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FACULDADE DE ENGENHARIA DE ALIMENTOS
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PREPARAÇÃO, PROPRIEDADES E APLICAÇÃO DE BIOFILMES COMESTÍVEIS À BASE DE GLÚTEN DE TRIGO

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“As gold breeds misery
Misery breeds light
That makes the stones glare
For the pauper’s delight.

Light is but the pauper’s gold
Stones are but rocks
That pave the way where run
God’s miserable flocks.

The world has many rocks
God has many flocks
God’s a shepherd, I was told
God is made of gold.”

Vinicius de Moraes

“Great opportunities await those who give more than what is asked. I encourage you to put the uncommon touch on even the most common task. We may never have the opportunity to do great things in a great way, but we all have the chance to do small things in a great way.”

Dedico ao Vesa e à Amanda
E aos meus pais Alzira e Jorge
Com todo amor e carinho.

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RESUMO

Recentemente surgiu um grande interesse no desenvolvimento de biofilmes comestíveis ou degradáveis biologicamente, principalmente devido à demanda por alimentos de alta qualidade e preocupações ambientais sobre o descarte de materiais não renováveis de embalagem para alimentos. Os biofilmes são geralmente produzidos com macromoléculas, como polissacarídeos, proteínas, lipídios e derivados. O objetivo desse trabalho foi o desenvolvimento e caracterização de biofilmes simples de glúten de trigo, composto e de duas camadas contendo cera de abelha e ácidos palmítico e esteárico, bem como testar a aplicação dos filmes e das coberturas em frutas frescas. A solução formadora de filme foi preparada usando glúten de trigo, etanol, glicerol e hidróxido de amônio (6N) para ajustar o pH da solução. As composições das formulações dos filmes foram calculadas segundo um planejamento fatorial 2^4. As propriedades funcionais utilizadas para verificar os efeitos dos fatores foram as propriedades mecânicas (resistência à tensão e elongação na ruptura), permeabilidades ao vapor d'água e ao oxigênio e solubilidade em água. A partir da formulação que apresentou as melhores propriedades funcionais, foram desenvolvidos e caracterizados os filmes compostos e de duas camadas contendo glúten de trigo, cera de abelha e mistura 1:1 de ácidos palmítico e esteárico em várias concentrações. Outras análises como efeito da umidade relativa sobre as propriedades mecânicas dos filmes e análises microestruturais através da microscopia eletrônica de varredura foram realizadas. A seguir, realizou-se o estudo da aplicação das coberturas e filmes comestíveis à base de glúten em morangos frescos mantidos sob refrigeração, através das análises de: perda de peso, retenção da firmeza, cor, acidez titulável, sólidos solúveis e totais, açúcares redutores e totais e análise sensorial de aparência para determinar a vida de prateleira dos morangos e de degustação para verificar a aceitação das coberturas à base de glúten. Para complementar o estudo, foi realizada uma reação de lipofilização do glúten de trigo, verificada através da porcentagem de grupos amino livres pelo método de TNBS. A partir do glúten modificado, o biofilme foi preparado e caracterizado, comparando-se com o filme de glúten não modificado. Outro trabalho complementar foi a preparação e caracterização de biofilmes preparados a partir de farinhas de trigo brasileiras e também a comparação das propriedades com os filmes de glúten vital (comercial). Os resultados obtidos indicaram que as propriedades mecânicas dos filmes simples foram influenciadas pelas concentrações de glúten e glicerol, sendo o filme mais resistente o de maior concentração de glúten e menor de glicerol. A menor permeabilidade ao oxigênio foi obtida no filme com menor concentração de glúten, glicerol e etanol e a permeabilidade ao vapor d'água mais baixa foi no filme com menor concentração de glicerol. Os filmes compostos e de duas camadas ficaram opacos e o filme de duas camadas com 10% de ácidos esteárico e palmítico e cera de abelha apresentou a menor permeabilidade ao vapor d'água, porém a resistência à tensão e a elongação foram baixas. A morfologia observada através do microscópio eletrônico de varredura para o filme simples de glúten foi completamente modificada quando se adicionou a mistura de ácidos palmítico e esteárico e cera de abelha. O estudo da aplicação dos biofilmes e coberturas em morangos mostrou que a cobertura de duas camadas com glúten e lipídios teve efeito na retenção da firmeza e na diminuição da taxa de perda de peso nas frutas cobertas. As frutas embaladas com o filme de glúten retardaram a senescência por apresentarem os menores parâmetros (a/b) em relação à cor da fruta. A análise sensorial dos morangos indicou que as coberturas simples de glúten e composta com lipídios mantiveram a qualidade dos morangos durante o tempo de armazenamento em termos de aparência e que o sabor dos morangos com a cobertura simples de glúten foi aceito pelos consumidores. A reação de lipofilização modificou o glúten através da diminuição da porcentagem de grupos amino livres, porém o filme preparado com o glúten lipofilizado apresentou propriedades mecânicas e de barreira inferiores ao filme com glúten sem modificação. Os filmes preparados a partir das farinhas de trigo "semi-duras" brasileiras apresentaram propriedades mecânicas e de barreira semelhantes ao filme com glúten vital (comercial), podendo até serem usadas em substituição ao glúten comercial.

SUMMARY

Recently interest in the development of edible films and coatings for foods has grown considerably due to consumer demand for high quality foods and environmental concerns over the disposal of non-renewable food packaging materials. Polysaccharides, proteins and lipids can be used as edible film-forming agents. The objective of this work was the development and characterization of simple, composite and bilayer wheat gluten films with lipids (beeswax and stearic and palmitic acids) and the application of the films and coatings on fresh fruits. The base film was prepared from a solution of gluten, absolute ethanol, glycerol and 6 N ammonium hydroxide to adjust the pH. The composition of the film formulations was calculated according to a 2^4 factorial planning. The functional properties used to verify the factor effects were mechanical properties (tensile strength and percent elongation at break), oxygen and water vapor permeabilities and solubility in water. The simple film with the best functional properties was used to prepare the composite and bilayer films of wheat gluten, beeswax and a 1:1 blend of stearic and palmitic acid. Other analysis as effect of the relative humidity on the mechanical properties and morphology of the surface by scanning electron microscopy were effected. After this, the application of the gluten-based coatings and films was effected on fresh strawberries maintained upon refrigeration, and the analysis of weight loss, retention of the texture, color, titratable acidity, soluble and total solids, reducing and total sugars and sensory evaluation of appearance to determine the shelf life of the strawberries and of taste to verify the acceptance of the gluten-based coatings, were effected. To complement the study, a reaction of lipofilization of wheat gluten was effected, and it was verified through the percentage of free amino groups by the TNBS method. Biofilms were prepared from the modified gluten and were characterized and compared with the film from unmodified gluten. Another complementary work was the preparation and characterization of biofilms prepared from Brazilian wheat flours and also comparison of the properties with the films from vital gluten (commercial). The results showed that the mechanical properties of the simple films had been influenced by the concentrations of gluten and glycerol, being the most resistant film with higher concentration of gluten and less glycerol. The lowest oxygen permeability was obtained in the film with lower concentration of gluten, glycerol and ethanol and the lowest water vapor permeability was in the film with lower concentration of glycerol. The composite and bilayer films were opaque and the bilayer film with 10% blend of stearic and palmitic acids and beeswax showed the lowest water vapor permeability, however the mechanical resistance and the elongation were low. The morphology observed through the scanning electron microscopy for the simple film of gluten was completely modified when the mixture of palmitic and stearic and beeswax was added. The application of biofilms and coatings in strawberries showed that the bilayer coating of gluten and lipids had a significant effect in the retention of the texture and the reduction of weight loss in the coated fruits. The senescence was decreased in fruits packed with the gluten film verified by the lower parameters in relation to the color of the fruit. The sensory evaluation of the strawberries indicated that the simple coating of gluten and the composite with lipids had kept the quality of the strawberries during the time of storage in terms of appearance and the consumers accepted the flavor of the strawberries with the simple coating. The lipofilization reaction really modified the gluten through the reduction of the percentage of free amino groups, however the film prepared with the lipophilized gluten presented lower mechanical and barrier properties comparing to the film with unmodified gluten. The films prepared from Brazilians “semi-hard” wheat flours had presented similar mechanical and barrier properties than the film from vital gluten (commercial), being able to be used in substitution.

INTRODUÇÃO GERAL

Recentemente, surgiu um grande interesse no desenvolvimento de biofilmes (filmes biodegradáveis), principalmente devido à demanda por alimentos de alta qualidade, preocupações ambientais sobre o descarte de materiais não renováveis de embalagem para alimentos e oportunidades para criar novos mercados para matérias-primas formadoras de filme, provenientes de produtos agrícolas. Os biofilmes podem ser de dois tipos: coberturas, quando são aplicadas diretamente nas superfícies dos alimentos, e filmes, que possuem a capacidade de formar estruturas próprias independentes. Coberturas e filmes comestíveis podem ser definidos como uma fina camada contínua formada ou depositada no alimento preparada a partir de materiais biológicos que agem como barreira a elementos externos (fatores como umidade, óleo e gases) e, consequentemente, protegem o alimento, aumentando a sua vida de prateleira. Algumas possíveis propriedades funcionais dos biofilmes são: reduzir a migração de umidade, de gases (O_2, CO_2), de óleo ou gordura e de soluto; oferecer uma integridade estrutural adicional aos alimentos; reter compostos aromáticos e poder carregar aditivos alimentícios.

A grande vantagem dos biofilmes e coberturas comestíveis é que estes podem ser consumidos junto com o alimento, além de serem produzidos a partir de componentes biodegradáveis, e também por atuarem como suporte de nutrientes e/ou aditivos que melhoram as características nutricionais e sensoriais do alimento.

Os biofilmes são geralmente produzidos com macromoléculas como polissacarídeos, proteínas, lipídios e derivados. Os biofilmes produzidos a partir de polissacarídeos ou proteínas possuem boas propriedades mecânicas mas alta permeabilidade ao vapor d’água. Ao contrário, os filmes de lipídios apresentam boas propriedades de barreira ao vapor d’água, mas são opacos e relativamente inflexíveis. É de nosso interesse o desenvolvimento de um biofilme composto contendo uma proteína e uma mistura de lipídios e sua aplicação em frutas frescas. A proteína estudada neste trabalho é o glúten de trigo. Glúten de trigo é um termo geral para as proteínas insolúveis em água da farinha de trigo. A coesividade e elasticidade do glúten dão integridade e facilitam a formação de filme. A adição de lipídios poderá diminuir a permeabilidade ao vapor d'água do filme de glúten de trigo.

Este trabalho será apresentado em capítulos que são descritos sinteticamente a seguir.

O Capítulo 1, "**Biofilmes comestíveis: formação e propriedades físicas**", apresenta uma sintética revisão bibliográfica de biofilmes comestíveis, incluindo sua formação e propriedades físicas. Nesse capítulo, aborda-se os mecanismos de formação de biofilmes, com maior enfoque para os biofilmes a base de glúten de trigo e as propriedades físicas dos filmes, como permeabilidade ao vapor d'água e oxigênio e propriedades mecânicas. Revisão bibliográfica adicional encontra-se na introdução de cada capítulo.

O Capítulo 2, "**Aplicação de biofilmes e coberturas comestíveis em frutas frescas**", é uma revisão que aborda os materiais de formação e as propriedades funcionais dos biofilmes e coberturas, e as aplicações em frutas frescas. Discute-se as características de barreira (ao vapor d'água e a gases) dos biofilmes e os estudos de aplicação de biofilmes e coberturas em diversas frutas, como abacates, mangas, bananas, morangos e goiabas.

O Capítulo 3, "**Edible wheat gluten films: development, mechanical and barrier properties and application to strawberries (*Fragaria ananassa*)**", mostra o desenvolvimento e a caracterização das propriedades de biofilmes de glúten de trigo e a aplicação da cobertura em morangos frescos. Descreve-se o planejamento experimental 2^4 completo com as variáveis independentes: pH, concentrações de glúten, glicerol e etanol e as variáveis respostas do planejamento foram as permeabilidades ao vapor d’água e oxigênio, as propriedades mecânicas (resistência à tensão e porcentagem de elongação na ruptura) e solubilidade em água. Também apresenta um estudo preliminar da aplicação das coberturas à base de glúten em morangos.

O Capítulo 4, “**Wheat gluten composite and bilayer edible films: effect of lipid addition**”, aborda o desenvolvimento e caracterização de biofilmes compostos e de duas camadas de glúten de trigo e lipídios. Descreve-se as propriedades mecânicas (resistência à tensão e porcentagem de elongação na ruptura), solubilidade em água, as permeabilidades ao vapor d’água e oxigênio e as características morfológicas dos filmes.

O Capítulo 5, “**Effect of edible wheat gluten-based films and coatings on refrigerated strawberry quality**”, mostra o efeito da aplicação de coberturas e filmes à base de glúten de trigo em morangos frescos. Realizou-se o acompanhamento da vida útil dos morangos através de análises da fruta (perda de peso, retenção da firmeza, cor, acidez total, açúcares redutores e totais, ^{0} Brix, pH e sólidos totais) e da análise sensorial de aparência e degustação.

O Capítulo 6, “**Chemical lipophilization of wheat gluten and its effects on the properties of edible films**”, aborda o ensaio de lipofilização do glúten de trigo com o cloreto do ácido esteárico analisado pelo método de porcentagem de grupos amino livres (TNBS). Também, foi incluída a caracterização do filme comestível preparado a partir do glúten lipofilizado e comparado com o filme sem modificação química através das propriedades mecânicas (resistência à tensão e porcentagem de elongação na ruptura), solubilidade em água, permeabilidades ao vapor d’água e oxigênio e características morfológicas dos filmes.

O Capítulo 7, “**Development and characterization of edible gluten films from hard and soft Brazilian wheat flours**”, apresenta o desenvolvimento e caracterização de biofilmes de glúten extraídos de 4 tipos de farinha de trigo (sendo 2 “fracas” e 2 “semi-fortes”) e a comparação das propriedades (mecânicas, permeabilidades ao vapor d’água e oxigênio, solubilidade em água e características morfológicas) com os biofilmes de glúten vital (comercial). Também caracterizou-se a extensibilidade e a resistência à tensão dos glútens extraídos das farinhas.

A seguir, são apresentadas as conclusões gerais, onde todos os resultados obtidos estão analisados conjuntamente.

Os anexos apresentam informações adicionais e fotos da análise sensorial dos morangos, as isotermas de sorção e a temperatura de transição vítrea determinadas para os filmes simples, compostos e de duas camadas de glúten e lipídios. Também dois trabalhos extras realizados com a aluna Farayde M. Fakhouri sobre o desenvolvimento e caracterização de filmes compostos de glúten de trigo e gelatina e de glúten e celulose acetato ftalato, são apresentados.

O Anexo 1, “**Análise sensorial de morangos cobertos com filmes e coberturas a base de glúten de trigo**”, contém resultados e discussões adicionais ao capítulo 5 e, também, fotos da análise sensorial de aparência e degustação dos morangos.

O Anexo 2, “**Isotermas de sorção e temperatura de transição vítrea de biofilmes comestíveis à base de glúten de trigo**”, apresenta as isotermas de adsorção e a temperatura de transição vítrea dos biofilmes simples, compostos e de duas camadas de glúten de trigo e lipídios (cera de abelha, ácidos palmítico e esteárico). As isotermas de adsorção de água foram realizadas nos três filmes nas temperaturas de 25 e 45 ^{0}C e as temperaturas de transição vítrea foram determinadas através de análise mecânica-dinâmica DMA.

O Anexo 3, “**Tabelas com as espessuras de todos os filmes estudados**” apresenta as espessuras médias dos filmes.

O Anexo 4, “**Characterization of composite biofilms of wheat gluten and cellulose acetate phthalate**”, mostra o desenvolvimento e a caracterização de filmes compostos de celulose acetato ftalato e glúten de trigo em três espessuras diferentes. As características analisadas para os filmes foram as propriedades mecânicas (resistência à tensão e porcentagem de elongação na ruptura), solubilidades em água e ácido e permeabilidades ao vapor d’água e oxigênio.

O Anexo 5, “**Filmes biodegradáveis – extensão da vida útil de frutas tropicais**”**,** apresenta o desenvolvimento e a caracterização de filmes compostos de gelatina e glúten de trigo e celulose acetato ftalato e glúten. As características analisadas para os filmes biodegradáveis foram propriedades mecânicas (resistência à tensão e porcentagem de elongação na ruptura), solubilidades em água e ácido, permeabilidades ao vapor d’água e oxigênio e características morfológicas dos filmes. Também foi estudada a aplicação dessas coberturas em goiabas brancas com análises de perda de peso e retenção da textura.

O Anexo 6 apresenta uma listagem dos “posters” apresentados e aceitos em congressos ao longo do desenvolvimento desta tese.

CAPÍTULO 1

BIOFILMES COMESTÍVEIS: FORMAÇÃO E PROPRIEDADES FÍSICAS

BIOFILMES COMESTÍVEIS: FORMAÇÃO E PROPRIEDADES FÍSICAS

1. Introdução

O uso de biofilmes comestíveis em produtos alimentícios parece recente, mas vários alimentos já foram recobertos com coberturas comestíveis há muitos anos atrás. As ceras são usadas para retardar a desidratação de frutas cítricas desde os séculos XII e XIII. As aplicações mais importantes de filmes comestíveis, desde 1930, estão no uso de emulsões de ceras e óleos em água aspergidas sobre as frutas para melhorar sua aparência, incluindo o brilho e a cor, e para controlar o amadurecimento e prevenir a perda de peso. Várias coberturas comestíveis à base de polissacarídeos, incluindo alginatos, carragenas, ésteres de celulose, pectina e derivados de amido, foram usadas para melhorar a qualidade de carne (DEBEAUFORT et al., 1998).

Recentemente, surgiu um grande interesse no desenvolvimento de biofilmes (filmes biodegradáveis), principalmente devido à demanda por alimentos de alta qualidade, preocupações ambientais em relação ao descarte de embalagens não renováveis e oportunidades para criar novos mercados para as matérias-primas formadoras de filme, provenientes de produtos agrícolas. Biofilme é um filme fino preparado a partir de materiais biológicos que age como barreira a elementos externos (fatores como umidade, óleo e gases) e, consequentemente, protege o produto, aumentando a sua vida de prateleira. Os filmes também podem ajudar a manter características de qualidade desejáveis aos alimentos, como cor, aroma, acidez, doçura, teor de tempero ou sal, etc. Algumas possíveis propriedades funcionais dos biofilmes são: retardar a migração de umidade, de gases (O_2 e CO_2), de óleo ou gordura, de soluto; oferecer uma integridade estrutural adicional aos alimentos; reter compostos aromáticos e funcionar como suporte para aditivos. Além disso, podem prover nutrientes, melhorar as características sensoriais do produto e incorporar aromas, pigmentos ou outros aditivos, incluindo agentes antioxidantes e antimicrobianos. Os biofilmes podem funcionar como embalagens ativas porque podem interagir diretamente com os alimentos, controlando suas mudanças fisiológicas, microbiológicas e físico-químicas para preservar e estender a sua vida de prateleira. Isso é obtido através do controle da troca de gás com o alimento e da modificação e controle das condições da superfície dos alimentos (DEBEAUFORT et al., 1998).

Os biofilmes são geralmente produzidos com materiais biológicos como polissacarídeos, proteínas, lipídios e derivados. Entre as proteínas que podem formar filmes estão a zeína do milho, o glúten do trigo, as proteínas do leite e da soja, entre os polissacarídeos estão os derivados de celulose, amido, alginatos, pectinas e diferentes gomas e entre os lipídios, estão ceras, ácidos graxos e monoglicerídeos. Os filmes protéicos e com polissacarídeos podem ser usados em aplicações onde o objetivo não é o controle da migração de vapor d'água. Esses filmes agem como boas barreiras a gases, como oxigênio e gás carbono e também a lipídios. Os filmes com lipídios são usados para melhorar a barreira ao vapor d'água. Devido à sua baixa polaridade, a função principal do lipídio é bloquear a migração da umidade (OKAMOTO, 1978).

Muitos métodos têm sido desenvolvidos para a formação de coberturas diretamente em superfícies de alimentos, ou em separado, como filmes de auto-suporte. Neste trabalho, serão discutidos os métodos de formação de filme, como a remoção de solvente, gelatinização térmica e coacervação, e as suas propriedades físicas.

As propriedades requeridas para os biofilmes e coberturas comestíveis dependem principalmente das características do produto alimentício que será coberto ou embalado. Entretanto, baixa permeabilidade ao oxigênio é requerida em produtos sensíveis à oxidação como gorduras poliinsaturadas. Uma propriedade de transferência de massa desejada por exemplo, é permitir a respiração da fruta ou hortaliça (trocas de oxigênio, gás carbônico e etileno), e limitar sua desidratação durante a estocagem ou evitar a penetração de soluto durante a desidratação osmótica de frutas (DEBEAUFORT et al., 1998).

Além de eficientes à barreira, os filmes comestíveis devem ser compatíveis sensorial e funcionalmente com os alimentos. Embora as embalagens comestíveis ofereçam potenciais para melhorar a qualidade e a vida de prateleira de produtos alimentícios, ainda poucas aplicações industriais foram desenvolvidas. Na verdade, a formulação dessas embalagens permanece empírica e inacessível, o que dificulta seu uso e aplicação em alimentos. Maior esforço na pesquisa fundamental é necessária para melhor entender os mecanismos de transferência de solutos e voláteis através de polímeros de origem agrícola, como os filmes e coberturas comestíveis (DEBEAUFORT et al., 1998).

2. Formação de biofilmes

Vários métodos podem ser usados para formar os biofilmes, como os descritos a seguir.

(i) Remoção de solvente: Para materiais formadores de filme dispersos em soluções aquosas, a remoção de solvente é necessária para a formação de filmes sólidos. A taxa e temperatura de secagem podem influenciar a cristalinidade resultante e as propriedades mecânicas de vários materiais formadores de filme, incluindo filmes celulósicos (OKAMOTO, 1978).

(ii) Gelatinização térmica: Esse método de formação de filme deve envolver o aquecimento da proteína para provocar a desnaturação, seguida de gelatinização, ou o simples resfriamento de uma suspensão hidrocoloidal aquecida para causar uma transformação sol-gel. A formação de um gel à base de um biopolímero pode começar espontaneamente ou por mudança de temperatura ou composição da solução (pH, força iônica, tipo de íons, etc.). Como o solvente aplicado à solução polimérica evapora, o gel é formado a uma concentração crítica de sólidos durante a formação do filme (OKAMOTO, 1978, WALL e BECKWITH, 1969).

(iii) Coacervação: A coacervação pode ser simples ou complexa. Na coacervação simples, dois hidrocolóides são enriquecidos em fases separadas, e na complexa, duas soluções de hidrocolóides carregados com cargas opostas são combinados numa das fases de separação, causando interação e precipitação do polímero complexo (WALL et al., 1968). Coacervação envolve uma separação do material polimérico de cobertura de uma solução por aquecimento, mudança de pH, adição de solventes ou por alteração da carga do polímero envolvido.

Para uso em alimentos, os filmes são geralmente formados de uma solução ou dispersão da solução filme-formadora, seguida da técnica de formação de filme, como pulverização, imersão, extrusão, envoltura com o filme e obtenção do filme numa superfície após a evaporação do solvente da solução filme-formadora.

3. Biofilmes à base de glúten de trigo

Glúten de trigo é um termo geral para as proteínas insolúveis em água da farinha de trigo. É composto por uma mistura de moléculas polipeptídicas, consideradas como proteínas globulares. A coesividade e a elasticidade são características do glúten que facilitam a formação de filme (GENNADIOS e WELLER, 1990).

O glúten contém cerca de 80 a 85% das proteínas da farinha de trigo e pode ser obtido pressionando-se a massa aquosa da farinha e lavando-se delicadamente o amido e outros materiais solúveis numa solução diluída de ácido ou em excesso de água (KRULL e WALL, 1969; KRULL e INGLETT, 1971; KASARDA et al. , 1971).

O glúten pode ser separado em 2 frações de quantidades aproximadamente iguais. Uma fração é solúvel em etanol 70% e é chamada de gliadina, enquanto a outra fração, insolúvel em etanol, é chamada de glutenina

(WALL et al., 1968; KRULL e INGLETT, 1971; KASARDA et al., 1971). As proteínas da fração gliadina possuem peso molecular menor do que as proteínas da glutenina. De acordo com JONES et al. (1961), o peso molecular da gliadina está na faixa de 20.000 a 40.000 e da glutenina entre 50.000 e 1.000.000. As moléculas da glutenina são espirais longos e extensos, enquanto as moléculas da gliadina são esferas pequenas e intactas.

Para a formação de biofilmes, o glúten é inicialmente disperso em solução (etanol e água) por redução e quebra das ligações dissulfeto (S-S). A mistura mecânica ajuda na dispersão. A redução das ligações dissulfeto é atingida em ambiente alcalino (OKAMOTO, 1978) ou através da adição de agentes redutores como mercaptoetanol (WALL e BECKWITH, 1969). A quebra das ligações S-S pela sua redução em grupos SH resulta em cadeias polipeptídicas com menor peso molecular, desfazendo a elasticidade e a coesividade do glúten. O glúten disperso é então reoxidado no ar e a formação de novas ligações S-S resulta na estrutura do filme (OKAMOTO, 1978).

Um biofilme de glúten de trigo foi desenvolvido e os efeitos da concentração de glúten, concentração de etanol e pH da solução formadora de filme foram avaliados nas diversas propriedades do filme usando a metodologia de superfície de resposta (GONTARD et al., 1992). O pH e a concentração de etanol tiveram efeitos interativos na opacidade do filme, solubilidade em água e permeabilidade ao vapor d'água. Uma variação simultânea na concentração de etanol e no pH resultaram num filme homogêneo e transparente com solubilidade em água relativamente baixa. As propriedades mecânicas foram afetadas principalmente pela concentração de glúten e pelo pH. O filme mais resistente foi obtido com alta concentração de glúten (12.5%) e pH 5.

GENNADIOS et al. (1993a) investigaram o efeito do pH das soluções filme-formadoras de glúten de trigo na formação de filme. Os filmes de glúten de trigo se formaram a pH 2-4 e 9-13. A formação de filme foi inibida pela fraca dispersão da proteína ao redor da região do ponto isoelétrico do glúten de trigo (pH 7.6). Os filmes de glúten preparados em condições alcalinas apresentaram resistência à tração maior do que os filmes processados sob condições ácidas. Embora os filmes de glúten possuam pouca barreira ao vapor d'água, são efetivos à barreira de oxigênio. Assim, pode ser possível o uso desse filme como camada de barreira ao oxigênio, em materiais de embalagem com multicamadas, substituindo a poliamida e o etileno vinil álcool, que são barreiras ao oxigênio sensíveis à umidade. Muitos produtos alimentícios susceptíveis à oxidação lipídica poderiam ter o benefício da aplicação de filmes protéicos em combinação com uma barreira externa contra umidade.

Biofilmes foram também produzidos a partir de soluções formadoras de filme a base de glúten de trigo por GENNADIOS et al. (1993b). Um filme foi produzido como controle e outros três filmes foram obtidos sujeitando-se os filmes controle a diferentes tratamentos. Para todos os filmes foram medidas as propriedades físicas e de permeabilidade ao vapor d'água e oxigênio. Todos os filmes mostraram boa barreira ao oxigênio mas limitada barreira ao vapor d'água. Os filmes contendo queratina hidrolizada mostraram baixa permeabilidade ao oxigênio (83%) e baixa permeabilidade ao vapor d'água (23%). Os filmes contendo óleo mineral apresentaram baixa permeabilidade ao vapor d'água (25%). Os filmes umidecidos em solução de cloreto de cálcio e em solução tampão no ponto isoelétrico do glúten de trigo apresentaram alta resistência à tração (47% e 9%, respectivamente) e baixa permeabilidade ao vapor d'água (14% e 13% respectivamente). A adição de um agente redutor aumentou a resistência à tração (14%). O umidecimento em solução de ácido láctico não melhorou as propriedades do filme padrão.

HERALD et al. (1995) prepararam vários biofilmes a partir de glúten de trigo seco em spray-drier (SD) e flash-drier (FD) em diferentes pHs, níveis de sal e temperatura. As diferenças foram observadas nas propriedades do filme relacionadas às condições de processo. Os filmes preparados de glúten de trigo

SD foram mais espessos e tiveram maior resistência à tração e módulo de Young. As soluções de glúten de trigo SD aplicadas como material de cobertura resultaram em cascas de ovos de grau A, mantendo a sua qualidade por 30 dias em temperatura ambiente. Adicionalmente, a cobertura de glúten de trigo SD aumentou a estabilidade em água de embalagens à base de amido.

GONTARD et al. (1993) estudaram o efeito da água e do glicerol nas propriedades de barreira ao vapor d'água do biofilme de glúten de trigo. O glicerol melhorou a extensibilidade do filme mas reduziu a elasticidade, a força de ruptura e as propriedade de barreira ao vapor d'água. O efeito plastificante da água foi altamente dependente da temperatura. Durante a hidratação do filme de glúten, foi observado um decréscimo acentuado na força de ruptura e elasticidade, e um aumento da extensibilidade e da taxa de transmissão de vapor d'água nas temperaturas de 5, 30 e 50 ^{0}C. Isso está relacionado à quebra das pontes de hidrogênio do polímero e à temperatura de transição vítrea.

O efeito do aquecimento de soluções formadoras de filme de glúten de trigo nas propriedades físicas e moleculares foi determinado (ROY et al.,1999). Os filmes plastificados com glicerol foram preparados em pH alcalino (10) e aquecidos à temperatura de 55, 75 ou 95 ^{0}C por 10 minutos em solução aquosa-etanólica de glúten de trigo. A solubilidade protéica dos filmes em água aumentou ($p<0.05$) com o aumento da temperatura. Os cromatogramas em gel de permeação mostraram reduzida extração das frações protéicas do que ω-gliadinas dos filmes de glúten. Essa reduzida extração foi devida à formação de pontes dissulfeto. As bandas de SDS-PAGE do glúten nativo e das amostras de filme de glúten sugeriram um aumento de ligação cruzada através de pontes dissulfeto nos filmes preparados de soluções aquecidas a 75 ou 95 ^{0}C. Em futuras aplicações de embalagens de biofilme de glúten de trigo, a resistência à água poderia ser modificada pelo ajustamento da temperatura de tratamento.

Biofilmes compostos constituídos de glúten de trigo como matriz estrutural e diversas concentrações de lipídios diferentes como componentes de barreira à umidade foram testados para a permeabilidade ao vapor d'água, dispersão em água, opacidade e propriedades mecânicas (GONTARD et al., 1994). Os efeitos dos lipídios nas propriedades funcionais dos filmes compostos a base de glúten dependeram das características dos lipídios e das interações entre o lipídio e a matriz estrutural de proteína. A cera de abelha, um lipídio sólido e altamente hidrofóbico, foi o lipídio mais efetivo para melhorar as propriedades de barreira à umidade dos filmes; porém esses filmes foram opacos, frágeis e desintegravam facilmente na água. Combinando-se as proteínas do glúten de trigo com um éster de monoglicerídios de diacetil tartárico, reduziu-se a permeabilidade ao vapor d'água, aumentou-se a força e manteve-se a transparência.

GONTARD et al. (1995) investigaram várias formulações e métodos de produzir biofilmes de 2 camadas contendo glúten de trigo como camada estrutural e uma camada fina de lipídio como camada de barreira à umidade na permeabilidade ao vapor d'água. Os lipídios sólidos como a cera de abelha ou de parafina foram depositados no estado fundido sobre o filme base e foram os mais efetivos para a barreira ao vapor d'água. Um filme constituído de glúten de trigo, glicerol e éster de monoglicerídios de diacetil tartárico como uma camada e cera de abelha como outra camada resultou em uma permeabilidade ao vapor d'água menor do que o polietileno de baixa densidade.

Biofilmes de glúten de trigo foram preparados com várias concentrações de glicerol como plastificante (TANADA-PALMU et al., 2000). Permeabilidade ao vapor d'água e ao oxigênio, resistência à tração e porcentagem de elongação na ruptura em diferentes condições de atividade de água foram medidas. Os filmes com menor concentração de glicerol tiveram menores permeabilidades ao oxigênio e vapor d'água, maior resistência à tração e menor elongação na ruptura. Coberturas de glúten de trigo reduziram a perda de peso de caquis e tomates-caqui durante 2 semanas de armazenamento em comparação com controles sem cobertura. Uma cobertura de 2 camadas de glúten e cera de abelha significativamente reduziram a perda de peso de cubos de queijo em comparação com a cobertura de uma única camada.

4. Propriedades físicas dos filmes

Como componentes de um alimento, os filmes e coberturas comestíveis geralmente não devem apresentar sabor para não serem detectados durante o consumo do produto alimentício que foi coberto ou embalado. Quando os filmes ou coberturas comestíveis apresentarem um aroma ou sabor particular, suas características sensoriais devem ser compatíveis ao alimento (DEBEAUFORT et al., 1998).

Os filmes e coberturas comestíveis, por serem embalagens e componentes de um alimento, devem apresentar os seguintes requerimentos: boas qualidades sensoriais; alta eficiência à barreira e mecânica; estabilidade bioquímica, físico-química e microbiana; livre de toxicidade e seguros para a saúde; obtenção por tecnologia de processamento simples, não poluente e de baixo custo, tanto de matérias primas como de processo (DEBEAUFORT et al., 1998).

Os biofilmes devem apresentar propriedades funcionais específicas que são dependentes do tipo de material usado, sua formação e sua aplicação. Plastificantes, agentes reticulantes, antimicrobianos e antioxidantes, podem ser adicionados para melhorar as propriedades funcionais do filme. Em um biofilme polimérico está envolvida a força de coesão entre as moléculas formadoras de filme do polímero. O grau de coesão afeta as propriedades do filme como resistência, flexibilidade, propriedades de barreira a gases e aumento da porosidade. A coesão depende da estrutura do biopolímero, da presença de plastificantes e agentes reticulantes e da espessura final do filme. A coesão do filme é favorecida por polímeros de maior cadeia polimérica (GENNADIOS e WELLER, 1990).

Embalagens comestíveis devem apresentar propriedades funcionais e específicas. Em muitos casos, a eficiência à barreira de água é desejável para retardar a desidratação da superfície de produtos frescos (carne, frutas e verduras) ou congelados. O controle das trocas gasosas, particularmente do oxigênio, permite o melhor controle da maturação das frutas ou a redução da oxidação de alimentos sensíveis ao oxigênio e a rancidez de gorduras poliinsaturadas, por exemplo. A transferência de vapores orgânicos deve ser diminuída a fim de reter os compostos aromáticos no produto durante a estocagem ou previnir a penetração de solventes no alimento, o que pode envolver toxicidade ou sabor não característico ("off-flavor") (GENNADIOS e WELLER, 1990).

A aplicação de biofilmes depende fortemente de suas propriedades de barreira e mecânicas. Assim, foram desenvolvidas metodologias precisas para se medir as propriedades dos filmes, descritas a seguir.

O método mais comum usado para determinar a permeabilidade ao vapor d'água (WVP) é o método padrão para transmissão de vapor d'água de materiais (ASTM, 1990a). É um método gravimétrico que envolve a vedação do filme a ser testado em um prato parcialmente cheio com água ou solução saturada de sal (método da água) ou dessecante (método do dessecante), deixando um espaço de ar entre o filme e a solução de água, sal saturado ou dessecante. Em seguida, o prato é colocado em um ambiente com temperatura e umidade relativa controlada e a mudança de peso com o tempo, deve ser acompanhada para determinar o estado de fluxo estável de água para o filme. O fluxo é dividido pela diferença de pressão parcial sobre o filme durante o teste e multiplicado pela sua espessura para calcular os valores de WVP do filme (Mc HUGH et al., 1993).

A permeabilidade ao vapor d'água de filmes comestíveis é normalmente medida usando um gradiente de 100-0% de umidade relativa, assim como é medida para filmes sintéticos. Estudos recentes mostraram a necessidade de testar os filmes sob condições de temperatura e umidade relativa (RH) que serão encontrados na prática. Por exemplo, se o filme for usado entre uma peça de carne e um biscoito, o filme deve ser testado com gradiente de RH de 88-30%, já que isso representa as condições que existem entre os dois componentes. Em todos os casos, o pesquisador deve reportar o gradiente exato e as condições de temperatura do teste (DONHOWE e FENNEMA,1994).

Os pesquisadores de filmes comestíveis estão interessados na permeabilidade ao oxigênio e gás carbônico, já que esses dois gases influem nas taxas de oxidação e respiração de alimentos embalados. O método mais comum usado para determinar as propriedades de barreira ao oxigênio é em aparelhos Oxtran. Os sensores desse aparelho operam pelo princípio da lei de Faraday, onde quatro elétrons são liberados eletroquimicamente por cada molécula de oxigênio que passa pelo sensor. Esses elétrons produzem uma corrente, que passa por um resistor, criando assim uma voltagem que pode ser quantificada (DONHOWE e FENNEMA,1994). Esse método é aprovado pela ASTM (1990b) para determinação de transmissão de oxigênio em embalagens secas. Os novos sensores 2/20 do Oxtran são umidificados para prevenir a secagem do sensor. A sensibilidade dos sensores do Oxtran representa a maior desvantagem desse método em comparação com outros métodos que determinam a taxa de transmissão de oxigênio. Outra desvantagem do Oxtran é sua capacidade em controlar uma temperatura precisa e umidade relativa, e isso é importante quando se examinam propriedades de barreira de filmes comestíveis para serem usados em sistemas alimentícios sob temperaturas e condições de umidade relativa diferentes.

Um filme com boas propriedades de barreira, pode ser ineficiente se suas propriedades mecânicas não permitirem a manutenção da integridade do filme durante o processo de manipulação, empacotamento e transporte. Assim, a resistência mecânica e a deformabilidade dos biofilmes deve ser determinada. O teste mais usado para medir a força mecânica é o teste de tração, onde podem ser derivadas as propriedades de resistência à tração, elongação, força resultante e módulo de elasticidade. As propriedades mecânicas são medidas por deformação de uma amostra e monitoração da força e deformação até que esta se rompa (OKAMOTO, 1978). A resistência à tração é medida pela força máxima de tração que o filme pode sustentar. Elongação é geralmente tirada do ponto de quebra e é expressa como porcentagem de aumento do comprimento original da amostra. Força resultante é a força de tração na qual ocorre o primeiro sinal de deformação não elástica. O módulo de elasticidade ou módulo de Young mede também a resistência do filme. Na literatura, as propriedades mecânicas são frequentemente reportadas como resistência à tração e elongação (CHEN, 1995). Materiais diferentes exibem padrões de tração diferentes. Por exemplo, um filme de polissacarídeo possui alta resistência à tração e pouca elongação, enquanto que filmes protéicos apresentam moderada resistência à tração e grande elongação. Essas diferenças podem ser devidas à estrutura molecular diferente. A estrutura da cadeia polimérica de polissacarídeos é linear, enquanto que as proteínas apresentam uma estrutura complexa devido às interações inter e intramoleculares dos grupos radicais (CHEN, 1995). As medidas de resistência à tração e elongação são geralmente conduzidas de acordo com o método padrão de propriedades de tração de filme plástico fino (ASTM, 1993). Utiliza-se um instrumento universal de teste mecânico, como o Instron ou o texturômetro TA-XT2.

Outras características dos biofilmes são também estudadas, especialmente para entender melhor as propriedades mecânicas e de barreira dos filmes. Essas características são espessura, isotermas de sorção de água, solubilidade em água, difusividade de solutos entre os filmes, cor e opacidade, etc.

Atualmente, tem sido estudado o efeito de plastificantes como glicerol, xilitol e sorbitol nas propriedades físicas (como permeabilidade ao vapor d'água, resistência mecânica e temperatura de transição vítrea) de filmes à base de isolado protéico de soro de leite (SHAW et al., 2002). O aumento da concentração de glicerol e sorbitol provocou um aumento da permeabilidade e da elongação, mas uma diminuição da resistência mecânica e da temperatura de transição vítrea (Tg). Já o aumento da concentração de xilitol não provocou efeito na permeabilidade e na Tg, mas diminuiu as propriedades mecânicas. Essas diferenças nas propriedades físicas podem ser atribuídas às diferenças nas propriedades cristalinas e higroscópicas dos plastificantes.

Outros estudos que têm sido realizados envolvem a incorporação de lipídios em filmes a fim de melhorar as propriedades de barreira ao vapor d'água (QUEZADA-GALLO et al., 2000 e DEBEAUFORT et al., 2000). Nestes trabalhos, estudaram os efeitos da hidrofobicidade e do estado físico nas propriedades de filmes compostos e de duas camadas à base de polissacarídios. Tanto nos filmes de duas camadas quanto nos compostos, a resistência mecânica foi atribuída à matriz de metilcelulose, porém a permeabilidade ao vapor d'água foi substancialmente reduzida com o acréscimo de lipídios.

GARCÍA et al. (2000) também estudaram a adição de lipídios em filmes e coberturas à base de amido. A adição de óleo de girassol nos filmes diminuiu a permeabilidade ao vapor d'água e as análises calorimétricas mostraram que houve uma diminuição na razão cristalino-amorfo dos filmes sem lipídios.

Para filmes de glúten de trigo, um trabalho recente mostrou os efeitos de tratamentos químicos (vapores de formaldeído) e físicos (temperature, UV e radiação γ) na resistência mecânica e na permeabilidade ao vapor d'água (MICARD et al., 2000). O tratamento térmico severo (acima de 110 ^{0}C por 15 min.) e com formaldeído melhorou a resistência mecânica dos filmes e também provocou uma grande mudança nos parâmetros de cor, porém não afetou a permeabilidade ao vapor d'água.

5. Conclusão

Embora os filmes comestíveis ofereçam muitas oportunidades para manter ou melhorar a qualidade, estabilidade ou segurança de alimentos, ainda não foram testadas muitas aplicações comerciais. Isso é devido ao sucesso da indústria de polímeros em oferecer materiais de embalagem à indústria de alimentos com muitas propriedades funcionais e vantagens econômicas. Considerando as vantagens da tecnologia de filme comestível sobre as embalagens tradicionais, assim como o grande aumento das pesquisas em materiais de embalagem alternativos, é possível predizer que o futuro das embalagens alimentícias pertença aos filmes biodegradáveis (GENNADIOS e WELLER, 1990). Para isso, faz-se necessário entender claramente a influência da estrutura da proteína formadora de filme e suas propriedades químicas, e da técnica de formação do filme na permeabilidade, flexibilidade e adesividade do filme. Esses estudos são importantes para delinear as propriedades funcionais dos filmes comestíveis para as diversas aplicações possíveis.

6. Referências bibliográficas

ASTM. 1990a. Standard test method for water vapor transmission of materials. Designation E-96-90, **ASTM Book of Standards**, ASTM, Philadelphia, PA, pp. 834-841.

ASTM. 1990b. Standard test methods for oxygen transmission rate through dry packages using a coulometric sensor, Designation F 1307-90, **ASTM Book of Standards**, ASTM, Philadelphia, PA, pp. 1177-1182.

ASTM. 1993. Standard test methods for tensile properties of thin plastic sheeting, Designation D 882-91, **ASTM Book of Standards**, ASTM, Philadelphia, PA, pp. 316.

CHEN, H. 1995. Functional properties and application of edible films made of milk protein. **Journal of Dairy Science** 78: 2563-2583.

DEBEAUFORT, F.; QUEZADA-GALLO, J.-A. e VOILLEY, A. 1998. Edible films and coatings: tomorrow's packagings: a review. **Critical Reviews in Food Science** 38 (4): 299-313.

DEBEAUFORT, F.; QUEZADA-GALLO, J.-A.; DELPORTE, B. e VOILLEY, A. 2000. Lipid hydrophobicity and physical state effects on the properties of bilayer edible films. **Journal of Membrane Science** 180 (2000): 47-55.

DONHOWE, I.G. e FENNEMA, O.R. 1994. Edible films and coatings: characteristics, formation, definitions, and testing methods, Ch. 1, in **Edible Coatings and Films to Improve Food Quality**, Krochta, J.M.; Baldwin, E.A. & Nisperos-Carriedo, M. (eds), Technomic Publishing Company, Inc., Lancaster, PA, pp. 1-24.

GARCÍA, M.A.; MARTINO, M.N. e ZARITZKY, N.E. 2000. Lipid addition to improve barrier properties of edible starch-based films and coatings. Journal of Food Science 65 (6): 941-947.

GENNADIOS, A. e WELLER, C.L. 1990. Edible films and coatings from wheat and corn proteins. **Food Technology** 44 (10): 63-69.

GENNADIOS, A.; BRANDENBURG, A.H.; WELLER, C.L. e TESTIN, R.F. 1993a. Effect of pH on properties of wheat gluten and soy protein isolate films**. Journal of Agricultural and Food Chemistry** 41: 1835-1839.

GENNADIOS, A.; PARK, H.J. e WELLER, C.L. 1993b. Relative humidity and temperature effects on tensile strength of edible protein and cellulose ether films. **Transactions of ASAE** 36: 1867-1872.

GONTARD, N.; GUILBERT, S. e CUQ, J.L. 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. **Journal of Food Science** 57 (1): 190-199.

GONTARD, N.; GUILBERT, S. e CUQ, J.L. 1993. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. **Journal of Food Science** 58 (1): 206-211.

GONTARD, N.; DUCHEZ, C.; CUQ, J.L. e GUILBERT, S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. **International Journal of Food Science and Technology** 29: 39-50.

GONTARD, N.; MARCHESSEAU, S.; CUQ, J.L. e GUILBERT, S. 1995. Water vapor permeability of edible bilayer films of wheat gluten and lipids. **International Journal of Food Science and Technology** 30: 49-56.

HERALD, T.J.; GNANASAMBANDAM, R.; MCGUIRE, B.H. e HACHMEISTER, K.A. 1995. Degradable wheat gluten films: preparation, properties and aplications. **Journal of Food Science** 60 (5): 1147-1150, 1156.

JONES, R.W.; BABCOCK, G.E.; TAYLOR, N.W. e SENTI, F.R. 1961. Molecular weights of wheat gluten fractions. **Arch. Biochem. Biophys**. 94: 483.

KASARDA, D.D.; NIMMO, C.C. e KOHLER, G.O. 1971. Principal chemical components of wheat and flour. In **Wheat Chemistry and Technology**; Pomeranz, Y. American Association of Cereal Chemists Inc., p. 227.

KRULL, L.H. e WALL, J.S. 1969. Relationship of amino acid composition and wheat protein properties. **Baker's Digest** 43 (4): 30.

KRULL, L.H. e INGLETT, G.E. 1971. Industrial uses of gluten. **Cereal Science Today** 16 (8): 232.

McHUGH, T.H.; AVENA-BUSTILLOS, R.J e KROCHTA, J.M. 1993. Hydrophilic edible films: Modified procedure for water vapor permeability and explanation of thickness effects. **Journal of Food Science** 58 (4): 899-903.

MICARD, V.; BELAMRI, R.; MOREL, M.-H. e GUILBERT, S. 2000. Properties of chemically and physically treated wheat gluten films. **Journal of Agricultural and Food Chemistry** 48: 2948-2953.

OKAMOTO, S. 1978.Factors affecting protein film formation. **Cereal Foods World** 23: 256-262.

QUEZADA-GALLO, J.-A.; DEBEAUFORT, F.; CALLEGARIN, F. e VOILLEY, A. 2000. Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion-based edible films. **Journal of Membrane Science** 180 (2000): 37-46.

ROY, S.; WELLER, C.L.; GENNADIOS, A.; ZEECE, M.G. e TESTIN, R.F. 1999. Physical and molecular properties of wheat gluten films cast from heated film-forming solutions. **Journal of Food Science.** 64 (1) 57-60.

SHAW, N.B.; MONAHAN, F.J.; O'RIODAN, E.D. e O'SULLIVAN, M. 2002. Physical properties of WPI films plasticized with glycerol, xylitol or sorbitol. **Journal of Food Science** 67 (1): 164-167.

TANADA-PALMU, P.S., HELÉN, H. e HYVÖNEN, L. 2000. Preparation, properties and applications of wheat gluten edible films. **Agricultural and Food Science in Finland** 9 (2000): 23-35.

WALL, J.S e BECKWITH, A.C. 1969. Chemical modification of wheat gluten proteins and related model systems. **Cereal Science Today** 14 (1): 16-21.

WALL, J.S.; FRIEDMAN, M.; KRULL, L.H.; CAVINS, J.F. e BECKWITH, A.C. 1968. Relationships between structure and rheological properties of gluten properties. **Journal of Polymer Science**: Part C 24: 147.

CAPÍTULO 2

APLICAÇÃO DE BIOFILMES E COBERTURAS COMESTÍVEIS EM FRUTAS FRESCAS

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APLICAÇÃO DE BIOFILMES E COBERTURAS COMESTÍVEIS EM FRUTAS FRESCAS

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RESUMO

A importância do desenvolvimento de materiais biodegradáveis de embalagem que possam substituir os materiais sintéticos convencionais deve-se ao grande aumento da procura pelos consumidores por produtos com alta qualidade e maior vida de prateleira. As coberturas comestíveis são aplicadas diretamente na superfície dos alimentos e podem criar uma atmosfera modificada (provendo uma barreira semi-permeável a gases e vapor d'água) que resultam no aumento da vida de prateleira. Tais coberturas podem reduzir a respiração e perda de água e podem ser usadas como carregadores de preservativos e antioxidantes. Esse trabalho mostra uma visão do desenvolvimento e aplicação de filmes e coberturas comestíveis em frutas frescas.

SUMMARY

APPLICATION OF EDIBLE FILMS AND COATINGS TO FRESH FRUITS. The importance of the development of biodegradable packaging material that can replace conventional synthetic materials is due to the increased consumer demand

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for high quality and long shelf-life foods. Edible coatings are applied directly on the food surface and can create a modified atmosphere (providing a semipermeable barrier to gases and water vapor), resulting in a prolonged shelf life. Such coatings can reduce respiration and water loss, and be used as carriers of preservatives and antioxidants. An overview of the development and application of edible films and coatings to fresh fruits is given in this chapter.

Palavras-chave: biofilmes, coberturas, frutas, permeabilidade.

Introdução

As perdas pós-colheita de frutas frescas são enormes, cerca de 25 a 80%, dependendo do tipo de fruta e do uso de tecnologias de operações pós-colheita [15]. Isso reflete a falta de conhecimento dos manipuladores de frutas sobre os fatores biológicos e das condições do ambiente envolvidos na deterioração, ou falta de tecnologias pós-colheita requeridas na preservação da qualidade das frutas.

Trabalhos recentes estão explorando o potencial das coberturas comestíveis de superfície para manter e estender a qualidade e a vida útil de produtos frescos e reduzir a quantidade de embalagens descartáveis não-biodegradáveis. Tradicionalmente, os filmes são usados para reduzir a perda de peso, alterando a permeabilidade e criando um efeito de “atmosfera modificada” sobre as frutas frescas. Coberturas foram aplicadas em frutas como bananas [3], frutas cítricas [8], maçãs [4], mangas [6], peras [1], abacates [11], morangos [9] e goiabas [14]. As coberturas semi-permeáveis apresentaram influência na fisiologia das frutas, no atraso do amadurecimento e no metabolismo pós-colheita. Essa revisão discute os principais fatores no desenvolvimento de biofilmes comestíveis e aplicações em frutas frescas.

Materiais e propriedades funcionais dos filmes/coberturas

Biofilme é um filme fino preparado a partir de materiais biológicos que age como barreira a elementos externos (fatores como umidade, óleo e gases) e, consequentemente, protege o produto e aumenta a sua vida de prateleira. Os biofilmes são geralmente produzidos a partir de macromoléculas como polissacarídeos, proteínas, lipídios e derivados. As coberturas comestíveis são aplicadas e formadas diretamente no produto alimentício, enquanto os biofilmes são aplicados após serem formados separadamente.

As coberturas e biofilmes à base de lipídios produzidos de ceras e óleos são efetivos como barreira à umidade e reduzem a troca de gases, devido à hidrofobicidade. As coberturas e biofilmes feitos de polissacarídeos (celulose, pectina, amido, alginatos, quitosana e gomas) possuem boa barreira a gases (O_2 e CO_2), mas não à água, provavelmente relacionada à alta polaridade do filme. As coberturas e biofilmes protéicos (caseína, gelatina, soja, zeína, glúten e albumina de ovo) são boas barreiras ao O_2 e CO_2 em ambientes com baixa umidade relativa, mas não em alta umidade devido à susceptibilidade do filme em absorver umidade e se dissolver. Filmes e coberturas compostas ou de duas camadas estão sendo investigados, a fim de melhorar as características de permeabilidade, força, flexibilidade e valor nutricional [2].

Filmes/coberturas comestíveis devem apresentar barreira à umidade e gases, solubilidade/insolubilidade em água e lipídios, cor e aparência, características mecânicas e reológicas adequadas, não-toxicidade, etc. A cobertura/biofilme ideal deve criar uma barreira que possa retardar a perda de vapor d'água e aromas voláteis, enquanto restringe a troca de O_2 e CO_2, criando uma atmosfera modificada (AM) com CO_2 relativamente elevado e O_2 reduzido. Essa AM deve diminuir a taxa de respiração e produção e ação do etileno. Baixa permeabilidade ao vapor d'água depende da temperatura e umidade relativa, e pode ser melhorada pela inclusão de lipídios na solução filmogênica [12]. As propriedades mecânicas (resistência à tração e elongação na ruptura) dependem do tipo de material de formação do filme e condições de formação (tipo de processo e solvente, resfriamento ou taxa de evaporação e técnica de cobertura) e especialmente de sua coesão estrutural.

Aplicação de filmes e coberturas comestíveis em frutas frescas

A aplicação de alguns filmes/coberturas em frutas frescas e hortaliças é antiga, e entre eles, as ceras são aplicadas para limitar os processos de deterioração fisiológica (através do controle da troca de gases) e contaminação microbiana. Aplicações em frutas frescas foram revisadas e são apresentadas a seguir.

Bender *et al.* [5] cobriu abacates com "NatureSeal", um filme comestível a base de polissacarídeos e conseguiu retardar o amadurecimento. Os abacates cobertos tiveram menor taxa de respiração e produção de etileno e maior firmeza do que as frutas sem cobertura. Uma cobertura permeável foi produzida nas superfícies de frutas pela imersão em uma dispersão aquosa de "Pro-long", uma mistura de ésteres de sacarose de ácidos graxos e sal de sódio de carboximetilcelulose [7]. Mangas tratadas com suspensões aquosas de Pro-long produziram um aumento da vida de prateleira das frutas, retardando o amadurecimento e reduzindo a perda de peso, sem afetar a qualidade sensorial da fruta. Mangas cobertas com o filme comestível "Semperfresh", uma mistura de ésteres de mono e diglicerídeos com sacarose e carboximetilcelulose, tiveram reduzida perda de água e mudança de cor e amadurecimento estendido [6]. Kittur *et al*. [13] estudaram coberturas à base de polissacarídeos (quitosana, amido e celulose) em bananas e mangas e observaram que as coberturas à base de quitosana foram superiores em prolongar a vida de prateleira e a qualidade das frutas. Coberturas à base de amido em morangos armazenados a 0 ^{0}C reduziram o número de frutas infectadas e estenderam a vida de prateleira dos morangos pelo atraso da senescência. Os morangos cobertos tiveram menor perda de peso e mudança de cor e mantiveram a firmeza do que os morangos sem cobertura. As modificações dos parâmetros fisiológicos, como o conteúdo de antocianina, açúcares redutores e não-redutores, acidez e pH foram diminuídas nas frutas cobertas [9]. Biofilmes de fécula de mandioca utilizados na conservação de morangos armazenados em condição ambiente (21 ^{0}C e 64,5% UR) apresentaram diminuição na perda de peso, aumento de textura, prolongando em até 5 vezes a vida pós-colheita dos frutos não sendo detectado sabor ou aroma estranho devido ao filme [10]. Oliveira e Cereda [14] constataram que a utilização de película de mandioca em goiabas acarretou numa diminuição da perda de peso e da taxa respiratória dos frutos em relação ao tratamento sem cobertura.

CONCLUSÕES

O potencial de utilização de biofilmes/coberturas comestíveis em frutas frescas é crescente, por aumentar a sua vida útil, não apresentar toxicidade e também por não prejudicar o meio ambiente. A possibilidade de misturas entre polímeros naturais e modificações químicas e enzimáticas sobre os mesmos é ainda muito pouco explorada. A busca de informações básicas sobre suas características físico-químicas e funcionais no melhor aproveitamento de frutas frescas deve ser incentivada.

REFERÊNCIAS

[1] AMARANTE, C. V. T. Gas exchange, ripening behaviour and postharvest quality of coated pears. Palmerston North, 1998. PhD. Dissertation, Massey Univ., New Zealand.

[2] AMARANTE, C. and BANKS, N. H. Postharvest physiology and quality of coated fruits and vegetables. Horticultural Reviews, v. 26, p. 161-238, 2001.

[3] BANKS, N. H. Some effects of TAL Pro-Long coating on ripening bananas. J. Expt. Bot., n. 35, p. 127-137, 1984.

[4] BANKS, N. H., CHENG, Q., NICHOLSON, S .E., KINGSLEY, A. M., JEFFERY, P. B. Variation with temperature in effects of surface coatings on gas exchange of apples. Int. Cong. Plastics Agric., Tel Aviv, Israel, 9-15 March 1997.

[5] BENDER, R. J., BRECHT, S. A., SARGENT, J. C., NAVARRO, J.C., CAMPBELL, C. A. Ripening initiation and storage performance of avocados treated with an edible-film coating. Acta Hort., v. 343, p. 184-186, 1993.

[6] CARRILO-LOPEZ, A., RAMIREZ-BUSTAMANTE, F., VALDEZ-TORRES, J. B. and ROJAS-VILLEGAS. Ripening and quality changes in mango fruit as affected by coating with an edible film. J. Food Quality, v. 23, p. 479-486, 2000.

[7] DHALLA, R. and HANSON, S. W. Effect of permeable coatings on the storage life of fruits. II. Pro-long treatment of mangoes (*Mangifera indica* L. cv. Julie). Int. J. Food Sci. Technol., v. 23, p. 107-112, 1988.

[8] HAGENMAIER, R. D. and BAKER, R. A. Reduction in gas exchange of citrus fruit by wax coatings. J. Agric. Food Chem., v. 42, p. 899-902, 1993.

[9] GARCÍA, M. A., MARTINO, M. N. and ZARITZKY, N.E. Starch-based coatings: effect on refrigerated strawberry (*Fragaria ananassa*) quality. J. Sci. Food Agric., v. 76, p. 411-420, 1998.

[10] HENRIQUE, C. M. and CEREDA, M. P. Utilização de biofilmes na conservação pós-colheita de morango (*Fragaria ananassa*) cv IAC Campinas. Ciênc. Tecnol. Aliment., v. 19, n. 2, p. 231-233, 1999.

[11] JOHNSTON, J. W. and BANKS, N. H. Selection of a surface coating and optimization of its concetration for use on “Hass” avocado (*Persea amaricana*) fruit. New Zealand L. Crop Hort. Sci., v. 26, p. 143-151, 1998.

[12] KESTER, J. J. and FENNEMA, O.R. Resistance of lipid films to water vapor transmission. J. Am. Oil Chem. Soc., v. 66, p. 1139-1146, 1989.

[13] KITTUR, E. S., SAROJA, N., HABIBUNNISA, THARANATHAN, R. N. Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. Eur Food Res. Technol., v. 213, p. 306-311, 2001.

[14] OLIVEIRA, M. A. and CEREDA, M. P. Efeito da película de mandioca na conservação de goiabas. Braz. J. Food Technol., v. 21, n. 2, p. 97-102, 1999.

[15] WILLS, R. H., LEE, T. H., GRAHAM, D., MACGLASSON, W.B. and HALL, E. G. 1981. Postharvest: an introduction to the physiology and handling of fruit and vegetables. AVI Publ. Co., Westport, CT.

CAPÍTULO 3

EDIBLE WHEAT GLUTEN FILMS: DEVELOPMENT, MECHANICAL AND BARRIER PROPERTIES AND APPLICATION TO STRAWBERRIES (*Fragaria ananassa*)

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EDIBLE WHEAT GLUTEN FILMS: DEVELOPMENT, MECHANICAL AND BARRIER PROPERTIES AND APPLICATION TO STRAWBERRIES (*Fragaria Ananassa*)

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Films from wheat gluten were prepared with different pH values and concentrations of gluten, ethanol and glycerol. Their effects on oxygen and water vapor permeabilities, water solubility, tensile strength and percent elongation at break were evaluated using Response Surface Methodology. The lowest oxygen permeability would be expected at low concentrations of glycerol, gluten and ethanol. The mechanical properties were mainly affected by gluten and glycerol concentrations and the most resistant film was obtained at high gluten and low glycerol concentrations. Wheat gluten coating applied to fresh strawberries reduced weight and firmness losses during storage as compared to uncoated controls.

KEY WORDS: EDIBLE FILMS, WHEAT GLUTEN, STRAWBERRIES, PERMEABILITY, MECHANICAL PROPERTIES.

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1 INTRODUCTION

Recently there has been considerable interest in edible films for foods (TANADA-PALMU & GROSSO, 2002; PARK, 1999; AMARANTE & BANKS, 2001). 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 (DONHOWE & FENNEMA, 1992). They may also improve the recyclability of some packaging applications avoiding the disposal of non-renewable materials.

Polysaccharides, proteins and lipids can be used as edible film-forming agents (KESTER & FENNEMA, 1986; GENNADIOS & WELLER, 1991; AVENA-BUSTILLOS & KROCHTA, 1993; McHUGH, AUJARD & KROCHTA, 1994). In general, mechanical and barrier properties of biological films are highly associated with the polarity of film constituents (DEBEAUFORT, MARTIN-POLO & VOILLEY, 1993). In protein-based films, the film-forming ability may be influenced by amino acid composition, their distribution and polarity which determine cross links between amino acids, hydrogen and dissulfide bondings (GENNADIOS & WELLER, 1991). Due to the unique cohesive and elastic properties of gluten (WALL & HUEBNER, 1981), good film-forming properties may be expected. Biodegradable and edible films from wheat proteins are useful in food packaging provided they are flexible, strong, heat sealable, and relatively transparent (RAYAS, HERNANDEZ & NG, 1997). The mechanical and barrier properties of wheat gluten films have been studied (GENNADIOS *et al.*, 1993; GONTARD, GUILBERT & CUQ, 1993; CHERIAN *et al.*, 1995). An edible wheat gluten film was developed and the effects of gluten and ethanol concentrations and pH of the film-forming solution on various film properties were evaluated (GONTARD, GUILBERT & CUQ, 1992).

HERALD *et al*., (1995) compared wheat gluten films prepared at various pH values, as a result films prepared at pH 3.3 showed higher water vapor permeability, presumibly due to the unfolding conformation of protein molecules and exposure of hydrophilic groups. Edible wheat gluten films were prepared with various amounts of glycerol (TANADA-PALMU, HELÉN & HYVÖNEN, 2000). Films with low amounts of glycerol had lower water vapor and oxygen permeabilities, higher tensile strength and lower elongation at break.

The objective of this work was the preparation of edible wheat gluten films and the evaluation of film forming conditions (pH, concentrations of gluten, ethanol and glycerol) on the film properties (water solubility, oxygen and water vapor permeabilities and mechanical properties). Applications of such films as coatings for strawberries, a typical soft fruit having a high physiological post-harvest activity, were also investigated.

2 MATERIALS AND METHODS

2.1 Materials

Vital wheat gluten (Rhodia, Campinas, Brazil), beeswax (Chemco, São Paulo, Brazil), glycerol (Merck, Darmstadt, Germany), ammonium hydroxide (Synth, São Paulo, Brazil), calcium chloride (Ecibra, São Paulo, Brazil), sodium chloride (Synth, São Paulo, Brazil), magnesium nitrate (Ecibra, São Paulo, Brazil), solid paraffin (Chemco, São Paulo, Brazil) and fresh strawberries (Campinas free market).

2.2 Film preparation

The gluten concentration (g/100 mL solution), ethanol concentration (mL/100 mL solution), glycerol concentration (g/100 mL solution), and the pH of the solution (adjusted with ammonium hydroxide), used to prepare the films varied according to the values presented in Table 1. The film was prepared from a solution of gluten/absolute ethanol, ammonium hydroxide and distilled water. All components were mixed under magnetic stirring until the temperature of the mixture reached 70 ^{0}C and the solution centrifuged at 5856 g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a teflon covered glass surface and dried at room temperature for 24 h (modified method from GONTARD, GUILBERT & CUQ, 1993). The quantity of solution (vol.) poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for experiments were equilibrated at 52% relative humidity (RH), using a saturated solution of $Mg(NO_3)_2$ at 25 ^{0}C for 48 hours (h) before being tested.

2.3 Film thickness

Film thickness was measured using a micrometer (Model MDC-25M, Mitutoyo, MFG, Japan). The thickness of individual film samples was determined as an average of five measurements.

2.4 Water vapor permeability

The water vapor transmission rate of the films was determined gravimetrically at 25 ^{0}C using a modified American Society for Testing and Materials (ASTM) Standard Method E-96 according to TANADA-PALMU, HELÉN & HYVÖNEN, (2000). The samples, four replicates of each film, were conditioned for 2 days at 52% RH before measurement. The water vapor permeability (WVP) was calculated according to the ASTM method.

2.5 Oxygen permeability

The oxygen transmission rates were determined using a modification of the ASTM Standard Method D 3985-81 with an Ox-Tran apparatus (Mocon, Inc., Minneapolis, USA) at 25 ^{0}C. The samples, two replicates of each film, were conditioned for 2 days at 52% RH before measurement. The oxygen permeability (OP) was calculated by dividing the oxygen transmission rate by the oxygen pressure and multiplying by the mean thickness.

2.6 Tensile strength and percent elongation at break

Film tensile strength (TS) and percent elongation at break (ELO) were determined using a Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), operated according to the ASTM Standard Method D 882-83 (25 ^{0}C and 52% RH, initial grip separation = 50 mm and cross head speed = 100 mm/min). The peak loads and extension at break were recorded for tested film specimens (100 mm long and 25.4 mm wide). The films were conditioned for 2 days at 52% RH before measurement. Six specimens of each film were measured. The tensile strength and percent elongation at break were calculated according to the ASTM method.

2.7 Solubility in water

The percent of solubility was the percentage of dry matter of the film solubilized after 24 h immersion in water. The percentage of initial dry matter of each film was determined at 105 ^{0}C for 24 h. Two discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water and slowly and periodically agitated for 24 h at 25 ^{0}C. The pieces of film were then taken out and dried (105 ^{0}C for 24 h) to determine the weight of dry matter, which was not solubilized in water. The weight of dry matter solubilized was calculated by subtracting the weight of dry matter not solubilized from the weight of initial dry matter and reported on an initial dry weight basis.

2.8 Statistical analyses

The 2^4 complete factorial planning with 3 repetitions in the central point was used in this experiment. The data were analyzed to fit the following second order equation to all dependent Y variables:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2$$

Where b_n are constant regression coefficients and X_1, X_2, X_3 coded independent variables. The Statistica 5.5 (Statsoft, USA) program was used for the analysis of variance, regression coefficient calculation and response surfaces.

2.9 Application of the edible film to fresh strawberries

The edible wheat gluten film was applied to coat fresh strawberries to evaluate the influence of the film on the weight and firmness losses of the product. Four treatments were effected to cover fresh strawberries (triplicates) with the gluten film. In treatment 1, fresh strawberries were dipped with pliers into the film-forming solution (7.5 g gluten + 1.87 g glycerol + 45 mL ethanol/100 mL solution, pH 10) at room temperature for 1 minute (min). In treatment 2, strawberries were dipped into the same film-forming solution as treatment 1 for 1 min and allowed to dry for 1 h (hanging up by their calyces); then they were dipped again into the same solution for 1 min in order to form a bilayer gluten film over the strawberries. In treatment 3, strawberries were treated as in treatment 2, but the second layer was formed by dipping the strawberries into molten beeswax at 70 ^{0}C for a few seconds. In treatment 4 (control), strawberries were dipped into distilled water for 1 min. Strawberries were stored in the refrigerator (7 ^{0}C) for two weeks and during this time, the weight loss was followed and the firmness loss was registered by the Texture Analyzer (the compression force of the strawberry flesh was measured using a Texture Analyzer TA.XT2 (SMS, Surrey, UK) equipped with a compression cell of 5 kg and a cylindrical acrylic probe of 1 cm in diameter, using a 1 mm/s crosshead speed and 75% strain).

3 RESULTS AND DISCUSSION

3.1 Statistical analysis and response surfaces

The experimental data presented on Table 1 and 2 shows the results of the analysis of variance for each of the dependent variables, with corresponding coefficients of multiple determination (R^2) and F values. The model for water vapor permeability (Y_2) showed a high lack of fit, considering there were no significant p and F values, indicating that data variations could not be adequately explained (THOMPSON, 1982). An investigation of the residual errors (difference between predicted and experimental Y-variables values) indicated that a higher order model would be necessary to more adequately describe the data. However, this model could be used for trend analysis. The models for oxygen permeability, tensile strength and percent elongation at break seemed adequate, with satisfactory R^2 or significant p or F values.

The estimated partial regression coefficients (RC), pure error (PE) and level of significance (p) for the five quadratic models and the results of significance tests on the coefficients are indicated in Table 2. The three variables (gluten, glycerol and ethanol) appeared to affect film properties. From the significant p values, gluten (glu), glycerol (gly) and ethanol (et) concentrations of the film-forming solution were the most important factors influencing oxygen permeability and mechanical properties. The interaction between independent and dependent variables is represented by the three-dimensional response surfaces (Figs. 1 and 2) generated by the regression equations developed from the mathematical analysis.

Table 1 – RESPONSES OF DEPENDENT VARIABLES TO THE FILM-FORMING CONDITIONS

	Independent variables[a]				Dependent variables				
Films	X_1(pH)	X_2(glu)	X_3(gly)	X_4(et)	Y_1(OP)	Y_2(WVP)	Y_3(SOL)	Y_4(TS)	Y_5(ELO)
1	9.5 (-1)	6.0 (-1)	1.50 (-1)	32.5 (-1)	24.97	8.48	15.8	2.07	170.6
2	10.5 (+1)	6.0 (-1)	1.50 (-1)	32.5 (-1)	21.03	8.67	13.3	1.98	143.5
3	9.5 (-1)	9.0 (+1)	1.50 (-1)	32.5 (-1)	39.86	10.20	22.0	3.91	184.1
4	10.5 (+1)	9.0 (+1)	1.50 (-1)	32.5 (-1)	41.02	8.61	22.7	5.25	215.3
5	9.5 (-1)	6.0 (-1)	1.50 (-1)	57.5 (+1)	24.40	12.95	16.3	1.19	170.0
6	10.5 (+1)	6.0 (-1)	1.50 (-1)	57.5 (+1)	26.79	11.61	16.1	1.33	160.5
7	9.5 (-1)	9.0 (+1)	1.50 (-1)	57.5 (+1)	28.30	9.48	26.0	5.38	140.5
8	10.5 (+1)	9.0 (+1)	1.50 (-1)	57.5 (+1)	36.95	8.58	11.1	7.17	138.6
9	9.5 (-1)	6.0 (-1)	2.25 (+1)	32.5 (-1)	25.85	19.77	12.4	0.45	222.9
10	10.5 (+1)	6.0 (-1)	2.25 (+1)	32.5 (-1)	33.20	14.89	24.1	0.42	163.4
11	9.5 (-1)	9.0 (+1)	2.25 (+1)	32.5 (-1)	42.20	10.29	13.6	1.49	139.1
12	10.5 (+1)	9.0 (+1)	2.25 (+1)	32.5 (-1)	42.30	9.63	12.4	1.65	175.1
13	9.5 (-1)	6.0 (-1)	2.25 (+1)	57.5 (+1)	38.70	13.57	25.4	0.30	277.2
14	10.5 (+1)	6.0 (-1)	2.25 (+1)	57.5 (+1)	28.30	12.48	27.0	0.42	282.8
15	9.5 (-1)	9.0 (+1)	2.25 (+1)	57.5 (+1)	30.45	8.18	16.6	0.72	187.9
16	10.5 (+1)	9.0 (+1)	2.25 (+1)	57.5 (+1)	36.40	8.19	17.4	0.70	273.9
17	9.0 (-2)	7.5 (0)	1.87 (0)	45.0 (0)	46.58	12.69	23.3	1.03	263.9
18	11.0 (+2)	7.5 (0)	1.87 (0)	45.0 (0)	34.76	8.53	20.9	1.70	232.6
19	10.0 (0)	4.5 (-2)	1.87 (0)	45.0 (0)	29.16	9.61	13.5	0.37	302.3
20	10.0 (0)	10.5 (+2)	1.87 (0)	45.0 (0)	34.47	8.65	16.2	1.39	282.0
21	10.0 (0)	7.5 (0)	1.87 (0)	20.0 (-2)	41.93	11.07	26.5	0.88	234.1
22	10.0 (0)	7.5 (0)	1.87 (0)	70.0 (+2)	49.68	14.91	22.5	0.87	196.8
23	10.0 (0)	7.5 (0)	1.12 (-2)	45.0 (0)	35.82	5.72	17.4	2.36	184.1
24	10.0 (0)	7.5 (0)	2.62 (+2)	45.0 (0)	38.64	10.46	12.7	0.55	336.4
25(c)	10.0 (0)	7.5 (0)	1.87 (0)	45.0 (0)	39.19	13.60	21.8	0.84	207.6
26(c)	10.0 (0)	7.5 (0)	1.87 (0)	45.0 (0)	41.25	12.56	16.3	1.22	216.5
27(c)	10.0 (0)	7.5 (0)	1.87 (0)	45.0 (0)	38.56	7.89	21.0	1.60	236.8

[a] values between brackets are the coded symbols of the levels of the independent variables; “(c)”- central points of the plan; X_1(pH) - pH of the film-forming solution; X_2(glu) - gluten concentration (g/100 mL solution); X_3(gly) – glycerol concentration (g/100 mL solution); X_4(et) – ethanol concentration (mL/100 mL solution); Y_1(OP) – oxygen permeability ($cm^3\mu m/m^2dkPa$); Y_2(WVP) – water vapor permeability (gmm/m^2dkPa); Y_3(SOL) – solubility in water (%); Y_4(TS) – tensile strength (MPa); Y_5(ELO) – percent elongation at break (%).

Table 2– REGRESSION COEFFICIENTS, PURE ERROR AND LEVEL OF SIGNIFICANCE OF THE SECOND ORDER POLYNOMIALS[a] FOR 5 RESPONSE VARIABLES

	Oxygen permeability (Y_1)			Water permeability (Y_2)			Solubility in water (Y_3)			Tensile strength (Y_4)			Elongation at break (Y_5)		
	RC	PE	p	RC	PE	p	RC	PE	p	RC	PE	p	RC	PE	P
constant (b_0)	39.67	0.81	**0.0004**	11.35	1.75	**0.0231**	19.70	1.71	**0.0075**	1.220	0.22	**0.0308**	220.30	8.64	**0.0015**
linear															
pH (b_1)	-1.03	0.57	0.2143	-1.55	1.24	0.3386	-0.73	1.21	0.6069	0.39	0.15	0.1254	-0.09	6.11	0.9890
glu (b_2)	7.07	0.57	**0.0065**	-2.60	1.24	0.1714	-0.27	1.21	0.8464	1.68	0.15	**0.0084**	-36.84	6.11	**0.0264**
gli (b_3)	3.31	0.57	**0.0288**	2.32	1.24	0.2020	-0.32	1.21	0.8185	-2.14	0.15	**0.0052**	80.74	6.11	**0.0057**
et (b_4)	-0.39	0.57	0.5702	0.18	1.24	0.8971	0.97	1.21	0.5091	-0.01	0.15	0.9886	10.19	6.11	0.2374
quadratic															
pH x pH(b_{11})	-1.61	0.61	0.1183	-0.11	1.32	0.9390	0.91	1.29	0.5517	0.41	0.16	0.1311	-12.27	6.48	0.1874
glu x glu(b_{22})	-6.04	0.61	**0.0100**	-0.85	1.32	0.5833	-2.71	1.29	0.1696	0.17	0.16	0.4192	9.17	6.48	0.2925
gli x gli(b_{33})	-3.33	0.61	**0.0319**	-1.37	1.32	0.4064	-0.32	1.29	0.1794	0.45	0.16	0.1103	-6.77	6.48	0.4055
et x et(b_{44})	0.96	0.61	0.2566	1.08	1.32	0.4996	2.11	1.29	0.2423	0.16	0.16	0.4250	-29.17	6.48	**0.0460**
interactions															
pH x glu(b_{12})	2.56	0.70	0.0680	0.50	1.52	0.7746	-3.15	1.48	0.1681	0.39	0.19	0.1757	30.31	7.48	0.0559
pH x gli(b_{13})	-0.66	0.70	0.4486	-0.37	1.52	0.7941	3.72	1.48	0.1290	-0.37	0.19	0.1918	9.34	7.48	0.3382
pH x et(b_{14})	0.24	0.70	0.7655	0.45	1.52	0.8293	-2.67	1.48	0.2136	0.08	0.19	0.7106	12.53	7.48	0.2360
glu x gli(b_{23})	-2.95	0.70	0.0523	-2.45	1.52	0.8227	-6.15	1.48	0.0537	-1.52	0.19	**0.0152**	7.60	7.48	0.4164
glu X et(b_{24})	-5.80	0.70	**0.0144**	-0.39	1.52	0.2488	-2.35	1.48	0.2545	0.42	0.19	0.1584	-53.48	7.48	**0.0190**
gli x et(b_{34})	0.09	0.70	0.9074	-2.35	1.52	0.2619	3.52	1.48	0.1410	-0.47	0.19	0.1384	86.28	7.48	**0.0074**
%variability explained-R^2		0.61639			0.72556			0.79565			0.76931			0.67388	
F		1.38			2.27			**3.34**			**2.86**			**3.39**	

[a] Model on which X_1=pH, X_2=gluten concentration, X_3=glycerol concentration, X_4=ethanol concentration of the film-forming solution is:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2$$

RC: regression coefficients

PE: pure error

p: level of significance

F from table:2.62

Bold numbers: significant at 5% level

3.2 Water vapor permeability

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).

According to BANKER, (1966), plasticizers are added to films to reduce brittleness, increase toughness, strength, tear and impact resistance and impart flexibility. Usually the addition of the plasticizer increase gas, water vapor and solute permeabilities and decrease the tensile strength of the films. From the results (Table 1), water vapor permeability (WVP) increased with glycerol concentration in accordance with GONTARD, GUILBERT & CUQ, (1993); PARK *et al.*, (1994a) and TANADA-PALMU, HELÉN & HYVÖNEN, (2000). The plasticizers achieve such changes probably through interactions between plasticizer and polymer and the reduction of cumulative intermolecular forces along polymer chains, leading to “softening” of the film structure (GENNADIOS *et al.*, 1993). Additionally this could be explained by the modifications of the protein structure, which might become less dense, and by the hydrophilicity of glycerol, which favors adsorption and desorption of water molecules (GONTARD, GUILBERT & CUQ, 1993). Thus, the lowest WVP was obtained from film 23, the one with the lowest glycerol concentration.

Increasing the ethanol concentration of the film-forming solution increased WVP (Table 1). At high ethanol concentration the film became heterogeneous and this could explain the feasibility of water transmission.

3.3 Oxygen permeability

Good oxygen barrier properties of edible films can be used in food packaging and preservation. As an example, coating foods susceptible to lipid oxidation with protein films, in combination with an external conventional moisture barrier package seems to be possible. Oxygen permeability of all the wheat gluten films were similar to the values obtained by PARK & CHINNAN, (1990) for zein:glycerin films (13.0-44.9 $cm^3 \mu m/m^2 dkPa$) and gluten:glycerin films (9.6-24.2 $cm^3 \mu m/m^2 dkPa$) at 30 ^0C and 0% RH. Oxygen permeability of all films was low, maybe due to their polar nature and linear structure, which leads to a high cohesive energy density and a low free volume (TANADA-PALMU, HELÉN & HYVÖNEN, 2000).

The shape of the response surfaces (Figs. 1A and 2A) was characteristic of the interaction between gluten, glycerol and ethanol concentrations and showed that the lowest oxygen permeability value would be expected with low concentrations of these three variables.

3.4 Mechanical properties

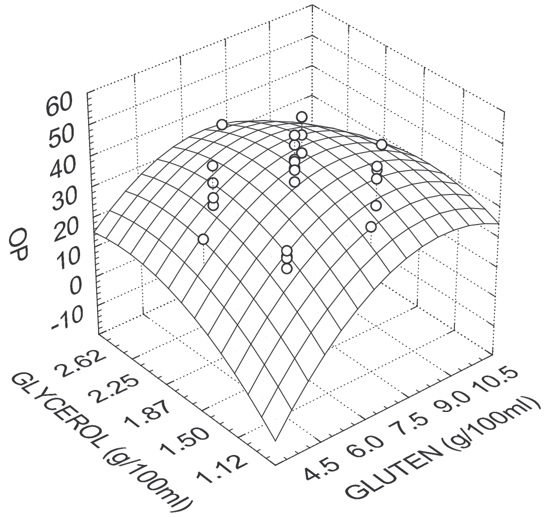
An edible film should be resistant in order to withstand the manipulation during its application and to maintain its integrity and also its barrier properties. Tensile strength decreased and percent elongation at break increased with an increase in glycerol concentration (Figs. 1B and 1C), in accordance with TANADA-PALMU, HELÉN & HYVÖNEN, (2000). This similar behavior has been reported for other films (PARK *et al.*, 1993; McHUGH & KROCHTA, 1994; GENNADIOS *et al.*, 1996). Unplasticized gluten film has low flexibility due to highly cooperative protein-protein interaction, mainly because of the high glutamine content which is responsible for numerous hydrogen bonds between protein chains. Glycerol is a small hydrophilic molecule which could be inserted between protein chains. With glycerol in the protein network the distance between protein chains was increased and direct interactions were reduced.

The response surface showed that at high gluten concentrations films became more resistant with a high tensile strength (Figs. 1B and 2B). Percent elongation at break decreased as gluten concentration increased and the concentration of ethanol decreased (Figs. 1C and 2C). The mechanism to form a resistant film could involve a higher number and/or a better localization of bonds between protein chains. During the drying of the film-forming solution, ethanol and ammonium hydroxide were evaporated, allowing the formation of bonds between protein chains. During this stage, the proximity of protein chains induced by high gluten concentrations could form such cross-bonds. During film formation, polymerization occurs through intermolecular disulfide, hydrophobic and hydrogen bondings (WALL & BECKWITH, 1969). An alkaline environment and heating are necessary to denature the gluten protein, thus disrupting protein structure, breaking existing disulfide intramolecular bonds and exposing sulphydryl and hydrophobic groups, making them available for bonding. The cleavage of disulfide bonds results in polypeptide chains with lower molecular weights, destroying elasticity and cohesiveness of gluten. Upon casting and drying, sulphydryl groups reform disulfide bonds by air oxidation, which leads the film structure (GENNADIOS *et al*., 1993). Similar observations (MUNOZ, PANGBORN & NOBLE, 1986; BANKER, 1966; GUILBERT & BIQUET, 1989) have been made with protein (gelatin) gels which had lower puncture forces at low protein concentration.

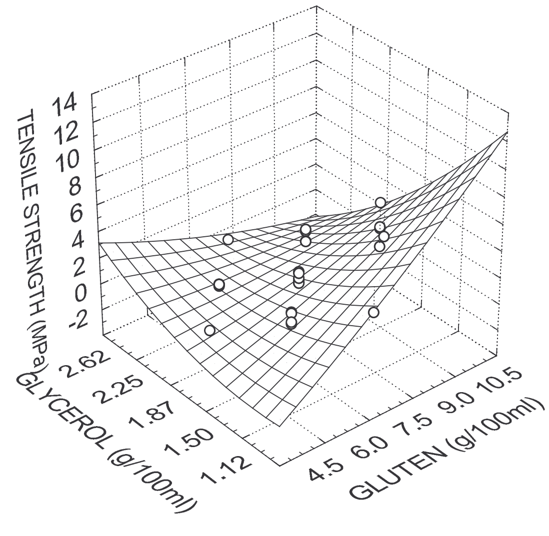
3.5 Solubility in water

Water resistance is an important property of edible films for applications in food protection, where water activity is high, or when the film must be in contact with water during processing of the coated food to avoid exudation of fresh or frozen products. Therefore, 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). Solubility in water was low for all films, compared to zein films (solubility about 90%, F5000, Freeman Industries, NY) which disintegrate easily in water.

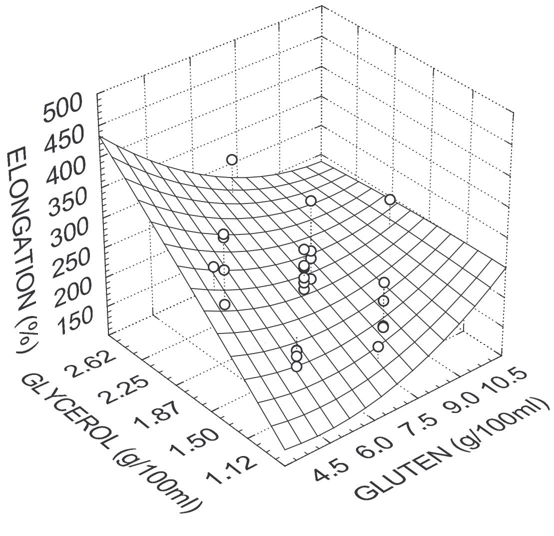
FIGURE 1 – RESPONSE SURFACE FOR THE EFFECT OF GLUTEN AND GLYCEROL CONCENTRATIONS OF THE FILM-FORMING SOLUTION ON OXYGEN PERMEABILITY (A), TENSILE STRENGTH (B) AND ELONGATION AT BREAK (C)



A

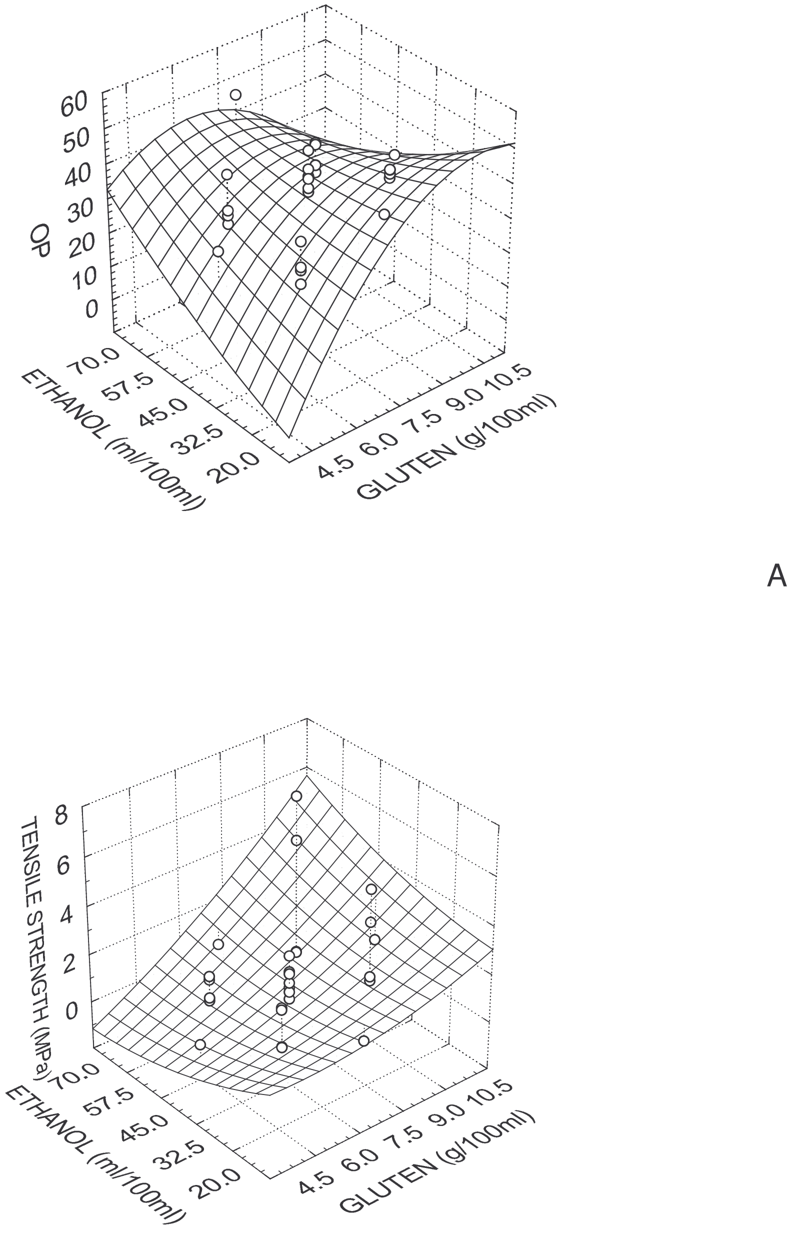

TENSILE STRENGTH (MPa)
14
12
10
8
6
4
2
0
-2
2.62
2.25
1.87
1.50
1.12
GLYCEROL (g/100ml)
4.5
6.0
7.5
9.0
10.5
GLUTEN (g/100ml)

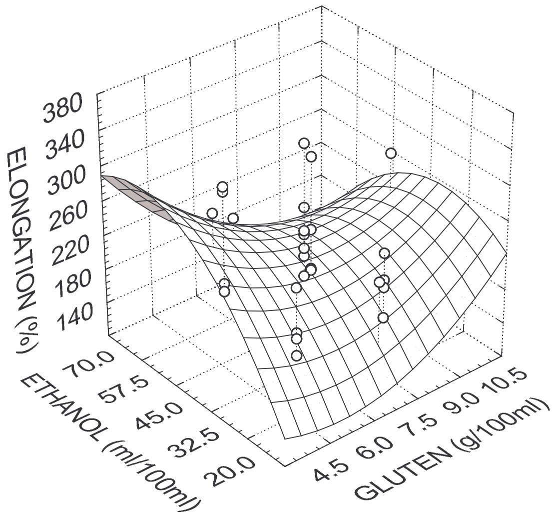
B


ELONGATION (%)
500
450
400
350
300
250
200
150
2.62
2.25
1.87
1.50
1.12
GLYCEROL (g/100ml)
4.5
6.0
7.5
9.0
10.5
GLUTEN (g/100ml)

C

FIGURE 2 – RESPONSE SURFACE FOR THE EFFECT OF GLUTEN AND ETHANOL CONCENTRATIONS OF THE FILM-FORMING SOLUTION ON OXYGEN PERMEABILITY (A), TENSILE STRENGTH (B) AND ELONGATION AT BREAK (C)





3.6 Application of wheat gluten to strawberries

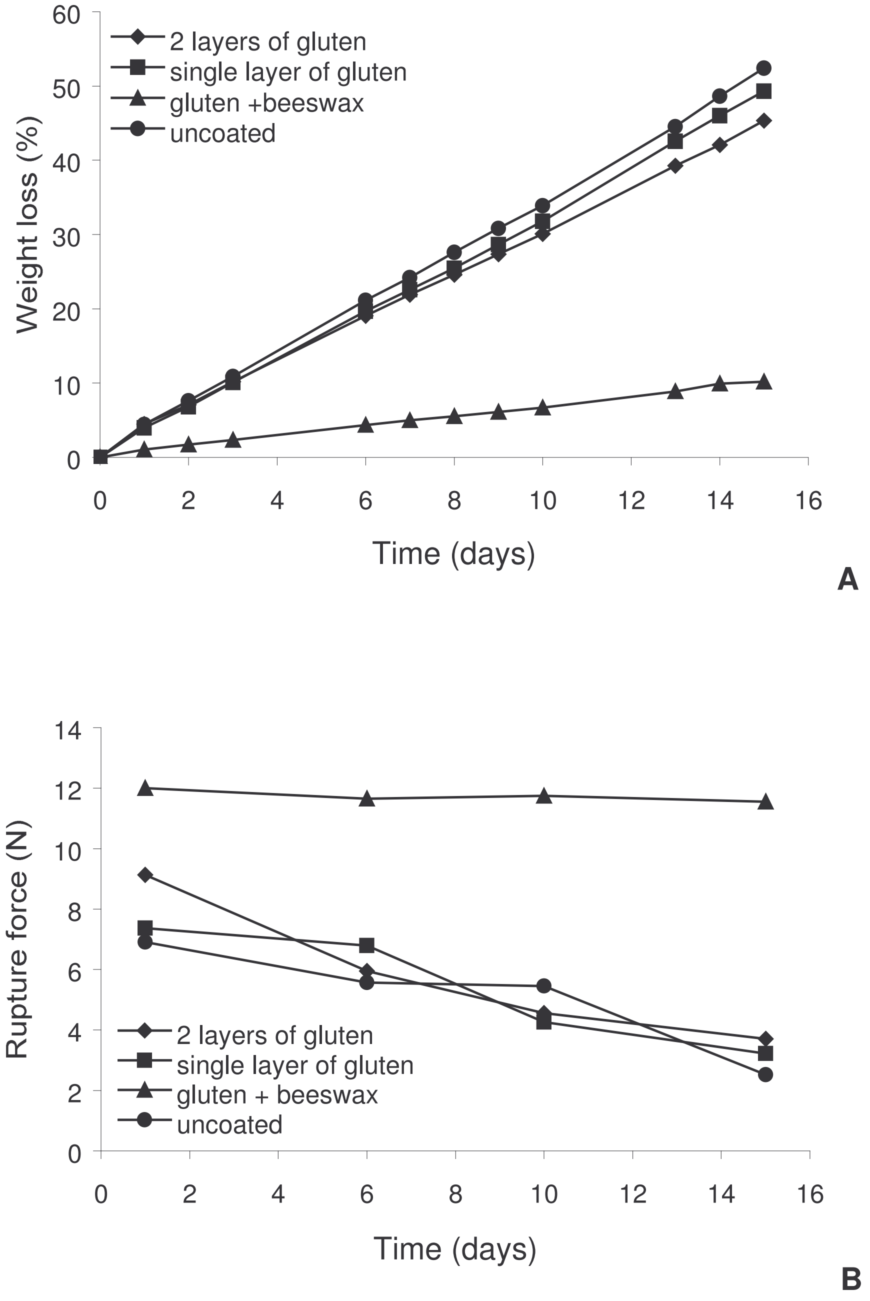
Edible wheat gluten coatings were applied in order to reduce moisture and firmness losses during storage. Moisture and firmness losses, in terms of % weight loss and rupture force (N), between controls and coated products during two weeks of storage are illustrated in Fig. 3.

Uncoated strawberries lost weight faster and showed higher firmness loss than the coated samples with wheat gluten and beeswax during storage. However, there was no great difference in weight and firmness losses of the gluten coated strawberries (1 or 2 layers) and the controls. The strawberries coated with a bilayer coating, dipped in wheat gluten film-forming solution and with a second layer of beeswax, had relatively low weight and firmness losses. Texture modifications in fruits and vegetables are related to the composition of the cell wall, enzyme activity, metabolic changes and water content. The rate and extension of firmness loss during ripening of soft fruits like strawberries is the main factor which determines fruit quality and post-harvest shelf life. According to MANNING, (1993), fruit softening is attributed to the degradation of cell wall components due to specific enzyme activity.

The wheat gluten coating reduced moisture and firmness losses from strawberries. TANADA-PALMU, HELÉN & HYVÖNEN, (2000) showed that gluten coating reduced weight loss of cherry tomatoes and sharon fruits and a bilayer coating of gluten and beeswax significantly reduced the weight loss of cheese cubes comparing to a single layer of gluten coating. PARK, CHINNAN & SHEWFELT, (1994b) reported that a corn zein film behaved similarly with tomatoes. LERDTHANANGKUL & KROCHTA, (1996) observed that a mineral-oil-based coating reduced moisture loss from green bell peppers. Starch-based films reduced moisture loss from strawberries (GARCíA, MARTINO & ZARITZKY, 1998a,b).

A bilayer coating of wheat gluten and beeswax showed the best results to keep the quality of strawberries during storage. Thus, a second layer of lipid is necessary to improve the water vapor barrier capacity of the wheat gluten coatings in order to control the moisture and firmness losses from coated products.

FIGURE 3 – WEIGHT (A) AND RUPTURE FORCE (B) OF GLUTEN COATED AND UNCOATED STRAWBERRIES DURING STORAGE



4 CONCLUSIONS

Wheat gluten can be used as a source of protein-based biodegradable films and coatings. Response surface methodology seemed to be an effective tool for studying film-formation conditions. Concentrations of gluten, glycerol and ethanol influenced oxygen permeability and mechanical properties (tensile strength and percent elongation at break) of the gluten films. Gluten based coatings could be used to protect perishable foods such as strawberries. However, an additional lipid layer is necessary to reduce moisture and firmness losses of the coated products. Possibilities of improving wheat gluten film properties and evaluating new applications of the gluten coating should be explored.

Resumo

FILMES COMESTÍVEIS DE GLÚTEN DE TRIGO: DESENVOLVIMENTO, PROPRIEDADES DE BARREIRA E MECÂNICAS E APLICAÇÃO NA COBERTURA DE MORANGOS (*Fragaria Ananassa*).

Filmes de glúten de trigo foram preparados em diferentes valores de pH e concentrações de glúten, etanol e glicerol, e seus efeitos sobre permeabilidade ao vapor de água e oxigênio, solubilidade em água, força de tensão e porcentagem de elongação foram avaliados usando Metodologia de Superfície de Resposta. Menor permeabilidade ao oxigênio foi observada a menores concentrações de glicerol, glúten e etanol. As propriedades mecânicas foram principalmente afetadas pelas concentrações de glúten e glicerol e o filme mais resistente foi obtido quando maior concentração de glúten e menor de glicerol foram utilizadas. Coberturas de glúten de trigo aplicadas em morangos frescos reduziram as perdas de firmeza e de peso durante a estocagem comparado a um controle não revestido.

PALAVRAS CHAVES: FILMES COMESTÍVEIS, GLÚTEN DE TRIGO, MORANGOS, PERMEABILIDADE, PROPRIEDADES MECÂNICAS.

REFERENCES

1. AMARANTE C. and BANKS N. H. Postharvest physiology and quality of coated fruits and vegetables. **Horticultural Reviews,** v.26, p. 161-238, 2001.

2. ASTM. Standard test methods for water vapor transmission of materials. Method E-96. In **Annual Book of ASTM Standards**. American Society for Testing and Materials. Conshohocken, PA, 1980.

3. ASTM. Standard test methods for gas transmission rate of plastic film and sheeting. Method D 3985-81. In **Annual Book of ASTM Standards**. American Society for Testing and Materials. Conshohocken, PA, 1980.

4. ASTM. Standard test methods for tensile properties of thin plastic sheeting. Method D 882-83. In **Annual Book of ASTM Standards**. American Society for Testing and Materials. Conshohocken, PA, 1980.

5. AVENA-BUSTILLOS, R. J. and KROCHTA J. M. Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. **Journal of Food Science**, v.58, n. 4, p. 904-907, 1993.

6. BANKER, G. S. Film coating theory and practice. **Journal of Pharmacy Science** v. 55, p. 81-89, 1966.

7. CHERIAN, G.; GENNADIOS, A.; WELLER, C. L. and CHINACHOTI P.1. Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. **Cereal Chemistry**, v. 72, p. 1-6, 1995.

8. DEBEAUFORT, F.; MARTIN-POLO, M.; and VOILLEY, A. Polarity, homogeneity and structure affect water vapor permeability of model edible films. **Journal of Food Science**, v.58, p. 436-434, 1993.

9. DONHOWE, G. and FENNEMA, O. The effect of relative humidity gradient on water vapor permeance of lipid and lipid-hydrocolloid bilayer films. **Journal of American Chemistry Society**, v.6, p.1081-1087, 1992.

10. GARCÍA, M. A.; MARTINO, M. N. and ZARITZKY, N. E. Starch-based coatings: effect on refrigerated strawberry (*Fragaria ananassa*) quality. **Journal of the Science of Food and Agriculture**, v.76, p. 411-420, 1998a.

11. GARCÍA, M. A.; MARTINO, M. N. and ZARITZKY, N. E. Plasticized starch-based coatings to improve strawberry (*Fragaria ananassa*) quality and stability. **Journal of Agriculture and Food Chemistry**, v.46, p. 3758-3767, 1998b.

12. GENNADIOS, A. and WELLER, C. L. Edible films and coatings from soymilk and soy protein. **Cereal Foods World**, v.36, p. 1004-1009, 1991.

13. GENNADIOS, A.; BRANDENBURG, A. H.; WELLER, C. L. and TESTIN, R. F. Effect of pH on properties of wheat gluten and soy protein isolate films. **Journal of Agriculture and Food Chemistry**, v.41, p.1835-1839, 1993.

14. GENNADIOS, A.; WELLER, C. L.; HANNA, M. A. and FRONING, G. W. Mechanical and barrier properties of egg albumen films. **Journal of Food Science,** v.61, n. 3, p. 585-589, 1996.

15. GONTARD, N.; GUILBERT, S. and CUQ, J. L. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. **Journal of Food Science**, v.57, n.1, p. 190-199, 1992.

16. GONTARD, N.; GUILBERT, S. and CUQ, J. L. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. **Journal of Food Science**, v.58, n.1, p. 206-211, 1993.

17. GUILBERT, S. and BIQUET, B. Les films et enrobage comestibles. In L'EMBALLAGE des denrées alimentaires de grande consommation. Technique et Documentation, Lavoisier Apri: Paris France, 1989. p.320.

18. HERALD, T. J.; GNANASAMBANDAM, R.; MCGUIRE, B. H. and HACHMEISTER, K. A. 1995. Degradable wheat gluten films: preparation, properties and aplications. **Journal of Food Science**, v.60, n.5, p. 1147-1150, 1156, 1995.

19. KESTER, J. J. and FENNEMA, O. Edible films and coatings: A review. **Food Technology**, v.40 n.12, p. 47-59, 1986.

20. LERDTHANANGKUL, S. and KROCHTA, J. M. Edible coating effects on postharvest quality of green bell peppers. **Journal of Food Science**, v.61, n.1, p. 176-179, 1996.

21. MANNING, K. Soft fruits. In BIOCHEMISTRY of fruit ripening**.** London, UK: Chapman & Hall, 1993. p. 347-373.

22. MCHUGH, T. H.; AUJARD, J. F. and KROCHTA, J. M. Plasticized whey protein edible films: water vapor permeability properties. **Journal of Food Science**, v.59, p. 416-419, 423, 1994.

23. MCHUGH, T. H. and KROCHTA, J. M. Sorbitol vs glycerol plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. **Journal of Agriculture and Food Chemistry**, v.42, n.4, p.841-845, 1994.

24. MILLER, K. S. and KROCHTA, J. M. Oxygen and aroma barrier properties of edible films: A Review. **Trends in Food Science and Technology**, v.8, p.228-237, 1997.

25. MUNOZ, A. M.; PANGBORN, R. M. and NOBLE, A. C. Sensory and mechanical attributes of gel texture. 1. Effect of gelatin concentration. **Journal of Texture Studies**, v.17, p.1, 1986.

26. PARK, H.J. and CHINNAN, M.S. Properties of edible coatings for fruits and vegetables, **ASAE** Paper n^0 90-6510, presented at the 1990 International Winter Meeting, American Society of Agricultural Engineers, 1990 December 18-21, Chicago, IL.

27. PARK, H. J.; WELLER, C. L.; VERGANO, P. J. and TESTIN, R. F. Permeability and mechanical properties of cellulose-based edible films. **Journal of Food Science**, v.58, p.1361-1364, 1993.

28. PARK, H. J.; BUNN, J. M.; WELLER, C. L.; VERGANO, P. J. and TESTIN, R. F. Water vapor permeability and mechanical properties of grain protein-based films as affected by mixtures of polyethylene glycol and glycerin plasticizers. **Transactions of the ASAE**, v.37, p.1281-1285, 1994a.

29. PARK, H.J.; CHINNAN, M.S. and SHEWFELT, R.L. Edible coating effects on storage life and quality of tomatoes. **Journal of Food Science**, v.59, n.3, p.568-570, 1994b.

30. PARK, H. J. Development of advanced edible coatings for fruits. **Trends in Food Science and Technology** v.10, p. 254-260, 1999.

31. RAYAS, L. M.; HERNANDEZ, R. J. and NG, P. K. W. Development and characterization of biodegradable/edible wheat protein films. **Journal of Food Science**, v.62, n.1, p.160-162, 189, 1997.

32. STATISTICA. Kernel release 5.5 A. 1984-2000 by StaSoft, Inc., USA.

33. TANADA-PALMU, P. S., HELÉN, H. and HYVÖNEN, L. Preparation, properties and applications of wheat gluten edible films. **Agriculture and Food Science in Finland** v.9, p.23-35, 2000.

34. TANADA-PALMU, P. S. and GROSSO, C. R. F. Wheat gluten composite and bilayer edible films: effect of lipid addition. In RESEARCH Advances in Food Agriculture and Chemistry, Kerala, Índia: Global Research Network. 2002. p.53-60.

35. THOMPSON, D. R. Response surface experimentation. **Journal of Food Process and Preservation**, v.6, p. 155, 1982.

36. WALL, J. S. and BECKWITH, A. C. Relationships between structure and rheological properties of gluten properties. **Cereal Science Today** v.14, n.1, p.16-21, 1969.

37. WALL, J. S. and HUEBNER, F. R. Adhesion and cohesion. In PROTEIN functionality in foods. Washington, D.C: American Chemical Society, 1981. p.111.

CAPÍTULO 4

WHEAT GLUTEN COMPOSITE AND BILAYER EDIBLE FILMS: EFFECT OF LIPID ADDITION

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WHEAT GLUTEN COMPOSITE AND BILAYER EDIBLE FILMS: EFFECT OF LIPID ADDITION

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ABSTRACT: Improvement of the moisture barrier properties of edible wheat gluten films by combining both wheat gluten proteins and lipids (beeswax, stearic and palmitic acids), and the effects of different types of lipids at various concentrations on film water vapor permeability (WVP), water solubility, mechanical properties and surface characteristics, were analyzed. Composite films were prepared using gluten, glycerol and lipids as emulsions, and bilayer films were prepared by depositing a thin layer of lipid on the surface of a dried base film of gluten and glycerol. All bilayer and composite films of gluten and lipids were opaque. Bilayer films were more brittle and difficult to handle than composite films. The bilayer film with a 10% mixture of stearic and palmitic acids (S-P) and beeswax had the lowest WVP. The water solubility of all bilayer films with 10% lipids (except the film with a 10% mixture of S-P and beeswax) was lower, and increased as the concentration of lipids increased. All composite and bilayer films with more than 10% lipids (except the S-P composite film) had lower tensile strength and percent elongation at break, compared to gluten films with no lipids. Composite and bilayer films of wheat gluten and lipid materials can be prepared and improve water vapor permeability, but they decrease mechanical resistance and the films become more opaque.

Running title: Wheat gluten composite and bilayer films

INTRODUCTION

Recently there has been considerable interest in edible films for foods (1,2,3). Such films can maintain food quality and improve stability and shelf life by retarding unwanted mass transfer in food products (4). They may also improve the recyclability of some packaging applications, avoiding the disposal of non-renewable materials.

Polysaccharides, proteins and lipids can be used as edible film-forming agents (1,5,6,7). Due to the unique cohesive and elastic properties of gluten (8), good film-forming properties may be expected. The mechanical and barrier properties of wheat gluten films have been studied (9,10,11). Edible wheat gluten films were prepared with various amounts of glycerol (12). Films with low amounts of glycerol had lower water vapor and oxygen permeabilities, higher tensile strength and lower elongation at break.

Moisture exchange with the surrounding environment is a critical factor affecting the sensory quality and shelf life of food products. Control of moisture loss has been achieved through synthetic film packaging. However, biodegradable edible films with sufficient mechanical and barrier properties could be used to replace synthetic films. Bilayer or composite films that combine proteins with emulsions or lipid layers can be interesting, since the lipids reduce the water vapor permeability and the proteins give strength to the film, improving its structural integrity. Among the lipid materials investigated, waxes and long-chain saturated fatty acids and fatty alcohols were the most effective in improving the moisture barrier properties of hydrophilic films (13).

The objective of this work was the development of edible bilayer and composite films of wheat gluten and lipids (beeswax, stearic and palmitic acids), and the evaluation of the film forming conditions (type of film, concentration and type of lipids) on the film properties (water solubility, water vapor permeability, mechanical properties and surface characteristics).

MATERIALS AND METHODS

Materials

Vital wheat gluten (Rhodia, Campinas, Brazil), beeswax (Chemco, São Paulo, Brazil), glycerol (Merck, Darmstadt, Germany), ammonium hydroxide (Synth, São Paulo, Brazil), calcium chloride (Ecibra, São Paulo, Brazil), sodium chloride (Synth, São Paulo, Brazil), magnesium nitrate (Ecibra, São Paulo, Brazil), lithium chloride (Ecibra, São Paulo, Brazil), magnesium chloride (Synth, São Paulo, Brazil), potassium chloride (Synth, São Paulo, Brazil), solid paraffin (Chemco, São Paulo, Brazil), stearic acid (Vetec, São Paulo, Brazil) and palmitic acid (Vetec, São Paulo, Brazil).

Base film preparation

The base film was prepared from a solution of gluten (9.0 g/100 mL solution), absolute ethanol (32.5 mL/100 mL solution), glycerol (1.5 g/100 mL solution), ammonium hydroxide and distilled water. All components were mixed using magnetic stirring until the temperature of the mixture reached 70 ^{0}C, when the solution was centrifuged at 5856 g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a teflon covered glass surface and dried at room temperature for 24 h [modified method of Gontard and others (10)]. The quantity of solution (vol.) poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for experiments were equilibrated at 52% RH and 25 ^{0}C for 48 h before being tested.

Bilayer and composite film preparation

The composite film was prepared with the addition of 10, 20 and 30% (lipid weight/gluten weight) of: a) beeswax; b) 1:1 blend of stearic and palmitic acid (S-P); c) beeswax with a 1:1 blend of stearic and palmitic acids, incorporated into the base film-forming solution. This solution with lipids was mixed vigorously using magnetic stirring and heated to melt the lipids. The film-forming solution was then immediately poured and spread evenly over a Teflon covered glass surface and dried at room temperature for 24 h [modified method of Gontard and others (14)].

The bilayer film was prepared by depositing a thin layer of melted lipids (beeswax, stearic and palmitic acids) onto the surface of a dried preformed and preheated base film consisting of gluten and glycerol. A thin teflon sheet was carefully placed over the whole surface of the film to uniformly smooth the surface. Films were cooled before removal [method of Gontard and others (14)].

Film thickness

Film thickness was measured using a micrometer (Model MDC-25M, Mitutoyo, MFG, Japan). The thickness of individual film samples was determined as an average of five measurements.

Water vapor permeability

The water vapor transmission rate through the films was determined gravimetrically at 25 ^{0}C using a modification of the American Society for Testing and Materials [ASTM (15)] Standard Method E-96, and the water vapor permeability (WVP) was calculated according to the method. The samples, four replicates of each film, were conditioned for 2 d at 52% RH before measurement. The relative humidity gradient of the test was 0% RH ($CaCl_2$ desiccant in the test cup) and 75% RH (saturated NaCl solution in the desiccator).

Tensile strength and percent elongation at break

Film tensile strength (TS) and percent elongation at break (ELO) were determined using a Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), operated according to the ASTM (16) Standard Method D 882-83 (initial grip separation = 50 mm and cross head speed = 100 mm/min). Six specimens (100 mm long and 25.4 mm wide) of each film were measured. The samples were conditioned for 2 d at 52% RH before measurement. The peak loads and extension at break were recorded for tested film specimens. The tensile strength and percent elongation at break were calculated according to the ASTM method. Before the tests with different relative humidity conditions, films were conditioned for seven days under each relative humidity condition. Saturated salt solutions in desiccators were used to obtain the following relative humidity conditions at 25^0 C: 11% with LiCl, 33% with $MgCl_2$, 52% with $Mg(NO_3)_2$, 75% with NaCl and 86% with KCl. Ten specimens of each film at each relative humidity were measured.

Solubility in water

The percentage of initial dry matter of each film was determined at 105 ^{0}C for 24 h. Two discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water and slowly and periodically agitated for 24 h at 25 ^{0}C. The pieces of film were then taken out and dried (105 ^{0}C for 24 h) to determine the weight of dry matter, which was not solubilized in water (17).

Scanning electron microscopy

Film samples were examined for surface characteristics using a Jeol (JMS-T330) scanning microscope (Jeol Ltd., Tokyo, Japan) operated at 10 kV. Film samples were left in a desiccator with silica gel for 7 days, then fraturated and affixed to aluminum stubs with cupper tape. The samples were then sputter-coated with gold in a Balzers evaporator (SDC 050, Baltec, Lichtenstein) for 180 seconds at 40 mA.

RESULTS AND DISCUSSION

Film formation

All the bilayer and composite films obtained were opaque due to the presence of the lipids. The bilayer films were more brittle and difficult to handle than the composite films. The composite films showed a more homogeneous surface and were more flexible.

A difficult task when preparing an edible bilayer or composite film with good water vapor barrier properties is the formation of a continuous layer of lipid (18). On account of its excellent barrier properties to water vapor permeability (19,20), beeswax was chosen for studying the development of edible composite and bilayer films composed of a wheat gluten based film and lipid materials.

The maximum concentration of lipids that could be added to wheat gluten films was 30% of the gluten weight (lipid/gluten). Films with a higher concentration of lipids showed an irregular lipid distribution, which is probably due to the limited dispersion capability of the lipids (13).

Water vapor permeability and solubility in water

The water vapor permeability (WVP) 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 (21).

Water permeation through the film usually occurs through the hydrophilic part of the composite and bilayer films, thus the relation of the hydrophilic/hydrophobic portions is important to determine WVP. WVP tends to increase with polarity, degree of unsaturation and degree of ramification of the lipids used, besides the effect of the properties of water molecule sorption from the polar part of the film (17).

The effect of lipid concentration on the water vapor permeability and solubility in water is shown in Figs. 1 and 2. Both sides of the bilayer films (lipid layer upside and downside on the test cup) were tested for WVP and no difference in values was found. The bilayer film with 10% mixture of S-P and beeswax had the lowest WVP (1.01 gmm/m^{2}dkPa) of all the films tested. A study with composite gellan-based films (13) showed that the beeswax and S-P mixture effectively reduced WVP, however beeswax was more effective, which was also observed for caseinate-lipids films by Kester and Fennema (19). The best results in reducing the WVP of the films were obtained with the mixture S-P and beeswax, over the films with beeswax. This could be due to the formation of a more irregular surface containing micropores and/or little cracks in the films with beeswax, while films with the mixture S-P and beeswax were shown to be apparently more regular and easier to manipulate. Another possible explanation may be that the S-P mixture lowered the melting point of the beeswax and thereby aided in remelting the lipids during the drying of the film (18). The composite (comp) film with 30% mixture of S-P and beeswax showed one of the lowest WVP (1.65 gmm/m^{2}dkPa) of all the films tested. The composite film with 30% beeswax had a WVP (5.01 gmm/m^{2}dkPa) similar to the WVP (4.72 gmm/m^{2}dkPa, at 30 ^{0}C and 100% RH) obtained by Gontard and others (17) for a gluten composite film with the same concentration of beeswax.

The WVP values of all composite and bilayer films were lower than the WVP of the gluten film with no lipids (8.61 gmm/m^{2}dkPa), showing that the lipids decreased the WVP of the wheat gluten films. The WVP of a similar gluten film prepared by Gontard and others (17) was lower (5.78 gmm/m^{2}dkPa), however the film thickness was lower too, resulting in this difference. The WVP of the films changed slightly as the concentration of lipids increased. However, another study (22) showed that the addition of increasing amounts of lipids in edible starch films, increased the WVP.

The water resistance of edible films is an important property in applications such as food protection, when the water activity is high or when the film has to be in contact with water during processing of the coated food (e.g., to avoid exudation of fresh or frozen products). Conversely, edible films with high water solubility may be required, for instance, to contain premeasured portions, which will be dissolved in water or hot food (3). All bilayer films with 10% lipids, except the film with the mixture S-P and beeswax, and the composite film with 10% S-P and beeswax, had lower solubility in water and this increased as the concentration of the lipids increased, in agreement with Gontard and others (17). Above 20% lipids, the presence of lipid components probably destabilized the protein matrix (reduction of intermolecular interactions between protein chains), or decreased water resistance when the lipid component was hydrophilic (17). Furthermore, the solubility in water of all composite and bilayer films was lower than in the film with no lipids, but it increased when the concentration of lipids increased from 10 to 20% and from 20 to 30%.

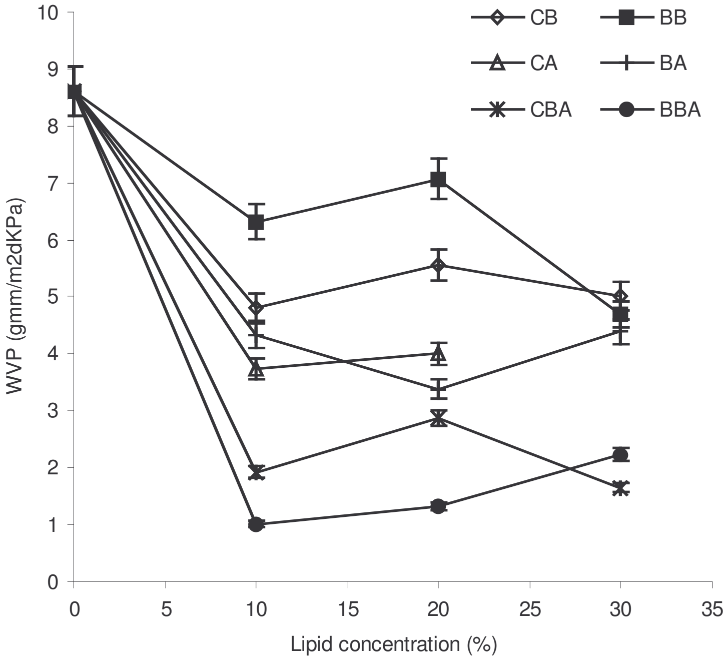

Figure 1 – Water vapor permeability (WVP) of wheat gluten/lipid composite and bilayer films (and standard deviation) as a function of lipid concentration (CB=composite beeswax, CA=composite acids, CBA=composite beeswax+acids, BB=bilayer beeswax, BA=bilayer acids, BBA=bilayer beeswax+acids).

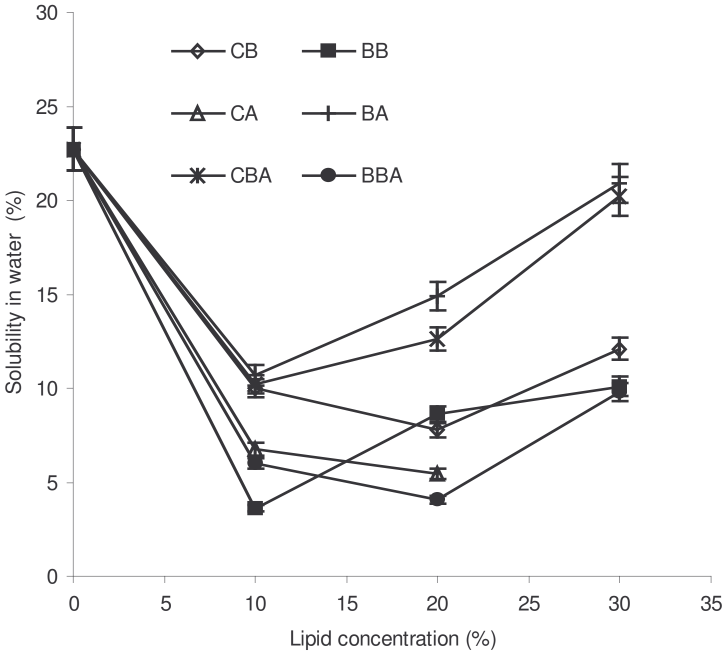

Figure 2 – Solubility in water of wheat gluten/lipid composite and bilayer films (and standard deviation) as a function of lipid concentration.

Mechanical properties

An edible film should be resistant in order to withstand manipulation during its application and to maintain its integrity and also its barrier properties.

The effect of lipid concentration on tensile strength (TS) and percent elongation at break (ELO) of the films is shown in Figs. 3 and 4. All composite and bilayer films with more than 10% lipids (except the S-P composite film) had lower tensile strength and percent elongation at break, compared to the gluten film with no lipids (5.25 N and 184.1%, respectively). The films were brittle, implying a low TS and were not flexible, which resulted in a low ELO. The TS of the films decreased as the concentration of the lipids increased, in accordance with Yang and Paulson (13). The TS was higher for all composite and bilayer films with S-P or beeswax as compared to the films with the mixture S-P and beeswax. The ELO was lower for all films with 10% lipids, increased greatly with 20% lipids and decreased slightly with 30% lipids (except the composite film with beeswax). The beeswax showed a negative effect on the mechanical properties of the composite and bilayer films, as compared to the film with no lipids. The lipid compounds had an anti-plasticizing effect. This effect could be related to the mechanical properties of this compound (wax is very brittle) and its hydrophobic characteristics, which could reduce the water concentration of the film as compared to the gluten film with no lipids. The high molecular weight of the wax could cause a reduction in the free volume and also modify the mechanical properties of the protein structural matrix (17). Another possibility could be that the intensity of the interactions between lipid-polymer was lower than the interactions between polymer-polymer (13).

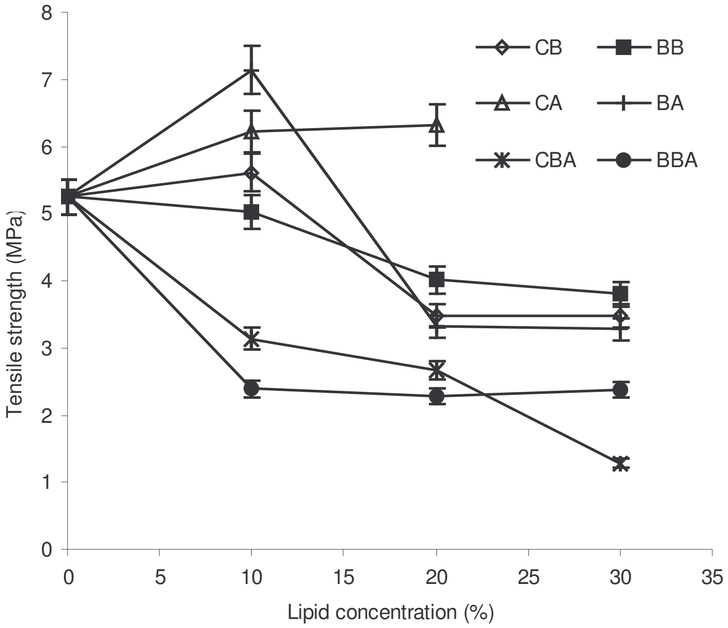

Figure 3 – Tensile strength (TS) of wheat gluten/lipid composite and bilayer films (and standard deviation) as a function of lipid concentration.

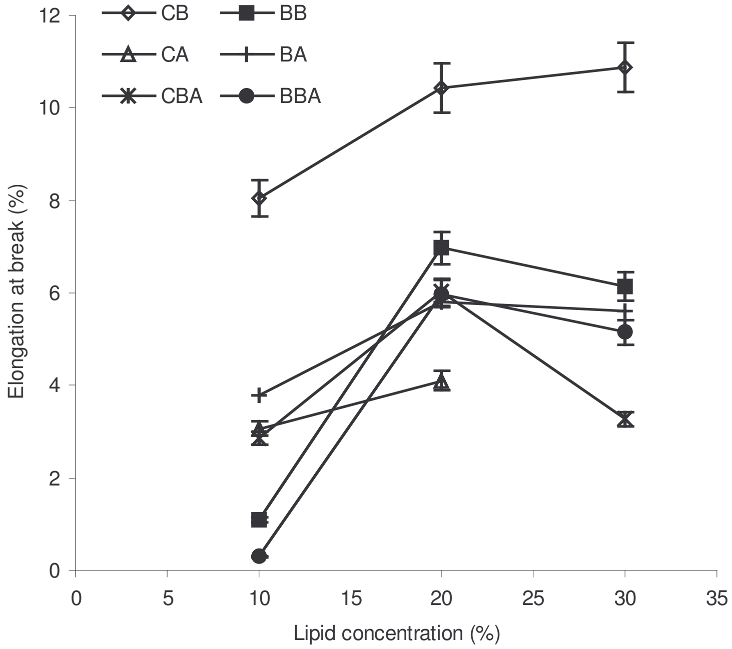

Figure 4 – Percent elongation at break (ELO) of wheat gluten/lipid composite and bilayer films (and standard deviation) as a function of lipid concentration.

Surface characteristics

Scanning electron micrographs (SEM) of the films are shown in Fig. 5 (a, b and c). SEM was used to examine the composite and bilayer films (lipid-enriched side upside the stub) with a 10.0% mixture of S-P and beeswax and also the gluten film without lipids. Although bubbles of lipids were observed in both composite and bilayer films, apparently greater compaction occurred in the composite film (Fig. 5b) as compared to the gluten film without lipids (Fig. 5a), and in the bilayer film (Fig. 5c) as compared to the composite film. The morphology observed in the gluten film was completely modified when the mixture beeswax and S-P was added (Fig. 5b). This new structural matrix conformation seems to determine a lower WVP for both the composite and bilayer films as compared to the gluten film.

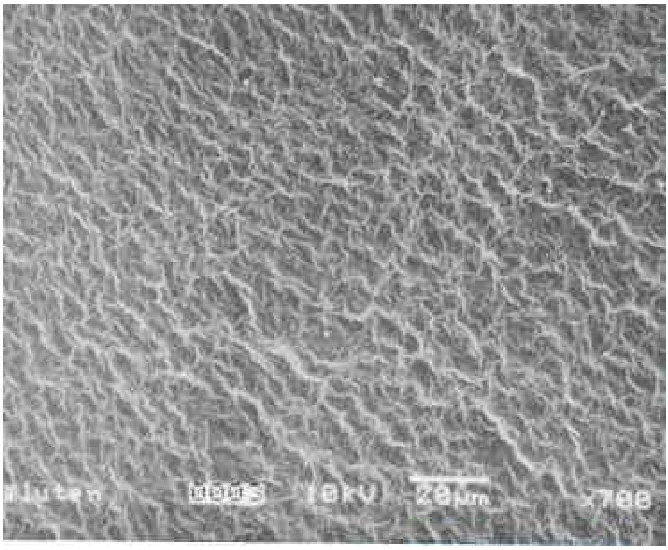

Figure 5a- SEM of a gluten film.

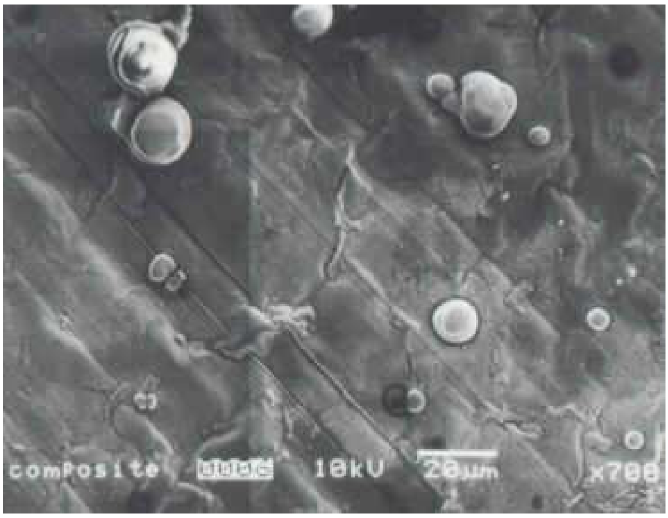

Figure5b–SEM of a composite film

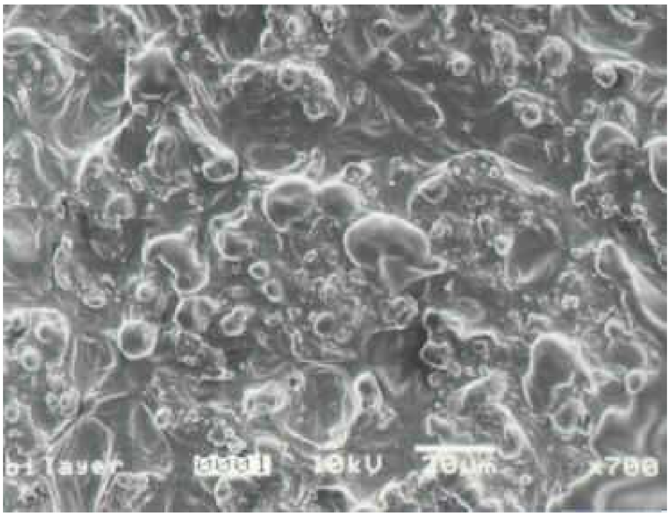

Figure 5c- SEM of a bilayer film.

Effect of water activity on the mechanical properties of wheat gluten/lipids composite and bilayer films

The mechanical properties of the wheat gluten composite and bilayer films with 20.0 and 30.0% beeswax and S-P acids, over a wide range of water activity (a_w), were examined. The mechanical properties of the films can be strongly affected by environmental conditions such as relative humidity (RH). The effect of a_w on the tensile strength and percent elongation at break is shown in Figs. 6 and 7.

The tensile strength decreased and percent elongation at break increased with increasing a_w for all the bilayer and composite films, in agreement with the results obtained by Tanada-Palmu and others (12) for wheat gluten film with no lipids. Although the composite and bilayer films became more flexible (higher ELO) with an increase in a_w, the TS decreased. At a_w values lower than 0.52, composite and bilayer films with beeswax showed higher TS than the composite and bilayer films with the mixture S-P and beeswax, except for the bilayer film with 20% mixture, which showed a higher TS. The same films also had a higher ELO than the composite and bilayer films with the mixture S-P and beeswax at higher conditions of a_w studied (0.75 and 0.86). Composite and bilayer films of wheat gluten and beeswax showed better mechanical properties as compared to films with the mixture S-P and beeswax, when the a_w was increased.

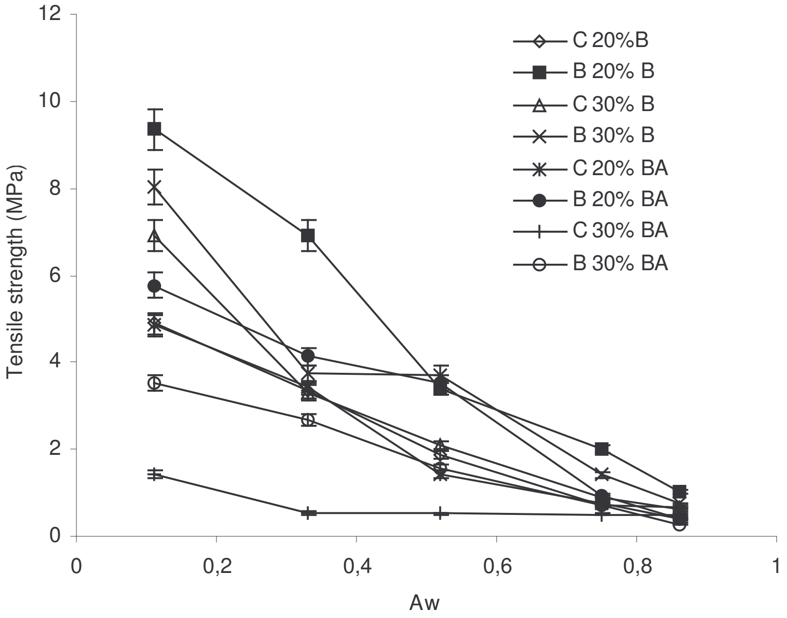

Figure 6 – Tensile strength of wheat gluten/lipids composite and bilayer films with 20.0 and 30.0% lipids (and standard deviation), as a function of water activity of the films.

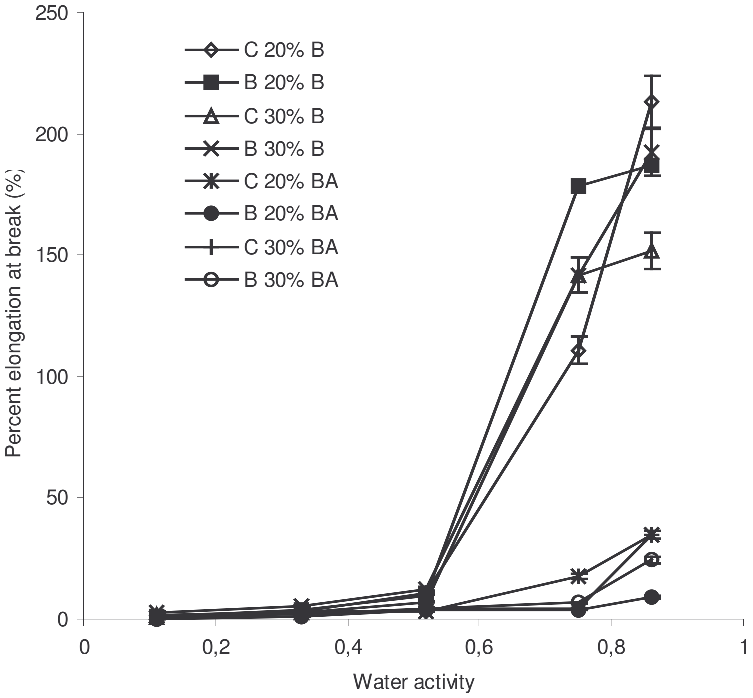

Figure 7 – Percent elongation at break of wheat gluten/lipids composite and bilayer films with 20.0 and 30.0% lipids (and standard deviation) as a function of the water activity of the films.

CONCLUSION

The effects of lipid materials on the functional properties of gluten-based composite and bilayer films are complex and depend on the lipid and on the interactions between the lipid and the structural protein matrix. Wheat gluten/lipid composite and bilayer films with improved water vapor permeability relative to lipid-free films can be made. Composite films were easier to manipulate as compared to bilayer films with gluten, beeswax and lipids (stearic and palmitic acids). Bilayer films were brittle and difficult to apply as edible coatings. Despite the better barrier to water vapor permeability, with the addition of beeswax and fatty acids the films became more opaque and with inferior mechanical properties as compared to the gluten film with no lipids.

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REFERENCES

1- Kester JJ and Fennema O. 1986. Edible films and coatings: A review. *Food Tech* 40 (12): 47-59.

2- Guilbert S. 1986. Technology and application of edible protective films. In *Food Packaging and Preservation*. Mathlouthi M, Editor. p. 371-394. Elsevier Applied Science Publishers. London UK.

3- Guilbert S and Biquet B. 1989. Les films et enrobage comestibles. In *L'emballage des denrées alimentaires de grande consommation*. Bureau G and

Multon JL, Editors. p.320. Technique et Documentation, Lavoisier, Apria, Paris France.

4- Miller KS and Krochta JM. 1997. Oxygen and aroma barrier properties of edible films: A Review. *Trends Food Sci Tech* 8: 228-237.

5- Gennadios A and Weller CL. 1991. Edible films and coatings from soymilk and soy protein. *Cereal Foods World* 36: 1004-1009.

6- Avena-Bustillos RJ and Krochta JM. 1993. Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. *J Food Sci* 58 (4): 904-907.

7- McHugh TH, Aujard JF and Krochta JM. 1994. Plasticized whey protein edible films: water vapor permeability properties. *J Food Sci* 59: 416-419, 423.

8- Wall JS and Huebner FR. 1981. Adhesion and cohesion. In *Protein Functionality in Foods*, J.P. Cherry, Editor. p.111. American Chemical Society, Washington, D.C.

9- Gennadios A, Brandenburg AH, Weller CL and Testin RF. 1993. Effect of pH on properties of wheat gluten and soy protein isolate films. *J Agric Food Chem* 41: 1835-1839.

10- Gontard N, Guilbert S and Cuq JL. 1993. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J Food Sci* 58 (1): 206-211.

11- Cherian G, Gennadios A, Weller CL and Chinachoti P. 1995. Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. *Cereal Chem* 72: 1-6.

12- Tanada-Palmu PS, Helén H and Hyvönen L. 2000. Preparation, properties and applications of wheat gluten edible films. *Agric Food Sci Finland* 9: 23-35.

13- Yang L and Paulson AT. 2000. Effect of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Res Inter* 33: 571-578.

14- Gontard, N, Marchesseau, S, Cuq, JL, Guilbert, S. 1995. Water vapour permeability of edible bilayer films of wheat gluten and lipids. *Intern J Food Sci Tech* 30: 49-56.

15- ASTM. 1980. Standard test methods for water vapor transmission of materials. Method E-96. In *Annual Book of ASTM Standards. American Society for Testing and Materials*. Conshohocken, PA.

16- ASTM. 1980. Standard test methods for tensile properties of thin plastic sheeting. Method D 882-83. In *Annual Book of ASTM Standards. American Society for Testing and Materials*. Conshohocken, PA.

17- Gontard N, Duchez C, Cuq JL and Guilbert S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. *Intern J Food Sci Tech* 29: 39-50.

18- Greener IK and Fennema OR. 1989. Barrier properties and surface characteristics of edible, bilayer films. *J Food Sci* 54: 1393-1399.

19- Kester JJ and Fennema O. 1989. Resistance of lipid films to water vapor transmission. *JAOCS* 66(8): 1139-1146.

20- Kamper SI and Fennema OR. 1984. Water vapor permeability of edible bilayer films. *J Food Sci* 49: 1478-1481.

21- Gontard N, Guilbert S and Cuq JL 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *J Food Sci* 57 (1): 190-199.

22- Garcia MA, Martino MN and Zaritzki NE. 2000. Barrier properties of edible starch-based films and coatings. *J Food Sci* 65 (6): 941-947.

CAPÍTULO 5

EFFECT OF EDIBLE WHEAT GLUTEN-BASED FILMS AND COATINGS ON REFRIGERATED STRAWBERRY (*FRAGARIA ANANASSA*) QUALITY

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EFFECT OF EDIBLE WHEAT GLUTEN-BASED FILMS AND COATINGS ON REFRIGERATED STRAWBERRY (*FRAGARIA ANANASSA*) QUALITY

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Abstract: Edible coatings and films can provide an alternative for extending the post-harvest life of fresh fruits and vegetables. The effect of different wheat gluten-based coating formulations and films on refrigerated strawberry quality, was studied. Fruit quality was evaluated by weight loss, firmness retention, microbial decay, surface color development, titratable acidity, total and soluble solids, sugar content and a sensory evaluation. The bilayer coating of wheat gluten and lipids (beeswax, stearic and palmitic acids) had a significant effect on the retention of firmness and reduced the weight loss of the coated fruits. Packed strawberries coated with gluten film produced the lowest ratios for the chromaticity parameters as compared to the control fruits, thus indicating that senescence was retarded. The sensory evaluation of the strawberries showed that the gluten and the composite coatings maintained the quality of the fruits in terms of appearance during the storage time, and the taste of the strawberries with the gluten coating was accepted by the consumers.

Keywords: strawberry, coating, film, gluten, shelf life, quality.

Running title: Wheat gluten films and coatings on strawberry

INTRODUCTION

Recent works are exploring the potential of edible coatings to extend and maintain the quality and shelf life of fresh products and to reduce the amount of disposable non-biodegradable packaging materials[1]. Edible coatings can provide an alternative to extend the post-harvest life of fresh fruits and vegetables and can also result in the same effect as modified atmosphere storage in modifying the internal gas composition[2]. Traditionally, films and coatings have been used to reduce water loss, but new film materials and edible coatings formulated with a wider range of permeability characteristics facilitate achieving a “modified atmosphere” effect in fresh fruits[3]. Several researchers have studied the application of coatings to vegetables such as tomato, cucumber and red peppers[4,5] and fruits like bananas[6], apples[7], mangoes[8], pears[9], citric fruits[10], avocado[11], strawberries[12,13] and guavas[14]. Highly perishable fruits like berries and tropical fruits are the most appropriate products to protect with coatings, because of the additional cost resulting from coating treatments. Strawberries, being typical soft fruits, have high physiological post-harvest activity. As a consequence, they have short ripening and senescence periods that make the commercialization of high quality fruit a challenge. The objectives of this work were to study the ability of gluten-based coatings to extend the shelf life of refrigerated strawberries, and to study the influence of different types of coatings on the quality attributes of strawberries, such as weight loss, firmness, surface color development, titratable acidity, sugar content and sensory evaluation.

MATERIALS AND METHODS

Materials

Vital wheat gluten (Rhodia, Campinas, Brazil), ethanol (Synth, São Paulo, Brazil), beeswax (Chemco, São Paulo, Brazil), glycerol (Merck, Darmstadt, Germany), ammonium hydroxide (Synth, São Paulo, Brazil), stearic acid (Vetec, São Paulo, Brazil), palmitic acid (Vetec, São Paulo, Brazil), PVC film (Reds 2000, São Paulo, Brazil) and strawberries direct from the producer (Jarinu, São Paulo, Brazil).

Film preparation

The film was prepared from a solution of gluten (9.0 g/100 mL solution), absolute ethanol (32.5 mL/100 mL solution), glycerol (1.50 g/100 mL solution), ammonium hydroxide to adjust to pH 10 and distilled water. All components were mixed under magnetic stirring until the temperature of the mixture reached 70 ^{0}C and the solution centrifuged at 5856 g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a teflon covered glass surface and dried at room temperature for 24 h (modified method of Gontard *et al.*[15]). The quantity of solution (vol.) poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for the experiments were equilibrated at 52% RH and 25 ^{0}C for 48 h before being tested.

Sample preparation

Strawberries (*Fragaria ananassa*) at the commercially ripe stage, of uniform size, free of physical damage and fungal infection, were used. Strawberries were dipped in chlorinated water (0.25 g Cl_2/L), dried and dipped in the formulated suspension (film-forming solution) at room temperature and dried or packed with the gluten film. Eight treatments were effected to cover fresh strawberries (triplicates) with the gluten film and coating. In treatment 1, fresh strawberries were held with pliers and dipped into the wheat gluten film-forming solution at room temperature for 1 min. In treatment 2, the strawberries were dipped into the warmed composite film-forming solution of gluten, beeswax and a 1:1 blend of stearic and palmitic acids for 1 minute. In treatment 3, strawberries were dipped into the same solution as in treatment 1, for 1 min and allowed to dry for 1 h; they were then dipped into the molten beeswax and 1:1 blend of stearic and palmitic acids for a few seconds, in order to form a bilayer film on the strawberries. In treatment 4 (control), the strawberries were dipped into distilled water for 1 min. In treatment 5, the strawberries were individually enveloped in the gluten film pouches (sealed) and in treatment 6, the strawberries were individually wrapped with the gluten film. In treatment 7, some strawberries were put in trays and each tray was packed with the gluten film and in treatment 8, each tray was packed with a thin PVC film (synthetic). The strawberries were then stored in the refrigerator at 7 ^{0}C and 42% RH (measured with a higrometer) for 16 days to follow the shelf life.

Strawberry decay

The fruits were inspected after 1, 6, 12 and 16 days of storage and were considered infected when a visible lesion was observed. The results were expressed as the percentage of infected fruits[12].

Weight loss

The same fruits were weighed at the beginning of the experiment and after 1, 6, 12 and 16 days of storage. The results (with standard deviation) were expressed as percentage loss of initial weight.

Firmness

The compression force of the strawberry flesh was measured using a Texture Analyzer TA.XT2 (SMS, Surrey, UK) equipped with a compression cell of 5 kg and a cylindrical acrylic probe of 1 cm in diameter, using a 1 mm/s crosshead speed and a 75% strain to penetrate the fruit[12]. Determinations (with standard deviation) were performed after 1, 6, 12 and 16 days of storage. Strawberries of uniform size from which the calyces had been removed to obtain even surfaces, were used to determine the break force (peak height).

Surface color development

Colorimetric measurements of the fruits surface were carried out with a Hunterlab colorimeter equipped with an optical sensor (ColorQuest II, Hunter Associates Laboratory, Fairfax, VA, USA). The Hunter scale was used: lightness (L) and the chromaticity parameters *a* and *b* were registered after 1, 6, 12 and 16 days of storage. The ratio of the cromaticity parameters (*a*/*b*) was calculated (with standard deviation).

Determination of reducing and total sugar contents, pH and titratable acidity

For each determination, ten fruits were processed after different storage times. A blender (Fisatom 70 W, Brazil) was used to obtain homogenates for the pH, titratable acidity, soluble solids, total solids and sugar content determinations. The pH of the homogenates (triplicates) was determined in a pHmeter (Digimed DM 20, Brazil).

The acidity was determined using a 10 g aliquot of the homogenate made up to 100 mL with distilled water, which was titrated with 0.1 N NaOH to an end point of pH 8.1[16]. The titratable acidity was expressed as g of citric acid per 100 g fruit. Three samples were analyzed for each storage time (1, 6, 12 and 16 days).

For the reducing sugars determination, 5 g aliquots of the homogenates (triplicates) were transferred to a becker and 50 mL distilled water added. The mixture was heated in a water bath for 5 min and then filtered into a 100 mL volumetric flask. The flask was completed to 100 mL with distilled water. Aliquots of 10 mL were titrated according to the Fehling method[17]. Aliquots of 50 mL from the flask were used for the total sugar determinations. The samples were hydrolyzed with HCl concentrated (5 mL) and were heated in a water bath for 15 minutes. After cooling, the samples were neutralized to pH 7 and the flasks completed to 100 mL with distilled water. Aliquots of 10 mL were titrated according to the Fehling method. Sugars were expressed as grams of glucose per 100 g of fruit.

Determination of soluble solids (^{0}Brix), total solids and moisture

The soluble solids were determined in a refractometer (Hegerstelit, Germany) and were expressed as ^{0}Brix.

To determine the total solids, 10 g aliquots of the homogenates were transferred to previously dried and weighed porcelain dishes containing treated sand. The sand was used to increase the surface area for drying. The samples (triplicates) were dried in a vacuum oven at 70 ^{0}C to constant weight. The total solids were calculated dividing the residue weight by the sample weight (x 100). The moisture content was obtained by difference (100 - total solids).

Sensory evaluation of the strawberries

For the sensory evaluation, the consumers evaluated 4 samples of strawberry: sample 1 was the strawberry with no coating (control - treatment 4), sample 2 with the gluten coating (treatment 1), sample 3 with the composite coating (treatment 2) and sample 4 with the bilayer coating (treatment 3). The samples were prepared as explained above. The sensory evaluation of the strawberries during the shelf life was effected by 40 consumers, in the age range from 20 to 45 years old. The order of the samples was randomized for each consumer, according to Macfie and Bratchell[17] to balance the effect of order of presentation and first-order carry-over effects. The strawberries were evaluated after 1, 6 12 and 16 days of storage in the refrigerator. The attributes analyzed were global appearance, color, brightness and intention to buy. The sensory evaluation of taste and flavor of the strawberries, also evaluated by 40 consumers, was effected after 5 days of storage, so that the coating was well adhered to the fruits, and the attributes were taste, flavor, texture and intention to buy.

Statistical analyses

The Statistica 5.5 (StatSoft, USA) program was used to calculate the analysis of variance (ANOVA) and the Tukey test was used to determine the significant differences of all the film properties at a 95% confidence interval.

RESULTS AND DISCUSSION

Film and coating characterization

The coatings were well adhered to the strawberry surfaces, the gluten and composite coatings were transparent, but the bilayer coating of gluten, beeswax and a 1:1 mixture of stearic and palmitic acids (S-P) was opaque white. Comparing the coated and control strawberries on day 1 and day 16, all the strawberries shrank and lost their brightness, however the control fruits were smaller and more opaque than the other fruits on day 16. The strawberries with the bilayer coating almost maintained their original day 1 size, showing that this coating could maintain the quality of the fruits during the time of storage. The strawberries covered with the gluten film maintained good visual quality up to day 16, the wrapped and the sealed films maintained the quality up to day 12, and the fruits covered with the PVC film remained good throughout the whole experiment. The gluten films maintained the quality of the strawberries for a longer time than the coatings.

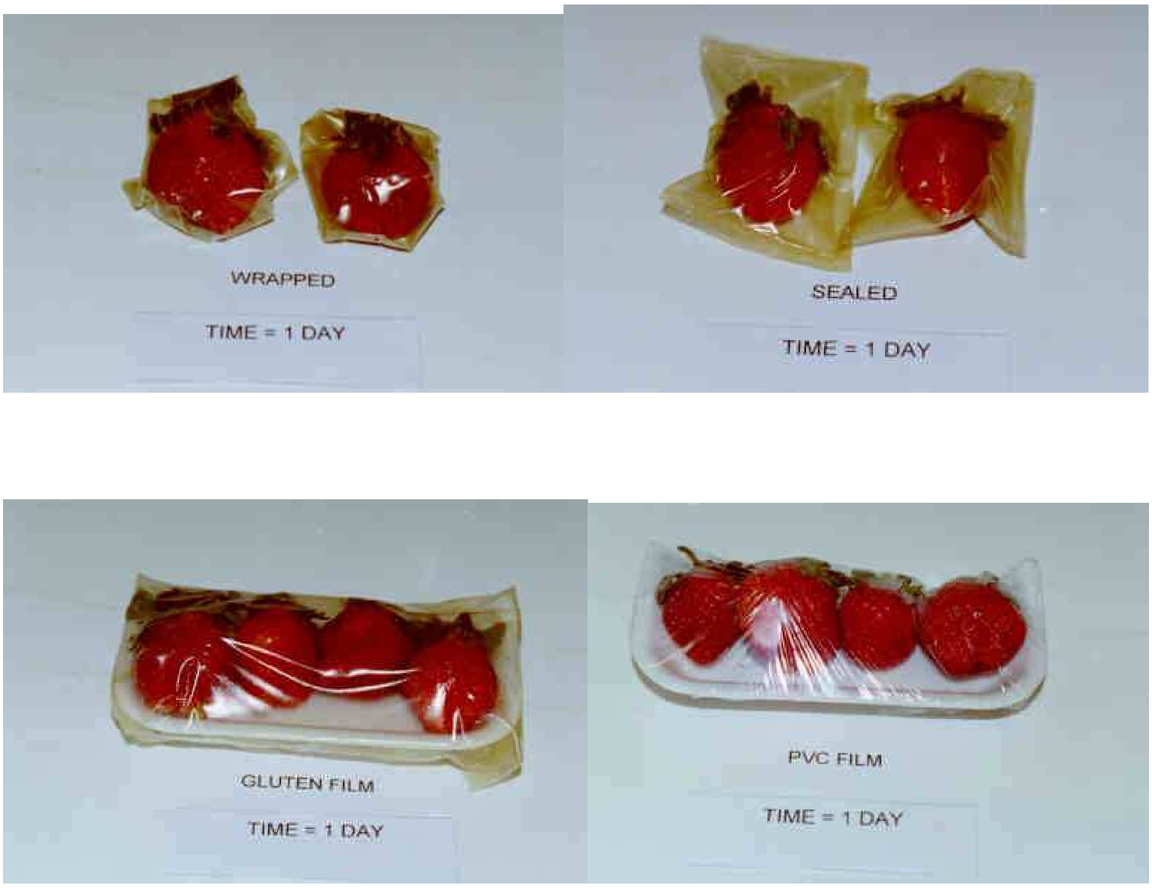

Figure 1 – Strawberries with the gluten and PVC films on the day 1.

Figure 2 - Strawberries with the gluten and PVC films on the day 6.

Figure 3 - Strawberries with the gluten and PVC films on the day 12.

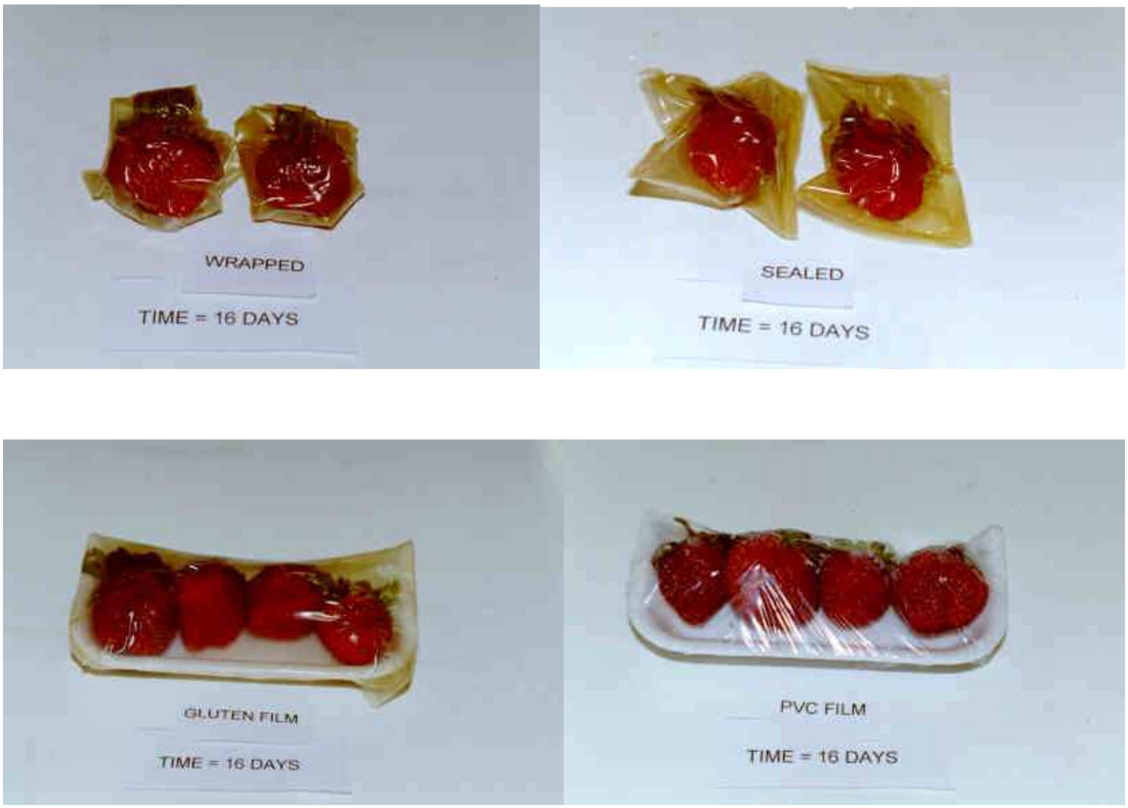

Figure 4 - Strawberries with the gluten and PVC films on the day 16.

Microbial decay

Strawberry is a highly perishable fruit and the shelf life usually ends due to fungal infection[18]. The microbial attack of the fruit was characterized as brown spots and a softening of the injured zone. In this work, the maximum storage life was defined as the time elapsed between the application of the coating and the visualization of fungal attack. The gluten film in pouches and the film that covered the fruits in a tray, reduced the number of infected fruits in comparison with the control fruits (Fig. 5b). The gluten, composite and bilayer coatings and the wrapped film did not reduce the number of infected fruits at the end of the storage time (Fig. 5a). However the composite and bilayer coatings and the film that covered the fruits in a tray were effective in extending fruit storage life by a factor of 2.67 X with reference to the control fruits.

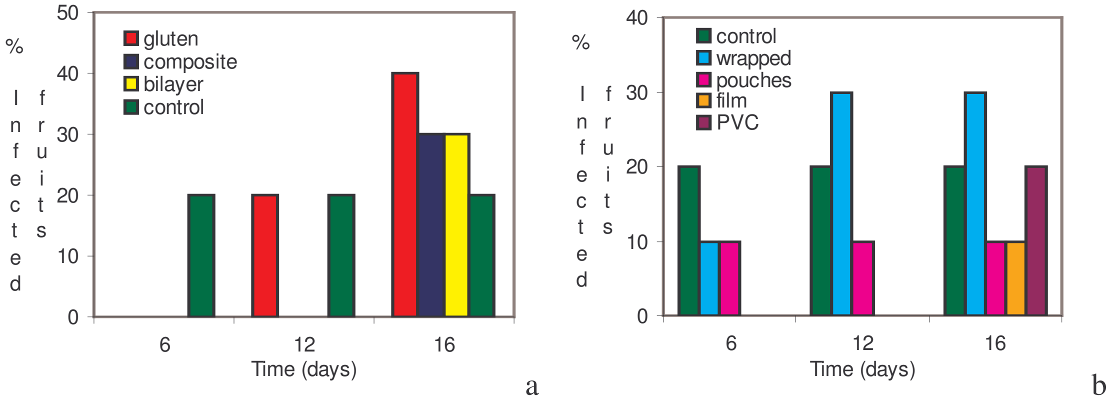

Figure 5 – Microbial decay of coated (a) and packed (b) strawberries expressed as percentage of infected fruits during storage time.

Weight loss

Weight loss during storage due to transpiration was observed for all fruits. The weight loss of fruits increased with storage time for the control, coated and packed fruits. Fig. 6 shows that the fruits coated with the bilayer coating of gluten and a mixture of beeswax and S-P had the lowest weight losses, and the fruits coated with the composite coating also had low weight losses. However, the fruits coated with the gluten coating had higher weight losses than the control fruits. All the fruits packed with gluten films had lower weight losses as compared to the control (Fig. 7), but the fruits packed with the PVC film had the lowest weight loss. Of the gluten films tested, the fruits covered with the gluten film in a tray had the lowest weight loss. The bilayer coating of gluten and lipids and the gluten film effectively reduced the weight loss of the strawberries during storage, being the most effective of all films and coatings with a weight loss lower than 10%. A similar effect was observed by García *et al.*[12,13] for strawberries coated with starch-based coatings. The water vapor permeability of the bilayer coating was low (1.01 gmm/m^{2}dkPa), as reported by Tanada-Palmu and Grosso (2002, unpublished), which can explain the best effect of this coating in reducing the weight loss of the strawberries.

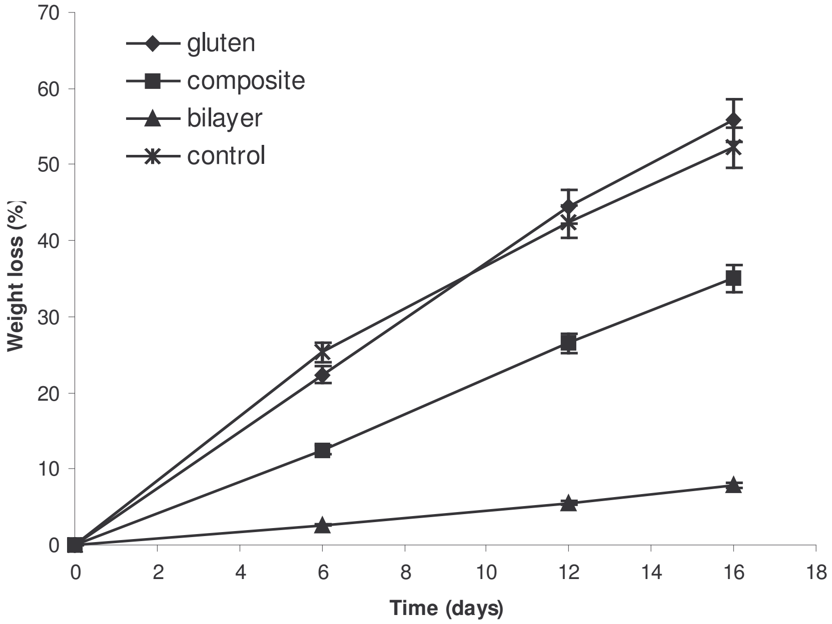

Figure 6 – Effect of gluten based coatings on weight loss of strawberries during storage.

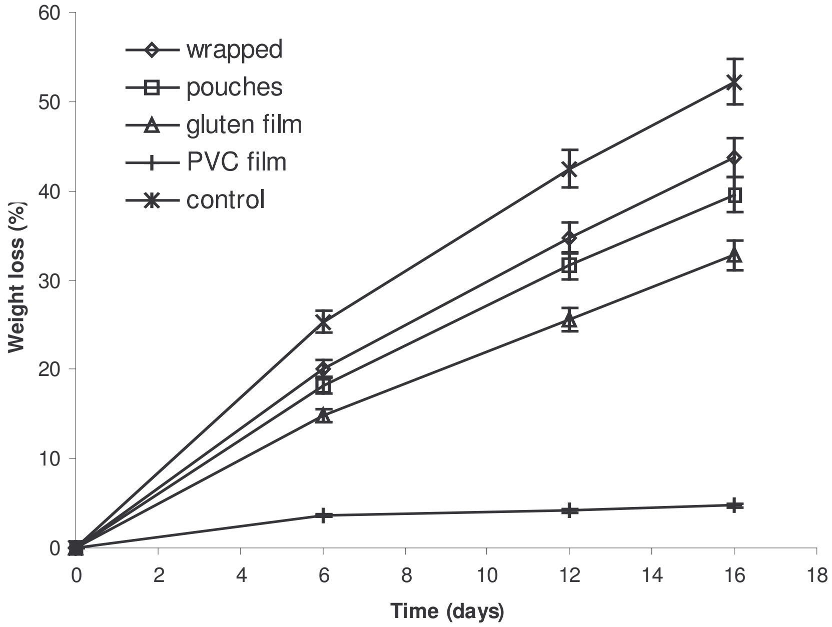

Figure 7 – Effect of gluten based films on weight loss of strawberries during storage.

Firmness

During prolonged storage, changes in appearance and texture occur in fruits and vegetables due to metabolic changes and water content[12]. One of the main factors used to determine fruit quality and postharvest shelf life is the rate and extension of the loss of firmness during the ripening of soft fruits, such as strawberries. According to Manning[19], fruit softening is attributed to the degradation of cell wall components, mainly pectins, due to the action of specific enzymes such as polygalacturonase.

Break force decreased as a function of storage time for the control, coated and packed fruits, as shown in Figs. 8 and 9. All coatings and films, except the film wrapping, showed a beneficial effect on firmness retention, the best results being obtained with bilayer and composite coatings. At the end of the experiment (16 days), break forces for composite and bilayer coatings were higher than those of gluten films, however, the fruits packed with the PVC film had the highest break forces.

As observed with weight loss, the bilayer coating also showed the best result with respect to the retention of fruit firmness.

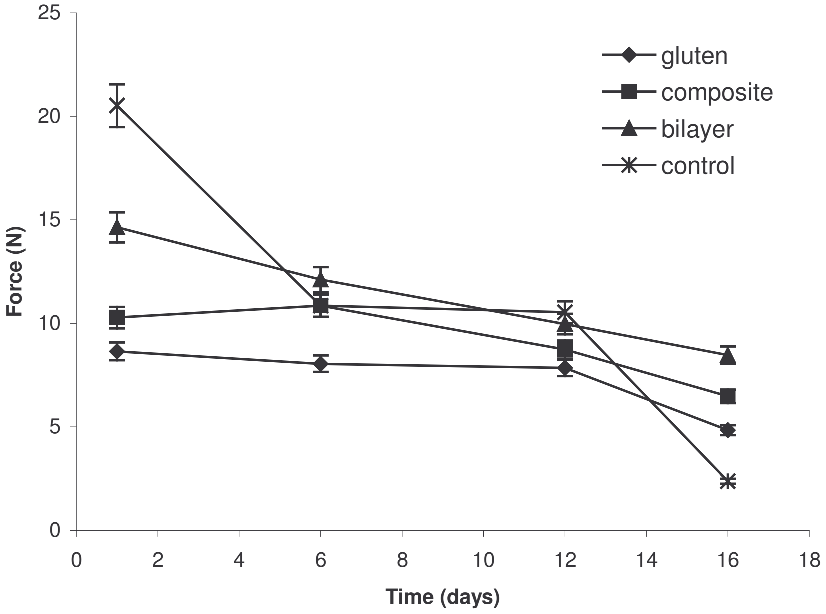

Figure 8 – Effect of coatings on firmness (Newton) of strawberries during storage.

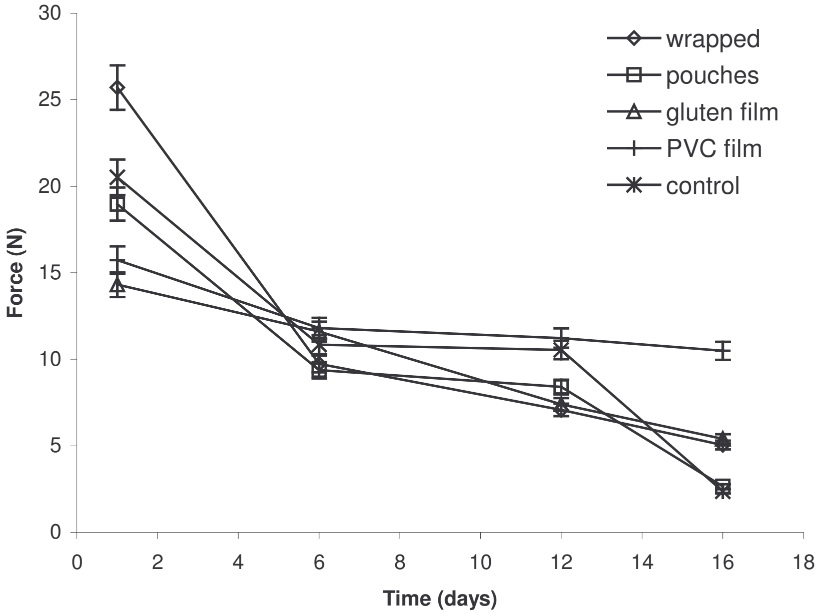

Figure 9 – Effect of films on firmness (Newton) of strawberries during storage.

Surface color development

Color changes during post-harvest ripening were evidenced by a decrease in lightness (*L*), an increase in redness (*a*) and a decrease in yellowness (*b*). Changes were evaluated by the *a*/*b* ratio.

As shown in Figs. 10 and 11, the *a*/*b* ratios increased with storage time (while the parameter *a* increased, *b* decreased). Control fruits showed the highest *a*/*b* values in comparison with coated and packed fruits. Thus the gluten coating and the film package were effective in the delay of senescence, as evidenced by the decrease in color changes, in accordance with García *et al.*[12].

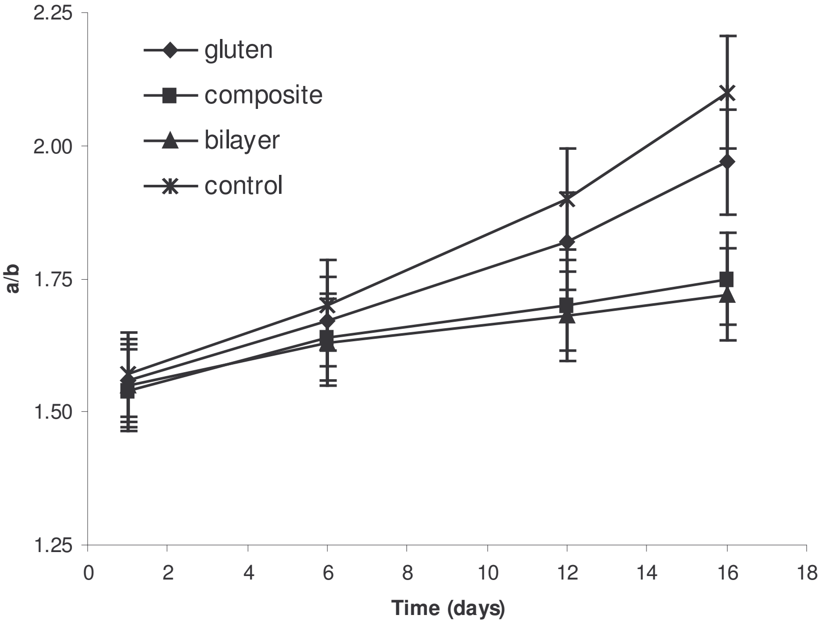

Figure 10 – Surface color changes of coated strawberries during storage.

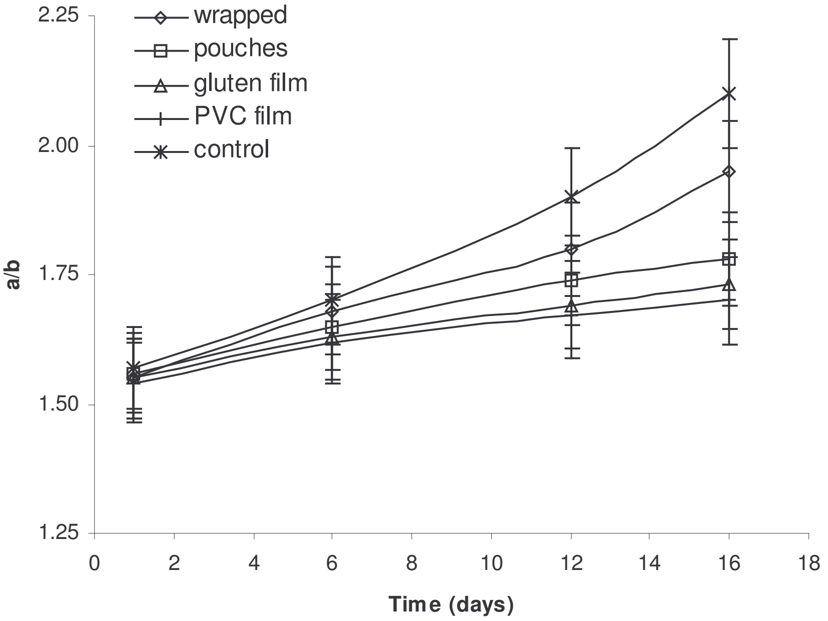

Figure 11 – Surface color changes of packed strawberries during storage.

Changes in titratable acidity and the pH of the fruits

The most abundant acid in strawberries is citric acid, followed by malic acid[12]. As seen in Table 1, titratable acidity decreased as a function of storage time, with the exception of the fruits covered with the bilayer film. The decrease in acidity demonstrates maturity development. The titratable acidity did not change with storage time in the fruits covered with the bilayer coating, though this coating delayed the maturation process. These results are in accordance with El Gaouth *et al.*[4] for strawberries coated with a chitosan-based formulation and with García *et al.*[12,13], for strawberries coated with starch-based coatings. The pH values increased slightly during the storage time for control, wrapped and fruits in pouches.

Soluble and total solids and moisture content

The solids content of soft fruits is generally related to the size and weight of the seeds. Although high in number, strawberry achenes do not contribute to the solids content[20]. During storage, the moisture decreased in strawberries and as a consequence, the total solids increased. The soluble solids increased with storage time, with the exception of the fruits covered with the bilayer film. These results occurred in all coated, packed and control fruits.

Table 1 – Changes in pH, titratable acidity, soluble and total solids and moisture in control, coated and packed strawberries

		storage time at 7^{0}C			
parameter	sample	1 day	6 days	12 days	16 days
pH*	gluten	3.83 ± 0.04^{abA}	3.82 ± 0.06^{aA}	3.82 ± 0.01^{aA}	3.86 ± 0.05^{aA}
	composite	3.81 ± 0.06^{abcA}	3.87 ± 0.07^{aA}	3.90 ± 0.01^{aA}	3.98 ± 0.09^{aA}
	bilayer	3.86 ± 0.14^{aA}	3.83 ± 0.04^{aA}	3.90 ± 0.03^{aA}	3.98 ± 0.11^{aA}
	control	3.63 ± 0.05^{bcdB}	3.88 ± 0.17^{aAB}	3.93 ± 0.04^{aA}	3.93 ± 0.08^{aA}
	wrapped	3.60 ± 0.08^{cdB}	3.79 ± 0.07^{aAB}	3.86 ± 0.03^{aA}	3.88 ± 0.08^{aA}
	pouches	3.58 ± 0.04^{dB}	3.91 ± 0.03^{aA}	3.94 ± 0.01^{aA}	3.95 ± 0.06^{aA}
	gluten film	3.87 ± 0.08^{aA}	3.92 ± 0.07^{aA}	3.95 ± 0.04^{aA}	3.96 ± 0.07^{aA}
	PVC film	3.95 ± 0.07^{aA}	3.95 ± 0.08^{aA}	3.95 ± 0.02^{aA}	3.95 ± 0.07^{aA}
Titratable acidity (g citric acid /100g)*	gluten	1.13 ± 0.09^{bA}	0.97 ± 0.06^{bcAB}	0.80 ± 0.04^{bcBC}	0.67 ± 0.07^{bcC}
	composite	0.90 ± 0.08^{bcA}	0.85 ± 0.07^{bcdA}	0.71 ± 0.03^{bcA}	0.35 ± 0.01^{dB}
	bilayer	0.59 ± 0.08^{dA}	0.60 ± 0.09^{dA}	0.59 ± 0.02^{cA}	0.62 ± 0.08^{cA}
	control	1.60 ± 0.12^{aA}	1.07 ± 0.06^{abcB}	1.07 ± 0.02^{aB}	0.88 ± 0.09^{aB}
	wrapped	1.46 ± 0.06^{aA}	1.33 ± 0.12^{aAB}	1.10 ± 0.08^{aBC}	0.89 ± 0.04^{aC}
	pouches	1.42 ± 0.09^{aA}	1.11 ± 0.07^{abB}	0.91 ± 0.02^{abC}	0.82 ± 0.10^{abC}
	gluten film	1.00 ± 0.12^{bcA}	0.97 ± 0.11^{bcAB}	0.75 ± 0.02^{bcAB}	0.73 ± 0.11^{abcB}
	PVC film	0.81 ± 0.08^{cdA}	0.81 ± 0.13^{cdA}	0.69 ± 0.03^{bcA}	0.64 ± 0.04^{bcA}
Soluble solids (^{0}Brix)*	gluten	7.75 ± 0.25^{dD}	10.00 ± 0.25^{bcC}	11.75 ± 0.25^{abB}	13.00 ± 0.25^{abA}
	composite	8.00 ± 0.25^{cdC}	9.50 ± 0.25^{bcB}	12.00 ± 0.25^{aA}	11.50 ± 0.25^{cdA}
	bilayer	8.35 ± 0.25^{bcA}	7.75 ± 0.25^{dA}	8.25 ± 0.25^{dA}	7.75 ± 0.25^{eA}
	control	9.00 ± 0.25^{abA}	10.00 ± 0.25^{bcB}	11.00 ± 0.25^{bcC}	12.00 ± 0.25^{bcD}
	wrapped	8.00 ± 0.25^{cC}	10.00 ± 0.25^{bcB}	11.00 ± 0.25^{bcB}	12.50 ± 0.25^{abcA}
	pouches	8.00 ± 0.25^{cD}	9.00 ± 0.25^{cC}	12.00 ± 0.25^{aB}	13.00 ± 0.25^{abA}
	gluten film	9.50 ± 0.25^{aD}	11.25 ± 0.25^{aC}	12.25 ± 0.25^{aB}	13.50 ± 0.25^{aA}
	PVC film	9.00 ± 0.25^{abB}	10.25 ± 0.25^{abA}	10.50 ± 0.25^{cA}	10.75 ± 0.25^{dA}
Total solids (g/100g)*	gluten	8.36 ± 0.08^{dD}	11.33 ± 0.25^{cdC}	14.05 ± 0.35^{bB}	14.85 ± 0.18^{bA}
	composite	8.41 ± 0.08^{dD}	10.86 ± 0.25^{deC}	14.11 ± 0.15^{bA}	12.88 ± 0.16^{dB}
	bilayer	9.52 ± 0.06^{cC}	12.28 ± 0.30^{abB}	12.19 ± 0.31^{cB}	13.26 ± 0.32^{cdA}
	control	9.55 ± 0.17^{cC}	12.15 ± 0.22^{abB}	12.15 ± 0.28^{cB}	14.97 ± 0.13^{bA}
	wrapped	8.31 ± 0.16^{dC}	8.81 ± 0.15^{fC}	12.39 ± 0.29^{cB}	13.06 ± 0.25^{cdA}
	pouches	9.70 ± 0.27^{cC}	12.52 ± 0.26^{aB}	13.54 ± 0.36^{bB}	14.69 ± 0.11^{bcA}
	gluten film	12.99 ± 0.13^{aB}	11.87 ± 0.25^{bcB}	15.12 ± 0.48^{aA}	16.32 ± 0.66^{aA}
	PVC film	12.37 ± 0.21^{bB}	10.61 ± 0.15^{eC}	12.32 ± 0.18^{cB}	13.89 ± 0.16^{cA}
Moisture (g/100g)*	Gluten	91.64 ± 0.56^{abA}	88.67 ± 0.33^{bcdB}	85.95 ± 0.15^{bC}	85.15 ± 0.29^{cdC}
	composite	91.59 ± 0.61^{abA}	89.14 ± 0.45^{bcB}	85.89 ± 0.29^{bD}	87.12 ± 0.24^{aC}
	bilayer	90.48 ± 0.23^{abA}	87.72 ± 0.31^{deB}	87.81 ± 0.28^{aB}	86.74 ± 0.25^{abC}
	control	90.45 ± 0.46^{abA}	87.85 ± 0.57^{deB}	87.85 ± 0.12^{aB}	85.03 ± 0.23^{dC}
	wrapped	91.69 ± 0.38^{aA}	91.19 ± 0.54^{aA}	87.61 ± 0.28^{aB}	86.94 ± 0.51^{abC}
	pouches	90.30 ± 0.48^{bA}	87.48 ± 0.15^{eB}	86.46 ± 0.15^{bB}	85.31 ± 0.19^{cdC}
	gluten film	87.01 ± 0.67^{cA}	88.13 ± 0.65^{cdeB}	84.88 ± 0.14^{cC}	83.68 ± 0.28^{eD}
	PVC film	87.63 ± 0.39^{cB}	89.39 ± 0.52^{bA}	87.68 ± 0.24^{aB}	86.11 ± 0.17^{bcC}

*Mean ± standard deviation of three replicates. Means for the same parameter in the same column ($^{a\text{-}f}$) or in the same line ($^{A\text{-}D}$) with different superscript letters are significantly different ($p<0.05$), according to ANOVA and the Tukey test.

Changes in the concentration of reducing and total sugars

Generally, in ripe strawberries, the glucose and fructose concentrations are 2.3 and 2.2g/100 g of fresh fruits, respectively, which corresponds to 83% of the total sugar content[19]. The reducing sugar content of all coated, packed and control fruits increased with storage time, in accordance with García *et al.*[12]. The reducing sugars contents of fruits coated with bilayer coatings were lower than the others treatments (Table 2). Total sugars also increased with storage time for all coated, packed and control fruits (Table 2).

Table 2 - Changes in reducing and total sugars in the control, coated and packed strawberries

		storage time at 7^0C			
parameter	sample	1 day	6 days	12 days	16 days
Reducing sugars (g glucose/100g fruit)*	gluten	3.67 ± 0.05^{bD}	3.30 ± 0.12^{dC}	4.20 ± 0.07^{eB}	5.57 ± 0.04^{eA}
	composite	2.60 ± 0.02^{dD}	2.17 ± 0.04^{fC}	4.90 ± 0.02^{cB}	4.60 ± 0.04^{fA}
	bilayer	2.73 ± 0.06^{dB}	2.60 ± 0.08^{eB}	2.37 ± 0.08^{gC}	3.03 ± 0.07^{hA}
	control	3.75 ± 0.03^{bD}	4.17 ± 0.05^{aC}	5.20 ± 0.03^{bB}	6.00 ± 0.05^{cA}
	wrapped	3.47 ± 0.04^{cD}	3.63 ± 0.04^{cC}	4.53 ± 0.06^{dB}	6.73 ± 0.03^{aA}
	pouches	3.69 ± 0.02^{bB}	3.83 ± 0.12^{bcB}	5.60 ± 0.03^{aA}	5.80 ± 0.05^{dA}
	gluten film	4.33 ± 0.09^{aB}	4.23 ± 0.03^{aBC}	4.07 ± 0.08^{eC}	6.27 ± 0.08^{bA}
	PVC film	4.37 ± 0.09^{aA}	3.90 ± 0.08^{bB}	3.60 ± 0.04^{fC}	4.40 ± 0.04^{gA}
Total sugars (g glucose/ 100g fruit)*	gluten	5.23 ± 0.05^{aD}	5.40 ± 0.07^{abB}	5.60 ± 0.06^{abcA}	6.27 ± 0.06^{bcC}
	composite	5.20 ± 0.02^{aD}	5.33 ± 0.04^{abC}	5.57 ± 0.06^{abcB}	6.13 ± 0.05^{dA}
	bilayer	5.27 ± 0.07^{aC}	5.28 ± 0.04^{bC}	5.49 ± 0.07^{cB}	6.18 ± 0.03^{cdA}
	control	5.15 ± 0.04^{aD}	5.43 ± 0.05^{aC}	5.56 ± 0.07^{bcB}	6.25 ± 0.02^{bcA}
	wrapped	5.13 ± 0.05^{bD}	5.37 ± 0.05^{abC}	5.63 ± 0.03^{abcB}	6.32 ± 0.03^{abA}
	pouches	5.29 ± 0.07^{aD}	5.40 ± 0.07^{aC}	5.72 ± 0.04^{aB}	6.17 ± 0.05^{cdA}
	gluten film	5.27 ± 0.05^{aD}	5.45 ± 0.06^{aC}	5.67 ± 0.04^{abB}	6.40 ± 0.02^{aA}
	PVC film	5.18 ± 0.06^{aD}	5.38 ± 0.04^{abC}	5.53 ± 0.05^{bcB}	6.36 ± 0.06^{abA}

*Mean ± standard deviation of three replicates Means for the same parameter in the same column ($^{a-h}$) or in the same line ($^{A-D}$) with different superscript letters are significantly different ($p<0.05$), according to ANOVA and the Tukey test.

Sensory evaluation of the strawberries

The sensory evaluation of the shelf life of the strawberries showed that the consumers liked the global appearance, color and brightness of samples 1 (control), 2 (gluten) and 3 (composite) (Fig. 12 and Table 3) on day 1, but they disliked sample 1 on day 16 because the color was too dark red, like an over-ripe fruit. Sample 4 (bilayer) was disapproved by the consumers on all days tested because they thought this sample was artificial, no characteristic color, opaque, waxy aspect and with this coating, it was difficult to see the natural color of the fruit.

In the sensory evaluation of the strawberries (Fig. 13 and Table 4), the consumers most liked the flavor and texture of samples 1, 2 and 3, and the taste of samples 1 and 2. Some consumers detected a residual taste in samples 2 and 3, especially in sample 3, and they disliked sample 4 because the coating was hard and difficult to bite and the taste was not pleasant. For the intention to buy, the consumers would buy samples 1 and 2.

From the results of the sensory evaluation of the shelf life of the strawberries, it can be seen that the gluten and the composite coating of gluten and lipids, effectively improved the shelf life of the strawberries, maintaining the quality (global appearance, color and brightness) as compared to the fruits with no coating. From the results of the sensory evaluation for taste of the strawberries, the gluten coating did not affect the flavor and taste of the strawberries.

The consumers disapproved the appearance and the taste of the bilayer coating of gluten and lipids, but this coating was the best to maintain the quality of the strawberries during the whole storage time (demonstrated by the analysis of the fruits and visual analysis during the sensory evaluation).

Table 3 – Mean of the attributes in the sensory evaluation for the shelf life of 4 samples of strawberry stored at 7 ^{0}C

	Glob. Appearance*				Color*				Brightness*				Buy*			
Day	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	7.2^{a}	7.7^{a}	7.4^{a}	3.0^{b}	7.2^{a}	7.7^{a}	7.4^{a}	3.0^{b}	6.5^{b}	7.5^{a}	7.2^{ab}	2.7^{c}	3.9^{a}	4.4^{a}	4.2^{a}	1.8^{b}
6	6.8^{a}	7.4^{a}	6.6^{a}	3.4^{b}	6.9^{a}	7.4^{a}	7.0^{a}	3.3^{b}	6.0^{b}	7.5^{a}	7.2^{a}	2.7^{c}	3.6^{b}	4.3^{a}	4.1^{ab}	1.8^{c}
12	2.7^{b}	6.4^{a}	6.8^{a}	3.5^{b}	3.6^{b}	7.0^{a}	6.7^{a}	3.7^{b}	2.6^{c}	7.0^{a}	7.0^{a}	3.2^{b}	1.4^{b}	3.9^{a}	3.9^{a}	2.0^{b}
16	3.0^{b}	6.5^{a}	7.1^{a}	4.0^{b}	3.2^{c}	6.7^{a}	7.5^{a}	4.3^{b}	2.7^{b}	6.0^{a}	6.9^{a}	3.4^{b}	1.5^{b}	3.6^{a}	4.2^{a}	2.0^{b}

*Mean of 40 consumers for each sample. $^{a\text{-}c}$ Means with different superscript letters for the same parameter and day of test are significantly different ($p<0.05$), according to ANOVA and the Tukey test.

Samples: 1=control; 2=gluten; 3=composite; 4=bilayer.

Scores for the global appearance: color, brightness, flavor, taste and texture: 1=Disliked extremely; 2=Disliked very much; 3=Disliked moderately; 4=Disliked slightly; 5=Liked/disliked; 6=Liked slightly; 7=Liked moderately; 8=Liked very much; 9=Liked extremely.

Scores for the intention to buy: 1=Certainly would not buy; 2=Possibly would not buy; 3=Maybe would buy/maybe not; 4=Possibly would buy; 5=Certainly buy.

Table 4 - Mean of the attributes in the sensory evaluation for the taste and flavor of 4 samples of strawberry after 5 days at 7 ^{0}C

Sample	Flavor*	Taste*	Texture*	Buy*
1	7.2^{a}	7.2^{a}	7.2^{a}	3.9^{a}
2	6.6^{a}	6.1^{ab}	6.9^{a}	3.4^{ab}
3	6.4^{a}	5.5^{b}	7.1^{a}	3.0^{b}
4	4.4^{b}	3.4^{b}	3.7^{b}	1.5^{b}

*Mean of 40 consumers for each sample. $^{a\text{-}b}$ Means with different superscript letters in the same parameter are significantly different ($p<0.05$) according to ANOVA and the Tukey test.

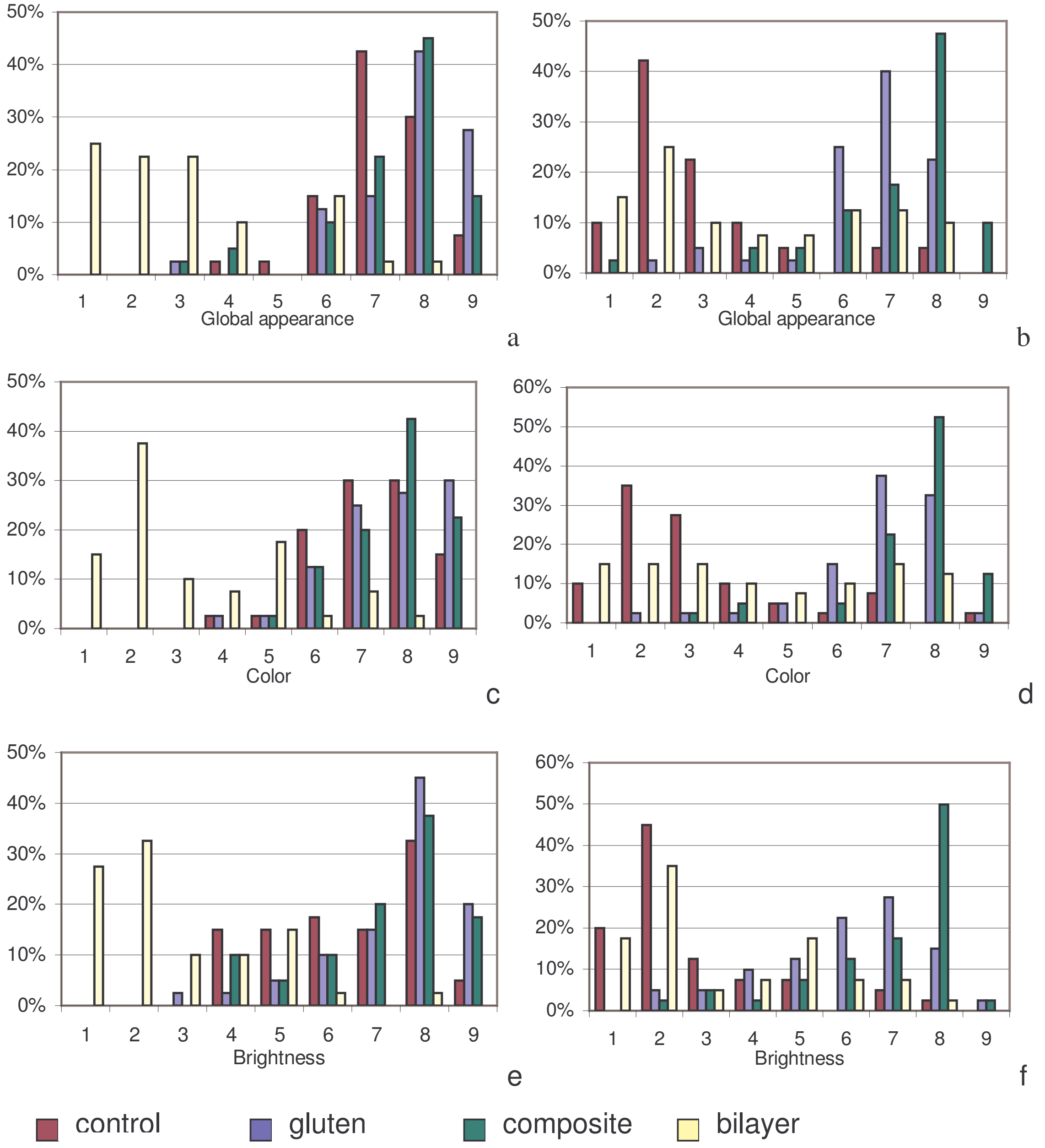

Figure 12 – Histograms for global appearance on day 1 (a) and day 16 (b), color on day 1 (c) and day 16 (d) and brightness of the strawberries on day 1 (e) and on day 16 (f).

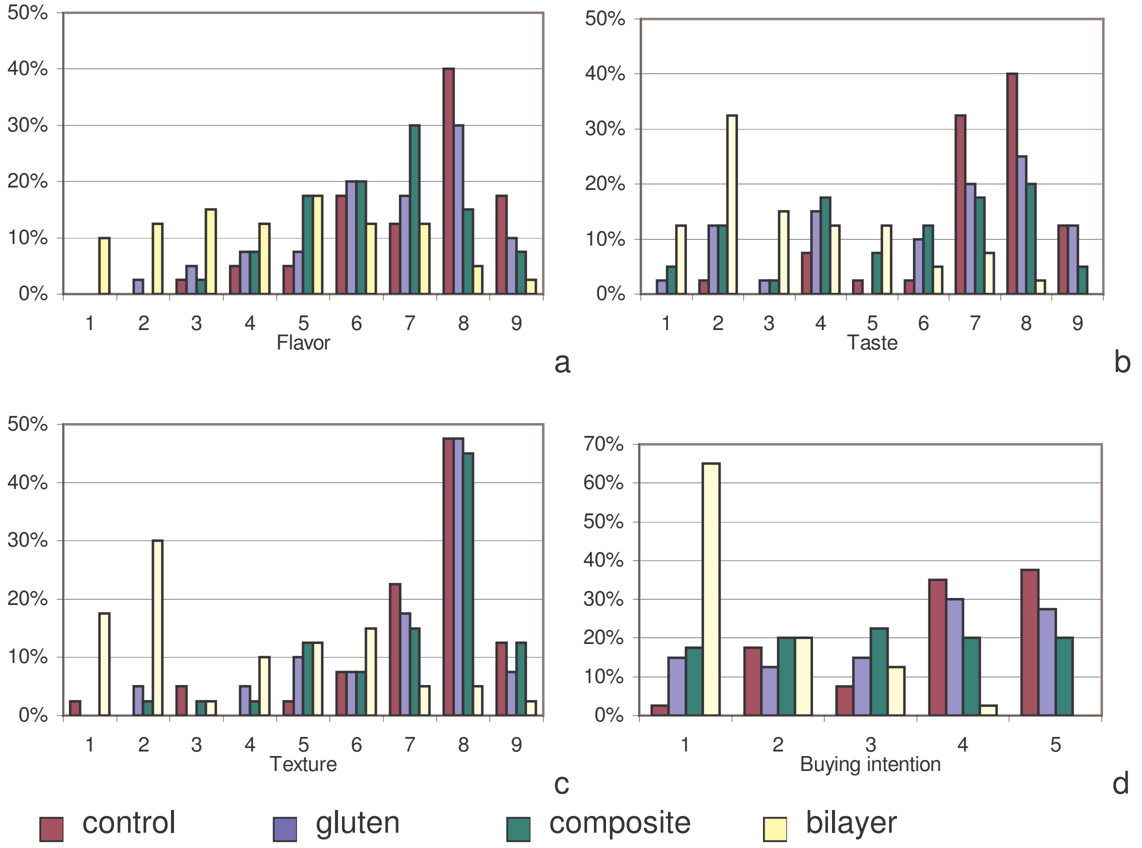

Figure 13 – Histograms for flavor (a), taste (b), texture (c) and buying intention (d) of the strawberries.

CONCLUSIONS

Wheat gluten coatings and films extended the storage life of strawberries and retarded the senescence process. The addition of lipids to the gluten coatings showed a beneficial effect on firmness retention and reduced weight loss of the strawberries. The gluten film retarded senescence as the packed strawberries produced the lowest *a*/*b* ratios as compared to the control fruits. Although the composite and bilayer coatings and the film that covered the fruits in a tray were effective in extending fruit storage life in comparison to the control fruits, a low percentage of the fruits were infected. A further study in quantify the microbial decay of the coated and packed strawberries should be investigated. The sensory evaluation of the strawberries showed that the fruits with gluten and the composite coating maintained the quality during the shelf life and the consumers approved their taste and flavor.

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REFERENCES

1 Rose P, Never mind the food, the packaging tastes great. *Food Manuf Int* **9**: 27, 29-30 (1992).

2 Park HJ, Development of advanced edible coatings for fruits. *Trends Food Sci Tech* **10**: 254-260 (1999).

3 Smith SM, Geeson J, Stow J, Production of modified atmospheres in deciduous fruits by the use of films and coatings. *HortSci* **22**: 772-776 (1987).

4 El Gaouth A, Arul J, Ponnampalam R, Boulet M, Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. *J Food Proc Preserv* **15**: 359-368 (1991).

5 El Gaouth A, Arul J, Ponnampalam R, Castaigne R, Arul J , Chitosan coating to extend the storage-life of tomatoes. *HortSci* **27**: 1016-1018 (1992).

6 Banks NH, Some effects of TAL Pro-Long coating on ripening bananas. *J. Expt. Bot.* **35**: 127-137 (1984).

7 Banks NH, Cheng Q, Nicholson SE, Kingsley AM, Jeffery PB, Variation with temperature in effects of surface coatings on gas exchange of apples. *Int. Cong. Plastics Agric.*, Tel Aviv, Israel (1997).

8 Dhalla R, Hanson SW, Effect of permeable coatings on the storage life of fruits. II. Pro-long treatment of mangoes (*Mangifera indica* L. cv. Julie). *Int J Food Sci Technol* **23**: 107-112 (1988).

9 Amarante CVT, Gas exchange, ripening behaviour and postharvest quality of coated pears. Palmerston North, PhD. Dissertation, Massey Univ., New Zealand (1998).

10 Hagenmaier RD, Baker RA, Reduction in gas exchange of citrus fruit by wax coatings. *J Agric Food Chem* **42**: 899-902 (1993).

11 Bender RJ, Brecht SA, Sargent JC, Navarro JC, Campbell CA Ripening initiation and storage performance of avocados treated with an edible-film coating. *Acta Hort* **343**: 184-186 (1993).

12 García MA, Martino MN, Zaritzky NE, Starch-based coatings: effect on refrigerated strawberry (*Fragaria ananassa*) quality. *J Sci Food Agric* **76**: 411-420 (1998a).

13 García MA, Martino MN, Zaritzky NE, Plasticized starch-based coatings to improve strawberry (*Fragaria ananassa*) quality and stability. *J Agric Food Chem* **46**: 3758-3767 (1998b).

14 Oliveira MA, Cereda MP, Efeito da película de mandioca na conservação de goiabas*. Braz J Food Technol* **21** (2): 97-102 (1999).

15 Gontard N, Guilbert S, Cuq JL, Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J Food Sci* **58** (1): 206-211 (1993).

16 AOAC, *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC, USA (1980).

17 Macfie HJ, Bratchell N, Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Studies* **4**: 129-148 (1989).

18 Maas JL, Postharvest diseases of strawberry. In *The Strawberry – cultivars to mar*keting, Ed Childers N F. Horticultural Publications, Gainesville, FL, USA, pp 329-353 (1981).

19 Manning K, Soft fruits. In *Biochemistry of Fruit Ripening*, Ed Seymour GB, Taylor JE, Tucker GA. Chapman & Hall, London, UK, p. 347-373 (1993).

20 Green A, Soft fruits. In *Food Science and Technology. The Biochemistry of Fruits and Their Products*, Ed Hulme AC. Academic Press, London, UK, p. 373-410 (1971).

CAPÍTULO 6

CHEMICAL LIPOPHILIZATION OF WHEAT GLUTEN AND ITS EFFECT ON THE PROPERTIES OF EDIBLE FILMS

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CHEMICAL LIPOPHILIZATION OF WHEAT GLUTEN AND ITS EFFECT ON THE PROPERTIES OF EDIBLE FILMS

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Abstract: Chemical modification of wheat gluten protein can be effected to change the structure of the protein and so its functional properties. Wheat gluten was lipophilized by adding stearyl chloride to aqueous protein dispersion. This study showed that it was possible to lipophilize proteins in an aqueous medium. The wheat gluten lipophilization was detected by the TNBS (trinitrobenzenesulfonic acid dihydrate) determination. There was about 32.5% less free amino groups for lipophilized gluten than for unmodified gluten. The edible films from lipophilized gluten have not improved water vapor permeability and mechanical resistance in comparison to films from unmodified gluten probably because the stearic acid presented low affinity toward the wheat gluten.

Keywords: wheat gluten, lipophilization, edible film, properties.

Introduction

Proteins and lipids can be chemically modified by lipophilization under mild conditions to improve the functional properties of these mixtures in edible film systems. Lipoproteins have important functions in many membrane and plant systems, however they have not been explored as application in edible films and coatings. Lipophilization increases the surface hydrophobicity of the protein molecule by covalent attachment of hydrophobic ligands, such as fatty acids.

Lipophilization has been performed on casein/palmitic acid mixtures, β-lactoglobulin/steric acid mixtures and soy glycinin with lauric, myristic, palmitic and stearic acid mixtures. Resulting lipoproteins have exhibited reduced allergenicity and improved functional properties (HAQUE and KITO, 1983). This type of covalent attachment may be beneficial in improving the properties of edible films.

The term lipophilization refers to the general phenomenon of an increase in the hidrophobocity of proteins modified by binding lipidic components, resulting in an increase in their affinity with apolar compounds. The nature and length of the chains of the hydrophobic groups bound to proteins are directly linked to the final structural and functional properties of so modified proteins. Until now, such transformations have been achieved by chemical means, generally involving fatty acid anhydrides or fatty esters of N-hydroxysuccinimide (ROUSSEL-PHILIPPE et al., 2000).

DJAGNY et al. (2001) effected chemical modification of gelatin with lauric, myristic and stearic acid, and stearic acid presented the highest affinity toward the gelatin. According to them, fatty acids with different chain-lengths differ in their chemical and physical properties including their capacity of reacting with other compounds. ROUSSEL-PHILIPPE et al. (2000) investigated chemical lipophilization of soy protein isolate and wheat gluten with different levels of lauroyl chloride. This study showed that it was possible to lipophilize proteins, even with significantly high protein concentration.

The utilization of fatty acid chloride has already been employed to modify enzymes in diluted medium like *Mucor miehei* aspartyl-proteinase (SMITH and YADA, 1991), trypsin and carboxypeptidase A (KOELSH et al., 1981).

Lipophilization of wheat gluten is carried out in an alkaline medium using stearyl chloride, according to the Schotten-Baumann’s reaction (SCHOTTEN, 1844; BAUMANN, 1886 apud ROUSSEL-PHILIPPE et al., 2000) as follows:

$P\text{-}NH_2 + R\text{-}CO\text{-}Cl + NaOH \rightarrow P\text{-}NH\text{-}CO\text{-}R + NaCl + H_2O$

The objective of this work was to promote chemical modification of wheat gluten in order to investigate the influence of this modification on the properties of edible films.

Materials and Methods

Materials

Wheat gluten (Rhodia, Brazil), stearic acid (Vetec, Brazil), thionil chloride (van de Riedel), chloride acid (Merck, Germany), sodium hydroxide (Synth, Brazil), n-hexane (Merck, Germany), glycerol (Merck, Germany), ammonium hydroxide (Synth, Brazil), calcium chloride (Ecibra, Brazil), sodium chloride (Synth, Brazil), magnesium nitrate (Ecibra, Brazil) and solid paraffin (Chemco, Brazil).

Schotten-Baumann's reaction

A 15% (w/v) wheat gluten was poured into a 0.5 N NaOH and the medium was stirred at 40 ^{0}C for 15 min. These media were adjusted to pH 9 with 6N HCl; then stearyl chloride (obtained from the reaction of stearic acid and thionil chloride) was added while stirring and maintaining pH 9 with 2N NaOH addition until all acyl chloride has reacted as indicated by no more change in pH. The "stearyl chloride/wheat gluten" weight ratio was 0.25. The reaction mixtures were brought to pH 4.3 with 6 N HCl for precipitation and centrifuged at 5.000 g for 10 min. To wash and remove excess salt, water was added to the precipitate to make a 50% dispersion. This dispersion was centrifuged and the residue was lyophilized and stored in a dessicator. To remove the excess of fatty reagent, the lipophilized wheat gluten was extracted with n-hexane in a soxhlet for 4 hours, and dried in a dessicator (ROUSSEL-PHILIPPE et al., 2000).

Analysis of free amino group content by TNBS method

The TNBS (trinitrobenzenesulfonic acid dihydrate) reaction was carried out as follows, according to ADLER-NISSEN (1979): 0.250 mL of a sample was mixed in a test tube with 2.0 mL of phosphate buffer at pH 8.2. Two milliliters of 0.1% TNBS solution was added and the test tube was shaken and placed in a water bath at 50 ^{0}C for 60 min. After the 60 min, 4.0 mL of 0.1 N HCl was added to terminate the reaction, and the test tube was allowed to stand at room temperature for 30 min before the absorbance was read against blank at 340 nm. The reactions on the blank and the standard solutions were carried out by replacing the sample with 1% sodium dodecyl sulfate (SDS) and 1.5 x 10^{-3} M L-leucine in 1% SDS, respectively.

Film preparation

The film was prepared from a solution of unmodified (UG) and lipophilized (LG) wheat gluten (9.0 g/100 mL solution), absolute ethanol (32.5 mL/100 mL solution), glycerol (1.5 g/100 mL solution), ammonium hydroxide (to adjust to pH 10) and distilled water, according to TANADA-PALMU and GROSSO (2002b). All components were mixed using magnetic stirring until the temperature of the mixture reached 70 ^{0}C, when the solution was centrifuged at 5856 g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a Teflon covered glass surface and dried at room temperature for 24 h (modified method of GONTARD et al., 1993). The quantity of solution poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for experiments were equilibrated at 52% RH and 25 ^{0}C for 48 h before being tested.

Film thickness

Film thickness was measured using a micrometer (Model MDC-25M, Mitutoyo, MFG, Japan). The thickness of individual film samples was determined as an average of five measurements.

Water vapor permeability

The water vapor transmission rate through the films (triplicate) was determined gravimetrically at 25 ^{0}C using a modification of the American Society for Testing and Materials (ASTM, 1980) Standard Method E-96 according to TANADA-PALMU et al. (2000), and the water vapor permeability (WVP) was calculated according to the method.

Oxygen permeability

The oxygen transmission rate though the films (duplicate) was determined using a modification of the ASTM Standard Method D 3985-81 with an Ox-Tran apparatus (Mocon, Inc., Minneapolis, USA) at 25 ^{0}C. The oxygen permeability (OP) was calculated by dividing the oxygen transmission rate by the oxygen pressure and multiplying by the mean thickness.

Tensile strength and percent elongation at break

Film tensile strength (TS) and percent elongation at break (ELO) were determined using a Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), operated according to the ASTM (1980) Standard Method D 882-83 (initial grip separation = 50 mm and cross head speed = 100 mm/min). Six specimens (100 mm long and 25.4 mm wide) of each film were measured. The tensile strength and percent elongation at break were calculated according to the ASTM method.

Solubility in water

The percentage of initial dry matter of each film was determined at 105 ^{0}C for 24 h. Three discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water and slowly and periodically agitated for 24 h at 25 ^{0}C. The pieces of film were then taken out and dried (105 ^{0}C for 24 h) to determine the weight of dry matter, which was not solubilized in water (GONTARD et al., 1993).

Scanning electron microscopy

Film samples were examined for surface characteristics using a Jeol (JMS-T330) scanning microscope (Jeol Ltd., Tokyo, Japan) operated at 10 kV. Film samples were left in a desiccator with silica gel for 7 days, then fraturated and affixed to aluminum stubs with cupper tape. The samples were then sputter-coated with gold in a Balzers evaporator (SDC 050, Baltec, Lichtenstein) for 180 seconds at 40 mA.

Results and Discussion

Wheat gluten lipophilization

Chemical lipophilization of wheat gluten can be used to investigate the influence of the chemical composition and the structure of the proteins on the functional properties and to adjust these properties to meet specific demands.

Wheat gluten lipophilization was carried out using stearyl chloride, obtained from stearic acid. Stearic acid was used in this lipophilization since a previous work (TANADA-PALMU and GROSSO, 2002a) reported its use in composite and bilayer films with gluten (its blend with palmitic acid could improve the properties of the films compared to simple films of gluten).

The wheat gluten lipophilization was detected by the TNBS determination. There was about 32.5% less free amino groups for lipophilized gluten (degree of hydrolisis (DH) of 3.72%) than for unmodified gluten (DH=5.51%). It demonstrates the presence of fatty acid chains in the esterified gluten molecules. It is expected that the free amino groups decrease with the lipophilization reaction due to their binding with the lipids. The lipophilization provoked a decrease in the percentage of free amino groups of the LG in relation to the UG, suggesting an occurrence of binds of lipid to the protein in function of the decrease of the free amino groups.

The fatty acid incorporation into the wheat gluten proteins is not high according to ROUSSEL-PHILIPPE et al. (2000) because of the incomplete reaction of the available lysines due to the specific structure of wheat proteins (gliadines and glutenines) and to a special distribution of the amino group, among them a certain amount is embedded in a hydrophobic environment. This result is in accordance with BARBER and WARTHESEN (1982), who observed that the succinylation rate of gluten was lower than that for other proteins. The specific structure of the gluten may prevent acylation of the amino groups that were inaccessible by steric and hydrophobic hindrance.

Properties of edible films from unmodified and lipophilized gluten

Proteins and lipids are capable of interacting in a many different ways to form effective edible films and coatings. Covalent bonding of lipids to proteins through lipophilization offers unique opportunities for film formation with improved properties (McHUGH, 2000).

The edible film made with lipophilized gluten (LG) was transparent like the film with unmodified gluten (UG), but more fragile and showed a less homogeneous surface and spots. It was harder to prepare the films with LG than films with UG because the dissolution of the component materials of the film was difficult. Moreover, it was hard to obtain films from LG with homogeneous thickness.

The films prepared with LG showed higher values for water vapor permeability (11.05 gmm/m^{2}dKPa) as compared to films with UG (8.61 gmm/m^{2}dKPa), as shown in Table 1. Also the solubility in water was higher in LG than in UG films (30.4 and 22.7%, respectively).

The LG films showed lower oxygen permeability than the UG films probably due the linear structure of the stearic acid, which leads to a high cohesive energy density and a low free volume (TANADA-PALMU et al., 2000).

In relation to mechanical properties, the films with LG showed lower tensile strength (1.37 MPa) and elongation at break (130.8%) then films with UG (5.25 MPa and 215.3%, respectively).

There was significant difference between LG and UG films in all the properties measured.

The LG films have not displayed improved water vapor permeability and mechanical resistance probably due to the low affinity of stearic acid toward wheat gluten. It seems from this study that the wheat gluten does not easily react with long-chain fatty acids, in contrast of what DJAGNY et al. (2001) observed for gelatin. Such behavior maybe the result of the structure of wheat gluten protein, which cannot accommodate a long-chain fatty acid like stearic acid.

Scanning electron micrographs were used to examine the surface of UG and LG films and are shown in Figs. 1 and 2. The morphology observed in the UG film (Fig. 1) was completely modified when the stearic acid was added. Residues of stearic acid were observed in the LG film micrograph (Fig. 2), probably showing that the lipid was not completely reacted with the gluten or the crystallization of the lipid material upon cooling. Apparently greater compaction occurred in the LG film compared to the UG film.

Table 1 – Properties of the lipophilized and unmodified gluten films

Film	Water vapor permeability (gmm/m^2dKPa)*	Solubility in water (%)*	Oxygen permeability ($cm^3\mu m/m^2dKPa$)*	Tensile strength (MPa)*	Elongation at break (%)*
UG	8.61 ± 1.03^b	22.7 ± 4.1^b	41.02 ± 2.30^b	5.25 ± 0.25^a	215.3 ± 12.2^a
LG	11.05 ± 1.24^a	30.4 ± 1.1^a	19.90 ± 2.00^a	1.37 ± 0.16^b	130.8 ± 4.67^b

*Mean and standard deviation of replicates. $^{a\text{-}b}$ Means with different superscript letters in the same column are significantly different ($P<0.05$) according to the ANOVA and Tukey tests.

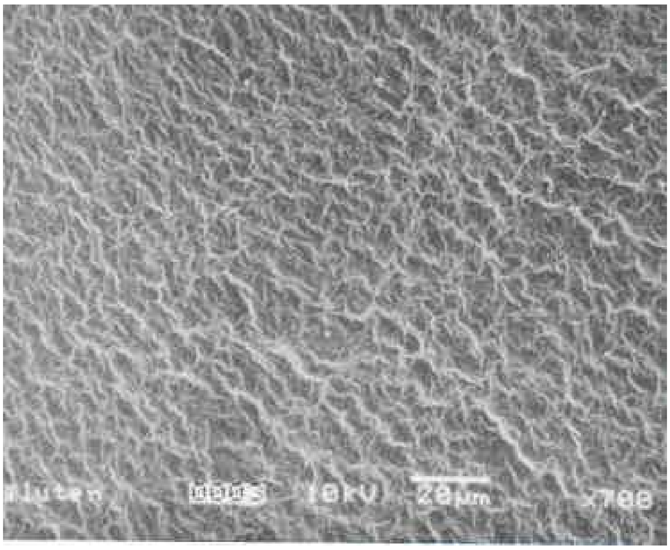

Figure 1 – Micrograph of the surface of the UG film

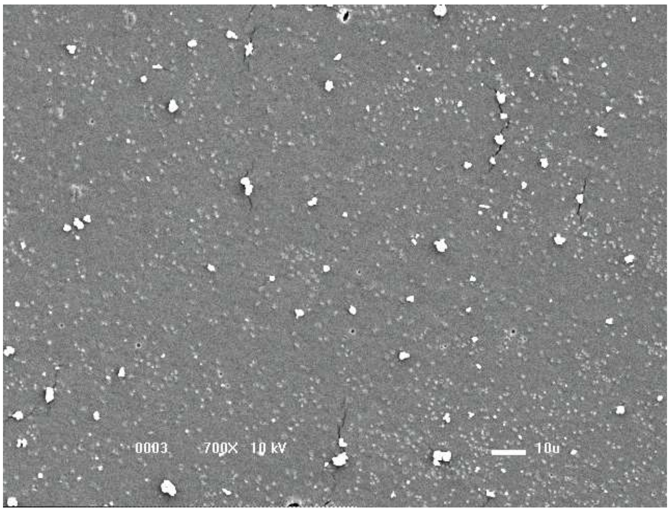

Figure 2 – Micrograph of the surface of the LG film

Conclusion

It was possible to lipophilize wheat gluten in an aqueous medium, as detected by TNBS determinations. However, edible films prepared with lipophilized gluten have not displayed better water vapor barrier and mechanical resistance than films with unmodified gluten. Further research is necessary to elucidate the changes in structure and functional behavior of the lipophilized gluten. Moreover edible film research is trying to find formulations that contain lipids to improve barrier and mechanical properties, so improving its performance in a way that can compete with classical synthetic packagings.

References

ADLER-NISSEN J. 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. **J. Agric. Food Chem**. 27(6): 1256-1262.

ASTM. 1980. Standard test methods for gas transmission rate of plastic film and sheeting. Method D 3985-81. In **Annual Book of ASTM Standards**. American Society for Testing and Materials. Conshohocken, PA.

ASTM. 1980. Standard test methods for tensile properties of thin plastic sheeting. Method D 882-83. In **Annual Book of ASTM Standards**. American Society for Testing and Materials. Conshohocken, PA.

ASTM. 1980. Standard test methods for water vapor transmission of materials. Method E-96. In **Annual Book of ASTM Standards**. American Society for Testing and Materials. Conshohocken, PA.

BARBER K J and WARTHESEN J J. 1982. Some functional properties of acylated wheat gluten. **J. Agric. Food Chem**. 30: 930-934.

BAUMANN E. 1886. Über eine einfache methode der darstellung von benzoesäureäther. **Berichte** 11: 3218-3222.

DJAGNY K B, WANG Z and XU S. 2001. Chemical modification of pigskin gelatin: factors affecting the esterification of gelatin with fatty acid. **J Food Sci** 66 (9): 1326-1330.

GONTARD N, GUILBERT S and CUQ JL. 1993. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. **J Food Sci** 58 (1): 206-211.

HAQUE Z and KITO M. 1983. Lipophilization of α_{s1}-casein.1.Covalent attachment of palmitoyl residue. **J. Agric. Food Chem**. 31: 1225-1230.

KOELSH R, LASCH J, KLIBANOV A L and TORCHILIN V P. 1981. Incorporation of chemically modified proteins into liposomes. **Acta Biol. Med. Ger**. 40: 331-346.

McHUGH T H. 2000. Protein-lipid interactions in edible films and coatings. **Nahrung** 44: 148-151.

ROUSSEL-PHILIPPE C, PINA M and GRAILLE J. 2000. Chemical lipophilization of soy protein isolates and wheat gluten. **Eur. J. Lipid Sci. Technol**. 2000: 97-101.

SCHOTTEN C. 1844. Über die oxidation des piperidins. **Berichte** 17: 2544-2547.

SMITH J L and YADA R Y. 1991. Lipophilization of *Mucor miehei* aspartyl proteinase: effect on structure-function and stability. **J. Food Biochem**. 30: 481-486.

TANADA-PALMU PS, HELÉN H and HYVÖNEN L. 2000. Preparation, properties and applications of wheat gluten edible films. **Agric Food Sci Finland** 9: 23-35.

TANADA-PALMU PS and GROSSO CRF. 2002a. Wheat gluten composite and bilayer films: effect of lipid addition in **Research Advances in Agriculture & Food Chemistry**, ed. Global Research Network, Kerala, Índia, p. 19-26.

TANADA-PALMU PS and GROSSO CRF. 2002b. Edible wheat gluten films: development, mechanical and barrier properties and application to strawberries (*Fragaria ananassa).* **Boletim do CEPPA** 20 (2): 291-308.

CAPÍTULO 7

DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILMS BASED ON GLUTEN FROM SEMI-HARD AND SOFT BRAZILIAN WHEAT FLOURS

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DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILMS BASED ON GLUTEN FROM SEMI-HARD AND SOFT BRAZILIAN WHEAT FLOURS

(Development of films based on gluten from wheat flours)

Patrícia S.Tanada-Palmu[4] AND Carlos R.F.Grosso[1]*

ABSTRACT

Edible films based on gluten from four types of Brazilian wheat gluten (2 "semi-hard" and 2 "soft") were prepared and mechanical and barrier properties were compared with those of wheat gluten films with vital gluten. Water vapor, oxygen permeability, tensile strength and percent elongation at break, solubility in water and surface morphology were measured. The films from "semi-hard" wheat flours showed similar water vapor permeability and solubility in water to films from vital gluten and better tensile strength than the films from "soft" and vital gluten. The films from vital gluten had higher elongation at break and oxygen permeability than the films from the wheat flours. In spite of the vital gluten showing greater mechanical resistance, for the purpose of developing gluten films, Brazilian "semi-hard" wheat flours can be used instead of vital gluten, since they showed similar barrier and mechanical properties.

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RESUMO

DESENVOLVIMENTO E CARACTERIZAÇÃO DE FILMES COMESTÍVEIS DE GLÚTEN DE FARINHAS SEMI-FORTES E FRACAS DE TRIGOS BRASILEIROS

Filmes a base de glúten de quatro tipos de farinhas de trigo brasileiras (2 "semi-fortes " e 2 "fracas") foram preparados e suas propriedades mecânicas e de barreira foram comparadas com filmes à base de glúten vital (comercial). Permeabilidade ao vapor d'água e oxigênio, resistência à tração, porcentagem de elongação na ruptura, solubilidade em água e morfologia de superfície foram avaliadas. Filmes das farinhas "semi-fortes" mostraram permeabilidade ao vapor d'água e solubilidade em água similares aos filmes de glúten vital e melhor resistência à tração do que os filmes das farinhas "fracas" e glúten vital. O filme de glúten vital apresentou maior elongação na ruptura e permeabilidade ao oxigênio do que os filmes das farinhas brasileiras. Apesar do glúten vital ter uma grande resistência mecânica, para o propósito do desenvolvimento de filmes comestíveis, as farinhas de trigo "semi-duras" brasileiras podem ser usadas ao invés do glúten vital, já que elas mostraram propriedades mecânicas e de barreira similares.

KEY-WORDS – PALAVRAS-CHAVE. Mechanical properties, permeability, vital gluten - Propriedades mecânicas, permeabilidade, glúten vital.

INTRODUCTION

Recently interest in edible films and coatings for foods has grown considerably (Park 1999, Amarante and Banks 2001). Such films can control mass transport between the components of a product, as well as between the product and its surrounding environment (Garcia and others 2000). The kinetics of chemical and enzymatic reactions, microbial growth, texture behavior and the physical stability of foods are all strongly influenced by their moisture contents and this can vary drastically as a result of processing, packaging and shelf life conditions (Gontard and others 1994). Other factors contributing to renewed interest in the development of edible films include consumer demand for high quality foods, environmental concerns over the disposal of non-renewable food packaging materials, and opportunities for creating new market outlets for film-forming ingredients derived from agricultural products.

Polysaccharides, proteins and lipids can be used as edible film-forming agents (Kester and Fennema 1986, Gennadios and Weller 1991, Avena-Bustillos and Krochta 1993, McHugh and others 1994). The use of commercially available proteins for the preparation of edible films has been increased in the past decade (Gennadios and others 1993, Sanches and others 1998). They show good properties in the preparation of biofilms, like the ability to form networks and also plasticity and elasticity. The great advantage of the commercial proteins is that they are easily available. Different kinds of vegetal origin proteins have been used, such as soy proteins (Brandenburg and others 1993), wheat gluten (Gontard and Guilbert 1994), cotton seed protein (Marquie and others 1995), peanuts, corn zein and peas (Guilbert and others 1997, Cuq and others 1998) and whey proteins (Sothornvit and Krochta 2000). Due to the unique cohesive and elastic properties of gluten (Wall & Huebner 1981), good film-forming properties may be expected. Biodegradable and edible films from wheat proteins are useful in food packaging provided they are flexible, strong, heat sealable, and relatively transparent (Rayas and others 1997). The mechanical and barrier properties of wheat gluten films have been studied (Gennadios and others 1993, Gontard and others 1993, Cherian and others 1995). An edible wheat gluten film was developed and the effects of gluten and ethanol concentrations and pH of the film-forming solution on various film properties, were evaluated (Gontard and others 1992). Herald and others (1995) compared wheat gluten films prepared at various pH values, as a result films prepared at pH 3.3 showed higher water vapor permeability, presumibly due to the unfolding conformation of protein molecules and exposure of hydrophilic groups. Edible wheat gluten films were prepared with various amounts of glycerol (Tanada-Palmu and others 2000). Films with low amounts of glycerol had lower water vapor and oxygen permeability, higher tensile strength and lower elongation at break. The absence of studies about edible films from some varieties of wheat flours can justify this work. Our objectives were to develop edible gluten films from four types of Brazilian wheat (2 “semi-hard” and 2 “soft”) and compare them with some functional properties of wheat gluten films prepared with vital gluten, which is the product usually used in research involving edible gluten films.

MATERIALS AND METHODS

Materials

Vital wheat gluten (Rhodia, Campinas, Brazil), glycerol (Merck, Darmstadt, Germany), ammonium hydroxide (Synth, São Paulo, Brazil), calcium chloride (Ecibra, São Paulo, Brazil), sodium chloride (Synth, São Paulo, Brazil), magnesium nitrate (Ecibra, São Paulo, Brazil), solid paraffin (Chemco, São Paulo, Brazil), 2 types of Brazilian “semi-hard” wheat (Embrapa 22 and Embrapa 42) and 2 types of Brazilian “soft” wheat flour (Trigo BR 23 and Embrapa 119) from Embrapa (Rio Grande do Sul, Brazil) were used in this study. “Semi-hard” wheat Embrapa 22 is “harder” than Embrapa 42 and the “soft” wheat Embrapa 119 is “harder” than Trigo BR 23.

Gluten extraction

The wheat was milled in a Brabender mill (Quadrumat Senior, OHG Duisburg, Germany) to obtain the wheat flour. The gluten was extracted from the wheat flour (200 g) by adding distilled water and mixing vigorously to form a mass. This mass was washed exhaustively until the water became transparent, the remaining mass being the gluten. The gluten was dried at 40 ^{0}C in a vacuum oven (20 in Hg) for 24 h, broken up in a blender (Philips, São Paulo, Brazil) and ground in a small Brabender mill (type 881400, OHG Duisburg, Germany) to obtain gluten powder.

Gluten tensile strength and extensibility

The gluten from the “hard” and “soft” wheat flours was prepared in the Glutomatic (Gluten Index type 2200, Perten Instruments, Huddinge, Sweden), according to Smewing (1997). These glutens and the vital gluten were transferred to 50 mL centrifuge tubes, distilled water added (in a quantity sufficient to cover the samples) and the tubes then centrifuged at 2789 g for 5 minutes at room temperature to disrupt any bubbles formed during the process. The gluten was then transferred to a convenient mold and allowed to rest for 40 minutes at 30 ^{0}C. The tensile strength (TSg) and extensibility of the gluten were then measured in the Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK). The probe used was specifically for the gluten extensibility test (dough and gluten extensibility rig) and the cross head speed was 3.3 mm/s. Six samples of each kind of gluten were measured.

Film preparation

The film was prepared from a solution of gluten (7.5 g/100 mL solution), absolute ethanol (45.0 mL/100 mL solution), glycerol (1.12 or 1.50 g/100 mL solution), ammonium hydroxide (to adjust to pH 10.0) and distilled water. All components were mixed and heated using a magnetic stirrer until the temperature of the mixture reached 70 ^{0}C. The solution was then centrifuged at 5856 g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a teflon covered glass surface and dried at room temperature for 24 h (according to the modified method of Gontard and others 1993). The quantity of solution poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for experiments were equilibrated at 52% RH (saturated $Mg(NO_3)_2$ solution in the dessicator) at 25 ^{0}C for 48 h before being tested.

Film thickness

Film thickness was measured using a micrometer (Model MDC-25M, Mitutoyo, MFG, Japan). The thickness of individual film samples was determined as a random average of five measurements.

Water vapor permeability

The water vapor transmission rate of the films was determined gravimetrically at 25 ^{0}C using a modified American Society for Testing and Materials Standard Method E-96 (ASTM 1980) and the water vapor permeability (WVP) was calculated according to this method. The samples, triplicates of each film, were conditioned for 2 days at 52% RH before measurement. The relative humidity gradient of the test was 0% RH ($CaCl_2$ desiccant in the test cup) and 75% RH (saturated NaCl solution in the desiccator) on both sides of the film.

Oxygen permeability

The oxygen transmission rates were determined using a modification of the ASTM Standard Method D 3985-81 (ASTM 1980) with an Ox-Tran apparatus (Mocon, Inc., Minneapolis, USA) at dry condition and 25 ^{0}C. The samples, duplicates of each film, were conditioned for 2 days at 52% RH before measurement. The oxygen permeability (OP) was calculated by dividing the oxygen transmission rate by the oxygen pressure and multiplying by the mean thickness.

Tensile strength and percent elongation at break

Film tensile strength (TS) and percent elongation at break (ELO) were determined using a Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), operated according to the ASTM (ASTM 1980) Standard Method D 882-83 (initial grip separation = 50 mm and cross head speed = 100 mm/min). Six specimens (100 mm long and 25.4 mm wide) of each film were measured. The peak loads and extension at break were recorded for tested film specimens. The tensile strength and percent elongation at break were calculated according to the ASTM method.

Solubility in water

The percentage of initial dry matter of each film was determined at 105 ^{0}C for 24 h. Two discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water and slowly and periodically agitated for 24 h at 25 ^{0}C. The pieces of film were then taken out and dried (105 ^{0}C for 24 h) to determine the weight of dry matter not solubilized by the water (Gontard and others 1993).

Scanning electron microscopy

Film samples were examined for surface characteristics using a scanning electron microscope (Jeol, JMS-T330, Tokyo, Japan) operating at 10 kV. Film samples were left in a desiccator with silica gel for 7 days, then fraturated and affixed to aluminum stubs with cupper tape. The samples were then sputter-coated with gold in a Balzers evaporator (SDC 050, Baltec, Lichtenstein) for 180 seconds at 40 mA.

Statistical analyses

The Statistica 5.5 (StatSoft, USA) program was used to calculate the analysis of variance (ANOVA) and a Tukey test was used to determine the significant differences of all film properties at a 95% confidence interval.

RESULTS AND DISCUSSION

Gluten

Gluten tensile strength and extensibility

This test was carried out to investigate the resistance of the gluten, thus comparing the gluten tensile strength (TSg) of the vital (commercial) gluten and that of the glutens extracted from the wheat flours. The TSg of the gluten from the "soft" wheat flours (Trigo BR 23 and Embrapa 119) was indeed significantly lower than that of the gluten from the "semi-hard" wheat flours (Embrapa 22 and Embrapa 42) as can be seen in Table 1. However, there was no significant difference in TSg between the two types of "semi-hard" wheat flours, or between the "soft" flours. The TSg of the vital gluten was very high (279.50 g), showing significant differences from the other glutens tested, which could indicate that this gluten was obtained from a "hard" wheat flour. On the other hand, its extensibility (4.82 cm) was lower than the gluten from the "semi-hard" wheat flours (Embrapa 22 and Embrapa 42) and gluten from Trigo BR 23 (7.64 cm). The gluten from Embrapa 22 and Embrapa 42 wheat flours had high extensibility (12.23 and 9.29 cm, respectively) and Embrapa 119 had the lowest extensibility (1.90 cm). These mechanical characteristics are important when the gluten is used in bakery products.

Table 1 – Properties of resistance and extensibility of gluten

Type of gluten	Tensile strength (g)*	Extensibility (cm)*
Vital gluten	279.50 ± 26.78^{a}	4.82 ± 0.68^{b}
Embrapa 22	69.46 ± 3.08^{b}	12.23 ± 1.45^{a}
Embrapa 42	58.13 ± 3.03^{b}	9.29 ± 1.34^{a}
Trigo BR 23	21.38 ± 6.06^{c}	7.64 ± 2.92^{a}
Embrapa 119	22.17 ± 4.30^{c}	1.90 ± 0.29^{c}

*Mean and standard deviation of six replicates. $^{a\text{-}c}$ Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

Films

Water vapor permeability

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 and others 1992). The films prepared with gluten extracted from both "soft" wheat flours (Trigo BR 23 and Embrapa 119) with a higher concentration of glycerol, showed higher values for WVP (15.37 and 14.65 gmm/m^{2}dKPa) as compared to films from vital gluten (11.35 gmm/m^{2}dKPa) and from the "semi-hard" wheat flours Embrapa 22 and Embrapa 42 (9.02 and 11.89 gmm/m^{2}dKPa, respectively), as shown in Table 2. As the glycerol concentration decreased from 25 to 15%, a decrease in the WVP values was observed, in agreement with other researchers (Gontard and others 1993, Tanada-Palmu and others 2000). According to Banker (1966), plasticizers are added to films to reduce brittleness, increase toughness, strength, tear and impact resistance and impart flexibility. Usually, the addition of a plasticizer increase the permeability of gas, water vapor and solute and decreases the tensile strength of the films.

For the films with 15% glycerol, the WVP values were in the following order (Table 2): vital gluten < Embrapa 22 ("semi-hard") < Embrapa 42 ("semi-hard") < Trigo BR 23 ("soft") < Embrapa 119 ("soft"). Although the film from vital gluten had the lowest WVP, there were no significant differences ($P<0.05$) between the other films with 15% glycerol. The gluten films from Brazilian “semi-hard” wheat flours showed better barrier to water vapor properties than films from “soft” flours, and similar efficiency to those from vital (commercial) gluten.

Solubility in water

Water resistance is an important property of edible films for applications in food protection, where water activity is high, or when the film must be in contact with water during processing of the coated food, to avoid exudation of fresh or frozen products. Therefore, edible films with high water solubility may be required, for example, to contain pre-measured portions, which will be dissolved in water or in hot food (Guilbert and Biquet 1989). For all the films tested, the highest solubility in water was observed when the higher concentration (25%) of glycerol was used. Films with glutens from the “soft” flours and 25% glycerol showed higher values for solubility in water and the film with vital gluten had the lowest value. For all the films with 15% glycerol, the values for solubility in water were not significantly different ($P<0.05$) and the lowest value was observed for the film with gluten from Embrapa 42 wheat flour (“semi-hard”).

Oxygen permeability

Good oxygen barrier properties of edible films are desired in food packaging and preservation. As an example, coating foods susceptible to lipid oxidation with protein films, in combination with an external, conventional moisture barrier package seems to be possible. Oxygen permeability of the films from wheat flour with the lower concentration of glycerol could not be measured because they were too brittle and cracked in the equipment. The film with gluten from Embrapa 22 “semi-hard” wheat flour had the lowest oxygen permeability (24.26 $cm^3\mu m/m^2dkPa$) of all the films tested. Oxygen permeability values (Table 2) of all the wheat gluten films tested (24.26-39.67 $cm^3\mu m/m^2dkPa$) were similar to the values obtained by Park and Chinnan (1990) for zein:glycerin films (13.0-44.9 $cm^3\mu m/m^2dkPa$) and gluten:glycerin films (9.6-24.2 $cm^3\mu m/m^2dkPa$) at 30 ^{0}C and 0% RH and was low compared to polysaccharide-based edible films (149.0-910.0 $cm^3\mu m/m^2dkPa$) and non-edible films like high density polyethylene (427 $cm^3\mu m/m^2dkPa$), maybe due to their polar nature and linear structure, which leads to a high cohesive energy density and a low free volume (Tanada-Palmu and others 2000).

Table 2 – Water vapor and oxygen permeability and solubility in water of films from vital gluten and gluten extracted from wheat flour ("semi-hard" and "soft")

Film	Water vapor permeability (gmm/m^2dKPa)*	Solubility in water (%)*	Oxygen permeability ($cm^3\mu m/m^2dKPa$)*
Trigo BR23 25% glycerol	15.37 ± 1.10^{a}	71.1 ± 7.1^{a}	34.30 ± 1.25^{ab}
Embrapa 119 25% glycerol	14.65 ± 0.97^{ab}	55.8 ± 6.1^{ab}	35.14 ± 2.43^{a}
Embrapa 22 25% glycerol	9.02 ± 2.54^{bcd}	36.8 ± 1.8^{bcd}	24.26 ± 3.22^{b}
Embrapa 42 25% glycerol	11.89 ± 1.24^{abc}	39.3 ± 5.4^{bc}	36.88 ± 5.70^{a}
Vital gluten 25% glycerol	11.35 ± 0.35^{abc}	19.7 ± 0.6^{cd}	39.67 ± 1.41^{a}
Trigo BR23 15% glycerol	8.70 ± 1.02^{cd}	31.4 ± 0.8^{cd}	-
Embrapa 119 15% glycerol	9.56 ± 0.14^{bcd}	22.4 ± 1.0^{cd}	-
Embrapa 22 15% glycerol	7.29 ± 0.82^{cd}	23.4 ± 0.9^{cd}	-
Embrapa 42 15% glycerol	7.82 ± 0.16^{cd}	16.8 ± 0.3^{d}	-
Vital gluten 15% glycerol	5.72 ± 0.03^{d}	17.4 ± 0.2^{d}	35.82 ± 2.87^{a}

*Mean and standard deviation of replicates. $^{a\text{-}d}$ Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

Mechanical properties

An edible film should be resistant in order to withstand manipulation during its application and to maintain its integrity and also its barrier properties. Table 3 shows that films prepared with glutens from "semi-hard" wheat flours (Embrapa 22 and Embrapa 42) and low concentrations of glycerol had greater tensile strength (7.80 and 10.89 MPa, respectively) than films from other wheat flours (2.00 and 3.71 MPa for Trigo BR 23 and Embrapa 119, respectively), vital gluten (2.36 MPa) and gluten:glycerol (2.5:1) films from Gennadios and others 1993 (2.60 MPa), showing that films from this wheat flour had better tensile strength than the other films. The films from one of the "semi-hard" wheat flours (Embrapa 42) showed a higher percent elongation at break (184.20%) when compared to the films from the other Brazilian wheat flours (2.21-25.50%). All films with low amounts of glycerol had higher tensile strength and lower percent elongation at break, in agreement with Tanada-Palmu and others (2000). This similar behavior has been reported for other films (Park and others 1993, McHugh and Krochta 1994, Gennadios and others 1996). Films with vital gluten and both concentrations of glycerol (15 and 25%) showed significantly higher percent elongation at break than the films made with gluten from Brazilian wheat flours.

Table 3 – Mechanical properties of films from vital gluten and gluten extracted from wheat flour (“semi-hard” and “soft”)

Film	Tensile strength (MPa)*	Elongation at break (%)*
Trigo BR23 25% glycerol	0.78 ± 0.23^{d}	24.20 ± 0.25^{b}
Embrapa 119 25% glycerol	0.91 ± 0.06^{cd}	25.50 ± 0.92^{b}
Embrapa 22 25% glycerol	1.39 ± 0.96^{cd}	13.96 ± 1.12^{b}
Embrapa 42 25% glycerol	2.41 ± 0.23^{cd}	184.20 ± 1.15^{a}
Vital gluten 25% glycerol	1.22 ± 0.47^{cd}	220.30 ± 1.56^{a}
Trigo BR23 15% glycerol	2.00 ± 0.28^{cd}	3.50 ± 2.00^{b}
Embrapa 119 15% glycerol	3.71 ± 0.67^{c}	14.00 ± 3.00^{b}
Embrapa 22 15% glycerol	7.80 ± 1.07^{b}	2.21 ± 1.56^{b}
Embrapa 42 15% glycerol	10.89 ± 1.56^{a}	4.81 ± 0.99^{b}
Vital gluten 15% glycerol	2.36 ± 0.02^{cd}	184.10 ± 8.40^{a}

*Mean and standard deviation of replicates (relative humidity of 52%). $^{a-d}$ Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

Scanning electron microscopy

The micrographs obtained from the film surfaces are presented in Figs. 1, 2 and 3. The use of scanning electron microscopy to observe the morphology of the surface of the film was efficient in evaluating the films containing mixtures of polysaccharide-fatty acids or polysaccharide-beeswax and their functional properties (Yang and Paulson, 2000). In this case for films from different glutens, it was not possible to observe any significant morphological difference between “semi-hard” Embrapa 22 (Fig. 1) and vital gluten (Fig. 3), but the “soft” gluten film showed a more open matrix surface morphology which could be capable of explaining functional differences such as the worst barrier properties or mechanical resistance compared to the other films.

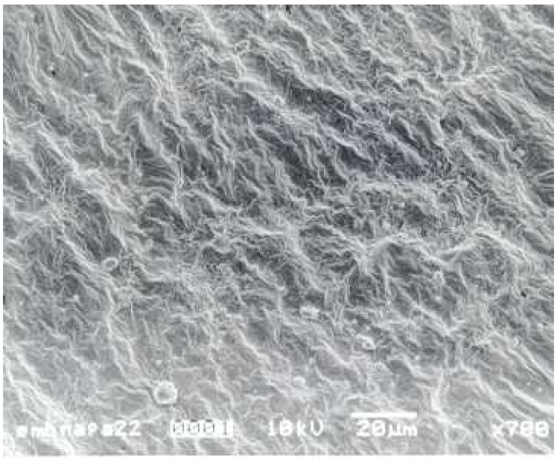

Figure 1 – Micrograph of the film from a “semi-hard” gluten (Embrapa 22)

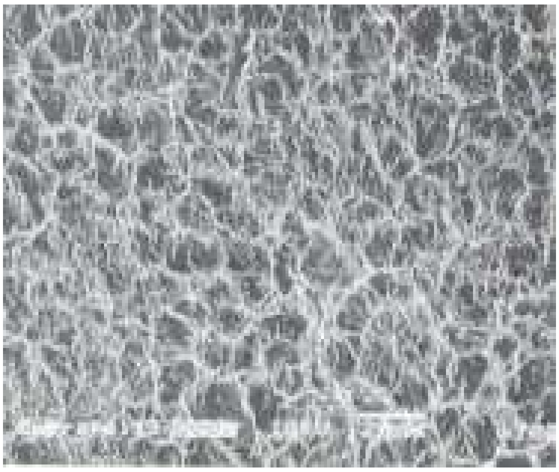

Figure 2 - Micrograph of the film from a “soft” gluten (Embrapa 119)

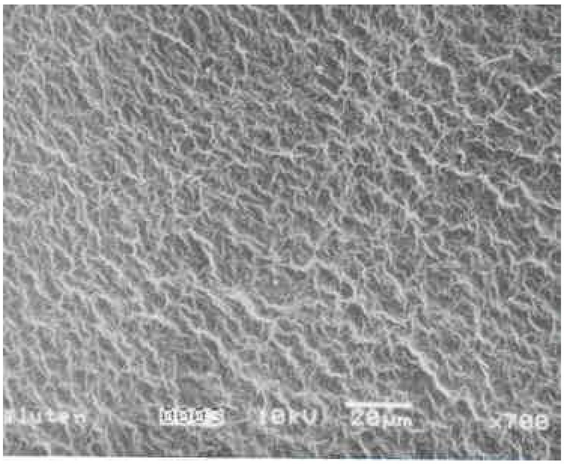

Figure 3 – Micrograph of the film from the vital gluten

CONCLUSIONS

Wheat gluten is a good source of protein-based biodegradable films. There was no significant difference between the gluten films obtained from the two types of Brazilian "semi-hard" wheat flours or between those obtained from the two "soft" flours. Films prepared with glutens from "semi-hard" wheat flour and the lower concentration of glycerol showed better properties as compared to films from the "soft" wheat flour. Brazilian "semi-hard" wheat flours can be used to develop edible gluten films instead of vital (commercial) gluten, since they showed similar barrier and mechanical properties. The possibility of improving the wheat gluten film properties by promoting crosslinking via enzymatic and chemical protein treatments, should be explored.

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REFERENCES

Amarante C and Banks N H. 2001. Postharvest physiology and quality of coated fruits and vegetables. *Horticultural Reviews* 26: 161-238.

ASTM. 1980. Standard test methods for gas transmission rate of plastic film and sheeting. Method D 3985-81. In *Annual Book of ASTM Standards*. American Society for Testing and Materials. Conshohocken, PA.

ASTM. 1980. Standard test methods for tensile properties of thin plastic sheeting.

Method D 882-83. In *Annual Book of ASTM Standards*. American Society for Testing and Materials. Conshohocken, PA.

ASTM. 1980. Standard test methods for water vapor transmission of materials. Method E-96. In *Annual Book of ASTM Standards*. American Society for Testing and Materials. Conshohocken, PA.

Avena-Bustillos RJ and Krochta JM. 1993. Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. *J Food Sci* 58 (4): 904-907.

Brandenburg AH, Weller CL, Testin RF. 1993. Edible films and coatings from soy proteins. *J Food Sci* 58: 1086-1089.

Banker GS. 1966. Film coating theory and practice. *J Pharmacy Sci* 55: 81-89.

Cuq B, Gontard N and Guilbert S. 1998. Protein as agricultural polymers for packaging production. *Cereal Chem* 75: 1-9.

Cherian G, Gennadios A, Weller CL and Chinachoti P.1995. Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. *Cereal Chem* 72: 1-6.

Garcia MA, Martino MN and Zaritzki NE. 2000. Barrier properties of edible starch-based films and coatings. *J Food Sci* 65 (6): 941-947.

Gennadios A and Weller CL. 1991. Edible films and coatings from soymilk and soy protein. *Cereal Foods World* 36: 1004-1009.

Gennadios A, Brandenburg AH, Weller CL and Testin RF. 1993. Effect of pH on properties of wheat gluten and soy protein isolate films. *J Agric Food Chem* 41: 1835-1839.

Gennadios A, Weller CL, Hanna MA and Froning GW. 1996. Mechanical and barrier properties of egg albumen films. *J Food Sci* 61 (3): 585-589.

Gontard N, Guilbert S and Cuq JL. 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *J Food Sci 57* (1): 190-199.

Gontard N, Guilbert S and Cuq JL. 1993. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J Food Sci 58* (1): 206-211.

Gontard N, Duchez C, Cuq JL and Guilbert S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. *Intern J Food Sci Tech* 29: 39-50.

Gontard N and Guilbert S. 1994. Edible wheat gluten films: optimization of the main process variables and improvement of water vapor barrier properties by combining gluten proteins with lipids. *Dev Food Eng Proc* 2: 790-972.

Guilbert S. 1986. Technology and application of edible protective films. In *Food Packaging and Preservation*. Mathlouthi M, Editor. p. 371-394. Elsevier Applied Science Publishers. London UK.

Guilbert S, Cuq B and Gontard N. 1997. Recent innovations in edible and/or biodegradable packaging materials. *Food Addit Contam* 14: 741-751.

Herald TJ, Gnanasambandam R, McGuire BH and Hachmeister KA. 1995. Degradable wheat gluten films: preparation, properties and aplications. *J Food Sci* 60 (5): 1147-1150, 1156.

Kester JJ and Fennema O. 1986. Edible films and coatings: A review. *Food Tech* 40 (12): 47-59.

Marquie C, Aymard C, Cuq, JL and Guilbert S. 1995. Biodegradable packaging made from cottonseed flour: formation and improvement by chemical treatments with gossipol, formaldehyde, and glutaraldehyde. *J Agric Food Chem* 43: 2762-2767.

McHugh TH and Krochta JM. 1994. Sorbitol vs glycerol plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. *J Agric Food Chem* 42 (4): 841-845.

McHugh TH, Aujard JF and Krochta JM. 1994. Plasticized whey protein edible films: water vapor permeability properties. *J Food Sci* 59: 416-419, 423.

Park HJ and Chinnan MS. 1990. Properties of edible coatings for fruits and vegetables . *American Society of Agriculture Engineers Meeting Presentation*, Paper 90-6510, St. Joseph, MI.

Park HJ, Weller CL, Vergano PJ and Testin, RF. 1993. Permeability and mechanical properties of cellulose-based edible films. *J Food Sci* 58: 1361-1364.

Park H J 1999 Development of advanced edible coatings for fruits. *Trends Food Sci Tech* 10: 254-260.

Rayas LM, Hernandez, RJ and NG PKW. 1997. Development and characterization of biodegradable/edible wheat protein films. *J Food Sci* 62(1): 160-162, 189.

Sanches AC, Popineau Y, Mangavel C, Larré C and Gueguen J. 1998. Effect of different plasticizers on the mechanical and surface properties of wheat gliadin films. *J Agric Food Chem* 46: 4539–4544.

Smewing. 1997. Analyzing the texture of pasta for quality control. *Cereal Foods World* 42 (1): 8-12.

Sothornvit R and Krochta JM. 2000. Water vapor permeability and solubility of films from hydrolyzed whey protein. *J Food Sci* 65 (4): 700-703.

Tanada-Palmu PS, Helén H and Hyvönen L. 2000. Preparation, properties and applications of wheat gluten edible films. *Agric Food Sci Finland* 9: 23-35.

Wall JS and Huebner FR. 1981. Adhesion and cohesion. In *Protein Functionality in Foods*. J.P. Cherry, Editor. p.111. American Chemical Society, Washington, D.C.

Yang L and Paulson AT. 2000. Effect of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Res Inter* 33: 571-578.

CONCLUSÕES GERAIS

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1) As concentrações de glúten, glicerol e etanol influenciaram a permeabilidade ao oxigênio e as propriedades mecânicas dos filmes a base de glúten de trigo. Um dos filmes mais resistente (filme 4, com TS=5,25 MPa) foi o com maior concentração de glúten (9,0 g/100 mL) e menor de glicerol (1,50 g/100 mL). Esta formulação de filme é a que foi escolhida para o trabalho da adição de lipídios e também para a cobertura dos morangos, por apresentar a maior resistência mecânica entre os filmes testados e baixa permeabilidade ao vapor d'água (8,61 gmm/m^2dkPa). A menor permeabilidade ao oxigênio (21,03 $cm^3\mu m/m^2dkPa$) foi obtida no filme 2, com menor concentração de glúten (6,0 g/100 mL), glicerol (1,50 g/100 mL) e etanol (32,5 mL/100 mL).

2) A permeabilidade ao vapor d'água do filme simples de glúten (8,61 gmm/m^2dkPa) pôde ser melhorada com a adição de lipídios, o que foi observada tanto no filme composto (1,65 gmm/m^2dkPa) como no de duas camadas (1,01 gmm/m^2dkPa). Porém, a adição de lipídios tornou os filmes opacos e com propriedades mecânicas (elongação menor que 10%) inferiores aos filmes simples. A morfologia da superfície observada através do microscópio eletrônico de varredura para o filme simples de glúten foi completamente modificada quando se adicionou a mistura de ácidos palmítico e esteárico e cera de abelha.

3) A aplicação dos biofilmes e coberturas à base de glúten de trigo em morangos aumentou sua vida-de-prateleira e retardou seu processo de senescência. A cobertura de duas camadas com glúten e lipídios teve efeito na retenção da firmeza e na diminuição da perda de peso nas frutas cobertas.

4) A análise sensorial dos morangos indicou que as coberturas simples de glúten e composta com lipídios mantiveram a qualidade dos morangos durante o tempo de armazenamento em termos de aparência e que o sabor

dos morangos com a cobertura simples de glúten foi aceito pelos consumidores.

5) A reação de lipofilização modificou o glúten de trigo (como visto pela diminuição de 32,5% dos grupos amino livres no glúten lipofilizado), porém o filme preparado com o glúten lipofilizado apresentou propriedades mecânicas (TS=1,37 MPa) e de barreira (WVP=11,05 gmm/m^2dkPa) inferiores ao filme com glúten sem modificação (TS=8,61 MPa e WVP=8,61 gmm/m^2dkPa).

6) Com relação aos filmes de glúten de trigo obtidos das farinhas brasileiras, observou-se que os filmes produzidos das farinhas “semi-duras” apresentaram propriedades mecânicas e de barreira similares aos filmes de glúten vital (comercial) e superiores aos filmes das farinhas “fracas”.

SUGESTÕES

1) Maior esforço na pesquisa fundamental para melhor entender os mecanismos de transferência de solutos e voláteis através de polímeros de origem agrícola, como os filmes e coberturas comestíveis.

2) Estudar a formulação de filmes ou coberturas comestíveis em escala comercial.

3) Mais estudos de aplicação dos biofilmes e coberturas em alimentos, como frutas frescas e minimamente processadas, separação de um recheio de uma massa, queijos, etc.

ANEXO 1

ANÁLISE SENSORIAL DE MORANGOS COBERTOS COM FILMES E COBERTURAS A BASE DE GLÚTEN DE TRIGO

Poster aceito para o 5th Pangborn Sensory Science Symposium – Boston – USA, julho 2003.

ANÁLISE SENSORIAL DE MORANGOS COBERTOS COM FILMES E COBERTURAS A BASE DE GLÚTEN DE TRIGO

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Resumo: Trabalhos recentes estão explorando o uso de filmes comestíveis na extensão da vida de prateleira e na manutenção da qualidade de produtos frescos. Filmes comestíveis podem prover uma alternativa na extensão da vida pós-colheita de frutas frescas. O objetivo desse trabalho foi o estudo da habilidade de coberturas comestíveis à base de glúten de trigo em estender a vida de prateleira de morangos através da análise sensorial de aparência durante um tempo de armazenamento e também verificar a aceitação pelos provadores do sabor das frutas cobertas. Os atributos avaliados na análise sensorial de vida de prateleira foram: aparência global, cor, brilho e intenção de compra e para a aceitação do sabor foram: aroma, sabor, textura e intenção de compra. Dos resultados da análise sensorial de vida de prateleira, foi observado que as coberturas de glúten e composta efetivamente aumentaram a vida de prateleira e mantiveram a qualidade das frutas quando comparada às frutas controle. Dos resultados da análise sensorial de degustação, a cobertura de glúten não afetou o sabor nem o aroma dos morangos. Os provadores não aceitaram nem a aparência e nem o sabor da cobertura de duas camadas.

INTRODUÇÃO

O sucesso de um produto alimentício no mercado está diretamente ligado à aceitação do mesmo pelo consumidor, o que também dita os seus padrões de qualidade (MUÑOZ et al., 1992). De acordo com FU & LABUZA (1993), a vida de prateleira de um alimento, nos últimos anos, têm sido determinada através de testes de aceitação, onde, segundo LABUZA & SCHMIDL (1988) apud CLEMENTE (1998), o parâmetro avaliado é a queda da aceitação do produto durante o armazenamento, medida através de uma escala hedônica de nove pontos que avalia o quanto o consumidor "gosta" ou "desgosta" do produto nos diferentes tempos de armazenamento. Para esses mesmos autores, a determinação da vida útil de produtos alimentícios é obtida pela recusa de compra de um produto, em função das suas características sensoriais.

A avaliação da qualidade e aceitação do produto estão diretamente ligadas à cor e a aparência dos alimentos (LITTLE, 1975). Esta última, freqüentemente, influencia a atitude de compra do mesmo (BAARDESTH et al., 1988).

O objetivo deste trabalho foi a determinação da vida de prateleira de morangos e sua aceitação quanto as propriedades sensoriais através da análise sensorial. Os atributos sensoriais avaliados neste trabalho incluem: aparência global, cor, brilho e intenção de compra, avaliados de seis em seis dias durante os dezesseis dias de estocagem, além da degustação das amostras, realizada no 6^{o} dia de armazenamento, onde os atributos sensoriais avaliados foram: aroma, sabor, textura e intenção de compra, para as amostras cobertas, lavadas e para a fruta controle.

MATERIAIS E MÉTODOS

Os materiais e a metodologia da análise sensorial dos morangos já foram descritos anteriormente no capítulo 5 - "Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality".

RESULTADOS E DISCUSSÃO

Todas as amostras de morango foram bem aceitas no dia 1 de análise sensorial, com exceção da fruta coberta com 2 camadas (glúten e lipídios), onde 70% dos provadores aferiram notas de 1-3. Já as outras amostras, incluindo a fruta controle e as demais coberturas (glúten e composta), obtiveram mais de 80% dos provadores dando notas entre 7-9, quanto à aparência global da amostra (Fig. 1). Pode se concluir que o brilho adicional dado pelas coberturas aos morangos agradaram os provadores, onde 20% a mais dos provadores deram notas superiores a 7 para esse parâmetro (Fig. 3).

Já no dia 6 de análise, as frutas cobertas (glúten e composta) continuaram a ser bem aceitas com mais de 72,5% dos provadores gostando das amostras (notas de 7-9) em relação à aparência global, porém houve uma diminuição da aceitação da fruta controle, onde 65% dos provadores gostaram da amostra.

A fruta controle foi rejeitada no dia 12 de análise (Fig. 4), já que 87,5% dos provadores não comprariam mais essa amostra (notas 1-2), além de não terem gostado da cor (60% dos provadores com notas 1-3, Fig. 2) e nem do brilho (75%). As outras frutas cobertas (glúten e composta) ainda continuaram a ter boa aceitação.

No dia 16 de análise, a amostra coberta com glúten teve uma diminuição na aceitação com relação ao brilho, onde somente 45% dos provadores deram notas superiores a 7, e com relação à intenção de compra (55% dos provadores com notas 1-2).

Durante todo o armazenamento, a amostra preferida pelos provadores foi a composta, já que foi bem aceita pelos provadores em todos os parâmetros estudados, tendo a maior porcentagem de provadores que aferiram notas superiores a 7. A amostra com 2 camadas não foi aceita pelos provadores em todas as análises, já que 82,5% dos provadores não comprariam essa amostra (notas 1-2 na intenção de compra) desde o dia 1 de análise e essa porcentagem pouco alterou com o tempo de análise.

Com relação à análise sensorial de degustação dos morangos (Fig. 5), os morangos controle foram mais bem aceitos em todos os parâmetros do que as frutas cobertas, com mais de 70% dos provadores aferindo notas 7-9. Porém as frutas cobertas com glúten também foram bem aceitas.

Pelas fotos dos morangos durante a análise sensorial de aparência (Figs. 6-9), comprova-se os resultados dos provadores, ou seja, o morango controle no dia 12 foi rejeitado, já que apresentava a cor vermelha “passada” e o tamanho bem menor do que as outras frutas. Os morangos com cobertura de glúten tinham boa aparência até o dia 16, assim como os morangos com cobertura de 2 camadas (“bilayer”).

As fichas da análise sensorial de aparência (Fig. 10) e de degustação (Fig. 11) encontram-se abaixo.

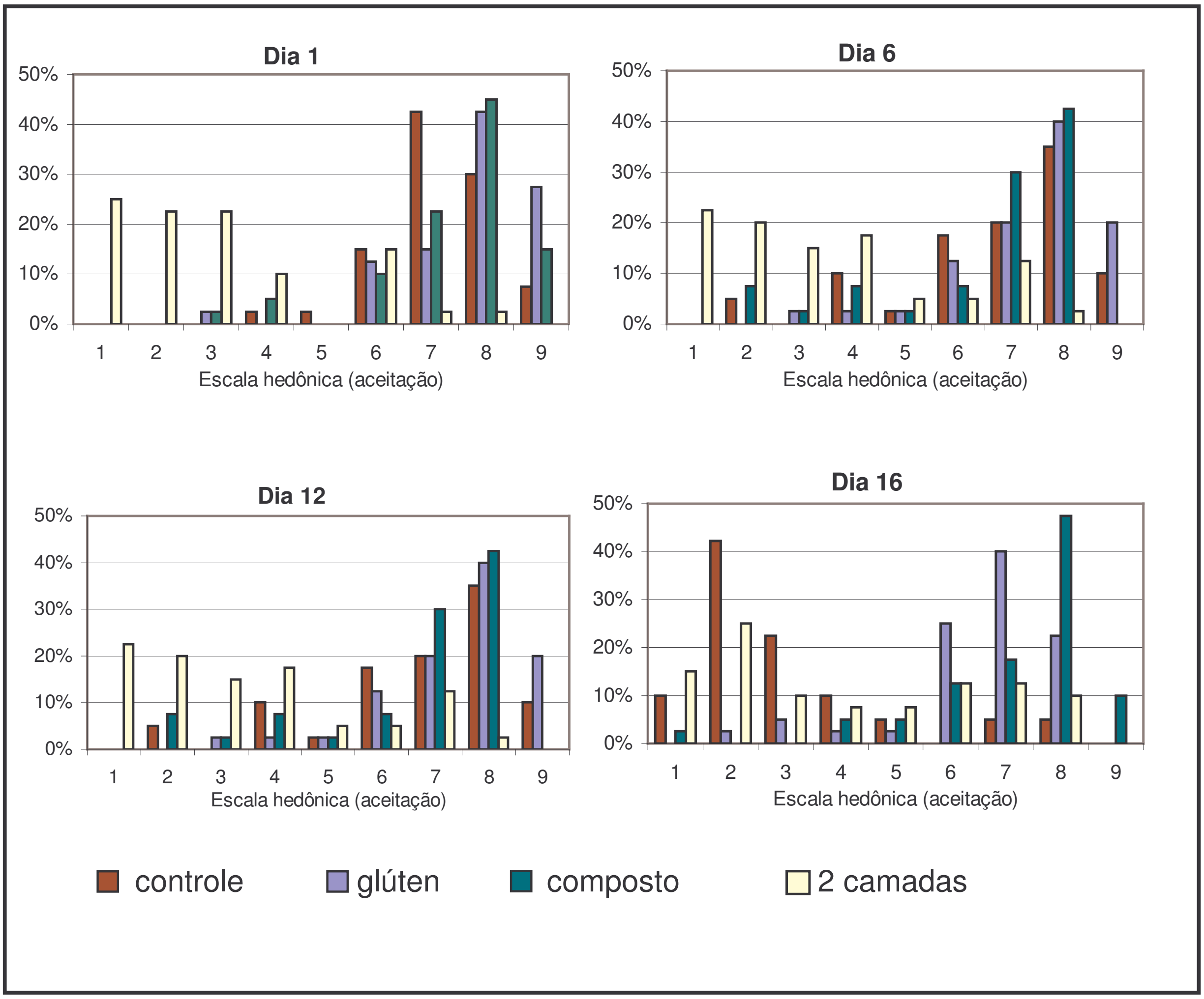


Figura 1 – Aparência global dos morangos durante o armazenamento.

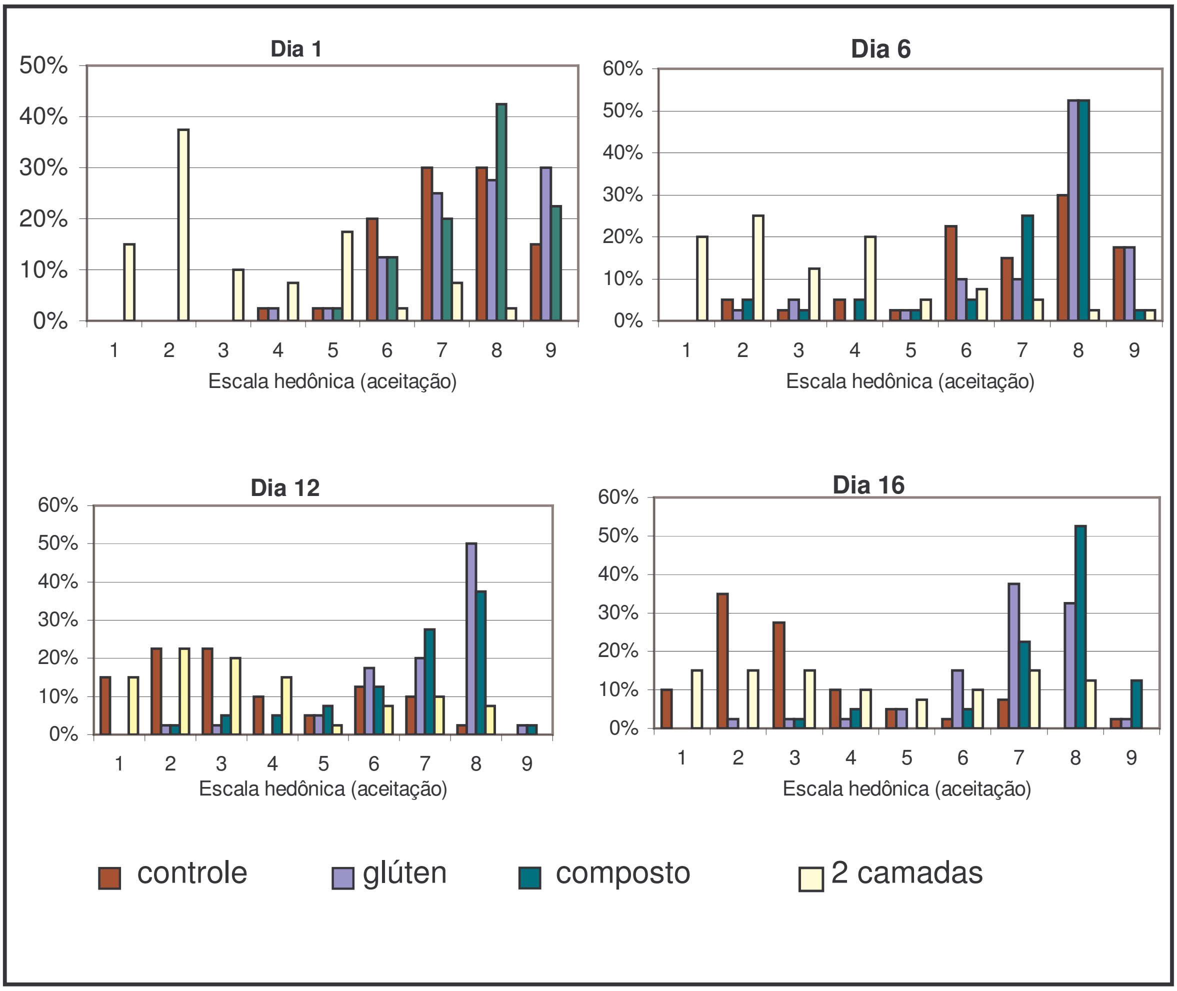


Figura 2 – Cor dos morangos durante o armazenamento.

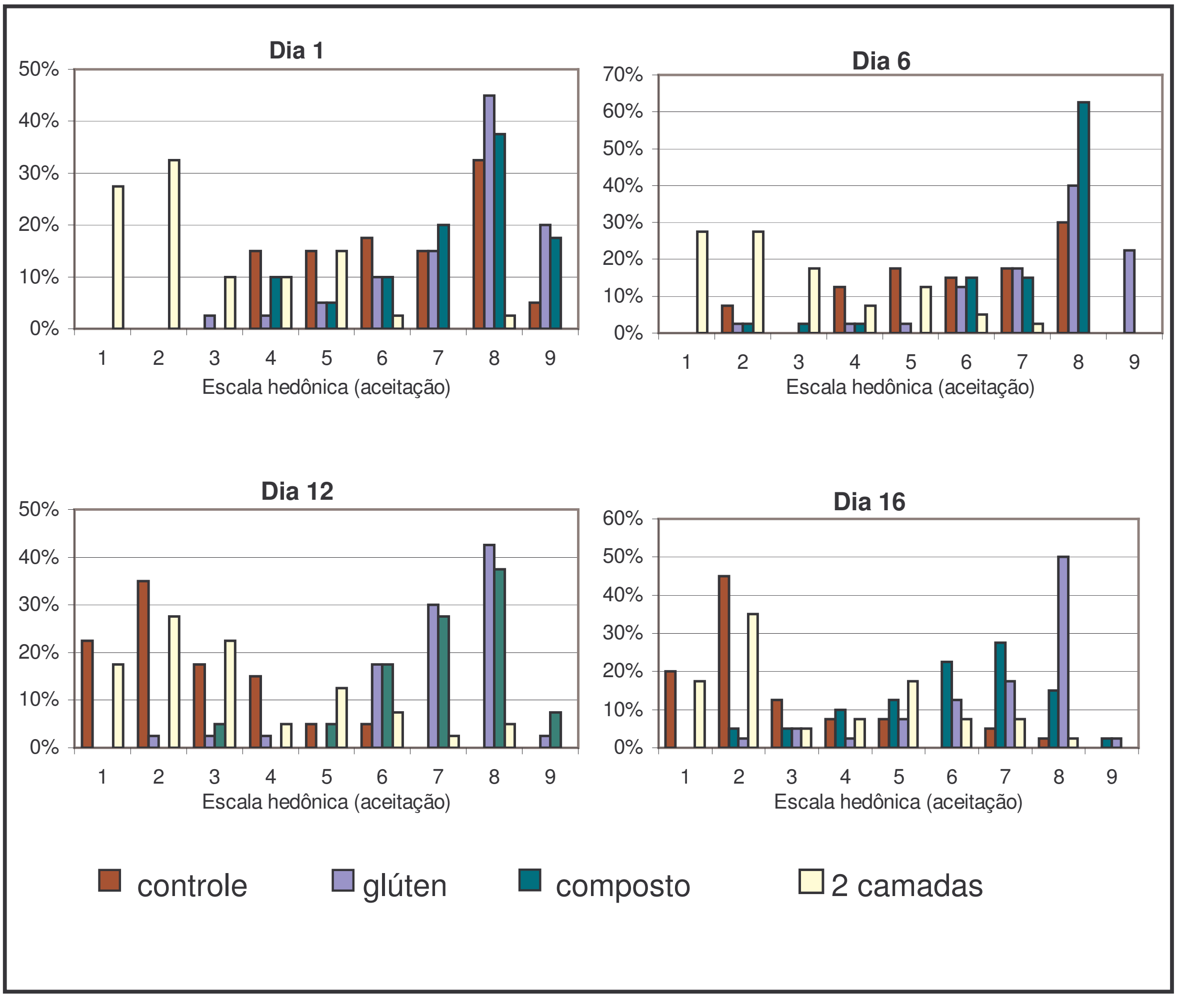


Figura 3 – Brilho dos morangos durante o armazenamento.

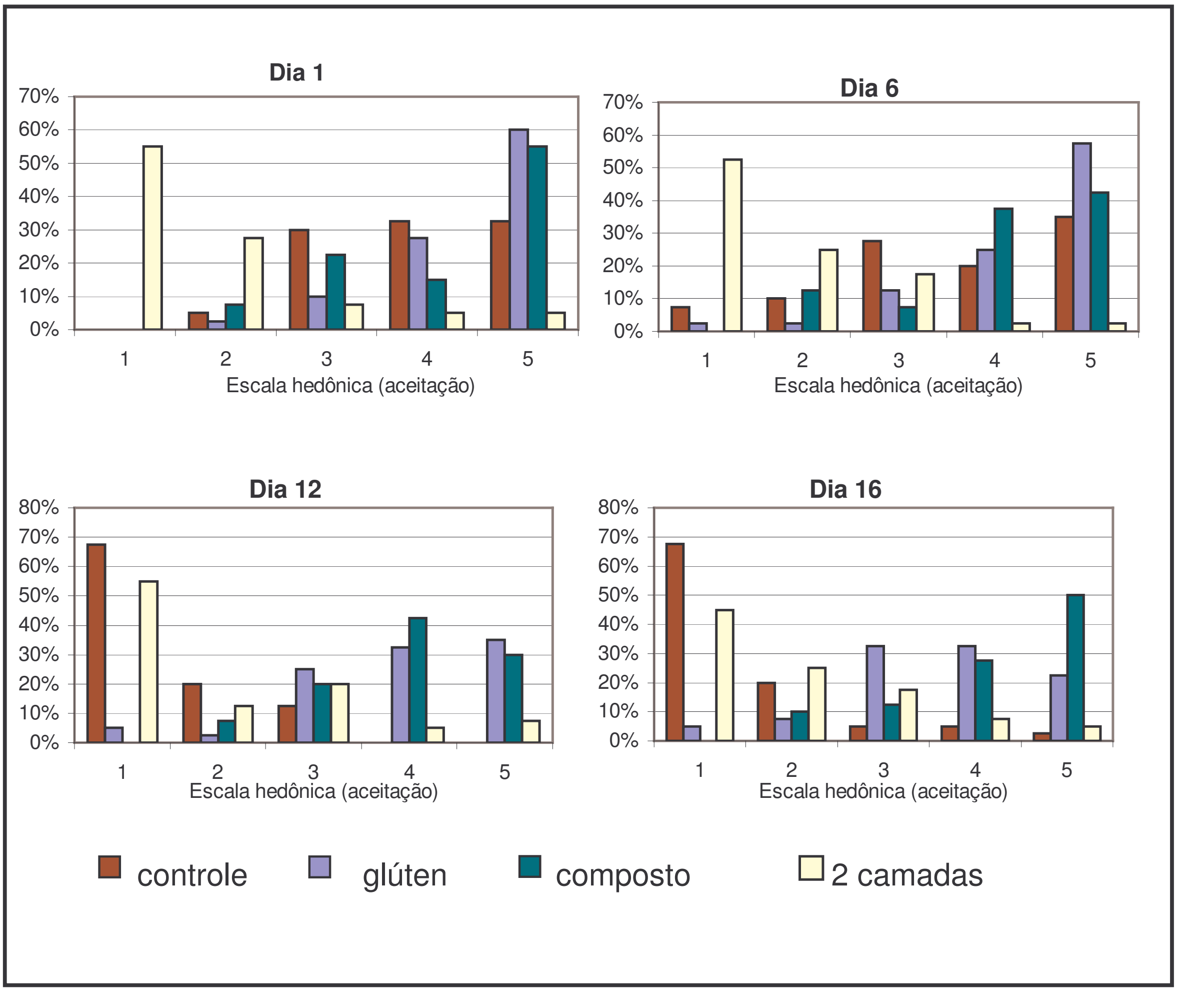


Figura 4 – Intenção de compra dos morangos durante o armazenamento.

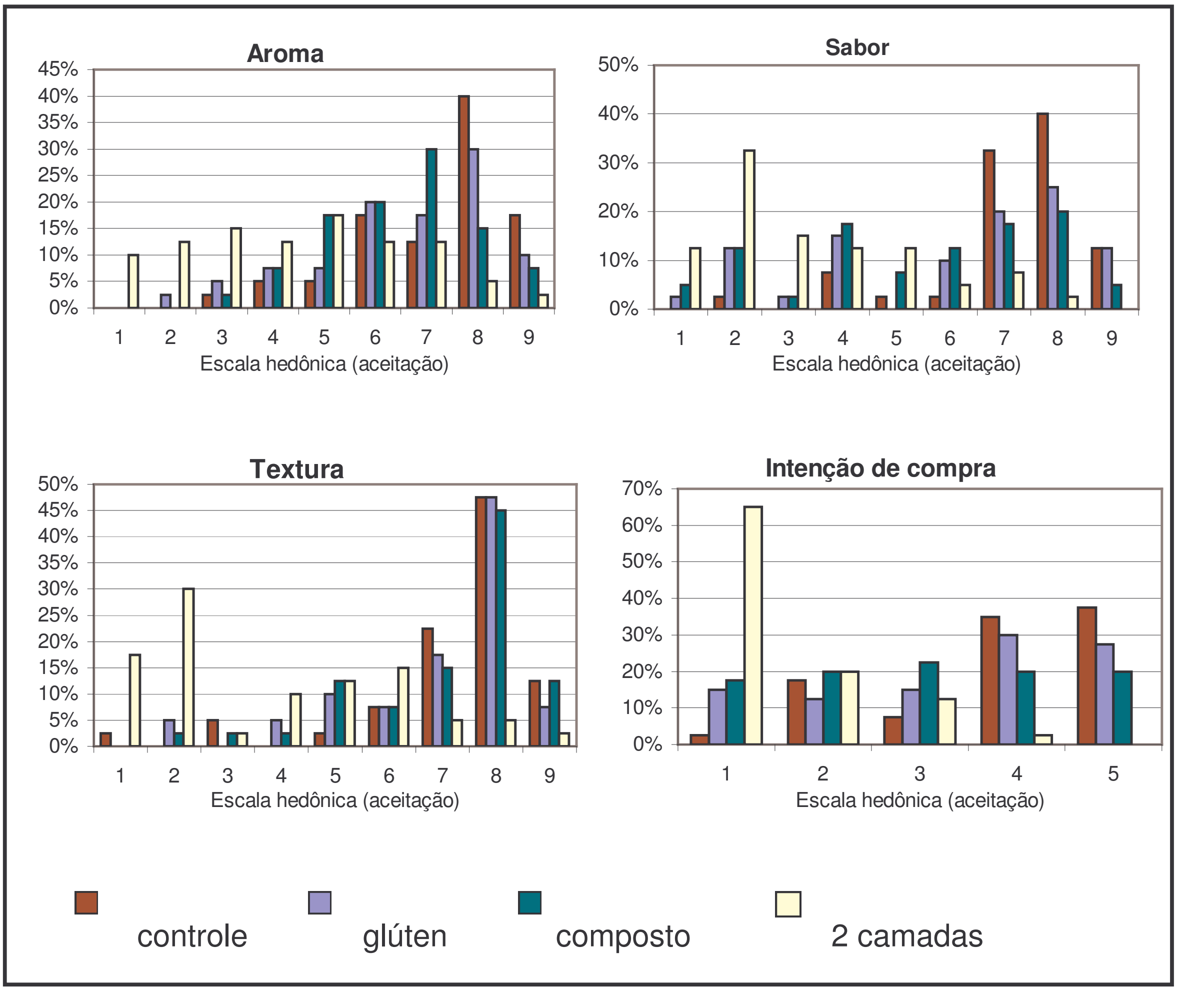


Figura 5 – Análise sensorial de degustação dos morangos.

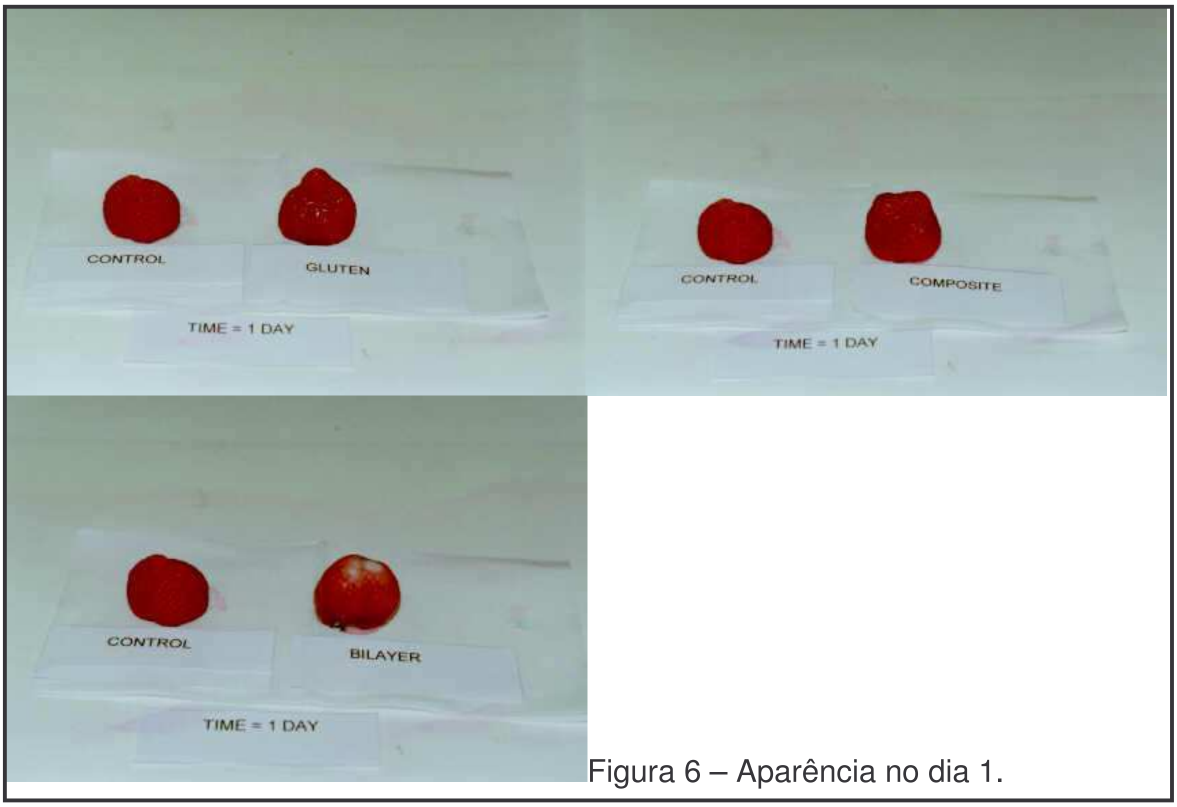


Figura 6 – Aparência no dia 1.

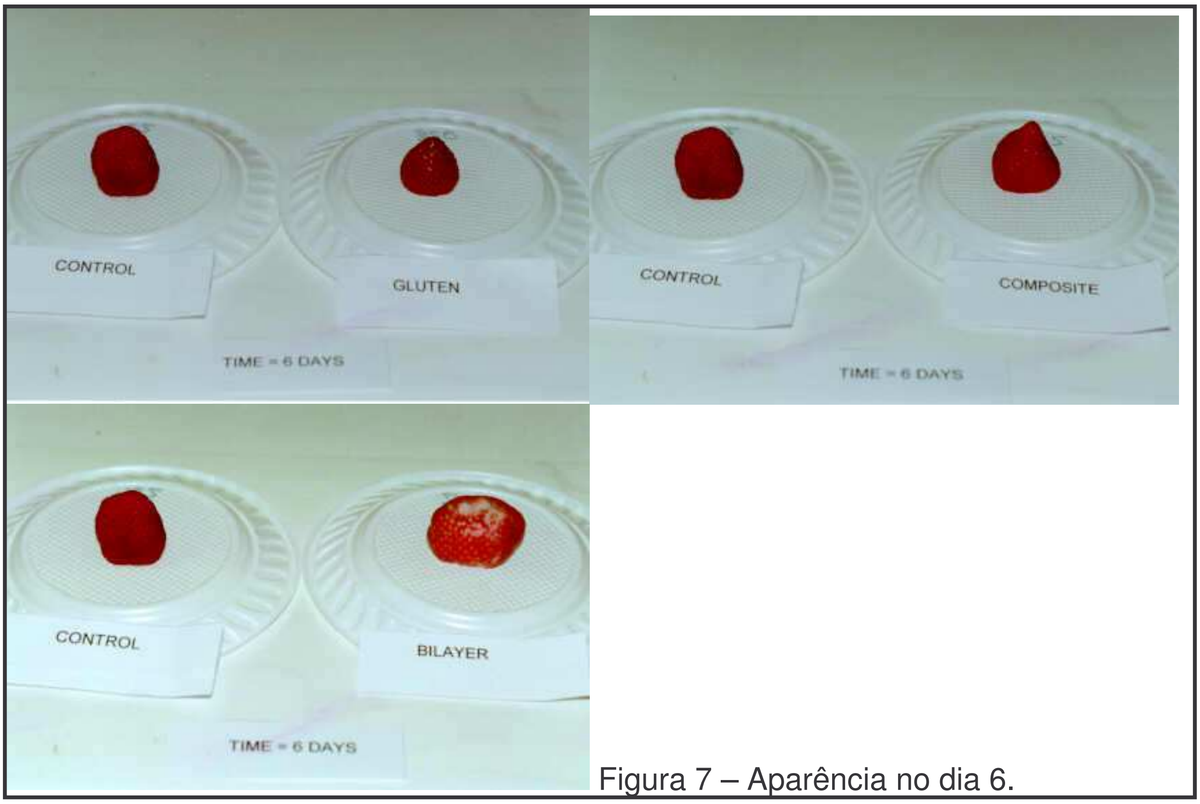


Figura 7 – Aparência no dia 6.

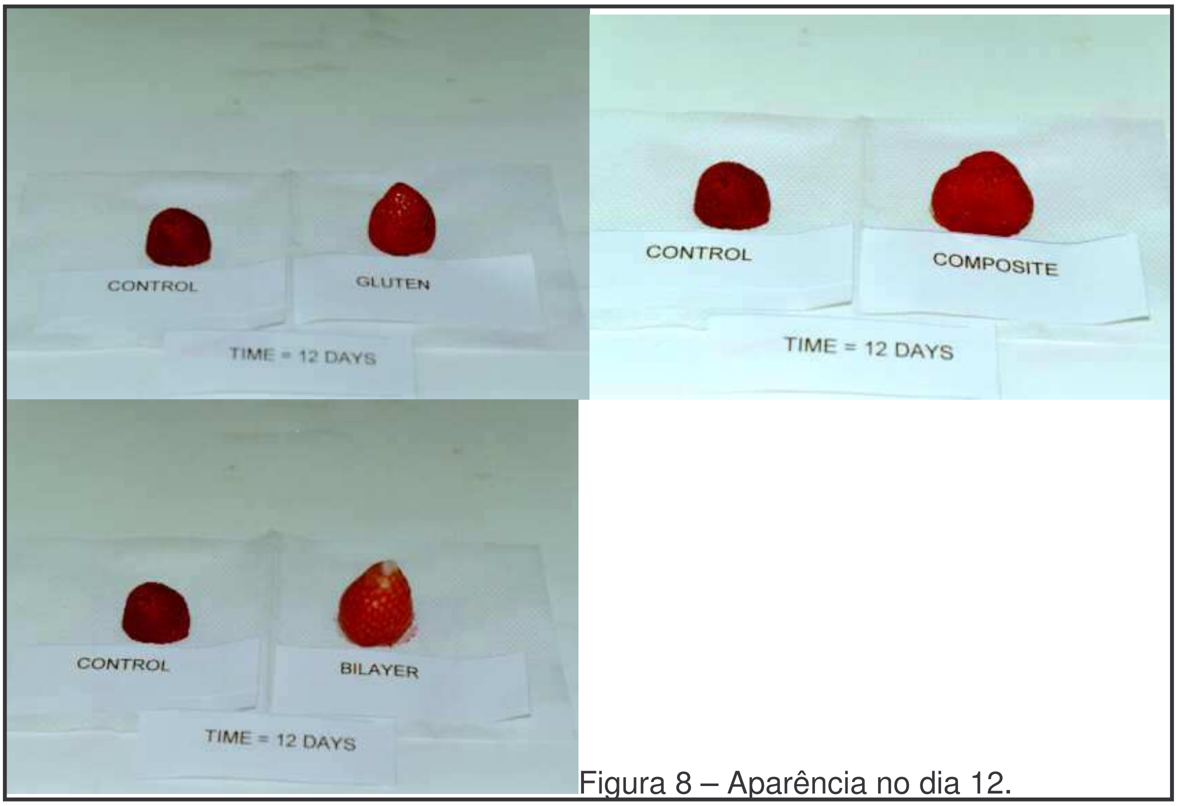


Figura 8 – Aparência no dia 12.

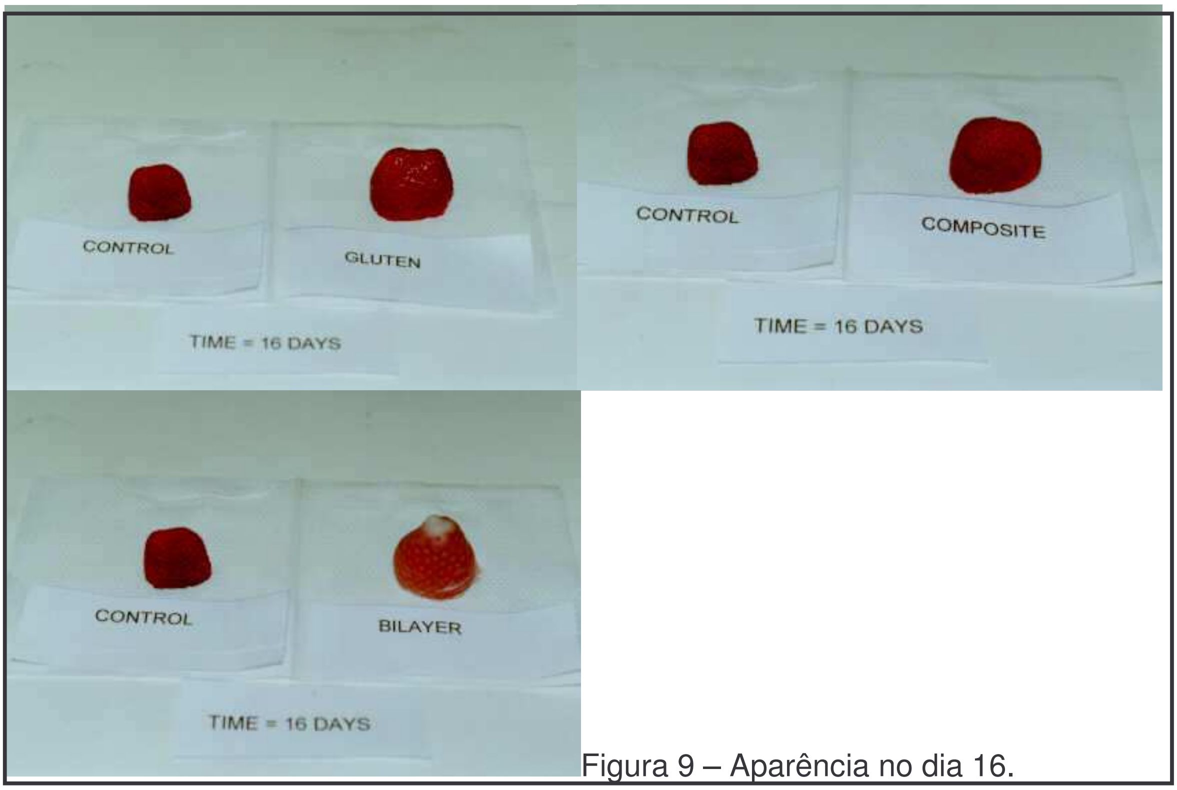


Figura 9 – Aparência no dia 16.

AVALIAÇÃO SENSORIAL DE MORANGO

Nome:___________________________________ **Idade:**______________
E-mail:____________________________________ **Data:** _____________
Telefone:______________ **Lab:**___________ **Ramal:**______________
Amostra: __________

1.Você está recebendo uma amostra codificada de MORANGO. Por favor, avalie a APARÊNCIA GLOBAL da amostra e indique na escala abaixo o quanto você gostou ou desgostou da APARÊNCIA GLOBAL da amostra:

9. Gostei extremamente
8. Gostei muito
7. Gostei moderadamente
6. Gostei ligeiramente
5. Nem gostei / nem desgostei
4. Desgostei ligeiramente
3. Desgostei moderadamente
2. Desgostei muito
1. Desgostei extremamente

2. Comente o que você mais gostou ou menos gostou na APARÊNCIA GLOBAL da amostra:

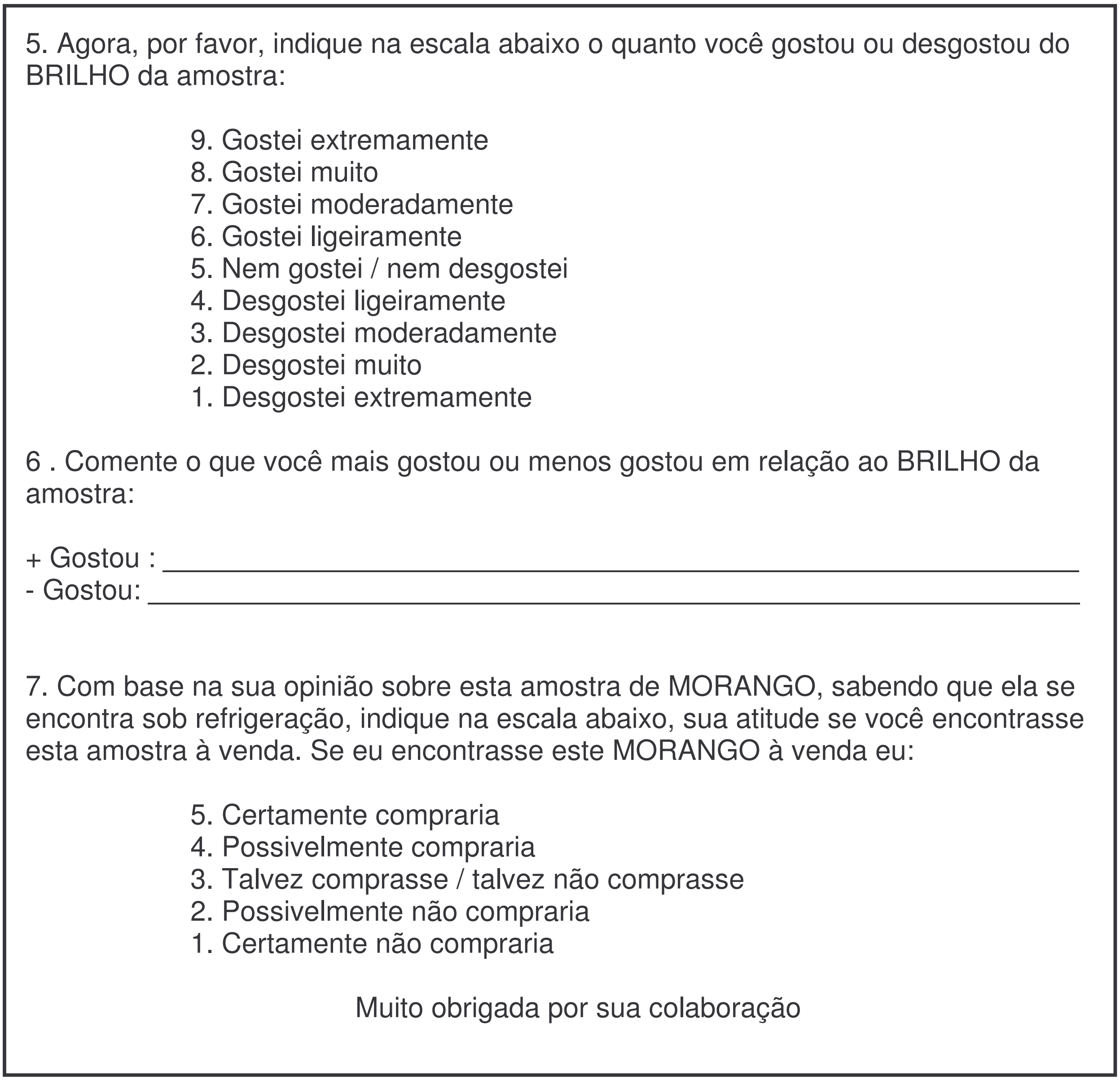
+ Gostou :____
- Gostou: ____

3. Avalie a COR da amostra e indique na escala abaixo o quanto você gostou ou desgostou da COR da amostra:

9. Gostei extremamente
8. Gostei muito
7. Gostei moderadamente
6. Gostei ligeiramente
5. Nem gostei / nem desgostei
4. Desgostei ligeiramente
3. Desgostei moderadamente
2. Desgostei muito
1. Desgostei extremamente

4. Comente o que você mais gostou ou menos gostou na COR da amostra:

+ Gostou :____
- Gostou: ____



5. Agora, por favor, indique na escala abaixo o quanto você gostou ou desgostou do BRILHO da amostra:

9. Gostei extremamente
8. Gostei muito
7. Gostei moderadamente
6. Gostei ligeiramente
5. Nem gostei / nem desgostei
4. Desgostei ligeiramente
3. Desgostei moderadamente
2. Desgostei muito
1. Desgostei extremamente

6 . Comente o que você mais gostou ou menos gostou em relação ao BRILHO da amostra:

+ Gostou : ____
- Gostou: ____

7. Com base na sua opinião sobre esta amostra de MORANGO, sabendo que ela se encontra sob refrigeração, indique na escala abaixo, sua atitude se você encontrasse esta amostra à venda. Se eu encontrasse este MORANGO à venda eu:

5. Certamente compraria
4. Possivelmente compraria
3. Talvez comprasse / talvez não comprasse
2. Possivelmente não compraria
1. Certamente não compraria

Muito obrigada por sua colaboração

Figura 10 – Ficha da análise sensorial de aparência dos morangos.

AVALIAÇÃO SENSORIAL DE MORANGO

Nome:____________________________________ **Idade:**_______________
E-mail:____________________________________ **Data:** ______________
Amostra: ___________

1.Você está recebendo uma amostra codificada de MORANGO. Por favor, indique na escala abaixo o quanto você gostou ou desgostou do AROMA da amostra:

9. Gostei extremamente
8. Gostei muito
7. Gostei moderadamente
6. Gostei ligeiramente
5. Nem gostei / nem desgostei
4. Desgostei ligeiramente
3. Desgostei moderadamente
2. Desgostei muito
1. Desgostei extremamente

2. Comente o que você mais gostou ou menos gostou na AROMA da amostra:

+ Gostou : ____
- Gostou: ____

3. Por favor, agora prove a amostra e indique na escala abaixo o quanto você gostou ou desgostou do SABOR da amostra:

9. Gostei extremamente
8. Gostei muito
7. Gostei moderadamente
6. Gostei ligeiramente
5. Nem gostei / nem desgostei
4. Desgostei ligeiramente
3. Desgostei moderadamente
2. Desgostei muito
1. Desgostei extremamente

4. Comente o que você mais gostou ou menos gostou no SABOR da amostra:

+ Gostou : ____
- Gostou: ____

5. Por favor, prove novamente a amostra e indique na escala abaixo o quanto você gostou ou desgostou da TEXTURA da amostra:

9. Gostei extremamente
8. Gostei muito
7. Gostei moderadamente
6. Gostei ligeiramente
5. Nem gostei / nem desgostei
4. Desgostei ligeiramente
3. Desgostei moderadamente
2. Desgostei muito
1. Desgostei extremamente

6. Comente o que você mais gostou ou menos gostou na TEXTURA da amostra:

+ Gostou : ____
- Gostou: ____

7. Com base na sua opinião sobre esta amostra de MORANGO, sabendo que esta se encontra sob refrigeração ,indique na escala abaixo, sua atitude se você encontrasse esta amostra à venda. Se eu encontrasse este MORANGO à venda eu:

5. Certamente compraria
4. Possivelmente compraria
3. Talvez comprasse / talvez não comprasse
2. Possivelmente não compraria
1. Certamente não compraria

Muito obrigada por sua colaboração

Figura 11 - Ficha da análise sensorial de degustação dos morangos.

CONCLUSÕES

As coberturas à base de glúten de trigo aplicadas nos morangos conseguiram estender a vida de prateleira das frutas em 4 dias no período estudado, já que a recusa de compra das frutas controle pelos provadores ocorreu no dia 12 de análise, e segundo LABUZA & SCHIMIDL (1988) esse fato determina a vida útil de alimentos. Pela análise sensorial de aparência dos morangos, a cobertura composta foi a mais bem aceita pelos provadores em todo o armazenamento, mantendo as características de aparência global, cor e brilho dos morangos, porém na análise sensorial de degustação, essa amostra não foi bem aceita devido ao sabor residual da mistura de lipídios no morango. Já a cobertura simples de glúten foi aceita na análise sensorial de degustação, podendo assim ser usada na cobertura de morangos para a extensão de sua vida de prateleira e manutenção da qualidade. A cobertura de duas camadas não foi aceita pelos provadores tanto na aparência quanto na degustação.

REFERÊNCIAS

BAARDSETH, P.; SKEREDE, G.; NAES, T.; THOMASSEN, M. S.; IVERSEN, A.; KAABER, L. A comparison of CIE (1976) L* a* b* values obtained from two different instruments on general food commodities. **J Food Sci** 53 (6): 1737-1742, 1988.

CLEMENTE, E. S. **Caracterização química, nutricional, física e sensorial de dois cultivares de brócolis (*Brassica oleracea* L. var. *itálica* baron e *Brassica oleracea* L. var. *itálica* ramoso – Piracicaba) : um estudo de vida-de-prateleira**. Campinas, 1998, 169 p. (Mestrado), Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas.

FU, B. & LABUZA, T. P. Shelf life prediction: theory and application. **Food Control** 4 (3): 125-133, 1993.

LABUZA, T. P. & SCHMIDL, M K. Use of sensory data in the shelf life testing of foods: principles and graphical methods for evaluation. **Cereal Foods World** 33 (2): 193-203, 1988.

LITTLE, A. C. Off on a tangent. **J Food Sci** 40 (2): 410-412, 1975.

MUÑOZ, A. M.; CIVILLE, G. V.; CARR, B T. **Sensory evaluation in quality control**. New York: Van Nostrand Reinhold, 1992. 240 p.

ANEXO 2

ISOTERMAS DE SORÇÃO E TEMPERATURA DE TRANSIÇÃO VÍTREA DE BIOFILMES COMESTÍVEIS À BASE DE GLÚTEN DE TRIGO

Resumos enviados ao IV CIBIA (Congreso Iberoamericano de Ingeniería de Alimentos) - Chile, outubro 2003.

ISOTERMAS DE SORÇÃO E TEMPERATURA DE TRANSIÇÃO VÍTREA DE BIOFILMES COMESTÍVEIS À BASE DE GLÚTEN DE TRIGO

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Resumo: O objetivo desse trabalho foi obter e comparar as isotermas de adsorção e a temperatura de transição vítrea dos biofilmes simples, compostos e de duas camadas de glúten de trigo e lipídios (cera de abelha, ácidos palmítico e esteárico). As isotermas de adsorção de água foram realizadas nos três filmes nas temperaturas de 25 e 45 ^{0}C e as temperaturas de transição vítrea foram medidas através de análise dinâmica-mecânica DMA. Em relação às isotermas de adsorção, os biofilmes compostos e de duas camadas adsorveram menos umidade nas duas temperaturas estudadas e o modelo de GAB foi o melhor para ajustar os dados experimentais dos biofilmes estudados. Com relação à temperatura de transição vítrea, observou-se que a adição de lipídios ao filme simples de glúten aumentou a Tg, apresentando o filme de duas camadas a maior temperatura, seguida do filme composto.

INTRODUÇÃO

As propriedades funcionais de biofilmes sofrem grande influência das condições ambientais, temperatura e umidade relativa. As proteínas são sensíveis à umidade devido à característica hidrofílica das macromoléculas, que tornam as propriedades funcionais dos biofilmes dependentes da umidade relativa do ambiente. As isotermas de sorção dos biofilmes comestíveis, como proteína do amendoim, isolado de soja, zeína do milho e glúten de trigo, variam de acordo com suas propriedades de permeabilidade ao vapor d'água e natureza hidrofílica. As isotermas de sorção obtidas de dados experimentais resultam em uma estimativa do conteúdo de umidade em equilíbrio, que é necessário para predizer as propriedades dos filmes em diferentes ambientes em suas aplicações (JANGCHUD e CHINNAN, 1999).

A migração de água em alimentos ocorre, principalmente, devido à diferença de pressão de vapor d'água (KAREL, 1975). A diferença da pressão de vapor d'água entre componentes pode ser reduzida com o uso de plastificante, que fixa as moléculas de água no componente de maior atividade de água reduzindo a pressão de vapor (BANKER, 1966). Porém, o plastificante aumenta mobilidade da cadeia polimérica, gerando uma maior absorção de vapor d'água.

A absorção de vapor d'água pelo biopolímero pode resultar no aumento de volume e mudança de conformação na estrutura molecular (WATT,1983). O conhecimento das isotermas de sorção dos biofilmes é necessário para se saber o quanto de água ele pode ganhar ou perder do ambiente, ou de um sistema alimentício, e assim, estabelecer as condições adequadas de armazenamento e prever as propriedades dos biofilmes.

As relações entre estrutura e propriedades de biopolímeros hidratados podem ser entendidas melhor com a teoria de transição vítrea usada na ciência de polímeros, que está relacionada com as variáveis críticas de tempo, temperatura e conteúdo de diluente (SLADE et al., 1989). A transição vítrea de um polímero amorfo ou parcialmente amorfo é tipicamente descrita como uma transição do estado vítreo para um estado altamente viscoso ou gomoso (SLADE e LEVINE, 1991). A transição vítrea separa dois domínios de acordo com as diferenças estruturais, e as propriedades dos polímeros são fortemente modificadas quando a temperatura está acima da temperatura de transição vítrea (Tg). O valor de Tg é governado primariamente pela composição química e presença de plastificantes, e secundariamente pelas características de estrutura, como ramificação da cadeia, ligação cruzada e cristalinidade (ROGERS, 1985). Apesar da grande variedade de estrutura, refletindo a diversidade de aminoácidos de que são construídos, é estabelecido que a maioria das proteínas passam pela transição vítrea (SLADE et al., 1989). Plasticização pela água, polióis, mono, di ou oligossacarídios afeta a Tg de proteínas amorfas ou parcialmente amorfas, resultando em queda de Tg. As propriedades mecânicas e de barreira dos polímeros em qualquer temperatura são afetadas pelo valor de Tg. Por exemplo, é esperado que a permeação de gás e moléculas de vapor através do filme é maior acima da Tg, onde as cadeias do polímero estão mais móveis (ROGERS, 1985). Para o caso do glúten de trigo, uma variedade de técnicas, incluindo calorimetria diferencial de varredura (DSC) e análise dinâmica-mecânica (DMA) podem mostrar que o glúten é um sistema multipolimérico amorfo, que é plasticizado pela água, mas não solúvel (SLADE et al., 1989).

O objetivo desse trabalho foi o de obter e comparar as isotermas de adsorção e a Tg dos biofilmes simples, compostos e de duas camadas de glúten de trigo e lipídios (cera de abelha, ácidos palmítico e esteárico).

MATERIAIS E MÉTODOS

Formação do filme simples de glúten de trigo

A solução formadora de filme foi preparada usando glúten de trigo (9g/100mL), glicerol (1,5g/100mL), etanol (32,5mL/100mL), água destilada e hidróxido de amônio 6N para ajustar a solução a pH 10. Todos os componentes foram misturados sob agitação magnética a 70 ^{0}C. A solução filmogênica foi centrifugada à 5856 g por 6 minutos à temperatura ambiente. Após isso, a solução filmogênica foi colocada em superfície de teflon e seca à temperatura ambiente por aproximadamente 24 horas (método modificado a partir do método de GONTARD et al., 1993).

Formação do filme composto e de duas camadas de glúten de trigo e lipídeos

Para o filme composto, a cera de abelha (4,5g/100mL), o ácido esteárico (2,75g/100mL) e o ácido palmítico (2,75g/100mL) foram adicionados à solução formadora de filme (preparada como descrita anteriormente). Essa solução foi agitada e aquecida para a fusão dos lipídios a cerca de 100 ^{0}C. Após isso, a solução filmogênica foi colocada em superfície de teflon e seca à temperatura ambiente por aproximadamente 24 horas (GONTARD et al., 1994).

Para o filme de duas camadas, os mesmos lipídios foram fundidos a cerca de 100 ^{0}C e um volume de 5 mL foi espalhado sobre um filme previamente seco de glúten e pré-aquecido. Uma folha de teflon foi colocada sobre a superfície do filme para espalhar homogeneamente a mistura de lipídios sobre o filme (GONTARD et al., 1994).

Isotermas de adsorção de água

Os biofilmes foram cortados em pedaços pequenos (aproxidamente 2 x 2 cm), pesados (cerca de 0,5 g) e acondicionados em ambientes com diferentes umidades relativas conhecidas (Tabela 1) e em temperaturas controladas de 25 e 45 ^{0}C. Uma vez alcançado o equilíbrio, num período aproximadamente de 20 dias, pesou-se para se obter o peso final e calcular as isotermas de adsorção. Os testes de adsorção foram feitos em triplicata.

Tabela 1 – Umidades relativas de equilíbrio de soluções salinas saturadas

	Temperatura (^{0}C)	
Sal	25	45
LiCl	11,4	10,3
$MgCl_2$	32,9	30,8
K_2CO_3	44,3	43,0
$Mg(NO_3)_2$	53,6	49,7
NaCl	76,2	72,6
$BaCl_2$	90,4	90,0

Fonte: LOMAURO et.al (1985).

Modelos de isotermas de adsorção

Os modelos GAB (Guggenheim-Anderson-De Boer) e BET (Brunauer-Emmett-Tetter) mostrados a seguir, foram ajustados aos dados experimentais através do programa Water Analyzer Series – Isotherm/BET/GAB Program version 2.05p. (Macintosh) para estimar as constantes dos modelos.

GAB $$X = \frac{xm.c.k.aw}{(1-k.aw)(1-k.aw+c.k.aw)}$$

BET $$X = \frac{xm.c.aw}{1+aw(c-1)(1-aw)}$$

O modelo de BET fornece um bom ajuste para uma variedade de alimentos na região de atividade de água entre 0,05 e 0,45. O modelo de GAB tem sido sugerido como modelo matemático mais versátil para a sorção de alimentos numa faixa de atividade de água entre 0,1 e 0,9.

Determinação da temperatura de transição vítrea (Tg)

Para a temperatura de transição vítrea (Tg) dos filmes foi usado um analisador dinâmico-mecânico (Dynamic Mechanical Analysis DMA 2980, TA Instruments). Os filmes foram cortados em tiras. A amostra de filme foi presa na garra (“clamp”) do aparelho de DMA. A amostra foi aquecida de –20 a 120 ^{0}C a uma taxa de aquecimento de 2 ^{0}C/min. A frequência foi de 1 Hz, a amplitude de 10 µm e a força de 0,5 N. As medidas de módulo de armazenamento (E’), módulo de perda (E”) e ângulo de perda (tan δ) foram registradas e graficadas contra a temperatura para a análise de transição térmica. As medidas foram realizadas em duplicata e o valor de Tg foi medido como sendo o pico de temperatura da tan δ.

RESULTADOS E DISCUSSÃO

Isotermas de adsorção

Os dois modelos matemáticos (BET e GAB) utilizados ajustaram os resultados em toda faixa de atividade de água estudada. Os valores de R^2, que ajustam os dados às equações de isotermas de cada filme nas duas temperaturas estudadas, encontram-se nas Tabelas 2 e 3. Foi observado que o modelo de GAB apresentou os mais altos valores de R^2, o que indica que esse modelo se ajustou melhor aos dados experimentais, segundo LOMAURO et al. (1985). A equação de GAB também foi o modelo que melhor representou a isoterma de sorção de filmes de glúten de trigo e zeína de milho (GENNADIOS e WELLER, 1994).

Na temperatura de 25 ^{0}C não foi possível obter a umidade em atividade de água maior do que 0,8 porque os filmes apresentaram fungos.

As isotermas de adsorção dos biofilmes à base de glúten de trigo apresentaram a forma similar à sigmoidal (Figuras 1 e 2), como de polímeros sensíveis ao vapor d'água (LIOUTAS et al., 1988). Geralmente, sistemas com alto teor protéico apresentam maior incidência na forma sigmoidal como as proteínas do trigo (GONTARD et al., 1993).

Em todos os biofilmes testados, observa-se nos valores obtidos da equação de GAB, que o teor de umidade da monocamada (x_m) diminui à medida que a temperatura aumenta (Tabela 2 e 3). Esta é uma tendência geralmente verificada em biofilmes (CRUZ et al., 2001).

O biofilme simples de glúten de trigo adsorveu mais umidade do que os outros biofilmes em atividade de água maior do que 0,6 nas duas temperaturas estudadas (Figs. 1 e 2). Os biofilmes compostos e de duas camadas adsorveram menos umidade. Todos os biofilmes adsorveram menos umidade em atividade de água menor do que 0,6, mas houve um grande aumento na adsorção em atividades de água elevadas.

Tabela 2 – Constantes e coeficiente de determinação (R^2) de cada modelo de isoterma de adsorção para os filmes a 25 ^{0}C

Modelo	Constante ou R^2	Filme		
		Glúten	Composto	2 Camadas
BET	x_m	0,0682	0,0532	0,0651
	c	193,77	38,43	45,67
	R^2	0,9684	0,9739	0,9876
GAB	x_m	0,0703	0,0578	0,0785
	k	0,96	0,93	0,80
	c	182,31	99,08	113,60
	R^2	0,9954	0,9915	0,9932

Tabela 3 – Constantes e coeficiente de determinação (R^2) de cada modelo de isoterma de adsorção para os filmes a 45 ^{0}C

Modelo	Constante ou R^2	Filme		
		Glúten	Composto	2 Camadas
BET	x_m	0,0619	0,0430	0,0509
	c	79,43	44,13	33,16
	R^2	0,9670	0,9573	0,9662
GAB	x_m	0,0522	0,0469	0,0611
	k	1,01	0,99	0,84
	c	57,08	20,84	68,57
	R^2	0,9994	0,9976	0,9959

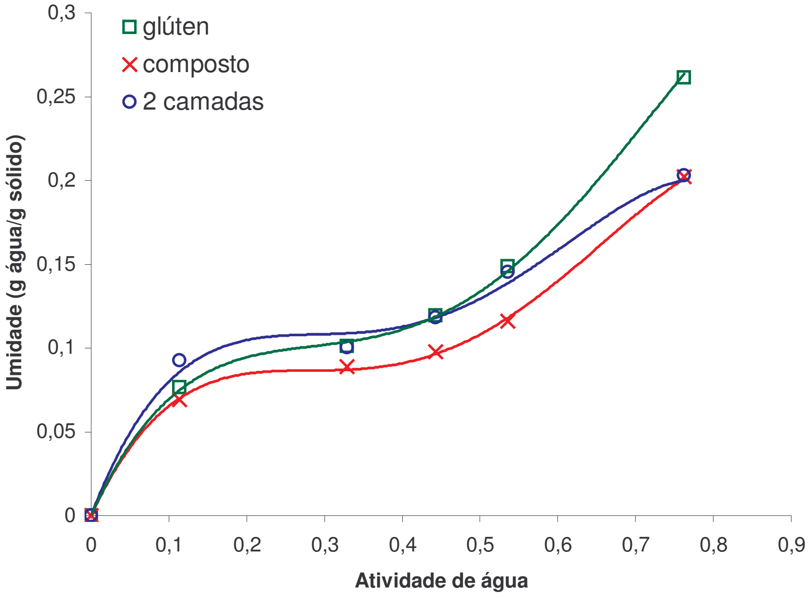

Figura 1 – Isotermas de adsorção dos biofilmes à base de glúten à temperatura de 25 ^{0}C (ajustadas de acordo com o modelo de GAB).

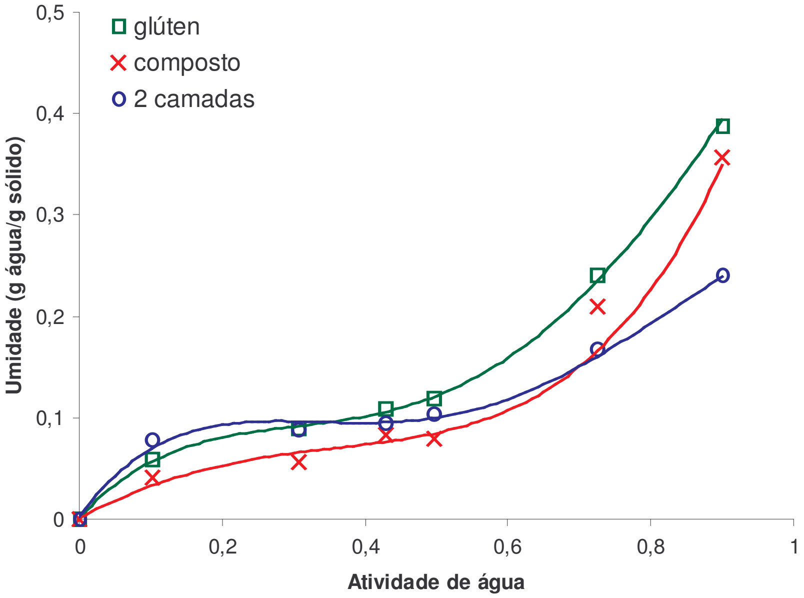

Figura 2 - Isotermas de adsorção dos biofilmes à base de glúten à temperatura de 45 ^{0}C (ajustadas de acordo com o modelo de GAB).

Análise da temperatura de transição vítrea (Tg)

Gráficos típicos de DMA dos filmes à base de glúten de trigo, mostrando a tan δ (módulo de perda/módulo de armazenamento), o módulo de perda e o módulo de armazenamento em função da temperatura, estão apresentados nas Figs. 3, 4 e 5. Os filmes à base de glúten mostraram o comportamento de um polímero amorfo, onde com o aumento da temperatura, as proteínas amorfas passam por uma região vítrea, uma região de transição (no pico da tan δ) e uma região gomosa. O tamanho do pico da tan δ, que representa a fração do volume do material que sofre a transição (WETTON, 1986), é similar ao pico da tan δ típico de outros biopolímeros amorfos, como a elastina (LILLIE e GOSLINE, 1993), gelatina, amilopectina, caseína (KALICHEVSKY et al., 1993) ou glúten de trigo nativo (KALICHEVSKY et al., 1992). Pela Fig. 3 e Tabela 3, observou-se que o filme de glúten (amostra 1) teve a menor temperatura de transição vítrea (29,07 ^{0}C), seguido do filme composto de glúten e lipídios (amostra 2) com temperatura de 53,67 ^{0}C e do filme de duas camadas (amostra 3) com temperatura de 63,32 ^{0}C. Porém, os picos da transição foram suaves em todos os filmes, possivelmente devido à mistura de vários materiais em cada filme estudado, o que dificultou a detecção de um pico de transição bem definido. Pela Fig. 4, através do módulo de perda também pode se observar uma região de transição, onde o pico pode representar a Tg. O filme de glúten apresentou Tg de 20,87 ^{0} C, semelhante à Tg observada através da tan δ. Já os filmes compostos e de duas camadas apresentaram temperaturas de transição bem menores do que as observadas pela tan δ e transições também suaves. Na Fig. 5, através do módulo de armazenamento, as faixas de transições não foram bem definidas, assim não se pode observar a Tg. A Tg dos filmes pode ser melhor observada através do gráfico de tan δ, porém as transições foram pouco perceptíveis, talvez devido à mistura de vários componentes em cada filme estudado.

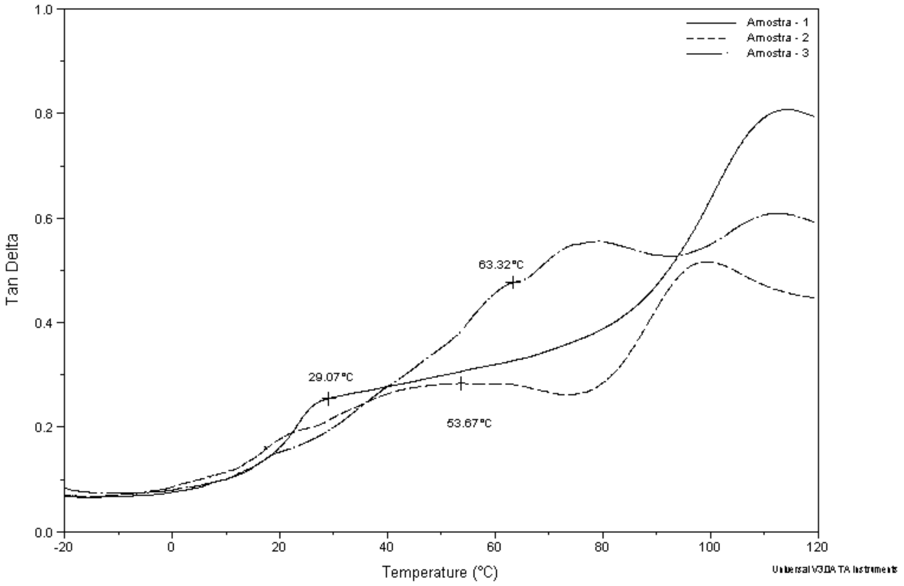

Figura 3 – Tan δ dos filmes de glúten, composto e duas camadas.

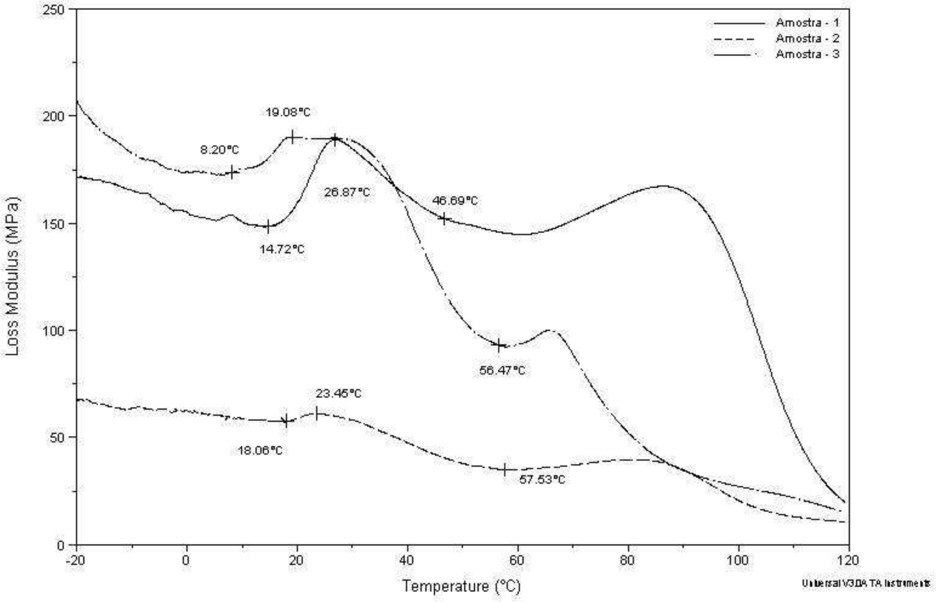

Figura 4 – Módulo de perda dos filmes de glúten, composto e duas camadas.

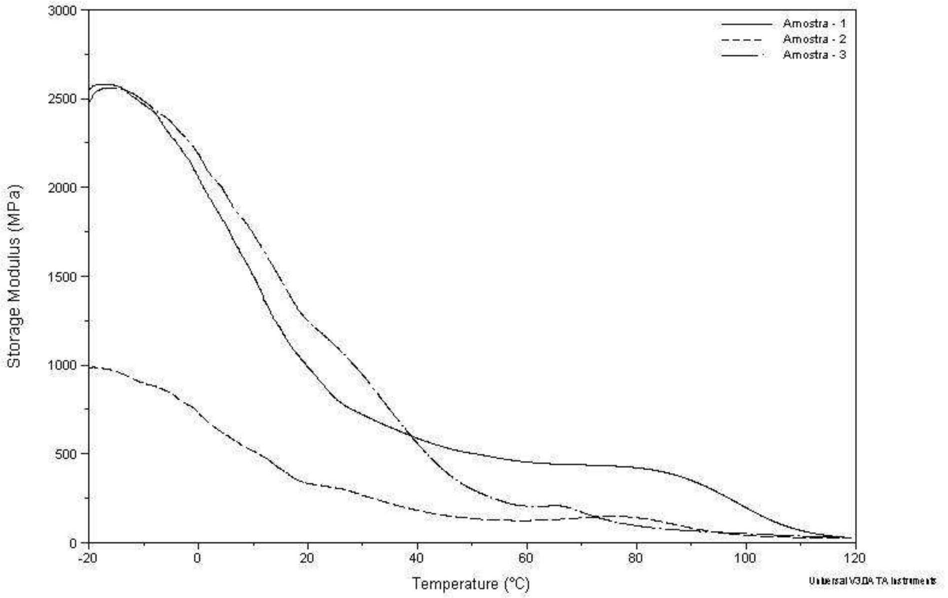

Figura 5 – Módulo de armazenamento dos filmes de glúten, composto e duas camadas.

Tabela 4 – Temperatura de transição vítrea e umidade dos filmes de glúten, composto e duas camadas.

Tipo de filme	Tg pelo tan δ (^{0}C)	Tg pelo módulo de perda (^{0}C)	Umidade (%)
Filme glúten	29,07 ± 0,12	26,87 ± 0,08	10,56 ± 0,24
Filme composto	53,67 ± 0,15	23,45 ± 0,11	9,88 ± 0,32
Filme duas camadas	63,32 ± 0,19	19,08 ± 0,09	10,10 ± 0,28

Média e desvio de padrão de duplicatas.

CONCLUSÕES

Os resultados apresentados mostraram que a temperatura provocou o efeito esperado sobre as isotermas, ou seja, o teor de umidade diminuiu à medida que a temperatura aumentou. Este estudo demonstrou que o modelo de GAB foi o melhor para ajustar os dados experimentais de biofilmes à base de glúten de trigo. Os biofilmes compostos e de duas camadas adsorveram menos umidade nas duas temperaturas estudadas (25 e 45 ^{0}C). Essa diferença nas isotermas entre o filme simples de glúten e os filmes com lipídios, provavelmente deve-se a possíveis modificações ocorridas na estrutura molecular da proteína ou devido ao lipídio ser massa inerte, por isso diminuiu-se a umidade (não absorveu água). Com relação à temperatura de transição vítrea, observou-se que a adição de lipídios ao filme simples de glúten aumentou a Tg, apresentando o filme de duas camadas a maior temperatura, seguida do filme composto. Porém, as transições foram bem suaves possivelmente devido à mistura de vários materiais em cada filme estudado. Correlacionando a isoterma de sorção com a Tg do filme simples de glúten, percebe-se que a Tg é menor do que 25 ^{0}C e houve maior adsorção de umidade, já que estava na região onde há maior mobilidade molecular. As Tgs dos filmes composto e duas camadas foram maiores do que 45 ^{0}C, assim as temperaturas das isotermas estudadas (25 e 45 ^{0}C) estavam na região de menor mobilidade molecular, o que reduziu a adsorção de água desses filmes.

REFERÊNCIAS

BANKER, G. S. 1966. Film coating theory and practice. **J Pharmacy Sci** 55: 81-89.

CRUZ, G.V.; TORRES, J.A. e MARTÍN-POLO, M.0. Temperature effect on the moisture sorption isotherms for methylcellulose and ethylcellulose films. **J Food Eng** 49 (1): 91-94, 2001.

GENNADIOS, A. e WELLER, C.L. Moisture sorption by grain protein films. **Transactions of American Society of Agricultural Enginners** 37 (2): 535-539, 1994.

GONTARD, N.; GUILBERT, S. e CUQ, J. L. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. **J Food Sci** 58 (1): 206-211, 1993.

GONTARD, N.; DUCHEZ, C.; CUQ, J. L. e GUILBERT, S. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. **International Journal of Food Science and Technology** 29: 39-50, 1994.

JANGCHUD, A. e CHINNAN, M. S. Properties of peanut protein film: sorption isotherm and plasticizer effect. **Lebensm. Wiss. U. Technolo**. 32: 89-94, 1999.

KALICHEVSKY, M. T.; JAROSZKIEWICZ, E. M.; BLANCHARD, J. M. Glass transition of gluten. 1: gluten and gluten-sugar mixtures. **Int. J. Biol. Macromol**. 14 (10): 257-266, 1992.

KALICHEVSKY, M. T.; BLANCHARD, J. M.; MARSH, R. D. Application of mechanical spectroscopy to the study of glassy biopolymers and related systems. In **The glassy state in foods**; Blanshard, J. M. V., Lillford, P. J., Eds.; Nottingham University Press: Nottingham, pp 133-156, 1993.

KAREL, M. Effect of water activity and food preservation. In: **Principles of food science**. M. Karel; O. R. Fennema and D. B. Lud, Eds., Marcel Dekker, New York, 1975.

LILLIE, M. A.; GOSLINE, J. M. The effect of swelling solvents on the glass transition in elastin and other proteins. In **The glassy state in foods**; Blanshard, J. M. V., Lillford, P. J., Eds.; Nottingham University Press: Nottingham, pp 281-302, 1993.

LIOUTAS, T. S.; BECHTEL, P. J.; STEINBERG, M. P. Desorption and adsorption isotherms of meat-salt mixture. **Journal of Agricultural and Food Chemistry** 32 (6): 1382-1385, nov./dez. 1988.

LOMAURO, C.J.; BAKSHI, A.S.; LABUZA, T.P. Evaluation of food moisture sorption isotherm equations. Part I: fruit, vegetable and meat products. **Lebensm. Wiss. U. Technolo.** 18 (2): 11-117, 1985.

ROGERS, C. E. Permeation of gases and vapors in polymers. In: **Polymer permeability**. J. Comyn, Ed. Elsevier Applied Science: London, pp. 11-73, 1985.

SLADE, L.; LEVINE, H.; FINLEY, J. W. Protein-water interactions: water as a plasticizer of gluten and other protein polymers. In: **Protein quality and the effects of processing**. R. D. Phillips and J. W. Finley, Eds. Marcel Dekker: New York, pp. 9-24, 1989.

SLADE, L.; LEVINE, H. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. **Critical review in food science and nutrition** 30 (2,3): 115-360, 1991.

WATT, I. C. The theory of wate sorption by biological materials. In: **Physical properties of foods**, sds. R. Jowitt, F. Escher, B. Hallström, H. F. T. Meffert, W. E. L. Spiess e G. Vos. Londres, Inglaterra, ed. Applied Science Publishers, 27-41, 1983.

WETTON, R. E. Dynamic mechanical analysis of polymers and related systems. In **Developments in polymer characterization**; Dawkins, J. V., Ed.; Elsevier Science Publishers: Amsterdam, 1986; pp. 179-221.

ANEXO 3

TABELAS COM AS ESPESSURAS DE TODOS OS FILMES ESTUDADOS

CAPÍTULO 3

Filme	Espessura média (μm)
1	93 ± 12
2	92 ± 18
3	104 ± 10
4	104 ± 14
5	91 ± 10
6	97 ± 18
7	107 ± 12
8	101 ± 12
9	99 ± 12
10	101 ± 16
11	109 ± 14
12	105 ± 14
13	96 ± 13
14	96 ± 12
15	104 ± 11
16	101 ± 12
17	103 ± 10
18	107 ± 12
19	92 ± 14
20	108 ± 10
21	103 ± 17
22	94 ± 17
23	103 ± 16
24	103 ± 11
25	102 ± 16
26	107 ± 15
27	103 ± 16

Média e desvio padrão de 5 medidas em cada filme

CAPÍTULO 4

Filme	Espessura média (μm)		
	10% lipídios	20% lipídios	30% lipídios
CB	116 ± 10	154 ± 12	171 ± 13
BB	170 ± 19	174 ± 18	186 ± 20
CA	133 ± 11	156 ± 15	-
BA	166 ± 12	176 ± 18	207 ± 15
CBA	139 ± 17	161 ± 17	198 ± 21
BBA	202 ± 20	211 ± 23	221 ± 18

Média e desvio padrão de 5 medidas em cada filme

CAPÍTULO 7

Filme	Espessura média (µm)
Trigo BR23 25% glicerol	91 ± 13
Embrapa 119 25% glicerol	94 ± 16
Embrapa 22 25% glicerol	93 ± 16
Embrapa 42 25% glicerol	98 ± 13
Vital glúten 25% glicerol	104 ± 17
Trigo BR23 15% glicerol	89 ± 18
Embrapa 119 15% glicerol	80 ± 13
Embrapa 22 15% glicerol	97 ± 17
Embrapa 42 15% glicerol	103 ± 15
Vital glúten 15% glicerol	103 ± 12

Média e desvio padrão de 5 medidas em cada filme

ANEXO 4

CHARACTERIZATION OF COMPOSITE BIOFILMS OF WHEAT GLUTEN AND CELLULOSE ACETATE PHTHALATE

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CHARACTERIZATION OF COMPOSITE BIOFILMS OF WHEAT GLUTEN AND CELLULOSE ACETATE PHTHALATE

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Abstract: The objective of this research was to develop and characterize composite biofilms produced using wheat gluten and cellulose acetate phthalate. Biofilms show barrier properties against moisture and oxygen diffusion through the film. The films were prepared with different thicknesses and component concentrations and were analyzed for water vapor and oxygen permeabilities, water and acid solubilities and mechanical properties. The results showed that the mixture improved the film characteristics as compared to the individual components. The 1:1 mixture showed better properties against water and oxygen permeabilities. The composite films were completely soluble in water and acid, except the film with a greater gluten concentration that was 50% soluble in water and acid. With respect to tensile strength, an increase in the gluten concentration in the composite films resulted in a decrease in strength. Considering elongation at break, there was no significant difference between the composite films. With respect to thickness, no difference was detected. The results showed that the mixture improved the characteristics as compared to the individual components.

Key words: biofilm, gluten, cellulose, properties.

INTRODUCTION

The edible films are considered an alternative to increase the shelf life of fruits and vegetables, protecting them from the effect of humidity and oxygen, thus delaying their deterioration. The use of these films is not recent. In China, in centuries XI and XIII, the wax coating was used in oranges and lemons to avoid moisture loss (HARDENBURG, 1997). Edible films from carnauba wax have been commercialized since 1930 in the covering of fresh fruits and vegetables (KAPLAN, 1986). The edible films are used to control the water vapor migration, oxygen permeability, carbon dioxide and lipids migration in a food. Moreover, they can introduce additives in the food, such as antioxidant, antimicrobial and flavors, thus improving the mechanical integrity, facilitating handling and keeping the quality of the food (KROCHTA & JOHNSON, 1997). Biofilms are produced from proteins, hydrocolloids, lipids or the combination of them. The composite films have an advantage of combining the positive points of each component used. The objective of this work was the comparison of films from wheat gluten, cellulose acetate phthalate and the mixture of both in relation to the water vapor and oxygen permeability, mechanical properties, and solubility in water and acid.

MATERIALS AND METHODS

Materials

Vital wheat gluten (Rhodia, Campinas, Brazil), glycerol (Merck, Darmstadt, Germany), ammonium hydroxide (Synth, São Paulo, Brazil), calcium chloride (Ecibra, São Paulo, Brazil), sodium chloride (Synth, São Paulo, Brazil), magnesium nitrate (Ecibra, São Paulo, Brazil), cellulose acetate phthalate (Eastman Kodak, USA), monobasic sodium phosphate (Synth, São Paulo), tribasic sodium phosphate (Synth, São Paulo) and solid paraffin (Chemco, São Paulo, Brazil).

Gluten film preparation

The film was prepared from a solution of gluten (9.0 g/100 mL solution), absolute ethanol (32.5 mL/100 mL solution), glycerol (1.50 g/100 mL solution), distilled water and ammonium hydroxide to adjust to pH 10. All components were mixed under magnetic stirring until the temperature of the mixture reached 70 ^{0}C and the solution centrifuged at 5856 g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a Teflon covered glass surface and dried at room temperature for 24 h (modified method from GONTARD et al.[15]). All films used for experiments were equilibrated at 52% RH at 25 ^{0}C for 48 h before being tested.

Cellulose acetate phthalate (CAP) film preparation

The CAP film was prepared solubilizing 10 g of CAP in a buffer solution of sodium phosphate pH 8.0 in mechanical agitator and then adding 0.5 g of glycerol. Aliquots of 8 mL and 16 mL were distributed in 15 cm plaxi-glass plates and left to dry at room temperature for 24h.

Composite films preparation

The composite films were prepared mixing the CAP film-forming solution to the wheat gluten film-forming solution under magnetic agitation, in the ratios of 1:1, 1:4 and 4:1, then the mixtures were distributed in plaxi-glass plates in the aliquots of 8 mL, 12 mL and 16 mL.

Water vapor permeability

The water vapor transmission rate of the films was determined gravimetrically at 25 ^{0}C using a modified American Society for Testing and Materials (ASTM) Standard Method E-96 according to TANADA-PALMU et al.

(2000). The samples, triplicates of each film, were conditioned for 2 d at 52% RH before measurement. The water vapor permeability was calculated according to the ASTM method.

Oxygen permeability

The oxygen transmission rates were determined using a modification of the ASTM Standard Method D 3985-81 with an Ox-Tran apparatus (Mocon, Inc., Minneapolis, USA) at 25 ^{0}C. The samples, duplicates of each film, were conditioned for 2 d at 52% RH before measurement. The oxygen permeability (OP) was calculated by dividing the oxygen transmission rate by the oxygen pressure and multiplying by the mean thickness.

Tensile strength and percent elongation at break

Film tensile strength and percent elongation at break were determined using a Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), operated according to the ASTM Standard Method D 882-83 (25 ^{0}C, initial grip separation = 50 mm and cross head speed = 100 mm/min). The peak loads and extension at break were recorded for tested film specimens (100 mm long and 25.4 mm wide). The films were conditioned for 2 d at 52% RH before measurement. Six specimens of each film were measured. The tensile strength and percent elongation at break were calculated according to the ASTM method.

Solubility in water

The percent of solubility was the percentage of dry matter of the film solubilized after 24 h immersion in water. The percentage of initial dry matter of each film was determined at 105 ^{0}C for 24 h. Two discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water and slowly and periodically agitated for 24 h at 25 ^{0}C. The pieces of film were then taken out and dried (105 ^{0}C for 24 h) to determine the weight of dry matter which was not solubilized in water. The weight of dry matter solubilized was calculated by subtracting the weight of dry matter not solubilized from the weight of initial dry matter and reported on an initial dry weight basis.

Solubility in acid

The same procedure as the solubility in water, except that the films were immersed in HCl 1 N solution for 24 h at 25 ^{0}C.

Statistical analyses

The Statistica 5.5 (Statsoft, USA) program was used to determine the significant differences of all film properties at a 95% confidence interval.

RESULTS AND DISCUSSION

The composite films of wheat gluten and CAP were opaque due to cellulose and fragile, specially the thinner films. Due to the brittleness of the films, it was not possible to characterize the composite films with higher concentration of gluten. The composite films of gluten and cellulose 1:1 and 1:4 of all thicknesses presented excellent water vapor barrier (Table 1), compared to films of hydroxipropilmetilcellulose (HPMC) and metilcellulose (MC) with values of 1.92 and 1.48 gmm/m^{2}dkPa, respectively (KAMPER and FENNEMA, 1984; GREENER and FENNEMA, 1989). In the composite film with higher concentration of gluten, the water vapor permeability increased. The composite films were completely soluble in water, as well as the simple films of cellulose. The composed film with higher concentration of gluten showed 50% of solubility in acid and the composite films with the other concentrations of gluten and cellulose were completely soluble in acid. The tensile strength (Table 2) reduced with increase of gluten concentration in the composite films. In comparison with the simple film of cellulose, the addition of gluten until the concentration of 25% in the composite films increased the tensile strength, however with higher concentration, the strength was reduced. For the elongation at break, there was not significant difference in the composite films, the film with higher concentration of cellulose showed the highest elongation, but low in comparison with the simple film of gluten. With relation to the thickness of the films, there was not significant difference in any property measured. The oxygen permeability could only be measured in the composite film of cellulose and gluten 1:1 (16 mL) because the other films were very fragile and brittle. This permeability was low compared to the simple film of gluten and the values from PARK and CHINNAN (1990) for films of zein:glycerol (13.0-44.9 cm^3mm/m^2dkPa) and films of gluten:glycerol (9.6-24.2 cm^3mm/m^2dkPa) at 30 ^{0}C and 0% of relative humidity.

Table 1 – Water vapor permeability and solubility in water and acid of the films

Film	Water vapor permeability (gmm/m^2dkPa)*	Solubility in water (%)*	Solubility in acid (%)*
CAP:glu (1:4)/8ml	-	-	-
CAP:glu (1:4)/12ml	-	-	-
CAP:glu (1:4)/16ml	12.19 ± 0.25^a	100.00^a	50.69 ± 1.64^b
CAP:glu (1:1)/8ml	3.75 ± 0.17^b	100.00^a	100.00^a
CAP:glu (1:1)/12ml	3.83 ± 0.13^b	100.00^a	100.00^a
CAP:glu (1:1)/16ml	4.11 ± 0.20^b	100.00^a	100.00^a
CAP:glu (4:1)/8ml	3.81 ± 0.01^b	100.00^a	100.00^a
CAP:glu (4:1)/12ml	3.64 ± 0.0^b	100.00^a	100.00^a
CAP:glu (4:1)/16ml	3.76 ± 0.26^b	100.00^a	100.00^a
CAP/8ml	6.03 ± 0.35^{ab}	100.00^a	30.50 ± 1.56^c
CAP/16 ml	10.05 ± 0.47^a	100.00^a	33.14 ± 1.87^c
Gluten	8.61 ± 1.03^{ab}	22.70 ± 4.10^b	-

*Mean and standard deviation of replicates. $^{a-c}$ Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

Table 2 – Mechanical properties and oxygen permeability of the films

Film	Tensile strenght (MPa)*	Elongation at break (%)*	Oxygen permeability ($cm^3\mu m/m^2dkPa$)*
CAP:glu (1:4)/8ml	-	-	-
CAP:glu (1:4)/12ml	-	-	-
CAP:glu (1:4)/16ml	1.32 ± 0.11^{e}	4.06 ± 0.78^{e}	-
CAP:glu (1:1)/8ml	7.57 ± 0.18^{de}	4.65 ± 1.63^{de}	-
CAP:glu (1:1)/12ml	10.89 ± 2.19^{cd}	5.27 ± 0.85^{cde}	-
CAP:glu (1:1)/16ml	16.18 ± 3.11^{bc}	4.91 ± 1.01^{de}	$22.21 \pm 1,23^{b}$
CAP:glu (4:1)/8ml	12.87 ± 2.41^{cd}	4.79 ± 1.96^{de}	-
CAP:glu (4:1)/12ml	22.60 ± 1.63^{ab}	6.79 ± 0.98^{bd}	-
CAP:glu (4:1)/16ml	23.78 ± 5.12^{a}	8.82 ± 0.68^{b}	-
CAP/8ml	11.08 ± 3.29^{cd}	7.86 ± 1.23^{bc}	-
CAP/16 ml	11.76 ± 4.31^{cd}	8.60 ± 1.36^{b}	-
Gluten	5.25 ± 0.24^{de}	215.30 ± 12.20^{a}	41.02 ± 0.86^{a}

*Mean and standard deviation of replicates. $^{a-e}$ Means with different superscript letters in the same column are significantly different ($p<0.05$) according to the ANOVA and Tukey tests.

CONCLUSION

Composite films of wheat gluten and cellulose acetate phthalate had lower water vapor permeability than simple films of gluten or cellulose, showing an excellent water vapor barrier. With increase of gluten concentration in the composite films, the solubility in acid and water and the mechanical resistance of the films decreased.

REFERENCES

ASTM. Methods D 3985-81, D 882-83 and E 96. In *Annual Book of Standards*. American Society for Testing Materials. Philadelphia, PA (1980).

GONTARD, N.; GUILBERT, S. & CUQ, J.L. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science* 58 (1): 206-211 (1993).

GREENER, I.K. & FENNEMA, O. Barrier properties and surface characteristics of edible, bilayer films. *Journal of Food Science* 54 (6): 1393-1399 (1989).

HARDENBURG R.E. Wax and related coatings for horticultural products. A bibliography. *Agriculture Research Service Bulletin* 51-15, Unites States Department of Agriculture, Washington, DC (1997).

KAMPER, S.L. & FENNEMA, O. Water vapor permeability of edible bilayer films. *Journal of Food Science* 49: 1478-1481, 1485 (1984).

KAPLAN H.J. *Washing, waxing and color-adding in fresh citrus fruit,* ed W.F.Wardowski, S. Nagy and W.Grierson, p379. A VI, Westport, CT (1986).

KROCHTA J.M. & JOHNSON C.M. Edible and biodegradable polymer films: Challenges and opportunities. *Food Technology* 51 (2): 60-74 (1997).

PARK, H.J. & CHINNAN, M.S. Properties of edible coatings for fruits and vegetables, *ASAE* Paper n^{0} 90-6510, presented at the 1990 International Winter Meeting, American Society of Agricultural Engineers, 1990 December 18-21, Chicago, IL.

TANADA-PALMU, P.S., HELÉN, H. & HYVÖNEN, L. Preparation, properties and applications of wheat gluten edible films. *Agricultural and Food Science in Finland* 9 (2000): 23-35.

ANEXO 5

FILMES BIODEGRADÁVEIS – EXTENSÃO DA VIDA ÚTIL DE FRUTAS TROPICAIS

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ANEXO 6

POSTERS APRESENTADOS E ACEITOS EM CONGRESSOS

LISTA DE POSTERS APRESENTADOS E ACEITOS EM CONGRESSOS DURANTE O DOUTORADO

1. Obtenção de filmes de glúten e utilização na extensão da vida útil de morangos frescos - XVII Congresso de Ciência e Tecnologia de Alimentos – Fortaleza, agosto 2000.
2. Preparation and properties of wheat gluten edible films - III Congreso Iberoamericano de Ingeniería de Alimentos (CIBIA) – Valencia, Espanha, março 2001.
3. Mechanical properties of edible bilayer and composite films of wheat gluten and lipids - III Congreso Iberoamericano de Ingeniería de Alimentos (CIBIA) - Valencia, Espanha, março 2001.
4. Application of wheat gluten film to strawberries - 2001 IFT Annual Meeting – New Orleans, Estados Unidos, junho 2001.
5. Properties of composite and bilayer films of wheat gluten and lipids - IV Simpósio Latino Americano de Ciência de Alimentos, Campinas, novembro 2001.
6. Desenvolvimento e caracterização de biofilmes compostos de glúten de trigo e gelatina - IV Simpósio Latino Americano de Ciência de Alimentos, Campinas, novembro 2001.
7. Development, characterization and comparison of edible gluten films from Brazilian wheat and vital gluten - 2002 IFT Annual Meeting, Anaheim, Estados Unidos, junho 2002.
8. Application of composite films of wheat gluten and gelatin to guavas - 2002 IFT Annual Meeting, Anaheim, Estados Unidos, junho 2002.
9. Aplicação de biofilmes e coberturas comestíveis em frutas frescas - XVIII Congresso Brasileiro de Ciência e Tecnologia de Alimentos, Porto Alegre, agosto 2002.

10. Characterization of edible films obtained from the mixture of wheat gluten and cellulose acetate phtalate - XIV Congresso Brasileiro de Engenharia Química, Natal, agosto 2002.
11. Development to methodologies to elaborate lipid-based edible films - apresentação oral no IV Brazilian Meeting on Chemistry of Food and Beverages, Campinas, dezembro 2002.
12. Edible wheat gluten-based coatings: application on refrigerated strawberries – apresentação oral aceita para o 2003 IFT Annual Meeting, Chicago, Estados Unidos, julho 2003.
13. Edible wheat gluten-based films: application on refrigerated strawberries – poster aceito para o IUFoST 12th World Congress of Food Science and Technology, Chicago, Estados Unidos, julho 2003.
14. Sensory evaluation of shelf life and taste of strawberries coated with edible wheat gluten-based coatings – poster aceito para o 5th Pangborn Sensory Science Symposium, Boston, Estados Unidos, julho 2003.