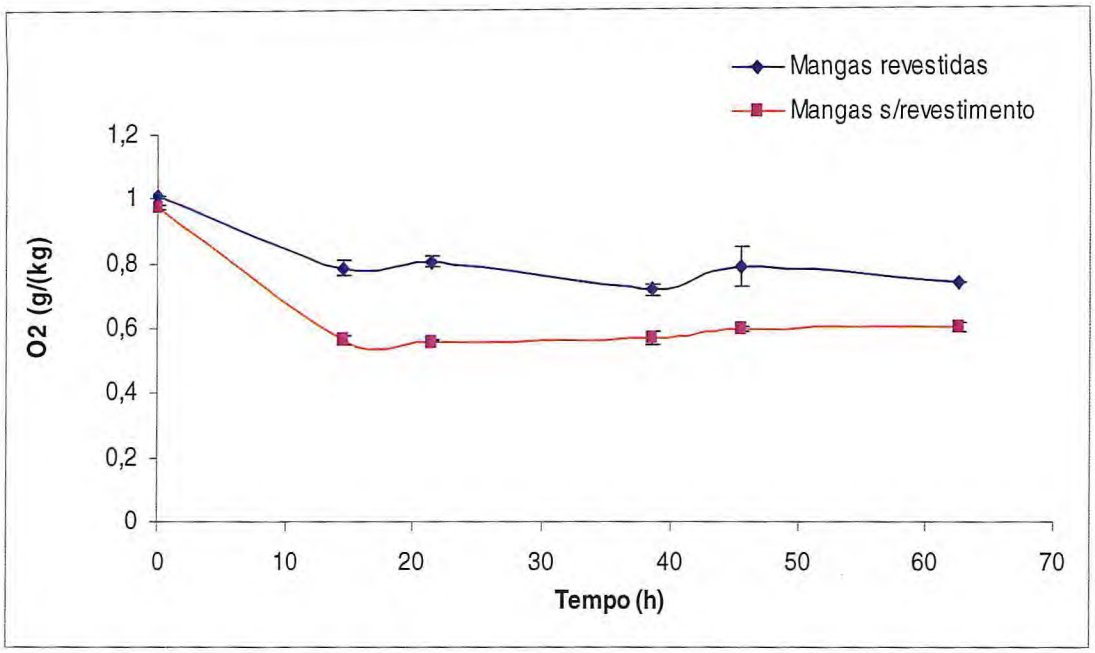


Figura 31 – Curva de consumo de O_2 ao longo do tempo em mangas revestidas com solução filmogênica (0,5% de galactomanana de *A. pavonina,* 1,5% de Colágeno e 1,5% de glicerol) e sem revestimento.

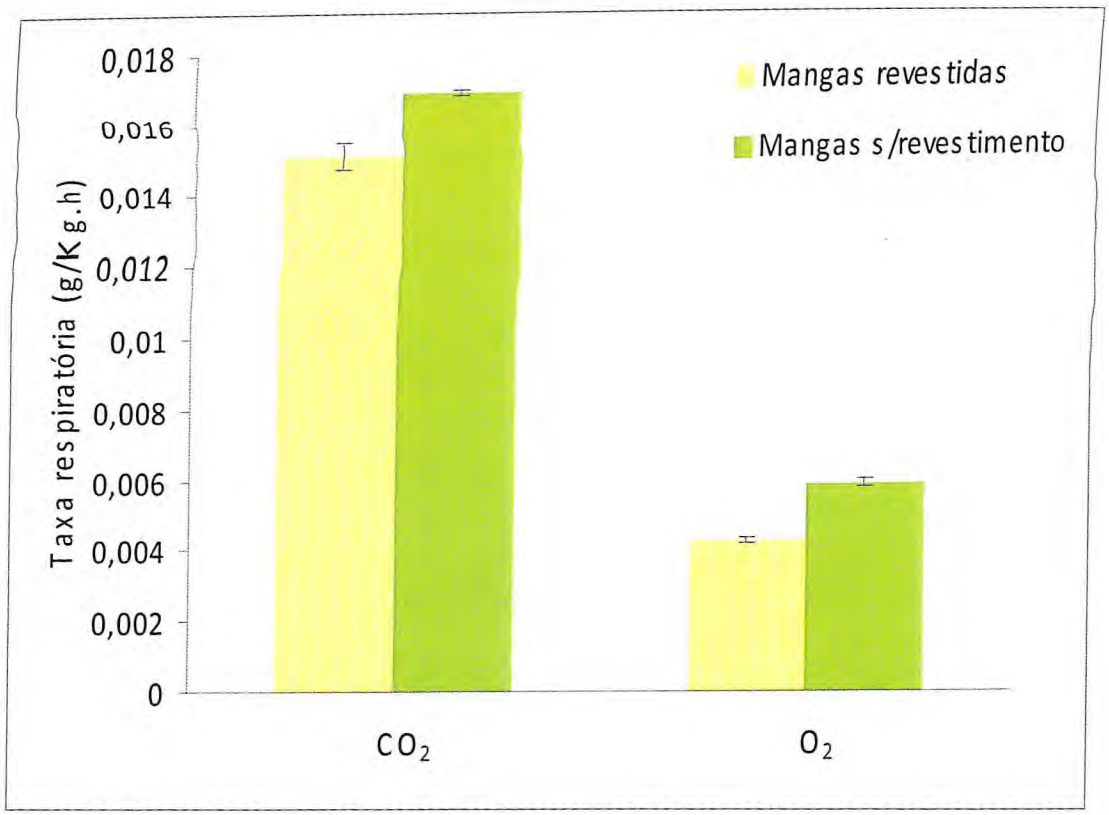


Figura 32 – Taxas de transferências de O_2 e CO_2 em mangas revestidas e sem revestimento.

Os valores obtidos estão de acordo com outros valores obtidos previamente para outros frutos e hortaliças (tabela 17). Os valores mais elevados apresentados neste trabalho estão justificados pela temperatura mais elevada (20°C), em que os experimentos foram conduzidos. Outros trabalhos já demonstraram que o aumento de temperatura tem uma grande influência nas taxas de respiração.

Tabela 17 - Taxas respiratórias dos frutos estudados e previamente estudados por outros autores.

Fruta	RO_2 ($ml \cdot kg^{-1} \cdot h^{-1}$)	RCO_2 ($ml \cdot kg^{-1} \cdot h^{-1}$)	Condições	Referência
Manga	26.4	68.7	21 °C, revestida	Este trabalho
Manga	36.6	77.1	21 °C, sem revestimento	Este trabalho
Maçã	14.9	16.1	21 °C, revestida	Este trabalho
Maçã	30.2	31.6	21 °C, sem revestimento	Este trabalho
Manga	14.5	16.5	5 °C	RAVINDRA;GOSWAMI, 2008
Maçã	19	22	20 °C	MAHAJAN; GOSWAMI, 2001
Maçã	-	1.0	0 °C	KIM; SMITH;LEE, 1993
Fatias de batata	5.4	9.4	4 °C	ANGÓS; VÍRSEDA; FERNÁNDEZ, 2008
Couve galega	5.6 - 161	7.9 - 153	1 – 20 °C	FONSECA *et al.*, 2002

6 – RESUMO DE RESULTADOS

Quando avaliadas as propriedades de superfície, os frutos em estudo apresentaram valores mais altos do componente dispersivo quando comparados ao componente polar, indicando a habilidade destes frutos em formar interações hidrofóbicas. Acerola e seriguela foram os frutos que apresentaram maiores valores de interações polares, enquanto a manga apresentou menores valores deste tipo de interação. A tensão superficial crítica destes frutos variou entre 9,39 $mN \cdot m^{-1}$ e 23,92 $mN \cdot m^{-1}$, sendo assim superfícies de baixa energia (inferiores a 100 $mN \cdot m^{-1}$) e passíveis de aplicação do método de Zisman para análise destes frutos.

A capacidade molhante foi estudada através da determinação dos valores do coeficiente de espalhamento (*Ws*). Os resultados obtidos mostraram que os valores de *Ws*, são muito dependentes tanto da fonte como da concentração de galactomanana, e também variam de acordo com o fruto testado. As soluções com concentrações mais baixas de galactomanana de *Adenanthera pavonina* apresentaram melhores (mais altos) valores de *Ws* ($p<0,05$) para os frutos com valores maiores do componente polar (acerola e seriguela). Para galactomanana de *Caesalpinia pulcherrima* os melhores valores de *Ws* para manga (valores de componente polar menores) foram encontrados em uma concentração de 1,5% de galactomanana. Estes resultados mostram que há uma relação direta com a polaridade das soluções aquosas, onde um aumento na concentração de galactomanana reduz a polaridade das soluções tornando-as mais adequadas no revestimento de superfícies apolares (como ocorre com a superfície da manga).

Com relação aos estudos de permeabilidades, as formulações que apresentaram menor permeabilidade ao vapor de água foram: 0,5% de galactomanana de *C.pulcherrima* – 1% de glicerol; 1,5% de galactomanana de *C.pulcherrima* – 2% de glicerol; 0,5% de galactomanana de *A.pavonina* – 1% de glicerol; 1% de galactomanana de *A.pavonina* – 1% de glicerol e 1,5% de galactomanana de *A.pavonina* – 1% de glicerol. Os valores mais baixos de permeabilidade ao oxigênio foram obtidos para estas três formulações: 1% de galactomanana de *A. pavonina* – 1% de glicerol; 1,5% de galactomanana de *A.*

pavonina – 1% de glicerol; 1,5% de galactomanana de *A. pavonina* – 1,5% de glicerol. Os valores mais altos de permeabilidade ao CO_2 foram obtidos para a formulação de 1,5% de galactomanana de *A. pavonina* – 1% de glicerol.

A avaliação das propriedades mecânicas mostrou que filmes menos concentrados de galactomanana de *C. pulcherrima* (0,5%) são tão resistentes quanto filmes mais concentrado de galactomanana de *A. pavonina* (1,5%). Este fato pode ser explicado devido a um menor grau de substituição da cadeia principal de manana da galactomanana de *C. pulcherrima* (razão manose/galactose 2,88) se sobrepondo a galactomanana de *A. pavonina* (razão manose/galactose 1,35). Com relação aos valores de *E* (elasticidade até ruptura) o que se pôde observar é que os filmes de *C. pulcherrima* apresentaram valores mais altos de *E* , o que pode ser explicado por um maior conteúdo de glicerol, mas também pela maior flexibilidade de estruturas menos substituídas (galactomanana de *C. pulcherrima*) quando comparada com estruturas mais substituídas (galactomanana de *A. pavonina*).

Foram ainda desenvolvidas blendas de galactomanana – colágeno e glicerol, que foram avaliadas com relação as suas propriedades de revestimento em mangas e maçãs. Os melhores valores de coeficiente de espalhamento, em manga, foram obtidos com blendas de 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol (-29,07 mN . m^{-1}). Os melhores valores de coeficiente de espalhamento, em maçã, foram obtidos com blendas de 0,5% de galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol (-42,79 mN . m^{-1}).

O filme contendo 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol é menos permeável ao oxigênio que o filme contendo 0,5% de galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol. Resultados semelhantes foram obtidos para a permeabilidade ao dióxido de carbono. O filme de galactomanana de *A. pavonina* é aproximadamente 18 vezes menos permeável ao dióxido de carbono que o filme de galactomanana de *C. pulcherrima*. Com relação a permeabilidade ao vapor de água o inverso ocorre quando comparado as permeabilidades ao O_2 e CO_2. O revestimento com 0,5% de

galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol é aproximadamente 60% menos permeável a água que o filme contendo 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol.

O filme contendo 0,5% de galactomanana de *C. pulcherrima* e 1,5% de colágeno possui valores mais altos de *TS* (tração) e valores mais baixos de *E* (elasticidades até ruptura) quando comparado ao filme de *A. pavonina*, colágeno e glicerol. Quando comparado com outros filmes, as blendas de galactomanana - colágeno são mais resistentes que filmes de amido e filmes de galactomanana.

O estudo comparativo da respiração dos frutos de maçãs revestidos com a blenda, e frutos não revestidos mostrou que as maçãs que foram revestidas apresentaram uma menor troca gasosa. A produção de CO_2 e o consumo de O_2 é aproximadamente 50% menor em maçãs com o revestimento, quando comparadas a maçãs que não foram revestidas.

O mesmo experimento foi repetido para mangas e assim como ocorreu com as maçãs, as mangas que foram revestidas apresentaram uma menor troca gasosa. Seu consumo de O_2 foi 28% menor que em mangas sem revestimento, e a produção de CO_2 foi 11% menor que em mangas não revestidas.

Tanto os filmes compostos de galactomanana e glicerol, como as blendas de galactomanana e colágeno, mostraram-se alternativas promissoras no desenvolvimento de revestimentos comestíveis de frutos, para sua utilização no aumento da vida de prateleira destes frutos.

7 – CONCLUSÕES

- Tendo por base os estudos de propriedades de superfície e permeabilidade dos revestimentos, quatro se mostraram os que mais se adequam a cobertura de frutos: 0,5% de galactomanana de *A. pavonina* e 1% de glicerol (para acerola); 1% de galactomanana de *A. pavonina* e 1% de glicerol (para cajá); 1,5% de galactomanana de *A. pavonina* e 1% de glicerol (para manga e pitanga) e 0,5% de galactomanana de *C.pulcherrima* e 1,5% de glicerol.
- Os filmes apresentaram boas características mecânicas, sendo compatíveis com filmes de outros biopolímeros previamente estudados.
- Para blendas de galactomanana/colágeno/glicerol duas formulações se apresentaram mais efetivas no revestimento de mangas e maçãs. Blendas contendo 0,5% de galactomanana de *A. pavonina*, 1,5% de colágeno e 1,5% de glicerol foram selecionadas para revestirem mangas e blendas contendo 0,5% de galactomanana de *C. pulcherrima*, 1,5% de colágeno e sem glicerol foram selecionadas para revestirem maçãs.
- A blenda de galactomanana de *C. pulcherrima*/colágeno/glicerol mostrou-se menos permeável ao vapor de água e mais permeável ao oxigênio e ao dióxido de carbono quando comparada à blenda de galactomanana de *A. pavonina*/colágeno/glicerol.
- Mangas revestidas com a blenda selecionada apresentaram um consumo 28% menor de O_2 e uma produção 11% menor de CO_2 quando comparadas a mangas não revestidas.
- Em maçãs, as frutas revestidas com a blenda apresentaram aproximadamente um consumo de O_2 e uma produção de CO_2, 50% inferior quando comparadas a maçãs não revestidas.

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Anexos

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Extraction, purification and characterization of galactomannans from non-traditional sources

Miguel A. Cerqueira [a], Ana C. Pinheiro [a], Bartolomeu W.S. Souza [a], Álvaro M.P. Lima [b], Clara Ribeiro [e], Cândida Miranda [e], José A. Teixeira [a], Renato A. Moreira [b], Manuel A. Coimbra [c], M. Pilar Gonçalves [d], António A. Vicente [a,*]

[a] IBB – Institute for Biotechonolgy and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Departamento de Bioquímica e Biologia Molecular Universidade Federal do Ceará, CEP 60451-970 Fortaleza (CE-Br), Brazil
[c] Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[d] REQUIMTE – Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[e] Research and Development Department, Frulact, S.A., Rua do Outeiro, 589, Gemunde, 4475-150 Maia, Portugal

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ABSTRACT

This work presents a methodology for the extraction of galactomannans from seeds of four different species of Leguminosae (*Adenanthera pavonina, Caesalpinia pulcherrima, Gleditsia triacanthos* and *Sophora japonica*) to be used e.g. in the food and biomedical industries. The galactomannans were obtained by aqueous extraction followed by a precipitation with ethanol. This methodology is simpler and easier to perform than other existing extraction and purification methodologies, and because it avoids the use of organic solvents (other than ethanol), it is able to generate food grade substances and is environmentally friendlier. The yield of extraction in different stages of the process, monosaccharide composition, as well as physical and chemical parameters of the isolated galactomannans were determined and compared with previously published results. The mannose/galactose ratio of the extracted galactomannans ranged from 1.35 (*A. pavonina*) to 5.75 (*S. japonica*). The intrinsic viscosity ranged from 11.34 dL/g (*C. pulcherrima*) to 8.74 dL/g (*S. japonica*), while the viscosity average molecular mass ranged between 1.81×10^6 Da and 1.17×10^6 Da (*A. pavonina* > *C. pulcherrima* > *G. triacanthos* > *S. japonica*). The results confirm the suitability of the extraction and purification procedure to obtain galactomannans from non-traditional sources.

1. Introduction

In the last decade there has been a growing interest in the development of thermoplastic materials from biodegradable polymers, particularly those derived from renewable resources (Paes, Yakimets, & Mitchell, 2008; Petersen et al., 1999).

Biobased packaging is defined as packaging containing raw materials originating from agricultural sources, i.e. produced from renewable, biological raw materials such as starch and bioderived monomers. To date, biodegradable packaging has attracted great attention, and numerous projects are under way in this field. One important reason for this attention is the marketing of environmentally friendly packaging materials. Furthermore, the use of biodegradable packaging materials has the greatest potential in countries where landfill is the main waste management tool.

The biodegradable polymers have also interest for biomedical engineering, featuring 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 (Chu, 2003).

Galactomannans are present in the endosperm of numerous plants, particularly the Leguminosae, and they have several functions, including reserve of carbohydrates (Reid & Edwards, 1995). Galactomannans are polysaccharides built up of a β-(1–4)-D-mannan backbone with single D-galactose branches linked α-(1–6). Their mannose/galactose (M/G) ratios differ according to the species (Kök, Hill, & Mitchell, 1999). They are water soluble hydrocolloids which form highly viscous, stable aqueous solutions (Neukom, 1989). Galactomannans can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries (Srivastava & Kapoor, 2005; Vieira, Mendes, Gallão, & de Brito, 2007). Most galactomannans used in pharmaceutical technology and cosmetics are usually unpurified gums (Üner & Altinkurt,

* Corresponding author. Tel.: +351 253 604419; fax: +351 253 678986.
E-mail address: avicente@deb.uminho.pt (A.A. Vicente).

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2004). In some occasions galactomannans have been used in binary mixtures with other polysaccharides such as: xanthan gum, agar and kappa-carrageenan, to form gels with new properties (Bresolin, Milas, Rinaudo, Reicher, & Ganter, 1999; Fernandes, Gonçalves, & Doublier, 1991; Vendruscolo, Andreazza, Ganter, Ferrero, & Bresolin, 2005).

The three major galactomannans of commercial importance in food and non-food industries are guar gum (GG, *Cyamopsis tetragonolobo*, M/G ratio: 2:1), tara gum (TG, *Caesalpinia spinosa*, M/G ratio: 3:1) and locust bean gum (LBG, *Ceratonia siliqua*, M/G ratio: 3.5:1 (Dakia, Blecker, Roberta, Watheleta, & Paquota, 2008).

Currently the international trends demand the introduction of alternative sources of seed gums (Joshi & Kapoor, 2003) and it is therefore important to search for alternative renewable sources for e.g. the production of edible and biodegradable films and coating materials. In particular, Latin American sources of galactomannans are not well known, in spite of the rich biodiversity of the local flora and of the favorable climate for their production (Azero & Andrade, 2002).

Mikkonen et al. (2007) show the use of galactomannan sources as an alternative for film formation. The mechanical and thermal properties from different film formulations (containing GG and LBG with glycerol and sorbitol) show that galactomannans with different M/G ratio have different behaviours, pointing at a promising use of biobased-galactomannan films and coatings as edible packaging materials (Mikkonen et al., 2007).

In the present work, four non-traditional galactomannans were isolated from seeds of *Adenanthera pavonina, Caesalpinia pulcherrima, Gleditsia triacanthos* and *Sophora japonica* and a simple methodology using only ethanol and water as solvents was developed for their extraction in view of their use e.g. in the demanding area of food industry. All the galactomannans from those plants were obtained by aqueous extraction followed by precipitation with ethanol, but the extraction procedure of galactomannan from the seeds of *S. japonica* required an acidic pretreatment in order to effectively separate the hull from the endosperm. Other works used solvents as chloroform (Mirzaeva, Rakhmanberdyeva, Kristallovich, Rakhimov, & Shtonda, 1998) and petroleum ether (Amin, Ahmad, Yin, Yahya, & Ibrahim, 2007; Üner & Altınkurt, 2004) to extract galactomannans, which are not authorized in the food industry (List of Codex Specifications For Food Additives, 2008). The extraction yield of each of the galactomannans was determined, as well as their monosaccharide composition, M/G ratio, purity, intrinsic viscosity and viscosity average molecular mass, thus providing a convenient measure of the hydrodynamic volume of individual polymer coils.

2. Materials and methods

2.1. Plant material

The pods of *A. pavonina and C. pulcherima* were collected in Fortaleza, Federal University of Ceará (Ce-Br) during January 2006. The pods of *G. triacanthos* and *S. japonica* were collected in the Botanic Garden in Porto, Portugal, during April 2006. The seeds were manually separated and kept in a cool, dry place until further use.

Adenanthera pavonina is a plant from the family Leguminosae, native from tropical Asia. It is used in reforestation, as ornamental plant and constitutes an important source of wood. The baking of the seeds and of the wood allows its use in the treatment of pulmonary infections, and also in the treatment of chronic ophthalmia (Fonseca & Perez, 2003).

Caesalpinia pulcherrima is an ornamental plant found throughout India, but it can be found in other countries as well, especially in Brazil. It also belongs to the family Leguminosae. Some of the constituents extracted from *C. pulcherrima* were found to possess anti-tumour (Che, McPherson, Cordell, & Fong, 1986; Patil et al., 1997) and antimicrobial properties (Ragasa, Ganzon, Hofilena, Tamboong, & Rideout, 2003).

Gleditsia triacanthos belongs to the family Leguminosae, grows in America, Middle Europe and Mediterranean area (Üner & Altınkurt, 2004). Galactomannans are the main polysaccharide constituents from the endosperm of the seed of *G. triacanthos* (Manzi, Mazzini, & Cerezo, 1984).

Sophora japonica, also a Leguminosae, is native from China where it is widely cultivated and used as a hemostatic agent in traditional Chinese medicine (Ishida, Umino, Tsuji, & Kosuge, 1989; Tang, Lou, Wang, & Zhuang, 2001). This tree is currently spread all over the world, including Portugal. Chemical constituents of the seeds of these plants such as triterpenes, phospholipids, alkaloids, amino acids, polysaccharides and fatty acids have been reported (Grishkovets & Gorbacheva, 1995; Mukhamedova & Glushenkova, 1997). According to previous studies (Smirnova, Mestechkina, & Sherbukhin, 2004), the galactomannan from *S. japonica* exhibits a high M/G ratio (greater than 5). Its unusually low content in galactose can be very interesting for possible commercial application of this gum because it has been recognised that galactomannans which are less substituted show a greater synergistic effect with e.g. xanthan gum (Fernandes, 1995; Scborsch, Gamier, & Doublier, 1997).

2.2. Polysaccharide extraction

The polysaccharide extraction of *A. pavonina, C. pulcherrima* and *G. triacanthos* was performed with ethanol and distilled water. In this process, the seeds were removed from the pods, cleaned and placed in a blender, where they were mechanical broken. Following this operation, the endosperm was manually separated from the germ and the hull, suspended in ethanol (purity 99.8%, Riedel-de Haën, Germany) in a proportion 1:3 (seeds:ethanol) at 70 °C during 15 min to inactivate the enzymes and eliminate low-molecular-weight compounds (Egorov, Mestechkina, & Shcherbukhin, 2003; Egorov, Mestechkina, & Shcherbukhin, 2004). The ethanol was decanted and distilled water was added in a proportion of 1:5 (endosperm:water), the suspension was left to rest for approximately 24 h. Then water, in a proportion of 1:10, (suspension:water) was added and mixed in a blender for 5 min.

Only in the case of the *S. japonica* seeds this procedure was not efficient because the black hull remained attached to the endosperm and consequently the gum contained a high level of impurities and was brownish. In order to obtain a purer gum, two different de-hulling pre-treatments (procedures A and B) of the *S. japonica* seeds with acid were tested, as described below.

2.2.1 Procedure A

The seeds of *S. japonica* were peeled using sulfuric acid (purity 98%, Fluka, Germany) (1:1) in a water bath at 100 °C for 1.5 h. This treatment with acid at an elevated temperature carbonized the hull which was removed by successively rinsing firstly with water (3 × 200 mL) and further with ethanol (250 mL) (purity 99.8%, Riedel-de Haën, Germany) until the husks and acid were mainly removed. After this pre-treatment, the procedure was similar to the one performed for the other seeds, differing only in the temperature of the extraction process. In this case, after adding the water in a proportion of 1:5 (endosperm: water), the mixture was heated at 80 °C during 2 h and left to rest approximately 22 h at room temperature. Da Silva and Gonçalves (1990) and Smirnova et al. (2004) showed that the increase of temperature in extraction pro-

cedures of galactomannans with high M/G ratio originates higher extraction yields and leads to galactomannan with different intrinsic viscosities.

2.2.2. Procedure B

This method differs from procedure A only in the bath temperature and in its duration. Thus, procedure B consisted in placing the seeds in sulfuric acid (1:1) in an oil bath at 120 °C for 10 min. After this pre-treatment, the procedure was similar to procedure A.

2.3. Polysaccharide purification

The endosperm mixed in the blender was filtered through a nylon net followed by a centrifugation step at 3800*g* (Sigma 4K, B. Braun, Germany) during 20 min at 20 °C. The precipitation of the galactomannan was achieved by adding the supernatant to ethanol (purity 99.8%, Riedel-de Haën, Germany) at a ratio of 1:2. The ethanol was decanted and the precipitated galactomannan was lyophilized and kept in a dry place until further use.

Fig. 1 shows the flow chart representative of the extraction and purification processes for the galactomannans of the four seeds considered in this work.

2.4. Determination of polysaccharide yield

The yield is one of the most economically important aspects of polysaccharide extraction and purification, and it was determined in three stages of the process (*Y1*, *Y2* and *Y3*), for an initial mass of 50 g seeds of each species. *Y1* was calculated dividing the mass of recovered dry endosperm (m_r) by the initial mass of seeds (m_i) thus determine the yield in the stage where the hull and the germ are removed manually, it represents the yield of the pre-treatment process. *Y2* was calculated dividing the result of the difference between the mass of recovered endosperm (m_r) and the mass obtained from the filtration and centrifugation (after drying in a oven until constant weight at 105 °C) (m_f) by the mass of recovered endosperm (m_r), it represents the yield of the purification process. *Y3* represents the total yield of the extraction and purification

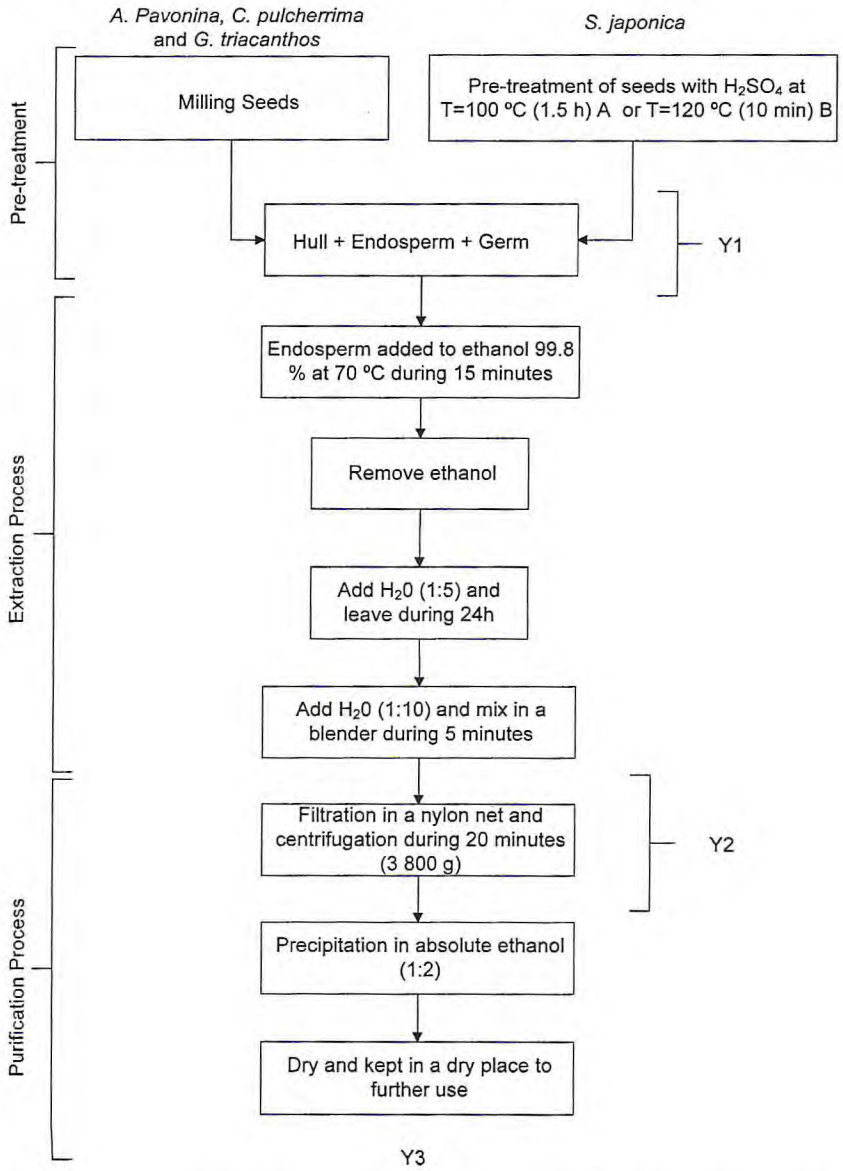

Fig. 1. Flow chart representative of the extraction and purification processes of the galactomannans. *Y1*, *Y2* and *Y3* are the points of the procedure where the yield was calculated.

processes and was calculated dividing the mass of lyophilized galactomannan (m_l) by the initial mass of the seeds (m_i).

2.5. Polysaccharide analyses

Polysaccharide analyses were performed as described in Ferreira, Mafra, Soares, Evtuguin, and Coimbra (2006). Neutral sugars (2 mg) were released through an acid treatment using 0.2 mL 11 M H_2SO_4 for 3 h at 20 °C followed by 2.5 h in 1 M H_2SO_4 at 100 °C, reduced with sodium borohydride, acetylated with acetic anhydride using methylimidazole as catalyst, and the alditol acetates formed were analyzed by gas chromatography (Carlo Erba 6000, Carlo Erba, Milan, Italy) with a split injector (split ratio 1:60) and a flame ionization detector. The column was a DB-225 (J & W, USA) with 30 m × 0.25 mm and film thickness of 0.25 μm; the oven temperature program was: 220 °C during 5 min, being then the temperature raised at a rate of 20 °C min^{-1} to 230 °C and maintained at this temperature for further 6 min. The flow rate of the carrier gas (H_2) was set at 1 mL/min at 220 °C. The injector temperature was 220 °C and the flame ionization detector temperature was 230 °C. The hydrolysis of all samples was performed in duplicate and each one was injected twice.

Uronic acids were determined by the 3-phenylphenol colorimetric method as described in Ferreira et al. (2006). Samples were prepared in duplicate by hydrolysis in 0.2 mL 11 M H_2SO_4 for 3 h at 20 °C followed by 1 h in 1 M H_2SO_4 at 100 °C. The uronic acids determined were quantitatively accounted as galacturonic acid.

The purity of the polysaccharides was evaluated both by the total amount of monosaccharides obtained in the monosaccharide composition and by the amount of manose + galactose present per mg of sample.

2.6. Macromolecular characterization

Viscosities of dilute solutions were measured at 25 ± 0.1 °C with a Cannon Fenske capillary viscometer (ASTM-D2515, Series 100), using exactly 10 mL of solution sample.

Solutions were prepared to have relative viscosities, η_{rel}, from about 1.2–2.0, to assure good accuracy and linearity of extrapolation to zero concentration. The intrinsic viscosity, $[\eta]$, was determined from Huggins' (Eq. (1)) and Kramer's (Eq. (2)) equations, where k_H and k_K are the Huggins' and Kramer's coefficients, respectively, η_{sp} is the specific viscosity and C is the solution concentration.

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \quad (1)$$

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k''[\eta]^2 C \quad (2)$$

Viscosity average molecular masses, M_v, were calculated using the Mark–Houwink relationship given by Doublier and Launay (1981) for guar gum as modified by Gaisford, Harding, Mitchell, and Bradley (1986) to take into account the different values of M/G of the galactomannans.

$$\eta = 11.55 \times 10^{-6}[(1-\alpha) \times \overline{M_v}]^{0.98} \quad (3)$$

Where $\alpha = 1/[(M/G) + 1]$ and $[\eta]$ is expressed in dL/g.

3. Results and discussion

3.1. Pre-treatment, extraction, purification and global yield

The extraction yield was measured for the processes of polysaccharide pre-treatment (*Y1*), extraction and purification (*Y2*) and for the global process (*Y3*). Table 1 shows the results of the yields *Y1*, *Y2* and *Y3*.

Y1 is the yield after pre-treatment, where the hull and germ are removed from the endosperm. This yield is a measure of the ease with which hull and germ can be separated from the endosperm. It is also a measure of the relative amount of endosperm in the seed. The highest value of *Y1* (67.13%) was obtained for *G. triacanthos*, while *A. pavonina* and *C. pulcherrima* had similar values (43.73% and 45.27%, respectively). These results show that the pre-treatment was more effective in removing the hull and germ from the seeds of *A. pavonina* and *C.pulcherrima* than for the seeds of *G. triacanthos*.

The yield *Y2* was measured after the filtration and centrifugation processes; here most of the hull that is still attached to the endosperm after the extraction process is removed. In this stage, *C. pulcherrima* seeds showed the highest value of yield (66.19%), while *G. triacanthos* presented the lowest value (42.40%). This is a direct consequence of the previous pre-treatment step: *G. triacanthos* endosperm was carrying much more attached material than *A. pavonina* and *C. pulcherrima*, therefore such material was now removed in more significant amounts, thus decreasing the yield value obtained for the first species. The opposite has happened with *C. pulcherrima*.

The best global yield was obtained for *G. triacanthos* and *C. pulcherrima*, which have values close to 25% (24.73% and 25.70%, respectively).

In the extraction of *S. japonica* galactomannan, the acid pre-treatment caused a progressive hydrolysis from external to internal components of the seed. The extraction yield obtained with pre-treatment B (3.33%) was significantly lower than that obtained with pre-treatment A (9.22%). During the pre-treatments it was observed that procedure B caused the carbonization of hull and also

Table 1
Pre-treatment, extraction and purification and global yields

Species	*Y1* (%)	*Y2* (%)	*Y3* (%)
G. triacanthos	67.13 ± 0.64	42.40 ± 3.51	24.73 ± 2.08
A. pavonina	43.73 ± 1.75	44.67 ± 6.54	17.11 ± 4.15
C. pulcherrima	45.27 ± 0.50	66.19 ± 5.89	25.70 ± 3.20
S. japonica (A)	–	–	9.22 ± 0.53
S. japonica (B)	–	–	3.33 ± 0.21

Table 2
Physicochemical composition of the studied polysaccharides in other works

Species	M/G	η (dL/g)	$M_v \times 10^6$ (Da)	Yield (%)	References
G. triacanthos	1.5–2.6	–	–	15.4	Manzi et al. (1984)
	4.6	–	–	18	Mirzaeva et al. (1998)
	1.48–3.12	–	–	11.90–34.16	Sciarini et al. (2008)
	3.2–3.5	–	–	15–20	Leschziner & Cerezo (1970)
A. pavonina	1.8	–	–	–	Tavares (1999)
C. pulcherrima	2.8	13.75	2.1	25	Andrade et al. (1999)
S. japonica	4.8–5.3	10.29–12.11	1.19–1.40	2.5–10.38	Smirnova et al. (2004)
	5.26	–	–	4.3	Kooiman (1971)

damaged the endosperm, decreasing the extraction yield. This clearly shows that the extraction global yield is very much dependent on the pre-treatment step. These yield values are in close agreement with those reported in other works (see Table 2).

The extraction yields obtained for the galactomannan of *S. japonica* are much lower than those obtained for the other galactomannans, and this may be related with the temperature of the extraction process. Although the extraction of the galactomannan from *S. japonica* included 2 h in hot water, the other steps were performed at room temperature. This may explain the lower extraction yields once the high M/G ratio of the galactomannan from *S. japonica* lowers its solubility in cold water (Smirnova et al., 2004).

Finally, the variability of the yield results is a direct consequence of the use of different species (Fernandes, 1995).

The final milled galactomannan of *S. japonica* was a white mucilage containing low remains of the husk and germ fractions. The other galactomannans presented a light yellow colour; in fact, even when performing the precipitation in ethanol, some parts of the germ and pigments from the hull pass to the polysaccharide. This observation has been described by other authors, who reported the passage of pigment and tannins from the hull or from the germ to the endosperm (Avallone, Plessi, Baraldi, & Monzani, 1997; Dakia et al., 2008). The final stage of the global process was the drying step that can determine the color of the end product. In the present work the polysaccharides were lyophilized, thus minimizing browning and moisture absorption during long-term storage. In other works, as those of Dakia et al. (2008) and Sciarini, Maldonado, Ribotta, Pérez, and Léo (2008), the final product was dried in a oven at 100 °C and 35 °C, respectively; the combination of a relatively low water activity and high temperature can enhance the Maillard reaction which provokes browning of the galactomannan, thus changing the galactomannan's chemical properties which in turn may potentially have negative health effects such as those reported to occur in coffee and bread crust, related with acrylamide formation (Frank & Hofmann, 2000).

3.2. Polysaccharide composition

Table 3 shows the results of polysaccharide analyses which confirmed that mannose (Man) and galactose (Gal) are the major monosaccharides present in the polysaccharide material extracted from *G. triacanthos* (66.9% and 23.7%, respectively), *A. pavonina* (52.8% and 39.2%), *C. pulcherrima* (69.1% and 24.0%) and *S. japonica* (81.5% and 14.2%; 81.9% and 14.5%, for gums obtained by procedure A and B, respectively). All the extracted galactomannans contain minor amounts of other monosaccharides such as rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl) and glucose (Glc). In the polysaccharides of *G. triacanthos*, *A. pavonina* and *C. pulcherrima* there are significant values of Ara monosaccharides (3.0–4.5%). This presence was also showed by Navarro, Cerezo, and Stortz (2002) in the galactomannan of *G. triacanthos* and by Nunes, Domingues, and Coimbra (2005) in galactomannans from green and roasted coffee. The presence of these minor components could be attributed to a more complex polysaccharide composition, as single Ara side chains such as those occurring in coffee (Nunes et al., 2005), and/or to contaminants proceeding from the seed coat (Da Silva & Gonçalves, 1990; Dakia et al., 2008).

The value of M/G ratio obtained for *G. triacanthos* (M/G = 2.82) is in agreement with the values reported in the literature: 3.2 (Leschziner & Cerezo, 1970) and 1.48–3.12 (Sciarini et al., 2008). For *A. pavonina* the value of M/G reported by Tavares (1999) was high (1.8) when compared with the one obtained in this work (1.35); factors such as the degree of maturation of the seeds, the place of cultivation and differences in the extraction and purification procedures are known to play a determinant role in the M/G ratio and may justify the differences found in diverse literature sources. This means that comparisons such as the one made here are useful but should be made with these restrictions in mind. The value of M/G obtained for *C. pulcherrima* (2.8) is close to the value obtained by Andrade, Azero, Luciano, and Gonçalves (1999), where the extraction process was quite different, with the use of solvents as toluene, acetone and diethyl ether, and with a drying temperature of 35 °C. The M/G values obtained for the galactomannans of *S. japonica* extracted by procedure A and procedure B (M/G = 5.75 and M/G = 5.66, respectively) are statistically equal ($p < .05$) and are in good agreement with those reported by Smirnova et al. (2004) (M/G = 5.30) and by Kooiman (1971) (M/G = 5.28). These values are however very much lower than the M/G = 8 reported for green coffee (Nunes et al., 2005) and up to M/G = 20 and 45 in light and dark roasted coffee infusions (Nunes, Reis, Domingues, & Coimbra, 2006).

Anyway, the galactomannan of *S. japonica* exhibits a high M/G compared with the other galactomannans, thus rendering this galactomannan especially suitable for possible synergistic interactions. In general, galactomannans with higher relative values of Gal monosaccharides are readily soluble in H_2O but have less ability to form gels, while galactomannans with higher relative Man content have the tendency to interact with gelling polysaccharides. The galactomannan from *S. japonica* presents the lower value of Gal and the highest value of Man (see Table 3); this observation allows the establishment of the hypothesis that such galactomannan consists of long blocks of unsubstituted Man units, and is the most interesting galactomannan in terms of the possibility of interaction with other polysaccharides (e.g. covalent interactions and chemical bonds) (Srivastava & Kapoor, 2005).

Gleditsia triacanthos and *C. pulcherrima* have values of M/G very similar to the commercial tara gum (3.0) (Dakia et al., 2008), that is widely used as a thickening agent and stabilizer for food applications.

The results also show (see Table 3) that the extraction and purification process presented here allows purity values between 80.8% and 98.4%, as evaluated from the total monosaccharide content of the sample.

Table 3
Polysaccharide composition of the galactomannans from the four studied species

Species	Monosaccharide composition (% mol)							Total Man + Gal	Total (μg/mg)	M/G
	Man	Gal	Rha	Fuc	Ara	Xyl	Glc			
G. triacanthos	66.9 ± 0.9	23.7 ± 0.8	0.0 ± 0.0	1.3 ± 0.6	4.5 ± 0.5	1.4 ± 0.2	1.4 ± 0.2	741.6	808	2.82 ± 0.05
A. pavonina	52.8 ± 0.4	39.2 ± 1.1	1.0 ± 0.3	0.9 ± 0.2	4.2 ± 1.0	1.2 ± 0.2	0.8 ± 0.1	754.7	811	1.35 ± 0.03
C. pulcherrima	69.1 ± 1.5	24.0 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	3.0 ± 1.4	1.2 ± 0.0	1.2 ± 0.2	841.7	897	2.88 ± 0.07
S. japonica (A)	81.5 ± 0.1	14.2 ± 0.4	0.9 ± 0.0	0.7 ± 0.2	0.7 ± 0.4	1.2 ± 0.1	0.9 ± 0.0	946.5	984	5.75 ± 0.13
S. japonica (B)	81.9 ± 0.8	14.5 ± 0.1	0.5 ± 0.0	0.8 ± 0.3	0.8 ± 0.5	0.5 ± 0.2	1.1 ± 0.1	882.6	913	5.66 ± 0.08

Table 4
Physical–chemical parameters of galactomannans extracted from the species considered in the present work (M_v – viscosity average molecular mass)

Species	Intrinsic viscosity Huggins extrapolation (dL/g)	Intrinsic viscosity Kraemer extrapolation (dL/g)	Intrinsic viscosity (average values) (dL/g)	Huggins coefficient, k_H	M_v ($\times 10^{-6}$)
G. triacanthos	10.06	10.78	10.42	1.13	1.62
A. pavonina	8.85	9.37	9.11	1.10	1.81
C. pulcherrima	10.91	11.77	11.34	1.27	1.75
S. japonica (A)	9.58	10.43	10.01	1.33	1.34
S. japonica (B)	8.93	8.55	8.74	1.25	1.17

3.3. Macromolecular characterization

The determination of intrinsic viscosity provides a measurement of the hydrodynamic volume occupied by the isolated polymer chains in a given solvent and depends primarily on the molecular structure and molecular weight of the polysaccharides as well as on the solvent quality.

The values for k_H depend on solute-solvent interactions and on the state of aggregation of the macromolecules; in a good solvent and for flexible macromolecules, $k_H = 0.3$; however it can be higher than one in case of aggregation (Sittikijyothin, Torres, & Gonçalves 2005).

Table 4 shows the values of physical–chemical composition of the studied galactomannans; k_H values of 1.10–1.33 probably reflect some intermolecular aggregation in the samples.

In a previous work, Andrade et al. (1999) showed that the galactomannan of *C. pulcherrima* has an intrinsic viscosity of 13.75 dL/g and a viscosity average molecular mass of 2.10×10^6 Da (Andrade et al., 1999); however, lower values were found in this work. These differences can be mainly explained by the extraction and purification processes, which are known to influence the intrinsic viscosity and, therefore, the viscosity average molecular mass. This is notorious, e.g., in the results shown for *S. japonica* extracted by pretreatments A and B; in this case, the processing at a higher temperature has eventually lead to a more extensive degradation of the polymer chains, which has in turn lowered the intrinsic viscosity and, together with it, the viscosity average molecular mass.

The galactomannans of *G. triacanthos* and *C. pulcherrima* have similar M/G ratios (2.82 and 2.88, respectively) but exhibit different intrinsic viscosities (10.42 and 11.34, respectively). For a certain M/G ratio, the galactomannans can differ in the distribution of galactose units along the mannan backbone. This distribution, though not fully understood yet, is believed to be important for the functional properties of these polysaccharides (Dakia et al. 2008).

4. Conclusions

Galactomannans are used by the industry in commercial form as, e.g., Locust Bean Gum, Guar Gum and Tara Gum, and new sources are important as an alternative to these traditional galactomannan sources. In this work galactomannans were extracted and purified from four species of seeds of plants from the family Leguminosae through an improved extraction and purification procedure which uses only ethanol and water. It is simpler and easier to perform than most of the published procedures and, as it avoids the use of non-food grade solvents it is food grade itself and environmentally friendlier.

This procedure allows a galactomannan yield of 24.73% – 25.70 starting from the seeds of *G. triacanthos* and *C. pulcherrima*, 17.11% for *A. pavonina* and 3.33–9.22% for *S. japonica*. The polysaccharide composition features a high content of Man and Gal, being the M/G ratio between 1.35 and 5.75 (*A. pavonina* < *G. triacanthos* < *C. pulcherrima* < *S. japonica* (B) < *S. japonica* (A)). *G. triacanthos* and *C. pulcherrima* present values of the M/G ratio very close to the commercial Tara Gum, and *S. japonica*, with a high Man monosaccharide content (leading to M/G over 5) can be interesting for possible synergistic interactions with other polysaccharides.

The fact that this extraction and purification methodology has been applied with success to four galactomannans with very different M/G ratios gives a clear indication that it may be used with other galactomannans as well. The results have also shown that the extracted galactomannans show adequate characteristics to be used in the food industry.

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Suitability of novel galactomannans as edible coatings for tropical fruits

Miguel A. Cerqueira [a], Álvaro M. Lima [b], José A. Teixeira [a], Renato A. Moreira [c], António A. Vicente [a,*]

[a] IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Departamento de Bioquímica e Biologia Molecular, Federal University of Ceará, Campus do Pici, CEP 60451-970 Fortaleza, CE, Brazil
[c] Centro de Ciências da Saúde, Universidade de Fortaleza, Av. Washington Soares, 1321 Bairro Edson Queiroz, 60811-905 Fortaleza, CE, Brazil

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ABSTRACT

The main objective of this work was to determine the optimal composition of edible coatings in view of their application to extend the shelf life of several tropical fruits. Coatings constituted by galactomannans from different sources (*Caesalpinia pulcherrima* and *Adenanthera pavonina*) and glycerol were characterized as coatings for 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 and different aqueous galactomannan solutions (0.5%, 1.0% and 1.5%) with glycerol (1.0%, 1.5% and 2.0%) were tested for their wettability on fruits. For the solutions having a better wettability, films were casted and water vapour permeability, oxygen permeability, carbon dioxide permeability, tensile strength and elongation at break were determined. Taking into account the surface and permeability properties of the obtained films, four compositions were selected as the best coatings to the studied fruits: acerola – 0.5% of *A. pavonina* galactomannan and 1.0% of glycerol; cajá – 1.0% of *A. pavonina* galactomannan and 1.0% of glycerol; mango and pitanga – 1.5% of *A. pavonina* galactomannan and 1.0% of glycerol; and seriguela – 0.5% of *C. pulcherrima* galactomannan and 1.5% of glycerol. For the coating, the values of the measured properties were as follows: wettability ranged from -36.33 ± 3.39 to -26.45 ± 4.58 mN · m^{-1}; water vapour permeability ranged from 4.89 ± 0.11 to $6.25 \pm 0.20 \times 10^{-11}$ g m^{-1} s^{-1} Pa^{-1}; oxygen permeability ranged from 0.31 ± 0.01 to $0.99 \pm 0.13 \times 10^{-15}$ g m (Pa s m^2)$^{-1}$; carbon dioxide permeability ranged from 28.81 ± 3.08 to $61.19 \pm 1.44 \times 10^{-15}$ g m (Pa s m^2)$^{-1}$; tensile strength ranged from 2.56 ± 0.15 to 3.96 ± 0.43 MPa; and elongation at break ranged from 28.26 ± 4.53% to 46.36 ± 2.29%.

1. Introduction

During the last decades, there has been an increasing demand for fresh fruits and vegetables forcing the food industry to develop new and better methods for maintaining food quality and extending shelf life. Great losses (from 20% to 80%) in the quality of fresh fruits occur from harvesting to final consumption and the fruits short shelf life is an important drawback concerning distribution chains. On the other hand, consumers around the world demand food of high quality, without chemical preservatives and with extended shelf life. As a consequence, an increased effort has been made to discover new natural preservatives and antimicrobials (Chien et al., 2007).

Packaging plays a decisive role in the improvement of fruits' shelf life and new packaging materials are being developed, most of them are derived from renewable resources (Lin and Zhao, 2007). Edible coatings act by creating a modified atmosphere surrounding the commodity, similar to that achieved by controlled or modified atmospheric storage conditions. The modified atmosphere created by edible coatings protects the food from the moment it is applied until it reaches the final consumer (Diab et al., 2001; Durango et al., 2006; Ribeiro et al., 2007).

Several researchers studied the application of coatings in fruits such as apples (Rojas-Grau et al., 2007), strawberries (Mali and Grossmann, 2003; Tanada-Palmu and Grosso, 2005; Ribeiro et al., 2007), mango (Srinivasa et al., 2002; Chien et al., 2007; Dang et al., 2008) and kiwi (Xu et al., 2001). Polysaccharide-based coatings are colourless and have an oil-free appearance, and can be used to increase the shelf life of fruits, vegetables, shellfish or meat products to avoid dehydration, and to reduce the oxidative rancidity and darkening of the surface to some extent. Other characteristics that make them attractive are their transport properties (permeability to CO_2, O_2 and water vapour), the reduction of materials weight loss and the reduction of the microbial spoilage of the fruits (Petersen et al., 1999; Dang et al., 2008). However, the effectiveness of edible coatings for fruits preservation depends, in a first stage, on the control of the wettability of the coating in order to ensure a uniformly coated surface. Other factors also affect the effectiveness of the coating, such as transport (permeability) and mechanical properties; these must also be considered in order to:

* Corresponding author. Tel.: +351 253 604 419; fax: +351 253 678 986.
E-mail address: avicente@deb.uminho.pt (A.A. Vicente).

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- Decrease the water loss in the fruits (i.e., lower water vapour permeability values).
- Decrease the O_2 permeability (i.e., lower O_2 permeability values) as a lower O_2 concentration prolongs the shelf life by delaying the oxidative breakdown of the complex substrates (Farber et al., 2003), and reduce the production of ethylene, a key element of the ripening and maturation process (Lee et al., 1996; Zagory, 1995).
- Increase the shelf life of fruits, by increasing the lag-phase and generation time during the logarithmic growth phase of spoilage microorganisms (Farber et al., 2003; Phillips, 1996), which is accomplished by keeping high CO_2 permeability values.
- Improve the mechanical resistance of the films/coatings in order to preserve their integrity as much as possible during the fruits' shelf life.

Galactomannans are present in the endosperm of numerous plants, particularly the *Leguminosae*, and they have several functions, including being a reserve of carbohydrates (Reid and Edwards, 1995). Galactomannans are polysaccharides built up of a β-(1–4)-D-mannan backbone with single D-galactose branches linked α-(1–6). Their mannose/galactose (M/G) ratios differ according to the species (Kök et al., 1999). Galactomannans can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries (Srivastava and Kapoor, 2005; Vieira et al., 2007).

In this work, coatings of galactomannans from two plant species (*Adenanthera pavonina* and *Caesalpinia pulcherrima*) were evaluated. *A. pavonina*, a plant from the *Leguminosae* family, native from tropical Asia, is used in reforestation and as an ornamental plant and is also an important source of wood. *C. pulcherrima* is also a plant from the *Leguminosae* family being found throughout India and other regions of the globe where it is used as an ornamental plant. Galactomannan from *A. pavonina* presents a ratio mannose/galactose of 1.35, while the galactomannan obtained by *C. pulcherrima* seeds presents a ratio mannose/galactose of 2.88 (Cerqueira et al., 2009). These polysaccharides are a cheap alternative to the existing (mostly synthetic) substances and have the advantage of being produced locally, near the harvesting sites of the studied fruits. The development of these applications from natural products and their use in the production sites to increase the fruits' shelf life can be an important contribution to the economy of countries such as Brazil.

This work aims at assessing the suitability of galactomannans from seeds of *A. pavonina* and *C. pulcherrima* to be used as edible coatings for different tropical fruits: acerola (*Malpighia glabra*), cajá (*Spondias lutea*), mango (*Mangifera indica*), pitanga (*Eugenia uniflora*) and seriguela (*Spondias purpurea*) and to determine which formulation is the most adequate to coat these fruits. This has been done by evaluating the fruits' surface properties following the application of different coatings (formed by mixtures of each of the polysaccharides at different concentrations and plasticizer) and by optimizing the composition of the coating in terms of its wettability and permeability properties. To evaluate the mechanical properties of the selected coatings the tensile strength and elongation at break were measured.

2. Materials and methods

2.1. Raw material

The seeds of *A. pavonina* (AP) and *C. pulcherima* (CP) were collected in the Federal University of Ceará, Campus of Pici, Fortaleza, CE – Brazil during January 2006 and after being cleaned they were maintained in a cool, dry place until further use.

The polysaccharide extraction was performed as described in Cerqueira et al. (2009). The seeds were removed from the pods, cleaned and placed in a blender, where they were mechanically broken. Following this, the endosperm was manually separated from the germ and the hull and was suspended in ethanol (purity 99.8%, Riedel-de Haën, Germany) at 70 °C for 15 min. The ethanol was decanted, distilled water was added in a 1:5 (endosperm:water), this suspension was left to rest for approximately 24 h. Water in a 1:10 (suspension:water) volumetric ratio was added and the obtained suspension was mixed in a blender for 5 min. The blended suspension was filtered through a nylon net followed by a centrifugation step at 3800 *g* (Sigma 4 K, B. Braun, Germany) for 20 min at 20 °C. The precipitation of the galactomannan was achieved by adding the supernatant to ethanol (purity 99.8%, Riedel-de Haën, Germany) at a ratio of 1:2. The ethanol was decanted and the precipitated galactomannan was lyophilized (Christ, alpha 2–4, Germany) and maintained in a dry place until further use.

The materials used to prepare the edible coating solutions were: galactomannan (as extracted from *A. pavonina* and *C. pulcherrima* seeds), glycerol 87% (Gly) (Panreac, Spain) and distilled water.

2.2. Coatings and films preparation

The coating formulations were based in a three-level factorial design with galactomannan concentrations of: 0.5%, 1.0% and 1.5% (w/v) and glycerol concentrations of: 1.0%, 1.5% and 2.0% (v/v). The number of replications is specified when describing each of the methodologies used. The concentrations were chosen based on preliminary experiments (data not shown) where it was determined that for galactomannan contents above 1.5% (w/v) their dissolution was extremely difficult; also for glycerol, previous studies indicated that a maximum of 2.0% would be necessary, while for values lower than 0.5% the film would be too brittle.

The coating solutions were prepared by dissolving the lyophilized galactomannan in distilled water (20 °C) and addition of the plasticizer. Each mixture was stirred for 2 h at room temperature (20 °C) and left to stabilize for 10 more minutes at the same temperature. The films were prepared with a constant amount (28 mL) of solution which was cast onto a 9 cm diameter glass plate. The films were dried in an oven at 35 °C for 16 h and maintained at 20 °C and 50% RH until their characterization.

2.3. Fruits and their preparation for contact angle measurements

Acerola, pitanga, seriguela, cajá and mango were purchased from a local supermarket (Fortaleza, CE – Brazil). All fruits were maintained at 8–10 °C until further use. The fruits were selected for their uniformity, size, colour (paying particular attention at their ripeness state) and the absence of damage and fungal infection. Before testing, the fruits were left at room temperature (20 °C) and their surface was cleaned with distilled water. Thin portions of the outer surface (skin) of the fruits were cut with a knife and placed on a glass plate for contact angle measurements.

2.4. Critical surface tension of fruits skins

According to Zisman (1964), in systems having a surface tension lower than 100 mN m^{-1} (low-energy surfaces), the contact angle formed by a drop of liquid on a solid surface will be a linear function of the surface tension of the liquid, γ_{LV}, (where phase *V* is air saturated with the vapour of liquid, *L*). The Zisman method is applicable only for low-energy surfaces; therefore it is necessary to determine the surface energy of the fruits.

For a pure liquid, if polar (γ_L^p) and dispersive (γ_L^d) interactions are known, and if θ is the contact angle between that liquid and a solid, the interaction can be described in terms of the reversible work of adhesion, W_a, as:

$$W_a = W_a^d + W_a^p \Longleftrightarrow W_a = 2 \cdot \left(\sqrt{\gamma_S^d \cdot \gamma_L^d} + \sqrt{\gamma_S^p \cdot \gamma_L^p}\right) \quad (1)$$

where γ_S^p and γ_S^d are the polar and dispersive contributions of the surface of the studied solid. Rearranging Eq. (1), yields:

$$\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^p} \cdot \sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_S^d} \quad (2)$$

The contact angle determinations (please see Section 2.5) of at least three pure compounds: bromonaphthalene (Merck, Germany), formamide (Merck, Germany) and ultra pure water, on the surface of the fruit (fruit skin) combined with the each dispersive and polar component value, will allow the calculation of both the independent variable, $\left(\sqrt{\frac{\gamma_L^p}{\gamma_L^d}}\right)$, and the dependent variable, $\left(\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}}\right)$, from Eq. (2).

The surface tension, the dispersive and the polar component were, respectively, 72.10, 19.90 and 52.20 mN m^{-1} for water, 44.40, 44.40 and 0.00 mN m^{-1} for bromonaphthalene and 56.90, 23.50 and 33.40 mN m^{-1} for formamide (Busscher et al., 1984).

The estimation of the critical surface tension (γ_C) was performed by extrapolation from Zisman plots (Zisman, 1964). Zisman plots have long been used to characterize the wettability of low-energy surfaces. Zisman plots are obtained by plotting the cosine of the contact angle of pure liquids on a solid surface to be studied against the surface tension of the same series of liquids. The intercept of these curves with $\cos\theta = 1$ is known as the critical surface tension (γ_C). The critical surface tension is an imaginary point of the γ_{sv} value and it is frequently used to describe the wettability of a surface. It represents the value of γ_{LV} of a liquid above which the spreading of this liquid in a solid surface is complete. The critical surface tension (γ_C) is defined as:

$$\gamma_C = \lim \gamma_{LV} \quad \text{as } \theta \to 0 \quad (3)$$

2.5. Wettability

The wettability was studied by determining the values of the spreading coefficient (W_s) and the works of adhesion (W_a) and cohesion (W_c). The adhesive forces promote the liquid spreading in a solid surface and the cohesive forces promote their contraction. The wetting behaviour of the solutions will mainly depend on the balance between these forces. The surface tension of the coating solution was measured by the pendant drop method using the Laplace–Young approximation (Song and Springer, 1996).

The contact angle (θ) of a liquid drop on a solid surface is defined by the mechanical equilibrium of the drop under the action of three interfacial tensions: solid–vapour (γ_{SV}), solid–liquid (γ_{SL}), and liquid–vapour (γ_{LV}). The equilibrium spreading coefficient (W_s) is defined by Eq. (4) (Rulon and Robert, 1993) and can only be negative or zero:

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{SL}. \quad (4)$$

where W_a and W_c are the works of adhesion and cohesion, defined by Eqs. (5) and (6), respectively

$$W_a = \gamma_{LV} + \gamma_{SV} - \gamma_{SL} \quad (5)$$

$$W_c = 2 \cdot \gamma_{LV}. \quad (6)$$

Contact angle (θ) and liquid–vapour surface tension (γ_{LV}) were measured in a face contact angle meter (OCA 20, Dataphysics, Germany). The samples of the coatings were taken with a 500 μL syringe (Hamilton, Switzerland), with a needle of 0.75 mm of diameter. The contact angle at the fruit surfaces was measured by the sessile drop method (Newman and Kwok, 1999), in which a droplet of the tested liquid was placed on a horizontal surface and was observed with a face contact angle meter. Measurements were made in less than 30 s. Ten replicates of contact angle and surface tension measurements were obtained at 21.3 ± 0.5 °C.

2.6. Film thickness

The film thickness was measured with a digital micrometer (Mitutoyo, Japan). Five thickness measurements were taken on each testing sample in different, randomly chosen points. The mean value was used to calculate water vapour permeability (*WVP*), oxygen permeability (O_2P), carbon dioxide permeability (CO_2P) and tensile strength (*TS*).

2.7. Water vapour permeability measurement (WVP)

The measurement of water vapour permeability (*WVP*) was determined gravimetrically based on ASTM E96-92 method (McHugh et al., 1993; Guillard et al., 2003). 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 weighed 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 (McHugh et al., 1993). The slope of weight loss versus time was obtained by a linear regression. Three replicates were obtained for each sample.

2.8. Oxygen and carbon dioxide permeability

Oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P) were determined based on the ASTM D 3985-02 (2002) method. The films were sealed between two chambers, having each one two channels. In the lower chamber O_2 (or CO_2) was supplied at a controlled (J & W Scientific, ADM 2000, USA) flow rate to maintain its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at controlled flow. Nitrogen acted as a carrier for the O_2 (or the CO_2).

In the case of O_2P measurement, the flow leaving this chamber was connected to an O_2 sensor (Mettler Toledo, Suisse) which measured the O_2 concentration in that flow on-line. In the case of CO_2P measurement the flow leaving this chamber was collected in a syringe for CO_2 quantification. To determine CO_2 concentration, 1 mL of sample was injected in a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110 °C with a column Porapak Q 80/100 mesh 2 m × 1/8″ × 2 mm SS, using a flame ionization detector (FID) at 110 °C. Helium at 23 mL min^{-1} was used as carrier gas. A standard mixture containing 10% CO_2, 20% O_2 and 70% N_2 was used for calibration.

The flows of the two chambers were connected to a manometer to ensure the equality of pressures (both at 1 atm) between both compartments. As the O_2 (and the CO_2) was carried continuously by the nitrogen flow, it was considered that partial pressure of O_2 (and the CO_2) in the upper compartment is null, therefore ΔP is equal to 1 atm. Three replicates were obtained for each sample, in each case (O_2P and CO_2P).

2.9. Mechanical properties – tensile strength (TS) and elongation at break (E)

TS and *E* were measured with an Instron Universal Testing Machine (Model 4500, Instron Corporation) following the guidelines of ASTM D 882-91 (1991). The initial grip separation was set at 30 mm and the crosshead speed was set at 5 mm min^{-1}. *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. *E* was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (30 mm) and expressed as a percentage. According to the ASTM standard, film strips with a length of 45 mm and a width of 20 mm were used. *TS* and *E* tests were replicated ten times for each sample.

2.10. Statistical analysis

The statistical analyses of the data were performed using Analysis of Variance (ANOVA), Tukey mean comparison test ($p < 0.05$) and regression analysis (SigmaStat, trial version, 2003, USA).

3. Results and discussion

3.1. Surface tension and critical surface tension of fruits skins

The surface tension of the fruits is very similar, but there are differences between the polar and the dispersive components (Table 1). All the fruits present a higher dispersive component, which shows the ability of the fruit surface to participate in non-polar interactions. This was also demonstrated by Ribeiro et al. (2007) in strawberry, where the dispersive component was higher than the polar component. A surface with these characteristics interacts with liquid primarily by dispersion forces that can influence the effective spreading of the coating on the surface of the fruits: the compatibility of the polarity (apolar or polar) of the surface and of the coating may play therefore an important role in the wettability of a surface by a liquid and may condition the composition of the latter. In the present work, the fruits presenting the higher values of polar interactions were acerola and seriguela, while mango displayed the lowest value of the polar component, therefore presenting the lowest ability to participate in polar interactions. These differences, despite being statistically significant, are presumably not sufficient to impose a very different behaviour of the surface towards the coating solution. This hypothesis is supported by the results presented in, e.g. Table 2, where no clear difference is shown between the values of W_s for the fruits and coatings evaluated in this work.

Table 1 also shows the value of the critical surface tension obtained for each fruit, which varies between 9.39 and 23.92 mN m^{-1}. Acerola presented the lowest value and cajá the highest. The obtained values are close to the critical surface tension of the apple (18.70 mN m^{-1}) and of the orange (20.00 mN m^{-1}) presented by Choi et al. (2002), exception made to acerola and pitanga that present a lower value. Also, the values of the critical surface tension must be lower than the values of the surface tension (Dann, 1970), which holds true for all the fruits used in this study. In all cases, it is possible to conclude that the studied fruits have low-energy surfaces (i.e., below 100 mN m^{-1}) meaning that the Zisman method is applicable.

3.2. Wettability

Wettability determinations were performed with different galactomannan concentrations for varying plasticizer concentrations. The wettability was studied by determining the values of the spreading coefficient (W_s). The spreading coefficient (W_s) of the solutions of galactomannans and glycerol applied on each fruit was analysed and presented in Tables 2 and 3. For each fruit, the best (higher) value of W_s for the respective galactomannan was determined (Tukey test, $p < 0.05$) and is filled in gray. When no statistically significant differences between galactomannan solutions are observed, it has been assumed that both were equally good in terms of wettability and that their differentiation must be made based on other criteria (such as permeability to gases, which will be presented subsequently).

The results show that the values of W_s are quite dependent on both the source and concentration of galactomannan and the fruit tested. The solutions with lower values of *A. pavonina* (AP) galactomannan concentration present better (higher) values ($p < 0.05$) of W_s for those fruits with higher values of the polar component (ace-

Table 1
Values of critical surface tension, surface tension and its polar and dispersive components for the analysed fruits.

Fruit	Critical surface tension (mN m^{-1})	Surface tension (mN m^{-1})	Polar component (mN m^{-1})	Dispersive component (mN m^{-1})
Acerola	9.39 ± 0.07^{a}	27.94 ± 0.03^{a}	4.35 ± 0.01^{a}	23.59 ± 0.02^{a}
Cajá	23.92 ± 0.10^{b}	30.15 ± 0.02^{b}	2.29 ± 0.01^{b}	27.86 ± 0.01^{b}
Mango	22.68 ± 0.09^{c}	29.04 ± 0.02^{c}	1.47 ± 0.01^{c}	27.57 ± 0.01^{c}
Pitanga	13.42 ± 0.09^{d}	26.95 ± 0.02^{d}	3.07 ± 0.01^{d}	23.88 ± 0.01^{d}
Seriguela	19.62 ± 0.09^{e}	31.48 ± 0.05^{e}	4.59 ± 0.03^{e}	26.89 ± 0.02^{e}

$^{a-e}$Means ($n = 10$) in the same column with different superscripts are significantly different ($p < 0.05$).

Table 2
Spreading coefficient (W_s) obtained for solutions of *A. pavonina* galactomannan and glycerol on the analysed fruits.

Gal. (w/v)	Glycerol (v/v)	Acerola	Cajá	Mango	Pitanga	Seriguela
0.5	1.0	-29.92 ± 2.10a	-36.50 ± 3.05^{a}	-30.97 ± 2.17^{ade}	-28.17 ± 7.27^{a}	-29.15 ± 2.78^{a}
0.5	1.5	-36.35 ± 3.95^{b}	-35.32 ± 3.74^{a}	-31.40 ± 2.97^{ade}	-31.71 ± 6.11^{a}	-23.72 ± 2.01^{b}
0.5	2.0	-42.38 ± 3.58^{c}	-27.84 ± 2.89^{b}	-37.91 ± 3.80^{b}	-39.13 ± 7.15^{bcd}	-28.95 ± 3.74^{a}
1.0	1.0	-42.11 ± 3.03^{c}	-30.80 ± 2.96^{b}	-34.37 ± 2.43^{abc}	-38.53 ± 3.91^{bcd}	-31.11 ± 2.79^{ad}
1.0	1.5	-46.71 ± 2.91^{d}	-36.96 ± 4.37^{a}	-30.93 ± 3.62^{ade}	-38.18 ± 3.78^{bcd}	-37.46 ± 2.65^{c}
1.0	2.0	-47.09 ± 4.54^{d}	-32.00 ± 3.44^{c}	-35.39 ± 1.75^{bc}	-44.22 ± 6.98^{c}	-36.86 ± 3.15^{c}
1.5	1.0	-41.57 ± 5.04^{c}	-32.85 ± 2.67^{c}	-29.18 ± 3.57^{e}	-26.45 ± 4.58^{a}	-32.35 ± 2.96^{d}
1.5	1.5	-41.06 ± 3.04^{c}	-31.24 ± 2.91^{bc}	-33.71 ± 2.95^{acd}	-32.75 ± 3.27^{ca}	-32.54 ± 3.70^{d}
1.5	2.0	-42.68 ± 1.41^{c}	-31.54 ± 3.21^{bc}	-30.38 ± 2.39^{ade}	-35.15 ± 2.68^{cd}	-37.90 ± 2.26^{c}

$^{a-e}$Means ($n = 10$) in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3
Spreading coefficient (W_s) obtained for solutions of *C. pulcherrima* galactomannan and glycerol on the analysed fruits.

Gal. (w/v)	Glycerol (v/v)	Acerola	Cajá	Mango	Pitanga	Seriguela
0.5	1.0	−42.68 ± 6.50^{a}	−27.69 ± 3.73^{a}	−51.29 ± 4.14^{a}	−40.76 ± 4.61^{a}	−40.57 ± 3.10^{a}
0.5	1.5	−39.83 ± 5.95^{a}	−31.85 ± 2.37^{b}	−65.48 ± 4.57^{b}	−35.54 ± 5.39^{b}	−36.33 ± 3.39^{b}
0.5	2.0	−32.59 ± 4.65^{b}	−38.86 ± 5.14^{ce}	−49.73 ± 6.09^{a}	−39.70 ± 3.71^{a}	−40.66 ± 2.82^{a}
1.0	1.0	−44.70 ± 4.42^{a}	−45.34 ± 4.46^{d}	−68.82 ± 6.38^{c}	−47.91 ± 6.25^{c}	−45.27 ± 3.17^{c}
1.0	1.5	−43.47 ± 3.37^{a}	−45.08 ± 3.54^{d}	−77.83 ± 5.87^{d}	−50.01 ± 4.73^{c}	−51.16 ± 3.82^{d}
1.0	2.0	−41.36 ± 3.32^{a}	−47.48 ± 4.31^{d}	−66.24 ± 7.72^{ec}	−57.02 ± 2.86^{d}	−49.87 ± 3.51^{d}
1.5	1.0	−42.38 ± 6.32^{a}	−40.06 ± 6.04^{ce}	−64.36 ± 7.84^{ec}	−41.88 ± 4.30^{a}	−41.44 ± 4.72^{a}
1.5	1.5	−40.60 ± 3.77^{a}	−37.55 ± 2.59^{c}	−62.81 ± 4.26^{e}	−43.01 ± 5.46^{a}	−42.29 ± 3.37^{a}
1.5	2.0	−58.65 ± 5.65^{c}	−43.58 ± 3.72^{ced}	−45.20 ± 4.49^{f}	−58.83 ± 5.31^{d}	−47.81 ± 3.59^{d}

$^{a-f}$Means (n = 10) in the same column with different superscripts are significantly different ($p < 0.05$).

rola and seriguela). In the case of *C. pulcherrima* galactomannan the better value of W_s in mango (lower polar component) was achieved for a galactomannan concentration of 1.5%. These results are related to the polarity of the aqueous solutions; with the increase in the galactomannan concentration the polarity of the solutions decreases rendering them more able to coat non-polar surfaces (such mango surface).

Table 2 displays the values of W_s obtained using the galactomannan of (AP). Acerola presents the best value of W_s when coated with solutions containing 0.5% galactomannan and 1.0% glycerol, seriguela presents the best value with the same concentration of galactomannan and 1.5% of glycerol. For mango, pitanga and cajá solutions with 0.5%, 1.0% and 1.5% of galactomannan do not show a statistically significant difference, presenting good values of W_s for the different concentrations of galactomannan and glycerol used.

When the galactomannan of *C. pulcherrima* (CP) was used, the values of W_s (Table 3) present statistically significant differences for each fruit; a single solution was found in each case, having the lower value of W_s. In all cases, with the exception of mango (the best W_s value was obtained with 1.5% of galactomannan) the best value of W_s was obtained with solutions containing 0.5% of galactomannan.

The best coatings of *A. pavonina* and *C. pulcherrima* in terms of wettability (represented by the spreading coefficient – W_s) were subsequently analysed for water vapour, oxygen and carbon dioxide permeability, according to the below mentioned criteria.

3.3. Water vapour permeability (WVP)

The water vapour permeability is the most extensively studied property of edible films mainly because of the importance of the water in deteriorative reactions. Table 4 shows that the values of *WVP* change with the use of different concentrations of glycerol in the galactomannan solutions as for higher concentrations of glycerol an increase in *WVP* occurs. These differences are statistically significant when the plasticizer concentration is increased from 1.0% to 2.0%. Gontard et al. (1993) explained that the effect of glycerol can be attributed to the hydrophilic properties of this compound which favour the adsorption of water molecules. Glycerol, through its plasticizing action, changes the polymer network creating mobile regions with larger interchain distances, promoting water clustering by competing with water at active sites of the polymer matrix and the formation of microcavities in the polymer network structure (Diab et al., 2001). The increase in *WVP* of edible films supported by increasing concentration of plasticizer has also been reported to happen in starch-based films (Mali et al., 2006), pullulan films (Diab et al., 2001), wheat gluten films (Gontard et al., 1993; Cherian et al., 1995), cellulose based films (Park and Chinnan, 1995) locust bean gum films (Aydinli and Tutas, 2000) and sodium caseinate/starch blends (Arvanitoyannis and Biliaderis, 1998).

An increase in the concentration of AP and CP galactomannans, for the same concentration of glycerol, corresponds to a decrease in *WVP*, possibly due a stronger gel network, where the polysaccharide molecules are closer, forming a more cohesive film structure. These changes present statistically significant differences when increasing of galactomannan concentration from 0.5% to 1.5% (Table 4). The formulations featuring lower values of *WVP* were: 0.5% CP – 1.0% Gly, 1.5% CP – 2.0% Gly, 0.5% AP – 1.0% Gly, 1.0% AP – 1.0% Gly and 1.5% AP – 1.0 Gly.

The *WVP* values obtained for the galactomannans films are in agreement with those reported for other galactomannan and polysaccharide films. Aydinli and Tutas (2000) obtained *WVP* values ranging between 3.2 and 1.8 (10^{-11} g m^{-1} s^{-1} Pa^{-1}) for films of locust bean gum (≈1.0% w/v) with polyethylene glycol (≈0.4% and 1.7% (v/v)). In other work, corn starch-based films (5%) with glycerol (1.4%) presented a *WVP* value of 8.7 (10^{-11} g m^{-1} s^{-1} Pa^{-1}) (Garcia et al., 2006).

Table 4
Values of *WVP*, O_2P, CO_2P and thickness of galactomannan films.

Samples	$WVP \times 10^{-11}$ (g m^{-1} s^{-1} Pa^{-1})	$O_2P \times 10^{-15}$ (g m (Pa s m^2)$^{-1}$)	$CO_2P \times 10^{-15}$ (g m (Pa s m^2)$^{-1}$)	Thickness $\times 10^{-4}$ (m)
0.5CP–1.0Gly	5.25 ± 0.60^{de}	0.97 ± 0.02^{a}	37.57 ± 2.75^{c}	0.028 ± 0.004^{a}
0.5CP–1.5Gly	6.25 ± 0.20^{cd}	0.99 ± 0.13^{ab}	28.81 ± 3.08^{d}	0.030 ± 0.002^{ab}
0.5CP–2.0Gly	7.70 ± 1.00^{ab}	1.10 ± 0.14^{a}	4.10 ± 1.67^{g}	0.032 ± 0.003^{ab}
1.5CP–2.0Gly	5.12 ± 0.50^{de}	1.04 ± 0.11^{a}	14.95 ± 4.57^{ef}	0.035 ± 0.005^{abc}
0.5AP–1.0Gly	5.33 ± 0.01^{e}	0.50 ± 0.01^{c}	47.85 ± 3.00^{b}	0.030 ± 0.001^{a}
0.5AP–1.5Gly	6.98 ± 0.27^{b}	0.53 ± 0.02^{c}	17.40 ± 1.21^{e}	0.031 ± 0.003^{ab}
0.5AP–2.0Gly	8.10 ± 0.50^{a}	0.86 ± 0.02^{b}	10.94 ± 1.44^{f}	0.032 ± 0.003^{ab}
1.0AP–1.0Gly	5.02 ± 0.03^{e}	0.37 ± 0.02^{de}	43.13 ± 2.21^{b}	0.035 ± 0.002^{bc}
1.0AP–1.5Gly	6.47 ± 0.43^{bcd}	0.43 ± 0.02^{d}	29.29 ± 1.51^{d}	0.034 ± 0.002^{b}
1.5AP–1.0Gly	4.89 ± 0.11^{e}	0.31 ± 0.01^{e}	61.19 ± 1.44^{a}	0.036 ± 0.001^{c}
1.5AP–1.5Gly	6.18 ± 0.08^{c}	0.41 ± 0.12^{cde}	47.08 ± 1.94^{b}	0.038 ± 0.003^{c}
1.5AP–2.0Gly	6.81 ± 0.21^{bc}	0.49 ± 0.03^{cd}	30.26 ± 1.14^{d}	0.037 ± 0.001^{c}

$^{a-g}$Means (n = 10) in the same column with different superscripts are significantly different ($p < 0.05$).

3.4. Oxygen permeability (O_2P)

Oxygen is the key factor for oxidation, which is responsible for changes in food odour, colour, flavour and nutrients deterioration. Therefore, films that provide a proper oxygen barrier can help in improving food quality and extending food shelf life. Table 4 presents the O_2P as measured for the selected coatings of AP and CP. It is known that the increase in galactomannan concentration contributes to the decrease of permeability, while it is normally accepted that a higher concentration of glycerol increases O_2P (as it does with *WVP*, as shown before) (Caner et al., 1998; Kester and Fennema, 1986). In general, the samples with higher concentration of plasticizer have higher values of O_2P than the samples with a lower plasticizer concentration. These results can be explained by the apolar nature of the oxygen molecule that does not interact with the polar properties of the glycerol molecule, increasing the film permeability to the oxygen. Similar results were also obtained by Caner et al. (1998) and Kester and Fennema (1986) as the plasticizer decreases the intermolecular attractions between polymeric chains, facilitating the penetration of gas molecules. In the present case, the effect of the galactomannan source seems to have surpassed the effect of glycerol concentration, having statistically significant differences been found between O_2P from films of AP and CP galactomannan. These differences might be explained by the different polymer chain constitution of the two galactomannans; AP galactomannan has a mannose/galactose ratio lower than that of CP galactomannan: 1.35 and 1.85, respectively (Mikkonen et al., 2007; Cerqueira et al., 2009). The lowest values of O_2P were obtained with three formulations: 1.0% AP – 1.0% Gly, 1.5% AP – 1.0% Gly and 1.5% AP – 1.5% Gly.

3.5. Carbon dioxide permeability (CO_2P)

Carbon dioxide is very important to the respiration of living tissues and a higher value of CO_2P can delay fruits softening (Holcroft and Kader, 1999). Table 4 shows CO_2P values for the tested polysaccharide solutions. The results seem to indicate that solutions with a higher concentration of plasticizer produce films with a lower value of CO_2P. The effect of glycerol concentration seems to be, by far, the most important one affecting CO_2P. An opposite effect of glycerol concentration has been noticed for *WVP* and O_2P; those films showing a lower O_2P are the ones that show a higher CO_2P. When glycerol concentration increases the *WVP* and O_2P increased and CO_2P decreased probably as a result of the polar and hydrogen-bonding properties of the glycerol molecule. The highest value of CO_2P was obtained with the formulation: 1.5% AP – 1.0% Gly.

Based on the previously presented criteria – high values of wettability, low water vapour permeability, a low O_2P and a high CO_2P – the following coating/film compositions were chosen to be the most adequate for each fruit: acerola – 0.5% AP and 1.0% Gly; cajá – 1.0% AP and 1.0% Gly; mango and pitanga – 1.5% AP and 1.0% Gly; and seriguela – 0.5 CP and 1.5 Gly (filled in gray in Table 4).

3.6. Mechanical properties

The values of *TS* and *E* were measured in the films selected for each fruit (corresponding to the gray cells in Table 4). Fig. 1 shows that the values of *TS* increase with the increase in galactomannan concentrations for AP films. Comparing the *TS* values between films from AP and CP, Fig. 1 shows that the films of CP with a concentration of 0.5% of galactomannan and 1.5% of glycerol are not statistically different ($p > 0.05$) from AP films with concentrations of 1.5% of galactomannan and 1.0% of glycerol; this may be due to the less substituted structure of the galactomannan of CP (mannose/galactose ratio of 2.88) when compared with the structure of

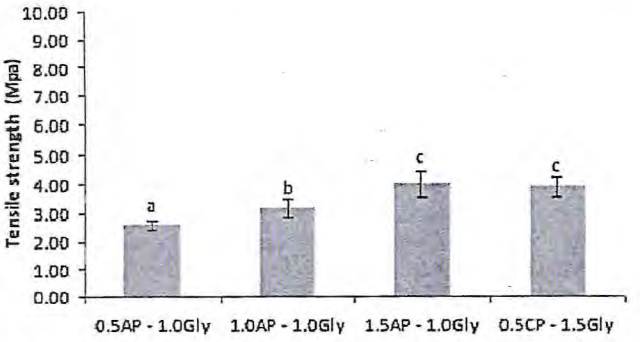

Fig. 1. Tensile at break for the film samples. $^{a-c}$Means ($n = 3$) with a different superscript are significantly different ($p < 0.05$).

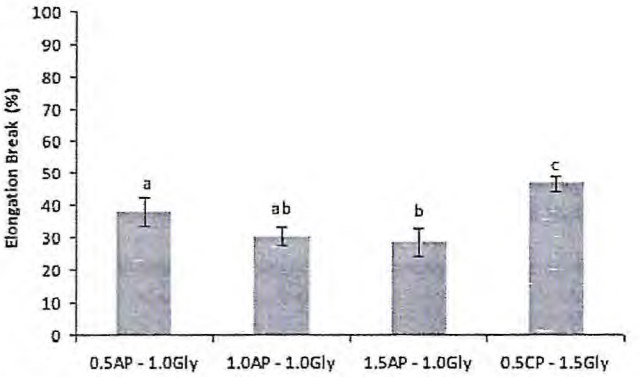

Fig. 2. Elongation at break for the film samples. $^{a-c}$Means ($n = 3$) with different superscripts are significantly different ($p < 0.05$).

AP (mannose/galactose ratio of 1.35); due to its less branched nature, the former may be more prone to establish intermolecular bonds than the latter, therefore providing a film with similar *TS* values despite the lower galactomannan concentration. Similar results were obtained by Mikkonen et al. (2007) when comparing the *TS* of locust bean gum (mannose/galactose ration of 3.5) and guar gum (mannose/galactose ration of 1.5) films. The CP film presents the higher value of *E* (Fig. 2), which may be explained by the higher content of glycerol, but also by the more flexible, less substituted structure of the CP galactomannan when compared to that of the AP galactomannan; however, from the data it is not possible to conclude which of the two effects is the one most influencing the *E* value. A similar trend has been reported by Mikkonen et al. (2007), who have shown that locust bean gum films are more flexible than the guar gum films.

The obtained values are in agreement with other reported studies that use polysaccharide films. Srinivasa et al. (2007) showed that chitosan films, with glycerol as plasticizer, have values of 14.14 MPa and 34.00%, for *TS* and *E*, respectively. Garcia et al. (2006) showed that films with different ratios of corn starch, chitosan and glycerol have values ranging from 60.7 to 7.1 MPa for *TS* and values ranging between 22.5 and 3.0% for *E*, with the film formed by corn starch and glycerol presenting *TS* and *E* values closer to the films of galactomannan.

4. Conclusion

This work describes a methodology to optimize the composition of edible coatings to be applied in different tropical fruits taking into account parameters such as wettability, permeability to gases and mechanical properties.

All fruits were shown to have the ability to participate in non-polar interactions, as a consequence of the higher values of the dispersive component of the surface tension. Different formulations of galactomannan and glycerol showed good values of W_s, being the best values related to the dispersive component of the fruits surface.

The best coating formulation for each of the studied fruits was: acerola – 0.5% of *A. pavonina* and 1.0% of glycerol; cajá – 1.0% of *A. pavonina* and 1.0% of glycerol; mango and pitanga – 1.5% of *A. pavonina* and 1.0% of glycerol; and seriguela – 0.5% of *C. pulcherrima* and 1.5% of glycerol. These formulations should be either applied by immersion or sprayed on the fruits and let dry at room temperature during 3 h.

It has been shown that these novel galactomannan extracted from AP and CP can be applied on the studied fruits based on their surface properties. Future work should include shelf-life studies in order to demonstrate the positive expected effects of the application of these coatings on the fruits: shelf-life extension with improved sensory quality.

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Functional Polysaccharides as Edible Coatings for Cheese

MIGUEL A. CERQUEIRA,*,† ÁLVARO M. LIMA,‡ BARTOLOMEU W. S. SOUZA,† JOSÉ A. TEIXEIRA,† RENATO A. MOREIRA,§ AND ANTÓNIO A. VICENTE†

IBB, Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal, Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Campus do Pici, 60451-970 Fortaleza (CE), Brazil, and Centro de Ciências da Saúde, Universidade de Fortaleza, Av. Washington Soares, 1321 Bairro Edson Queiroz, 60811-905 Fortaleza (CE), Brazil

The objective of the present study was to apply the polysaccharides from different nontraditional sources for cheese coatings. Chitosan, galactomannan from *Gleditsia triacanthos*, and agar from *Glacilaria birdiae* were tested, with different formulations and with the addition of plasticizer and corn oil. The surface properties of the cheese and the wetting capacity of the coatings on the cheese were determined. The three best solutions for each polysaccharide were chosen, further films were cast, and permeability to water vapor, oxygen, and carbon dioxide was determined, along with opacity. The solutions of *G. triacanthos* (formulation: 1.5% of galactomannan, 2.0% of glycerol, and 0.5% of oil) presented the best properties to coat the cheese: -38.76 mN·m^{-1} for wettability; 3.24×10^{-11} (g·(m·s·Pa)$^{-1}$) for water vapor permeability; 0.94×10^{-15} and 15.35×10^{-15} (g·m(Pa·s·m^2)$^{-1}$) for oxygen and carbon dioxide permeabilities, respectively; and opacity values of 5.27%. The O_2 consumption and CO_2 production rates of the cheese with and without coating were evaluated, showing a decrease of the respiration rates when the coating was applied. The uncoated cheese had an extensive mold growth at the surface when compared with the coated cheese. The results show that these coatings can be applied as an alternative to synthetic coatings.

KEYWORDS: Edible coatings; galactomannan; agar; chitosan; cheese

INTRODUCTION

Consumers and food and packaging industries have joined their efforts to reduce the amount of food packaging materials, because of environmental protection. As an answer to that concern, several issues have to be addressed in order to foster the commercial use of biobased primary food packaging materials. These issues include degradation rates under various conditions, changes in mechanical properties during storage, potential for microbial growth, and release of harmful compounds into the packaged food product (*1*). However, consumers around the world demand for food of high-quality, without chemical preservatives, and with an extended shelf life. Therefore, an increased effort has been made to develop new natural preservatives and antimicrobials (*1*).

The future generation of packaging materials will be derived from renewable resources. These materials will ideally be biodegradable. However, natural polymeric materials vary in their rate of degradation in the environment, and some proteins, for example, cannot presently be classified as degradable because of standard definitions (*1*). Edible films and coatings can improve shelf life and food quality by providing good and selective barriers to moisture transfer, oxygen uptake, lipid oxidation, losses of volatile aromas and flavors (*2*), better visual aspect, and reduction of microbiological contamination (*3*). The use of coatings creates a modified atmosphere surrounding the commodity similar to that achieved by controlled or modified atmospheric storage conditions. The modified atmosphere created by edible coatings can protect the food from the moment it is applied, through transportation to its final retail destination, and in the home of the consumer (*1*, *4*).

Cheese is a complex food product consisting mainly of casein, fat, and water. Several researchers have recommended that fresh cheeses (e.g., cream cheese, decorated cream cheese, soft cheese, and cottage cheese) are packaged in modified atmosphere with N_2 and/or CO_2 replacing the O_2 in the package (*5*). However, spoilage caused by yeast and especially bacteria may still occur even at very low O_2 and elevated CO_2 levels (*6*). Semisoft and hard cheeses (whole, sliced, or shredded) have a relatively high respiration rate, which require a packaging material somewhat permeable to CO_2 to avoid an expansion of the packaging.

* To whom correspondence should be addressed. Phone: +351.253.604400. Fax: +351 0.253.678986. E-mail: miguelcerqueira@deb.uminho.pt.
† Universidade do Minho.
‡ Universidade Federal do Ceará.
§ Universidade de Fortaleza.

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Table 1. Spreading Coefficient (*Ws*) Obtained for the Tested Polysaccharide Solutions on Cheese

solution	polysacch. solutions (w/v)	glycerol (w/v)	glycerol/ sorbitol (w/v)	oil (w/v)	spreading coefficient (*Ws*) chitosan[a]	galactomannan from *G. triacanthos*[a]	agar from *G. birdiae*[a]
1	0.5	0.5			**−28.97 ± 1.62** a	−42.94 ± 2.52 a	−45.85 ± 3.27 a
2	0.5	2.0			**−29.81 ± 1.66** a	−57.84 ± 4.87 b	**−36.49 ± 2.65** bc
3	0.5	0.5		0.5	−34.50 ± 1.50 b	**−37.05 ± 2.59** c	−55.46 ± 2.33 d
4	0.5	2.0		0.5	−35.76 ± 2.99 bc	−41.69 ± 2.85 ae	−47.37 ± 1.81 ae
5	0.5		0.5		−34.46 ± 2.33 b	−49.69 ± 4.03 d	−49.62 ± 1.62 e
6	0.5		2.0		**−29.96 ± 3.10** a	−54.79 ± 0.78 b	−45.69 ± 2.46 f
7	0.5		0.5	0.5	−36.62 ± 1.89 bcd	−51.01 ± 2.37 d	−52.81 ± 2.34 d
8	0.5		2.0	0.5	−36.49 ± 2.19 bcd	−41.93 ± 2.77 ae	−47.97 ± 1.81 e
9	1.5	0.5			−38.31 ± 2.11 cde	−58.97 ± 3.65 b	−39.24 ± 1.83 gh
10	1.5	2.0			−38.95 ± 1.65 de	−59.53 ± 3.65 b	−37.61 ± 2.16 cgh
11	1.5	0.5		0.5	−34.65 ± 2.22 b	−59.03 ± 1.86 b	**−30.45 ± 1.39** j
12	1.5	2.0		0.5	−40.13 ± 2.84 e	**−38.76 ± 3.38** ce	−37.52 ± 1.38 cg
13	1.5		0.5		−36.11 ± 1.98 bc	−56.12 ± 2.30 b	−43.97 ± 2.85 fi
14	1.5		2.0		−49.56 ± 0.76 f	−55.99 ± 1.28 b	−46.87 ± 1.50 a
15	1.5		0.5	0.5	−37.74 ± 2.48 cde	**−40.16 ± 1.40** ace	−34.50 ± 3.41 bj
16	1.5		2.0	0.5	−40.31 ± 2.64 e	−41.45 ± 2.59 ae	−40.88 ± 1.14 hi

[a] Values reported are the means ± standard deviations ($n = 20$, 95% confidence interval, at 21.4 ± 0.5 °C). Different letters in the same column indicate a statistically significant difference (Tukey test $p < 0.05$). Bold values are the best values for the same group of polysaccharides.

Meanwhile, O_2 must be kept out to avoid fungal spoilage and oxidation of the cheese. Instead, these products require a balanced oxygen and carbon dioxide atmosphere to prolong their shelf life (*7*).

In semihard cheeses, the factor that most affects cheese stability is water activity (a_w), which depends mainly on moisture and salt contents. During ripening, a_w is not constant but decreases until the cheese surface is in equilibrium with the surrounding atmosphere, thus influencing the microbiological and chemical evolution of the cheese (*8*). Additional environmental factors must be considered in selecting a material for cheese coating (e.g., the light). All of these factors affect not only the cheese's physical characteristics but also its flavor during storage. In fact, many different compounds contribute to cheese flavor, and most of them form during cheese ripening (*9*).

The cheese studied in this work is a cylindrical, yellow, and semihard cheese; it is sold unpackaged, covered with a synthetic/antibiotic coating, and under normal storage conditions, it suffers excessive water loss. The present work evaluates the possibility of using functional polysaccharides as coatings on semihard cheese. The choice of the best coating is made taking into consideration its wettability, permeability, and opacity properties. The coating was applied on a cheese without any previous treatment or ripening period. Extreme conditions were used (cheese without ripening, nor treatment; ambient temperature of approximately 22 °C) to evaluate how the coating can improve respiration, water loss, and surface spoilage of the cheese.

MATERIALS AND METHODS

Materials. Edible coating solutions were prepared with chitosan with a degree of deacetylation of approximately 90% (Aqua Premier Co., Thailand); galactomannan extracted from *Gleditsia triacanthos* seeds (collected in the Botanic Garden, in Oporto, Portugal, in 2006); agar extracted from *Glacilaria birdiae* seaweed (specimens of the red seaweed *G. birdiae* were collected in 2006 on the Atlantic coast of Brazil, Fleixeiras, Trairi − Ceará); corn oil (Sovena, Portugal); 87% glycerol (Panreac, Spain) and 97% sorbitol (Acros Organics, Belgium); Tween 80 (Acros Organics, Belgium); lactic acid (Merck, Germany); and distilled water. A commercial semihard cheese was obtained from Queijo Saloio S.A. (Portugal) without any previous treatment (without ripening and coating) two days after production, the samples being stored at 5 °C and 80% RH until further use. *Regional Saloio* cheese is a full fat cheese produced with a mixture of caprine, bovine, and ovine pasteurized milk, which, after coating with a synthetic coating and an antibiotic protector, is submitted to a short ripening period at low temperatures (5 and 12 °C in different stages of the ripening process). It requires conditions of 0−22 °C for sale. The cheese's physicochemical composition is as follows: moisture, 46%; fat, 25%; protein, 18.4%; total ash, 3.58%; chlorides, 1.54; pH 4.8; and total acidity, 1.40 (*10*).

Polysaccharide Extraction. *Galactomannan Extraction (G. triacanthos).* The polysaccharide extraction was performed as described in Cerqueira et al. (*11*).

Agar Extraction (G. birdie). The red seaweed was cultivated in the sea using seedlings collected during low tide. The seedlings were cleaned and then tied in a structure made of string, which was placed in the sea, where it was anchored and submerged for two months. The polysaccharide extraction was performed with ethanol (purity 99.8%, Riedel-de Haën, Germany) and distilled water as described by Noseda et al. (*12*).

Coating and Film Preparation. The coating formulations were based on a two level factorial design with polysaccharide concentrations of 0.5% and 1.5% (w/v), plasticizer concentrations of 0.5 and 2.0% (v/v), and oil concentrations of 0 and 0.5% (w/v). The coating solutions were prepared dissolving the chitosan (0.5 or 1.5% w/v) in a 1.0% (v/v) lactic acid solution with agitation using a magnetic stirrer during 2 h at room temperature (20 °C); Tween 80 was also added as a surfactant at concentrations of 0.2% (w/v). Corn oil was added in concentrations of 0.5% (w/v), with agitation during 20 min at 60 °C. As plasticizers, glycerol and a mixture of glycerol/sorbitol (50:50) were added at concentrations between 0.5 and 2.0% (w/v).

The coating solutions from galactomannan of *G. triacanthos* (GT) were prepared by dissolving the galactomannan (0.5 or 1.5% w/v) in distilled water with agitation using a magnetic stirrer during 2 h at room temperature (20 °C). As plasticizers, glycerol and a mixture of glycerol/sorbitol (50:50) were added at concentrations between 0.5−2.0% (w/v). Corn oil was added at concentrations of 0.5% (w/v), with agitation during 20 min at 60 °C.

The coating solutions from agar of *G. birdiae* (GB) were prepared dissolving the agar (0.5 or 1.5% w/v) in distilled water with agitation using a magnetic stirrer during 20 min at 60 °C. As plasticizers, glycerol and a mixture of glycerol/sorbitol (50:50) were added at concentrations between 0.5 and 2.0% (w/v). Corn oil was added at a concentration of 0.5% (w/v).

In all cases, a constant amount (13 mL) of solution was cast onto a 5.7 cm diameter glass plate in order to maintain film thickness. The films were dried in an oven at 35 °C during 16 h. These solutions correspond to solutions 1−16, in **Table 1**.

Films were maintained at 20 °C and 50% RH before permeability and opacity tests. (These were the average conditions at the laboratory, as maintained by the existing temperature and humidity control system).

Film Thickness. The film thickness was measured with a digital micrometer (No. 293-561, Mitutoyo, Japan). Five thickness measurements were taken on each testing sample at different points, and the mean values were used for the calculation of water vapor permeability (*WVP*), oxygen permeability (O_2P), and dioxide carbon permeability (CO_2P).

Critical Surface Tension and Surface Tension of Cheese Skin. According to Zisman (*13*), in systems having a surface tension lower than 100 $mN \cdot m^{-1}$ (low-energy surfaces), the contact angle formed by a drop of liquid on a solid surface will be a linear function of the surface tension of the liquid, γ_{LV} (where phase *V* is air saturated with the vapor of liquid, *L*). The Zisman method, briefly described below, is applicable only for low energy surfaces; therefore, it is necessary to determine the surface energy of the cheese.

For a pure liquid, if polar (γ_L^p) and dispersive (γ_L^d) interactions are known, and if θ is the contact angle between that liquid and a solid, the interaction can be described in terms of the reversible work of adhesion, *Wa*, as follows:

$$W_a = W_a^d + W_a^p \Leftrightarrow W_a = 2(\sqrt{\gamma_s^d \cdot \gamma_L^d} + \sqrt{\gamma_s^p \cdot \gamma_L^p}) \quad (1)$$

where γ_S^p and γ_S^d are the polar and dispersive contributions of the surface of the studied solid. Rearranging eq 1 yields

$$\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_s^p} \cdot \sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_s^d} \quad (2)$$

The contact angle determinations of at least three pure compounds, bromonaphthalene (Merck, Germany), formamide (Merck, Germany), and ultra pure water, on the surface of the cheese (cheese skin) combined with the values presented below will allow the calculation of both the independent variable, $(\gamma_L^p)/(\gamma_L^d)^{1/2}$, and the dependent variable, $(1 + \cos\theta)/(2) \cdot (\gamma_L)/(\gamma_L^d)^{1/2})$, from eq 2.

The surface tension and the dispersive and the polar component were, respectively, 72.10, 19.90, and 52.20 $mN \cdot m^{-1}$ for water, 44.40, 44.40, and 0.00 $mN \cdot m^{-1}$ for bromonaphtalene and 56.90, 23.50, and 33.40 $mN \cdot m^{-1}$ for formamide (*14*).

The estimation of the critical surface tension (γ_C) was performed by extrapolation from Zisman plots (*13*). Zisman plots have long been used to characterize the wettability of low-energy surfaces. Zisman plots are obtained by plotting the cosine of the contact angle of pure liquids on a solid surface to be studied against the surface tension of the same series of liquids. The intercept of these curves with $\cos\theta = 1$ is known as the critical surface tension (γ_C). The critical surface tension is an imaginary point of the γ_{sv} value, and it is frequently used to describe the wettability of a surface. It represents the value of γ_{LV} of a liquid above which the spreading of this liquid in a solid surface is complete. The critical surface tension (γ_C) is defined as follows:

$$\gamma_C = \lim \gamma_{LV} \text{ as } \theta \to 0 \quad (3)$$

All experiments were performed at 21.3 ± 0.2 °C with 20 replicates for each of the compounds used.

Wettability. The wettability was studied by determining the values of the spreading coefficient (*Ws*) and the works of adhesion (*Wa*) and cohesion (*Wc*). The adhesive forces promote the liquid spreading on a solid surface and the cohesive forces promote their contraction. The wetting behavior of the solutions will mainly depend on the balance between these forces. The surface tension of the coating solution was measured by the pendant drop method using the Laplace–Young approximation (*15*).

The contact angle (θ) of a liquid drop on a solid surface is defined by the mechanical equilibrium of the drop under the action of three interfacial tensions: solid–vapor (γ_{SV}), solid–liquid (γ_{SL}), and liquid–vapor (γ_{LV}). The equilibrium spreading coefficient (*Ws*) is defined by eq 4 (*16*) and can only be negative or zero.

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{SL} \quad (4)$$

where *Wa* and *Wc* are the works of adhesion and cohesion, defined by eqs 5 and 6, respectively.

$$W_a = \gamma_{LV} + \gamma_{SV} - \gamma_{SL} \quad (5)$$

$$W_c = 2 \cdot \gamma_{LV} \quad (6)$$

Contact angle (θ) and liquid–vapor surface tension (γ_{LV}) were measured by a face contact angle meter (OCA 20, Dataphysics, Germany). The samples of the coatings were taken with a 500 μL syringe (Hamilton, Switzerland), with a needle of 0.75 mm diameter. The contact angle at the cheese surface was measured by the sessile drop method (*17*). Measurements were made in less than 30 s. Ten replicates of contact angle and surface tension measurements were obtained at 21.3 ± 0.5 °C.

Water Vapor Permeability Measurement (WVP). The measurement of water vapor permeability (*WVP*) was determined gravimetrically on the basis of the ASTM E96-92 method (*18*). The film was sealed on the top of a permeation cell containing distilled water (100% RH; 2337 Pa vapor pressure at 20 °C), placed in a desiccator at 20 °C and 0% RH (0 Pa water vapor pressure) containing silica. The cells were weighed at intervals of 2 h during 10 h. Steady-state and uniform water pressure conditions were assumed by keeping 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 film.

Oxygen and Carbon Dioxide Permeability. Oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P) were determined on the basis of the ASTM D 3985-02 (2002) method (*19*). The films were sealed between two chambers, each one having two channels. In the lower chamber, O_2 (or CO_2) was supplied at a controlled (J & W Scientific, ADM 2000, USA) flow rate to keep its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at controlled flow. Nitrogen acted as a carrier for the O_2 (or the CO_2).

In the case of the O_2P measurement, the flow leaving this chamber was connected to an O_2 sensor (Mettler Toledo, Suisse), which measured the O_2 concentration in that flow online. In the case of the CO_2P measurement, the flow leaving this chamber was collected in a syringe for CO_2 quantification. To determine CO_2 concentration, 1 mL of sample was injected in a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110 °C with a column Porapak Q 80/100 mesh 2 m × 1/8″ × 2 mm SS, using a flame ionization detector (FID) at 110 °C. Helium at 23 $mL \cdot min^{-1}$ was used as carrier gas. A standard mixture containing 10% CO_2, 20% O_2, and 70% N_2 was used for calibration.

The flows of the two chambers were connected to a manometer to ensure the equality of pressures (both at 1 atm) between both compartments. As the O_2 (and the CO_2) was carried continuously by the nitrogen flow, it was considered that O_2 (and the CO_2) partial pressure in the upper compartment is null, and therefore, ΔP is equal to 1 atm. Three replicates were obtained for each sample, in each case (O_2P and CO_2P).

Opacity. The opacity of the samples was determined according to the Hunter laboratory method, with a Minolta colorimeter (CR 300; Minolta, Japan), as the relationship between the opacity of each sample on the black standard (Y_b) and the opacity of each sample on the white standard (Y_w).

Cheese Coating. The semihard cheeses, with approximately 270 g, were coated with the selected solution by brushing the surface until all of it was covered, the residual coating being allowed to drip off. Cheeses were left for 4 h at 4 °C until the coating was dry.

O_2 and CO_2 Exchange Rates. The closed system method with air as initial atmosphere was used for the measurement of the gas exchange rate of the whole cheese. The whole cheese was placed inside a hermetic jar at a temperature of 21.86 ± 0.76 °C and an initial relative humidity of 70%. The jar was closed, and air circulation was promoted inside it by using a miniature fan. The atmosphere inside the jar was measured by drawing gas samples with a 1 mL syringe through a septum fitted in the jar lid. The O_2 and CO_2 contents in the jar were determined using a gas chromatograph (Chrompack 9001, Middelburg, Netherlands)

at 110 °C with a column molecular sieve 5A 80/100 mesh 1 m × 1/8″ × 2 mm to separate the O_2 and a column Porapak Q 80/ 100 mesh 2 m × 1/8″ × 2 mm SS to separate the CO_2 using a flame ionization detector (FID) at 110 °C. Helium at 23 mL·min^{-1} was used as carrier gas. A mixture containing 10% CO_2, 20% O_2, and 70% N_2 was used as the standard for calibration. Two replicates of each condition were measured during 48 h.

The O_2 consumption and CO_2 production rates were determined applying eqs 7 and 8 (*20*), developed for a closed system impermeable to gases.

$$dy_{O_2} = -R_{O_2}\frac{w}{V_f}dt \quad (7)$$

$$dy_{CO_2} = R_{CO_2}\frac{w}{V_f}dt \quad (8)$$

where, R_{O2} is the O_2 consumption rate, mL[O_2]·kg^{-1}·h^{-1}, R_{CO2} is the CO_2 production rate, mL[CO_2]·kg^{-1}·h^{-1}, w (kg) is the weight of the cheese, and V_f (mL) is the free volume of the container. The free volume V_f of the package is calculated by

$$V_f = V_p - \frac{w}{\rho_{ch}} \quad (9)$$

where, V_p (mL) is the total volume of the container, w (kg) is the weight of the cheese, and ρ_{ch} is the true density of the cheese, in this case 1.095 × 10^{-3} kg·mL^{-1}, obtained experimentally following the method described by Owolarafe et al. (*21*). The graph of O_2 consumed versus time or CO_2 produced versus time was used to calculate the slopes, which correspond to the derivatives, dy_{O_2}/dt (or dy_{CO_2}/dt).

Weight Loss and Relative Humidity. The weight loss and relative humidity were measured in parallel to the measurements of O_2 and CO_2 exchange rates. The cheese was weighed at the beginning of the experiment (*IW*) and at the end (*FW*), the results expressed as the relative weight loss (*RWL*) defined as

$$RWL = \frac{IW - FW}{IW} \cdot 100 \quad (10)$$

The change in relative humidity (*RH*) of the atmosphere of the jar was followed using a sensor (hygrometer HD 8501 H) fitted inside the jar.

Cheese Surface. The surface of the cheese was inspected for the appearance of molds at the end of the O_2 and CO_2 exchange rate determination (*22, 23*).

Statistical Analyses. Statistical analyses were performed using Analysis of Variance (ANOVA) and linear regression analysis. The Tukey test ($\alpha = 0.05$) was used to determine any significance of differences between specific means (SigmaStat, trial version, 2003, USA).

RESULTS AND DISCUSSION

Critical Surface Tension and Surface Tension of Cheese. The determination of the surface tension and of the critical surface tension of the cheese allows the characterization of the surface of its skin. According to Zisman (*17*), in systems having a surface tension lower than 100 mN·m^{-1} (low energy surfaces), the contact angle formed by a drop of liquid on a solid surface will be a linear function of the surface tension of the liquid, γ_{LV} (where phase V is air saturated with the vapor of liquid, L), which allows the application of the method to determine the wettability.

The surface from the cheese displays values of critical surface and surface tension of 18.33 ± 0.10 mN·m^{-1} and 37.79 ± 0.76 mN·m^{-1}, respectively. The cheese surface is a low-energy surface (<100 mN·m^{-1}) and presents a higher dispersive component (29.93 ± 0.41 mN·m^{-1}), which shows its ability to participate in nonpolar interactions, and a low polar component (7.87 ± 0.37 mN·m^{-1}). A surface with these characteristics interacts with liquid primarily by dispersion forces, influencing the effective spreading of the coating on the cheese surface. The compatibility of the polarity (apolar or polar) of the surface and of the coating may therefore play an important role in the wettability of the surface. The cheese, being very rich in apolar components (e.g., fat), features a significant apolar influence.

Wettability. The wettability was studied by determining the values of the spreading coefficient (*Ws*). Wettability is one of the most important properties when evaluating the capacity of a solution to coat a designated surface. In practical terms, the closer the *Ws* values are to zero, the better a surface will be coated. The results show (**Table 1**) that depending on the amount of polysaccharide, plasticizer, and oil added, the values of *Ws* are statistically different. Considering the solutions tested, the best (higher) value of *Ws* on the cheese surface was determined for each polysaccharide (Tukey test, $p < 0.05$). The best values are shown in bold. (When two or more values are shown in bold for the same polysaccharide, it means that those values are statistically equal).

In chitosan coating solutions, the use of Tween 80 was necessary to increase the otherwise very low values of *Ws* (results not shown). The improvement of *Ws* with the addition of Tween 80 was also shown by Ribeiro et al. (*4*). Tween 80 acts by reducing the superficial tension of the liquid and by increasing the value of *Ws*, therefore improving the compatibility between the solution and the cheese surface. The results obtained demonstrate that chitosan solutions with lower concentration of chitosan and without oil present better values of *Ws*. Solutions 1, 2, and 6 do not present a statistically significant difference (**Table 1**). The higher values of *Ws* of the solutions with lower chitosan concentrations can be explained by the high ratio between the concentration of Tween 80 (which acts by reducing the superficial tension of the liquid) and the concentration of chitosan. The incorporation of oil to the solution of chitosan, in the presence of Tween 80, will form a micellar structure, the interaction between chitosan and oil made through the hydrophilic and hydrophobic parts of the Tween 80 molecule, respectively; this will contribute to the increase of the superficial tension of the liquid once Tween 80 molecules are occupied in the micelles and are no longer available to reduce the superficial tension of the liquid.

In the case of *G. triacanthos*, the solutions with higher values of *Ws* were those containing oil. Solutions 3, 12, and 15 (**Table 1**) do not present a statistically significant difference. The presence of oil in *G. triacanthos* coatings decreased the values of *Ws*. The partly hydrophobic surface of the cheese, as explained previously, presents a good adhesion to the solutions of *G. triacanthos* containing oil, eventually due to the ability of the solution with oil (more hydrophobic) to interact with the cheese surface (*24*).

For the solutions made with *G. birdiae*, solution 11 was the best, presenting statistically significant differences from the other samples (**Table 1**). As in previous cases, the solutions containing oil present the best value of *Ws*.

When there were no statistically significant differences between polysaccharide solutions, it has been assumed that both were equally good in terms of wettability and that their differentiation must be made on the basis of other criteria (such as water vapor, O_2, and CO_2 permeability and opacity).

Water Vapor Permeability (*WVP*). The water vapor permeability is the most extensively studied property of edible films mainly because of the importance of the water in deteriorative reactions. The three best solutions of chitosan (C) in terms of wettability were subsequently analyzed for *WVP*. **Table 2** shows

Table 2. Values of Water, O_2, CO_2 Permeability, and Opacity of the Films

solution		$WVP^a \times 10^{-11}$ (g·(m·s·Pa)$^{-1}$)	$O_2P^a \times 10^{-15}$ (g·m·(Pa·s·m^2)$^{-1}$)	$CO_2P^a \times 10^{-15}$ (g·m·(Pa·s·m^2)$^{-1}$)	opacity[a] (%)
Chitosan	C1	**3.22 ± 0.22** ac	2.35 ± 0.17 a	10.35 ± 0.32 a	2.74 ± 0.21 a
	C2	4.05 ± 0.31 b	1.82 ± 0.19 b	6.85 ± 0.78 b	2.45 ± 0.19 a
	C6	**3.29 ± 0.34** ac	2.26 ± 0.15 a	6.73 ± 0.49 b	2.82 ± 0.03 a
G. triacanthos	GT3	3.93 ± 0.17 b	1.61 ± 0.12 b	34.88 ± 2.17 c	5.62 ± 0.68 c
	GT12	**3.24 ± 0.23** ac	**0.94 ± 0.15** c	15.35 ± 0.99 d	5.27 ± 0.15 c
	GT15	**2.69 ± 0.23** a	2.43 ± 0.29 a	12.84 ± 0.91 da	8.82 ± 0.40 d
G. birdiae	GB2	6.21 ± 0.52 d	**0.95 ± 0.08** c	**41.71 ± 1.80** e	5.27 ± 0.49 c
	GB11	3.79 ± 0.40 bc	**0.61 ± 0.13** c	5.55 ± 0.53 b	9.89 ± 0.61 d
	GB15	4.14 ± 0.24 b	**0.55 ± 0.14** c	3.66 ± 0.54 f	**13.03 ± 0.29** e

[a] Values reported are the means ± standard deviations ($n = 5$, 95% confidence interval). Different letters in the same column indicate a statistically significant difference (Tukey test $p < 0.05$). Bold values are the best values.

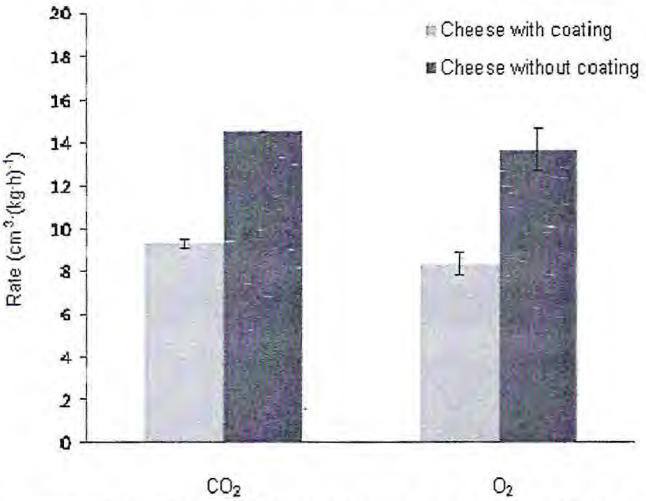

Figure 1. O_2 and CO_2 transfer rates in cheese at 21.86 ± 0.76 °C ($n = 2$, 95% confidence level).

that the values of *WVP* change with the integration of sorbitol and with different concentrations of glycerol. With the addition of sorbitol, the *WVP* decreases, and this observation is in agreement with the conclusions of Garcia et al. (*25*) and Hernandez-Muñoz et al. (*26*). **Table 2** shows that *WVP* for films from solution C2 is statistically significant different from that of the other two (C1 and C6), presenting a higher value of *WVP*. Although an increase of the mean value of *WVP* is observable due to the increase of glycerol concentration (from solution C1 to solution C2), the difference is statistically significant.

The same procedure was adopted for *G. triacanthos* (GT) solutions GT3, GT12, and GT15. Films from solutions GT12 and GT15 showed a lower value of *WVP* without a statistically significant difference, while the value of *WVP* for the films from solution GT15 is significantly different from those obtained with solution GT3 (**Table 2**). An increase of the concentration of GT galactomannan corresponds to a decrease of *WVP*, presumably due to a stronger gel network, where the polysaccharide molecules are closer together. Furthermore, the solution with sorbitol (GT15) showed the lowest value of *WVP*; this observation may be explained by the larger size and lower hygroscopicity of the sorbitol compared to those of glycerol, reducing its ability to affect hydrogen bonding between polysaccharide chains (*27*).

Table 2 also shows the values of *WVP* for the best solutions of *G. birdiae* (GB2, GB11, and GB15). The lower *WVP* values were registered for films from solutions GB11 and GB15, which are not statistically different but have a statistically significant difference with solution GB2. In parallel to what happened with the films from solutions of *G. triacanthos*, increasing the concentration of *G. birdiae* led to lower values of *WVP*.

The addition of oil promoted a decrease of *WVP* in both *G. triacanthos* and *G. birdiae* films. In this line, Hernandez-Muñoz et al. (*26*) indicated that *WVP* occurs through the hydrophilic portion of the film; therefore, depending on the hydrophilic–hydrophobic ratio of the films, Avena-Bustillos et al. (*28*) showed that *WVP* decreases with the addition of beeswax to sodium caseinate films. Also, Péroval et al. (*29*) showed that arabinoxylan films with hydrogenated palm oil have lower *WVP* values than films without oil. Pranoto et al. (*30*) showed similar results with alginate-based films containing garlic oil.

Oxygen Permeability (O_2P). Oxygen is the key factor in cheese preservation. Films that provide a proper oxygen barrier can help improve food quality and extending food shelf life. **Table 2** presents the values of O_2P of the analyzed samples. In the case of chitosan films, the samples with higher concentration of plasticizer have statistically higher values of O_2P than the samples with lower concentration, which were also shown by Caner et al. (*31*). The plasticizer decreases the intermolecular attractions between polymeric chains, facilitating the penetration of gas molecules (*2*). However, the partial replacement of glycerol by sorbitol provoked an increase of the O_2P value, as can be observed when comparing the results for films from solutions C2 and C6. As mentioned before, this difference can be explained by the different molecular size and hygroscopicity of sorbitol and glycerol (*27*).

Films from solution GT12 show the lower value (significantly different) of O_2P, corresponding to the higher concentration of plasticizer and also to the higher concentration of *G. triacanthos* galactomannan. It is known that the increase of galactomannan concentration contributes to the decrease of permeability, while it is normally accepted that a higher concentration of glycerol increases O_2P. In the present case, the effect of the galactomannan concentration seems to have surpassed the effect of glycerol concentration, contrary to what has been observed for the solutions of chitosan. Garcia et al. (*25*) found similar results for starch-based films and explained their results by stating that the addition of plasticizer decreases the presence of pores and cracks, improving dispersion and decreasing gas permeability. There were no statistically significant differences for the films from solutions of *G. birdiae* in terms of O_2P (**Table 2**), having lower values when compared with the films of GT and C.

Carbon Dioxide Permeability (CO_2P). **Table 2** shows the comparison of CO_2 permeability values for the different polysaccharides. The chitosan films displayed lower values of CO_2P, and the different films of C do not present a statistically

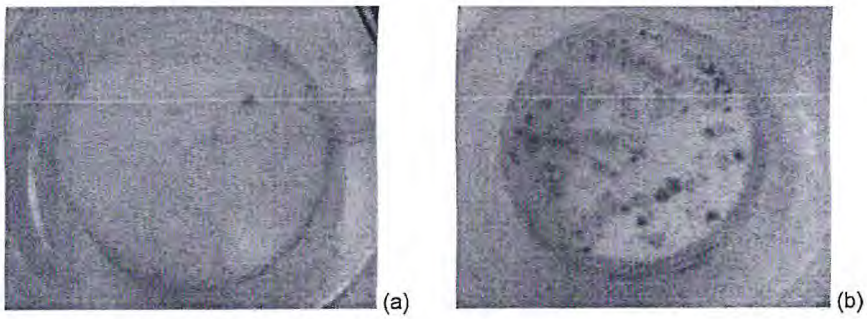

Figure 2. Cheese in a jar, with coating (a) and without coating (b).

significant difference. The film from solutions C2, however, shows the lower value. These results seem to indicate that solutions with a higher concentration of plasticizer produce films with a lower value of CO_2P. The addition of plasticizer decreases the presence of pores and cracks, improving the dispersion and decreasing the gas permeability (*25*).

For *G. triacanthos* films, the increase of the polysaccharide concentration and the addition of sorbitol provoked a decrease of CO_2P. Films from solution GT3 show a statistically significant difference from those of solutions GT12 and GT15 (**Table 2**).

G. birdiae films display a very significant decrease of the value of CO_2P with the increase of polysaccharide concentration. Also here, the addition of sorbitol decreases the value of CO_2P, as shown by Garcia et al. (*25*). The effect of polysaccharide concentration seems to be, by far, the most important one affecting CO_2P.

Opacity. The opacity means a smaller transparency, important to control the incidence of light on the cheese (*32*). Opacity values increase with the concentration in polysaccharide for films from solutions of GT and GB, the solutions with sorbitol and oil being those with a higher value of opacity. The addition of lipid caused the films to become whitish. **Table 2** shows that the incorporation of corn oil in the films increased the opacity. Yang et al. (*33*) demonstrated that gellan film also has increased opacity with the increase of lipid concentration.

Criteria for Choosing a Coating. When choosing an adequate coating composition for the cheese under consideration, there are a number of criteria that should be met. Some of those (such as wettability) have already been considered. Others, such as gas transport properties and opacity, should be met in order to (i) decrease the water loss of the cheese (i.e, lower *WVP* values); (ii) decrease the O_2 permeability (i.e., lower O_2P values), once the oxygen in contact with the cheese contributes to the oxidation of fats and to the growth of undesirable microorganisms (*13*); (iii) increase the shelf life of cheese, by increasing the lag-phase for the growth of coliforms (and other Gram-negative spoilage bacteria), yeasts and molds (*9*), i.e., high CO_2P values; and (iv) decrease the light incidence in the cheese (light promotes fat oxidation) (*13*), i.e., high values of opacity. Having in mind the criteria explained above, it is possible to select the best values of the permeability for water vapor, O_2, and CO_2, and opacity (**Table 2**).

In **Table 2**, the variables (*WVP*, O_2P, and CO_2P, opacity) were placed by decreasing order of importance, and solution GT12 was chosen as the best option for coating cheese despite the fact that its value of CO_2P was not the highest among those determined in this work. In fact, previous works have shown that there are advantages and disadvantages both for low and high CO_2P values (*34*), thus justifying the choice for an intermediate one.

O_2 and CO_2 Transfer Rates in Cheese. To understand how the GT coating solution can improve water loss and gas exchange, the whole cheese was coated using a solution with the formulation of GT12, and O_2 and CO_2 transfer rates were compared with those of the cheese without coating.

The concentration of the gases was measured during 48 h, the gas transfer rate was calculated, and the results are presented in **Figure 1**. The coated cheese clearly displays a lower gas exchange rate, and it is also clear that the rate of CO_2 production is higher than that of O_2 consumption.

The obtained values for the O_2 consumption rate ranged between 13.65 and 8.33 $mL \cdot kg^{-1} \cdot h^{-1}$, while the CO_2 production rate ranged between 14.52 and 9.27 $mL \cdot kg^{-1} \cdot h^{-1}$ for uncoated and coated cheese, respectively. These values are high when compared with those of other cheese types. Fedio et al. (*35*) studied the gas exchange in Swiss cheese, and they found values ranging from 1 to 2 $mL \cdot kg^{-1} \cdot h^{-1}$. These values are difficult to compare, however, because of differences in cheese composition and in the extent of cheese maturation (e.g., ours was not subjected to maturation). The presence of molds in the surface of the cheese may also be related to the differences found: the coated cheese with less molds at the surface showed lower values of RO_2 and RCO_2.

Weight Loss and Surface Evaluation. The coated cheese presents a relative weight loss of $0.11 \pm 0.04\%$, while the cheese without coating loses $0.84 \pm 0.07\%$. Therefore, the coating allows a decrease in weight loss (ca. 8-fold the value in the absence of coating).

During the experiments, the values of relative humidity inside the jar increased rapidly, and at the end of the experiment, a value of 100% was reached. After 48 h from the beginning of the experiment, the cheese began to show fungal growth at the surface, mostly occurring on the uncoated cheese. Visual evaluation confirmed that the uncoated cheese had extensive mold growth with almost the entire surface covered with mold colonies after only 48 h (**Figure 2**). The coating solution GT12 appears to have inhibited the growth of molds, when compared with uncoated cheese. Further work has to be made to confirm the suitability of this coating to increase the shelf life of cheese after ripening and at different storage temperatures.

In conclusion, the cheese with coating has lower gas transfer rates as well as a decrease of the relative weight loss (ca. 8-fold less the value in the absence of coating). Visual evaluation also confirmed that the uncoated cheese suffered from an extensive mold growth when compared with the coated cheese.

The present work can serve as a guide for the use of new coatings for cheese as alternatives to synthetic coatings and may also be a guide for the study of future new materials for this purpose.

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New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits – Influence on fruits gas transfer rate

Álvaro M. Lima [a], Miguel A. Cerqueira [b], Bartolomeu W.S. Souza [b], Ed Carlos M. Santos [a], José A. Teixeira [b], Renato A. Moreira [c], António A. Vicente [b,*]

[a] Departamento de Bioquimica e Biologia Molecular, Federal University of Ceará, Campus do Pici, CEP 60451-970 Fortaleza, CE, Brazil
[b] IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[c] Centro de Ciências da Saúde, Universidade de Fortaleza, Av. Washington Soares, 1321 Bairro Edson Queiroz, 60811-905 Fortaleza, CE, Brazil

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ABSTRACT

The objective of this work was to produce new edible coatings, based on a mixture of galactomannans from novel sources (seeds of *Adenanthera pavonina* and *Caesalpinia pulcherrima*), collagen and glycerol, and to determine their influence in gas transfer rates when they are applied on mangoes and apples. The first part of the work consisted in obtaining coating solutions with the convenient values of wettability for each fruit; such coating solutions were then characterized in terms of their permeability (to CO_2, O_2 and water vapour), mechanical properties, colour and opacity. Gas transfer rates from mangoes coated with a solution of *A. pavonina* galactomannan (0.5%), collagen (1.5%) and glycerol (1.5%) were compared with those of mangoes without coating: 28% less O_2 consumption and 11% less CO_2 production were observed in coated mangoes. The same procedure was performed in apples (in this case using *C. pulcherrima* galactomannan (0.5%), collagen (1.5%) and no glycerol); the CO_2 production and the O_2 consumption was approximately 50% lower in apples with coating than in apples without coating. The results suggest that these coatings can reduce gas transfer rates in these fruits, and can be therefore important tools to extend their shelf life.

1. Introduction

One of the most important problems in fruit trading is their short shelf life. Fruits are living organisms which continue to respire after harvesting. Shelf-life can be extended by reducing gases transfer rates and by controlling factors such as the gas composition (O_2, CO_2 and ethylene), of the gas surrounding the fruit, water vapour permeability, temperature, relative humidity and light. An important strategy to control some of these factors is the use of modified atmospheres (Lin and Zhao, 2007). When the fruit is harvested, there is a change of the gaseous balance between the consumption of oxygen and the production of carbon dioxide. In this new condition the cells are not renewed and the gas transfer rates increase, causing a metabolic loss and taking the fruit to a gradual maturation and eventual senescence. The gas transfer rates depend of both of internal and external factors. The internal factors include the species, the cultivar, and the growth state, while the external factors include the atmospheric composition O_2, CO_2 and ethylene ratios, the temperature and other stress factors (Kluge et al., 2002).

Edible films and coatings act as semipermeable barriers which may be able to keep the quality of the food. Being biodegradable they, offer alternative packaging systems which cause reduced environmental damages. The modified atmosphere created by the coating generates a physical capture of CO_2 inside the fruit and a partial sealing of the pores, reducing the gaseous exchange and reducing the gas transfer rates.

Edible films and coatings can consist of three types of biological materials: polysaccharides, proteins and lipids (Lin and Zhao, 2007). In general, due to their hydrophilic nature, polysaccharide films generally exhibit poor water vapour barrier ability. However, certain polysaccharides, applied in the form of high moisture viscous coatings, can retard moisture loss from coated foods by functioning as sacrificing agents rather than moisture barriers (Kester and Fennema, 1986). Galactomannans, being reserve carbohydrates, are found in various albuminous or endospermic seeds. The physicochemical and conformational properties of the galactomannans are mainly related with the ratio mannose/galactose (M/G) and the distribution of galactose residues throughout the main chain (Cerqueira et al., 2009). The galactomannan species used in the present work were two plants of Leguminosae family, *Adenanthera pavonina* and *Caesalpinia pulcherrima*. *A. pavonina* is native from tropical Asia and is used in reforestation, as ornamental plant

* Corresponding author. Tel.: +351 253 604 419; fax: +351 253 678 986.
E-mail address: avicente@deb.uminho.pt (A.A. Vicente).

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and constitutes an important source of wood. The baking of the seeds and of the wood allows its use in the treatment of pulmonary infections, and also in the treatment of chronic ophthalmia (Fonseca and Perez, 2003). *C. pulcherrima* is an ornamental plant found throughout India, but it can be found in other countries as well, especially in Brazil. Some of the constituents extracted from *C. pulcherrima* were found to possess anti-tumour (Che et al., 1986; Patil et al., 1997) and antimicrobial properties (Ragasa et al., 2003). Galactomannans from *A. pavonina* and *C. pulcherrima* present a different mannose/galactose ratio. By using these two galactomannan species, the authors wanted to evaluate the effect of different monosaccharide composition in the coatings behaviour.

The polar nature of proteins confers to protein films the property of being excellent barriers to oxygen (apolar), possibly due to their impermeability to apolar substances and the high value of cohesive energy that they contain, however they are poor water vapour barriers (Kester and Fennema, 1986). Collagen is an abundant protein constituent of connective tissue in vertebrate (about 50% of total human protein) and invertebrate animals (Johnston-Banks, 1990).

Blending has acquired importance in improving the performance of the polymeric materials. It has become an economical and versatile way to obtain materials with a wide range of desirable properties. Biodegradable protein and polysaccharide films with satisfactory mechanical properties and good appearance are potential and ecological alternatives to synthetic packaging in pharmaceutical and food applications.

Based in this type of knowledge, a strategy deserving to be evaluated is the development of films produced from blends of galactomannans and collagen, with the purpose of improving coating properties through the possible synergism between them.

Apple is pomaceous fruit, of the species *Malus domestica*, from the rose family *Rosaceae*. It is one of the most widely cultivated fruits. Forty five million tons of apples were grown worldwide in 2002, with a value of about 10 billion USD. China produced almost half of this total. Argentina is the second leading producer, with more than 15% of the World production. The United States is the third leading producer, accounting for 7.5% of World production. France, Italy, South Africa and Chile are among the leading apple exporters (Dobrzanski et al., 2006).

Mango (*Mangifera indica*) is a tropical fruit that belongs to the genus *Mangifera* which consists of about 35 species, in the flowering plant family *Anacardiaceae*. Mango is one of the most appreciated fruits of tropical origin, being currently cultivated in practically all the countries of the tropical and equatorial zone of the World. In 1998, it was the fruit that more contributed to the Brazilian exports of fresh fruits (Souza, 2002). In 2003, Brazil was responsible for the production of 845,000 tons in an area of 67,000 hectares, occupying the second place as main exporting country in amount of mangoes (FAO, 2006).

Apple and mango are climacteric fruits. They suffer a predictable elevation in respiration rates and typically ripen and show an increase of ethylene production at or just prior to the onset of ripening and a necessity for ethylene to complete the process (Giovannoni, 2007).

Due to the economical relevance of these two fruits and the problems occurring in their preservation, they were selected as targets for the present work. Apples have a considerably longer shelf-life than those of many other climacteric species, nevertheless fruit quality deteriorates gradually after ripening (Wang et al., 2009). There are great differences in the rates of deterioration among cultivars, presenting loss of firmness, softness and mealiness as the main problems (Varela et al., 2008; Wang et al., 2009). Mango ripens quickly after harvest (between 3 and 9 days). This short period seriously restricts long distance marketing (Gomez-Lim, 1997). Sensitivity to disease as well as perishability due to ripening or softening of the fruit, limit its potential in terms of storage, packaging and transport (Mitra and Baldwin, 1997).

The objective of the present work was to produce new edible coatings, based in the mixture of novel galactomannans (extracted from seeds of *A. pavonina* and *C. pulcherrima*, two plants which are widespread in Brazil and have no commercial exploitation so far), collagen and glycerol, to characterize the coatings presenting the best coating ability (determined based on the wettability) in terms of their physical–chemical properties and to evaluate the use of these coatings on commercially important fruits (apple and mango) featuring different respiration patterns.

2. Materials and methods

2.1. Plant material

The seeds of *A. pavonina* and *C. pulcherrima* were collected in the Federal University of Ceará, Campus of Pici, Fortaleza, CE, Brazil during June 2006 and after being cleaned they were maintained in a cool, dry place until further use.

2.2. Animal material

The soluble anionic collagen was prepared by alkaline treatment of bovine intestinal submucosal tissue, at 20 °C for a period of 72 h, followed by homogenization in 0.5 mol L^{-1} acetic acid (Merck, Germany) solution and brought to a final collagen concentration of 10 g L^{-1} (Goissis et al., 1994).

2.3. Galactomannan extraction

The polysaccharide extraction was performed as described previously (Cerqueira et al., 2009). In this process, the seeds were removed from the pods, cleaned and placed in a blender, where they were mechanically broken. After this operation, the endosperm was manually separated from the germ and the hull and was suspended in ethanol (purity 99.8%, Riedel-de Haën, Germany) at 70 °C for 15 min. The ethanol was decanted and distilled water was added in a proportion of 1:5 (endosperm:water); the resulting suspension was left to rest for approximately 24 h. Then water, in a proportion of 1:10 (suspension:water) was added and mixed in a blender for 5 min. The endosperm mixed in the blender was filtered through a nylon net followed by a centrifugation step at 3800*g* (Sigma 4K, B. Braun, Germany) for 20 min at 20 °C. The precipitation of the galactomannan was achieved by adding the supernatant to ethanol (purity 99.8%, Riedel-de Haën, Germany) at a ratio of 1:2. The ethanol was decanted and the precipitated galactomannan was lyophilized (Christ, alpha 2–4, Germany) and maintained in a dry place until further use.

2.4. Coating and film preparation

The coating solutions (blends) were prepared dissolving the lyophilized galactomannans in distilled water followed by the addition of the collagen solution and the plasticizer (glycerol 87%, Panreac, Spain). Analyses were performed with different galactomannan concentrations (0.5%, 1.0% and 1.5% (w/v)) with different collagen concentrations (0.5%, 1.0% and 1.5% (w/v)), obtaining different galactomannan/collagen ratios, and varying plasticizer (glycerol) concentrations (0%, 0.5%, 1.0% and 1.5% (v/v)) (see Table 1 for details). The concentrations were chosen based on preliminary experiments. Galactomannan and collagen blends present good miscibility at the concentrations used. Solutions were homogenized during 5 min at room temperature (20 °C) and left to stabilize during 10 min. The wettability of these solutions was

Table 1
Galactomannan (from *A. pavonina* and *C. pulcherrima*), collagen, and glycerol concentrations used in coating formulation, and galactomannan/collagen ratios.

Sample	Galactomannan % (w/v)	Collagen % (w/v)	Galactomannan/ collagen ratio	Glycerol % (w/v)
S.1	0.5	1.5	1/3	0.0
S.2	1.0	1.0	1	0.0
S.3	1.5	0.5	3	0.0
S.4	0.5	1.5	1/3	0.5
S.5	1.0	1.0	1	0.5
S.6	1.5	0.5	3	0.5
S.7	0.5	1.5	1/3	1.0
S.8	1.0	1.0	1	1.0
S.9	1.5	0.5	3	1.0
S.10	0.5	1.5	1/3	1.5
S.11	1.0	1.0	1	1.5
S.12	1.5	0.5	3	1.5

determined (see Section 2.6) in order to select the blends with the best wettability values in mango and apple. The films were prepared with a constant amount (28 mL) of solution, which was cast onto a 9 cm diameter glass plate in order to maintain the film thickness. The films were dried in an oven at 35 °C during 16 h. Films were maintained at 20 °C and 50% RH, before permeability, colour and mechanical tests were performed.

2.5. Fruits conditioning

Mangoes and apples were purchased from a local supermarket (Braga, Portugal). All fruits were kept at 8–10 °C until further use. The fruits were selected for their uniformity, size, colour and absence of damage and fungal infection. Before testing, the fruits were left at room temperature (20 °C) and their surface was cleaned with distilled water. Thin portions of the outer surface (skin) of the fruits were cut with a knife and placed on a glass plate. Three fruits were used for each of the contact angle and surface tension measurements, providing a total of 20 skin portions.

2.6. Wettability

The wettability was studied by determining the values of the spreading coefficient (W_s) and the works of adhesion (W_a) and cohesion (W_c). The adhesive forces promote the liquid spreading in a solid surface and the cohesive forces promote their contraction. The wetting behaviour of the solutions will mainly depend on the balance between these forces. The surface tension of the coating solution was measured by the pendant drop method using the Laplace–Young approximation (Song and Springer, 1996).

The contact angle (θ) of a liquid drop on a solid surface is defined by the mechanical equilibrium of the drop under the action of three interfacial tensions: solid–vapour (γ_{SV}), solid–liquid (γ_{SL}), and liquid–vapour (γ_{LV}). The equilibrium spreading coefficient (W_s) is defined by Eq. (1) (Rulon and Robert, 1993) and can only be negative or zero.

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{SL} \quad (1)$$

where W_a and W_c are the works of adhesion and cohesion, defined by Eqs. (2) and (3), respectively.

$$W_a = \gamma_{LV} + \gamma_{SV} - \gamma_{SL} \quad (2)$$

$$W_c = 2\gamma_{LV} \quad (3)$$

To obtain this parameter it is necessary to determine the contact angles of the coating on the surface of the fruits. Contact angle (θ) and liquid–vapour surface tension (γ_{LV}) were measured in a face contact angle meter (OCA 20, Dataphysics, Germany). The samples of the coatings were taken with a 500 μL syringe (Hamilton, Switzerland), with a needle of 0.75 mm of diameter. The contact angle at the fruit surfaces was measured by the sessile drop method (Newman and Kwok, 1999). In order to do this, a droplet of the tested liquid was placed on a horizontal surface and observed with a face contact angle meter. Measurements were made in less than 30 s. Twenty replicates of contact angle and surface tension measurements were performed at (20 ± 1) °C.

An estimation of the critical surface tension (γ_c) of the fruits' surface was obtained by extrapolation from the Zisman plot (Zisman, 1964), which was built using water, formamide and bromonaphthalene as reference liquids. The Zisman method, described below, is applicable only to systems with a surface tension below 100 mN m^{-1} (low-energy surfaces); therefore it is necessary to determine the surface energy of apple and mango to confirm the applicability of that method.

Several authors had developed the idea that interfacial liquid–vapour tension can be separated in polar and dispersive components (Owens and Wendt, 1969; Kaelble, 1970).

The polar and dispersive contributions to the superficial tension are added, yielding:

$$\gamma_L = \gamma_L^d + \gamma_L^p \quad \gamma_S = \gamma_S^d + \gamma_S^p \quad (4)$$

For a pure liquid, polar (γ_L^p) and dispersive (γ_L^d) interactions are known, and if the contact angle between that liquid and a solid is obtained, the interaction can be described by:

$$W_a = W_a^d + W_a^p \Longleftrightarrow W_a = 2 \cdot \left(\sqrt{\gamma_S^d \cdot \gamma_L^d} + \sqrt{\gamma_S^p \cdot \gamma_L^p} \right) \quad (5)$$

$$\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^p} \cdot \sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_S^d} \quad (6)$$

The contact angle determinations of at least three pure compounds on the surface of vegetables will allow the calculation of both the independent variable, $\left(\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} \right)$, and the dependent variable, $\left(\frac{1+\cos\theta}{2} \cdot \frac{\gamma_L}{\sqrt{\gamma_L^d}} \right)$, from Eq. (6).

The surface tension, the dispersive and the polar component were, respectively, 72.10, 19.90 and 52.20 mN m^{-1} for water, 44.40, 44.40 and 0.00 mN m^{-1} for bromonaphthalene and 56.90, 23.50 and 33.40 mN m^{-1} for formamide (Busscher et al., 1984).

The estimation of the critical surface tension (γ_C) was performed by extrapolation from Zisman plots (Zisman, 1964). Zisman plots have long been used to characterize the wettability of low-energy surfaces. Zisman plots are obtained by plotting the cosine of the contact angle of pure liquids on a solid surface to be studied against the surface tension of the same series of liquids. The intercept of these curves with $\cos\theta = 1$ is known as the critical surface tension (γ_C). The critical surface tension is an imaginary point of the γ_{SV} value and it is frequently used to describe the wettability of a surface. It represents the value of γ_{LV} of a liquid above which the spreading of this liquid in a solid surface is complete. The critical surface tension (γ_C) is defined as:

$$\gamma_C = \lim \gamma_{LV} \quad \text{as } \theta \to 0 \quad (7)$$

2.7. Film thickness

The film thickness was measured with a hand-held digital micrometer (No. 293–561, Mitutoyo, Japan) having a sensitivity of 0.001 mm. This measurement was carried out at the end of the permeability test to avoid the effect of mechanical damage that could be caused on the film during the thickness measurement. Five thickness measurements were taken on each testing sample in different points and the mean values were used in permeability and mechanical calculations.

2.8. Oxygen and carbon dioxide permeability

Oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P) were determined based on ASTM D 3985-02 (2002) method. The films were sealed between two chambers, having each one two channels. In the lower chamber O_2 (or CO_2) was supplied at a controlled (J&W Scientific, ADM 2000, USA) flow rate to maintain its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at controlled flow. Nitrogen acted as a carrier for the O_2 (or the CO_2).

In the case of O_2P measurement, the flow leaving this chamber was connected to an O_2 sensor (Mettler Toledo, Switzerland) which measured the O_2 concentration in that flow on-line. In the case of CO_2P measurement the flow leaving this chamber was collected in a syringe for CO_2 quantification. To determine CO_2 concentration, 1 mL of sample was injected in a gas chromatograph (Chrompack 9001, Middelburg, The Netherlands) at 50 °C with a column Porapak Q 80/100 mesh 2 m × 1/8 in. × 2 mm SS, using a thermal conductivity detector (TCD) at 110 °C. Helium at 23 mL min^{-1} was used as carrier gas. A standard mixture containing 10% CO_2, 20% O_2 and 70% N_2 was used for calibration.

The flows of the two chambers were connected to a manometer to ensure the equality of pressures (both at 1 atm.) between both compartments. As the O_2 (and the CO_2) was carried continuously by the nitrogen flow, it was considered that partial pressure of O_2 (and the CO_2) in the upper compartment is null, therefore ΔP is equal to 1 atm. Three replicates were obtained for each sample, in each case (O_2P and CO_2P).

2.9. Water vapour permeability measurement

The water vapour permeability (WVP) of the films was determined gravimetrically based on the ASTM E96-92 method (Mc Hugh et al., 1993; Guillard et al., 2003). The test film was sealed on the top of a permeation cell containing distilled water (100% RH; 2.337 × 10^3 Pa vapour pressure at 20 °C), placed in a desiccator which was maintained at 20 °C and 0% RH (0 Pa water vapour pressure) with silica gel. The water transferred through the film and adsorbed by the desiccant was determined from the weight loss of the permeation cell. The cups were weighed 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 cup by using a fan inside the desiccator (Mc Hugh et al., 1993). The slope of weight loss versus time was obtained by a linear regression. The measured WVP of the films was determined as follows:

$$WVP = \frac{WVTR \cdot L}{\Delta P} \tag{8}$$

where WVTR is the water vapour transmission rate (g m^{-2} s^{-1}) measured through a film, L is the mean film thickness (m), and ΔP is the partial water vapour pressure difference (Pa) across the two sides of the film. The measurements were repeated three times for each film.

2.10. Colour and opacity

The colour and opacity of films were determined with a Minolta colorimeter (Cr 400; Minolta, Japan). A white standard colour plate (Y = 93.5, x = 0.3114, y = 0.3190) was used as a background for colour measurements of the coated films, and the L^*, a^*, b^* values of each film were evaluated by reflectance measurement.

The opacity of a material is an indication of how much light passes through it: the higher the opacity, the lower the amount of light that can pass through the material. Generally, the opacity is calculated from reflectance measurements. The opacity of the samples was determined according to the Hunter lab method, as the relationship between the opacity of each sample on a black standard (Y_b) and the opacity of each sample on the white standard (Y_w) (Eq. (9)). The measurements were repeated three times for each film.

$$\text{Opacity } (\%) = \frac{Y_b}{Y_w} \cdot 100 \tag{9}$$

2.11. Mechanical properties

TS and E were measured with an Instron Universal Testing Machine (Model 4500, Instron Corporation, USA) following the guidelines of ASTM method D 882-91 (1991). The initial grip separation was set at 30 mm and the crosshead speed was set at 5 mm min^{-1}. 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. E was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (30 mm) and expressed as a percentage. According to the ASTM standard, film strips with a length of 45 mm and a width of 20 mm were used. The measurements were repeated five times for each film.

2.12. Coating application

The fruits, after being cleaned with distilled water, were coated with the selected solution by brushing the surface until all of it was covered, being the residual coating allowed to drip off. Fruits were left during 4 h at 20 °C until the coating was dry. Two groups of coated fruits were prepared: with apples coated with 0.5% *C. pulcherrima* galactomannan, 1.5% collagen and no glycerol, an another with mangoes coated with 0.5% *A. pavonina* galactomannan, 1.5% collagen and 1.5% glycerol. Two control groups, one of each of the fruits, without coatings were also prepared. These fruits were used for the determination of O_2 and CO_2 transfer rates.

2.13. O_2 and CO_2 transfer rates

The O_2 and CO_2 production/consumption rates in apple and mango were measured by placing fruits inside a hermetic jar and closing it. The air circulation was promoted inside the jar by using a miniature fan. The atmosphere inside the jar was measured by drawing the gas samples with a 1 mL syringe through a septum fitted in the jar lid. The O_2 and CO_2 content in the jar was determined using a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 50 °C with a column mol sieve 5A 80/100 mesh 1 m × 1/8 in. × 2 mm to separate the O_2 and a column Porapak Q 80/100 mesh 2 m × 1/8 in. × 2 mm SS to separate the CO_2 using a thermal conductivity detector (TCD) at 110 °C. Helium at 23 mL min^{-1} was used as carrier gas. A mixture containing 10% CO_2, 20% O_2 and 70% N_2 was used as standard for calibration. All determinations were performed at 20 ± 1 °C during 60 h. For O_2 and CO_2 transfer rates determination three replicates were performed for each of the control group and for each of the coated fruits groups. In each of the replicates three fruits were used.

2.14. Statistical analyses

The statistical analyses of the data were performed using Analysis of Variance (ANOVA), Tukey mean comparison test ($p < 0.05$) and regression analysis (SigmaStat, Trial Version, USA). The W_s data presented in the surface plots was adjusted to polynomial equations using Statistica software (Release 7, Edition 2004, Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Surface and critical surface tension

The estimated values of the polar and dispersive components of the surface tension are 1.71 and 24.77 mN m^{-1} for mango, and 0.68 and 27.13 mN m^{-1} for apple, respectively. The surface tensions of the mango and apple are the sum of the two components (26.48 and 27.81 mN m^{-1}, respectively). Both fruits have therefore low-energy surfaces. This type of surface interacts with liquids primarily through dispersion forces (Rulon and Robert, 1993).

Once both values of the surface tension are lower than 100 mN m^{-1}, the Zisman method can be applied to estimate the critical surface tension by extrapolation from the corresponding Zisman plot. The values obtained in the present work were 19.5 mN m^{-1} for mango and 25.4 mN m^{-1} for apple, and are in agreement with those published in other works with values of 17.4 mN m^{-1}, 18.8 mN m^{-1} and 23 mN m^{-1} to tomato, strawberry and orange, respectively (Casariego et al., 2008; Hagenmaier and Baker, 1993). It must be noted that critical surface tension values should be lower than the surface tension values for a given surface (Dann, 1970). The results obtained in the present work are in agreement with this requirement.

3.2. Wettability

The optimization of the composition of the coating solutions based on their ability to spread over a surface can be made considering three parameters: the wettability, the adhesion and the cohesion coefficients. The control of the adhesion and cohesion coefficients is very important because if the former promotes the spreading of the liquid, the later promotes its contraction (Ribeiro et al., 2007) and an adequate equilibrium between these two forces is necessary. The wettability was evaluated by determining the values of the spreading coefficient (W_s). Wettability is one of the most important properties when evaluating the capacity of a solution to coat a designated surface.

Fig. 1a represents the variation of the spreading coefficient of the coating solutions on mango versus their glycerol concentrations, for different ratios of galactomannan of *C. pulcherrima* – collagen in the coating solution. The experiments were repeated with mango using the galactomannan of *A. pavonina*, in replacement of the galactomannan of *C. pulcherrima*; the results are shown in Fig. 1b.

Fig. 1a and b shows that W_s values increase (approaching zero) for higher glycerol concentrations when a galactomannan/collagen ratio of 1/3 was maintained. Similar results were observed for the coatings with a galactomannan/collagen ratio of 1. Results also show that the increase of galactomannan/collagen ratio (corresponding to an increase of galactomannan concentration and a decrease of collagen concentration) leads to lower values of W_s. This behaviour is clear from Eq. (10) that represents the surface plotted in Fig. 1a, where GCR stands for "galactomannnan/collagen ratio". The equation coefficients show that the glycerol concentration has a statistically significant effect on W_s ($p > 0.05$); only when multiplied by galactomannan/collagen ratio (GCR) or when its squared value is considered. Eq. (11) represents the surface fitted in Fig. 1b. In this case, glycerol concentration only exerts statistically significant influence ($p < 0.05$) when multiplied by the galactomannan/collagen ratio.

$$W_s = -42.7390 - 15.8008\,\mathrm{GCR} + 4.4881\,\mathrm{GCR}^2 + 4.0350\,\mathrm{glycerol}^2 - 5.2180\,\mathrm{GCR}\cdot\mathrm{glycerol} \quad (p < 0.05) \quad R^2 = 0.76 \tag{10}$$

$$W_s = -26.6101 - 31.0150\,\mathrm{GCR} + 8.1603\,\mathrm{GCR}^2 - 2.2649\,\mathrm{GCR}\cdot\mathrm{glycerol} \quad (p < 0.05) \quad R^2 = 0.77 \tag{11}$$

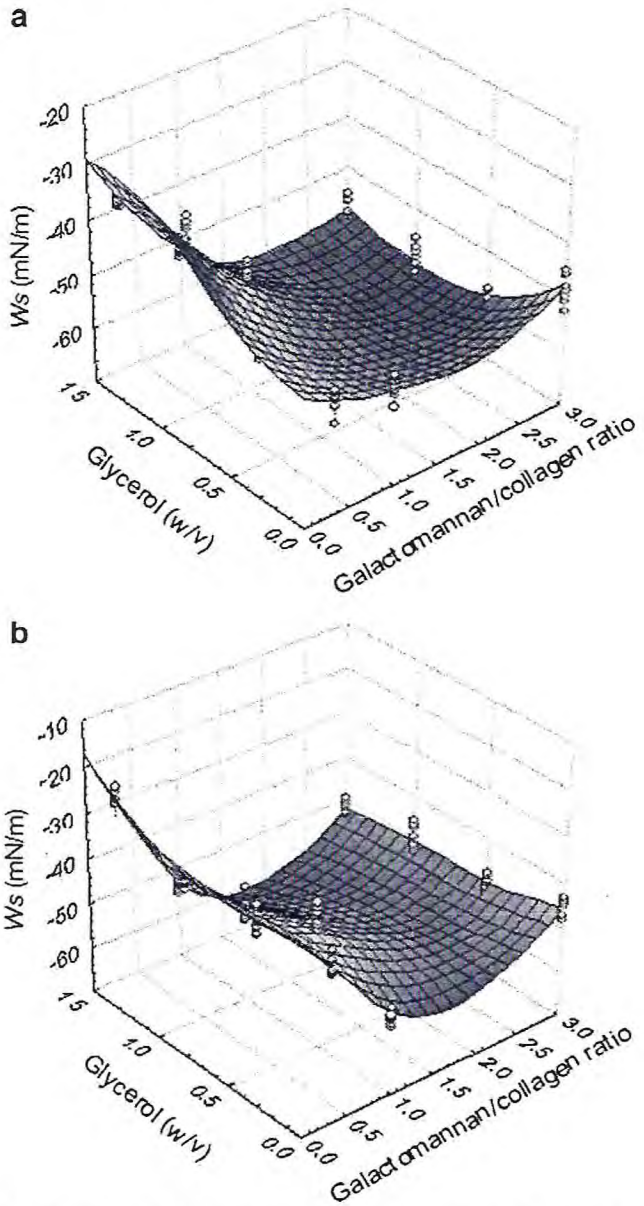

Fig. 1. Spreading coefficient (W_s) values (surface colours indicate different values of W_s) for different galactomannan of *C. pulcherrima* – collagen ratios and glycerol (a) and for the galactomannan of *A. pavonina* – collagen ratios and glycerol (b) on mango.

As shown in Eq. (1), W_s values are influenced by the values of the work of adhesion (W_a) and work of cohesion (W_c). While W_c presents a similar behaviour for all the studied coatings (results not shown), it was observed that W_a displays a similar trend to the values of W_s (results not shown). This means that the W_s values of the coatings on the studied fruits were mostly influenced by the adhesion of the coatings to the fruit surface, and not by the cohesion forces within the coating solution. These are usually influenced by the presence of surfactants, which were not used in this work (see e.g. Choi et al., 2002).

Fig. 1 shows that the coatings formed with galactomannan from *A. pavonina* present lower values of W_s when compared with coatings formed with galactomannan of *C. pulcherrima*. The W_s values ranged from −53.38 to −29.07 mN m^{-1} for *A. pavonina* coatings and between −59.63 and −36.59 mN m^{-1} for *C. pulcherrima* coatings. These differences may be explained by the different monosac-

charide composition of each galactomannan. *C. pulcherrima* galactomannan contains a higher relative amount of mannose and, when dissolved in aqueous solutions, it also presents higher values of intrinsic viscosity, when compared with solutions formulated with galactomannan of *A. pavonina* (Cerqueira et al., 2009). In some way these properties may explain the W_s values obtained for the coatings of these two galactomannans, considering that the higher viscosity possibly contributes to a decrease of the values of W_a.

Fig. 2a represents the variation of the spreading coefficient of the coating solutions on apple versus their glycerol concentrations, for different ratios of galactomannan of *C. pulcherrima* – collagen. Fig. 2b represents the variation of the spreading coefficient of the coating solutions on apple versus their glycerol concentrations, for different ratios of galactomannan of *A. pavonina* – collagen in the coating solutions. Fig. 2a and b shows that the best W_s values obtained for the coatings on apple correspond to the coating solutions without glycerol. This is possibly associated to the particularity that the apple surface presents a high dispersive component, denoting a predominance of apolar forces, while glycerol is a polar substance; therefore, its presence would decrease the spreadability of the coating. In the case of mango (see Fig. 1a and b), which has a lower dispersive component, the presence of glycerol improves the spreadability of the solution. Following this line of thought, it should be highlighted here that the values of W_s are closer to zero (thus corresponding to a better spreadability) when the coatings were applied on the mango surface. As explained above, W_c values present similar trends for all the studied coatings, but the adhesion forces (represented by W_a) present higher values for mango surfaces. This behaviour is consistent with the idea of the higher polar and lower dispersive component of the mango surface presenting a better interaction with the polar solutions used in the coatings' formulation.

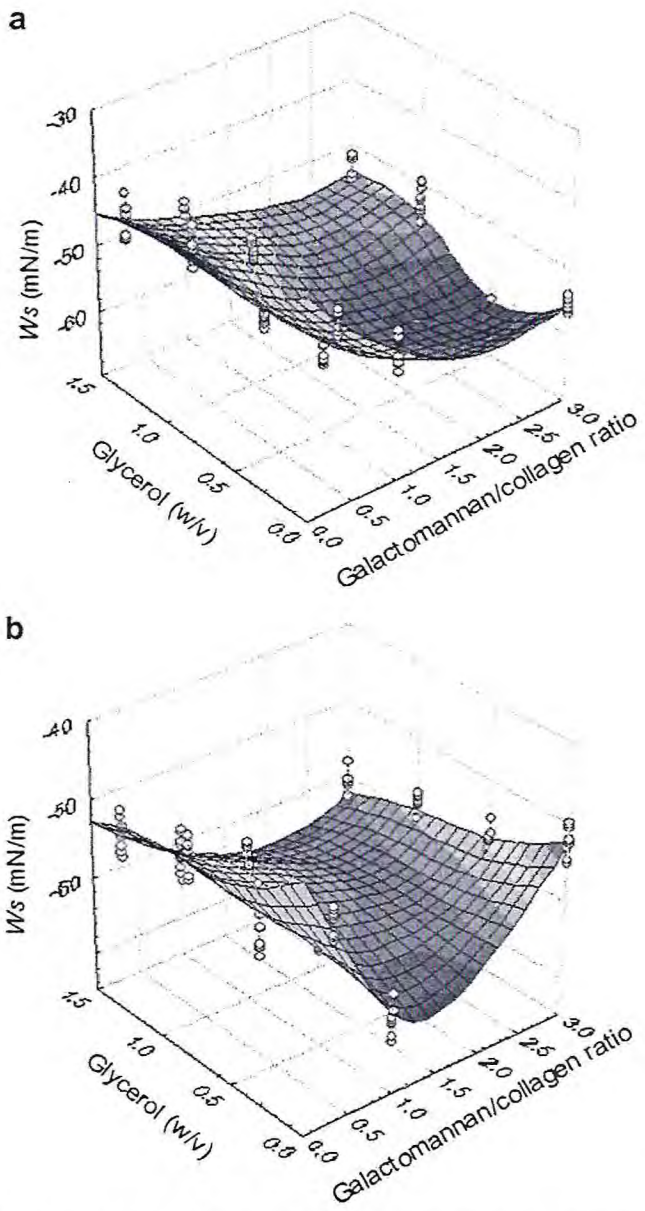

Fig. 2. Spreading coefficient (W_s) values (surface colours indicate different values of W_s) for different galactomannan of *C. pulcherrima* – collagen ratios and glycerol (a) and for different galactomannan of *A. pavonina* – collagen ratios and glycerol (b) on apple.

Eq. (12) represents the surface plotted in Fig. 2a, where GCR stands for "galactomannan/collagen ratio". This equation shows that all the factors are statistically significant ($p < 0.05$). When *A. pavonina* galactomannan is used in the coatings formulation, the fitted regression to the surface (represented in Fig. 2b) shows that glycerol concentration is significant only when multiplied by the galactomannan/collagen ratio (Eq. (13)).

$$W_s = -39.2385 - 14.4117\,\mathrm{GCR} + 2.7733\,\mathrm{GCR}^2 - 10.0921\,\mathrm{glycerol} + 5.3869\,\mathrm{glycerol}^2 + 1.7179\,\mathrm{GCR}\cdot\mathrm{glycerol} \quad (p < 0.05) \quad R^2 = 0.64 \tag{12}$$

$$W_s = -49.5081 - 15.5014\,\mathrm{GCR} + 4.6242\,\mathrm{GCR}^2 - 1.9550\,\mathrm{GCR}\cdot\mathrm{glycerol} \quad (p < 0.05) \quad R^2 = 0.53 \tag{13}$$

The best values of the spreading coefficients for mango were obtained with blends of 0.5% of galactomannan of *A. pavonina*, 1.5% of collagen and 1.5% of glycerol ($W_s = -29.07$ mN m^{-1}). The best values of the spreading coefficients for apple were obtained with blends of 0.5% of galactomannan of *C. pulcherrima*, 1.5% of collagen and no glycerol ($W_s = -42.79$ mN m^{-1}).

The best solutions in terms of wettability (represented by the spreading coefficient – W_s) were analyzed for the permeability to water vapour, oxygen and carbon dioxide and also characterized in terms of their mechanical properties.

3.3. Water vapour, oxygen and carbon dioxide permeability

Fig. 3 shows the differences of oxygen permeability (O_2P), carbon dioxide permeability (CO_2P) and water vapour permeability (WVP) between the films made with the coating solutions under consideration. The sample with 0.5% of *A. pavonina* galactomannan, 1.5% of collagen and 1.5% glycerol is less permeable to oxygen (O_2P) than the sample with 0.5% *C. pulcherrima* galactomannan, 1.5% of collagen and no glycerol with values of 1.08×10^{-15} and 4.07×10^{-15} g m Pa^{-1} s^{-1} m^{-2}, respectively. It is known that the addition of plasticizer decreases the presence of cracks and pores, improving the dispersion and decreasing the gas permeability (Garcia et al., 2000), thus the results shown here can be explained in light of these facts. Similar results were obtained for carbon dioxide permeability (CO_2P). For this particular property, the film with 0.5% of *A. pavonina* galactomannan, 1.5% of collagen and 1.5% glycerol is approximately 18 times less permeable to CO_2 than the one with 0.5% of *C. pulcherrima* galactomannan; 1.5% of collagen and no glycerol with values of 0.20×10^{-15} and 3.62×10^{-15} g m Pa^{-1} s^{-1} m^{-2}, respectively. The obtained values are in agreement with the results of other authors. Brindle and Krochta (2008) obtained values ranging between of 1.70×10^{-15} and 1.82×10^{-15} g m Pa^{-1} s^{-1} m^{-2} of O_2P for films made from blends

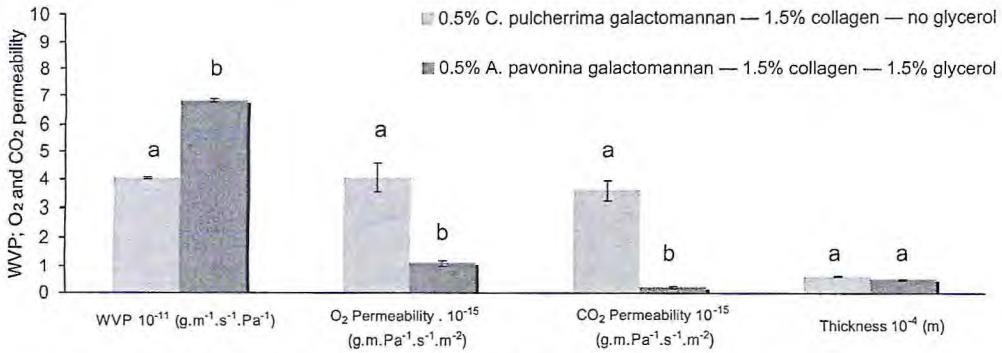

Fig. 3. Water vapour permeability (WVP), oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P) properties of coatings based on galactomannan–collagen blends and the respective standard deviations (Δ 0 5% *C. pulcherrima* galactomannan, 1.5% collagen, no glycerol; ▲ 0.5% *A. pavonina* galactomannan, 1.5% collagen, 1.5% glycerol). Different letters indicate a statistically significant difference (Tukey test, $p < 0.05$; values reported are the means ± standard deviations; $n = 3$, 95% confidence interval).

of whey protein and hydroxypropylmethylcellulose. Gounga et al. (2007) present values of O_2P ca. 5.79×10^{-15} g m Pa^{-1} s^{-1} m^{-2} for films with ratios of pullanan and whey protein isolate similar to those used in this work. In 2007, Han and Krochta reported that whey protein films have O_2P values of 2.09×10^{-15} g m Pa^{-1} s^{-1} m^{-2}.

In terms of the water vapour permeability (WVP) the opposite occurs as observed for CO_2 and O_2 permeability values. The coating with 0.5% *C. pulcherrima* galactomannan, 1.5% collagen and no glycerol is approximately 60% less permeable to water vapour than the coating with 0.5% *A. pavonina* galactomannan; 1.5% collagen and 1.5% glycerol, with the values of permeability decreasing from 6.79×10^{-11} to 4.06×10^{-11} g m^{-1} s^{-1} Pa^{-1}. The plasticizer decreases the intermolecular attractions between polymeric chains, facilitating the penetration of polar water vapour molecules (Kester and Fennema, 1986). Glycerol is a hydrophilic molecule (polar) and an increase of its concentration causes an increase of water vapour mass transfer. Being apolar, CO_2 and O_2 possibly do not penetrate so easily in such a polar moiety. Gómez-Estaca et al. (2009) obtained similar values for the WVP of bovine-hide and tuna-skin gelatine films (6.11×10^{-11} and 4.58×10^{-11} g m^{-1} s^{-1} Pa^{-1}, respectively). Fig. 3 shows the values for the thickness of the films. There is no statistical difference between them ($p > 0.05$) possibly due to the fact that the same concentrations of galactomannan and collagen were used.

3.4. Colour and opacity

The colour measurement was performed by determining the values of the parameters L^*, a^* and b^*, and the results are presented in Table 2. Comparing the two coatings it is possible to observe that the coating with 0.5% *A. pavonina* galactomannan, 1.5% collagen and 1.5% glycerol has higher values of L^* (94.52) and b^* (5.84). Its colour tended to yellowish as indicated by the increase of b^*. The coating with 0.5% *C. pulcherrima* galactomannan, 1.5% collagen and no glycerol presented a somewhat lower L^* value (91.85) and a higher a^* value (5.23) as compared to the *A. pavonina* coating. It indicates that the colour of this coating tends to be somewhat darker and reddish. These values are similar to the colour values obtained for films of whey protein isolate, with values of 97.09 and 3.54 to L^* and b^*, respectively. However, the films presented in the present work have shown a positive a^*, while the values of a^* for whey protein isolate films were negative; that can be explained by the presence of the polysaccharide, in one case, and the different origin of the protein, in the other (Sothornvit et al., 2009).

In terms of opacity, the coating with 0.5% *A. pavonina* galactomannan, 1.5% collagen and 1.5% glycerol is less opaque than the other coating tested. This characteristic is typical of protein-based coatings and it is one of the advantages of using collagen in the formulation, as transparency is a very valued property in films and coatings.

3.5. Mechanical properties

Mechanical properties can give good information on the compatibility of polymer mixtures. Normally, positive interactions between the components lead to a significant improvement in mechanical properties (Brindle and Krochta, 2008). The film with 0.5% *C. pulcherrima* galactomannan – 1.5% collagen – no glycerol has a higher value of tensile strength (TS) (117.56 MPa) and a lower value of elongation at break (E) (18.74%) when compared with the *A. pavonina* film (8.34 MPa and 47.17%, respectively). These results were expected due the presence of glycerol (plasticizer) that causes a reduction in the strength of the film although increasing its elasticity. Brindle and Krochta (2008) have shown a decrease of TS and an increase of E with the increase of glycerol and protein concentrations. The values of TS and E obtained for the film containing 0.5% *A. pavonina* galactomannan – 1.5% collagen – 1.5% glycerol are in agreement with the values obtained for similar

Table 2
Colour and opacity values of the selected films.

Film	L^* (black-white)	a^* (green-red)	b^* (blue-yellow)	Opacity (%)
0.5% C. pulcherrima Galactomannan – 1.5% Collagen – no glycerol	91.85 ± 0.38[a]	5.23 ± 0.01[a]	4.85 ± 0.12[a]	13.67 ± 0.01 [a]
0.5% A. pavonina Galactomannan – 1.5% Collagen – 1.5% glycerol	94.52 ± 0.66[b]	4.61 ± 0.04[b]	5.84 ± 0.03[b]	11.34 ± 0.01 [b]

* Values reported are the means and standard deviations ($n = 3$, 95% confidence interval). Different superscript letters in the same column indicate a statistically significant difference (Tukey test, $p < 0.05$).

amounts of whey protein: hydroxypropylmethylcellulose: glycerol, which were of 7.8 MPa and 47% for TS and *E*, respectively (Brindle and Krochta, 2008). Also, Osés et al. (2009) presented similar results of TS (11.5 MPa) for films of whey protein isolate and mesquite gum and sorbitol; on the other hand, a lower value of *E* (6.7%) was reported, which was explained by the lower value of plasticizer used in their work.

3.6. O_2 and CO_2 transfer rates in fruits

Apples were coated using a solution of 0.5% of *C. pulcherrima* galactomannan, 1.5% of collagen and no glycerol and their O_2 and CO_2 transfer rates were determined and compared with those of apples without coating. The coated apples were more glossy than the uncoated fruits. Visually inspection of the coated apples revealed a uniformly distributed coating, with no cracks or lamps, thus confirming the good wettability of the coating solution.

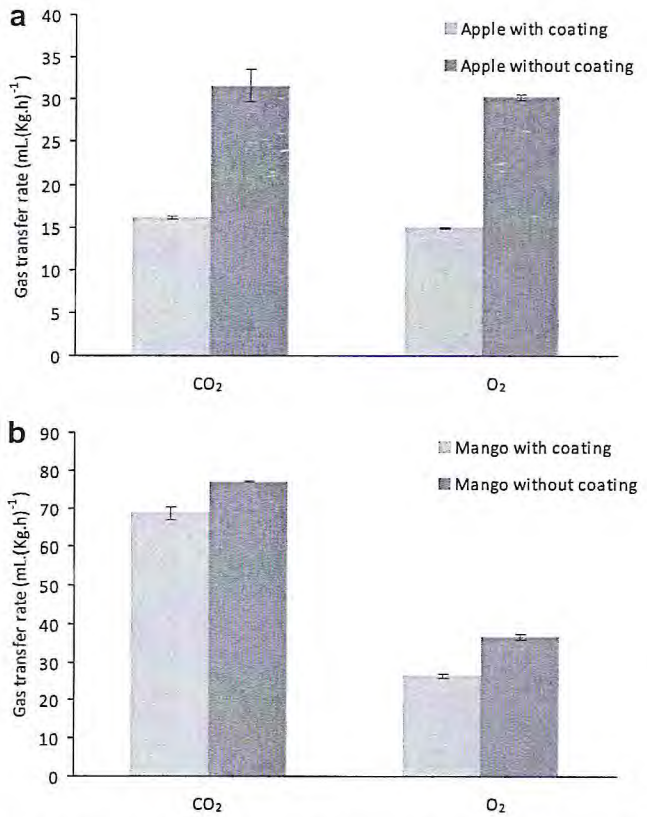

Fig. 4. O_2 and CO_2 transfer rate in coated and uncoated apples (a) and mangoes (b) (values reported are the means ± standard deviations; $n = 3$, 95% confidence interval).

The concentrations of the gases were measured during 60 h, the gas transfer rate was calculated and the results are presented in Fig. 4a. The coated apple permits a lower gas exchange. The CO_2 production and the O_2 consumption is approximately 50% lower in apples with coating than in apples without coating. The rate of CO_2 production is higher than that of O_2 consumption, both in coated an uncoated fruits. This is very important because it means that the presence of the coating does not alter the gas balance in the fruit; it just retards the gas transfer rates.

Mangoes were coated using a solution of 0.5% of *A. pavonina* galactomannan, 1.5% of collagen and 1.5% of glycerol. The mangoes were subjected to a visual inspection, similar to the one performed on apple. Also here the fruits were well coated, with a glossy appearance and no cracks or lumps were observed at the surface of the coating.

The O_2 and CO_2 transfer rates were compared with mangoes without coating. The gas concentrations were measured during 120 h, the gas transfer rate was calculated and the results are presented in Fig. 4b. The coated mango permits lower gas exchange rates. A 28% less O_2 consumption and 11% less CO_2 production is observed in coated mangoes when compared with mangoes without coating. Again, this is very important to maintain the gas balance inside the fruit.

The values obtained are in agreement with those reported in other works (Table 3). The higher values presented in this work are possibly related with the higher temperature at which the experiments were performed (20 °C), as the other works show that the increase of the temperature has a great influence in the values of the gas transfer rates.

4. Conclusions

This work shows how galactomannan–collagen blends can be used to decrease the fruits gas transfer rates, and how the wettability (W_s) can be used as a parameter for coating optimization. The fruits surfaces were found to be of low-energy and therefore the Zisman method was used to determine their wettability. Mango and apple fruits have the ability to participate in non-polar interactions, as a consequence of the higher values of the dispersive component, which was found to be higher in apples than in mangoes. The best values in terms of W_s were obtained for mango and apple with the following formulations, respectively: 0.5% of galactomannan of *A. pavonina*, 1.5% collagen and 1.5% of glycerol; and 0.5% of galactomannan of *A. pavonina*, 1.5% of collagen and no glycerol. This procedure is important in order to ensure that the application of the coating solutions on the fruits is made uniformly and easily, in view of future industrial uses. These two coatings were further characterized in terms of WVP, O_2P, CO_2P, TS, *E*, colour and opacity.

A 28% less O_2 consumption and 11% less CO_2 production were observed in coated mangoes when compared with mangoes without coating. In apples, the CO_2 production and the O_2 consumption was approximately 50% lower in the presence of the coating. It is important to note that in both cases (more in the case of apples

Table 3
Comparison of the gas transfer rates of different fruits and vegetables.

Fruit	RO_2 (mL kg^{-1} h^{-1})	RCO_2 (mL kg^{-1} h^{-1})	Conditions	Reference
Mango	26.4	68.7	20 °C, with coating	This study
Mango	36.6	77.1	20 °C, without coating	This study
Apple	14.9	16.1	20 °C, with coating	This study
Apple	30.2	31.6	20 °C, without coating	This study
Mango	14.5	16.5	5 °C, without coating	Ravindra and Goswami (2008)
Apple	19	22	20 °C, without coating	Mahajan and Goswami (2001)
Apple	-	1.0	0 °C, without coating	Kim et al. (1993)

than in the case of mangoes, but still relevant for both fruits), the gas balance inside the fruit was reasonably maintained due to reductions in both CO_2 production and O_2 consumption.

The results suggest that these coatings can reduce gas transfer rates in the studied fruits, and can therefore be important tools to extend their shelf-life.

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Effect of moderate electric fields in the permeation properties of chitosan coatings

B.W.S. Souza [a], M.A. Cerqueira [a], A. Casariego [a,b], A.M.P. Lima [a,c], J.A. Teixeira [a], A.A. Vicente [a,*]

[a] IBB-Institute for Biotechnology and Bioengineering, Center of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Instituto de Farmacia y Alimentos, Universidad de la Habana, La Habana, Cuba
[c] Depto de Bioquímica e Biologia Molecular, Centro de Ciências, Universidade Federal do Ceará Fortaleza, Ceará Brazil

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ABSTRACT

Edible films and coatings can provide additional protection for food, while being a fully biodegradable, environmentally friendly packaging system. Preliminary works have shown that the presence of a moderate electric field during the preparation of chitosan coating solutions may influence e.g. their transport properties. If such effect is confirmed, moderate electric fields could be used to tailor edible films and coatings for specific applications. The aim of this work was to determine the effect of field strength on functional properties of chitosan coatings (obtained from lobster from the Cuban coasts). Four different field strengths were tested (50, 100, 150, 200 V cm^{-1}) and, for each electric field treatment, the water vapor, oxygen and carbon dioxide permeabilities of the films formed were determined, together with their color, opacity and solubility in water. The surface microstructure of the films was analyzed using atomic force microscopy (AFM).

The results showed that ohmic heating had statistically significant effects on film's physical properties and structure. In general, the most pronounced effect of the field strength was observed for treatments made at 100 V cm^{-1} or higher, a positive correlation being found between the water vapor, oxygen and carbon dioxide permeability coefficients and field strength. The AFM results show that the surface of chitosan films is much more uniform when an electric field is applied, which may be related with a more uniform gel structure leading to the differences observed in terms of transport properties.

1. Introduction

Edible coatings can provide an alternative to extend the post-harvest life of fresh fruits and other vegetables and can also result in a similar effect as modified atmosphere storage in modifying the internal gas composition (Park, 1999). Indeed, this protective barrier can be formulated to prevent the transfer of moisture, gases, flavor or lipids, and thus to maintain or improve food quality and to increase food product shelf life (Krochta & De Mulder-Johnson, 1997). Carbohydrates (starches, polysaccharides), proteins, lipids, and combinations of these can be used to make edible films. Chitosan is a chitin derived polysaccharide and is one of the most abundant natural polymers, largely widespread in living organisms such as shellfish, insects, and mushrooms. It is a polysaccharide with linear structure constituted by a copolymer of β-(1-4)-linked D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) residues (Tharanathan & Kittur, 2003). It is obtained chiefly by homogeneous deacetylation of chitin with strong bases, rendering chitosans of different acetyl content or deacetylation degrees. Chitosan is a versatile biopolymer, having a broad range of applications in the food industry (Tharanathan & Kittur, 2003). The performance of edible coatings depends on their composition and the conditions in which they are used (e.g. relative humidity). A plasticizer is generally required for edible films to overcome film brittleness. Plasticizers could reduce the intermolecular forces and increase the mobility of polymer chains, therefore improving the flexibility and extensibility of the films. Nevertheless, the addition of plasticizers also increases (in general) gas and water vapor permeability of the film, and could possibly decrease the mechanical strength (Gontard, Duchez, Cuq, & Guilbert, 1994; Mali, Grossmann, García, Martino, & Zaritzky, 2004).

Ohmic heating is based on the passage of electrical current through a sample that has electrical resistance. The electrical energy is directly converted to heat and instant heating occurs, at a rate which depends on the intensity of the current passing through the material. There are practically no works dealing with the subject of producing edible films under an electric field; Lei, Zhi, Xiujin, Takasuke, and Zaigui (2007) wrote one of the very few,

* Corresponding author. Tel.: +351 253 604419; fax: +351 253 678986.
E-mail address: avicente@deb.uminho.pt (A.A. Vicente).

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where they reported that ohmic heating had many advantages in the production of protein-lipid film, including the improvement of the yield, film formation rate and rehydration capacity of protein-lipid films.

Atomic Force Microscopy (AFM) is one of the techniques that has been used to characterize the surface microstructure e.g. of plasticized soy protein isolate films (Ogale, Cunningham, Dawson, & Acton, 2000). AFM imaging modes can potentially provide structural information for a sample in its more natural state (without dehydration or coatings) (Lent, Vanasup, & Tong, 1998). Nanoscale measurements by AFM allow the influence of different factors on the hardness, elasticity and permeability of the film surface to be quantified, which is extremely useful for the design of high-performance edible food packaging systems (Herrmann, Yoshida, Antunes, & Marcondes, 2004). Measurements of the topography and roughness can be undertaken with extremely high resolution. This technique has been used to characterize the surface morphology of whey protein films (Herrmann et al., 2004; Lent et al., 1998).

The aim of this work was to study the effect of field strength on transport properties of chitosan coatings and film structure, therefore providing insight on the effect of the electric fields on films structure.

2. Materials and methods

2.1. Coating materials

The materials used to prepare the edible coating solutions were: chitosan (obtained in the Pharmaceutical Laboratories Mario Muñoz, Cuba) with a degree of deacetylation of 90% approximately, Tween 80 (Acros Organics, Belgium) as surfactant and lactic acid (Merck, Germany).

2.2. Film formation

The coating solutions were prepared dissolving the chitosan (1.5% w/v) in a 1% (v/v) lactic acid solution with agitation using a magnetic stirrer during 2 h at room temperature (20 °C); subsequently, Tween 80 was added as a surfactant at a concentration of 0.1% (w/w) (Casariego et al., 2008). After homogenizing, the chitosan solution was filtered to remove most of the undissolved impurities (<1% of the chitosan content). At the end of these treatments, a constant amount (28 mL) of chitosan solution was cast onto an 8 cm diameter glass plate in order to maintain the film thickness. The films were dried in an oven at 35 °C during 8 h. Dried films were peeled from the plate and cut in circles with 8 cm of diameter, approximately, for property testing.

2.3. Device description

A set of experiments was conducted to determine the effect of the application of a moderate electric field to chitosan solutions. The chitosan solution samples were treated in an ohmic heater using four different field strengths (from 50 to 200 V cm^{-1}) with a 2 cm gap between the electrodes, in all cases leading to an increase of temperature up to 60 °C. The heater and data acquisition system used are represented in Fig. 1 and consisted of a cylindrical glass tube of 30 cm total length and 2.3 cm inside diameter; two Titanium electrodes with Teflon pressure caps were placed at each end of the tube (for details of the apparatus please refer to Castro, Teixeira, Salengke, Sastry, & Vicente, 2004). Samples were heated using an alternating current source of 50 Hz, with different field strengths. Temperatures were monitored using a type-K thermocouple, placed at the geometrical centre of the chamber though the available opening. A data-logger was employed to record continuously and simultaneously, current intensity, voltage and temperature. In order to measure voltage across and current through the samples voltage and current transducers were used, respectively.

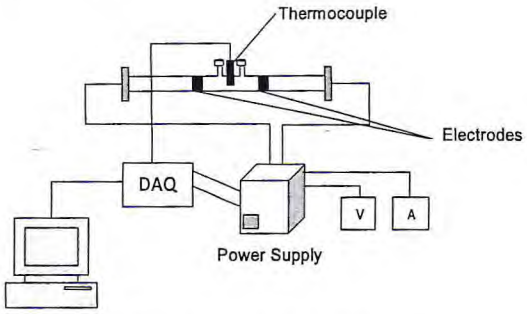

Fig. 1. Ohmic heater and data acquisition system.

In order to collect data for the conventional heating, 30 mL Falcon tubes containing the chitosan solution samples were placed in a temperature controlled water bath. The thermal history of the samples, until temperature stabilization, was monitored by the introduction of a thermocouple connected to the data acquisition system previously described; this treatment was used as control in order to discard the temperature effects and to evaluate only the effects of electric field.

2.4. Characterization of chitosan films

2.4.1. Conditioning

All chitosan films used for permeability tests were conditioned in desiccators, at 20 °C and 25% RH.

2.4.2. Thickness

Film thickness was measured with a hand-held digital micrometer (Mitutoyo, Japan) having a sensitivity of 0.001 mm. Ten thickness measurements were taken on each testing sample in different randomly chosen points and the mean values were used in permeability calculations.

2.4.3. Optical properties

The color of films was determined with a Minolta colorimeter (CR 300; Minolta, Japan). A white color plate ($Y = 93.5$, $x = 0.3114$, $y = 0.3190$) was used as standard for calibration. The CIELab scale was used to measure lightness (L) and chromaticity parameters a^* (red – green) and b^* (yellow – blue). Measurements were performed placing the film sample over the standard. Samples were analyzed in triplicate, recording four measurements for each sample.

The opacity of a material is an indication of how much light passes through it. How higher the opacity, lower the amount of light that can pass through the material. Generally, opacity is calculated from reflectance measurements. The opacity of the samples was determined, according to Hunter lab method, as the relationship between the opacity of each sample on a black standard (Y_b) and the opacity of each sample on the white standard (Y_w):

$$\text{Opacity} = \frac{Y_b}{Y_w} \times 100\% \quad (1)$$

where Y is the CIE tristimulus value.

2.4.4. Gases permeability

Oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P) were determined based on the ASTM (2002) method. A chitosan film was sealed between two chambers, having each one two channels to the exterior. In the lower chamber O_2 or CO_2 were supplied at a controlled flow rate to keep the pressure constant in that compartment. The upper chamber was purged by a stream of nitrogen, also at a controlled flow. This nitrogen acted as a carrier for the O_2 or CO_2 coming from the lower chamber though the film. The flows of the two chambers were connected to manometers to ensure the equality of pressures between both compartments, kept at 1 atm. As the O_2 or CO_2 were carried continuously by the nitrogen flow, it was considered that O_2 or CO_2 partial pressure in the upper compartments is null, therefore ΔP can be considered to be 1 atm O_2P was determined from the measurements of O_2 concentration in the nitrogen flow leaving the chamber with an O_2 sensor installed on-line. CO_2P was determined from the measurements of CO_2 concentration in a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) with a column Porapak Q 80/100 mesh–2 m × 1/8″ × 2 mm SS (Temperatures: Oven = 35 °C, detector and injector = 110 °C; flow of the carrier gas = 23 mL min^{-1}). In all cases the variation coefficient obtained between the three replicates made for each experiment was below 5%.

2.4.5. Water vapor permeability measurement

The water vapor permeability (*WVP*) of the films was determined gravimetrically based on the ASTM E96-92 method (Guillard, Broyart, Bonazzi, Guilbert, & Gontard, 2003; Mc Hugh, Avena-Bustillos, & Krochta, 1993). The test film was sealed on the top of a permeation cell containing distilled water (100% RH; 2.337×10^3 Pa vapor pressure at 20 °C), placed in a desiccator which was maintained at 20 °C and 0% RH (0 Pa water vapor pressure) with silica gel. The water transferred through the film and adsorbed by the desiccant was determined from weight loss of the permeation cell. The cups were weighed at intervals of 2 h during 10 h. Steady-state and uniform water pressure conditions were assumed by keeping the air circulation constant outside the test cup by means of a miniature fan placed inside the desiccators (Mc Hugh et al., 1993). The slope of the curve representing the weight loss versus time was obtained by linear regression. The measured (*WVP*) of the films was determined as follows:

$$WVP = (WVTR.L)/\Delta P \quad (2)$$

where *WVTR* is the measured water vapor transmission rate ($g \cdot m^{-2}\ s^{-1}$) through the film (calculated from the slope of the curve divided by the area of the film), L is the mean film thickness (m), and ΔP is the partial water vapor pressure difference (Pa) across the two sides of the film. For each type of film, *WVP* measurements were replicated three times and the variation coefficient obtained was at all times below 5%.

2.4.6. Film solubility

The film solubility in water was determined according to the method reported by Gontard et al. (1994). It was defined by the content of dry matter solubilized after 24 h immersion in water. The initial dry matter content of each film was determined by drying it to constant weight in an oven at 105 °C. Two disks of film (2 cm diameter) were cut, weighed, and immersed in 50 mL of water. After 24 h of immersion at 20 °C with occasional agitation, the pieces of film were taken out and dried to constant weight in an oven at 105 °C, to determine the weight of dry matter which was not solubilized in water. The variation coefficient obtained between the three replicates made for each experiment was below 5%.

2.4.7. Atomic force microscopy

The surface morphology of the films was analyzed by AFM with a Nanoscope III, Multimode (Digital Instruments) with a 10 μm × 10 μm scan size and a 3.5 μm vertical range. Measurements were taken from several areas of the film surface (10 μm·10 μm) using the tapping mode. The resulting data set for each sample was transformed into a 3D image. The average sample roughness (ASME B46.1, 1995) (*Ra*) was estimated with the aid of the built-in software of the equipment.

2.4.8. Statistical analysis

Analysis of Variance (ANOVA) and linear regression were the main statistical tools used for data analysis. The Tukey test ($\alpha = 0.05$) was also used to determine the significance of differences between specific means (SigmaStat 3.1, 2004, Excel, 2003, USA).

3. Results and discussion

3.1. Oxygen permeability (O_2P) and carbon dioxide permeability (CO_2P)

Permeability is a steady-state property that describes the extent to which a permeating substance dissolves and then the rate at which it diffuses through a film, with a driving force related to the difference in concentration of that substance between the two sides of the film (Gennadios, 2002).

Gas permeabilities of edible films and coatings depend on several factors such as the integrity of the film, the ratio between crystalline and amorphous zones, the hydrophilic-hydrophobic ratio and the polymeric chain mobility; the interaction between the film-forming polymer and the presence of a plasticizer or other additives are also important factors in film permeability (Garcia, Martino, & Zaritzky, 2000).

The measurement of the permeability of edible films to oxygen and carbon dioxide provides important information for the development of edible films. Oxygen is the key factor that might cause oxidation, inducing several unwanted food changes such as odor, color and flavor, as well as nutrients deterioration. Therefore, films providing a proper oxygen barrier can help improving food quality and extending food shelf life (Sothornvit & Pitak, 2007). Carbon dioxide is formed in some foods due to deterioration and respiration reactions. The produced CO_2 has to be removed from the package to avoid food deterioration and/or package destruction (Vermeiren, Heirlings, Devlieghere, & Debevere, 2003). Such films can maintain food quality and improve stability and shelf life by retarding unwanted mass transfer in food products (Miller & Krochta, 1997), including to retard transport of gases (O_2, CO_2) for fruits and vegetables, migration of moisture for dried and intermediate moisture foods, and migration of solutes for frozen foods.

Table 1 shows O_2P and CO_2P as measured for chitosan films formed from solutions subjected to electric fields of different intensities. The samples with treatments made at 100 V cm^{-1} or higher have lower values ($p < 0.05$) of O_2P and CO_2P.

The *AFM* observation of a regular surface of the chitosan films treated at 100 V cm^{-1} or above (confirmed by the *Ra* values) as opposed to a rougher surface of the untreated films indicates that the films structure might have been altered due to the application of the electric field during the preparation of the film-forming solution (see Table 1 and also Fig. 3). Wan, Creber, Preppley, and Bui (2003) observed that the crystallinity of the chitosan membranes increased gradually with increasing degree of deacetylation ranging from 70 to 90%. This can be attributed to the fact that chains of chitosan with higher degree of deacetylation are more compact thus facilitating hydrogen-bonding formation and consequently

Table 1
Comparison of roughness (*Ra*) values obtained from AFM images and water vapor permeability (WVP), CO_2 permeability and O_2 permeability for the films obtained with film-forming solutions subjected to different field strengths.

Electric field strength	*Ra* (nm)	*WVP* (g·(m.day.atm)$^{-1}$)	CO_2 Permeability 10^{14} (g.m·(Pa.s.m^2)$^{-1}$)	O_2 Permeability 10^{16} (g.m·(Pa.s.m^2)$^{-1}$)
0 V cm^{-1}	10.22	0.3228 ± 0.027^a	6.98 ± 0.030^a	10.60 ± 0.420^a
50 V cm^{-1}	20.13	0.3219 ± 0.022^a	6.97 ± 0.029^a	10.60 ± 0.450^a
100 V cm^{-1}	7.75	0.2740 ± 0.027^b	6.74 ± 0.037^b	9.54 ± 0.400^b
150 V cm^{-1}	4.71	0.2728 ± 0.030^b	6.72 ± 0.041^b	9.42 ± 0.540^b
200 V cm^{-1}	4.01	0.2667 ± 0.025^b	6.72 ± 0.040^b	9.62 ± 0.600^b

*Different letters in the same column correspond to statistically different samples ($p < 0.05$).

favoring crystallinity formation in the film. Furthermore, chitosan with a higher degree of deacetylation contains more glucosamine groups, which also facilitate the hydrogen-bonding formation; on the contrary, chitosan with a lower degree of deacetylation has more acetyl groups, which hinder the chitosan chain packing due to their rigidity and steric effect (Bangyekan, Aht-Ong, & Srikulkit, 2006). Lei et al. (2007) studied the effects of different heating methods on the production of protein-lipid film and concluded that the major advantage of ohmic heating is that the heat is dispersed uniformly throughout the whole liquid compared to water bath heating, and finally concluded that the film formation rate was higher when ohmic heating was applied. During the heating process, heat was uniformly applied to the whole volume of the film, accelerating the collisions between molecules. This process can provide an improvement in the crystallinity of the chitosan film, thus increasing the material's resistance to gas permeation. Balau, Lisa, Popa, Tura, and Melnig (2004) studied the X-ray difractogram of chitosan films, an almost amorphous structure; the films treated with an electric field of $E = 20$ kV cm^{-1}, developed a crystalline structure, while the films to which no electric field was applied displayed a significantly lower proportion of crystalline material, showing that the electric field plays an important role in the crystallization process.

3.2. Water vapor permeability

Water vapor permeability (*WVP*) is an important parameter commonly considered in food packaging. *WVP* comprises sorption, diffusion and adsorption and is largely governed by the interactions between the polymer and the water molecules (Nivedita, Sangaj, & Malshe, 2004). Water permeation through a film usually occurs through the hydrophilic part of the film, thus the relation of the hydrophilic/hydrophobic portions is important to determine *WVP*. Polymers with high hydrogen-bonding produce films that are susceptible to moisture while polymers with hydrophobic groups make excellent barrier to moisture. Generally, *WVP* is also depended on the pore size of the film (Paramawati, Yoshino, & Isobe, 2003). In fact, *WVP* tends to increase with polarity, degree of unsaturation and degree of ramification of the lipids used (if any), in addition to the effect of the water molecule sorption by the polar part of the film material (Gontard et al., 1994).

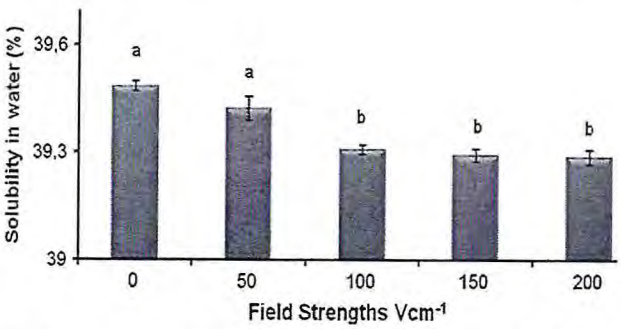

Fig. 2. Solubility in water of chitosan films treated different electrical field strengths. Different letters in the same column correspond to statistically different samples ($p < 0.05$).

Butler, Vergano, Testin, Bunn, and Wiles (1996) reported that chitosan films are highly impermeable to oxygen, however they have relatively poor water vapor barrier characteristics, which result from their hydrophilicity.

The water vapor permeability should be as low as possible since an edible film or coating should retard moisture transfer between the food and the environment, or between two components of a heterogeneous food product (Gontard, Guilbert, & Cuq, 1992).

The results obtained in this work show that *WVP* of chitosan films decrease (up to 17.3%) with the increase of the field strengths for values of 100 V cm^{-1} or higher. These films showed lower *WVP* values than those of other hydrocolloids films reported in literature (Bravin, Peressini, & Sensidoni, 2006; Mathew & Abraham, 2008; Olivas & Barbosa-Cánovas, 2008; Vargas, Albors, Chiralt, & González-Martínez, 2009; Ziani, Oses, Coma, & Maté, 2008).

There is a positive correlation between *WVP* and film surface roughness (*Ra*). The increase of the field strength seems to be correlated with the permeability and *Ra* (see Table 1). Herrmann et al. (2004) observed that an increase of protein concentration lead to an increase of the viscosity of the film-forming solution, which resulted in the incorporation of air bubbles; this formed non-homogeneous and non-compact film networks, increasing the roughness and, as a consequence, the value of *WVP*.

Anker, Stading, and Hermansson (2000) concluded that the reason for the increased *WVP* is probably the larger pores formed at high polymer concentration, compared to the smaller pores formed at low polymer concentration. The work of Miller and Krochta (1997) also points at the fact that the permeability is highly affected by how closely packed the polymer chains are, thus establishing a direct relationship between the crystallinity of the structure and permeability.

Table 1 summarizes the results for *Ra*, *WVP*, O_2P and CO_2P.

3.3. Solubility in water and optical properties

3.3.1. Solubility in water

Solubility in water is defined as the maximum percentage (by weight) of a substance that will dissolve in a unit volume of water at certain (usually room) temperature. It is an important property, which governs potential applications of these materials to food preservation. Films with low water solubility are necessary for the protection of foodstuffs with high or intermediate water activity (Sébastien, Stéphane, Copinet, & Coma, 2006). On the other hand, edible films with high water solubility may be required, for example, to contain premeasured portions which will be dissolved in water or in hot food (Guilbert & Biquet, 1989).

In the present work the solubility of the chitosan films was evaluated, and it is shown that the solubility of chitosan films

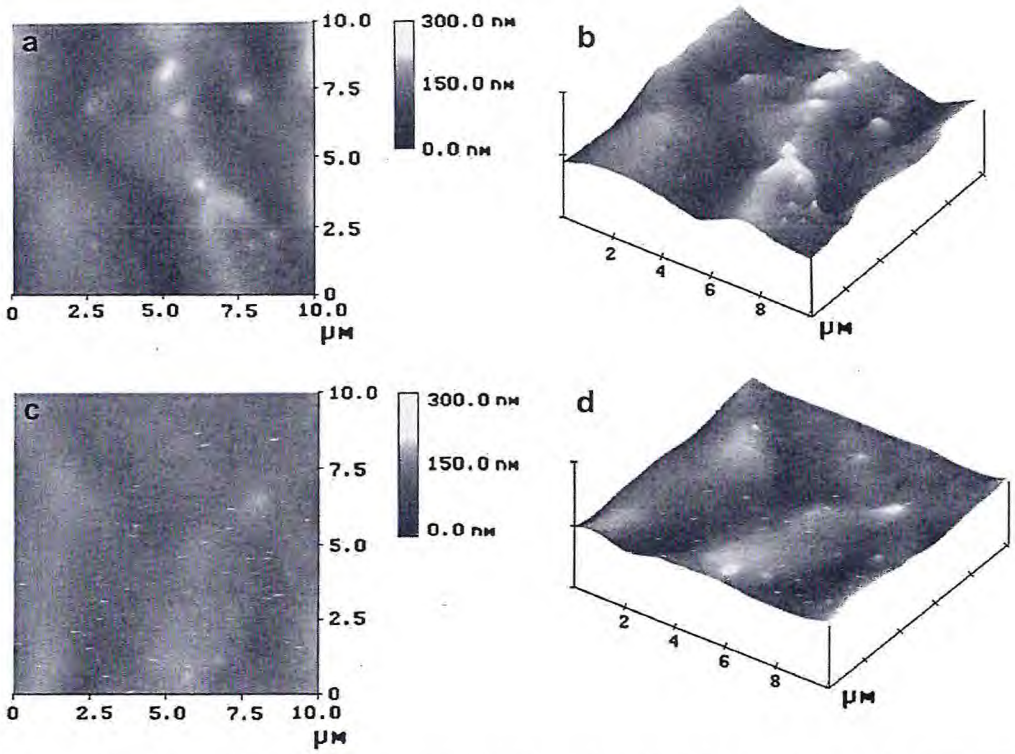

Fig. 3. AFM topographic images of (a) chitosan films obtained from film-formed solutions treated at a field strength of 50 V cm^{-1} (b) is three dimensional image. (c) AFM topographic images of chitosan films obtained from film-formed solution treated at a field strength of 100 V cm^{-1} (d) is three dimensional image.

decrease with the increase of the field strengths for values of 100 V cm^{-1} or higher (Fig. 2). Balau et al. (2004) showed that the electric field plays an important role in the crystallization process, which may also interfere in the water solubility of the films. In fact, similarly to what has been reported for gas permeability, the solubility of chitosan films has been associated to the crystallinity of the sample; a high crystallinity contributes to a higher insolubility (Du & Hsieh, 2007), and the poor solubility of chitosan has been attributed to its partially crystalline structure (Nishimura, Kohgo, Kurita, & Kuzuhara, 1991).

3.3.2. Optical properties

The results of the measurements of color are shown in Table 2. The films should be visually attractive, and should not change their color throughout the time of storage, in order not to harm the acceptance of the product on which they are applied.

The results show that the lightness of chitosan films is somewhat lower when an electric field is applied, but still high. For comparison, results for the lightness of albumen (from egg) range between 95.67 and 96.20 (Gennadios, Weller, Hanna, & Fronnig, 1996); such films were reported to be clearer and more transparent than films based on wheat, soy protein and corn zein, studied by the same authors. Further, the values of lightness for chitosan films are higher than those reported for wheat protein films, which presented values of L between 83.3 and 89.7 (Rayas, Hernandez, & Perry, 1997). The high values of the component b^* indicate the predominance of the yellow color in the chitosan films; this coincides with the data reported by Butler et al. (1996). Our results also indicate that an increase in the field strength leads to a significant increase of the values of b^* (see Table 2). In general, polysaccharide films are free from the color problems associated with protein (which can suffer Maillard reactions) and lipid (which can suffer oxidation) films (Trezza & Krochta, 2000).

The evaluation of the opacity of a material demonstrates its greater or lesser transparency. For the development of materials meant to be used as films or coatings for food, increased transparency tends to be better (Yang & Paulson, 2000) once the goal is to retain the original features of the product, such as color.

The values of opacity (Table 2) for the films under consideration did not differ significantly ($p < 0.05$); all films were transparent, meaning that there was no apparent effect due to the application of an electrical field.

Table 2
Optical properties of chitosan films.

Electric field strenght	L^* (lightness)	a^*	b^*	Opacity (%)
0 V cm^{-1}	93.80 ± 0.38[a]	4.04 ± 0.11[a]	11.01 ± 0.46[a]	4.98 ± 1.11[a]
50 V cm^{-1}	94.01 ± 0.72[a]	4.15 ± 0.17[a]	11.64 ± 0.56[a]	5.05 ± 0.97[a]
100 V cm^{-1}	93.68 ± 0.56[a]	4.29 ± 0.27[a]	20.73 ± 1.57[b]	5.07 ± 0.28[a]
150 V cm^{-1}	93.73 ± 0.91[a]	4.50 ± 0.40[a]	21.26 ± 1.64[b]	5.06 ± 0.61[a]
200 V cm^{-1}	93.84 ± 0.98[a]	4.00 ± 0.47[a]	20.34 ± 1.46[b]	4.89 ± 0.47[a]

*Different letters in the same column correspond to statistically different samples ($p < 0.05$).

4. Conclusions

The results obtained showed that the application of a moderate electric field to the film-forming solutions has statistically significant effects on the film's physical properties and structure. In general, the most pronounced effect of the field strength was observed for treatments made at 100 V cm^{-1} or higher. The solubility in water and the water vapor, oxygen and carbon dioxide permeability coefficients showed a positive correlation with the application of an electric field. The *AFM* results show that the surface of chitosan films is much more uniform when an electric

field is applied as shown by the roughness results obtained, which may be related to the differences observed in terms of transport properties.

In practice, the changes in the film properties induced by the application of the electrical field may translate in an improved shelf life of the products due to reduced water loss (calculated on the basis of the lower *WVP* values achieved) and reduced O_2 and CO_2 exchanges (due to the lower values of O_2P and CO_2P), which will mean a slower metabolism e.g. in fruits and vegetables (Casariego et al., 2008). Future work should be directed towards the confirmation of these effects in real food systems.

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