

CAROLINA PEÑA SERNA

Desenvolvimento de filmes biodegradáveis à base de zeína,
caracterização das propriedades funcionais e estruturais e
avaliação do uso como cobertura na conservação das
características físico-químicas do queijo Minas padrão

São José do Rio Preto
2015

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Tese apresentada como parte dos requisitos para
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Orientador:

Prof. Dr. José Francisco Lopes Filho

Co-orientadora:

Profa. Dra. Ana Lúcia Barretto Penna

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“The will to win, the desire to succeed, the urge to reach your full potential... these are the keys that will unlock the door to personal excellence”

Confucius

***Dedico este trabalho à minha família porque graças ao seu amor incondicional,
encorajamento e apoio, hoje concluo mais uma etapa na minha vida.***

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RESUMO

Devido à sua composição química, os alimentos podem sofrer alteração da qualidade e do seu conteúdo nutricional como consequência de fatores tais como a contaminação microbiológica, a contaminação com outros alimentos ou compostos químicos, a ação de agentes ambientais como radiação (UV), ar (oxigênio), câmbios de temperatura, entre outros. Por essa razão, a indústria dos alimentos, a fim de proteger e garantir a qualidade, a segurança e o conteúdo nutricional dos produtos alimentícios, é a indústria que mais usa embalagens não biodegradáveis.

No entanto, a preocupação mundial devido ao excessivo aumento da contaminação ambiental e acumulação de embalagens plásticas não biodegradáveis têm gerado a necessidade de desenvolver embalagens biodegradáveis que permitam garantir a qualidade do produto além de diminuir o impacto negativo ao ambiente.

Dessa forma, nos últimos tempos, as indústrias de alimentos e de embalagens, assim como as instituições de pesquisa, têm focado os seus estudos no desenvolvimento de embalagens biodegradáveis produzidas a partir de resíduos agrícolas e subprodutos da agroindústria que podem ser rapidamente degradados pela ação de microrganismos do solo. Essas embalagens, principalmente na forma de filmes e coberturas, são baseadas em biopolímeros como proteínas (zeína, colágeno, gelatina, entre outras), carboidratos (amido, quitosana, alginato, entre outras) e lipídeos (ceras e ácidos graxos).

A zeína, principal proteína do milho, tem sido usada em algumas aplicações na indústria de alimentos e farmacêutica, porém, o seu uso como embalagem ainda é um grande obstáculo visto que as suas propriedades físicas, mecânicas e estruturais são inferiores quando comparadas com as embalagens plásticas tradicionais. Baseado nisso, vários estudos têm demonstrado que a combinação de duas ou mais biomoléculas, além de agentes plastificantes, permite melhorar as propriedades de filmes de zeína.

Assim, na primeira parte deste trabalho, diferentes concentrações de etanol (solvente de zeína) e glicerol (agente plastificante) foram avaliadas com a finalidade

de produzir filmes de zeína homogêneos com boas propriedades funcionais e mecânicas, chegando-se aos resultados de que 10 % de glicerol e 95 % de etanol deveriam ser usados para preparar filmes compostos de zeína e ácido oleico.

Uma vez determinadas as concentrações de solvente e plastificante, na segunda parte do trabalho foram desenvolvidos filmes compostos de zeína e ácido oleico (Z-AO), zeína, ácido oleico e goma xantana (Z-AO-GX) e zeína, ácido oleico e gelatina (Z-AO-GE) visando obter filmes compostos com melhores propriedades comparadas com os filmes não compostos de zeína. De acordo com os resultados obtidos, não foi possível obter filmes compostos Z-AO-GE em razão da desnaturação da gelatina em contato com 95 % etanol. Por outro lado, o filme composto Z-AO-GX apresentou maior opacidade e resistência à tensão enquanto que o filme Z-AO exibiu menor solubilidade em água e maior flexibilidade. Os filmes Z-AO-GX e Z-AO apresentaram permeabilidade ao vapor de água e ao oxigênio e microestrutura semelhantes.

Baseado nisso, na terceira parte do trabalho, avaliou-se o efeito de ambas as embalagens, em forma de cobertura, sobre a conservação das características físico-químicas, cor e textura do queijo Minas padrão ao longo de 56 dias de armazenamento em comparação com o queijo com embalagem comercial (polietileno) e sem embalagem. As coberturas exibiram uma redução de 30 % na perda de peso, além de prevenir a contaminação microbiológica do queijo por mais de 50 dias com relação ao queijo sem embalagem. Porém, os queijos com e sem cobertura apresentaram grande perda de umidade comparados com queijos em embalagem comercial, o que influenciou outras características tais como textura (maior dureza), proteólise (menor atividade enzimática e bacteriana), alteração de cor superficial e teor lipídico (perda de lipídeos).

Durante o desenvolvimento desta parte do trabalho, considerou-se a possibilidade de que alguns compostos pequenos da matriz da cobertura pudessem migrar para o queijo e gerar problemas em sua qualidade. Assim, na quarta parte do trabalho, avaliaram-se as propriedades de difusão dos filmes Z-AO-GX e Z-AO em diferentes temperaturas e usando simuladores alimentícios como água, ácido acético 3 % e Tenax®. Além disso, produziram-se filmes Z-AO-GX e Z-AO com atividade antimicrobiana, mediante a adição de timol, e avaliou-se a liberação controlada do

timol ao longo do tempo em diferentes simuladores alimentícios e temperaturas. Os dados apresentados nesta parte do trabalho referem-se ao estágio de pesquisa realizado no departamento de *Compliance of Packaging Materials of the Fraunhofer Institute for Process Engineering and Packaging-IVV*, na Alemanha.

Palavras-chave: Filmes compostos à base de zeína. Propriedades de barreira. Propriedades mecânicas. Propriedades de difusão. Queijo Minas padrão. Qualidade do queijo. Embalagem ativa.

ABSTRACT

Due to their chemical composition, foodstuffs may undergo quality and nutritional content deterioration as a consequence of factors such as microbiological contamination, contact with other foodstuffs or chemical compounds, the action of environmental agents as radiation (UV), air (oxygen), temperature variations, among others. Based on that, the food industry, aiming to protect and assure the quality and nutritional content of foodstuffs, is the greatest user of non-biodegradable packaging. In spite of that, the world's environmental concern due to the increase of the contamination and the accumulation of non-biodegradable packaging has promoted the development of biodegradable materials that allows for reducing the negative environmental impact as well as assures the food quality and nutritional content of foodstuffs.

Thereby, food and packaging industries as well as several research institutions have focused their studies on the development of biodegradable packaging from sources such as agricultural residues and agroindustry by-products that are quickly degraded by soil microorganisms due to their natural origin. These packaging, mainly films and coatings, are based on biopolymers such as proteins (zein, collagen, gelatin, among others), carbohydrates (starch, chitosan, alginate, among others) and lipids (waxes and fatty acids).

Zein, the main corn protein, has been used for industrial food and pharmaceutical applications; nonetheless, its use as an industrial packaging is limited due to its inferior physical, mechanical and structural properties compared to traditional plastic packaging. Based on that, several studies have demonstrated that combining two or more biopolymers as well as including plasticizer agents into the matrix of the biodegradable materials allows for improving the functional properties of the zein-based films.

Thus, in the first part of this study, different concentrations of ethanol (zein solvent) and glycerol (plasticizer agent) were evaluated in order to produce homogeneous zein-based films with good functional and mechanical properties. Based on that,

better properties and homogeneity of blend films prepared from zein and oleic acid were obtained with glycerol and ethanol concentrations of 10 % and 95 %, respectively.

After adjusting the composition of the zein-based film, the second part of the research was focused on developing zein-based blend films aiming to improve their functional properties in comparison to simple zein films. Thus, blend films of zein and oleic acid (Z-OA), zein, oleic acid and xanthan gum (Z-OA-XG) as well as zein, oleic acid and gelatin (Z-OA-GE) were developed. The production of Z-OA-GE blend films was prevented due to gelatin denaturation caused by contact with 95 % ethanol. Otherwise, Z-OA-XG films exhibited higher opacity and tensile strength while Z-OA blend films displayed lower water solubility and higher flexibility. Both blend films, Z-OA-XG and Z-OA, showed similar water vapor and oxygen permeability as well as microstructure.

On the third part of the study, both biodegradable materials, Z-OA-XG and Z-OA, were tested as coatings on the conservation of the physicochemical, color and textural characteristics of “Minas padrão” cheese. Cheeses with biodegradable coatings in comparison to cheeses without packaging and with the commercial polyethylene packaging were evaluated throughout a storage period of 56 days. Cheeses with biodegradable coatings exhibited ca. 30 % lower weight loss and avoided microbiological contamination for more than 50 days compared to unpackaged cheeses. Nevertheless, the moisture loss of biodegradable-coated and unpackaged cheeses influenced the texture (high hardness), the proteolysis (lower enzymatic and bacterian activity) and changes on surface color and lipid content (lipid loss) of the cheese in comparison to the cheese with polyethylene packaging.

During this part of the study, the possible migration of small compounds from the coating matrix into the cheese was supposed, and for that reason, a fourth part of the study was accomplished in order to evaluate the diffusional properties of the Z-OA-XG and Z-OA blend films at different temperatures using food simulants such as water, 3 % acetic acid and Tenax®. Furthermore, Z-OA and Z-OA-XG films with antimicrobial activity were produced and the controlled release of thymol, as antimicrobial agent, was assessed along the time at different food simulants and temperatures. The data shown in this part of the study are the results of a research

internship performed in the department of Compliance of Packaging Materials of the Fraunhofer Institute for Process Engineering and Packaging-IVV in Germany.

Keywords: Zein-based blend films. Barrier properties. Mechanical properties. Diffusion properties. “Minas padrão” cheese. Cheese quality. Active packaging.

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1

Introdução geral

1.1 Embalagens para alimentos

A embalagem é essencial para o transporte e distribuição dos alimentos já que facilita sua manipulação, protege a integridade do produto diminuindo os danos físico-mecânicos, preserva a segurança e a qualidade do alimento limitando a contaminação externa, química ou microbiológica, estendendo sua vida de prateleira (VAN WILLIGE, 2002; COLES, 2003; THARANATHAN, 2003; DAINELLI et al., 2008; VALLE et al., 2008; RESTUCCIA et al., 2010).

O desenvolvimento de embalagens para alimentos é determinado pela proteção, higiene, qualidade do produto, conveniência e facilidade para o transporte e manipulação e, além disso, o seu desenho deve satisfazer as necessidades e desejos do consumidor garantindo sempre sua saúde e segurança (COLES, 2003). Nos últimos 30 anos, inovações tais como embalagens plásticas com atmosfera modificada, materiais que podem ser aquecidos em forno microondas e embalagens inteligentes que indicam o estado e a qualidade do alimento durante a vida de prateleira têm sido desenvolvidas com o intuito de garantir a qualidade do produto por maior tempo (DAINELLI et al., 2008) e facilitar o preparo para o consumo do alimento.

Ao longo da história, diferentes tecnologias de embalagens têm sido desenvolvidas de acordo com as necessidades e inovações do momento. Dentre os materiais de embalagens que continuam em uso até hoje destacam-se a folha de flandres, o vidro, o papel e o plástico, porém, na atualidade, o plástico é o material mais frequentemente usado para alimentos em razão de baixo preço, leveza, flexibilidade, resistência, termo-estabilidade e versatilidade, permitindo a elaboração de embalagens para todo tipo de produtos (líquidos, semi-sólidos, sólidos) e de diferentes formatos (sacolas, garrafas) (KIRWAN; STRAWBRIDGE, 2003; THARANATHAN, 2003; LAGARON; LÓPEZ, 2011).

Além das características anteriores, os plásticos apresentam boas propriedades funcionais, tais como resistência ao rasgo e à tração e barreira ao oxigênio e aromas, porém, apresentam maior permeabilidade ao vapor de água em comparação com embalagens como o vidro ou a lata. Além disso, os plásticos não

são biodegradáveis e, portanto, geram um aumento excessivo da contaminação ambiental como resultado da maior disposição desses materiais que demoram anos e até séculos em degradar-se (BÓSQUEZ, 2003; THARANATHAN, 2003; CUTTER, 2006; GHANBARZADEH et al., 2006; BALDWIN, 2007; VALLE et al., 2008).

Segundo a Agência de Proteção Ambiental (EPA) dos Estados Unidos de América (EUA), em 2010, os EUA produziram 250 milhões de toneladas de resíduos sólidos municipais dos quais somente 34,1 % foram reciclados. O material plástico somou 31,04 milhões de toneladas sendo 13,68 milhões de toneladas correspondentes às embalagens plásticas. De acordo com o relatório, os resíduos plásticos são o segundo material menos reciclado, com somente 8,2 % de reciclagem (EPA, 2013). O excessivo uso de embalagens plásticas e sua baixa taxa de reciclagem têm gerado preocupação em nível mundial, levando os governos e os consumidores a exigirem a diminuição do seu uso ou sua substituição para reduzir a acumulação de resíduos contaminantes do ambiente (COLES, 2003).

Esse problema repete-se em todos os países do mundo, portanto é necessário buscar novas fontes de materiais compostáveis, biodegradáveis ou comestíveis que tenham propriedades funcionais semelhantes ao plástico e permitam a substituição das embalagens plásticas. Baseado nisso, há mais de 30 anos, diferentes tipos de embalagens, degradáveis, biodegradáveis e/ou comestíveis têm sido desenvolvidas (AURAS et al., 2006; NOBILE et al., 2008; FAJARDO et al., 2010).

1.2 Embalagens degradáveis

Entende-se por degradação o processo sofrido pelos materiais que gera mudanças em sua estrutura química, nas propriedades físicas ou na aparência. Atualmente, existem alguns materiais que tem a característica de degradar-se no ambiente em um período de tempo menor que o plástico normal. De acordo com o padrão *American Society for Testing and Materials* (ASTM D883-11), esses materiais podem classificar-se em: compostáveis, biodegradáveis, fotodegradáveis, degradáveis hidroliticamente ou degradáveis oxidativamente.

Os materiais compostáveis são aqueles que sofrem degradação biológica durante compostagem e decompõem-se em biomassa, dióxido de carbono, minerais e água. Sua velocidade de degradação deve ser semelhante à velocidade de outro material compostável conhecido e além disso, não podem deixar resíduos visíveis ou tóxicos (ASTM D883-11).

Os materiais biodegradáveis correspondem àqueles que podem ser degradados em biomassa, dióxido de carbono e água por microrganismos presentes (não adicionados), como bactérias, algas ou fungos, sendo que as condições e tempo de decomposição são variáveis (ASTM D883-11).

Por sua vez, os materiais fotodegradáveis, degradáveis hidroliticamente ou degradáveis oxidativamente (oxo-degradáveis) são aqueles que decompõem-se pela ação de radiação solar, calor, água e/ou oxigênio (ASTM D883-11).

Os plásticos são considerados materiais não degradáveis devido à sua composição e origem (derivados do petróleo) e ao longo tempo de decomposição, que varia de séculos até milhares de anos; portanto, para diminuir o tempo de degradação e tornar o plástico degradável é necessário a adição de um aditivo químico ou iniciador de degradação.

Na atualidade, os materiais oxo-degradáveis são os mais usados na indústria e, como dito anteriormente, sua degradação é possível por meio da adição de um catalisador químico ou iniciador de degradação. O catalisador é, geralmente, um composto orgânico com metais de transição, tais como níquel, manganês, ferro ou cobalto que, por ação da radiação solar e do calor, acelera a reação oxidativa, que rompe as moléculas poliméricas (polietileno por exemplo) deteriorando sua resistência, tornando o material mais frágil e, em consequência, fracionando-o em pequenos pedaços (EUROPEAN BIOPLASTIC, 2009; DEFRA, 2010). Porém, segundo uma pesquisa desenvolvida pela *Loughborough University* e pelo *Department for Environment, Food and Rural Affairs* (DEFRA), o problema ambiental ainda continua, visto que esses pequenos fragmentos permanecem por muitos anos no ambiente onde são dispostos sem sofrer decomposição (DEFRA, 2010). Além disso, esses fragmentos não podem ser reciclados em razão da presença do

iniciador de degradação, que pode ativar-se e iniciar a degradação do material durante o processo de produção.

Devido a esses inconvenientes, os materiais biodegradáveis, comestíveis e compostáveis são as únicas alternativas viáveis que permitiriam reduzir significativamente o uso de embalagens plásticas, diminuindo a contaminação ambiental. Por essa razão, inúmeras pesquisas têm como finalidade desenvolver embalagens com características e propriedades funcionais semelhantes as do plástico, e que sejam aplicáveis na indústria de alimentos (KROCHTA, 2002; THARANATHAN, 2003; AURAS et al., 2006; NOBILE et al., 2008; FAJARDO et al., 2010).

1.3 Embalagens biodegradáveis: filmes e coberturas

Os materiais biodegradáveis, também conhecidos como biopolímeros ou bioplásticos, têm sido desenvolvidos em forma de filmes ou coberturas para serem aplicados, principalmente, na indústria de alimentos. Em geral, esses termos são usados indistintamente para indicar que a superfície de um alimento foi coberta com uma fina camada de material de composição específica, porém existe diferença entre filme e cobertura (PAVLATH; ORTS, 2009). Chama-se filme o material produzido de forma separada para um uso específico. Pode ser usado para cobrir, envolver ou como camada de separação e também permite que sejam avaliadas as propriedades funcionais e estruturais do material. Por outro lado, a cobertura é a camada de material formada diretamente sobre a superfície do produto que se deseja proteger, tornando-se parte do alimento (KROTCHA, 2002).

Estes materiais podem ser produzidos a partir de matéria prima (MP) de origem microbiana, agrícola ou agroindustrial. A MP de origem microbiana é obtida por meio de processos biotecnológicos nos quais um organismo vivo, seja bactéria, levedura, alga ou fungo, transforma um substrato (nutriente) em uma substância que pode ser usada para a fabricação de filmes ou coberturas. Destacam-se o ácido láctico produzido por bactérias ácido lácticas para a produção do bioplástico conhecido como PLA ou ácido polilático, a goma xantana, que é um biopolímero produzido pela

bactéria *Xanthomonas campestris*, o alginato obtido a partir das algas marrons, entre outros (KAMPF; NUSSINOVITCH, 2000; THARANATHAN, 2003; VEIGA-SANTOS et al., 2005a; AURAS et al., 2006; NETTLES, 2006).

A MP agrícola e agroindustrial é obtida dos produtos agrícolas, florestais, de coprodutos ou resíduos de processos industriais através de diferentes métodos de extração. Na Tabela 1.1 apresentam-se alguns produtos agrícolas e agroindustriais a partir dos quais extraem-se substâncias para a produção de filmes e coberturas biodegradáveis.

Tabela 1.1. MP agrícola e agroindustrial para obtenção de filmes e coberturas

MP	Fonte	Sustância extraída	Referencia
Soro de leite	Resíduo da indústria leite	Proteína do soro	Nettles (2006)
Milho, trigo, mandioca	Grãos, tubérculos	Amido	Nettles (2006), Dhanapal et al. (2012)
Milho	Coproducto da moagem	Zeína	Liu et al. (2005), Bourtoom (2008)
Esqueleto de crustáceos	Resíduo da indústria camarão	Quitossana	Tharanathan (2003), Nettles (2006), Dhanapal et al. (2012)
Pele, ossos	Resíduo da indústria carne e peixe	Colágeno, gelatina	Tharanathan (2003), Nettles (2006)
Árvores	Resíduo da indústria madeira, papel	Celulose e derivados	Nettles (2006), Dhanapal et al. (2012)
Trigo	Grão	Glúten de trigo	Nettles (2006)
Soja	Grão	Extrato proteína soja	Nettles (2006)
Casca frutas cítricas	Resíduo da indústria suco, polpa e frutas	Pectina	Tharanathan (2003), Nettles (2006), Dhanapal et al. (2012)
Sementes oleaginosas	Produto da indústria de óleos	Triglicerídeos, ácidos graxos	Nettles (2006), Dhanapal et al. (2012)

Ressalta-se que o uso de um resíduo ou coproduto da agroindústria para a produção de embalagens biodegradáveis apresenta grandes vantagens para a indústria, visto que elimina os problemas de disposição final do resíduo, agrega valor através da geração de um novo produto (DANGARAN et al., 2009) e evita a competição pelo uso de fontes usadas como alimento.

Como observado na Tabela 1.1, as substâncias extraídas e usadas para a produção de filmes e coberturas biodegradáveis são biomoléculas como polissacarídeos (quitosana, celulose, pectina, amido), proteínas (gelatina, colágeno, proteínas do soro, zeína, extrato de proteína de soja) e lipídeos (triglicerídeos e ácidos graxos). Assim, os filmes e as coberturas são classificados segundo o tipo de biomolécula usada para seu preparo conforme ilustra a Figura 1.1.

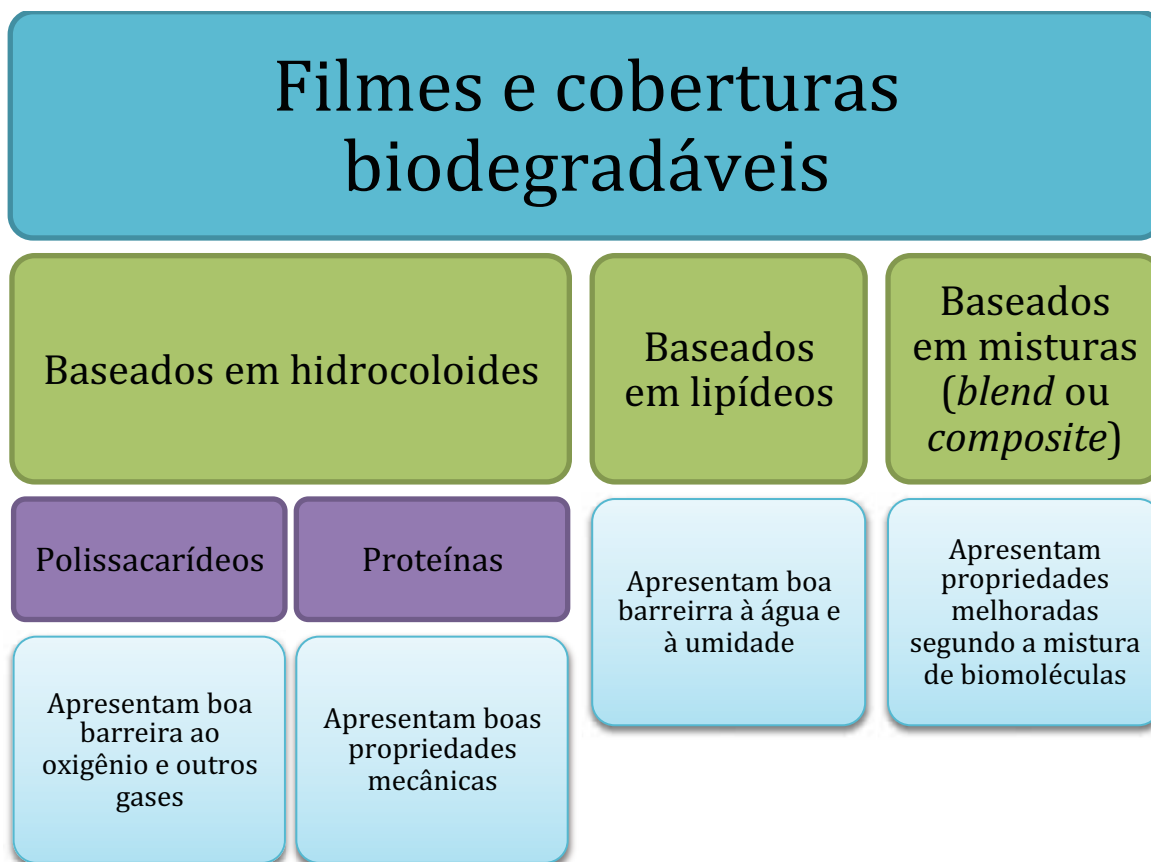


Figura 1.1. Classificação dos filmes e coberturas

Os filmes e as coberturas à base de hidrocoloides são formados a partir de polissacarídeos ou proteínas, os filmes e coberturas lipídicos são produzidos a partir de compostos do grupo dos lipídeos, e por último, os filmes e coberturas compostos (*blend* ou *composite*) são aqueles fabricados a partir da mistura de duas ou mais biomoléculas (KROTCHA, 2002; THARANATHAN, 2003; BOURTOOM, 2008; PAVLATH; ORTS, 2009; DHANAPAL et al., 2012).

Como toda embalagem, os filmes e as coberturas biodegradáveis devem satisfazer alguns requerimentos, tais como (COLES, 2003; THARANATHAN, 2003):

- Proteger o produto contra danos mecânicos e físicos durante as etapas de transporte e distribuição dos produtos.
- Preservar o produto de contaminação microbiana ou mudanças químicas ou bioquímicas durante sua vida de prateleira.
- Gerar uma barreira seletiva aos gases, lipídeos ou vapor de água, permitindo a conservação da qualidade do produto ao longo do tempo.
- Servir como meio de comunicação e *merchandising*, criando impacto visual no consumidor.

Assim, o desempenho dos filmes e das coberturas biodegradáveis está diretamente relacionado com as propriedades funcionais que dependem da estrutura e das propriedades físicas e químicas das macromoléculas a partir das quais são produzidos.

Dessa forma, os filmes e as coberturas à base de polissacarídeos são reconhecidos por apresentarem boa barreira contra o oxigênio e outros gases, permitindo gerar uma atmosfera modificada dentro da embalagem e estender a vida de prateleira do alimento, além de evitar a oxidação de lipídeos e o desenvolvimento do sabor de ranço. Porém, devido ao seu caráter polar, apresentam alta permeabilidade ao vapor de água (PAVLATH; ORTS, 2009; DHANAPAL et al., 2012). As propriedades funcionais desses filmes e coberturas são influenciadas pela quantidade de pontes de hidrogênio que formam entre as cadeias de polissacarídeos, pelas ramificações presentes em sua estrutura, pela presença de cargas elétricas e grupos substituintes de açúcares e pelo seu peso molecular. Em

geral, os polissacarídeos ramificados formam filmes e coberturas frágeis com baixas propriedades mecânicas, enquanto que os polissacarídeos lineares de alto peso molecular e de caráter não iônico formam, facilmente, filmes e coberturas mais fortes e com melhores propriedades mecânicas que os polissacarídeos ramificados (NIETO, 2009).

As propriedades funcionais dos filmes e coberturas produzidos a partir de proteínas são influenciadas pela composição e pela distribuição dos aminoácidos, da carga superficial, da hidrofobicidade ou hidrofiliabilidade, do peso molecular, do ponto isoelétrico e da estrutura terciária da proteína (DANGARAN et al., 2009). Esses tipos de filmes e coberturas são conhecidos por sua estabilidade física e mecânica (PAVLATH; ORTS, 2009), as quais são devidas à estabilidade da matriz do filme ou cobertura gerada por interações eletrostáticas, pontes de hidrogênio, forças de van der Waals, ligações covalentes e pontes dissulfeto (KROCHTA, 2002; PADUA; WANG, 2002; BÓSQUEZ, 2003; CUTTER, 2006; BOURTOOM, 2008; BOURTOOM, 2009; DANGARAN et al., 2009). Adicionalmente, as propriedades de barreira ao vapor de água, oxigênio e outros gases dos filmes e das coberturas à base de proteínas dependem diretamente do seu caráter polar, portanto, estas variam de uma proteína para outra e de um filme ou cobertura para outro (CUQ et al. 1995; KROCHTA, 2002; PADUA; WANG, 2002).

Os filmes e as coberturas à base de lipídeos são os materiais biodegradáveis mais antigos, usados inicialmente na China, no século XII, como recobrimento para frutas, principalmente laranja e limão, para evitar perda de água por transpiração (THARANATHAN, 2003). Exibem uma ótima barreira ao vapor de água em razão de seu caráter hidrofóbico e, por isso, são os materiais menos permeáveis à umidade (DEBEAUFORT; VOILLEY, 2009); porém, apresentam uma alta permeabilidade aos lipídeos e gases (PAVLATH; ORTS, 2009), sofrem oxidação gerando problemas de sabor a ranço, além de apresentarem problemas de homogeneidade, aparência oleosa (CUQ et al., 1995; GENNADIOS et al., 2007) e reduzidas propriedades mecânicas (BOURTOOM, 2008).

No último grupo de filmes e coberturas encontram-se os materiais compostos (*blends* ou *composites*) que são misturas entre as biomoléculas dos outros três grupos. Para obter um filme ou cobertura composto é necessário uma mistura de, no

mínimo, duas biomoléculas. Como mencionado, cada tipo ou grupo de filme e cobertura apresenta, pelo menos, uma propriedade funcional destacada com relação aos outros, contudo, também exhibe deficiências em suas outras propriedades. Por essa razão, quando duas ou mais macromoléculas são misturadas, é possível melhorar as propriedades funcionais (mecânicas e de barreira) dos filmes e coberturas produzidos, devido à combinação das propriedades individuais de cada macromolécula (BOURTOOM, 2008; DEBEAUFORT; VOILLEY, 2009). As macromoléculas a serem misturadas e suas concentrações dependerão da especificação do alimento onde será usado o filme ou a cobertura (THARANATHAN, 2003), o que permite uma enorme quantidade de possibilidades na fabricação de filmes e coberturas compostos para a indústria de alimentos.

1.4 Filmes e coberturas biodegradáveis à base de zeína

A zeína, principal proteína do milho, é extraída do coproduto conhecido como farinha de glúten de milho (*corn gluten meal*) obtida durante a moagem úmida do grão (PADUA; WANG, 2002) para a produção de etanol e amido (FORATO et al., 2004; GHANBARZADEH et al., 2006; ZHANG et al., 2011). Sua extração é desejável em razão do baixo preço da farinha de glúten, que é usada principalmente para alimentação animal, enquanto que a zeína possui um alto preço, agrega valor ao coproduto e contribui para melhorar o lucro da indústria (GHANBARZADEH et al., 2006).

A zeína tem sido usada desde há muito tempo na indústria de alimentos devido à sua habilidade para a formação de filmes e coberturas biodegradáveis (GENNADIOS et al., 1997; KROTCHA, 2002; PADUA; WANG, 2002; NOBILE et al., 2008; ZHANG et al., 2011; WU et al., 2012). Essa proteína apresenta características hidrofóbicas devido à sua grande proporção de aminoácidos não polares (LIU et al., 2005; BOURTOOM, 2008; DANGARAN et al., 2009; ZHANG et al., 2011) e à deficiência em aminoácidos carregados positiva ou negativamente (SHUKLA; CHERYAN, 2001; DANGARAN et al., 2009). É uma proteína tipo prolamina e, por esta razão, para a formação de filmes ou coberturas, a zeína deve ser solubilizada

em soluções aquosas de etanol ou isopropanol, pois não é solúvel em água (PADUA; WANG, 2002; FORATO et al., 2004; KIM et al., 2004; BOURTOOM, 2008; ZHANG et al., 2011; WU et al., 2012).

Os filmes e as coberturas produzidos a partir de zeína apresentam aparência brilhante, são resistentes, impermeáveis a compostos lipídicos (DANGARAN et al., 2009), hidrofóbicos, resistentes ao ataque microbiano, biodegradáveis, têm excelente compressibilidade (SHUKLA; CHERYAN, 2001; THARANATHAN, 2003; LIU et al., 2005; NOBILE et al., 2008), baixa permeabilidade ao vapor de água, comparados a outros filmes e coberturas biodegradáveis de origem agrícola (GHANBARZADEH et al., 2007; BOURTOOM, 2008; DANGARAN et al., 2009) e são pouco solúveis em água (KROTCHA, 2002), porém, são frágeis (PADUA; WANG, 2002; KIM et al., 2004; LAWTON, 2004; BOURTOOM, 2008).

A fragilidade dos filmes e coberturas à base de zeína pode ser diminuída mediante a adição de agentes plastificantes ou agentes de reticulação (*crosslinking*) que permitem torná-los mais flexíveis, afetando, assim, as propriedades mecânicas, além das propriedades de barreira (PADUA; WANG, 2002; KIM et al., 2004). O uso de agentes de reticulação, como por exemplo o formaldeído, é proibido para filmes e coberturas que serão usados na indústria de alimentos devido à sua toxicidade (BOURTOOM, 2008), portanto, a adição de agentes plastificantes é o mais recomendável. Os agentes plastificantes aprovados pela FDA (*Food and Drug Administration*) para serem usados em embalagens que estejam em contato com alimentos são glicerol, propilenoglicol, ácidos graxos (BOURTOOM, 2008; LAWTON, 2004) e açúcares (GHANBARZADEH et al., 2007).

O agente plastificante afeta as interações entre as moléculas de proteínas, diminuindo as forças intermoleculares das cadeias e, como consequência, modifica as propriedades do filme, diminuindo sua rigidez, incrementando sua flexibilidade, sua alongação e sua permeabilidade. O efeito nas propriedades do material depende da quantidade (PENA-SERNA, LOPES-FILHO, 2013) e do tipo de agente plastificante usado (KROCHTA, 2002). O plastificante mais usado nos filmes à base de zeína é o glicerol, contudo, a interação entre a zeína e o glicerol é muito frágil e o excesso de glicerol migra através do filme até a superfície, gerando uma aparência oleosa (PADUA; WANG, 2002; PENA-SERNA, LOPES-FILHO, 2013).

Conforme citado, os filmes e as coberturas de zeína apresentam baixa permeabilidade ao vapor de água quando comparados a outros filmes e coberturas biodegradáveis, porém, quando comparados ao polietileno de baixa densidade (LDPE) essa propriedade precisa ser melhorada (KROCHTA, 2002; PADUA; WANG, 2002).

Cuq et al. (1995) e Wang e Padua (2006) demonstraram que a adição de lipídeos, especificamente de ácido oleico, permite melhorar a barreira ao vapor de água dos filmes e coberturas de zeína, assim como sua flexibilidade. Dessa forma, a produção de filmes e coberturas compostos (*blends*) de zeína e ácido oleico apresentam propriedades funcionais (barreira ao vapor de água e propriedades mecânicas) melhoradas com respeito ao material produzido só com a zeína (KROCHTA, 2002; PADUA; WANG, 2002; WANG; PADUA, 2006).

Adicionalmente, a permeabilidade ao oxigênio também é uma propriedade importante para a conservação dos alimentos uma vez que o oxigênio pode gerar degradação dos componentes do alimento por oxidação, produzindo perda de qualidade nutricional, sabores desagradáveis ou mudanças de cor (BOURBON et al., 2011).

Os filmes e as coberturas de zeína são reconhecidos por serem boas barreiras à transferência de O₂ e CO₂ (THARANATHAN, 2003; BOURTOOM, 2008; TIHMINLIOGLU et al., 2010), apresentando permeabilidades menores que o LPDE ou PS (poliestireno) e muito semelhante às permeabilidades dos copolímero álcool de etileno vinil e cloreto de polivinilideno, que são os plásticos com as melhores barreiras ao oxigênio (KROCHTA, 2002). Porém, os filmes e as coberturas de zeína apresentam menor barreira a gases quando comparados aos materiais biodegradáveis produzidos a partir de polissacarídeos (PAVLATH; ORTS, 2009).

Por essa razão, a produção de filmes e coberturas compostos à base de zeína e de um polissacarídeo, como por exemplo a goma xantana, poderia ajudar a melhorar a barreira a gases. De Almeida et al. (2010) relataram que a adição de goma xantana ao filme de zeína-ácido oleico melhorou a homogeneidade do filme, porém não avaliaram as propriedades de barreira do filme.

1.5 Estudos realizados com coberturas à base de zeína em alimentos

Apesar de sua importância, poucos estudos têm sido realizados para avaliar o desempenho dos filmes e coberturas à base de zeína como embalagens na indústria de alimentos, demonstrando que esta área ainda continua em desenvolvimento. Ainda, os estudos estão focados, principalmente, na pesquisa básica, embora seja fundamental o conhecimento da eficácia e dos benefícios do uso desses tipos de materiais biodegradáveis e comestíveis na conservação da qualidade dos alimentos.

Zapata et al. (2008) recobriram tomates (*Solanum lycopersicum*) com coberturas preparadas a partir de zeína com adição de ácido oleico (8 % w/w) como agente plastificante e observaram que o processo de amadurecimento foi retardado devido à redução na taxa de respiração e produção de etileno. Também observaram a manutenção da concentração de açúcares e ácidos orgânicos, o que levou a uma maior aceitação dos frutos por parte dos consumidores.

Segundo o estudo realizado por Scramin et al. (2011) sobre a perda de massa de peras (*Pyrus communis* L.) recobertas com coberturas à base de zeína com ácido oleico (0,25 % w/w), as frutas revestidas mantiveram sua massa durante a vida de prateleira, demonstrando, assim, a eficácia da cobertura de zeína.

Lin et al. (2009) demonstraram que o recobrimento de almôndegas de peixe com coberturas de zeína adicionadas com propilenoglicol (PEG) como agente plastificante e três diferentes tipos de agentes antioxidantes permitiram reduzir significativamente a perda de massa em até 72 % num período de 15 dias de armazenamento.

De acordo com o reportado por Gennadios et al. (2008), a aplicação de coberturas de zeína em pedaços de carne de porco pré-cozido armazenados a 4 °C por 6 e 9 dias diminuiu a oxidação lipídica, mas não reduziu a perda de umidade.

Embora diversos estudos tenham sido realizados com diferentes alimentos, até o presente momento não foi localizado na literatura nenhum trabalho desenvolvido sobre a aplicação de coberturas a base de zeína para a conservação

da qualidade de produtos lácteos, tal como o queijo. Esse fato mostra um vasto potencial para pesquisas aplicadas, visto a importância da indústria de queijos no mundo, e especificamente, no continente sul americano.

1.6 Queijo e seu mercado

O termo queijo é o nome genérico dado a um grupo de produtos lácteos fermentados que são produzidos em diferentes texturas, formas, sabores, odores e cores. Acredita-se que existe no mundo, em torno de 1000 tipos de queijo.

De acordo com a Portaria nº 146 de 1996 do Ministério de Agricultura e Abastecimento e da Reforma Agrária, válida para os países membros do MERCOSUL (Mercado Comum do Sul), o queijo é o produto fresco ou maturado que se obtém por separação parcial do soro do leite ou leite reconstituído (integral, parcial ou totalmente desnatado), ou de soros lácteos coagulados pela ação física do coalho, de enzimas específicas, de bactérias específicas, de ácidos orgânicos, isolados ou combinados, todos de qualidade apta para uso alimentar, com ou sem agregação de substâncias alimentícias e/ou especiarias e/ou condimentos, aditivos especificamente indicados, substâncias aromatizantes e matérias corantes (BRASIL, 1996).

O queijo é um dos alimentos mais completos devido ao seu valor nutricional, contendo proteínas, vitaminas, lipídeos e minerais (BELITZ et al., 2009), principalmente cálcio e fósforo (PIOVESAN, 2004). Em geral, o queijo é rico em caseína e gorduras que estão presentes no leite, além de pequenas quantidades de constituintes solúveis em água como proteínas do soro, lactose e vitaminas hidrossolúveis, sendo que a maior parte dessas substâncias permanece no soro (O'CONNOR; O'BRIEN, 2000).

Segundo a Portaria nº 146 (BRASIL, 1996), os queijos podem ser classificados de acordo com o conteúdo de matéria gorda no extrato seco e o conteúdo de umidade, como é apresentado na Figura 1.2.

O consumo mundial de queijo tem sido incrementado ao longo do tempo em resposta à grande variedade de queijos disponível no mercado, seu alto valor nutricional e à compatibilidade deste alimento com as tendências atuais de consumo e processamento de alimentos preparados (O'CONNOR; O'BRIEN, 2000). Segundo projeções da OECD-FAO (*Organisation for Economic Co-operation and Development-Food and Agriculture Organization*), espera-se um aumento de 6 % no consumo per capita mundial até 2018 e um incremento de 15 % na produção (CREA, 2011). No caso da América do Sul, a produção de queijo aumentou 2,23 % em 2013 com relação ao ano anterior, onde a Colômbia e o Brasil contribuíram com o 4,78 % e 3,81 %, respectivamente (FAOSTAT, 2015).

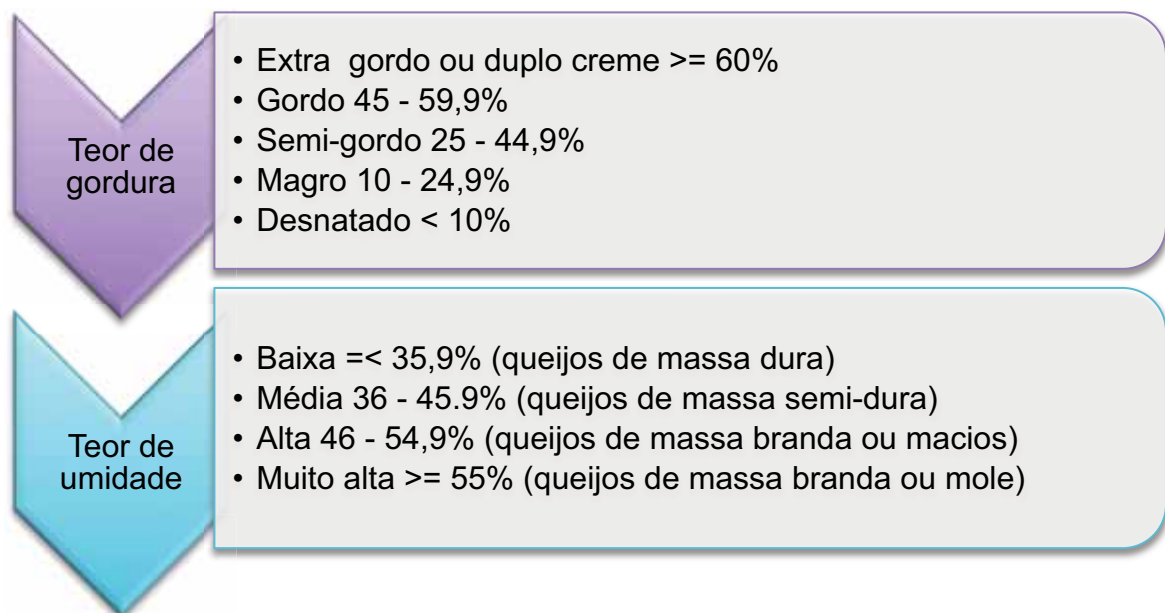


Figura 1.2. Classificação dos queijos de acordo com o teor de matéria gorda no extrato seco e umidade

Esses indicadores esboçam um mercado crescente, que apresenta grandes oportunidades para os países sul americanos, com possibilidade de incrementar a comercialização de seus produtos no exterior. Esse fato poderia permitir a ampliação da comercialização de produtos com denominação de origem e/ou produtos diferenciados que não sejam produzidos em outros países. Assim, queijos como o Minas Padrão, produto com denominação de origem, produzido unicamente no Brasil, poderia ter grande potencial no mercado exterior. Adicionalmente, a diferenciação dos produtos brasileiros mediante o uso de embalagens

biodegradáveis e comestíveis poderia ser uma outra opção viável para melhorar o comércio no mercado europeu, cujos consumidores apreciam os produtos ecológicos.

1.7 Queijo Minas Padrão

O queijo Minas Padrão, também conhecido como Minas curado, Minas prensado ou Minas pasteurizado (INMETRO, 2006) é um produto brasileiro desenvolvido localmente no estado de Minas Gerais. Além do queijo Minas Padrão, destacam-se os queijos Prato, Minas frescal, Minas meia cura, Reino, Requeijão e de coalho como queijos de origem brasileira (SEBRAE, 2008).

O Minas padrão é um queijo maturado produzido artesanalmente e, junto com o Minas frescal, foi um dos primeiros queijos produzidos no Brasil. Sua importância está ligada à fabricação do famoso pão de queijo mineiro (MARTINS, 2006; SEBRAE, 2008) e também por ter denominação de origem e reconhecido pelo IPHAN (Instituto do Patrimônio Histórico e Artístico Nacional) como patrimônio nacional brasileiro (MONTELEONE, 2008).

É produzido a partir de leite de vaca, é de massa crua, semi-duro, coagulado por adição de coalho, com um teor de umidade em torno de 42-44 %, consistência macia, seco e firme, de sabor pronunciado, levemente ácido, de cor branca a bege no interior, com uma fina casca amarelada formada nos primeiros 10 dias da maturação. Seu processo de maturação é de aproximadamente 30 dias (INMETRO, 2006; SEBRAE, 2008).

Na atualidade, o queijo Minas Padrão é comercializado e distribuído em embalagens plásticas de polietileno, capazes de assegurar a qualidade do queijo por um período de até 150 dias, tempo de validade do produto (segundo comunicações do Laticínios Balkis), porém, uma vez consumido o queijo, a embalagem torna-se um problema ambiental como consequência de sua não biodegradabilidade.

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2

Influence of ethanol and glycerol concentration over functional and structural properties of zein-oleic acid films

Abstract

This study was performed in order to determine the effect of the addition of different concentrations of glycerol and ethanol over functional and structural properties of zein-oleic acid films. Films were prepared from zein and oleic acid formulations, containing: 0, 10, 20 and 30 % (w/w) of glycerol as plasticizer and 75, 80, 85, 90 and 95 % (v/v) of ethanol as zein solvent. Water vapor permeability (WVP) at 4 and 24 °C, opacity, water solubility and structural behavior of the film were assessed. The film water barrier properties, WVP and water solubility, were increased when higher ethanol concentration and lower glycerol concentration were used. Furthermore, WVP at 4 °C was lower than WVP at 24 °C due to the crystalline solid state of oleic acid at lower temperatures. Likewise, opacity, homogeneity and structure of the composite film were improved as ethanol increased and glycerol lowered.¹

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2.1 Introduction

Non-biodegradable materials as plastics are frequently used for food packaging, increasing waste accumulation and intensifying environmental pollution. Nowadays, with the world's environmental concern, different kind of biodegradable packaging such as biodegradable and/or edible films have been developed (GHANBARZADEH et al., 2006).

Zein is the main corn protein and is one of the materials used to produce biodegradable films for different industrial applications (KROCHTA, 2002; RYU et al., 2002). The protein is commercially obtained from the corn wet-milling process and is an alcohol-soluble protein (LAWTON, 2004).

Several authors have reported zein solubilization in aqueous-ethanol solutions of 75 % (LAI; PADUA, 1998; WANG et al., 2005), 80 % (GHANBARZADEH et al., 2006), 90 % (KIM et al., 2004; LAWTON, 2004) and 95 % (WANG; PADUA, 2006; KU; SONG, 2007), nevertheless, its effect over some functional and structural properties have not been studied until now.

Also, several authors have demonstrated that the addition of other components such as: polyols, fatty acids, polysaccharides and others, allows for the formation of composite film with improved functional properties. Padua and Wang (2002) found that producing a composite film from zein and oleic acid allowed for the reduction of the film water solubility and water vapor permeability, therefore, improving the film water barrier properties.

Furthermore, glycerol is often used as plasticizer agent for reducing zein film brittleness (PADUA; WANG, 2002) and it is added at concentrations between 0 and 30 % (LAWTON, 2004; KU; SONG, 2007), nonetheless, plasticizer addition may affect film functional properties (KROCHTA, 2002; PADUA; WANG, 2002).

Based on the wide composition reported in literature to produce zein films, the aim of this work was to investigate the effect of different glycerol and ethanol concentrations over some functional and structural properties of zein-oleic acid film

such as: transparency/opacity, water solubility, water vapor permeability, homogeneity and, superficial and cross-section microstructure.

2.2 Material and methods

2.2.1 Material

Corn-zein was obtained from Sigma-Aldrich, ethanol (99.5 %) was supplied by Araçaprolab (Araçatuba, Brazil) and diluted with distilled water to prepare the different solvent concentrations. Glycerol, oleic acid, calcium nitrate and calcium bromide were analytical grade and were supplied by Araçaprolab (Araçatuba, Brazil). The emulsifier (Emulsibier, Brazil) was obtained at the local market.

2.2.2 Film composition and preparation

To prepare films 20 g of zein were added to 100 ml ethanol at 75, 80, 85, 90 and 95 % (v/v). Mixtures were mechanically stirred at 65 ± 0.5 °C for 5 min, then added with 70 g oleic acid, 5 g emulsifier and glycerol at 0, 10, 20 or 30 g, all of them by 100 g zein. Stirring was kept for another 10 min and then 50 ml solution was cast onto a rectangular glass plate. Films were peeled off after drying at room temperature for 24 h and conditioned in desiccator at 57.57 % relative humidity (%RH), 24 ± 2 °C and at least 40 h prior to analyses, according to the standard method of ASTM D618-08.

2.2.3 Measurement of film thickness

Film thickness was measured with a digital micrometer (Digimess, Brazil) with 0.001 mm precision. Five-thickness measurements were taken, one at the centre and four around the perimeter, then, the mean was used in calculations.

2.2.4 Transparency/opacity

Film opacity was determined in a spectrophotometer Bioespectro SP-220 at 600 nm. Film samples were cut into 9 mm × 43 mm rectangular shapes and placed on the internal side of the spectrophotometer cell (CAO et al., 2007; PÉREZ et al., 2009). Four replicates of each film were tested. Opacity was calculated according to Equation (2.1).

$$\text{Opacity} = \frac{A_{600}}{x} \quad (2.1)$$

Where A_{600} is the absorbance at 600 nm and x is the film average thickness.

2.2.5 Water solubility

Film water solubility was defined as the content of dried matter solubilized after 24 h of immersion in water. Film was cut into 2 cm (diameter) disk and dried in an oven at 105 ± 2 °C for 24 h, weighed (initial weight, w_i) and immersed into 50 ml distilled water at 27 ± 2 °C for 24 h under agitation in an orbital shaker MA-410 (Marconi, Brazil) at 76 rpm. After 24 h of immersion, disks were taken out and dried (final weight, w_f) at the same conditions stated before, in order to determine the weight of dried matter that was not solubilized in water (CERQUEIRA et al., 2007; PÉREZ et al, 2009). Three replicates of each film were tested and water solubility was calculated according to Equation (2.2).

$$\text{Water solubility (\%)} = \frac{w_i - w_f}{w_i} \times 100 \quad (2.2)$$

2.2.6 Water vapor permeability

Film water vapor permeability (WVP) was measured gravimetrically according to ASTM E 96/96M-10 desiccant method. Test cells were covered and sealed by the film samples and placed in a controlled chamber (desiccator) maintained at 51 %RH by a saturated solution of calcium nitrate. Silica gel activated at 200 °C was used to maintain 0 %RH inside the test cells. Desiccator was stored at 24±2 °C. Three replicates of each film were tested and WVP was calculated according to Equation (2.3).

$$\text{WVP} = \frac{\text{WVT}}{S(R_1 - R_2)} \times x \quad (2.3)$$

Where WVT is the water vapor transmission and calculated as Equation (2.4):

$$\text{WVT} = \frac{G}{t \times A} \quad (2.4)$$

G is the weight change of the test cell, t is the time during which G occurred and A is the test area (12.57 cm²). G/t was obtained from the slope of the linear portion of the plot of G vs. t . S is the saturation of vapor pressure at test temperature (2985 Pa at 24 °C), R_1 is RH in the test desiccator expressed as a fraction, R_2 is RH inside the test cell expressed as a fraction and x is the film average thickness.

Film WVP was also determined at refrigeration temperature, desiccator was stored at 4 °C. Saturation vapor pressure (S) at 4 °C is 813 Pa, R_1 was measured at 69 % and R_2 was assumed to be 0 %.

2.2.7 Optical microscopy

Optical microscopy was carried out in order to determine film superficial homogeneity. Film was cut in 1 cm² and stained with Xylidine Ponceau solution for 30 min, then washed in a bath of acetic acid 2.5 % for 10 min and finally washed in a distilled water bath for 10 min. The stained film was placed on a glass microscope slide and dried in an oven for 24 h at 37 °C. The dried stained film was fixated with Canada balsam and covered with the cover glass slide. The sample assembly was dried at room conditions for 24 h and then, analyzed with an optical microscope Olympus BX 60, equipped with an image capture system Olympus DP 71, using a magnification of 10x.

2.2.8 Scanning electron microscopy (SEM)

Film superficial and cross-section structure were observed under high vacuum and constant temperature using a Carl Zeiss EVO LS15 scanning electron microscope equipped with a secondary electron detector (SE). Film samples were covered with gold and then images were taken at 15-20 kV at different magnifications from 1000x to 10000x. Cross-section images were obtained by film cryogenic fracture, immersing samples into liquid nitrogen during 2 min. Film samples were fixated to the microscope stubs using a carbon duple face conducting tape.

2.2.9 Statistical Analysis

Statistical analysis was performed with the software Statgraphics centurion, version 16.1. The evaluated factors were ethanol and glycerol concentrations and the response variables were water solubility, opacity and water vapor permeability. Analysis of variance and LSD multiple comparison tests were performed to detect

significant differences in the properties of the films. The significance level used was 0.05.

2.3 Results and discussion

Zein-oleic acid films were smooth, flexible, without visible pores and homogeneous, with the exception of film made with ethanol 75 %, which was totally heterogeneous; hence, that treatment could not be evaluated.

Visual and touch perception of the film changed when ethanol and glycerol concentrations were altered. Higher glycerol addition caused a greasy film surface, which is an undesirable film characteristic. According to Padua and Wang (2002), this is a consequence of the weak interactions between glycerol and zein causing the excess of glycerol to migrate from the inside of the film matrix to the surface. On the other hand, higher ethanol concentration caused a visible higher film opacity and better zein solubilization allowing for a more homogeneous film.

Film transparency/opacity plays an important role when film is intended to cover light sensitive products (CERQUEIRA et al., 2007), in such a case, an opaque film is recommended.

The effect of ethanol and glycerol concentration over opacity can be observed in Figure 2.1. Average thickness of tested samples was 0.140 ± 0.022 mm. In general, zein-oleic acid films showed higher opacity when glycerol and ethanol concentrations were lower and higher, respectively. Analysis of variance showed that ethanol concentration, glycerol concentration and their interaction had a significant statistically effect over film opacity ($p < 0.05$).

The higher opacity displayed by films produced with higher ethanol concentrations is the result of the better zein solubilization which allowed to increase the amount of intermolecular forces between protein chains and between protein and the other film components, therefore, enabled the formation of a stronger and denser matrix which prevented the light to pass through the film.

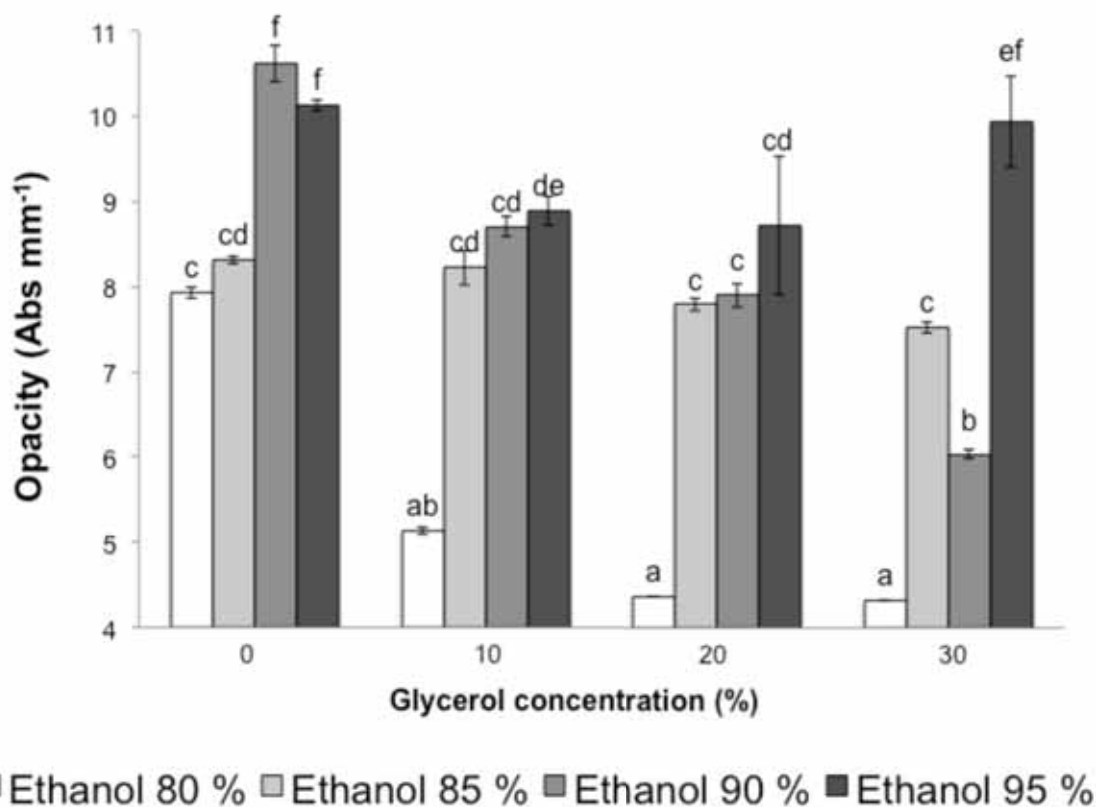


Figure 2.1. Opacity of the films. Reported values are mean \pm standard deviation. Histograms with different letter indicate a statistically significant difference ($p < 0.05$).

On the other hand, the lower opacity showed by zein-oleic acid films with higher glycerol addition was caused by the greater mobility of the protein chains since glycerol is added to create spaces (PADUA; WANG, 2002; TIHMINLIOGLU et al., 2010) that enable the light to pass through the film.

Pérez et al. (2009) reported slightly lower opacity values for blends of cod gelatin with sunflower oil compared with zein-oleic acid films evaluated in this study, in that case, zein-oleic acid film could be a better packing material for light sensitive products.

Keeping the product integrity and providing resistance against water, are important film features, therefore, low water solubility and WVP are extremely desirable (KROCHTA, 2002).

Film water solubility was influenced by ethanol and glycerol concentration as shown in Figure 2.2. In accordance to variance analysis both evaluated factors and their interaction had a statistically significant effect ($p < 0.05$) over the film water solubility. Figure 2.2 shows that film water solubility decreased with higher ethanol and lower glycerol concentration.

Ryu et al. (2002) reported an increment in the water solubility of zein-coated high-amylose cornstarch film when added a mixture of plasticizers (glycerol and polyethylene glycol-PEG). These results are in agreement with the behavior of the zein-oleic acid films when increasing glycerol (plasticizer) addition.

The water solubility of polysaccharide films made from chitosan, galactomannan and agar produced by Cerqueira et al. (2007) were in the order of 22.4-64.86 % and hence, zein-oleic acid films displayed lower water solubility.

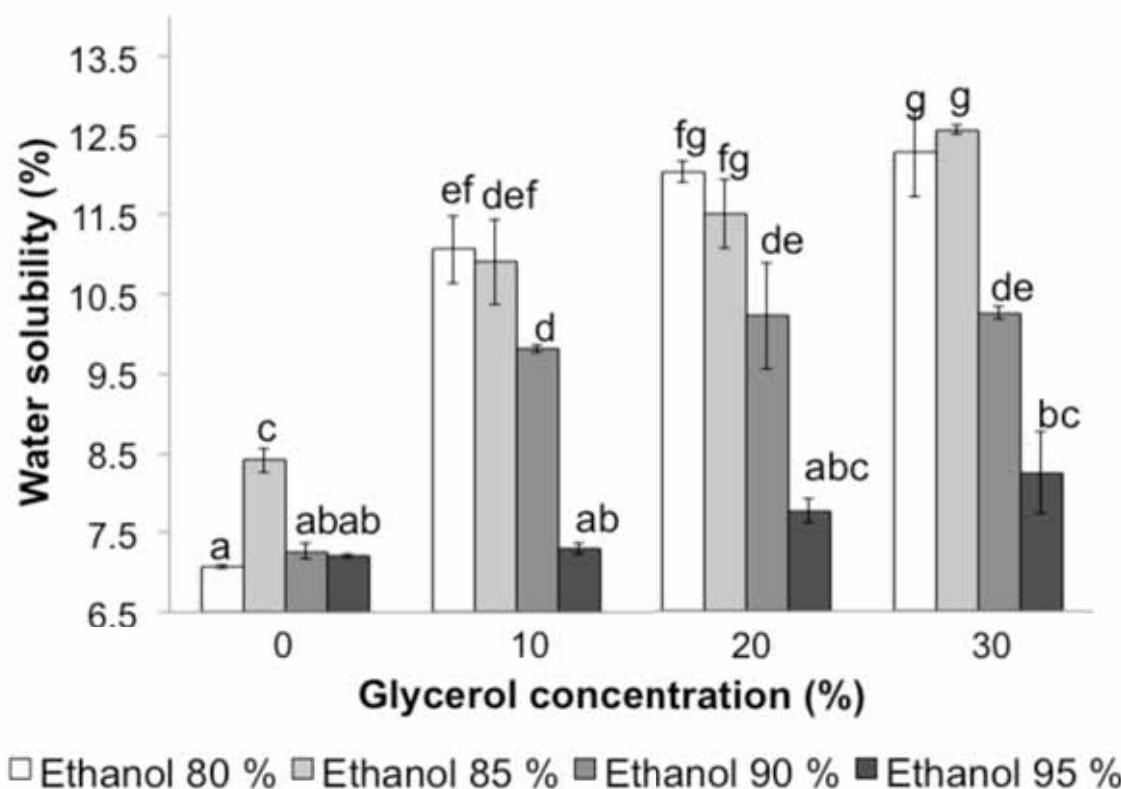


Figure 2.2. Water solubility of the films. Reported values are mean \pm standard deviation. Histograms with different letter indicate a statistically significant difference ($p < 0.05$).

Water solubility and contact angle are related to film hydrophobicity, thus, the greater contact angle the greater hydrophobicity (therefore, lower water solubility). Tihminlioglu et al. (2010) presented a decrease in contact angle for corn-zein coated polypropylene (PP) when increasing glycerol concentration (from 20 to 50 %) and ethanol concentration (from 70 to 95 %). The hydrophobicity of both films, corn-zein coated PP film and zein-oleic acid film, were decreased when higher glycerol concentration was added but presented an opposite behavior with respect to ethanol addition. We strongly believe that the water solubility of the film should decrease when a higher ethanol concentration was used, mainly due to the formation of a stronger and denser net of interactions between protein chains (TIHMINLIOGLU et al., 2010).

Likewise, water vapor permeability of the films is shown in Figure 2.3. Average thickness of tested samples at 24 °C and 4 °C were 0.158 ± 0.042 mm and 0.141 ± 0.027 mm, respectively. Results show that this functional property is influenced by ethanol concentration, glycerol concentration and also, storage temperature.

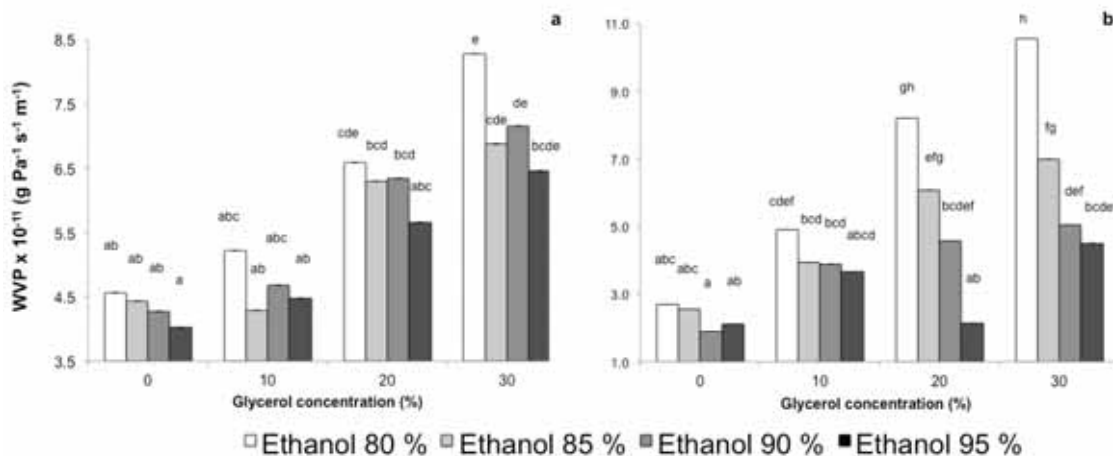


Figure 2.3. Water vapor permeability of the films at 24 °C (a) and 4 °C (b). Reported values are mean \pm standard deviation. Histograms with different letter indicate a statistically significant difference ($p < 0.05$).

In accordance to variance analysis both evaluated factors and their interaction had a statistically significant effect ($p < 0.05$) over the WVP at 4 °C, however, at 24 ± 2 °C only glycerol concentration affected the WVP.

WVP at 24 ± 2 °C are higher than WVP at 4 °C with the exception of films with 80 % ethanol/20 % glycerol and 80 % ethanol/30 % glycerol. Lower WVP at 4 °C was expected since oleic acid is a crystalline solid at lower temperatures while melts at 25 °C. The solid state of oleic acid may prevent the water vapor diffusion and in consequence, the film WVP should decrease (WANG; PADUA, 2006).

Both water barrier properties of the zein-oleic acid films, water solubility and water vapor permeability, were lower when films were produced with higher ethanol and lower glycerol concentration. The higher ethanol concentration allowed for the formation of greater intermolecular forces between protein chains preventing the water vapor migration through the film. Otherwise, glycerol addition increased water solubility and WVP due to the reduction of the film hydrophobicity as a result of the addition of a polar compound and besides, to the fact that glycerol works as a spacer between protein chains decreasing intermolecular forces and allowing for a greater mobility of the protein chains (TIHMINLIOGLU et al., 2010).

In accordance with the results presented by (TIHMINLIOGLU et al., 2010), WVP of corn-zein polypropylene film showed a similar behavior to the zein-oleic acid films, where film WVP decreased when the glycerol concentration was lower and ethanol concentration was higher.

Kokoszka et al. (2010) also reported an increment in the WVP of the whey protein isolate film when added glycerol as plasticizer. Besides, WVP of zein-oleic acid film was lower than the whey protein isolate film.

Zein-oleic acid films produced in the present work showed a similar WVP behavior compared with the zein-oleic acid films produced by (KU; SONG, 2007) which were prepared with 40 % zein, 70 % oleic acid, 4 % emulsifier, 0 % glycerol and diluted with ethanol 95%.

Film homogeneity assessed through optical microscopy was possible when samples were stained with Xylidine Ponceau, which allowed for protein detection and therefore, integration of film components was evaluated (DE ALMEIDA et al., 2010).

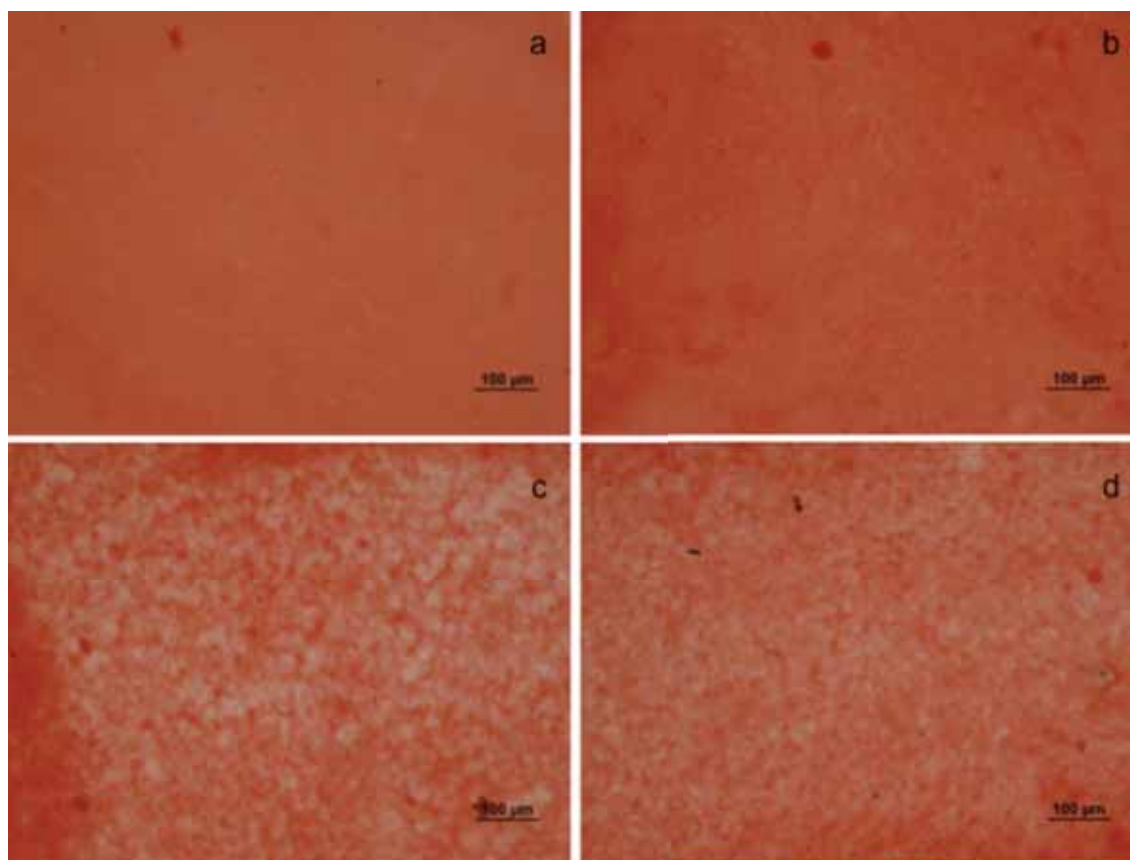


Figure 2.4. Optical micrograph of film surface, 10x. Films prepared with ethanol solution of 90 % and glycerol addition of 0 % (a), 10 % (b), 20 % (c) and 30 % (d).

As shown in Figure 2.4, zein-oleic acid films formed a more homogeneous matrix when added lower plasticizer (glycerol). The increment of glycerol concentration caused a heterogeneous film with a greater amount of white spots (Figures 2.4c and 2.4d). The weak interaction existing between glycerol and zein (PADUA; WANG, 2002) caused the excess of glycerol to be excluded from the matrix and the formation of the white spots as observed in Figure 2.4. For that reason, films with higher glycerol concentration also presented a greasy touch perception.

Likewise, zein-oleic acid films exhibited a more homogeneous microstructure when higher ethanol concentration was used. Figure 2.5 shows SEM micrographs of the surface of films prepared with no addition of plasticizer. In general, zein-oleic acid films presented a homogeneous and continuous matrix with some superficial solid material as indicated with the blue arrows.

That solid material appeared when ethanol increased (Figures 2.5b, 2.5c and 2.5d), thereby, indicating that some of the reagents used for preparing the films contained a compound that became insoluble at higher ethanol concentration and also that material was observed to be excluded from the zein-oleic acid matrix. Furthermore, as ethanol concentration increased, solid material occurrence increased as well, nevertheless, that fact did not deteriorate film functional properties given the better film behavior at higher ethanol concentration.

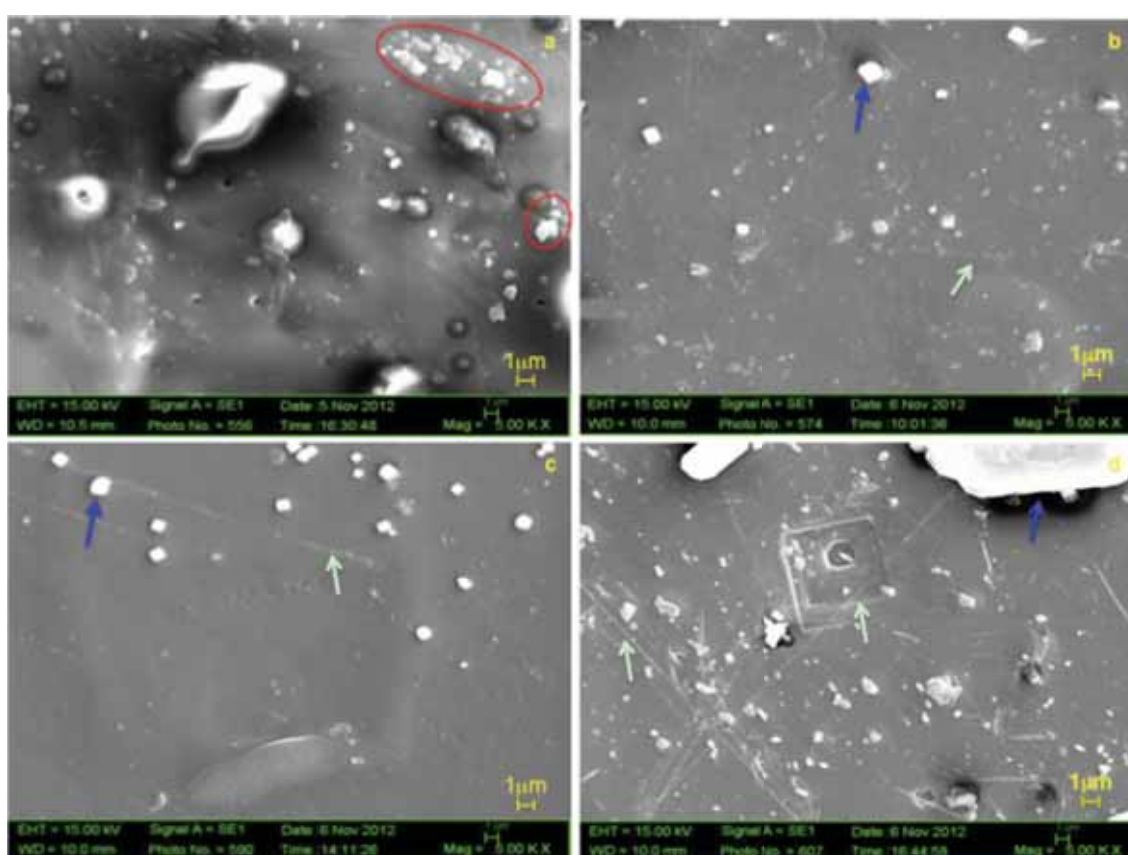


Figure 2.5. SEM micrograph of film surface, 5000x. Films prepared with glycerol addition of 0 % and ethanol solutions of 80 % (a), 85 % (b), 90 % (c) and 95 % (d).

A glossy film appearance was observed at Figure 2.5a when a lower ethanol concentration was used while the other ethanol concentrations showed an opaque appearance and a brittle behavior as indicated with the light green arrows (Figures 2.5b, 2.5c and 2.5d). Light green arrows mark some scratches and deformations suffered during film manipulation, indicating higher film brittleness with higher ethanol. Nonetheless, an oleic acid aggregation, indicated in red

circles, was produced when used ethanol 80 % (Figure 2.5a). Those aggregations cannot be glycerol aggregations since films shown in Figure 2.5 had no glycerol addition.

On the other hand, film cross-section shown in Figure 2.6 displays differences in film structure with variation of glycerol concentration, thus, when lower glycerol concentration was used, film cross-section structure presented greater amount of perforations or orifices.

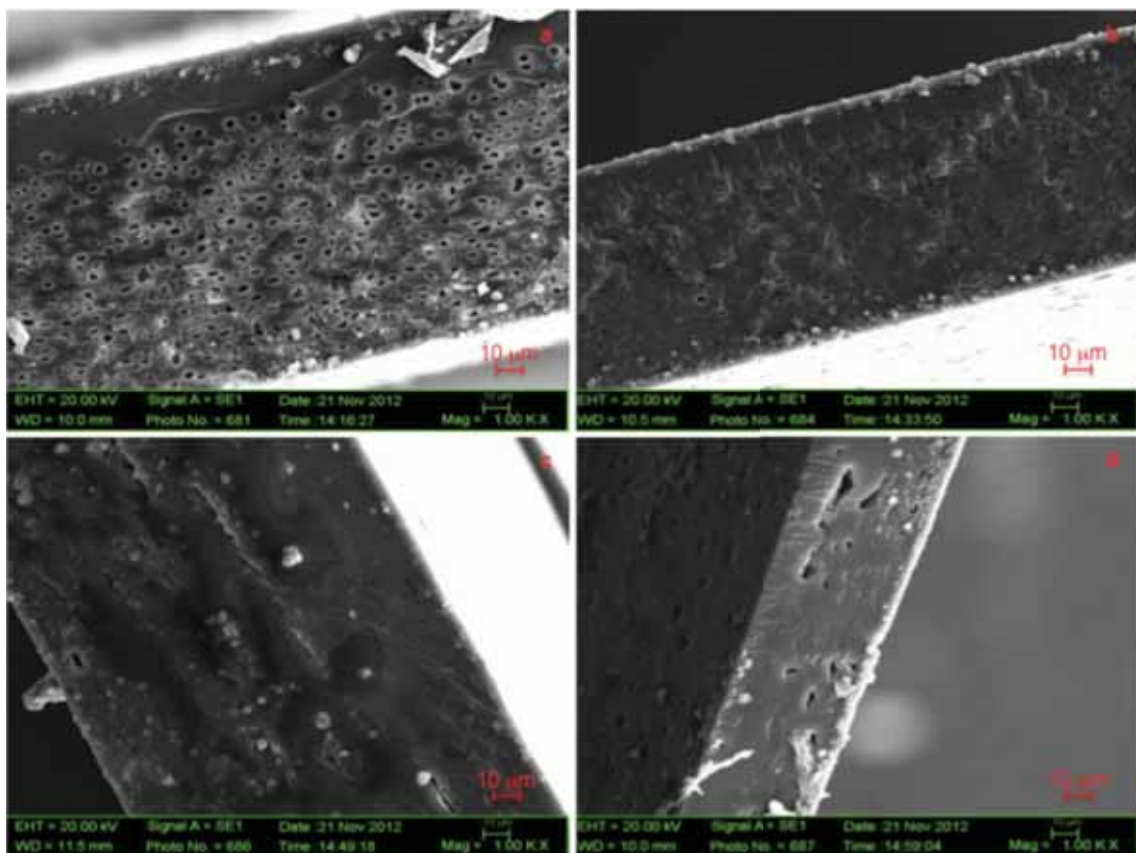


Figure 2.6. SEM micrograph of film cross-section, 1000x. Films prepared with ethanol solution of 80 % and glycerol addition of 0 % (a), 10 % (b), 20 % (c) and 30 % (d).

In this way, Figure 2.6a shows a film cross-section with more orifices as a result of ethanol evaporation during film drying stage. Otherwise, when glycerol concentration is increased, film orifices tend to disappear (Figures 2.6b, 2.6c and 2.6d). Glycerol addition generates spaces between protein chains, decreasing

intermolecular forces and allowing for the better mobility of the protein chains (TIHMINLIOGLU et al., 2010) and ethanol diffusion through the film.

Despite film cross-section structure appeared to be compacter with the addition of higher glycerol, film functional properties as well as film structure decayed with glycerol increment, therefore, addition of glycerol shall be keep as low as possible in order to allow for a better performance of the film.

Conclusions

The functional and structural properties of zein-oleic acid film were affected by changes of ethanol and glycerol concentrations, thereby, higher ethanol (95 %) and lower glycerol concentration (0 %) allowed for the improvement of water and light barrier properties as well as homogeneity and structure of the composite film. Nonetheless, in order to reduce film brittleness a low glycerol concentration as 10 % is required.

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3

Biodegradable zein-based blend films: structural, mechanical and barrier properties

Abstract

The effect of adding a hydrocolloid on the structural, mechanical and barrier properties of zein-based blend films was evaluated. Zein-oleic acid blend film added with xanthan gum (Z-OA-XG) showed higher water solubility (13.09 %) and opacity (8.49 AU/mm) than zein-oleic acid (Z-OA) film (10.80 % and 5.19 AU/mm, respectively). Furthermore, Z-OA film displayed higher flexibility with lower YM (5.02 MPa) and higher %E (10.62 %); nonetheless, less resistant to tension (TS of 8.5 MPa) compared to Z-OA-XG film that showed YM, %E and TS of 6.38 MPa, 6.66 % and 10.485 MPa, respectively. Both films, exhibited glossy and homogeneous structure with comparable water vapor and oxygen barrier properties around 4.39×10^{-11} g/(Pa·s·m) and 1.82×10^{-13} g/(Pa·s·m), respectively. Based on that, xanthan gum structure influenced mainly mechanical and light barrier properties of zein-oleic acid blend films.²

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3.1. Introduction

Packaging is essential for food transport and distribution, enabling quality preservation and protection against external chemical, physical and microbiological contamination (DAINELLI et al., 2008; RESTUCCIA et al., 2010; SOUZA et al., 2012). Nowadays, plastic materials are the most frequent used food packaging due to their thermo-stability, flexibility, lightness and low price (LAGARON; LOPEZ-RUBIO, 2011; SOUZA et al., 2012; SUN et al., 2013), nonetheless, as a result of the global environmental concern, new biodegradable materials, mainly films and coatings, have been developed in order to reduce plastic packaging usage (NOBILE et al., 2008; FAJARDO et al., 2010; KHAN et al., 2012). In comparison to plastics, biodegradable films and coatings are quickly degraded, by the action of microorganisms, as a result of their natural origin (NOBILE et al., 2008; SOUZA et al., 2012).

In general, biodegradable materials are prepared from biopolymers such as proteins, carbohydrates or lipids. For instance, zein, the main corn protein, is one of the materials that have been used to produce biodegradable films and coatings for food and pharmaceutical applications (NOBILE et al., 2008; ZHANG et al., 2011; WU et al., 2012). Zein-based materials have glossy appearance and low water solubility; these are tough, greaseproof, hydrophobic and resistant to microbial attack (THARANATHAN, 2003; NOBILE et al., 2008), though brittle (LAWTON, 2004; ARCAN; YEMENICIOĞLU, 2013).

Several studies have demonstrated that preparing biodegradable materials from two or more biopolymers, known as composites or blends, allow for improving their functional properties compared to single-biopolymer films (PÉREZ et al., 2009; KHAN et al., 2012; SUN et al., 2013). Some composite and blend biodegradable materials that have been recently reported are: a) cassava starch-based films with clay nanoparticles (SOUZA et al., 2012), b) pea starch and peanut protein isolate blend films (SUN et al., 2013), c) nanocrystalline cellulose reinforced chitosan based nanocomposite films (KHAN et al., 2012) and d) zein-wax composite and zein-fatty acid blend films (ARCAN; YEMENICIOĞLU, 2013). These composite and blend

materials showed better homogeneity or improved mechanical and water vapor barrier properties compared to the single-biopolymer ones.

Cuq et al. (1995) and, Wang and Padua (2006) reported that zein-based films blended with oleic acid displayed higher water vapor barrier and flexibility. De Almeida et al. (2010) reported the improvement of zein-oleic acid film homogeneity when different concentrations of xanthan gum were added; nevertheless, their effect on the film's functional properties was not evaluated. Following this idea, the aim of the current study was to evaluate the effect of adding xanthan gum with regard to the structural and functional properties of zein-oleic acid blend films.

3.2 Materials and methods

3.2.1 Materials

Corn-zein (Sigma, São Paulo, Brazil), 99.5 % ethanol (Synth, São Paulo, Brazil), glycerol (Dinâmica, Diadema, Brazil), oleic acid (Synth, São Paulo, Brazil), xanthan gum (ADM, Chicago, USA) and Emustab emulsifier (Duas Rodas, Jaraguá do Sul, Brazil) were used for preparing blend films. Calcium nitrate (Dinâmica, Diadema, Brazil) and sodium bromide salts (Synth, São Paulo, Brazil) were used for water vapor permeability tests and sample conditioning, respectively.

3.2.2 Film composition and preparation

Zein-based films blended with oleic acid (Z-OA) and zein-based films blended with oleic acid and xanthan gum (Z-OA-XG) were prepared by adding 20 % (by mass per volume) zein to 95 % aqueous ethanol during 5 min mechanical stirring at 65 ± 0.5 °C. Glycerol, emulsifier and oleic acid were added to the solution at 10 %, 5 % and 70 % (by mass), respectively; furthermore, 0.05 % (by mass per volume) xanthan gum

was added to the Z-OA-XG solution and then the Z-OA and Z-OA-XG solutions were mechanically stirred for 10 min. 50 mL of solution were poured onto a rectangular glass plate and air-dried at room conditions, overnight. Films were peeled off and conditioned according to ASTM D618-08 at 57.6 % relative humidity (% RH) and 24±2 °C for at least 40 h prior to analyses.

3.2.3 Film thickness

Thickness was measured with a digital micrometer (Digimes P54) with 0.001 mm precision. Five-thickness measurements were taken, one at the center and four around the perimeter. The thickness average value was used in further calculations.

3.2.4 Light permeability (opacity)

Opacity was assessed at 600 nm in a spectrophotometer (Bioespectro SP-220). Film samples were cut into 9 mm × 43 mm rectangular shapes and placed in the internal side of the spectrophotometer cell (CAO et al., 2007; PÉREZ et al., 2009). Six specimens of each film were tested and opacity was calculated according to Equation (3.1).

$$\text{Opacity} = \frac{A_{600}}{x} \quad (3.1)$$

Where x is the film average thickness and A_{600} is the absorbance at 600 nm.

3.2.5 Water solubility

For assessing the film solubility, film samples were cut into a 2 cm (diameter) disk and dried in oven at 105±2 °C for 24 h. Samples were weighed (initial weight, m_i)

and immersed into 50 mL distilled water at 27 ± 2 °C for 24 h under agitation in an orbital shaker MA-410 (Marconi, Brazil) at 76 rpm. After 24 h of immersion, the samples were taken out and dried (final weight, m_f) at the same conditions mentioned before (CERQUEIRA et al., 2007; PÉREZ et al., 2009). Three specimens of each film were tested and water solubility was calculated based on Equation (3.2).

$$\text{Water solubility (\%)} = \frac{m_i - m_f}{m_i} \times 100 \quad (3.2)$$

3.2.6 Water vapor permeability (WVP)

WVP of the films was measured gravimetrically according to the ASTM E 96/96M-10 standard, desiccant method. Test cells were covered and sealed by the film samples and placed in a desiccator with 51 % RH obtained by a saturated solution of calcium nitrate. Silica gel was activated at 200 °C and used to maintain 0 % RH inside the test cells. The desiccator was stored at 24 ± 2 °C and the weight change was measured until equilibrium. Three specimens of each film were tested and WVP was calculated according to Equation (3.3).

$$\text{WVP} = \frac{\text{WVT}}{S(R_1 - R_2)} \times x \quad (3.3)$$

Where WVT is the water vapor transmission, calculated with Equation (3.4).

$$\text{WVT} = \frac{G}{t \times A} \quad (3.4)$$

t is the measured time during the weight change occurred, A is the test area (12.57 cm^2) and G is the weight change of the test cell. G/t was obtained from the slope of the linear portion of the plot of G vs. t . R_1 is % RH in the test desiccator expressed as a fraction, R_2 is % RH inside the test cell expressed as a fraction, S is the saturation of vapor pressure at the test temperature (2985 Pa at 24 °C) and x is the film average thickness.

3.2.7 Oxygen permeability (O₂P)

Oxygen transmission rate (OTR) was measured according to the ASTM F1927-07 standard, using an oxygen permeation instrument (OXTRAN 2/20). Film OTR was determined under control conditions (23 °C and 0 % RH). The film was placed between two sides of the test chamber, one side was exposed to carrier gas containing 98 % N₂ and 2 % H₂ while the other side was exposed to test gas (pure O₂). The sensor monitored the exit port of the carrier gas side measuring the amount of oxygen present. OTR was calculated according to Equation (3.5).

$$\text{OTR} = \frac{\text{Conc. O}_2}{t \times A} \quad (3.5)$$

Where: *Conc. O₂* is the quantity of oxygen measured, *t* is the time required for reaching the stationary state and *A* is the transfer film area (100 cm²). Oxygen permeability was calculated using the Equation (3.6).

$$\text{O}_2\text{P} = \frac{\text{OTR}}{p} \times x \quad (3.6)$$

Where: *x* is the film average thickness and *p* is the oxygen partial pressure.

3.2.8 Mechanical test

Mechanical properties were evaluated according to the ASTM D882-10 standard. Elongation at break (%E), Tensile Strength (TS) and Young Modulus (YM) were tested with a texture analyzer (TA.XT *Plus*) using the Exponent software and the A/TG probe. Test films were cut with a guillotine according to ASTM D6287-09 standard into rectangular strips of 100 mm long and 15 mm wide. Thickness was measured in five points along the sample in order to assure thickness uniformity. Initial grip separation and crosshead speed were set at 50 mm and 500 mm/min, respectively. Stress and strain were recorded during the extension of the strips and

minimum 5 specimens were analyzed.

3.2.9 Microstructure

Film superficial and cross-section microstructure were observed using a scanning electron microscope-SEM (Carl Zeiss EVO LS15) equipped with secondary electron detector (SE). Cross-section images were obtained by cryogenic fracture by immersing the samples into liquid nitrogen for 2 min. Film samples were covered with gold and images were taken at 15-20 kV with magnification of 1000x and 5000x. Film samples were fixated to the microscope stubs using a carbon double face conducting tape.

3.2.10 Statistical Analysis

Statistical analysis was accomplished with Statgraphics centurion, v. 16.1. An analysis of variance and a Tukey multiple comparison test were performed to detect significant differences between Z-OA and Z-OA-XG film properties with 5 % significance level.

3.3 Results and discussion

Z-OA and Z-OA-XG blend films were homogeneous and smooth, without visible pores and without greasy touch perception; film components were visibly well integrated in the matrix. Z-OA-XG blend film showed glossy appearance and brittle touch perception probably due to the packed and arranged structure of the xanthan gum polysaccharide (BOURBON et al., 2011).

3.3.1 Light barrier (opacity)

Opacity is an indicator of the light barrier property of the film, representing the capacity of the film to protect the product against deterioration caused by light. The Z-OA-XG film exhibited an increase in the light barrier property over 60 % compared to the Z-OA film (Table 3.1). The addition of xanthan gum to zein-oleic acid blend film promoted the reduction of light transmission through the film, possibly, as a consequence of the polysaccharide's packed structure.

Blend films of soy protein isolate and gelatin reported by Denavi et al. (2009) exhibited lower opacity than Z-OA and Z-OA-XG blend films. Besides, the Z-OA-XG blend film of the current study showed higher light barrier compared to the same blend film reported by De Almeida et al. (2010). The higher opacity displayed by the current Z-OA-XG blend film is probably due to the difference in composition of both films. For instance, glycerol addition helps improving film flexibility by creating spaces into the matrix (PENA-SERNA; LOPES-FILHO, 2013), though decreasing the film light barrier. Thus, the lower glycerol concentration of the current Z-OA-XG film improved its opacity. Similarly, De Almeida et al. (2010) reported blend films prepared with 75 % ethanol while current Z-OA-XG blend films were prepared with 95 % ethanol. Higher ethanol concentration improves zein solubilization, increasing intermolecular interactions between protein chains and between protein and other film components; hence, creating a tight film matrix that prevents light transmission (PENA-SERNA; LOPES-FILHO, 2013).

Table 3.1. Water solubility and light barrier properties of zein-based blend films

Film	Thickness (mm)	Water solubility (%)	Opacity (AU/mm)
Z-OA	0.062±0.008 ^a	10.80±0.50 ^a	5.19±0.58 ^a
Z-OA-XG	0.062±0.011 ^a	13.09±1.40 ^b	8.49±0.38 ^b

Values are mean ± standard deviation. Different letters indicate statistically significant difference ($p < 0.05$).

3.3.2 Water solubility

Water solubility indicates the water affinity of the film, representing its water-resistant capacity. As shown in Table 3.1, Z-OA-XG film displayed higher solubility compared to Z-OA film possibly due to the higher polarity of the film caused by the addition of a hydrophilic compound (THARANATHAN, 2003; BOURBON et al., 2011).

Polysaccharide-based films produced from chitosan, galactomannan or agar exhibited water solubility over 22 % (CERQUEIRA et al., 2007). Similarly, the water solubility of gelatin-based films blended with 1 % sunflower oil was around 80 % (PÉREZ et al., 2009). Based on that, Z-OA and Z-OA-XG films showed higher water-resistant capacity compared to the films mentioned above, due to the hydrophobic nature of zein as well as the high oleic acid concentration of Z-OA and Z-OA-XG blend films.

3.3.3 Gas barrier properties

The gas barrier properties of the Z-OA and Z-OA-XG blend films that were assessed were water vapor and oxygen permeability (Table 3.2). Water vapor permeability (WVP) refers to the barrier property of the film, indicating the moisture transfer between the packaged product and the surroundings.

Table 3.2. Water vapor and oxygen permeability properties of zein-based blend films

Film	Thickness (mm)	WVP (g/(Pa·s·m))	O ₂ P (g/(Pa·s·m))
Z-OA	0.065±0.005 ^a	4.36x10 ⁻¹¹ ±7.29x10 ⁻¹² ^a	1.85x10 ⁻¹³ ±2.81x10 ⁻⁰⁸ ^a
Z-OA-XG	0.063±0.000 ^a	4.41x10 ⁻¹¹ ±5.53x10 ⁻¹² ^a	1.79x10 ⁻¹³ ±2.95x10 ⁻⁰⁸ ^a

Values are mean ± standard deviation. Same letter indicate no statistically significant difference ($p < 0.05$).

As shown in Table 3.2, both blend films, Z-OA and Z-OA-XG, displayed similar WVP values. Therefore, increasing film's polarity, by adding xanthan gum, had no effect on moisture barrier property as we expected (THARANATHAN, 2003; NIETO, 2009). That is probably a consequence of the low xanthan gum concentration added to Z-OA-XG film.

The blend film based on konjac glucomannan, chitosan and soy protein isolate reported by Jia et al. (2009), showed higher WVP (5.18×10^{-11} g/(Pa·s·m)) than Z-OA and Z-OA-XG blend films. Likewise, protein-based films produced from whey protein isolate (SOTHORNVIT et al., 2009) and blend films prepared from zein and wheat gluten (XINGFENG et al., 2012) displayed higher WVP values (66×10^{-9} and 5.0×10^{-11} g/(Pa·s·m), respectively) in comparison to Z-OA and Z-OA-XG films. Based on that, the Z-OA and Z-OA-XG blend films exhibited a better water vapor barrier property compared to other biodegradable films as a consequence of their higher hydrophobicity.

Similarly to WVP, oxygen permeability (O_2P) indicates the oxygen transfer between the packaged product and the surroundings. This film property is of great importance when the product might suffer oxidative reactions that deteriorate its composition and integrity.

As shown in Table 3.2, the xanthan gum addition to zein-oleic acid blend films had no impact on film oxygen permeability. Both films, Z-OA and Z-OA-XG, displayed similar O_2P values ($p < 0.05$) likely caused by the low concentration of xanthan gum that was added.

Z-OA and Z-OA-XG blend films exhibited higher O_2P in comparison to polysaccharide-based films reported by Cerqueira et al. (2007) and Matta et al. (2011), which showed oxygen permeability around 1×10^{-15} and 6.3×10^{-17} g/(Pa·s·m), respectively. This was probably an effect of the high oleic acid concentration, which increased oxygen solubilization and its diffusion through the film (KROCHTA, 2002). Furthermore, several studies have reported better oxygen barrier of polysaccharide-based films compared to protein-based and lipid-based films due to the structure and polarity of the polysaccharides (MILLER; KROCHTA, 1997).

3.3.4 Mechanical properties

Mechanical properties such as tensile strength, elongation at break and Young modulus represent the toughness and elasticity or brittleness of the film. Thus, as shown in Table 3.3, Z-OA-XG film displayed greater toughness (higher TS) compared to Z-OA due to the packed and arranged structure of xanthan gum; nevertheless, polysaccharide structure has increased the stiffness and brittleness of Z-OA-XG blend film (higher YM and lower %E, respectively).

Table 3.3 Mechanical properties of zein-based blend films

Film	Thickness (mm)	Tensile Strength (MPa)	% Elongation (%)	Young Modulus (MPa)
Z-OA	0.066±0.004 ^a	8.500±0.514 ^a	10.617±2.288 ^a	5.015±0.307 ^a
Z-OA-XG	0.065±0.004 ^a	10.485±0.635 ^b	6.656±1.264 ^b	6.378±0.501 ^b

Values are mean ± standard deviation. Different letters indicate statistically significant difference ($p < 0.05$).

For instance, gelatin films and 10 % zein-based films with 3 % glycerol showed higher tensile strength around 22 and 10 MPa, respectively (KU; SONG, 2007), in comparison to Z-OA and Z-OA-XG films. The lower TS exhibited by the current zein-based films was probably due to the higher glycerol concentration (10 %) added to the films as well as the high oleic acid concentration. Glycerol and oleic acid are plasticizer agents that reduce the tensile strength of the films (KROCHTA, 2002). Moreover, protein-based films produced from Cowpea protein isolate with 10 % PEG reported by Hewage and Vithanarachchi (2009) displayed lower tensile strength (6.6 MPa) compared to Z-OA and Z-OA-XG films.

3.3.5 Microstructure

Scanning electron microscopy allows for evaluating the distribution and integration of film components into the matrix. Z-OA and Z-OA-XG blend films displayed homogeneous surfaces with good integration of film components (Figures 3.1a and 3.1b). Film micrographs showed square-shape materials on Z-OA and Z-OA-XG film surface. As both films exhibited the same materials, we believed that these are due to contaminant substances contained in the reagents used for film preparation.

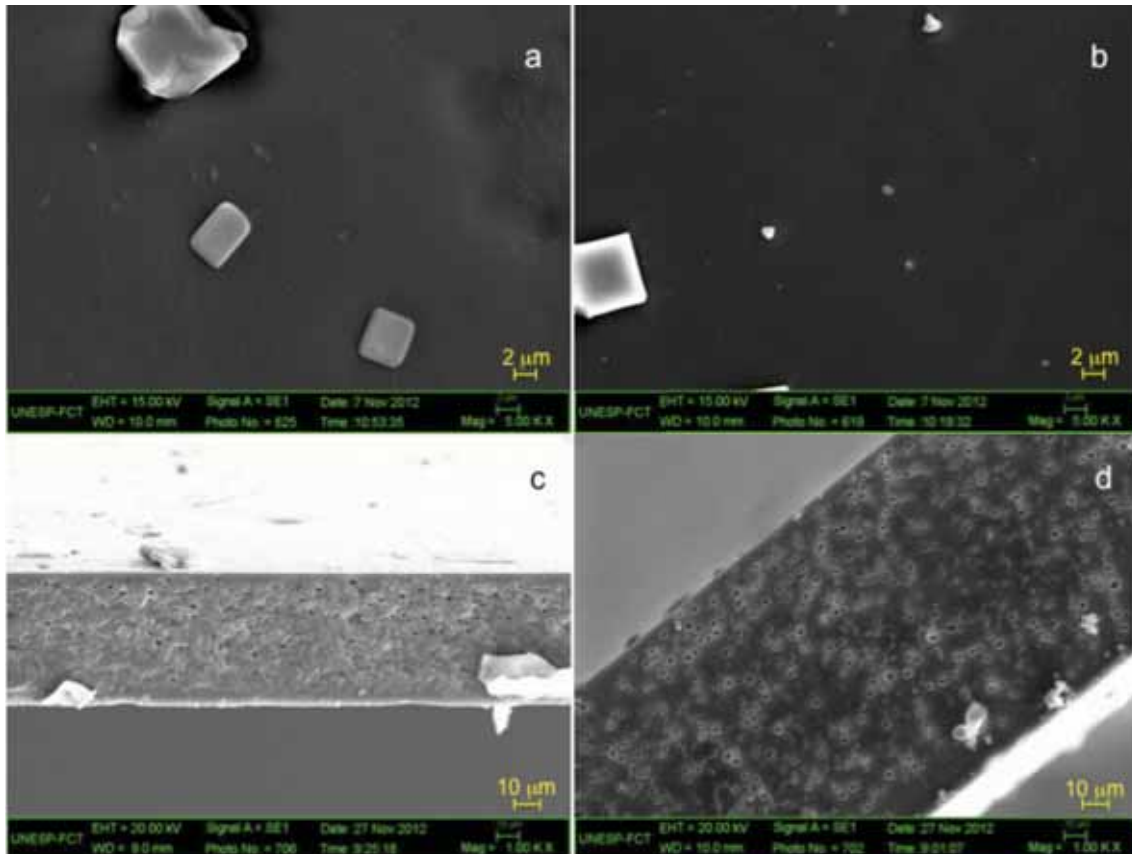


Figure 3.1. SEM micrographs of film surface at 5000x (a) Z-OA and (b) Z-OA-GX and film cross-section at 1000x (c) Z-OA and (d) Z-OA-GX.

The cross-section of Z-OA and Z-OA-XG films (Figures 3.1c and 1d) displayed porosity appearance or orifices caused by ethanol evaporation (SOTHORNVIT et al., 2009). Apparently, Z-OA-XG blend films exhibited higher

porosity as a consequence of the higher rigidity determined during the mechanical tests.

Conclusions

Performance of blend films based on zein-oleic acid and zein-oleic acid-xanthan gum were evaluated and compared. Z-OA and Z-OA-XG displayed homogeneous surfaces with good integration of film components. Z-OA exhibited lower water solubility whereas Z-OA-XG showed greater opacity. Hydrocolloid addition exhibited no effect on gas barrier properties since both blend films, Z-OA and Z-OA-XG, showed similar water vapor and oxygen permeability, nonetheless, xanthan gum structure influenced mechanical properties of the film, increasing the strength (higher TS) and rigidity (higher YM and lower %E). Hence, polysaccharides such as xanthan gum should be added to zein-oleic acid blend films, mainly when the food products tend to be oxidized as a consequence of the light action.

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4

Zein-based blend coatings: impact on the quality of a model cheese of short ripening period

Abstract

Two biodegradable zein-based blend coatings were evaluated according to the impact on the quality of the “Minas Padrão” cheese throughout a storage period of 56 days. Throughout the storage, the biodegradable-coated cheese samples showed similar physicochemical characteristics in comparison to unpackaged and plastic-packaged cheese samples in terms of chlorides, ash, protein and acidity. Besides that, cheese samples with biodegradable coatings exhibited ca. 30 % lower weight loss and avoided microbiological contamination for more than 50 days when compared to unpackaged cheese samples that exhibited contamination after 21 days. Although cheese samples with Z-OA and Z-OA-XG coatings exposed a positive behavior in comparison to unpackaged cheeses, some challenges remain and require further studies. For instance, as a consequence of the moisture loss, after 28 days of storage, the biodegradable-coated cheeses were 124 % harder, displayed 30 % less proteolysis and more than 50 % change in color surface than cheese samples with polyethylene packaging. Nonetheless, given the current characteristics exhibited by the developed Z-OA and Z-OA-XG coatings, these seem to be suitable for the industry of long ripened cheeses.³

³ This chapter was submitted as:

Carolina Pena Serna, Ana Lúcia Barretto Penna, José Francisco Lopes Filho. **Food Research International**. (April, 2015).

4.1 Introduction

“Minas Padrão” is a semi-hard cheese and one of the most important cheeses in Brazil. It is a traditional cheese, widely consumed and prepared from cow’s milk in few Brazilian states. According to *Laticínio Balkis*, the cheese supplier, “Minas Padrão” ripening is carried out after the cheese is salted in brine and packaged in a polyethylene packaging that undergoes thermal shrinkage. Cheese is ripened for 25 days under controlled conditions, 3.8 ± 0.5 °C and 85 % relative humidity; thereafter, the cheese is distributed to the supermarkets across the country.

The commercial “Minas Padrão” cheese is distributed and commercialized in plastic packaging, in order to avoid external chemical or microbiological contamination and to help preserving the cheese quality. Plastics are the most frequently used food packaging materials (LAGARON; LÓPEZ, 2011; SIRACUSA et al., 2008); nonetheless, due to environmental concern and their low recycling rate (SIRACUSA et al., 2008; SALARBASHI et al., 2014), new biodegradable and edible materials have been and are developed to reduce the plastic packaging usage (AURAS et al, 2006; FAJARDO et al., 2010; SALARBASHI et al., 2014).

One material that has been used to produce biodegradable and edible films and coatings is zein, the main corn protein (ZHANG et al., 2011; WU et al., 2012). Zein films have exhibited glossy appearance, toughness, low water solubility, resistance to microbial attack and high hydrophobicity (NOBILE et al., 2008). In spite of that, several authors have demonstrated that blend films, i.e. a combination of zein with other biomolecules, mainly lipids (CUQ et al., 1995; WANG; PADUA, 2006; ARCAN; YEMENICIOĞLU, 2013), allows for improving the functional properties of zein-based films.

In this regard, several studies have been focused on the development of new biodegradable and edible films, and the evaluation of their functional properties; nevertheless, few researches have explored their application to food (GENNADIOS et al., 1997; FAJARDO et al., 2010).

For instance, some biodegradable materials that have been reported as cheese coatings are mainly polysaccharide-based, e.g. chitosan (FAJARDO et al.,

2010; ZHONG et al., 2014), galactomannan (CERQUEIRA et al., 2010), alginate (ZHONG et al., 2014), carrageenan, gellan (KAMPF; NUSSINOVITCH, 2000), and few protein-based coating such as whey protein isolate-WPI (RAMOS et al., 2012) and soy protein isolate-SPI (ZHONG et al., 2014). In all the previously referenced cases, cheeses with biodegradable coatings exhibited better preservation of cheese physicochemical characteristics than the uncoated counterparts. Moreover, to the best of our knowledge, Ramos et al. (2012) reported the uniquely available study that has compared synthetic and biodegradable cheese packaging. According to Ramos et al. (2012), the synthetic nonedible coating made of polyvinyl acetate (PVA) and the WPI biodegradable coating displayed similar performance on cheese quality preservation; therefore, the WPI coating is a suitable alternative for cheese packaging.

Hereby, the current study aims to evaluate the impact of two zein-based blend coatings on the physicochemical characteristics of the “Minas Padrão” cheese, as a model for short ripened cheeses, in comparison to polyethylene packaged and unpackaged cheese samples.

4.2 Materials and methods

The following sections describe the materials and methods used within this study.

4.2.1 Materials

For the preparation of the zein-oleic acid (Z-OA) and zein-oleic acid-xanthan gum (Z-OA-XG) coatings, the following components were used: corn-zein (Sigma, São Paulo, Brazil), 99.5 % ethanol (Synth, São Paulo, Brazil), oleic acid (Synth, São Paulo, Brazil), glycerol (Dinamica, Diadema, Brazil), Emustab emulsifier (Duas Rodas, Jaraguá do Sul, Brazil) and xanthan gum (ADM, Chicago, USA). A

commercial brand of the “Minas Padrão” cheese was obtained from *Laticínios Balkis* (Santo Antônio do Aracanguá, Brazil) on the day of production.

4.2.2 Cheese sample preparation

After production, 84 semicircular cheeses with an approx. net weight of 0.4 kg each, were packaged in the regular polyethylene (commercial) packaging and transported under refrigeration from the dairy company to São Paulo State University in São Jose do Rio Preto. The amount of cheeses was randomly divided into four groups that were named as unpackaged cheese (negative control), commercially packaged cheese (positive control), Z-OA coated cheese and Z-OA-XG coated cheese; thus, each group was composed by 21 semicircular cheeses.

After 5 h from cheese production, cheeses were cut, in order to obtain experimental samples (that will be referred to as samples in the remainder of the document). Cheese cutting was made first in four similar pieces along the radius of the semicircular shaped cheese and then each piece was further cut transversally providing eight similar samples of approx. 0.05 kg.

4.2.3 Coating solution preparation

The two zein-based coating solutions were prepared by adding 20 g zein to 100 mL 95 % ethanol, and mechanically stirred for 5 min at 65 ± 0.5 °C. Glycerol (2 g), emulsifier (1 g) and oleic acid (14 g) were added to both solutions. Xanthan gum (0.05 g) was also added to the Z-OA-XG solution; afterwards, Z-OA and Z-OA-XG solutions were stirred for 10 min and these were allowed to reach room temperature before applying on the cheese surface.

4.2.4 Cheese coating procedure

After the cheese cutting, Z-OA and Z-OA-XG experimental samples were coated with a three-layer coating, as follows. One layer of the coating solution was brushed on the surface of the cheese sample and after 1 h of drying; a second layer was applied, repeating the procedure until completing three layers. Once the three layers were applied, the coating dried during 4 h at 24 °C and 50 % relative humidity (%RH). Thereafter, the samples of the four cheese groups were organized in plastic trays and stored under controlled conditions (3.8 °C, 85 %RH).

All the variables and conditions mentioned above (section 4.2.2 and 4.2.4) were established according to previous experiments (data not included for the lack of space) in which sample's size and shape, coating layer number, coating application method (dipping or brushing) and drying time and temperature were evaluated.

4.2.5 Cheese chemical composition and physicochemical analyses

Throughout 56 days of storage time, the “Minas Padrão” cheese samples were tested for physicochemical characteristics such as titratable acidity, moisture, total ash, chlorides, lipids, total nitrogen and proteolysis rate. The results of the tests were expressed on dry basis. Three replicates of 200 g each were used to perform the physicochemical analyses; based on that, each replicate was composed by four samples (4 x 0.05 kg) belonging to each cheese group. The three replicates were taken at 0, 7, 14, 21, 28, 42 and 56 days of coating application. Furthermore, weight loss analysis was performed twice a week during the storage period using eight samples (replicates) of each cheese group.

Titratable acidity was assayed and expressed as lactic acid (INSTITUTO ADOLFO LUTZ, 1985); moisture content was determined according to the 926.12 AOAC method by drying the cheese samples to constant weight at 70 °C in vacuum oven (AOAC, 1997); total ash content was analyzed by incineration at 550 °C according to the 935.42 AOAC method (AOAC, 1997); chloride content was

measured through an argentometric method using the total amount of ash previously obtained (INSTITUTO ADOLFO LUTZ, 1985); lipid content was tested by the Gerber-Van Gulik method (INSTITUTO ADOLFO LUTZ, 1985); weight loss was determined by weighing samples at the beginning (W_0) and throughout the storage period (W_i), the relative weight loss (ΔW) was calculated as follows $\Delta W = (W_0 - W_i) * 100 / W_0$; total nitrogen content was assessed by the micro-Kjeldahl method in accordance with the 960.52 AOAC method (AOAC, 1997); furthermore, the total protein content was calculated multiplying the nitrogen content by the conversion factor 6.38; cheese proteolysis was analyzed by separating the nitrogenous compounds into solvent-soluble and solvent-insoluble nitrogen, followed by the fractionation of the soluble components with discriminatory precipitants and quantification with the micro-Kjeldahl method. Thereby, soluble nitrogen at pH 4.6 (pH 4.6-SN) was obtained by precipitation with hydrochloric acid 1.41 N and trichloroacetic acid soluble nitrogen (TCA-SN) was obtained by precipitation with TCA 12 % (MERHEB-DINI et al., 2012).

4.2.6 Cheese surface color analysis

The color surface change of cheese samples was analyzed throughout the storage period with a Chroma meter ColorFlex EZ (HunterLab Inc., Reston, USA) using the CIELab color scale (where L = lightness, a = red-yellow color, and b = blue-green color) under daylight ($D65$ illuminant). The total color difference (ΔE) was calculated as follows $\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$, where L_0 , a_0 , and b_0 were the initial values (1 day of coating application) obtained for the samples of each cheese group, and L , a , b were the values measured during the storage period (CERQUEIRA et al., 2010; RAMOS et al., 2012). Three samples (replicates) belonging to each cheese group were used for the analysis and four-color readings were made on each one.

4.2.7 Cheese texture profile analysis (TPA)

The cheese texture profile was analyzed during the storage period using cylindrical cheese samples (25 mm diameter and 30 mm height). The analyses were made with a texture analyser TA.XT *plus* (Stable Micro Systems Ltd., Surrey, UK) equipped with 5 kg load cell and the 36R probe. The texture analyser was operated with two compression-decompression cycles and 2 mm s⁻¹ of crosshead speed (DIAMANTINO et al., 2014). Cheese textural parameters such as hardness, cohesiveness and gumminess were obtained using the texture profile function of the *Exponent* software. Nine samples (replicates) belonging to each cheese group were used for the measurements.

4.2.8 Experimental design and statistical analysis

A randomized repeated measures design was applied using the cheese packaging type as the factor under study with four different levels (unpackaged, Z-OA, Z-OA-XG and plastic). The statistical analysis was performed with Statgraphics centurion v. 16.1 software (Statpoint Technologies, Inc., Warrenton, USA), performing ANOVA and Tukey multiple comparison test functions to evaluate the significant mean difference ($p < 0.05$) between packaging.

4.3 Results and discussion

The impact of two biodegradable zein-based coatings on the physicochemical characteristics of “Minas Padrão” cheese was evaluated throughout 56 days of storage. Both coatings, Z-OA and Z-OA-XG, exhibited the similar effect on cheese physicochemical characteristics ($p > 0.05$).

After 21 days of storage, the visual examination showed that 33 % of unpackaged cheese samples displayed microbiological contamination on the “Minas Padrão” cheese surface whereas Z-OA and Z-OA-XG coated samples exhibited microbiological contamination after 50 and 53 days respectively. Therefore, the use of the Z-OA and Z-OA-XG biodegradable coatings allowed for extending cheese quality during the storage. Due to microbiological analyses were not the scope of this study, the identification and characterization of the detected microbiological contamination are subject of further studies and will be published elsewhere.

Physicochemical characteristics such as acidity (Figure 4.1a), ash (Figure 4.1b), chloride (Figure 4.1c) and total protein (Figure 4.1d) were similarly and stably preserved along the storage by the four-tested type of packaging ($p > 0.05$). The biodegradable coatings helped to decrease weight (Figure 4.2a) and moisture (Figure 4.2b) loss in comparison to the unpackaged cheese, due to the better water barrier property of the biodegradable coatings.

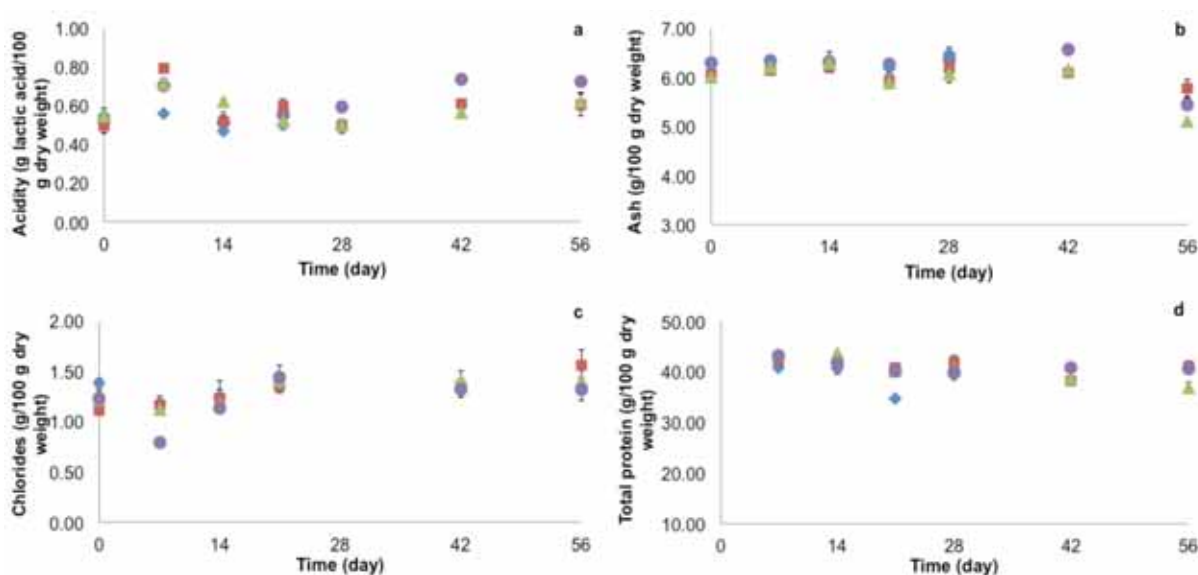


Figure 4.1. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on physicochemical characteristics of the “Minas Padrão” cheese throughout storage. Acidity (a), ash (b), chlorides (c) and total protein (d). Data are expressed on dry basis and values are mean \pm standard deviation.

Although the Z-OA and Z-OA-XG coatings showed a positive behavior when compared to unpackaged cheeses, the water vapor permeability of the coatings

requires further improvement, in order to achieve the performance of the polyethylene packaging. The higher water vapor transmission (WVT) of the biodegradable materials ($WVT = 6.3 \text{ g d}^{-1} \text{ m}^{-2}$) compared to polyethylene ($6 \text{ g d}^{-1} \text{ m}^{-2}$) caused the greater moisture loss of the coated samples (Figure 4.2b).

Cheese samples with biodegradable coating exhibited an initial moisture loss ($t = 0$) of ca. 10 % likely due to whey loss during the drying of the coating. Therefore, in order to overcome such problem further studies shall be conducted, aiming to test the use of Z-OA and Z-OA-XG biodegradable films instead of Z-OA and Z-OA-XG biodegradable coatings.

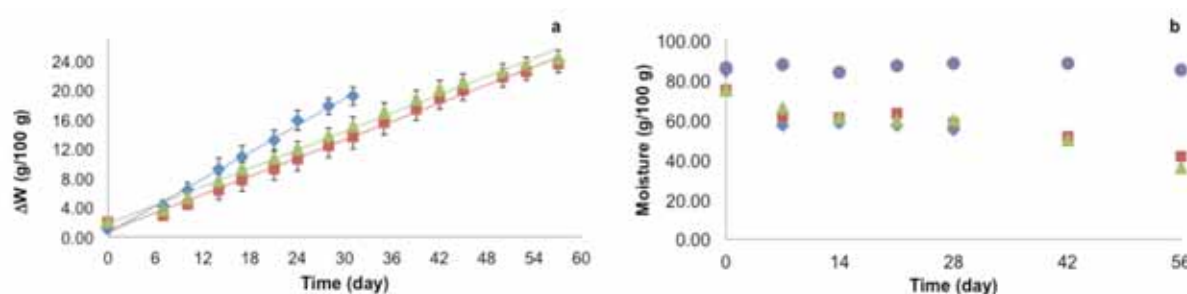


Figure 4.2. Effect of the packaging type (◇ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on physicochemical characteristics of the “Minas Padrão” cheese throughout storage. Weight loss (a) and moisture content (b). Values are mean \pm standard deviation.

The mozzarella cheese with SPI or chitosan coatings (ZHONG et al., 2014) and the “Salio” cheese coated with galactomannan (CERQUEIRA et al., 2010) or WPI (RAMOS et al., 2012) exhibited more than 15 % weight loss during 20 days of storage. Considering the same storage period, the “Minas Padrão” cheese with Z-OA and Z-OA-XG coatings displayed lower weight loss (ca. 9 %) due to the lower water vapor permeability of the zein-based materials in comparison to other biodegradable materials (PENA SERNA; LOPES FILHO, 2015). Furthermore, applying a three-layer coating on cheese surface instead of a single-layer coating helped to reduce cheese moisture loss as well.

Cheese characteristics such as proteolysis, lipid content, surface color and texture parameters were altered during the storage period as a result of either, cheese ripening or moisture loss (Figures 4.3, 4.4 and 4.5).

Ripening is a complex process where cheese flavor and texture are generated by means of the biochemical transformation of curd's triacylglycerides, casein and residual lactose. Thus, a softened cheese texture is obtained as a consequence of the casein hydrolysis as well as changes in pH and curd's water-binding ability. Factors such as cheese composition (especially moisture and salt content), temperature, storage time, type of microorganisms and the activity of the added enzymes, strongly influence this process (FARKYE; FOX, 1990; McSWEENEY, 2004).

Due to the several biochemical reactions involved in cheese ripening, there is a difficulty on measuring its evolution. Based on that, cheese proteolysis is used as one of the approaches to quantify cheese ripening. Proteolysis measures the degree of hydrolysis of the casein matrix; thus, the pH 4.6-SN, known as the extension index, allows for quantifying mainly the coagulant (rennet) enzymatic activity, i.e. casein hydrolysis into peptides of high molecular weight, whereas TCA-SN or depth index measures small peptides produced by the proteinases of the starter culture (McSWEENEY, 2004; PANIZZOLO et al., 2011).

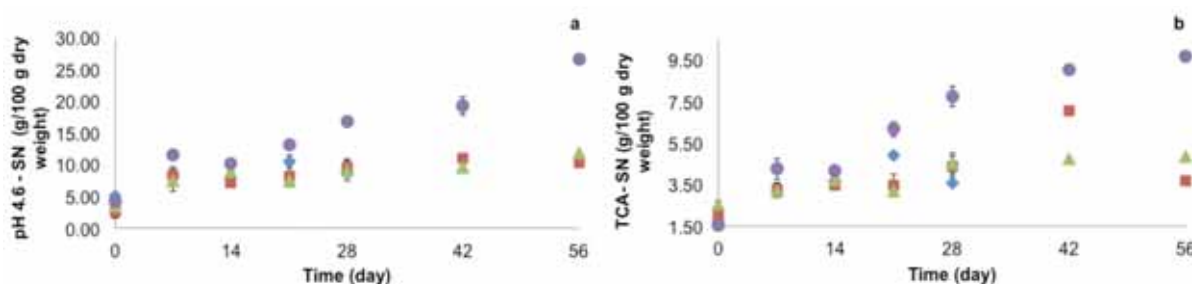


Figure 4.3. Effect of the packaging type (◇ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on the ripening of the “Minas Padrão” cheese throughout storage. Proteolysis indices of extension (a) and depth (b). Data are expressed on dry basis and values are mean \pm standard deviation.

The extension and depth of ripening (Figure 4.3a and 4.3b) tended to increase throughout the time, displaying the proteolysis evolution along cheese ripening and storage time. Proteolysis extent was influenced by packaging type ($p < 0.05$); thus, plastic-packaged samples exhibited greater proteolytic activity with higher extension and depth indices than biodegradable-coated and unpackaged cheese samples. As

an enzymatic process, cheese ripening depends on water activity and moisture content (CERQUEIRA et al., 2010); consequently, those factors influenced the proteolytic activity of coagulant and starter culture present in the “Minas Padrão” cheese.

The depth index of unpackaged and biodegradable-coated cheese samples showed a reduction on the measurements performed at 28 and 56 days respectively (Figure 4.3b). As previously mentioned, unpackaged and coated cheese samples displayed microbiological contamination after 21 and 50 days of storage, respectively. Such reduction was possibly caused by the contaminant microorganism, which likely degraded the small peptides produced during cheese ripening.

Cheese lipid content tended to decrease along time (Figure 4.4a) likely caused by the production of volatile flavor compounds during cheese ripening. Furthermore, at the end of the storage period, the lipid content of cheese samples with biodegradable coatings was lower compared to polyethylene-packaged samples ($p < 0.05$). The lipid loss displayed by the biodegradable-coated samples was probably a consequence of the greater affinity between the cheese lipids and the oleic acid-based (hydrophobic) coating (ZHONG et al., 2014). Thereby, cheese lipids migrate to cheese surface and may be lost during cheese manipulation and coating removal.

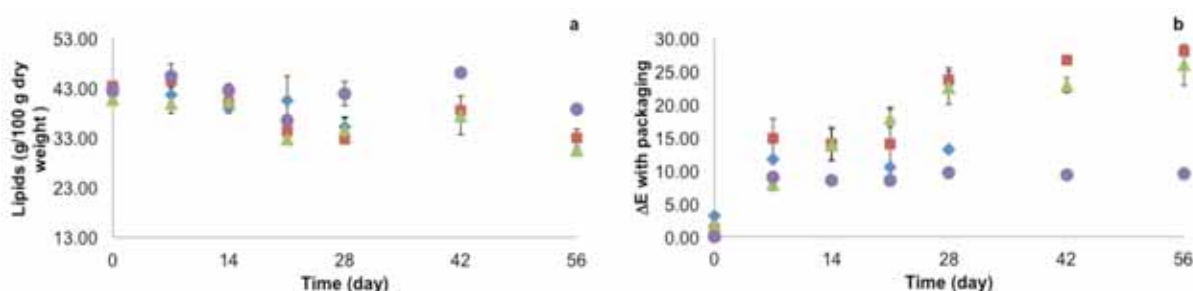


Figure 4.4. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on the “Minas Padrão” cheese lipid content (a) and surface color change (b) throughout storage. Data are expressed on dry basis and values are mean \pm standard deviation.

Furthermore, after 21 days of storage, Z-OA and Z-OA-XG coated cheeses exhibited a surface with greasy and wrinkled appearance (data not shown). Mozzarella cheese with SPI coating showed the same surface appearance after 7 days of storage (ZHONG et al., 2014).

The “Minas Padrão” cheese underwent color surface variation along the storage time (Figure 4.4b) and the packaging type influenced such characteristic ($p < 0.05$). Similarly, Ramos et al. (2012) and Zhong et al. (2014) reported color change throughout the storage on “Salio” cheese with WPI coating and Mozzarella cheese with SPI coating, respectively.

Initial CIELab color values for the “Minas Padrão” cheese were $L_0 = 84.91$, $a_0 = 7.7$ and $b_0 = 26.26$. Moreover, after one day of coating application, the measured CIELab values were $L = 73.68$, $a = 14.42$ and $b = 73.64$. According to these values, the coated cheese samples experienced color change immediately after coating application as a consequence of the yellow color of the Z-OA and Z-OA-XG coatings produced by the natural color of the zein. Hence, the Z-OA and Z-OA-XG coated cheese samples displayed higher color change compared to plastic-packaged and unpackaged cheese samples; nonetheless, unpackaged samples produced a yellow rind that influenced color surface and texture of the cheese (Figure 4.4b and 4.5).

The textural characteristics of the “Minas padrão” cheese were influenced by the packaging type ($p < 0.05$), as a result of the difference in the cheese moisture content and proteolysis extent (FARKYE; FOX, 1990; McSWEENEY, 2004). Thereby, biodegradable-coated and unpackaged cheese samples were harder, more rubbery and less cohesive than plastic-packaged cheese (Figure 4.5).

According to Figure 4.5a, the “Minas Padrão” cheese with Z-OA and Z-OA-XG coatings, exhibited 14 % hardness increase after 14 days of storage. During the same period of time, the Mozzarella cheese with SPI coating displayed an increase over 350 % (ZHONG et al., 2014). Similarly, the “Salio” cheese coated with WPI (RAMOS et al., 2012) and glucomannan (CERQUEIRA et al., 2010) coatings showed 400 % hardness increment. The better water barrier properties exhibited by the Z-OA and Z-OA-XG biodegradable materials compared to other polysaccharide and protein-based films (PENA SERNA; LOPES FILHO, 2015) allowed for decreasing

moisture loss, resulting in a softer and cohesive “Minas Padrão” cheese than Mozzarella and “Salio” cheeses reported by Zhong et al. (2014), Ramos et al. (2012) and Cerqueira et al. (2010).

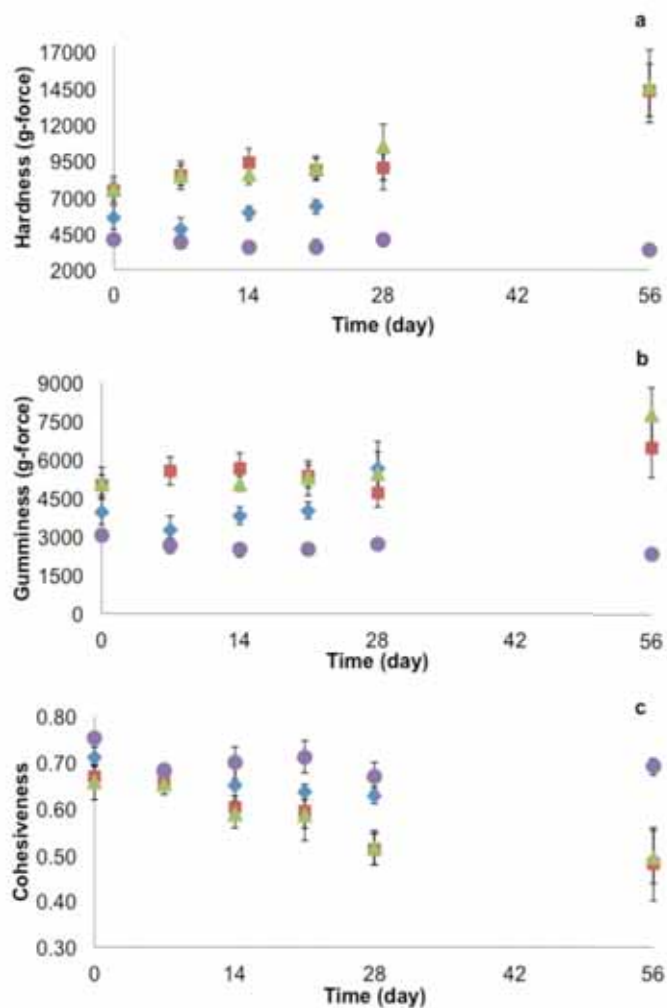


Figure 4.5. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on the texture of the “Minas Padrão” cheese throughout storage. Texture profile: hardness (a), gumminess (b) and cohesiveness (c). Values are mean ± standard deviation.

Given the harder texture as well as the higher color change exhibited by the biodegradable-coated samples in comparison to cheeses with polyethylene packaging; additional evaluations will be required in order to determine whether these cheese characteristics might cause consumer rejection. Otherwise, the current characteristics of the biodegradable-coated cheese samples may coincide with the

physicochemical characteristics of long ripened cheeses, based on that, Z-OA and Z-OA-XG biodegradable coatings could be successfully applied to the industry of long ripened cheeses, helping to reduce microbiological, chemical or environmental contamination during cheese ripening, storage and commercialization; nevertheless, further studies are required.

Conclusion

The impact of Z-OA and Z-OA-XG coatings on the quality of the “Minas Padrão” cheese was studied throughout 56 days of storage. The coated cheese samples exhibited similar preservation of physicochemical characteristics such as chlorides, ash, protein and acidity, compared to unpackaged and plastic-packaged cheese samples. The biodegradable coatings prevented the early microbiological contamination and decreased the cheese moisture loss in comparison to unpackaged samples. Although the Z-OA and Z-OA-XG coatings showed a positive behavior in comparison to unpackaged cheeses, some challenges remain and require further studies. For instance, the texture profile, surface color and proteolysis of the coated cheeses could be improved by decreasing the water vapor permeability of the coating or by using Z-OA and Z-OA-XG biodegradable films instead of the coatings. Given the current characteristics exhibited by the developed Z-OA and Z-OA-XG coatings, these seem to be suitable for the industry of long ripened cheeses.

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5

Zein-based blend films: diffusion of film components into food simulants

Abstract

The diffusion of small components of two biodegradable zein-based blend films into three food simulants (water, 3 % acetic acid and Tenax®) at 4 and 24 °C was evaluated. The migration of glycerol, thymol and oleic acid from the Z-OA and Z-OA-XG film matrix into the food simulants was confirmed and the release from the films into the food simulants were described satisfactorily by the Fick's second law. Glycerol exhibited the highest overall migration (above 80 %) in water whereas oleic acid showed higher overall migration into Tenax®. Temperature influenced the diffusion of the migrants; thus, higher diffusion coefficients were obtained at 24 °C. Furthermore, thymol releases from antimicrobial zein-based films (Z-OA_{Thy} and Z-OA-XG_{Thy}) was tested as well. The addition of thymol to Z-OA and Z-OA-XG films increased film's hydrophobicity, improving the affinity between oleic acid and Z-OA_{Thy} and Z-OA-XG_{Thy} compared to Z-OA and Z-OA-XG films (lower diffusion coefficients). Otherwise, thymol addition caused glycerol segregation, producing higher glycerol diffusion coefficients.⁴

⁴ This chapter will be submitted as:

Carolina Pena Serna, Frank Welle, José Francisco Lopes Filho. **Food Packaging and Shelf life.** (Under development).

5.1 Introduction

The global environmental concern caused by the increasing accumulation of plastic packaging waste (BALAGUER et al., 2013; KHAN et al., 2012) has triggered the development of new biodegradable materials, produced mainly from biopolymers such as proteins, polysaccharides or lipids (ARCAN; YEMENICIOĞLU, 2011; FAJARDO et al., 2010; SALARBASHI et al., 2014). For instance, blend films made from zein and oleic acid have exhibited better mechanical and water vapor barrier properties compared to zein films without the fatty acid (CUQ et al., 1995; WANG; PADUA, 2006; ARCAN; YEMENICIOĞLU, 2013) and other biodegradable films (PENA SERNA; LOPES FILHO, 2015).

In addition to the waste disposal and biodegradation issues, plastic packaging industry face the problem of food safety concern due to the possibility of chemical migration from the plastic packaging into the packaged food. These materials may contain substances of low molecular weight (< 1000 Dalton) that may diffuse within the polymer matrix, reaching the interface packaging-food and finally, dissolve in the food (GARCÍA et al., 2006; POÇAS; HOGG, 2007; SILVA et al., 2008).

These substances may be 1) non-intentionally added (referred as NIAS) such as impurities from the starting materials used to make the plastic polymer, reaction intermediates formed during the polymerization process, decomposition or reaction products formed during the polymerization or the thermal processing of the polymer to make the packaging, or 2) intentionally added as in the case of monomers and oligomers that remain not chemically bounded to the polymer molecules or additives used to improve the polymer properties (LAU; WONG, 2000; NERIN et al., 2013).

The migration of such substances into the food depends on the initial concentration and solubility in the packaging as well as the temperature and contact time between food and packaging (POÇAS et al., 2011).

In general, migration tests are accomplished using food simulants instead of foodstuffs due to the complexity of the food composition. Food simulants that are recognized by the Commission of European Communities (CEC) directives (COOPER; TICE, 2001; GROB, 2008) which have been used to test plastic

packaging migration are distilled water (simulant A), 3 % aqueous acetic acid solution (simulant B), 10 % aqueous ethanol solution (simulant C) and olive oil, sunflower oil, corn oil or synthetic triacylglycerides (Simulant D).

For instance, when there are technical problems with the analytical methods for migration test performed with fatty food simulants (D group), substitute media such as iso-octane, 95 % aqueous ethanol solution and modified polyphenylene oxide (Tenax®) are also recognized and allowed by the CEC directive 82/711/EEC (COOPER; TICE, 2001; GROB, 2008).

Contrary to plastic materials, biodegradable packaging are safe for human health due to the fact that the components used for preparing biodegradable films and coatings, intended to be in contact with foodstuffs, must be categorized by the FDA as generally recognized as safe (GRAS). Therefore, using biodegradable materials as food packaging, is not a risk for the consumer. Nonetheless, substances of low molecular weight, e. g., plasticizers and emulsifiers, may be present in the biodegradable films and coatings, thus, migration may occur and, consequently, cause variation on the food composition or physicochemical changes. For instance, off-flavors, changes in color, appearance, pH or texture may be produced causing food quality deterioration and consumer rejection.

Several studies have been developed in order to evaluate the release of active compounds, mainly antimicrobial agents, that were incorporated into biodegradable films. For instance, Sánchez-González et al. (2011) studied the release of limonene present in chitosan films, Fajardo et al. (2010), Bierhalz et al. (2012) and Silva et al. (2012) reported the migration of natamycin from alginate, alginate/pectin and alginate/chitosan films respectively. Besides, lysozyme (MECITOĞLU et al., 2006) and thymol (MASTROMATTEO et al., 2009; NOBILE et al., 2008) release was reported from zein films. Nevertheless, to the best of our knowledge, there is no study reported in the literature, aiming to evaluate the release of low molecular weight substances that were added to the biodegradable films in order to improve their functional properties. Thus, for the sake of food quality, migration evaluation of biodegradable materials should be accomplished.

Based on that, the aims of this study were (i) establish the components of the

Z-OA and Z-OA-XG films that may migrate into food simulants, (ii) evaluate the kinetic migration of the established migrants into water, 3 % aqueous acetic acid and Tenax® at 4 and 24 °C and (iii) produce Z-OA and Z-OA-XG films with antimicrobial activity, as active packaging, and evaluate the release of the antimicrobial substance (thymol).

5.2 Materials and methods

The following sections describe the materials and methods used within this study.

5.2.1 Materials

Corn-zein, 99.5 % ethanol, glycerol, oleic acid, xanthan gum and thymol supplied by Sigma-Aldrich (Munich, Germany), and Emustab® emulsifier kindly provided by Duas Rodas (Medellin, Colombia) were used for preparing zein-oleic acid (Z-OA) and zein-oleic acid-xanthan gum (Z-OA-XG) blend films. Food simulants were prepared from glacial acetic acid, distilled water and Tenax®, which were purchased from Sigma-Aldrich (Munich, Germany). LC-MS-grade methanol, distilled water supplied by Sigma-Aldrich (Munich, Germany) and diethyl ether picograde® purchased from LGC Standards GmbH (Wesel, Germany) were used for sample preparation and chromatographic analyses.

5.2.2 Film composition and preparation

For preparing Z-OA and Z-OA-XG blend films 20 g zein were added to 100 mL of 95 % ethanol under mechanical stirring (900-1000 rpm) for 5 min at 65 ± 0.5 °C. Glycerol, emulsifier and oleic acid were added to the solutions in a ratio of 10, 5 and 70 g/100 g zein, respectively. Xanthan gum (0.05 g) was also added to the Z-OA-XG

solution, and both solutions were stirred for another 10 min. Z-OA and Z-OA-XG solutions were cooled to room temperature before pouring 1 g solution into a polystyrene petri dish, allowing for air-drying overnight at room conditions. Blend films with antimicrobial activity (Z-OA_{Thy} and Z-OA-XG_{Thy}) were prepared as mentioned above, adding thymol (0.5 g) after cooling the solutions to room temperature and stirring them mechanically for about 10 min (NOBILE et al., 2008; MASTROMATTEO et al., 2009).

5.2.3 Migration experiments

The migration properties of four biodegradable materials were tested. Two of these materials were regular blend films referred as Z-OA and Z-OA-XG and the other two were blend films with antimicrobial activity referred as Z-OA_{Thy} and Z-OA-XG_{Thy}. Test samples were brought in contact with food simulants either by total immersion for liquid simulants (water and 3 % acetic acid) or by single side contact for solid simulant (Tenax®). The tests were accomplished in hermetically closed containers at 4 and 24 ± 1 °C (room temperature) and samples were taken at defined times throughout 81 h, which comprised short intervals at the beginning of the test and larger intervals after 9 h. For each time, three separate test set-up were assessed.

5.2.4 Quantification of migrants in food simulants and film samples

The quantification of migrant compounds into food simulant and film samples was accomplished using a gas chromatography with flame ionization detector-GC/FID (Agilent Mod. 6890); column: DB-1 30 m, 250 µm ID, 0.25 µm film thickness; temperature program: 50 °C (2 min) followed by heating at 10 °C min⁻¹ to 340 °C (9 min); pre-pressure: 50 kPa hydrogen and split ratio: 20.

5.2.4.1 Calibration procedure

A standard solution of the simulant compounds was prepared in 99.5 % ethanol in order to obtain a calibration curve. The calibration points were prepared by adding different aliquots of the standard solutions in water or 3 % acetic acid simulants. In the case of Tenax®, a special procedure was followed. Tenax® powder (1.14 g) was brought in contact with different aliquots of the standard solution for 12 h. Thereafter, the Tenax® was collected in a 250-mL Erlenmeyer and extracted three times with diethyl ether. The extractions were made adding 23.4 mL (first extraction) and 34 mL (second and third extractions) diethyl ether to the Tenax® powder and gently shaking for 1 min. The extraction solvent was carefully filtered in order to keep the powder into the Erlenmeyer, collecting the three solvent volumes in a 100-mL vial. Afterwards, the solvent was evaporated aided by N₂ and samples were analyzed by GC/FID. The calibration curves comprised points with concentrations from 5 to 2000 ppm with tert-butylhydroxyanisole (BHA) and Tinuvin 234 as internal standards.

5.2.4.2 Film sample preparation

For quantifying the migrant concentration present in the tested materials, the film samples were dissolved in 30 mL of 95 % aqueous ethanol and then 1-mL aliquots were analyzed by GC/FID.

5.2.4.3 Simulant sample preparation

The samples from liquid simulants were analyzed as they were taken during the migration experiments while Tenax® samples were extracted with diethyl ether prior to GC/FID analysis. 50 µL of BHA and Tinuvin 234 were added to both, film and simulant samples.

5.2.5 Mathematical modeling of the migrating film components

The release of substances of low molecular weight from a swelling polymeric matrix occurs when the water molecules penetrate into the matrix and cause the swelling of the polymer. As the water diffuses from the outer solution into the polymeric matrix, the polymer becomes more relaxed and wider, allowing for the substance to diffuse through the matrix into the outer solution until a thermodynamic equilibrium between the two phases is reached.

Mathematical modeling of the migration of substances dispersed in a polymeric matrix may be assessed according to the Fick's second law (Equation 5.1).

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (5.1)$$

Where C is the migrant concentration in the polymeric matrix (film) at time t and position x , and D is the diffusion coefficient of the substance in the film.

Crank (1975) has provided initial and boundary conditions in order to solve the partial differential equation, thus, the migration of a low molecular weight substance from a swelling homogeneous polymeric matrix, e. g., zein-based blend films, can be modeled according to Equation (5.2), considering that: 1) water diffusion and macromolecular matrix relaxation are faster than the dispersed substance diffusion through the swollen network, 2) the increase of the film size due to swelling is negligible, 3) the diffusion of the dispersed substance takes place in a homogeneous and symmetric medium, 4) the diffusion of the dispersed substance occurs in a plane sheet and is one-dimensional, 5) the dispersed substance is homogeneously distributed in the polymeric matrix at $t=0$, and 6) the total amount of the dispersed substance in the polymeric matrix and simulant remains constant during the migration process (NOBILE et al., 2008; MASTROMATTEO et al., 2009; FRANZ; BRANDSCH, 2012).

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[\frac{-(2n+1)^2 \pi^2 D t}{l^2}\right] \quad (5.2)$$

Where M_t is the mass of the migrating low molecular weight substance released from the film to the simulant and M_∞ is the mass of this substance released at equilibrium, D is the diffusion coefficient, l is the film thickness and t is time.

Hence, experimental data were fitted to Equation (5.2) by non-linear regression and the diffusion coefficient, assumed to be constant, was calculated.

5.3 Results and discussion

According to the preliminary tests accomplished in order to identify the film components that may migrate into the selected food simulants, glycerol and oleic acid with molecular weight of 92.09 and 282.46 g mol⁻¹ respectively, were found to be small enough to diffuse from the film matrix into the food simulants. Furthermore, thymol (150.22 g mol⁻¹), which was the antimicrobial agent added to the films migrated into the food simulant as well.

The initial amount of glycerol, thymol and oleic acid quantified into the matrix of the Z-OA, Z-OA-XG, Z-OA_{Thy} and Z-OA-XG_{Thy} films were 0.66 ± 0.04 mg cm⁻², 0.17 ± 0.02 mg cm⁻² and 4.2 ± 0.41 mg cm⁻², respectively.

In general, the four tested films exhibited an overall migration of glycerol above 80 % in water and 3 % aqueous acetic acid simulants while in Tenax®, the overall migration achieved a maximum of 5 %. Therefore, as expected, polar simulants caused higher glycerol migration than the non-polar one (Tenax®).

The high migration exhibited by glycerol in water and 3 % acetic acid was possibly a consequence of the low affinity of this component and the film matrix (POÇAS et al. 2011). Padua and Wang (2002) and Pena-Serna; Lopes-Filho (2013) reported a weak interaction between glycerol and zein which caused the migration of glycerol from the film matrix to the film surface.

Thymol, a hydrophobic migrant, exhibited higher overall migration in Tenax® than in the polar simulants. Thymol migrated above 90 % in Tenax® compared to an overall migration below 7 % in water and below 15 % in aqueous acetic acid.

Likewise, oleic acid, as a hydrofobic migrant, displayed higher overall migration in non-polar food simulant than in polar ones. Thus, this migrant achieved a maximum overall migration of 30 % in 3 % acetic acid, 18 % in water and 75 % in Tenax®. The greater molecular volume of the oleic acid compared to thymol, as well as the lack of a liquid phase that facilitates oleic acid diffusion might difficult its migration into the solid and hydrophobic simulant, Tenax® (POÇAS et al. 2011).

Some of the experimental and modeled migration kinetic of glycerol, thymol and oleic acid using the different simulants and temperatures are shown in Figures 5.1 to 5.5.

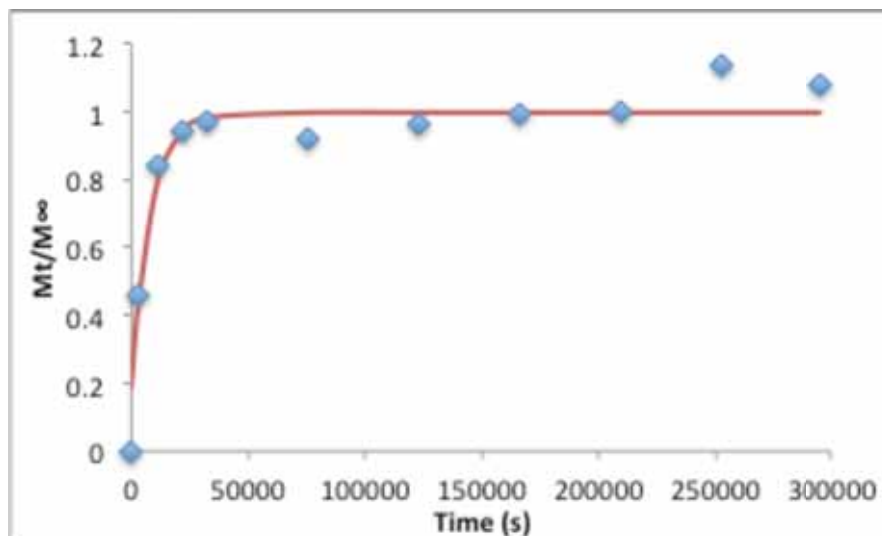


Figure 5.1. Experimental (♦) and simulated (–) migration of glycerol from Z-OA_{Thy} film into 3% aqueous acetic acid at 4 °C.

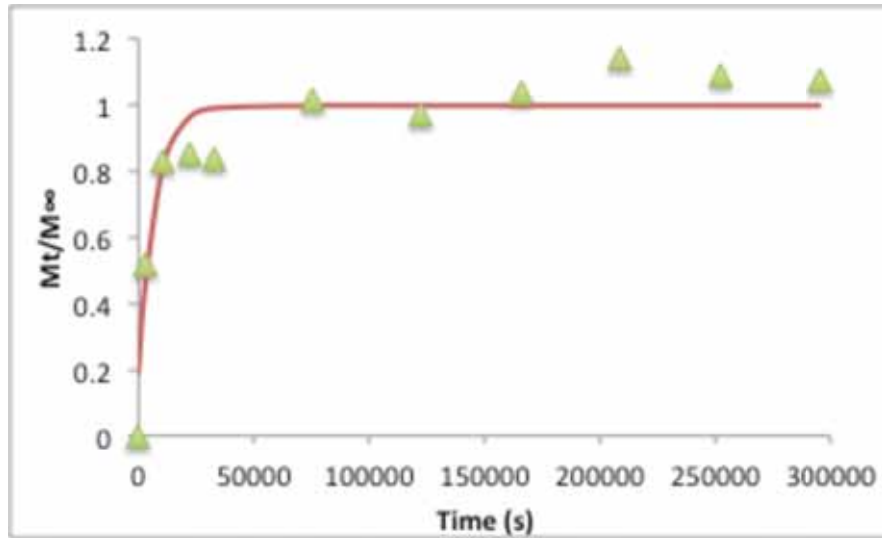


Figure 5.2. Experimental (\blacktriangle) and simulated (-) migration of thymol from Z-OA_{Thy} film into 3% aqueous acetic acid at 4 °C.

The simulated and experimental kinetics exhibited high accuracy, therefore, the proposed model satisfactorily described the experimental data.

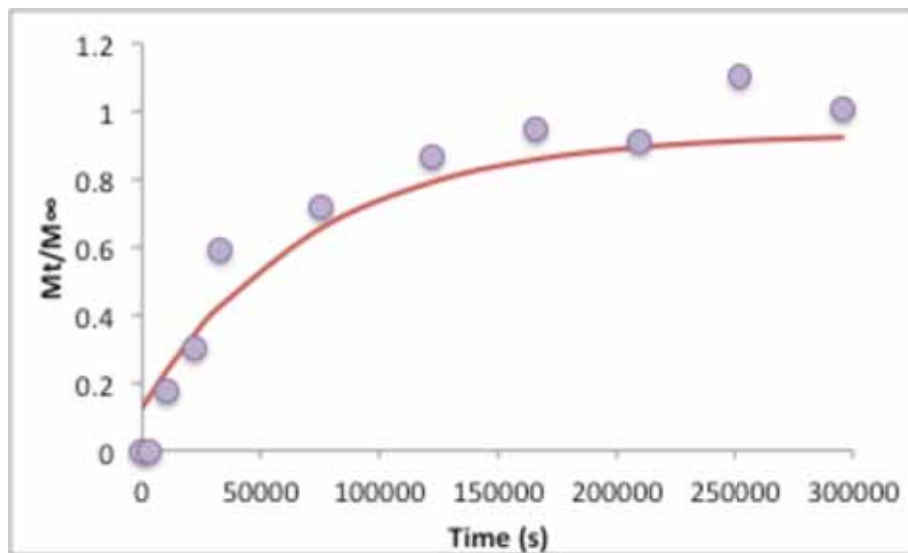


Figure 5.3. Experimental (\bullet) and simulated (-) migration of oleic acid from Z-OA_{Thy} film into 3% aqueous acetic acid at 4 °C.

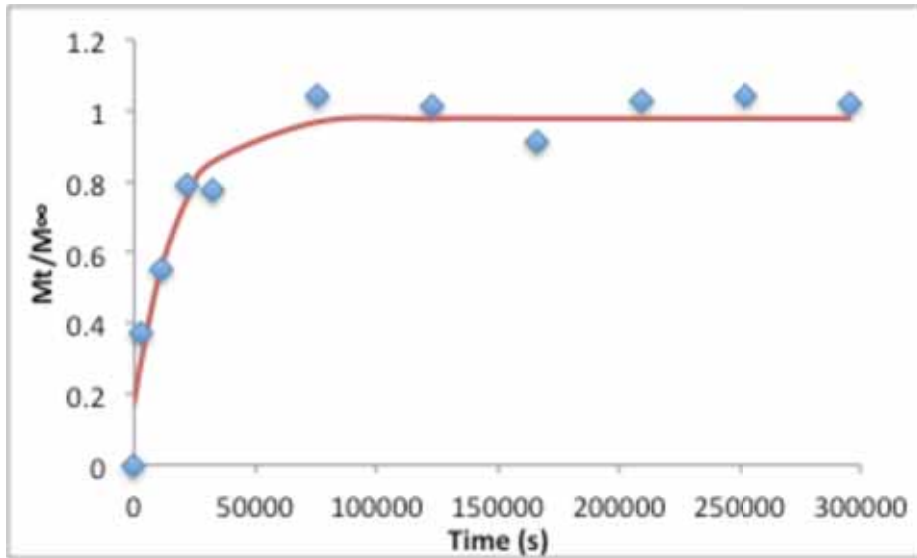


Figure 5.4. Experimental (♦) and simulated (-) migration of glycerol from Z-OA_{Thy} film into water at 24 °C.

According to these figures, the release of glycerol, oleic acid and thymol from the Z-OA, Z-OA-XG, Z-OA_{Thy} and Z-OA-XG_{Thy} films can be satisfactorily described by the Fick's second law.

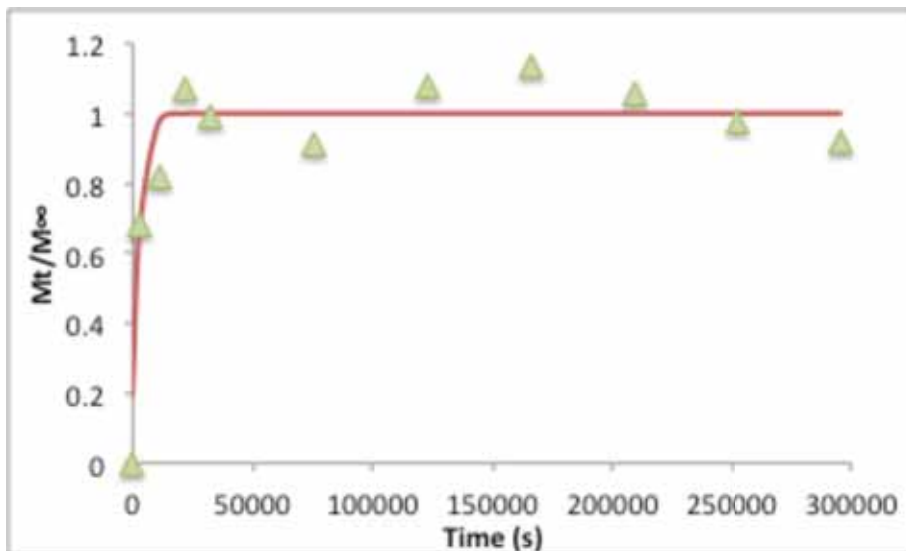


Figure 5.5. Experimental (▲) and simulated (-) migration of thymol from Z-OA_{Thy} film into water at 24 °C.

As observed in Figures 5.1 to 5.5, the migration of glycerol and thymol reached the equilibrium faster (< 21 h) than the oleic acid, possibly as a

consequence of the larger size and higher hydrofobicity of the oleic acid compared to the other migrant compounds.

The diffusion coefficient of glycerol (D_{Gly}), thymol (D_{Thy}) and oleic acid ($D_{Oleic\ acid}$) from the Z-OA, Z-OA-XG, Z-OA_{Thy} and Z-OA-XG_{Thy} films into water, 3 % acetic acid and Tenax® at 4 and 24 °C were calculated after fitting the experimental data to the Equation 5.2. The diffusion coefficient of the migrants at the specified conditions are presented in Tables 5.1 to 5.3.

Temperature influenced the migration of thymol from the Z-OA_{Thy} and Z-OA-XG_{Thy} films into 3 % acetic acid and Tenax® ($p < 0.05$). As reported by Welle and Franz (2012) and Poças et al. (2011), the diffusion process is temperature dependent; thus, as observed in Table 5.1, the higher the temperature, the greater the diffusion coefficient of thymol.

Table 5.1. Diffusion coefficient of thymol (D_{Thy}) into food simulants at 4 and 24 °C

Film type	Temperature (°C)	D_{Thy} (cm ² s ⁻¹)		
		3 % Acetic acid	Water	Tenax®
Z-OA _{Thy}	24 °C	8,410x10 ⁻⁹ ±	6,909x10 ⁻⁹ ±	3,861x10 ⁻¹⁰ ±
		3,491x10 ⁻¹⁹ a	4,283x10 ^{-20a}	1,745x10 ⁻²² a
Z-OA _{Thy}	4 °C	3,969x10 ⁻⁹ ±	5,732x10 ⁻⁹ ±	3,487x10 ⁻¹⁰ ±
		2,103x10 ⁻¹⁸ b	1,384x10 ⁻¹⁷ a	1,772x10 ⁻²¹ b
Z-OA-XG _{Thy}	24 °C	6,561x10 ⁻⁹ ±	4,162x10 ⁻⁹ ±	8,704x10 ⁻¹⁰ ±
		2,702x10 ⁻¹⁸ a	3,875x10 ⁻¹⁸ a	1,260x10 ⁻²³ a
Z-OA-XG _{Thy}	4 °C	4,749x10 ⁻⁹ ±	1,840x10 ⁻¹¹ ±	1,470x10 ⁻¹⁰ ±
		2,656x10 ⁻¹⁹ b	4,978x10 ⁻²¹ a	1,031x10 ⁻¹⁹ b

Values are mean ± standard deviation.

Different letters in the same column indicate statistically significant difference ($p < 0.05$).

Mastromatteo et al. (2009) studied the migration kinetic of thymol added to zein films brought into contact with water at room temperature. The study reported

diffusion coefficients of thymol between $2.33 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ and $6.81 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ for zein films with different thicknesses. The lower diffusion coefficient of the Z-OA_{Thy} film is due to the presence of oleic acid in the Z-OA film that caused an increase in the affinity between the Z-OA film and the thymol; therefore, decreasing its diffusion into the water (POÇAS et al., 2011).

Nobile et al. (2008) reported thymol diffusion coefficients between $7.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and $1.45 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for zein films with initial concentrations of thymol between 3.85 mg cm^{-2} and 26.92 mg cm^{-2} . The reported coefficients were obtained at room temperature and using water as food simulant. The higher diffusion coefficients reported by Nobile et al. (2008) were caused by the higher initial concentration of thymol added to the zein films compared to the thymol initial concentration added to Z-OA films (0.17 mg cm^{-2}). According to Crank (1975) and Poças et al. (2011), the initial concentration of the migrant into the polymer matrix influence the diffusion process.

Oleic acid and glycerol diffusion coefficients (Table 5.2 and 5.3) of the Z-OA, Z-OA-XG, Z-OA_{Thy} and Z-OA-XG_{Thy} films were influenced by both temperature and film type ($p < 0.05$); thus, at 24 °C higher $D_{\text{Oleic acid}}$ and D_{Gly} were obtained.

Besides, the addition of the antimicrobial agent (thymol) to the Z-OA and Z-OA-XG blend films influenced the diffusion process of the glycerol and oleic acid, may be as a consequence of structural changes caused to the film matrix when the thymol was added, or may be, by the increase of the hydrophobicity of the film.

Z-OA_{Thy} and Z-OA-XG_{Thy} films exhibited lower $D_{\text{Oleic acid}}$ (Table 5.2) in water and 3 % acetic acid (polar food simulants) compared to Z-OA and Z-OA-XG films. This behavior was possibly a consequence of the greater affinity of the oleic acid to the film when thymol (hydrophobic substance) was added. It is possible that the Z-OA_{Thy} and Z-OA-XG_{Thy} films were more hydrophobic than the Z-OA and Z-OA-XG films, though, further tests shall be conducted in order to establish that.

Table 5.2. Diffusion coeff. of oleic acid ($D_{\text{Oleic acid}}$) into food simulants at 4 and 24 °C

Film type	Temperature (°C)	$D_{\text{Oleic acid}}$ ($\text{cm}^2 \text{s}^{-1}$)		
		3 % Acetic acid	Water	Tenax®
Z-OA _{Thy}	24 °C	$6,403 \times 10^{-11} \pm$	$1,058 \times 10^{-10} \pm$	$3,179 \times 10^{-14} \pm$
		$7,605 \times 10^{-22} \text{ a}$	$5,630 \times 10^{-19} \text{ a}$	$2,780 \times 10^{-28} \text{ a}$
Z-OA _{Thy}	4 °C	$4,795 \times 10^{-12} \pm$	$1,733 \times 10^{-12} \pm$	ND
		$7,327 \times 10^{-24} \text{ a}$	$1,036 \times 10^{-22} \text{ a}$	
Z-OA-XG _{Thy}	24 °C	$6,881 \times 10^{-11} \pm$	$3,060 \times 10^{-11} \pm$	$1,011 \times 10^{-11} \pm$
		$8,414 \times 10^{-23} \text{ ab}$	$1,172 \times 10^{-18} \text{ a}$	$1,431 \times 10^{-22} \text{ a}$
Z-OA-XG _{Thy}	4 °C	$9,466 \times 10^{-11} \pm$	ND	ND
		$5,048 \times 10^{-21} \text{ ab}$		
Z-OA	24 °C	$8,149 \times 10^{-11} \pm$	$3,843 \times 10^{-11} \pm$	$3,674 \times 10^{-11} \pm$
		$5,949 \times 10^{-22} \text{ b}$	$2,149 \times 10^{-21} \text{ a}$	$2,976 \times 10^{-22} \text{ a}$
Z-OA	4 °C	$1,918 \times 10^{-10} \pm$	$3,542 \times 10^{-11} \pm$	$3,02989 \times 10^{-13} \pm$
		$1,407 \times 10^{-20} \text{ b}$	$1,811 \times 10^{-22} \text{ a}$	$3,30831 \times 10^{-26} \text{ a}$
Z-OA-XG	24 °C	$1,799 \times 10^{-10} \pm$	$5,831 \times 10^{-9} \pm$	$6,326 \times 10^{-11} \pm$
		$1,914 \times 10^{-21} \text{ b}$	$3,131 \times 10^{-19} \text{ b}$	$2,250 \times 10^{-22} \text{ a}$
Z-OA-XG	4 °C	$1,202 \times 10^{-10} \pm$	$6,461 \times 10^{-11} \pm$	$7,971 \times 10^{-10} \pm$
		$2,005 \times 10^{-21} \text{ b}$	$1,714 \times 10^{-21} \text{ a}$	$6,414 \times 10^{-20} \text{ b}$

ND: No migration was analytically detected.

Values are mean \pm standard deviation.

Different letters in the same column indicate statistically significant difference ($p < 0.05$).

Contrary to oleic acid, Z-OA_{Thy} and Z-OA-XG_{Thy} films displayed higher D_{Gly} (Table 5.3) compared to the Z-OA and Z-OA-XG films when brought in contact with polar simulants. As mentioned before, the possibly higher hydrophobicity of the Z-OA_{Thy} and Z-OA-XG_{Thy} films may be induced the segregation of the polar component (of the film), causing its higher diffusion.

Table 5.3. Diffusion coefficient of glycerol (D_{Gly}) into food simulants at 4 and 24 °C

Film type	Temperature (°C)	D_{Gly} ($\text{cm}^2 \text{s}^{-1}$)		
		3 % Acetic acid	Water	Tenax®
Z-OA _{Thy}	24 °C	$2,863 \times 10^{-9} \pm$	$8,144 \times 10^{-10} \pm$	$1,497 \times 10^{-9} \pm$
		$9,242 \times 10^{-19} \text{ a}$	$1,401 \times 10^{-20} \text{ a}$	$1,129 \times 10^{-18} \text{ c}$
Z-OA _{Thy}	4 °C	$1,721 \times 10^{-9} \pm$	$1,497 \times 10^{-9} \pm$	$1,631 \times 10^{-9} \pm$
		$1,797 \times 10^{-19} \text{ a}$	$3,202 \times 10^{-19} \text{ b}$	$6,918 \times 10^{-20} \text{ c}$
Z-OA-XG _{Thy}	24 °C	$1,683 \times 10^{-9} \pm$	$7,987 \times 10^{-10} \pm$	$5,182 \times 10^{-11} \pm$
		$2,204 \times 10^{-19} \text{ b}$	$2,892 \times 10^{-20} \text{ a}$	$1,249 \times 10^{-21} \text{ a}$
Z-OA-XG _{Thy}	4 °C	$1,009 \times 10^{-9} \pm$	$2,726 \times 10^{-9} \pm$	ND
		$1,962 \times 10^{-20} \text{ b}$	$3,379 \times 10^{-18} \text{ b}$	
Z-OA	24 °C	$5,338 \times 10^{-10} \pm$	$7,757 \times 10^{-10} \pm$	$3,442 \times 10^{-10} \pm$
		$3,265 \times 10^{-20} \text{ c}$	$1,215 \times 10^{-19} \text{ a}$	$9,092 \times 10^{-20} \text{ a}$
Z-OA	4 °C	$7,312 \times 10^{-10} \pm$	$2,109 \times 10^{-9} \pm$	$4,284 \times 10^{-9} \pm$
		$8,931 \times 10^{-20} \text{ c}$	$2,173 \times 10^{-21} \text{ b}$	$6,723 \times 10^{-19} \text{ d}$
Z-OA-XG	24 °C	$5,056 \times 10^{-10} \pm$	$8,151 \times 10^{-10} \pm$	$1,102 \times 10^{-10} \pm$
		$7,855 \times 10^{-20} \text{ bc}$	$6,505 \times 10^{-22} \text{ a}$	$8,896 \times 10^{-22} \text{ a}$
Z-OA-XG	4 °C	$9,769 \times 10^{-10} \pm$	$1,649 \times 10^{-9} \pm$	$5,809 \times 10^{-10} \pm$
		$1,574 \times 10^{-19} \text{ bc}$	$2,714 \times 10^{-20} \text{ b}$	$4,238 \times 10^{-19} \text{ b}$

ND: No migration was analytically detected.

Values are mean \pm standard deviation.

Different letters in the same column indicate statistically significant difference ($p < 0.05$).

Though the migration of small components present in the matrix of the zein-based blend films was confirmed, there are some issues that remain and require further studies. For instance, which effect will produce the diffusion of migrants on the foodstuff? The migration of the substances into the food will cause quality deterioration, physicochemical changes, organoleptic alterations or changes in the food composition? What happens to the film's microstructure and the diffusion of the small components present in the film matrix when thymol or any other active agent is added (in order to produce an active material)? The addition of the active agent will increase or reduce the diffusion of the small film components? In case of increasing the migration, which will be the impact on the food quality?

Conclusions

The diffusion of low molecular components of the Z-OA, Z-OA-XG, Z-OA_{Thy} and Z-OA-XG_{Thy} blend films into water, 3 % acetic acid and Tenax® at 4 and 24 °C was proved. The migration of glycerol and oleic acid from the film matrix into the food simulants was influenced by the temperature, the polarity of the simulant and the addition of the antimicrobial agent to the zein-based blend films. The higher temperature increased the diffusion of the migrants, exhibiting greater diffusion coefficients. The polar simulants caused higher migration of glycerol (polar migrant) while Tenax® produced higher diffusion of oleic acid and thymol (hydrophobic migrants). The addition of thymol to the zein-based blend films increased their hydrofobicity, causing higher affinity of the oleic acid to the films and the segregation of the glycerol from them. Although the diffusion of small components of the Z-OA, Z-OA-XG, Z-OA_{Thy} and Z-OA-XG_{Thy} films into food simulants was confirmed, some challenges remain and require further studies. Besides, evaluating the diffusion of the small components of Z-OA-XG and Z-OA blend films into foodstuffs instead of food simulants may give the chance for assessing a more realistic overall migration as well as evaluating the impact on the food organoleptic and physicochemical characteristics.

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Concluding remarks and recommendations

This study verified the influence of the ethanol and glycerol concentration, used as zein solvent and plasticizer agent, respectively, on the functional, mechanical and structural properties of zein-oleic acid blend films. Higher ethanol concentration (95 %) and lower glycerol concentration (0 %) allowed for improving water vapor and light barrier properties, homogeneity and microstructure of the Z-OA blend films, though film brittleness increased due to the lack of plasticizer. Therefore, 10 % glycerol shall be added in order to obtain Z-OA blend films with better mechanical, structural and functional properties.

In the second place, the current research verified the effect of adding xanthan gum (carbohydrate) or gelatin (protein) to the Z-OA film aiming to produce blend films with better functional, mechanical and structural properties compared to Z-OA films. Gelatin denaturation was proved when added to the blend solutions previously prepared in 95 % ethanol; therefore, no Z-OA-GE blend film was produced. This study established that Z-OA and Z-OA-XG blend films exhibited similar water vapor and oxygen permeability as well as microstructure. Nonetheless, the addition of xanthan gum influenced the light permeability and mechanical properties of the film, increasing the opacity, strength (higher TS) and rigidity (higher YM and lower %E) of Z-OA-XG compared to Z-OA blend films. Based on that, xanthan gum shall be added to zein-oleic acid blend films only in cases where the foodstuffs may be oxidized by the light action or when rigid food packaging are required.

Further studies testing blend films based on zein and any other hydrophobic protein may help to decrease the water vapor permeability of the current Z-OA blend film. Similarly, adding nano-clays to the Z-OA blend film may improve film's water barrier properties.

This study also verified the effect of using the Z-OA and Z-OA-XG coatings on the preservation of physicochemical, surface color and textural characteristics of the "Minas padrão" cheese throughout a storage period of 56 days. The Z-OA and Z-OA-XG coatings exhibited no difference on cheese preservation; thus, both biodegradable coatings displayed similar preservation of characteristics such as chlorides, protein, ash and acidity, compared to

unpackaged and polyethylene-packaged cheeses. The Z-OA and Z-OA-XG coatings helped to prevent early microbiological contamination and decrease moisture loss in comparison to unpackaged cheese. Nonetheless, the moisture loss influenced cheese characteristics such as texture, superficial color and proteolysis. Coated cheeses exhibited 10 % moisture loss during the process of drying the coating (on cheese surface). Based on that, accomplishing further tests using Z-OA and Z-OA-XG films instead of coatings may help to prevent cheese initial moisture loss, improving the performance of the Z-OA and Z-OA-XG materials as cheese packaging.

Supplementary studies aiming to evaluate the Z-OA blend film or coating on long ripened cheeses may prove the feasibility of using Z-OA biodegradable coatings or blends in the industry of long ripened cheeses.

Moreover, further analyses aiming to evaluate possible microbiological contamination or organoleptic (sensory) changes throughout the storage of the cheese or any other foodstuff are highly recommended.

Finally, this research verified the migration of small compounds present in the matrix of the Z-OA-XG and Z-OA blend films into food simulants at 4 and 24 °C, aiming to evaluate the diffusional properties of the films and the possible effect on food quality. The migration of glycerol and oleic acid from the film matrix into water, 3 % acetic acid and Tenax® (food simulants) was proved at 4 and 24 °C. Therefore, the evaluation of the diffusional properties of biodegradable materials aiming to be used as food packaging might be recommended, in order to avoid the deterioration of the food quality. Furthermore, evaluating the diffusion of small components of the Z-OA-XG and Z-OA blend film into foodstuffs instead of food simulants may help to assess the real migration and its impact in food organoleptic and physicochemical characteristics.

List of publications

Publications as abstracts in proceedings of symposia, conferences and congresses

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