LIVIO ANTONIO S. PEREIRA

DESENVOLVIMENTO DE REVESTIMENTO COMESTÍVEL DE ZEÍNA E BLENDA DE ÓLEOS ESSENCIAIS PARA APLICAÇÃO EM MOÇARELA

LAVRAS - MG

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Orientador

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Coorientadora

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DEVELOPMENT OF EDIBLE COATING ZEIN BASE AND BLEND OF ESSENTIAL OILS FOR USE IN MOÇARELA

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia de Biomateriais, área de concentração em Produtos e nanoprodutos alimentícios, para a obtenção do título de Mestre.

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À Deus ser supremo em minha vida,
aos meus pais e família pelo apoio, amor e dedicação,
à Priscila pelo amor e fortaleza.
Dedico

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RESUMO

A busca por conservantes naturais, aliado a qualidade e maior durabilidade de alimentos, gera a necessidade de utilização de embalagens biodegradáveis e otimizadoras de qualidade alimentícia. Atualmente, revestimentos comestíveis estão sendo estudados e fazem parte de um futuro promissor na área de alimentos. O uso de embalagens biopoliméricas com aditivos, como os óleos essenciais, são objeto de constantes pesquisas, devido às suas propriedades antimicrobianas e por atuarem como substitutos de conservantes. O presente estudo avaliou a zeína como matriz biopolimérica e uma blenda de óleo essencial de alho e tomilho como plastificante, flavorizante e antimicrobiano. Avaliou-se as propriedades antimicrobianas e flavorizantes deste revestimento aplicado em moçarelas com diferentes teores de sódio, além de sua aceitação pelo consumidor. A efetiva ação plastificante da blenda de óleos essenciais sobre os filmes de zeína a partir da concentração de 3% (v/v) foi observada. Na sequência, houve boa ação antimicrobiana dos filmes de zeína e blenda de óleos essenciais de alho e tomilho a partir da adição de 2% (v/v) de óleos frente as bactérias *Echerichia coli*, Enteropatogênica, *Listeria monocytogenes, Salmonella,* Enteritidise *Staphylococcus aureus*. Em relação a moçarela com 3% (v/v) de blenda de óleos, foi observado a diminuição da perda de água em até 50%, potencial da eficácia no controle de microrganismos aeróbios mesófilos e fungos e leveduras. Nas análises sensoriais foi observado boa aceitação de reduções de sódio em até 75% do padrão. A ação plastificante da blenda de óleos essenciais na matriz de zeína pode ser comprovada através da eliminação de trincas e diminuição da rigidez dos filmes, e foi detectada boa eficiência antimicrobiana para os microrganismos testados. De forma geral, conclui-se que os filmes à base de zeína e blenda de óleos essenciais são afetados pela adição de blenda de óleos essenciais, alterando as suas propriedades de engenharia e antimicrobianas, e atuando com eficácia na redução de sódio de moçarelas.

Palavras-chave: Revestimentos comestíveis. Zeína. Filmes antimicrobianos. Blenda de óleos essenciais. Redução de sódio.

ABSTRACT

The search for natural preservatives, allied to the higher quality and durability of food, creates the need for biodegradable and food quality optimizing packaging. Edible coatings are currently being studied and are a part of a promising future in the field of food. The use of biopolymer packaging with additives, such as essential oils, are constantly objects for researches, due to its antimicrobial properties and for acting as substitute for preservatives. In this study, we evaluated zein as biopolymeric matrix and a blend of garlic and thyme essential oil as plasticizer, flavoring and antimicrobial agent. By means of this coating, applied mozzarellas with different contents of sodium, the antimicrobial and flavoring properties, and consumer acceptance, were evaluated. We verified effective plasticizing action of the essential oils over zein films from the concentration of 3% (v/v). There was good antimicrobial action of the zein films and garlic and thyme essential oil blend with the addition of 2% (v/v) of oils, regarding bacteria Enteropathogenic *Echerichia coli*, *Listeria monocytogenes*, *Salmonella* Enteretidis and *Staphylococcus aureus.* Concerning the mozzarella with 3% (v/v) of oil blend, we verified a decrease in water loss of up to 50%, efficacy potential in the control of mesophle aerobic microorganisms, fungi and yeast. In sensorial analyses, we verified good acceptance of sodium reduction up to 75% of the standard. The plasticizing action of the essential oil blend in the zein matrix can be proven by means of eliminating cracks and reducing the rigidity of the films. We also found good antimicrobial efficiency for the tested microorganisms. In conclusion, the zein based films and essential oil blend are affected by the addition of essential oil blend, altering the engineering and antimicrobial properties and acting efficiently in reducing sodium from the mozzarellas. ~

Keywords: Edible coatings. Zein. Antimicrobial films. Essential oil blend. Sodium reduction.

LISTA DE ABREVIATURAS E SIGLAS

AFM	Microscopia de força atômica
ASTM	American Society for Testing and Materials
BHI	Caldo infusão de cérebro e coração
DMA	Dynamic Mechanical Analysis
DRX	Difração de Raio-X
DRBC	Dichloran Rose-Bengal Chloramphenicol
PCA	Plate Count Agar
DSC	Calorimetria exploratória diferencial
FTIR	Infravermelho por transformada de Furrier
KBr	Brometo de potássio
MEV	Microscopia eletrônica de varredura
NMP	Número mais provável
PET	Politereftalato de etileno
PVA	Permeabilidade a vapor de água
rpm	Rotações por minuto
TG	Termogravimetria
Tm	Temperatura de fusão
TSA	Agar Triptona de Soja
TSB	Caldo Triptona de Soja
UFC	Unidades formadoras de colônia
UFLA	Universidade Federal de Lavras

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Atualmente, há grande interesse por tecnologias que aumentem a produção e qualidade de alimentos. Segundo a *Food and drug admnistration*, um terço dos alimentos produzidos mundialmente vão para o lixo diariamente. Estes fatores são causados por diversas variáveis no processamento alimentício, como as más condições de conservação, que por sua vez levam ao aumento do número de microrganismos como bactérias e fungos (FERREIRA, 2008; FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2014).

Doenças causadas pelo consumo de alimentos contaminados ocorrem em nível mundial e são mais frequentes em alimentos de origem artesanal, embora não se deve desprezar sua ocorrência na indústria. Dentre as principais causas destaca-se as más condições sanitárias e contaminações cruzadas que ocorrem no processamento de matérias-primas e aditivos no processo (ORIANI et al., 2014).

Em muitos casos, podem ocorrer contaminações microbiológicas no armazenamento e estocagem, através de embalagens impróprias ou danificadas. Portanto, é de suma importância conhecer a natureza e as transformações que ocorrem no alimento e na embalagem aplicada. A utilização de embalagens é indispensável, visto que esta tem que agir como uma barreira entre o ambiente externo e o alimento sem compromete-lo. Entretanto, a maioria das embalagens são produzidas por materiais que não são biodegradáveis e estão envolvidas com problemas ambientais. Portanto, é indispensável o estudo de tecnologias que reduzem a utilização dessas embalagens. Além disso, deve-se ressaltar a importância dos tratamentos físicos, químicos e biológicos que auxiliam na preservação da integridade alimentícia. Dentre os tratamentos utilizados, há o uso de conservantes, como o sódio e seus derivados que, se utilizados em demasia, são prejudiciais à saúde (KHAN et al., 2014; RIGO, 2006).

A fim de reduzir os problemas mencionados, os biopolímeros tem demonstrado a possibilidade de criação de embalagens de vários materiais, especialmente baseados em polissacarídeos, lipídeos e proteínas. Diversos fatores são determinantes em relação as propriedades destas embalagens, como a natureza do polímero, sua formulação, seu processo de obtenção e a sua forma de aplicação no produto. Entretanto, as aplicações destes materiais estão demorando devido à sensibilidade de suas propriedades mecânicas e de barreira. Podem ainda oferecer algumas vantagens como: poderem ser consumidos em conjunto com o produto, serem capazes de reter compostos aromáticos e carregar aditivos alimentícios ou componentes com atividade antimicrobiana (BRITO et al., 2011; LUVIELMO; LAMAS, 2012).

Atualmente, estes biopolímeros estão sendo utilizados como revestimentos comestíveis sobre a superfície dos alimentos, principalmente embutidos como produtos cárneos e lácteos. Neste caso, os revestimentos têm o potencial de formar fina película sobre a superfície dos alimentos, atuando como agentes de proteção ao meio externo, aumentando a sua conservação. Há ainda a adição de aditivos naturais como óleos essenciais que podem gerar melhorias nas propriedades do revestimento e na inibição de crescimento de microrganismos, aumentando a vida útil do produto durante o armazenamento (FERREIRA, 2008; LUVIELMO; LAMAS, 2012; TEIXEIRA et al., 2014).

Nesse contexto, o objetivo deste trabalho engloba o desenvolvimento de revestimento comestível, tendo a zeína como matriz biopolimérica e uma blenda de óleos essenciais de alho e tomilho agindo como agente plastificante, flavorizante e antimicrobiano. Este revestimento, aplicado em moçarelas, foi investigado a fim de avaliar a eficácia da blenda de óleos essenciais como agente antimicrobiano em relação ao aumento de sua vida útil e agindo como agente flavorizante em moçarelas com sucessivas reduções de sódio, assim como avaliar a aceitação do consumidor frente as novas tecnologias na área de alimentos.

2 REVISÃO DE LITERATURA

2.1 Embalagens Comestíveis

Nos últimos anos, houve um grande interesse por materiais biodegradáveis e que sejam ambientalmente corretos. Nesse contexto, destacam-se muitos polímeros de origem natural como os derivados de polissacarídeos e proteínas (FORATO et al., 2013).

Hoje em dia, um grande público, assim como regulamentos internacionais, têm enfatizado a aplicação de materiais que mostram benefícios para a saúde e são ambientalmente corretos, especialmente em aplicações envolvendo alimentos. Revestimentos comestíveis provam-se promissores, estendendo o prazo de validade de diversos alimentos. São aplicados para proteger produtos de danos mecânicos e microbiológicos, para proporcionar uma aparência estética agradável, e para evitar a fuga de voláteis favoráveis ao aroma e conservação de substâncias do produto final. Estas embalagens são baseadas em materiais biodegradáveis e comestíveis a partir de fontes naturais e, portanto, satisfazem as leis ambientais por serem atóxicas e, na maioria dos casos, por se decomporem mais rápido na natureza do que os polímeros sintéticos convencionais (FERREIRA, 2008; KHAN et al., 2014).

A maioria dos estudos que investigam a aplicação de revestimentos comestíveis, utilizam compósitos contendo lipídios e proteínas. Os filmes feitos à base de polissacarídeos ou proteínas são chamados hidrocoloidais, e possuem excelentes propriedades mecânicas, ópticas e sensoriais. No entanto, deve-se considerar as iterações que ocorrem com o ambiente, relacionadas com a umidade, temperatura, pH, etc. As proteínas são indicadas para usos diversos, na indústria farmacêutica como material de encapsulamento de drogas, na impermeabilização de papeis e embalagens cartonadas e associadas a polímeros sintéticos para minimizações de contato e deteriorações em alimentos (FORATO et al., 2013; TRUJILLO-DE SANTIAGO et al., 2014b; YIN et al., 2014).

Filmes feitos de lipídios beneficiam-se de excelente barreira de água por serem hidrofóbicos e, assim, inibem a perda de água dos produtos revestidos. No entanto, são também menos permeáveis para gases, podendo levar ao acúmulo de CO_2 e demais gases liberados de alimentos, assim, causando o desenvolvimento de sabores estranhos (ALI et al., 2013; MOREIRA et al., 2014).

Sendo relativamente frágeis, são adicionados aos filmes de lipídios componentes auxiliares, tais como polissacarídeos e/ou plastificantes, que agregam propriedades de resistência mecânica. Revestimentos e compósitos naturais à base de amido, hidroxipropilmetilcelulose (HPMC), quitosana, pectina, zeína, dentre outros, estão sendo amplamente estudados no que diz respeito à melhoria da capacidade de armazenamento e qualidade de alimentos (ARNON et al., 2015; SWAIN; PATRA; KISKU, 2014).

Um fator que vem ganhando destaque nos últimos anos é o aproveitamento de materiais que são considerados como resíduos em muitos processos industriais. Entre os biopolímeros, um material de interesse para a confecção de filmes para emprego em embalagens de alimentos, destaca-se os derivados de zeínas (FORATO et al., 2013).

2.2 Biopolímeros - zeína

Zeína foi descrita pela primeira vez por John Gorham em 1821 depois que o mesmo isolou a proteína a partir do milho. Em 1909 foi concedida a primeira patente de preparação zeína termoplástica (ZHANG et al., 2015).

Zeínas são proteínas de reserva compostas por vários polipeptídeos que representam mais de 50% da massa total das proteínas presentes no endosperma do milho (*Zea mays*). O grão de milho destaca-se por ser um dos cereais mais cultivados em todo o mundo e é composto de 70 a 73 % de amido, 9 a 10% de proteínas, 4 a 5% de óleos, 1 a 2% de cinzas, 2% de açúcares e 9 a 10% de fibras. A obtenção de zeína é feita a partir da extração do glúten do milho, um dos subprodutos na produção (CARLESSO et al., 2012; CORR; LOPES FILHO, 2009).

Filmes de zeína são quebradiços, havendo muitas vezes a necessidade de adição de plastificantes, altamente hidrofóbico e resistente ao ataque microbiano, o que o torna aplicável em diversos setores da indústria (FORATO et al., 2013; PANKAJ et al., 2014).

As zeínas são consideradas proteínas globulares e constituídas de frações classificadas de acordo com a sua massa relativa e solubilidade, como zeínas α, γ, β e δ. As zeínas α são as de maior disponibilidade. Possuem 60% de proteínas e um terço de sua constituição é hidrofílica, e a restante é composta de resíduos de aminoácidos hidrofóbicos, apresentando hidrofobicidade média de 1,365 J/mol. É também composta por ácido glutâmico, grupos amino e outros constituintes como descrito na Tabela 1 (ARGÜELLO-GARCÍA et al., 2014; CARLESSO et al., 2012; DAUSCH; NIXON, 1990; TRUJILLO-DE-SANTIAGO et al., 2014b).

Tabela 1 - Aminoácidos presentes na zeína.

Aminoácidos apolares	Aminoácidos polares
Prolina	Treonina
Fenilanina	Cisteína
Metionina	Tirosina
Valina	Asparagina
Isoleucina	Histidina
Alanina	Ácido glutâmico
Leucina	Arginina
	Glutamina
	Glicina

Fonte: Arguello-Garcia et al. (2014)

Suas proteínas contêm 10 segmentos helicoidais sucessivos, dispostos de forma antiparalela, e que são estabilizados por ligações de hidrogênio. São ricas em resíduos de aminoácidos apolares, por isso, as zeínas α são altamente hidrofóbicas, ou seja, insolúveis em água, mas solúveis em meios alcoólicos. Por este motivo, filmes produzidos a partir da diluição de zeínas puras apresentam também caráter hidrofóbico, o que pode ser potencialmente interessante para aplicações como revestimentos ou barreiras à umidade e ao vapor de água em alimentos que exigem tais tratamentos. De forma geral, filmes à base de zeínas também apresentam boa barreira ao transporte de oxigênio, dióxido de carbono e demais compostos voláteis (ARGÜELLO-GARCÍA et al., 2014; FORATO et al., 2013).

2.2.1 Propriedades e custo

A zeína é um polímero completamente amorfo e sem plastificante, exibe a temperatura de transição vítrea (Tg) em torno de 165°C, entretanto, a sua Tg diminui significativamente com a adição de plastificante. É desejável uma diminuição da temperatura de transição vítrea em filmes e revestimentos de zeína, a fim de diminuir a sua rigidez e, consequentemente, melhorar a sua flexibilidade. Os plastificantes mais efetivos são aqueles que possuem grupos polares e apolares, tais como: trietil glicol, ácido oleíco e ácido dibutiltartárico.

A principal razão pela perda de mercado no final dos anos 50 e começo dos anos 60 foi o alto custo da zeína, além das propriedades superiores dos polímeros sintéticos (ERDOGAN; DEMIR; BAYRAKTAR, 2015).

A zeína possui boas propriedades de barreira ao oxigênio e gás carbônico, resistência às gorduras, e tem sido investigada quanto ao seu uso potencial como material estrutural em embalagens. Filmes e revestimentos baseados em zeína têm sido utilizados para diminuir a absorção de umidade em remédios, doces e alimentos perecíveis (CARLESSO et al., 2012).

Embora a zeína seja originada de resíduo industrial e possua grande potencial para substituir alguns polímeros convencionais, ela é ainda um polímero caro em comparação com o polietileno comum ou amido, devido ao seu processo de purificação. No entanto, existem grandes perspectivas para a diminuição do preço da zeína. É importante salientar que, no processo de extração da zeína, mesmo sem estar completamente purificada apresenta frações de ácido oleico em sua composição. Portanto, há muitas pesquisas desenvolvidas em relação à metodologia mais eficaz e barata de extração da zeína, e acredita-se que o seu custo pode ser minimizado, podendo aumentar a sua viabilidade como material plástico (CARLESSO et al., 2012; PANKAJ et al., 2014; SELLING; UTT, 2014).

2.2.2 Processamento e caracterização dos filmes

Filmes de zeína são obtidos pelo processo *casting*, o mais comumente utilizado para a formação de filmes e revestimentos. Em um processo *casting*, após a evaporação espontânea do solvente à temperatura ambiente (25 ± 3 ºC), os filmes são manualmente destacados (FORATO et al., 2013).

2.3 Óleos essenciais

Óleos essenciais (EOS) são líquidos oleosos aromáticos obtidos partir de material vegetal como flores, brotos, sementes, folhas, galhos, cascas, ervas, madeira, frutas e raízes. O termo "óleo essencial" é originado do nome inventado no século XVI por um pesquisador suíço, Paracelsus von Hohenheim, que nomeou o componente eficaz de fármaco de *Quinta essência*. Estima-se que mais de 3000 óleos essenciais são conhecidos, dos quais cerca de 300 são comercializados e destinados principalmente para o mercado de aromas e fragrâncias. Além de seu uso como flavorizante, muitos estudos já comprovam suas propriedades antimicrobianas, antioxidantes, antivirais e inseticidas (CALO et al., 2015; TUREK; STINTZING, 2013).

Os óleos essenciais são misturas naturais que podem se apresentar complexas, contendo de 10 a 60 componentes em distintas concentrações. São caracterizados por dois ou três componentes majoritários em concentrações bastante elevadas (20 a 70%) em comparação com outros componentes presentes em menores quantidades. Por exemplo, o carvacrol e timol são os componentes majoritários do *Origanum compactum*, linaloldo óleo essencial *Coriandrum sativum*, carvona e limoneno de sementes de *Anethum graveolens*. Os componentes majoritários geralmente definem as propriedades de um óleo essencial. Os componentes principais são compostos por aldeídos, fenóis, terpenos e terpenóides e um secundário aromático e compostos alifáticos, todos caracterizados por baixo peso molecular. Outros compostos importantes são hidratos de carbono, álcoois, éteres e cetonas (CORBO et al., 2009). A composição de óleos essenciais depende das estações de colheita e de fontes geográficas, assim como das diferentes partes das plantas (PERRICONE et al., 2015).

Há uma nova tendência em relação ao consumo de produtos naturais como óleos essenciais. Portanto, é importante desenvolver uma melhor compreensão de sua ação biológica para novas aplicações na saúde humana, agricultura e meio ambiente. Algumas dessas aplicações constituem alternativas eficazes na utilização de compostos sintéticos da indústria alimentícia (BAKKALI et al., 2008).

2.3.1 Óleo essencial de alho

O óleo essencial de alho provém de bulbos comestíveis da planta *Allium sativum* comumente utilizado na culinária mundial. O alho da terra, a cebolinha-de-cheiro e o alho-poró são espécies do mesmo gênero. A maior quantidade de fitoquímicos encontra-se nos bulbos, popularmente conhecidos como dentes de alho. Seu cultivo é adaptado às regiões mais amenas com período de dormência de dois meses. Quanto mais amena é a temperatura, maior é a concentração de fitoquímicos, pois depende da resposta da planta às agressões do meio ambiente (BHANDARI, 2012).

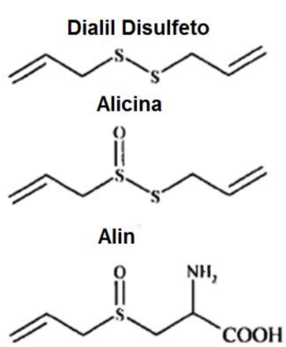
O alho tem sido utilizado como medicamento desde o tempo de Cristo. Há registros de seu uso como medicamento desde a época do Egito antigo. Atualmente, pesquisas têm demonstrado alguns desses efeitos, especialmente em relação à sua atividade imuno estimulante, antiaterosclerótica, anticancerígena e antimicrobiana. A valorização das suas propriedades tem se acelerado nos últimos anos por várias publicações científicas relacionadas ao seu uso na cura de doenças cardíacas e câncer. Embora alguns dos resultados ainda sejam conflitantes devido às falhas metodológicas, as evidências sugerem resultado positivo na cura de várias enfermidades (CHUNG, 2006; WILDMAN, 2006).

Estudos científicos descobriram a presença de vários compostos que possuem ação terapêutica no tratamento de parasitoses, desconfortos gastrintestinais, dislipidemias, verminoses intestinais, na doença hipertensiva, cardiovascular, câncer, além das atividades anti-inflamatória, antimicrobiana e antiasmática. É conhecido por uma infinidade de nomes como: alho-serpente, alho-bravo, alho-hortense, alho-manso, alho-ordinário e alho-do-reino. Esta erva bulbosa , cujo nome científico é *Allium sativum* , é pequena perene e com odor forte e característico de alimentos ricos em compostos sulfurados (CHARU; YOGITA; SONALI, 2014; DAUSCH; NIXON, 1990; DZIRI et al., 2014; TEIXEIRA et al., 2014).

2.3.1.1 Composição química

Apesar do alho ser ingrediente utilizado a milênios, o estudo de suas propriedades iniciou-se em períodos mais recentes. Em 1844, o químico alemão Wertheim realizou o processo de destilação a vapor no qual foi capaz de obter óleo de alho. Ele propôs o nome alilo para o hidrocarboneto contido no óleo, o qual é atualmente usado para descrever o grupamento CH_2 = $CHCH_2$ então presente em seus componentes (HARRIS et al., 2001).

Figura 1 - Principais componentes do óleo essencial de alho



Fonte: Dziri et al. (2014)

O alho contém em torno de 33 compostos de enxofre, assim como enzimas, aminoácidos e minerais como o selênio. Os componentes majoritários do óleo essencial de *Allium sativum* (alho) são divididos em dois grupos, componentes derivados de enxofre (84%) como o dialiltrisulfeto (37.3–45.9%), dialildisulfeto (17.5–35.6%), metil aliltrisulfeto (7.7–10.4%) e o 2-vinil-1,3-ditiano (3.9–5.9%) e terpenos como o γ-cadineno (4.3–6.8%) e bisaboleno (2.1–2.5%) (16%) (APARECIDA et al., 2010; BURT, 2004).

De todas as espécies do gênero *Allium*, o alho é o que contem maior concentração de enxofre em sua composição. O odor, descrito por muitos como desagradável, e muito de seus efeitos medicinais, advêm dos compostos de enxofre. Alicina é um dos principais compostos encontrados no alho, e a sua formação apenas ocorre após o esmagamento do alho. Ao esmagar o alho, a enzima alinase é ativada e metaboliza o alin à alicina que é posteriormente metabolizada. Alicina possui diversos efeitos antimicrobianos, porém, por sofrer diversas reações enzimáticas, derivados de alho como seu óleo essencial, não possui a mesma eficácia em relação ao alho in natura (BHANDARI, 2012; DZIRI et al., 2014).

2.3.1.2 Aplicações

O óleo essencial de alho pode retardar efetivamente a degradação por oxidação de lipídios e contribuir para o aumento da qualidade e valor nutricional dos alimentos aos quais são acrescentados. Especiarias e seus extratos têm tido cada vez mais interesse para a indústria de alimentos (BHANDARI, 2012; ASIK; CANDOGAN, 2014).

O alho aplicado em forma de óleo essencial pode também contribuir como fator essencial na área de alimentos, agindo como flavorizante e contribuindo para a redução de sódio e de outros aditivos alimentares. Atualmente, há vários estudos abordando sobre a redução de sódio em alimentos processados, com o objetivo de melhorar as dietas e promoção da saúde e qualidade de vida (LOPES et al., 2014).

A redução do teor de sódio dos alimentos é um processo oneroso por, na maior parte dos casos, os produtos reformulados serem rejeitados pelos consumidores. Entretanto, a utilização de especiarias representa um ponto chave para o desenvolvimento de produtos alimentícios com sódio reduzido. Especiarias são compostas de óleos essenciais, componentes químicos naturais tais como vitaminas A, E e C, e compostos fenólicos, os quais contribuem para a proteção do alimento e controle microbiológicos (LOPES et al., 2014; MANEESIN et al., 2014).

2.3.2 Óleo essencial de tomilho

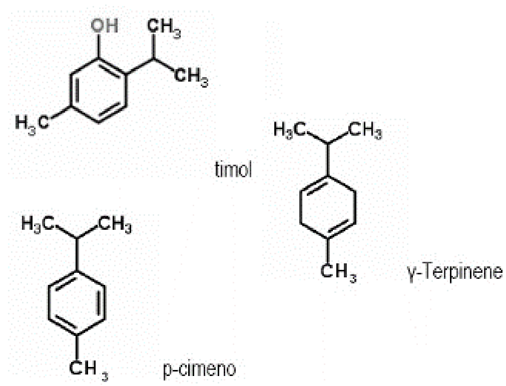
Tomilho *(Thymus vulgaris)*, é uma erva pungente da família de hortelã *(Lamiaceae)* conhecida pelo aroma e sabor de suas folhas secas e topos floridos. Tomilho é nativo da Eurásia e é cultivada em todo o mundo, sendo de maior ocorrência no mediterrâneo. É um pequeno arbusto e é comumente cultivado o ano todo. As hastes são lenhosas e as pequenas flores tubulares são em espirais ao longo das hastes e são normalmente roxas ou de cor branca. As abelhas são atraídas para as flores, o mel de tomilho da Sicília é famoso há centenas de anos. É usado para aromatizar vasta gama de alimentos, incluindo aves domésticas, recheios, peixes, ovos, carnes, molhos, sopas, legumes, queijos e massas (PAPAZOGLOU; TSIRAKI; SAVVAIDIS, 2012).

O principal componente do óleo essencial de tomilho é o timol, que é usado na fabricação de perfumes e cremes dentais. Óleo de tomilho possui atividades inibidoras contra várias bactérias e leveduras. O timol tem também demonstrado atividade antimicrobiana contra várias bactérias e fungos (BILENLER et al., 2015; BRITANNICA ACADEMIC, 2016).

2.3.2.1 Composição Química

Os componentes do óleo essencial de tomilho *(Thymus vulgaris)*, podem variar de acordo com o período de colheita, clima e estação, mas possuem em média: 54 a 58% de timol, 17 a 21% de *p*-cimeno, 6 a 7% de γ-terpineno e 2 a 11% de carvacrol. A maior parte da atividade antimicrobiana em óleo essencial de tomilho parece estar relacionada com os compostos fenólicos, por exemplo, o timol e o carvacrol (APARECIDA et al., 2010; BURT, 2004).

Figura 2 - Principais compostos do óleo essencial de tomilho.



Fonte: Bakkali et al. (2008)

2.3.2.2 Aplicações

O fato dos óleos essenciais serem considerados substâncias "naturais" torna-os altamente desejáveis para utilização em muitos produtos alimentares, uma vez que existe crescente demanda do consumidor por aditivos naturais em vez de sintéticos. Apesar das suas potenciais aplicações como componentes funcionais em alimentos e bebidas, a utilização de óleos essenciais é frequentemente limitada devido à sua relativa baixa solubilidade em água. Os tomilhos são muito apreciados em culinária por resistirem a cozedura prolongada e por realçarem o sabor de outros condimentos. Diversas espécies de tomilho são tradicionalmente utilizadas em salsicharia, em pão recheado com carne (bola de carne), terrinas ou recheios diversos, quer pelas suas propriedades conservantes, quer pelo sabor e aroma. Estas propriedades fazem do tomilho um aditivo natural de alto potencial na indústria alimentícia (CHANG; MCLANDSBOROUGH; JULIAN, 2015; MARTINS et al., 2015; PAPAZOGLOU; TSIRAKI; SAVVAIDIS, 2012).

Chang, Mclandsborough e Julian (2015), estudaram as propriedades físicas e antimicrobiana do óleo essencial de tomilho em nanoemulsões, tendo constatado haver mudanças na solubilidade em água e melhoria dos seus efeitos antimicrobianos e da sua estabilidade física. Papazoglou, Tsiraki e Savvaidis (2012) estudaram o efeito do óleo de tomilho na preservação de fígado de frango embalados a vácuo. Avaliaram parâmetros colorimétricos e microbiológicos, e obtiveram resultados satisfatórios em relação à conservação.

2.4 Moçarelas

O queijo, tido como uma das formas de conservar o leite, é considerado um dos alimentos mais antigos que se tem registro. Há registros de sua fabricação desde o Egito antigo e oriente. Provavelmente os queijos e leites fermentados tenham se originado devido ao armazenamento do leite em recipientes feitos de estômagos de ruminantes (MAGALHÃES, 2008).

A sua fabricação primitiva consiste basicamente na fermentação do leite, pela acidificação do leite pela microbiota e através da coagulação por uma enzima chamada renina ou quimosina, que converte o leite em coalhada e soro. Textura, sabor e demais propriedades podem variar de acordo com a produção e tipos de cultura de bactérias. Existem mais de 400 tipos de queijos, os quais são classificados de acordo com a sua textura, teor de umidade e sua maturação. Em média, é um produto de elevado valor nutritivo, com grandes quantidades de proteínas, vitaminas, sais minerais, fósforo e cálcio. A sua produção é geralmente feita com leite de vaca, porém, há também a produção de queijos de leite de cabra, búfala, ovelha, etc. (ZACARCHENCO et al., 2011).

A fabricação de queijos no Brasil firma-se do ponto de vista industrial em meados do século 20. A indústria queijeira representa importante segmento no setor lácteo no Brasil, e o Estado de Minas Gerais destaca-se por ser o maior produtor nacional, com 50% da produção nacional. Os queijos mais produzidos em Minas Gerais são o queijo minas, requeijão e a moçarela (TEIXEIRA; FONSECA, 2008; TEIXEIRA; FONSECA; MENEZES, 2007).

A produção de moçarelas possui microrganismos que podem contribuir na variação do seu sabor e textura, porém, podem ocorrer contaminações através de microrganismos deteriorantes ou patogênicos, os quais podem causar efeitos prejudiciais ao produto final, assim como provocar doenças em alguns casos. Grande parte do processo de contaminação em queijos moçarela ocorre superficialmente (PEREIRA, 2007).

A ingestão de queijos contaminados é um problema grave e de Saúde Pública. Dentre os microrganismos presentes no leite cru que podem ser associados ao consumo de queijo, destacam-se o *Staphylococcus aureus*, a *Escheriachia coli,* a *Listeria monocytogenes* e a *Salmonella* spp. Um dos fatores relevantes em relação à ocorrência de contaminações é a estocagem do produto final. Exposição a altas temperaturas, más condições de refrigeração assim como a iteração com o oxigênio podem comprometer gravemente a qualidade e a perenidade de queijos. Neste estágio, podem ocorrer contaminações através de diversos microrganismos (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2006; AVILA; BOHRZ; NOSKOSKI, 2011; LOGUERCIO; ALEIXO, 2001; MADIGAN, 2010).

Muitos desses microrganismos requerem oxigênio para o seu crescimento e desenvolvimento, o que faz com que cresçam na superfície de queijos que sofrem exposição ao ambiente. Nestes termos, não se espera o seu desenvolvimento em queijos embalados a vácuo, embora possa ocorrer em níveis de oxigênio residual. Neste estudo, justifica-se o uso de uma embalagem com propriedades antimicrobianas e flavorizantes e com características de um material biodegradável e atóxico aos seres humanos (ZACARCHENCO et al., 2011).

Há constantes estudos realizados com o objetivo de obter melhorias na conservação de produtos lácticos e minimizar contaminações microbiológicas. Em um estudo feito por de Cerqueira et al. (2009), foram aplicados diferentes polissacarídeos como revestimento em queijos Saloio, visando avaliar as interações dos diferentes revestimentos com o queijo. Resultados destacaram a diminuição das taxas de perda de água quando o revestimento foi aplicado, com a consequente diminuição de crescimento de fungos e leveduras. Pesquisas realizadas por Shin et al. (2012) avaliaram a eficiência de um filme de algas vermelhas com extrato de semente de uva. O material foi usado como revestimento para queijo e bacon, demonstrando eficácia em relação ao crescimento de bactérias patogénicas tais como a *Escherichia coli* e *Listeria monocytogenes.*

Nestes casos, o uso de óleos essenciais em embalagens ativas atrai um interesse particular, uma vez que estes compostos apresentam atividade antimicrobiana e antioxidante em sistemas alimentares, e aumentam os benefícios dos alimentos por causa de seus efeitos bioativos, além de serem potenciais na redução de conservantes como o sódio, através de combinações de óleos essenciais (ARCAN; YEMENICIOĞLU, 2014; CERQUEIRA et al., 2009; SHIN et al., 2012).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 ANTIMICROBIAL FILMS OF ZEIN PLASTICIZED WITH ESSENTIAL OILS OF GARLIC AND THYME

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ABSTRACT

Essential oils with antimicrobial properties are widely used in several applications such as in cosmetics and food industry. In food products, they are used for flavoring and preservation. In this study, the antimicrobial activity of a 1:1 (v/v) blend of essential oils of garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) was evaluated against various food pathogenic microorganisms, viz., *E. coli*, *L. monocytogenes*, *S. enteritidis* and *S. aureus*. The influence of the blend of essential oils in antimicrobial and mechanical properties of zein films was evaluated by adding 0, 2, 3, and 5% (v/v) in zein and cast to form films that were characterized by spectroscopy (FTIR), thermal analyses (TG and DSC), microscopy (SEM) and water solubility and absorption . Results showed that films incorporated with the blend of essential oils had antimicrobial activity against all tested microorganisms. Moreover, this blend acted as a plasticizer, as confirmed by a decrease in glass transition temperature and in the elastic moduli of the films. Oil addition also resulted in decreased solubility and water absorption. These films showed inhibitory activity on the bacteria tested from 2% (v/v) blend of essential oils, with halos above 6.5 mm. The results show that the coating can be applied as alternative to increased shelf life of various food products.

Keywords: antimicrobial coatings; zein; edible films; garlic oil; thyme oil.

INTRODUCTION

In recent years, research related to biodegradable packaging has increased, particularly due to disposal of non-renewable materials, which take hundreds of years to be fully degraded or decomposed. [1] The food packaging market has responded to the increasing demand for environmentally friendly materials, and the international regulatory agencies have issued guidelines for research in edible coatings emphasizing the use of materials that can bring benefit to health and to the environment.[2]

Most studies focusing on edible coatings have demonstrated the possibility of creating biopolymer packaging from various materials, especially from polysaccharides, lipids and proteins. These hydrophobic protein-based coatings are directly applied to the food surface, acting as a barrier to moisture, gas, oxygen, carbon dioxide, and fats.Among these materials, zein has been largely used in the preparation of films in food applications.[3–6]

Zein corresponds to 50% of maize endosperm proteins *(Zea mays*), and is extracted from the corn gluten meal, being therefore a byproduct. The presence of nonpolar aminoacids in Zein is responsible for its high hydrophobic character, what makes it soluble in nonpolar solvents such as ethanol. However, due to the brittle nature of zein, in applications where flexibility is required, plasticizers such as glycerol and poly(ethylene glycol) need to be added.[4] Although zein is resistant to attack by various microorganisms, the addition of essential oils can not only act as a plasticizer but also improve its antimicrobial properties[7].

Essential oils can be regarded as natural food preservatives, by means of its antimicrobial activity, being therefore an important aid in the control of foodborne pathogens and spoilage bacteria.[8] Among these, are garlic (*Allium sativum*) and thyme (*Thymus vulgaris*).[9–11] The use of antimicrobial coatings containing essential oils has proved to be effective in food packaging against several microorganisms including *E. coli*[12,13] and *Salmonella*[12], and *L. monocytogenes.*[13]

The major constituents of the essential oil of garlic are divided into two groups, sulfur containing substances such as cysteine derivatives (84%), diallyl trisulfide (37.3-45.9%), diallyl disulfide (17.5-35.6%), methyl allyl trisulfide (7.7-10.4%), 2-vinyl-1,3-dithiane (3.9-5.9%), and terpenes such as γ-cadinene (4.3-6.8%) and bisabolene (2.1-2.5%)[14] The major constituents of the essential oil of thyme (*Thymus vulgaris*) are: thymol (54-58%), p-cymene (17-21%), γ-terpinene (6-7%) and carvacrol (2-11%).[15] Most of the antimicrobial activity of thyme essential oil appears to be related to the presence of phenolic compounds thymol and carvacrol. Phytochemical components may vary according to the time of harvest, climate and season.[16,17]

In this context, edible coating films of zein containing essential oils of garlic and thyme were prepared and investigated in order to assess the effectiveness of the blend as a plasticizer and antimicrobial agent against the enteropathogenic bacteria *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus.*

EXPERIMENTAL

Materials. Zein from maize(Sigma-Aldrich Co., Saint Louis, Missouri, USA), ethanol (Vetec Fine Chemicals Ltd., Duque de Caxias, Rio de Janeiro, Brazil), garlic essential oil (Sigma-Aldrich, Saint Louis, Missouri, USA) and thyme essential oil (Ferquímica, Vargem Grande Paulista, São Paulo, Brazil) were used without further purification.

Preparation of zein films. Garlic and thyme (1:1 volume ratio) essential oils were mixed vigorously using a magnetic stirrer for 12h to form a homogenous mixture. Zein solutions were prepared by slowly adding 20 g of zein to 100 mL hydroalcoholic solution of ethanol (90 vol.%) at 50°C under vigorous stirring until forming a solution which was then cooled to room temperature prior to blending with essential oils at the following volumetric proportions: 0, 2, 3, and 5%.[18] Films of zein and zein/essential oil were produced by casting the solutions onto 50x10 mm Teflon plates and allowing the solvent to evaporate at room temperature.

ATR-FTIR. Attenuated total reflectance Fourier transform infrared spectra of essentials oils, oil blends and films with and without oils were recorded between 4000 and 500 cm^{-1} on a Shimadzu-IRAffinity (Shimadzu Co.,Kyoto, Japan) FT-IR spectrophotometer. Spectra were calculated from a total of 16 scans at a resolution of 4 cm^{-1}. A few drops of oil sample were positioned in contact with attenuated total reflectance (ATR) on a multi-bounce plate of crystal at room temperature.

Thermal analyses (TGA and DSC). The thermal stability of the materials was investigated using a model DTG-60AH thermogravimetric analyzer, TGA (Shimadzu Co., Kyoto, Japan). Samples were heated at a heating rate of 10°C.min^{-1} from 25 to 600°C in a platinum crucible under nitrogen flow rate of 50 mL.min^{-1}.Differential scanning calorimetry (DSC) was carried out for films with different concentrations of oil on a model DSC-60 calorimeter (Shimadzu Co., Kyoto, Japan). Each sample (3–5 mg) was heated in a crimped aluminum pan at a scanning rate of 5°C min^{-1} from 25 to 120°C under nitrogen atmosphere at a flow rate of 40 mL.min^{-1}. Reproducibility was checked by running the samples in triplicate.[19]

Scanning electron microscopy (SEM). Fracture surface was analyzed on a model LEO EVO 40 XVP scanning electron microscope (Carl Zeiss, Jena, Germany) equipped with secondary electron detector. Cross-section images were taken from cryogenic fractured samples which were previously immersing in liquid nitrogen for 2 min. Samples were mounted onto stubs using a double-sided adhesive carbon tape and sputtered with gold using a plasma sputter coater (Balzer, SCD 050). Images were taken at 15-20 kV at the magnifications of 1000 and 5000X. Pore size was measured using Image J 1.50i (Wayne Rasband, National Institute of Health, USA).

Water solubility (WS). Film solubility in water was analyzed according to the methodology described by Parris *et al*.[20] These experiments were used to determine the percentage of film solubilized after 24 h immersion in water at 23±2°C. Briefly, 3 cm film discs were weighed, dried at 50 °C for 24 h, and immersed in a petri dish containing 50 mL of water. Discs were removed from water and dried at 50°C for 24 h. The solubility in water (Ws) was calculated by:

$$WS(\%) = \frac{W - Wo}{Wo}.100 \quad (1)$$

Where W and W_0 are, respectively, the weight of disks before and after immersion in water.

Water absorption (WA). Water absorption was determined according to ASTM D 570-81 Standard Test Method.[21] Three discs of 3 cm in diameter were conditioned in an oven at 50°C for 24 h, cooled in a desiccator, and weighed. Conditioned specimens were entirely immersed in 50 mL distilled water at 25 °C. Specimens were removed from the soaking water at several time intervals, surface water was wiped off with a dry cloth, and the specimens were weighed. Water absorption (WA) was calculated by:

$$WA(\%) = \frac{w_w - w_c}{w_c}.100 \quad (2)$$

Where m_w and m_c are, respectively, the wet and conditioned mass of the samples. Values reported are an average of three determinations.

The kinetics of water absorption by zein films was determined according to Shi *et al*.[22].

$$y(t) = Ms(1 - e^{-t/T}) \quad (3)$$

Where y (t) is the rate of water absorption of the films (g/min), M_s is the capacity of water absorption, t is the immersion time in minutes and T is the temperature in Kelvin.

Mechanical properties. Tensile tests were determined with a model TA-XT2i texture analyzer (Stable Micro Systems, United Kingdom) according to Giménez *et al*.[23] 20mm x 100mm samples were tested using a load of 1 kN and a crosshead speed of 125 mm.sec^{-1}. Tensile strength and modulus of elasticity were average value of 10 measurements. Puncture testing was performed according to Zivanovic et al. [24] The films were cut into squares of 9 cm^2 and fixed in a holder with central opening. A spherical probe of 5.0 mm diameter was displaced perpendicularly to the film surface at a constant speed of 0.8 mm/s until the tube passes through the film. The puncture strength was calculated by dividing the load at the breaking point by the thickness of the film. Results were also an average of 10 measurements.

Microbiological analyzes. Microbiological tests were performed *Escherichia coli* (CDC O55), *Listeria monocytogenes* (ATCC 19117), *Salmonella enteritidis* (CDC S190) and *Staphylococcus aureus* (ATCC 5674). The stock cultures were stored in freezing medium (15 ml glycerol; 0.5 g of bacteriological peptone; 0.3 g of yeast extract; 0.5 g of NaCl and 100 ml distilled water at pH 7.0).

Cultures were reactivated according to the Clinical and Laboratory Standards Institute (CLSI) method inoculating 100 uL aliquots into tubes containing 10 ml of Tryptone Soya Broth (TSB) [25]. The cultures were incubated at 37 °C for 24h. TSB was added to standardization of the inoculum, which was performed by a growth curve. After the reaching an reactivation rate of 50 uL, the inoculum was transferred to 300 mL TSB and incubated at 37°C, periodic readings are made at one-hour intervals in a spectrophotometer, and plating on agar Tryptone Soy (TSA) with incubation at 37°C for 24h. The culture was standardized to10^8 CFU mL^{-1}.

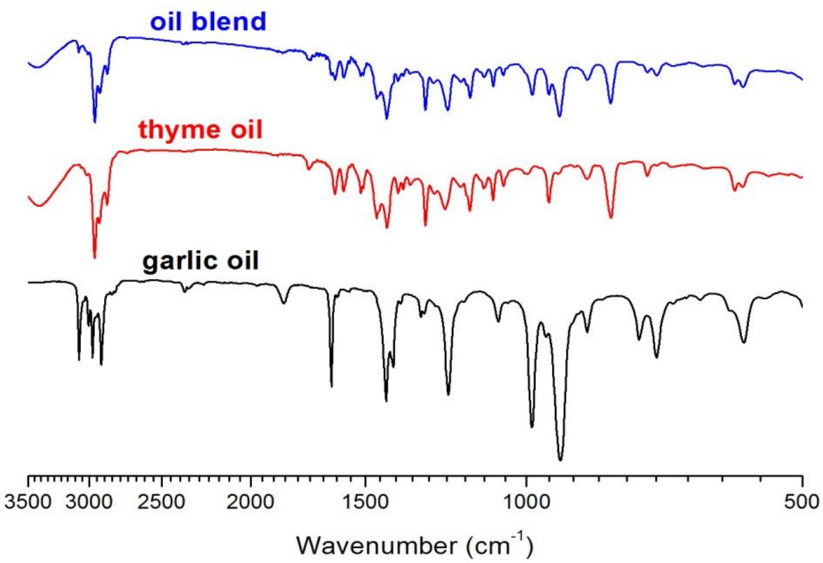
The minimum bactericidal concentration (MBC) of essential oils was determined using the microdilution technique according to CLSI standards with adaptations.[25] Solutions with different concentrations of essential oils were prepared in TSB, and 0.5% Tween 80 was added at concentrations of 5.00, 3.75, 3.0, 1.0, 0.5, 0.25 and 0.125 vol.%. Subsequently, aliquots of 150 uL of the different solutions were transferred to the wells of a microtiter plate and 10 uL of standard culture were inoculated into each well. Microplates were sealed and incubated at 37°C for 24 h. The experiment was conducted in triplicate with three replicates for each replica, using a positive control (TSB with 0.5% Tween 80 and culture) for each repetition.

The antimicrobial activity of the film-forming solution zein and oils blend against the selected microorganisms was studied using the agar diffusion method according with Gómez-Estaca *et al.* [26] with adaptations. Organism strains were cultured in TSB at 37°C and seeded in a Petri dish with 0.1 mL of the inoculum containing approximately 108 cfu/mL of the bacteria. 100 uL of different film-forming solutions were poured into 6 mm diameter TSA wells, and the plates were incubated at 37°C for 48 h, after which the diameter of the zone of inhibition was calculated. The positive control was the filmogenic solution without essential oils and a negative control was a filmogenic solution containing 1 vol.% chloramphenicol. Measurements were performed in triplicate.

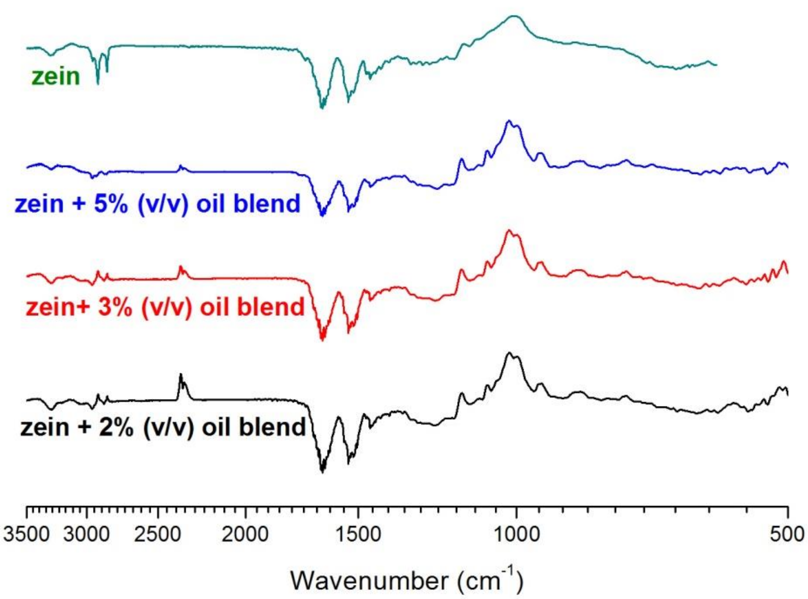
Statistical Analysis. One-way ANOVA and Tukey Test with a significance level set to $P < 0.05$ were used to analyze differences among groups, using Statistica 8.0 (Dell Statistica, Hoklahoma, USA). The data were presented as mean ± standard deviation.

RESULTS AND DISCUSSIONS

FTIR analyses of the essential oils, oil blend and zein films with and without oil blend are shown in Figure 1.



(a)



(b)

Figure 3. ATR-FTIR spectra of essential oils (a) and zein films (b).

Infrared spectra show that the main functional groups of essential oils of thyme garlic and found in this work are in accordance with the studies of Wang *et al*. [30], The presence of the major functional groups of the individual components are present in the garlic/thyme blend indicating some degree of interaction between these essential oils. In 3008 cm^{-1} is observed symmetry stretching vibration of $CH = CH_2$, in 1634 cm^{-1} there is an intense peak attributed to C = C in 1423 cm^{-1} $-CH_2$ at 918 cm^{-1} and 985 cm^{-1} CSC calls and peak SC at 723 cm^{-1}, assigned to components of the essential oil of garlic. The presence of essential oil components thyme is indicated by a long band at 3399 cm^{-1} due to axial deformation of OH on intermolecular bonds, bonds of metinic ring groups (-CH) are shown by aromatic axial deformation 2960 cm^{-1} at around 1667-1800 cm^{-1} combination bands are observed. In the range 1470- 1617 cm^{-1}, the axial deformation of the C=C aromatic ring; in the region 1225 cm^{-1}, the axial deformation of the linkage (C-O), and 720-810 cm^{-1}, metinic angular deformation out of plane.

The films present associated hydroxyl (O-H), or effecting hydrogen bonding, appearing as a band in the region around 3250 cm^{-1}. Aliphatic compounds (C-H) for the chain elongation generate multiple bands with peaks in the region 2918 cm^{-1} and extending to the concentration of blend oils. The same can be observed in changes in the region of 1640-1537 referring to the peaks of the amides present in the structure of zein. These changes are related to the interactions between zein and oil blend indicate changes in mechanical and morphological properties in the zein films, through the action of the oils blend as a plasticizer. These interactions are confirmed by the peak onset -OH at 1120 cm^{-1}, related to zein connections - oils blend. In the literature there are reports like this work in relation to interactions between proteins and plasticizers. Wongsasulak *et al*. [29] noted similar iterations of this work between zein and glycerol. From the region 1100 cm^{-1} for films with addition of the oils blend is observed the appearance of new peaks that increase in intensity with increasing concentration of blend oils. The appearance of new peaks is related to compounds found in thyme and sulfur compounds present in garlic oil, previously mentioned. Compounds of the oils blend in zein films, give evidence of retention effectiveness of the blend of compounds by zein matrix. The presence of these compounds interferes directly in the structural and functional properties of the coating.

Thermogravimetric analyzes were used to evaluate and compare the thermal stability and decomposition of the zein films incorporated with different blend concentrations of essential oils, as observed by Figure 2.

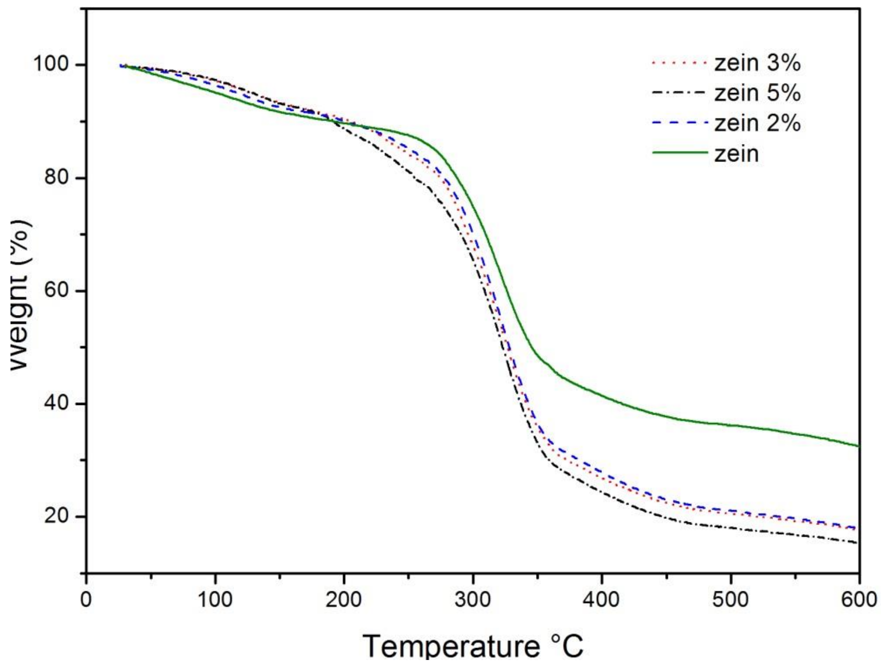


Figure 4. TGA Thermogram of zein films with different concentrations of oil blend.

Until the range of 175 °C, the film showed similar weight loss profile, subsequently at this temperature, thermal decomposition of different profiles can be observed between the neat film and films with essential oils blend. The loss of residual water from the films and volatiles to 120°C are observed, where neat film suffered a loss of 7% by weight and the films 2, 3 and 5% blend lost respectively 6, 5.2 and 5wt.%. The films with the addition of essential oil blend, exhibit weight loss of 9% at around 175 °C, related to the volatilization of essential oils, pure film has marked thermal decomposition at around 245 °C with 13% loss pasta. This difference is related to loss of volatile and constituents present in the oils blend where at adding the blend occur intermolecular interactions between the biopolymer matrix and essential oils reducing the thermal resistance of the film, showing plasticizer action efficiency by blend [30]. The results are consistent with studies of Karak et al. [31], which evaluated the profile of the thermal stability of different blends of chitosan, glycerol and polyethylene.

The films presented end of thermal decomposition near 373 °C. In this temperature range, it was observed different profiles of thermal degradation. The film showed 44% pure thermally stable mass, and films with 2, 3 and 5% oils blend was respectively 31, 29 and 27% of residual mass. The addition of oils in the blend films decreases the thermal stability. The results are consistent with the work of Altiok *et al*. [32] that a decrease in thermal stability of chitosan films incorporated with the essential oil of thyme was observed.

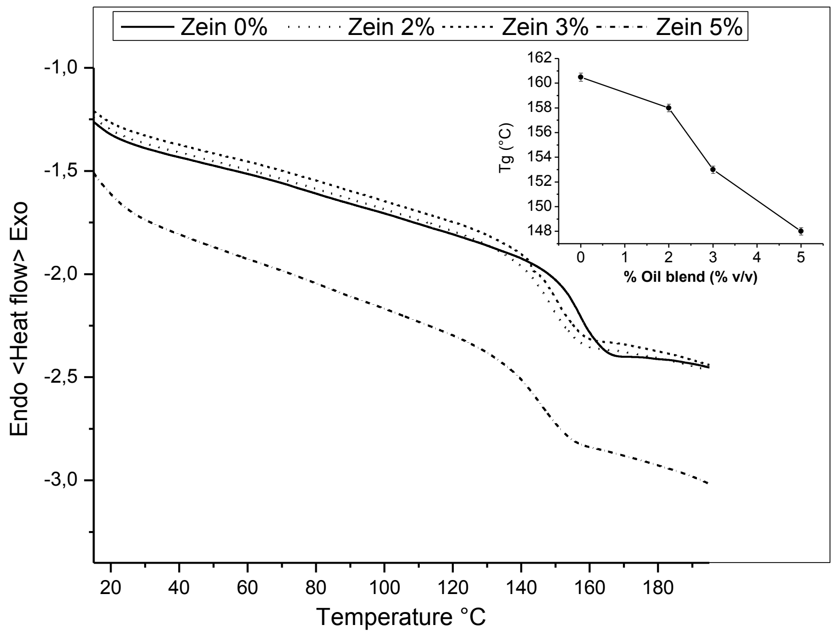


Figure 5. DSC thermograms of the zein films and different blend concentrations oils and their glass transition temperatures

DSC curves of films with and without essential oils can are shown in Figure 3. The inset shows the decrease in glass transition temperatures Tg with increasing oil content. Tg values for zein films with 0, 2, 3 and 5% of essential oils were respectively 160.5, 158, 153 and 148°C. This decrease in Tg for zein films with the addition of essential oils indicates that they acted as plasticizers, reducing intermolecular iterations between zein chains and improving flexibility of the films. Similar phenomena were observed in the study of Xu *et al.* [33] and Wang *et al.* [34] that examined the plasticizing effect of glycerol and fatty acids in zein films.

Figure 4 shows the SEM micrographs of the cross-sections of zein films with and without essential oils. The surface of zein films (Figure 4a) displays a brittle nature with several cracks, however, with increasing addition of garlic/thyme oil blend, there is a reduction in the number of cracks until a crack-free surface is formed for samples containing 3 and 5% oil blend (Figure 1c,d) due to the plasticizing effect of the essential oils on zein, in accordance with the literature.[7]

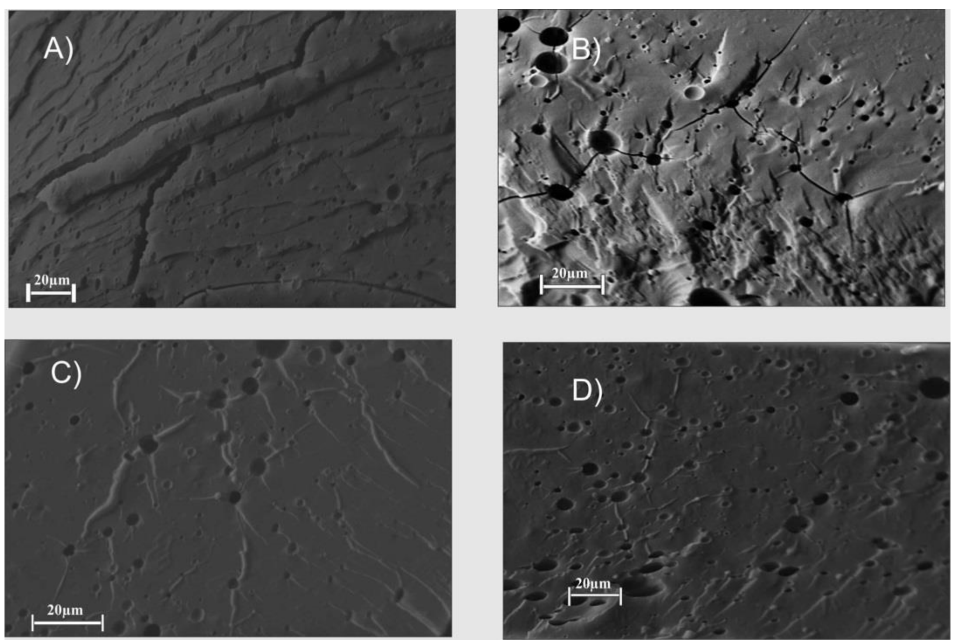


Figure 4. SEM images of the cross-sectional morphology of zein films with different concentrations of essential oil blend. A) Pure film of zein B) Film with 2% oil blend C) Film with 3% oil blend D) Film with 5% (5v/v) oil blend. Arrows indicate crack at the movies.

It can also be observed in Figure 4 that all films are porous due to the presence of the essential oils above the solubility limit, residues of fatty acids present in zein, and solvent evaporation during the drying process.[35] Moreover, as the concentration of oils increased, there was a gradual increase in these pores whose average diameter ranged from 2.9, 6.0, 4.8 and 5.7μm, respectively to films containing 0, 2, 3 and 5% of oil blend. Similar results were found by Nobile *et al*. [36] for zein films plasticized by thymol.

Table 1 shows the water absorption characteristics of zein films with and without essential oil blend. It can be observed that with increasing concentration oil there was a decrease in solubility and in water absorption rate. Zein had the highest solubility of 4.00±0.20% and decreased with increasing oil concentration to 0.54±0.03%. A similar behavior was found for the absorption rate. Both solubility and absorption rate are dependent of the diffusion of water molecules into the zein films. Since films become more hydrophobic, as the amount of garlic/thyme oil blend increases, the ultimate result is a reduction in solubility and in the diffusion of water molecules.[37]

Table 1. Water absorption for zein films

% Oil blend	Solubility (%)	Water absorption at 120 min (%)	Absorption rate (g/min)
0	4.00 ± 0.20	1.76 ± 0.52[a]	0.62 ± 0.18[a]
2	2.11 ± 0.10	1.59 ± 0.28[a,b]	0.56 ± 0.10[a,b]
3	0.83 ± 0.04	1.33 ±0.55[a,b]	0.47 ± 0.19[a,b]
5	0.54 ± 0.03	0.84 ± 0.15[b]	0.30 ± 0.05[b]

Values followed by different letters are significantly different $p \leq 0.05$ using the Tukey test followed by standard deviation.

Solubility in water is of key importance in food and coatings since it can determine the protective or coating efficiency of the films. In certain applications, low solubility becomes a prerequisite to enhance product integrity, confer better barrier properties to moisture and increase the shelf life. In others, however, low solubility is required, especially in applications such as in high-moisture foods.

After a period of 120 min, films became slightly opaque indicating excessive water absorption. The same phenomenon was also observed by Shi *et al*.[22] Significant differences ($p < 0.05$) between films without and with 5% oil blend were found. The other intervals showed no significant differences. However, there is a strong tendency in reducing water absorption with increasing concentration of essential oil blend.

The films of 3 and 5% of essential oil blend were those with the lowest absorption capacity of 1.74 and 1.63%, respectively. These differences are related to the spatial arrangement of the hydrophilic and hydrophobic groups in zein. In the process of absorption, exposed hydrophobic groups of the protein are likely to interact with water molecules. With increasing concentration of essential oils, hydrophobic groups repel water.

The addition of hydrophobic plasticizers such as oils and fats help maintain film integrity even in high humidity conditions, indicating therefore that such films can be potentially used as coating applications even where moisture is present. A similar behavior was fund by Santosa *& Padua*[38], where water absorption of zein films was modified by the addition of oleic and linoleic acids.

The mechanical properties of the films studied were affected by the addition of essential oil, corroborating the plasticizing effect as already discussed above in which Young's modulus (E), elongation at break, tensile strength and puncture resistance were affected by the presence of the plasticizer.

Table 2. Mechanical Properties of Zein films.

% Oil blend	Young's Modulus (E) (MPa)	% Elongation at break	Tensile strength (MPa)	Puncture resistance (MPa)
0	7.57 ± 1.30 [a]	1.37 ± 0.10[a]	6.20 ± 0.30 [a]	0.74 ± 0.08 [a]
2	4.30 ± 0.72[b]	0.80 ± 0.04[b]	4.83 ±0.25[b]	2.73 ±0.96[b]
3	4.38 ±0.03[b]	0.76 ± 0.04[b]	4.23 ±0.15[b]	3.27 ±0.26 [b]
5	3.41 ±0.06[b]	0.42 ± 0.03[c]	3.26 ±0.20[c]	2.67 ±0.79 [b]

Values followed by different superscript letters are significantly different p <0.05 using the Tukey test followed by standard deviation.

Despite the plasticizing effect of the garlic/thyme oil blend that decreased Young's modulus and tensile strength, the elongation at break decreased due to the presence of pores. These results are in agreement with the work of Altiok, et al. [32] that detected a decrease in percent elongation of zein films with addition of thymol due to the presence of pores.

The puncture resistance results were significantly affected with the addition of essential oil blend. The highest value was found to 3% oil blend (3.27 MPa) also in agreement with the SEM results, i.e., reduce pore size, absence of cracks and better dispersion. In general, mechanical properties of biopolymer films depend on a number of different factors. Nature of polymer, type of plasticizer and its concentration, crystallinity, chemical cross-linking, aging effects and microstructure are important factors that may modify the film strength.[33]

The minimum bactericidal concentration (MBC) of essential oils of thyme and garlic and its blend are shown in Table 3.

Table 3. Concentration inhibition of essential oil of garlic to *Escherichia coli* Enteropatogênica, *Listeria monocytogenes*, *Salmonella* Enteritidis e *Staphylococcus aureus*.

Microorganism	Garlic oil (%)	Thyme oil (%)	Oil blend (%)
	MBC		
Escherichia coli Enteropatogênica	5.00	0.5	1.0
Listeria monocytogenes	3.00	0.5	0.5
Salmonella Enteritidis	3.75	0.5	1.0
Staphylococcus aureus	3.75	0.5	1.0

The essential oil of garlic showed better inhibition bacteriological the 3.0 vol.% range in *Listeria monocytogenes*. Inhibition occurred at 3.75% for *Staphylococcus aureus* and *Salmonella* Enteritidis and 5.0% to *Escherichia coli*. This fact is explained by the existence of an outer membrane surrounding the cell wall of Gram-negative bacteria that restricts the diffusion of hydrophobic compounds through the cell wall. The essential oil of thyme presented a minimum bactericidal concentration 0.5% for all bacteria. These results are similar to those found in the literature.[39]

The antimicrobial blend of essential oils (Table 3) at concentrations as low as 1.0% inhibited the growth of *Escherichia coli*, *Salmonella* Enteritidis and *Staphylococcus aureus*. A fact that should be emphasized is the inhibition of 0.5% for *Listeria monocytogenes*, indicating a synergistic effect of essential oil blend. The addition of garlic intensifies the antibacterial effect on *Listeria monocytogenes* because the action of sulfite constituents are known to inhibit bacteria growth, being therefore potentialized by the presence of the essential oil of thyme. Although the synergistic effect is not clearly elucidated, it is understood that only physicochemical factors such as pH, temperature and water activity can increase or decrease the antimicrobial effect of essential oil blend. For the other bacteria, there was no synergistic effect. However, the addition of the essential oil of garlic down its bactericidal concentration is justified by its flavoring property in food applications. The zones of inhibition in millimeters for the different concentrations of essential oils in zein are shown in Table 4.

Table 4. Diameter of the zones of inhibition of zein films with of garlic/thyme essential oil blend.

	Inhibition zone (mm)			
% Oil blend in zein	*E.coli EPEC*	*L.monocytogenes*	*Salmonela* Enteritidis	*S. aureus*
0	n/i	n/i	n/i	n/i
2	6.46 a ±0.03 [a, a1]	6.75 ± 0.02 [a, a1]	6.62 ±0.015 [a, a1]	6.74 ± 0.01 [a, a1]
3	7.13 b ±0.015 [b, a2]	7.36 ±0.02 [b, b2]	7.10 ±0.04 [b, a2]	6.95 ± 0.01 [b, c2]
5	7.36 c ±0.04 [c, a3]	8.27 ±0.01 [c, b3]	8.18 ±0.02 [c, c3]	8.20 ± 0.005 [c, c3]
CP	20.06 ±0.27	21.02 ±0.39	19.34 ±0.71	19.48 ±0.05

Test carried out in triplicate. PC-Positive Control - Chloramphenicol. Zone of inhibition in mm. n / i - not inhibited. Values followed by different letters are significantly different, where the first letter represents the column and row corresponds to the second letter, at $p \leq 0.05$ using the Tukey test followed the standard deviation.

Results show that there were significant differences ($p < 0.05$) between the treatments, where the antimicrobial activity was directly proportional to the concentration of essential oils. All concentrations tested showed inhibition zone below the positive control (PC). The films containing 2% showed no significant difference for any of the bacteria tested. It is known that the antimicrobial effect of essential oils is somewhat reduced in a biopolymer matrix because of the different concentrations of the oils with zein, rate of oil release and microstructure developed during film processing. [41] However, for films with 3 and 5% significant differences were observed, where *Listeria monocytogenes* showed the largest zones of inhibition, confirming the synergistic effect found in the minimum bactericidal concentration test. These differences in antimicrobial growth inhibition can be explained by differences in cell wall composition and/or inheritance of genes in plasmids which can be easily transferred between bacterial strains.[40]

CONCLUSIONS

Results show the effectiveness of the blend of essential oils of garlic and thyme as plasticizer and as an antimicrobial agent to zein films. A reduction in glass transition temperature due to polymer blend-interaction, elimination of cracks and increased puncture resistance was fund with addition of oil. Zein films with essential oils were at a concentration above 2% inhibited the growth of the bacteria *Escherichia coli* Enteropathogenic, *Listeria monocytogenes*, *Salmonella* Enteritidis and *Staphylococcus aureus*. This study demonstrates the great potential of essential oils in improving biopolymers coating properties and, in turn, contributing to improve food quality and shelf life.

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ARTIGO 2 ZEIN EDIBLE ANTIMICROBIAL COATING FOR QUALITY CONTROL OF MOZZARELLA CHEESE

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Abstract

This study evaluates the influence of the blend of essential oils of garlic and thyme in zein films in relation to its action as an antimicrobial and flavoring agent in mozzarellas with reduced sodium content. It evaluated the influence of the coating on mozzarella's water loss and aerobic mesophilic count and fungi and yeasts. Moreover, it is performed the sensory analysis to assess consumer acceptance mozzarellas successive NaCl decreases to 0%, 25%, 50%, 75% and 100% (% w / v) coated with Zein, and 3% (% v / v) blend of essential oils. Mozzarellas submitted to the coating for 20 days, had 50% less water loss and indicated minor deteriorations for mesophilic aerobic and fungi and yeasts. Mozzarellas with reduction of 50% (% w / v) NaCl showed good acceptance rate, compared to standard mozzarella. Therefore, from the results of this study it was concluded that the coating of zein and essential oils blend, can be used efficiently and good acceptance in mozzarella, and demonstrates the potential application of zein coatings and natural additives in food preservation.

Keywords: edible coatings; garlic oil; thyme oil; Mozzarella cheese; essential oil blend.

1 Introduction

Currently there is a great interest in technologies that increase production and quality of food and minimize waste. According to the Food and Agriculture Organization of the United Nations, one third of the food produced worldwide, go to waste every day. These factors are caused by different variables in the food processing, such bad conditions, which in turn generates contamination by spoilage microorganisms such as bacteria and fungi (Liu & Tsao, 2009; Oriani, Molina, Chiumarelli, Pastore, & Hubinger, 2014).

Spoilage microorganisms in foods, characterized by decaying proteins, lipids and carbohydrates through the production of certain enzymes. Some of these microorganisms produce changes in appearance, odor and taste of food, and can cause pathogenesis (Sarkar & Shetty, 2014). Among the main spoilage microorganisms are aerobic mesophilic and fungi and yeasts. These microorganisms indicate the quality with which the food was processed, and the presence in high scores indicate inadequate hygiene procedures in processing (Cerna-Cortes et al., 2015).

In many cases may occur contamination in storage and storage of food through improper or damaged packaging, so it is very important to know the nature and changes that occur in food and packaging applied (Petrová et al., 2015).

In this context, cheeses, characterized as the most diverse group of dairy products, having different shapes, textures, aromas and flavors, are subject to a process of growth of mold and spoilage bacteria. For some cheeses such as mozzarella, used packaging is essential, as this has to act as a barrier between the external environment and food without spoiling them. The conservation Mozzarella cheese can be extended by lowering of its water loss rates, therefore, the application of a packing or coating becomes an effective strategy to diminish rates water loss (Ramos et al., 2012).

Nowadays, most of the flexible packaging are made with plastics, which despite having excellent functional properties are generally nonbiodegradable and are involved in environmental problems. The food industry and packaging are undergoing changes due to consumer demand for biodegradable materials and are able to extend food shelf life without the addition of chemical preservatives (Khan, Schutyser, Schroën, & Boom, 2014).

Several biopolymers are proving to be promising, because they are abundant, renewable and able to form continuous and sustainable arrays. They have highlighted the use of edible coatings on the surface of food, especially in embedded ones as meat and dairy products. In this case, the coatings have the potential of forming a thin film on the surface of the product act as protective agents to the external environment improving food preservation. Techniques are developed and improved every moment, such as using materials that control the water loss from the food during food storage in order to increase the retention as well as control and inhibition of microbial growth, extending the life of the product during storage (Ferreira, 2008; Luvielmo & Lamas, 2012).

Recently, this technique has received much attention due to their advantages over conventional packaging, such as being biodegradable, have lower cost, able to transport food additives that enrich nutritionally, such as essential oils and natural additives. (Ali, Chow, Zahid, & Ong, 2013; Teixeira et al., 2014)

Essential oils can improve the quality of storage and increase the shelf life of fruits, vegetables, grains and meats. However, the use of essential oils in food preservation remains limited, mainly because of its intense aroma, which could cause changes in the sensory properties of food. In order to minimize the concentrations, an alternative would be to use polymer matrices as carriers for these natural compounds. Due to the slow diffusion of the antimicrobial agent on the surface of the package, the compound is spread in a controlled manner on the surface of the product. This method becomes more efficient compared to application of the antimicrobial agent directly to the surface of the product (Muriel-Galet et al., 2012). Also, they act as antimicrobial agents, and flavoring, being promising in the replacement of currently used food preservatives such as sodium derivatives.

Exacerbated consumption derived from sodium as salt (NaCl) has been linked to several cardiovascular and hypertension problems (Arboatti et al., 2014). Therefore, it is crucial that the food industry to reduce the amount of salt added to food, since the sector is responsible for 80% of all salt ingested by people. In recent years, the consumption of mozzarella has shown significant growth, due to its wide application in pizzas, so it is very important the production of mozzarella with reduced salt levels (Ayyash & Shah, 2011).

In this context, the objective of this work involves the application of edible coating of zein and blend of essential oils of garlic and thyme, acting as flavoring and antimicrobial agent in mozzarellas with sodium reductions. The application of edible coating to prolong the shelf life of cheese has been little exploited. Fajardo et al. (2010) showed that chitosan coatings incorporated with lysozyme or natamycin can be applied effectively in mozzarellas to reduce the contamination of microorganisms. Zhong et al. (2014) investigated different coating application methods and their performance in mozzarella cheese.(Fajardo et al., 2010; Zhong, Cavender, & Zhao, 2014)

In this work, the coating applied in mozzarellas will be investigated in order to assess water loss reduction efficiency, antimicrobial action in preventing the emergence of mesophilic aerobic microorganisms and fungi and yeasts and acting as flavoring agent in reductions of sodium present in mozzarella and to evaluate consumer acceptance forward new technologies in the food area.

2 Materials and methods

2.1 Materials

Zein was purchased in powder from Sigma-Aldrich Co. (Saint Louis, Missouri, USA), ethanol 99.8 % (EtOH) was purchased by Vetec Química Fina Ltd. (Duque de Caxias, Rio de Janeiro, Brazil); garlic essential oil was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA) , thyme essential oil was purchased by Ferquímica (Vargem Grande Paulista, São Paulo, Brazil). Mozzarella cheese was acquired from Laticínios Vimilk Ltd. (Perdões, Minas Gerais, Brazil) and the pickle preparation was purchased NaCl 99,0% by Vetec Química Fina Ltd. (Duque de Caxias, Rio de Janeiro, Brazil).

2.2 Preparation of coating solutions

Casting method was made under the described in the methodology Pankaj et al. (2014), with some modifications and was employed to prepare zein and oils blend (3% , % v/v). Zein (20 g) was added slowly in 100 ml in hydroalcoholic solution of ethanol (90%, %v/v) by vigorous mixing at 50°C temperature, and finally, after cooling to 30 ° C temperature. Appropriate quantity of oils blend (3%; 1:1, %

w/v, EtOH) were poured to the zein solution for another 30 min to ensure the oil blend incorporation into the film (Pankaj, Bueno-Ferrer, Misra, Bourke, & Cullen, 2014).

2.3 Cheese Coating

The method of cheese coating was based for Cerqueira et al. (2009), with some modifications. The mozzarella cheeses, with approximately 25 g, were coated with the solution, by immersion the surface until all of it was covered, the residual coating being allowed to drip off. Cheeses were left for 4h at 7 °C until the coating was dry (Cerqueira et al., 2009).

2.4 Water loss

Water loss analysis was based of Pantaleão et al. (2007) with modifications. Standard mozzarella (20% NaCl;% w / v) with and without coating were stored in a chamber at 20 ° C in 7 days with weighings at intervals of 5 days. The analysis was performed in triplicates (Pantaleão, Pintado, & Poças, 2007).

2.5 Microbiological analysis

To evaluate the standard mozzarella life (20% NaCl;% w / v), measurements were made of mesophilic aerobic microorganisms and fungi and yeasts, based on the work of Peterkin (2001) and Teramura et al. (2015), with modifications. Weighed 25 g mozzarella was added solution of 225 ml sodium citrate (2%;% w / v). The material was homogenized with homogenizer type "stomacher" (490 strokes / min) for two minutes. From this initial dilution, dilutions were made with peptone water successively until dilution 10^{-8} (Peterkin, 2001; Teramura, Iwasaki, Ushiyama, & Ogihara, 2015).

For mesophilic microorganisms, and 100 ul aliquots of appropriate dilutions were transferred to plates containing PCA and incubated at 37 ° C / 48 h.

In fungi and yeast count of 100 uL aliquots of appropriate dilutions were transferred to Petri dishes containing DRBC and incubated at 25 ° C / 7days.

2.6 Sensory analysis

Mozzarella without added sodium chloride (NaCl) was subjected to salting method according to the methodology described by Rodrigues et al. (2014) with some modification. For the immersion in brine, 5 kg of mozzarella cheese was divided into 5 equal parts: (A)1 part is the traditional mozzarella cheese salted in brine containing 20% NaCl (%w/v), corresponding to a treatment of 100% NaCl (% w/g).The other parts were subjected to NaCl successive reductions, in different brines (75%,50%25% and 0%; %w/v) of NaCl, in a cold chamber at a temperature of 7°C for 8 h to yield the following 5 mozzarella cheese formulations: (B) mozzarella cheese salted in brine with a 15% in NaCl content, corresponding to a treatment of 75% NaCl (%w/g). (C) mozzarella cheese salted in brine with a 10% in NaCl content, corresponding to a treatment of 50% NaCl (%w/g). (D) mozzarella cheese salted in brine with a 5% in NaCl content, corresponding to a treatment of 25% NaCl (%w/g). (E) mozzarella cheese not immersed in brine solution, corresponding to a treatment of 0% (% w/v) of NaCl (Rodrigues, Gonçalves, Pereira, Carneiro, & Pinheiro, 2014).

The composition of the brine was determined using NaCl at different concentrations. It was measured the pH of mozzarella and adjusted the brine at the same pH of 5.4 and were used in the ratio of 3 L of brine per pound of cheese to be salted. After salting, the Mozzarella cheese was removed and dried in the same chamber for 48 h. After this period, the cheese was packaged in low-density polyethylene bags for later subjected a coating and sensorial analyses.

One hundred consumers of both sexes, aged 18-60 years and regular consumers of mozzarella were asked to perform sensory tests. Five samples of mozzarella with different concentrations of sodium were offered. Control sample with a standardized concentration of 20% (w / v) concentration corresponding to 100% NaCl and reduction NaCl in 75%, 50%, 25% and 0%. The samples were served in plastic cups containing 3 digits in balanced order according to the parameters by literature (Wakeling & MacFie, 1995). In all sessions, samples were served following the random order of presentation. It was performed range of the ideal test method salty taste and an acceptance test with respect to the overall printing attribute according works the literature (Amerine, Pangborn, & Poessler, 1967), using a hedonic scale of 9 points, ranging from 1 (disliked extremely) to 9 (I liked very much)

2.7 Statistical analysis

It is performed analysis of variance (ANOVA), and comparison of means by Tukey test with a significance level of 5% probability ($p < 0.05$)

by Statistica 8.0 software (Dell Statistica, Hoklahoma, USA) for absorption tests water, microbiological and sensory analysis. The sensory data were evaluated for map internal preference using SensoMaker software 1.9 (Informer Technologies).

3 Results and discussions

In Fig. 1 it can be seen the water loss profile of Mozzarella with and without coating.

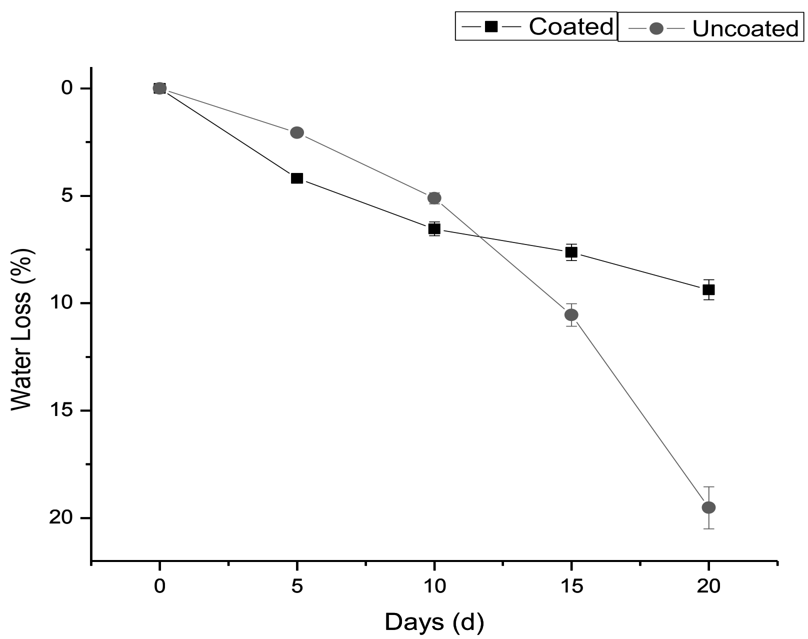

Fig. 1. Profile the water loss of mozzarella coated and uncoated by zein and 3% (%v/v) oil blend. Where $p \leq 0.05$ using the Tukey test.

It is very important to mention that the coating applied to the mozzarella had a mean thickness of 0.008 ± 0.002 mm. There were significant differences between the mozzarella coated and uncoated. The mozzarella subjected to the coating showed no significant differences in itself over time rated indicating barrier effectiveness water loss of mozzarella. The mozzarella uncoated showed 20% loss of water in twenty days and observed proportional to the decay days of storage. The mozzarella subjected to the coating showed 10% water loss no significantly diverging during this period, demonstrating efficacy relative to the barrier properties to water and other constituents of mozzarella. At the end of twenty days, mozzarella coated presented an aspect of apparent freshness compared to uncoated, as may be seen in Fig. 4.

The concentration of water is directly related to the physical-chemical and biological deterioration of food (Ramos et al., 2012). According to the water loss levels, microbial growth may differ in the types and species such as bacteria and fungi. Although there are many studies on the water barrier properties in the literature, searches numbers applicability concentrated in fruits and vegetables. In the works of Lerdthanangkul & Krochta, (1996), we investigated the properties of edible coatings for peppers and their effects on respiration rate, internal gases, and weight loss. The addition of mineral oil produced excellent barrier properties against moisture loss reductions. (Lerdthanangkul & Krochta, 1996)

The reduction of water loss demonstrates good compatibility between the coating and mozzarella, demonstrating the effectiveness of the coating as a water barrier property for sausage and cheese. These results come in line with the results found in the first article, where the zein coating and 3% (% v / v) blend of essential oils, showed low levels of water absorption of 1.33%, higher degree of hydrophobicity and better dispersion of the blend of essential oils in zein matrix. It should be noted the influence of iterations between the food and the coating in reducing water loss. The macromolecules of the zein coating are highly hydrophobic. When contacting the surface of the mozzarella, which has a similar nature, there are forces of attraction that increase the level of food surface hydrophobicity. These changes in the hydrophobicity, cause repulsion of water molecules present in mozzarella, causing the migration of water to the outside to be reduced, preserving the moisture of the food longer. Similar results are found in studies by Ayranci & Tunc, (2004), which evaluated the water loss properties for apricots and green peppers, in which the increase of hydrophobicity by means of hydrophobic additives cooperating with the reduction of water loss (Ayranci & Tunc, 2004).

In Fig. 2 can be seen mesophilic aerobic count evaluated in a total time of 20 days for mozzarella uncoated and coated, the results are presented in logarithmic profile.

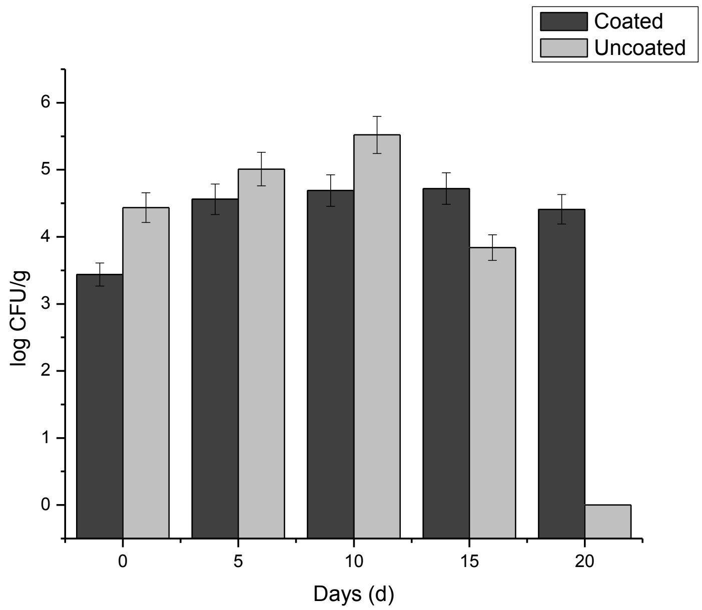

Fig. 2. Quantification of aerobic mesophilic microorganisms in mozzarella with zein coating containing 3% (% v/v) blends of EO and uncoated shown in Log CFU / g. Where $p \leq 0.05$ using the Tukey test.

The counts varied significantly ($p \leq 0.05$) at all time intervals. Initially, at time zero, they found 3.44 log CFU / g for mozzarella. For a range of foods that have no standards for the total microbial count, it is known that food products for human consumption with microbial populations on the order of 10^6 CFU / g or ml already in the degradation process, since there is a greater possibility of spoilage and / or pathogens, which can lead to organoleptic-characterization, loss of nutritional value and food attractiveness, and harm health (Ferrari, Winkler, & Oliveira, 2007). Therefore, the initial count of mozzarella presented 10^3 CFU / g, showing no high contamination in the product. However it is important to emphasize that the purpose of this analysis is to evaluate the front coating efficacy profile to spoilage agents of food, so it should be noted the comparison between mozzarella coated and uncoated. continued growth of colonies can be observed for both treatments until the tenth day, where there was a maximum population for mozzarella uncoated order of 10^5 CFU / g compared to mozzarella with coating that remained stable in the order of 10^4 CFU / g to the twentieth day. This stability in the growth of aerobic mesophilic the mozzarella coated, is an indicative of preserving food, thus delaying the process of deterioration. From the tenth day there was a continuous decay of mesophilic aerobic microorganisms for mozzarella uncoated until total absence of colonies on the twentieth day.

This has no relation with decreasing concentration of water present in mozzarella and indicates an advanced stage of food spoilage through growth of other microorganisms that develop on different conditions. These differences are related to the physico-chemical changes occurring in mozzarella and come into line with the results in water loss analysis, where in this same period, there was continual decay of concentrations of water contained in mozzarella without front coating stability water loss profile of mozzarella coated. However, these changes should be evaluated together with the growth profile of fungi and yeasts contained in mozzarella, shown in Fig. 3. As there is a decrease of colonies of mesophilic aerobic microorganisms, there is a growth of fungi and yeasts. These changes, again, are related to the water activity contained in mozzarella. According to Ramos et al. (2012) there is a lower moisture requirement for the growth of yeasts and molds as compared to bacteria. Accordingly, in a food which provides conditions for developing both groups of microorganisms, bacteria dominate the decay process while the moisture conditions are higher. As water loss occurs, fungi and yeasts dominate the process of deterioration (Ramos et al., 2012).

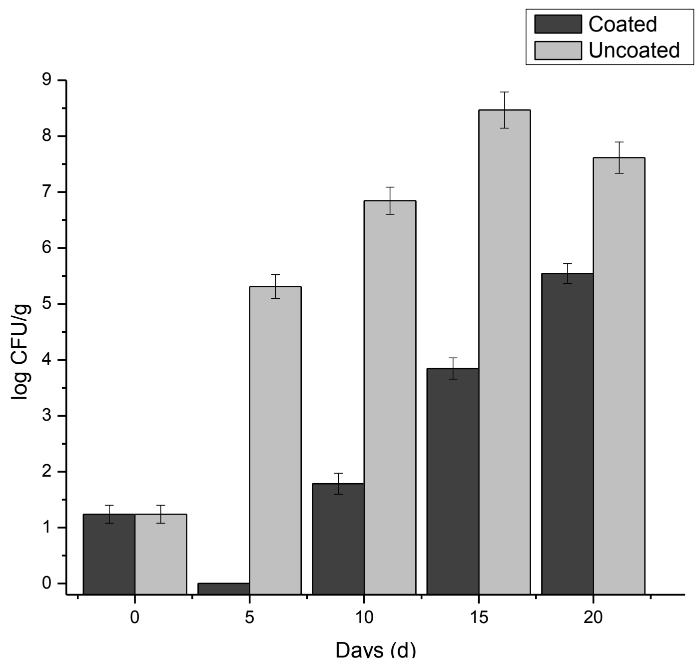

Fig. 3. Fungi and yeasts for mozzarella coated and uncoated. Where $p \leq 0.05$ using the Tukey test.

It can be seen in Fig. 3 that initially fungi and yeasts have a score of 1.23 log CFU / g or is in the order of 10^1. There was a significant difference ($p \leq 0.05$) for all intervals. With coating application on the fifth day is observed no growth of fungi and yeasts. This fact shows the antimicrobial effectiveness of the coating, which probably acted as mozzarella surface inhibiting the growth of fungi and yeasts. However for mozzarella uncoated there was a continued growth of microorganisms to the twentieth day, with number of colonies on the order of 10^7. With application of the coating, there was a significant decrease in growth, with the number of colonies in the order of 10^5.

In Fig. 4 can be seen the visual appearance of coated and uncoated mozzarella. It is not visually detected the presence of fungi and yeasts for mozzarella and zein coating blend of essential oils to the twentieth day.

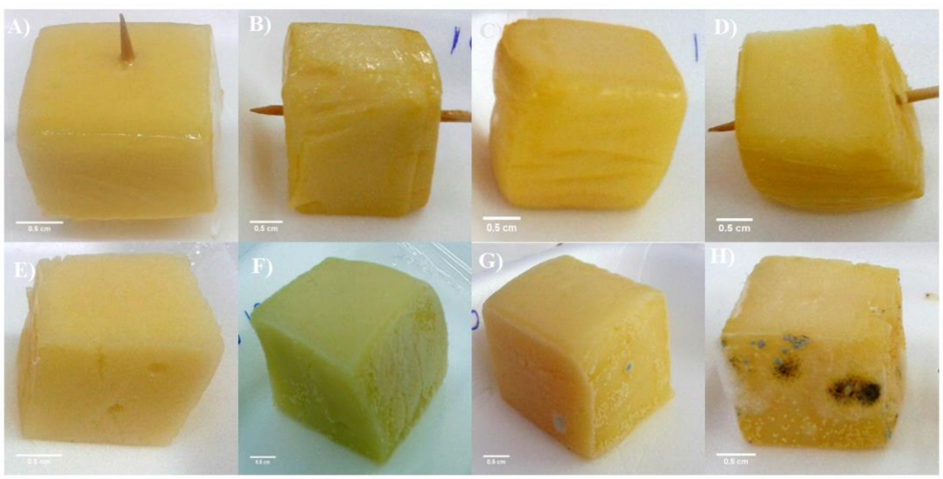

Fig. 4. Mozzarella lifetime. Coated A-D . Uncoated E-H. In the period 5 (A, E), 10 (B, F), 15 (C, G) and 20 (D, H) days.

Yet there are few studies in the literature regarding the coatings on cheese in order to increase the useful life and preserving its nutritional quality. Pena-Serna (2013) evaluated the quality of Minas standard cheese with the application of coating zein base throughout its lifetime. In his studies were detected minor changes in physical and chemical standards and reduced microbiological contamination with the application of zein coating, while bearing in accordance with the findings of this study (Pena-serna & Lopes-filho, 2013). Balaguer et al. (2013) studied the antifungal properties of cinnamaldehyde incorporated into gliadin films, for use in breads and cheeses. There was antifungal efficacy even in a period of 45 days (Balaguer, Lopez-Carballo, Catala, Gavara, & Hernandez-Munoz, 2013). These results show the importance of the application of edible coatings on food. It is detected increase on the lifetime of coated mozzarella by reducing the growth of spoilage microorganisms. It indicates potential application as a coating agent to reduce sodium and natural preservatives.

To assess the impact of the sodium reduction and of the film on mozzarella, an acceptance test was applied to mozzarella samples. The internal preference mapping (MDPRF) (Fig. 5) shows the results obtained for overall impression of the conventional and sodium-reduced mozzarella samples.

The first principal component (PC1) explained 53.72% and the second principal component explained (PC2) 17.55% and the two main components together explained 71.27% of the variation in acceptance of the samples as their overall impression relative to the sodium reduction and coating of zein and blends of essential oils.

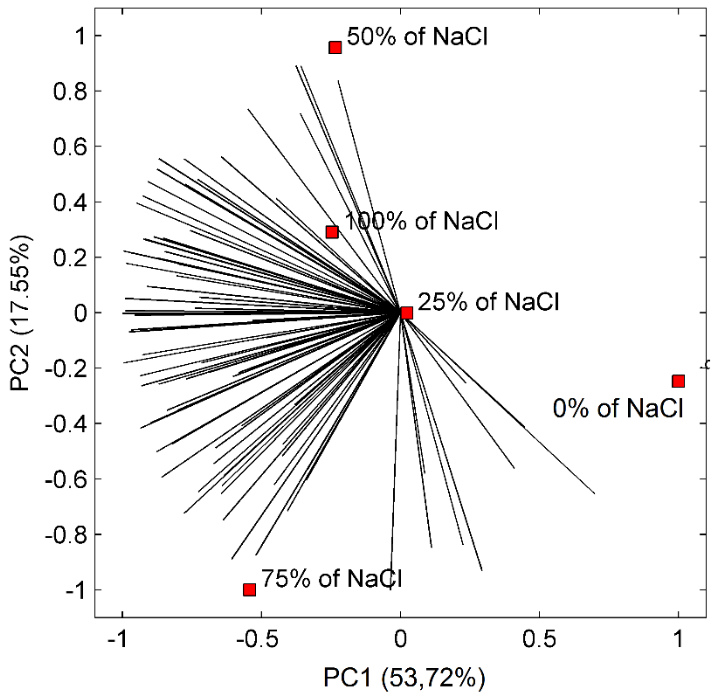

Fig. 5. Map preferably of different sodium concentrations of mozzarella.

In MDPRF, consumers are represented by vectors (MacFie, N.Bratchell, K.Greenhoff, & L.Vallis., 1989). Therefore, the higher concentration vectors near to the sample suggests that the sample has a greater acceptance. The spatial separation of the samples, suggest the existence of different groups in relation to preference. It also suggests that the sample with 75% (% w / v) NaCl was preferred, followed by samples 25, 50 and 100% (% w / v) NaCl. As the sample with 0% (% w / v) NaCl less preferred. This indicates that the use of the film allowed a reduction of up to 75% (% w / v) NaCl without loss of acceptance of the product.

For a better assessment of sensory acceptance of the samples, the results of mean and standard deviation found in sensory analysis as well as the result of ideal saltiness applied were shown in Table 1:

Table 1. Average sensory acceptance in relation to global and optimal print the salty taste.

Treatments - % NaCl	% NaCl (w/v) in mozzarella	Overall impression	Ideal (salty taste)
Control – 100	20	6.06 ± 1.79 b	4.09 ± 1.81 c
75	15	7.02 ± 1.05 c	4.00 ± 1.77 c
50	10	6.00 ± 1.77 b	4.04 ± 1.79 c
25	5	5.65 ± 1.86 b	3.20 ± 1.42 b
0	0	4.00 ± 1.86 a	1.80 ± 1.37 a

Letters equal in the same column indicate that there is no significant difference at 5% probability using the Tukey test followed by Standard Deviation.

Sensory analysis indicates a significant difference at 5% between samples. The mozzarella with 75% (% w / v) NaCl showed, in according to the evaluators, the best score between 7-8 and classified as "moderately good" to "very good". Regarding the control samples, 50% and 25% (% w / v) NaCl, there were no significant differences ($p \leq 0.05$), with grades ranging from "indifferent" to "moderately good". Treatment with 0% (% w / v) NaCl, ie; absence of NaCl had a 4 score, being classified as "slightly disgusted." Probably, with increasing NaCl concentration, the taste of the essential oils blend became more pronounced, reducing the preference for the control, reaching to equilibrium at 75% (% w / v) NaCl (25% reduction), with the preferred sample by consumers. The reduction NaCl in itself is an alternative to improve the quality of mozzarella, since industries using NaCl levels are often higher than those required for consumption (Henneberry, Wilkinson, Kilcawley, Kelly, & Guinee, 2015).

Furthermore, it was observed that up to 25% (% w / v) NaCl (75% reduction) did not cause loss of sensory acceptance of mozzarella compared to the control sample (100% (% w / v) NaCl). These results demonstrate the great potential of the blend of essential oils incorporated in the zein films, as additives that contribute flavoring action to reduce sodium and other preservatives in many foods. In studies of Arboatti et al. (2014) it assessed the sensory acceptance of mozzarella reduced sodium content. There was acceptance in reductions of up to 60% NaCl, smaller reductions compared to this study, which confirms the action of the coating (Arboatti et al., 2014). The sodium reduction effect in cheddar and mozzarella cheese, and the terms moisture and acceptability through sensorial analysis was evaluated by Ganesan et al. (2014). The results showed improved acceptance when applied together with spices and seasonings, low acceptability to samples with higher sodium reduction rates and a gradual reduction was required in the sodium reduction to obtain a better acceptance. This fact comes in line with the results presented in this paper, where the action of the zein coating and blend of essential oils made at concentrations of up to 25% NaCl there is good overall impression of notes demonstrating consumer approval for the application of the coating and the effectiveness of the blend as flavoring agent and sodium potential substituent (Ganesan, Brown, Irish, Brothersen, & McMahon, 2014).

The standard deviations shown in the table 1, is due to the fact that the great variation in people preference on foods with low or high concentrations of sodium. However, all values were below the ideal of salty taste, even for the control. Even in the range of 50% (% w / v) NaCl there were no significant differences compared to control, with scores slightly below the ideal. However, from 25% NaCl there was a considerable reduction of the ideal score 3.2, down to 0% NaCl with score 1.8. People do not usually eat mozzarella individually, as a way of tasting and it is more common the consumption with accompaniments in sandwiches, pizzas, pies and dishes in general, therefore, is justified levels below the ideal. Excessive exposure to foods with high salt concentrations can alter the perception of taste, which results in an increased consumption of NaCl to generate the feeling of salty taste. Over the past decades, there was overconsumption derivatives sodium by "fast food" and processed foods, changing the perception and taste of consumers, and therefore there is great resistance of the same in relation to reduction of the salty taste, and difficulty in identifying the taste of food more delicate flavor, such as mozzarella (McCarthy, Wilkinson, Kelly, & Guinee, 2015). Kim and Lee (2009), investigated the influence of the consumption of fast food relative to the increased preference for salty taste. The results were alarming, where most people evaluated did not identify saltiness into instant soups with normal levels of salinity (Kim & Lee, 2009). In studies by Rodrigues et al. (2014) mozzarella was developed with a mixture of salts (monosodium glutamate, potassium chloride and sodium chloride) to reduce sodium levels. sensory acceptance and time tests were done where, mozzarella with changes in NaCl composition obtained similar results to a standard formulation (Rodrigues et al., 2014).

Through the overall impression and ideal of the preference of salt, it is concluded that mozzarella with sodium reductions up to 50% (% w / v) showed good levels of acceptability. Using the zein coating and blends of essential oils showed good acceptance rate, for application in mozzarella. Other studies with descriptive tests should be done to complement this work.

4 Conclusion

Zein-based coating and 3% (% v/v) blend of essential oils were applied satisfactorily, acting as an antimicrobial agent and flavoring in mozzarellas. There was reduced growth of spoilage microorganisms, mesophilic aerobes and fungi and yeasts, increasing, therefore, the useful life of mozzarellas subjected to coating. With application of the coating a reduction in mozzarella NaCl in 50% (% w / v) afforded good acceptability compared to control mozzarella. The results show the effectiveness of the zein coating and blend of essential oils over the life of mozzarella demonstrating application to other foods through complementary studies.

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