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# REVESTIMENTO Á BASE DE PROTEÍNA DE ARROZ COMO ALTERNATIVA PARA PROLONGAR A VIDA DE PRATELEIRA DE OVOS

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# REVESTIMENTOS Á BASE DE PROTEÍNA DE ARROZ COMO ALTERNATIVA PARA PROLONGAR A VIDA DE PRATELEIRA DE OVOS[1]

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**Resumo:** A busca por tecnologias inovadoras que possam auxiliar na manutenção da qualidade interna dos ovos durante o armazenamento e também trazer melhoria nas propriedades da casca é de grande interesse por parte da indústria avícola. As vantagens do uso de revestimentos podem ser justificadas pela capacidade de proteger o alimento mecanicamente e ainda diminuir a degradação do produto, aumentando assim seu tempo de prateleira. O uso de diferentes revestimentos à base de proteína concentrada de arroz (PCA) (5, 10 ou 15%) e seus efeitos nas características de qualidade foram avaliadas em ovos convencionais (Experimento 1) e orgânicos (Experimento 2) e armazenados por oito semanas em temperatura ambiente. Posteriormente, a incorporação de extrato de própolis (0, 5 ou 10% – Experimento 3) ou 1% de óleos essenciais (copaíba, melaleuca ou tomilho – Experimento 4) aos revestimentos também foi avaliada, assim como o efeito de diferentes plastificantes (glicerol, propileno glicol ou sorbitol – Experimento 5). Em todos os experimentos foram utilizados ovos íntegros, não férteis e de um dia, distribuídos em delineamentos inteiramente casualizados, com 12 repetições cada. Foram avaliados a perda de peso (%), Unidade Hagh (UH), índice de gema (IG) e pH do albúmen e gema. Os dados foram analisados utilizando o programa estatístico SAS (9.4, SAS Inst. Inc., Cary, NC, Estados Unidos). Os dados foram submetidos à análise de variância utilizando o PROC GLM. Eventuais diferenças entre médias foram comparadas pelo teste de Tukey a 5% de probabilidade. No experimento 1 foi possível observar que ovos não-revestidos apresentaram a maior perda de peso (8,28%), enquanto a proteína do arroz a 5% (5,60%), 10% (5,45%) e 15% (5,54%) foram eficientes na prevenção da perda de peso ($P < 0,001$). Os ovos que não receberam nenhum tipo de tratamento apresentaram os piores valores de ($P < 0,001$) UH (54,45), IG (0,28) e pH do albúmen (9,18). O uso dos revestimentos à base de PCA preservaram a qualidade interna dos ovos por até 4 semanas a mais quando comparado a ovos não revestidos. Resultados semelhantes foram encontrados no experimento 2, onde os mesmos revestimentos foram testando em ovos orgânicos. O uso do revestimento de PCA pode preservar a qualidade interna dos ovos por até 3 semanas a mais do que os ovos não revestidos. Ovos revestidos com proteína de arroz com e sem própolis (experimento 3) apresentaram resultados com qualidade interna similar durante as 6 semanas de armazenamento. Os revestimentos com proteína de arroz e óleos essenciais (experimento 4) foram eficientes na preservação da qualidade interna dos ovos por até 3 semanas a mais que os ovos sem revestimento. Ovos não revestidos apresentaram maior perda de peso ($P < 0,001$) (5,43%) quando comparado com ovos revestidos com PCA (4,23%) ou PCA enriquecido com melaleuca (4,10%), copaíba (3,90%) ou tomilho (4,08%). O uso do sorbitol como plastificante é mais eficiente na manutenção do controle do aumento do pH do albumen (9,13) quando comparado aos ovos não

revestidos (9,52) ou revestidos com glicerol (9,20) ou propileno glicol (9,20) (P < 0,001) (experimento 5). A microscopia eletrônica de varredura demonstrou menor porosidade nas casca de ovos revestidos, indicando que o uso do revestimento pode fornecer uma barreira protetora contra a transferência de gases e umidade. O uso de revestimentos à base de proteína de arroz é uma opção interessante para auxíliar na manutenção da qualidade interna dos ovos armazenados uma vez que os resultados encontrados neste trabalho foram promissores.

Palavras-chave: Casca de ovos, óleos essenciais, própolis, revestimento proteico

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[1]Tese de Doutorado em Zootecnia - Produção Animal ou Plantas Forrageiras, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (160p.) Março, 2019.

# RICE PROTEIN-BASED COATINGS AS AN ALTERNATIVE TO PROLONG THE SHELF LIFE OF EGGS[1]

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**Abstract :** The search for innovative technologies that can help in maintaining the internal quality of the eggs during storage and also bring improvement in the properties of the shell is of great interest on the part of the poultry industry. The advantages of using coatings can be justified by the ability to mechanically protect the food and further decrease the degradation of the product, thereby increasing its shelf life. The use of different coatings based on rice protein concentrated (RPC) (5, 10 or 15%) and their effects on quality characteristics were evaluated in conventional and organic eggs and stored for eight weeks at room temperature. Later, the incorporation of propolis extract (0, 5 or 10%) or 1% of essential oils (copaiba, melaleuca or thyme) to the coatings was also evaluated, as well as the effect of different plasticizers (glycerol, propylene glycol or sorbitol) . Inbreeding eggs were used in a completely randomized design with 12 replicates each. The weight loss (%), Hagh Unit (UH), yolk index (GI) and albumin and gem pH were evaluated. Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). Data were submitted to analysis of variance using PROC GLM. Possible differences between averages were compared by the Tukey test at 5% probability.In the experiment 1, it was possible to observe that uncoated eggs had the highest weight loss (8.28%), while rice protein 5% (5.60%), 10% (5.45%) and 15% (5.54%) were efficient in preventing weight loss (P <0.001). The eggs that did not receive any treatment presented the worst values (P <0.001) HU (54,45), IG (0,28) and pH of the albumen (9,18). The use of PCA-based coatings preserved the internal quality of the eggs for up to 4 more weeks when compared to uncoated eggs. Similar results were found in Experiment 2, where the same coatings were tested on organic eggs. The use of the RPC coating can preserve the internal quality of the eggs for up to 3 weeks longer than uncoated eggs. Rice protein coated eggs with and without propolis (experiment 3) showed similar internal quality results during 6 weeks of storage. Rice protein coatings and essential oils (experiment 4) were efficient in preserving the internal quality of eggs for up to 3 weeks longer than uncoated eggs. Uncoated eggs presented greater weight loss (5.43%) when compared to eggs coated with RPC (4.23%) or RPC enriched with melaleuca (4.10%), copaiba (3.90%) or thyme (4%) (P <0.001). The use of sorbitol as a plasticizer is more efficient in maintaining control of the increase in albumen pH (9,13) when compared to uncoated eggs (9,52) or coated with glycerol (9,20) or propylene glycol (9, 20) (P <0.001) (experiment 5). Scanning electron microscopy demonstrated lower porosity in the coated eggshell, indicating that the use of the coating may provide a protective barrier against gas transfer and moisture. The use of rice protein coatings is an interesting option to help maintain the internal quality of stored eggs since the results found in this work were promising.

Key-words : eggshell, essential oil, propolis, protein coatings

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## LISTA DE ABREVIATURAS

COP: Copaíba
HU: Haugh unit
IG: Índice da gema
GLY: Glycerol
PCA: Proteína concentrado do arroz
PRO: Propylene glycol
RPC: Rice protein coating
RPC+P5: Rice protein coating with 5% of propolis
RPC+P10: Rice protein coating with 10% of propolis
SOR: Sorbitol
TEA: Tea tree
THY: Thymo
UH: Unidade Haugh
YI: Yolk index

# CAPITULO I

# 1. INTRODUÇÃO

O ovo é uma importante fonte de proteína, devido ao seu balanço de aminoácidos, sendo considerado representativo do modelo de proteína ideal. Além de apresentar proteínas de alto valor biológico, o ovo contém ainda vitaminas do complexo B, A, E, K; minerais como ferro, fósforo, selênio e zinco; e carotenóides como a luteína e zeaxantina. Além de ser equilibrado em sua composição nutricional, é uma fonte de proteína acessível pelo baixo valor econômico (Figueiredo, 2012).

Ovos são produtos perecíveis e perdem a qualidade caso não sejam manipulados e armazenados corretamente. O ovo, desde a oviposição, está sujeito a alterações físico-químicas do albúmen e da gema que podem resultar em alterações do sabor, frescor e palatabilidade. Quanto maior for o tempo de estocagem, maior é a deterioração da qualidade interna, pela maior movimentação de dióxido de carbono através da casca, principalmente em temperatura ambiente (Oliveira & Oliveira, 2013). Neste contexto, maior perda de qualidade poderia ser esperada em produtos cujo volume de negócios é menor, como os ovos orgânicos, que tendem a permanecer por um período mais elevado nas gôndolas de supermercados (Patterson et al., 2001; Hidalgo, 2008).

Estratégias para manutenção da qualidade dos ovos devem ser aplicadas pelo setor de postura devido à ausência da refrigeração dos ovos nos pontos de venda (Scatolini-Silva, 2013). Atualmente, há um crescente interesse no desenvolvimento de métodos eficazes para manter a qualidade interna do ovo e reduzir a porcentagem de quebra da casca (Wong, 1996; Xie, 2002). A utilização de revestimentos comestíveis é uma tecnologia simples e já demonstra resultados favoráveis. Revestimentos à base de quitosana (No, 2007; Jo, 2011), concentrado proteico de soro de leite (Caner & Yuceer, 2015) e proteína isolada de soja (Biladeau & Keener, 2009) foram eficazes em manter a qualidade interna dos ovos durante o armazenamento. Os benefícios da utilização do uso de própolis (Aygun, 2013; Akpinar, 2015) e de fitoquímicos (Upadhyaya et al., 2016) em revestimentos para ovos também já foram demonstrados anteriormente e estão provavelmente associados à presença de agentes antimicrobianos ativos, como os fenóis simples, ácidos fenólicos e polifenóis (Cowan, 1999).

Alguns estudos relatam a utilização de arroz como matéria-prima adequada para a preparação de filmes ou revestimentos comestíveis, dentre eles o farelo (Gnanasambandam, 1997), a proteína do farelo (Adebiyi, 2008), a farinha (Dias, 2010; Dias, 2011) e o amido de arroz (Dias, 2011; Das, 2013). Entretanto, não foram encontrados relatos da utilização de revestimentos à base de proteína de arroz para ovos.

A preparação de revestimentos comestíveis à base de proteína de arroz é uma alternativa de uso para a farinha de arroz, uma matéria prima de baixo custo que pode ser produzida a partir do arroz quebrado durante o beneficiamento do grão. Em paralelo, a utilização da própolis ou de óleos essenciais pode ser associada neste revestimento devido as suas características antibacterianas. Neste contexto, os trabalhos apresentados nesta tese foram desenvolvidos com o objetivo de avaliar o uso de revestimentos a base de proteína concentrada de arroz em ovos, assim como a

incorporação de extrato de própolis ou óleos essenciais nestes revestimentos. O problema de pesquisa e as questões a serem respondidas são apresentados nesta tese em um estudo bibliográfico e cinco artigos científicos.

# 2. REVISÃO BIBLIOGRÁFICA

Os ovos são uma fonte de proteína de excelente valor biológico (Yuceer & Caner, 2014) e podem ser considerados um dos alimentos mais nutritivos e completos da dieta humana, pois apresentam uma composição rica em vitaminas, minerais e ácidos graxos (Rêgo et al., 2012). Além disso, podem ser considerados alimentos funcionais, pois contêm componentes como colina, imunoglobulinas e lisozima. Apesar de suas excelentes características nutricionais, o ovo apresenta preço mais acessível quando comparado a outras proteínas de origem animal (Oliveira & Oliveira, 2013).

Em 2016, a produção mundial de ovos atingiu aproximadamente 96 milhões dúzias (USDA, 2018), dos quais aproximadamente 39 bilhões de unidades foram produzidas no Brasil. O consumo per capita de ovos pela população brasileira aumentou de 182 ovos em 2014 para 192 ovos em 2017 (ABPA, 2018). No Brasil, 92% dos ovos comercializados *in natura* estão expostos a temperatura ambiente e são resfriados apenas na casa do consumidor (Oliveira e Oliveira, 2013).

A qualidade dos ovos está relacionada com a aceitabilidade do consumidor e sua segurança alimentar. Assim como todo produto de origem animal, o ovo é um produto perecível. Por isso deveria ser mantido sob refrigeração desde a produção até o seu consumo, o que resultaria no aumento dos custos de produção e acréscimo no valor do produto final (Figueiredo, 2012). No mercado interno, a ausência de refrigeração de ovos ocorre por que este processo não é exigido, apenas sugerido durante sua estocagem doméstica, imediatamente após a aquisição, conforme Resolução RDC n. 35 de 17 de junho de 2009. Por isso, os ovos são acondicionados em temperatura ambiente, desde o momento da postura até a distribuição final (Figueiredo, 2012; Almeida, 2013). Assim, o prazo de validade de ovos com relação a sua qualidade físico-química e microbiológica irá depender de fatores extrínsecos, como condições ambientais de produção e armazenamento; e intrínsecos, como seus nutrientes, principalmente as proteínas, pH e atividade de água (Figueiredo, 2012).

## 2.1. Qualidade de ovos

A qualidade dos ovos é definida como o conjunto de características externas e internas que influenciam na aceitação do produto no mercado (Barbosa, 2008). A qualidade dos ovos está diretamente relacionada às características da poedeira, como linhagem, idade, condição nutricional e sanitária; e do sistema produtivo, como clima e manejo. Além disso, a qualidade dos ovos também pode sofrer alterações de acordo com as condições de estocagem após a postura (Oliveira & Oliveira, 2013).

As características externas de qualidade dos ovos estão relacionadas à qualidade da casca, ao considerar sua estrutura, resistência e higiene. Já as características internas estão relacionadas com aspectos do albúmen, gema, câmara de ar, cor, odor e sabor (Mendes, 2010). A legislação brasileira (BRASIL, 1997) determina condições mínimas de qualidade interna do ovo (câmaras de ar variando de 4 a 10 mm; gemas translúcidas, firmes, consistentes e sem germe desenvolvido; claras transparentes, consistentes,

límpidas, sem manchas e com as calazas intactas). No entanto, somente o peso e as características da casca têm sido considerados na prática.

## 2.2. Qualidade de ovos produzidos no sistema convencional ou em sistemas alternativos

A busca por alimentos produzidos em sistemas semiextensivos tem aumentado nos últimos anos, devido principalmente à preocupação com o uso de gaiolas na avicultura de postura convencional (Anderson, 2011). A indústria avícola respondeu a essa preocupação através do aumento da produção de ovos oriundos de poedeiras com acesso ao ar livre, como nos sistemas alternativos de produção (Patterson et al., 2001).

O tipo de sistema de criação das aves pode influenciar a quantidade e qualidade dos ovos produzidos (Van Den Brand et al., 2004; Hidalgo, et al., 2008). Estudos demonstraram que ovos oriundos de poedeiras criadas no sistema orgânico apresentam melhor qualidade interna quando comparados aos produzidos por poedeiras criadas no sistema convencional (Duric-Stojcic et al., 2009). A menor intensidade de postura nos sistemas orgânicos é um dos fatores que podem explicar esta variação. A melhora na qualidade dos ovos também pode ser explicada pela maior atividade motora exercida pelas poedeiras criadas em sistemas alternativos quando comparada com as aves criadas em gaiolas no sistema convencional (Van Den Brand et al., 2004; Singh et al., 2009).

O peso e os parâmetros relacionados ao tamanho do ovo (área de superfície, diâmetro e altura) também podem ser influenciados pelo sistema de produção. Resultados controversos sobre o peso de ovos produzidos nos diferentes sistemas foram encontrados, com vantagens produtivas relatadas para ovos de poedeiras criadas ao ar livre (Tumova & Ebeid, 2005; Pistekova et al., 2006) ou alojadas em gaiolas (Anderson & Adams, 1994; Leyendecker et al., 2001). Acredita-se que o menor peso de ovos de galinhas alojadas em gaiolas convencionais pode estar relacionado a uma maior produção de ovos, como verificado por Michel & Huonnic (2003) e Hulzebosch (2006).

Entretanto, algumas características de qualidade do ovo são superiores em ovos produzidos em gaiolas quando comparados com sistemas alternativos (Englmaierová, 2014). Melhor qualidade de casca foi verificada em ovos provenientes do sistema convencional (Tumova & Ebeid 2005; Tumova et al., 2009). Além disso, menor espessura da casca foi observada em ovos produzidos ao ar livre quando comparados aos ovos produzidos em gaiolas (Pavlovski et al., 2001).

Küçükyýlmaz et al. (2011) verificaram variação nos níveis de minerais em ovos comerciais e orgânicos, apesar dos níveis semelhantes de ingestão dietética e concluíram que isto poderia ser explicado pelo fato das aves criadas no sistema orgânico terem sido expostas a condições de maior estresse ambiental em comparação as aves criadas em gaiolas convencionais. Assim, as aves no sistema orgânico, supostamente utilizam maiores níveis de fósforo e zinco como nutrientes essenciais para manutenção do sistema imunológico. Além disso, a exigência de fósforo de poedeiras criadas em sistema orgânico pode ser subestimada devido às atividades físicas extras realizadas, devido ao acesso ao ar livre.

Ovos produzidos por aves mantidas ao ar livre apresentam maiores níveis de β-caroteno (Anderson, 2011), alfa-tocoferol, polifenóis e maior teor de carotenóides em comparação aos ovos de aves criadas em gaiolas (Mugnai, 2009). Além disso, aves criadas ao ar livre têm acesso a diferentes alimentos (principalmente forragem), o que pode influenciar na coloração da gema (Holt, 2010).

Com relação às propriedades funcionais, ovos orgânicos apresentam maior estabilidade de espuma, o que pode estar relacionado com o teor mais elevado de proteína e com a porcentagem de albúmen dos ovos orgânicos (Hidalgo, 2008). Ao investigar a qualidade de ovos produzidos em diferentes sistemas de produção (convencionais x orgânicos) e comercializados em um mesmo local, Hidalgo (2008) também verificou maior câmara de ar em ovos orgânicos e atribuiu esse efeito a aspectos de manejo no sistema orgânico, como possível atraso na coleta dos ovos e demora para distribuição dos ovos no varejo. Além disso, Patterson et al. (2001) relataram que ovos produzidos no sistema orgânico apresentam menor Unidade Haugh (UH), provavelmente como consequência de um volume de negócios mais lento, sugerindo que ovos orgânicos permanecem por um período mais elevado nas gôndolas de supermercados.

Por conta das variações existentes entre os sistemas de produção, é importante considerar que as tecnologias desenvolvidas para a indústria avícola devem ser testadas em ambos os sistemas. Entretanto, é comum que os produtos sejam preferencialmente testados nos sistemas convencionais, em detrimento dos extensivos.

## 2.3. Perda da qualidade dos ovos durante o armazenamento

Durante o armazenamento, o ovo sofre contínuas alterações físico-químicas do albúmen e gema que podem resultar em modificações do sabor, frescor e palatabilidade. A perda de qualidade é um fenômeno inevitável e contínuo e pode ser agravada por diversos fatores, como contaminação microbiológica, além da alta umidade e temperatura durante o armazenamento (Barbosa et al., 2008). Quanto maior o tempo de estocagem, maior será a deterioração da qualidade interna, pela movimentação de dióxido de carbono através da casca do ovo, principalmente em condições ambientais favoráveis aos processos.

Ovos frescos são caracterizados por albúmen límpido, transparente, consistente, denso e alto, com pequena porção mais fluída (Solomon, 1997). Conforme aumenta o tempo de estocagem, a proporção de albúmen líquido aumenta em detrimento da porção densa. A fluidificação e a perda da viscosidade do albúmen denso ocorrem em consequência da hidrólise das cadeias de aminoácidos, que ao serem degradadas liberam a água ligada a grandes moléculas de proteínas (Moreng & Avens, 1990). A liquefação do albúmen denso é evidenciada pela diminuição dos valores de UH. A legislação brasileira não utiliza a UH como parâmetro de avaliação da qualidade interna de ovos, entretanto países como Estados Unidos e México classificam ovos comerciais em diferentes classes de qualidade de acordo com a UH: excelente (AA ou México Extra), ovos com mais de 72 UH; boa (A ou México 1), entre 60 e 72 UH; e mediana (B ou México 2), entre 55 e 30 UH. Nos Estados Unidos, ovos com menos de 30 UH são classificados como de baixa qualidade (C),

enquanto no México estes são considerados impróprios para o consumo *in natura* (USDA, 2000; IMNC, 2004).

A gema também pode sofrer alterações durante o período de estocagem. A água liberada durante a reação de hidrólise dos aminoácidos do albúmen é transferida para a gema, que consequentemente aumenta de peso tornando-se descentralizada e menos densa (Ordónez, 2005; Oliveira & Oliveira, 2013). A gema de ovos frescos deve ser translúcida, consistente, centralizada na clara e bem fixada pelas chalazas, que são pequenos cordões laterais oriundos da própria clara. Gemas de ovos velhos são achatadas e flácidas, podendo apresentar manchas escuras. Além disso, a membrana vitelina, que circunda a gema, rompe-se com facilidade, deixando escorrer o conteúdo, o que prejudica a sua utilização (Solomon, 1997).

O índice de gema é um critério utilizado para determinar a firmeza desta estrutura e é calculado através da largura e altura da gema (Sharp & Powell, 1930). A faixa padrão para o índice de gema estabelecida para ovos frescos oscila entre 0,30 a 0,50. Ovos com índice da gema inferior a 0,25 possuem alta fragilidade desta estrutura, o que torna difícil a realização de medições sem rompimentos (Biagi, 1982). É importante destacar que o índice de gema diminuiu significativamente com o aumento do período de armazenamento (Caner, 2005; Canner & Yuccer, 2015).

A coloração da gema também pode sofrer alterações durante o período de estocagem. A cor da gema é influenciada pela dieta fornecida para a ave e é principalmente dependente do conteúdo de carotenóides (luteína, zeaxantina, β-criptoxantina e outros). Os carotenóides podem ser degradados pelo processo oxidativo, mudando de pigmentação durante o armazenamento (Caner, 2005). Redução linear na coloração de gemas de ovos com o aumento do tempo de armazenamento foi relatada em estudos prévios (Santos et al., 2009; Freitas et al., 2011). Além disso, ovos armazenados apresentam transferência de ferro da gema para o albúmen, ocasionando coloração rósea no albúmen e transferência de proteínas do albúmen para gema, ocasionando gema de coloração salmão (Sauveur, 1993).

A gema é rica em minerais, principalmente cálcio, cobre, ferro e manganês (Caner & Cansiz, 2007). Os minerais são geralmente estáveis, mas podem sofrer alterações devido às condições de armazenamento, além de reagirem com outros componentes alimentares, tais como proteínas e carboidratos. Variações nas concentrações de minerais em gemas de ovos durante o armazenamento foram relatadas em estudos anteriores (Manson et al., 1993; Caner & Cansiz, 2007).

O peso do ovo também é influenciado pelo tempo de armazenamento, mesmo quando os ovos são submetidos a ambientes com temperatura e umidade controladas (Moura, 2008). A perda de peso durante a estocagem é uma medida importante para monitorar as mudanças na qualidade da casca dos ovos frescos, uma vez que a diminuição de peso ocorre devido à transferência de umidade do albúmen para o ambiente externo por meio da casca (Scott & Silversides, 2000). A diminuição do peso do ovo pode também ser causada pela provável perda de amônia, nitrogênio e sulfeto de hidrogênio que são produtos da degradação química de seus constituintes orgânicos (Solomon, 1997). O conhecimento do conteúdo de sólidos totais dos ovos é importante, uma vez que essa variável determina o rendimento de ovos desidratados.

A densidade ou gravidade específica indica a qualidade da casca em relação aos demais componentes. Esta característica também pode ser alterada durante o armazenamento e está intimamente relacionada com a espessura de casca. A medida da gravidade específica é provavelmente uma das técnicas mais comumente utilizadas para determinar a qualidade da casca do ovo, devido a sua rapidez, praticidade e baixo custo. A densidade é obtida por imersão do ovo em diferentes concentrações salinas com densidades variando de 1,050 a 1,100. Quanto maior a densidade específica de um ovo maior é a sua qualidade (Haminton, 1982).

A perda de água que ocorre no ovo após da postura provoca um aumento progressivo da câmara de ar e consequentemente uma diminuição da gravidade específica do ovo (Santos, 2008). Quanto mais velho for o ovo, maior será a câmara de ar, devido à perda de vapor de água. Assim, ovos com pior qualidade de casca apresentam maior câmara de ar devido a maior perda de vapor de água. No Brasil, os regulamentos indicam variações de altura de no máximo 4 mm e até 10 mm em ovos de classe C. Ovos cuja altura da câmara de ar estejam acima de 10 mm são inviáveis para consumo (Oliveira & Oliveira, 2013).

O pH do albúmen e da gema pode sofrer alterações em decorrência das mudanças bioquímicas na gema e à transferência de água do albúmen. Silversides & Scott (2001), sugerem que o pH é mais adequado para a verificação da qualidade de ovos frescos do que a altura do albúmen ou UH, uma vez que esta medida é menos influenciada pela idade e linhagem da poedeira. O pH do albúmen de um ovo fresco pode variar de 7,6 até 8,5 podendo alcançar até 9,7 durante o período de estocagem (Oliveira & Oliveira, 2013). A perda de gás carbônico resulta em alteração no sabor do ovo em decorrência do aumento da alcalinidade (Moreng & Avens, 1990).

Durante o armazenamento do ovo, ocorre ainda a transformação da ovoalbumina em S-ovoalbumina e a dissociação do complexo ovomucina-lisozima, com destruição do gel de ovomucina. A ovoalbumina está relacionada com a estabilidade da espuma do albúmen (popularmente conhecida como “clara em neve”). Estas reações são importantes para a indústria em decorrência da perda das propriedades gelificantes e espumantes (Oliveira e Oliveira, 2013). A capacidade de uma proteína formar espuma refere-se à expansão de volume da dispersão proteica com a incorporação de ar por batimento, aeração ou agitação (Sgabieri, 1996).

## 2.4. Filmes e revestimentos comestíveis aplicados na conservação dos alimentos

O uso de filmes ou revestimentos comestíveis vem se tornando alvo de grande interesse, devido as suas características de biodegrabilidade e sua capacidade de evitar a deterioração dos alimentos (McHugh, 1996). As vantagens da utilização dos filmes e revestimentos podem ser justificadas pela capacidade de proteger os alimentos mecanicamente, já que atuam principalmente como barreira a gases e vapor de água, diminuindo a degradação e aumentando a vida de prateleira dos alimentos, além de atuarem como carreadores de compostos antimicrobianos, antioxidantes, entre outros (Maia et al., 2000).

Embora o emprego de filmes e revestimentos comestíveis em alimentos não seja um conceito novo, pesquisas nesta área têm se intensificado recentemente. Dentre os fatores que contribuem para a retomada de interesse nos revestimentos comestíveis incluem-se: demanda dos consumidores por alimentos de alta qualidade, necessidade de novas técnicas de armazenamento por parte das indústrias de alimentos, preocupações ambientais sobre a eliminação das embalagens produzidas a partir de matérias-primas não-renováveis e oportunidade para a criação de novos produtos através do uso de resíduos agrícolas (Gennadios, 2007).

Embora os termos sejam frequentemente utilizados como sinônimos, filmes e revestimentos comestíveis possuem diferentes apresentações. Filmes são pré-formados separadamente do alimento e posteriormente aplicada sobre ele. Os revestimentos comestíveis ou coberturas podem ser aplicadas diretamente sobre os alimentos em métodos de imersão ou aspersão, ocorrendo a formação de uma fina película sobre o alimento após a secagem (Gennadios & Weller, 1990).

À base dos filmes e revestimentos pode ser obtida através dos biopolímeros, como os polissacarídeos, as proteínas e os lipídeos. As proteínas são comumente usadas como materiais formadores de filmes e são macromoléculas com sequências de aminoácidos específicas e estruturas moleculares. As estruturas das proteínas podem ser facilmente modificadas para alcançar as propriedades desejáveis do filme (Han, 2014).

As proteínas são boas formadoras de filme, apresentando excelentes propriedades de barreira a oxigênio, dióxido de carbono e lipídios, particularmente em baixas umidades relativas (Lacroix, 2014). As proteínas já investigadas para o desenvolvimento de filmes incluem a caseína (Avena-Bustillos & Krochta, 1993), proteínas de soro de leite (Gago, 2006; Almeida, 2016), proteínas de soja (Brandenburget al., 1993; Stuchell & Krochta, 1994), glúten de trigo (Gennadios et al., 1993; Herald et al., 1995) e proteínas da farinha de arroz (Shih, 1996; Gnanasambandam, 1997).

Filmes formados à base de proteínas são extremamente frágeis e de baixa aderência. Nestas formulações, é indicado o uso de plastificantes para favorecer a adesão ao alimento (Assis & Britto, 2014). Plastificantes são substâncias não-voláteis que, ao serem adicionadas a um material alteram suas propriedades mecânicas e/ou físicas (Alleoni, 2006). Na preparação de filmes ou revestimentos comestíveis, um plastificante é frequentemente incorporado para induzir a flexibilidade (Wan et al., 2005). Plastificantes como glicerol, sorbitol e polietilenoglicol são comumente empregados nas formulações (Garcia et al., 1998) devido a sua capacidade de reduzir a fragilidade dos filmes.

## 2.5. Arroz e sua utilização na formulação de filmes e revestimentos comestíveis

O arroz (*Oryza sativa* L.) é uma das principais culturas alimentares no mundo, com uma produção anual global estimada em cerca de 480 milhões de toneladas métricas (base arroz moída, USDA, 2015). O Brasil se destaca como o principal produtor de arroz entre os países ocidentais, sendo o Rio Grande do Sul o maior estado produtor. A atividade registra tendência de crescimento, apesar da redução na produção em algumas safras devido às

condições climáticas. Para a próxima safra (2018/2019), a estimativa é de uma produção por volta de 10,7 milhões de toneladas (CONAB, 2019).

A farinha de arroz, obtida a partir dos grãos quebrados no processo de beneficiamento do arroz, possui baixo valor comercial no Brasil. Estima-se que são obtidos em média 14 kg de arroz quebrado a cada 100 kg de arroz em casca beneficiados. Este produto apresenta menor valor comercial, representando apenas cerca de 20% do valor em relação aos grãos inteiros (Dias, 2010; Oliveira, 2014).

O método mais conhecido para a obtenção de isolados proteicos de arroz consiste da extração alcalina de seus subprodutos, como a farinha comercial de arroz, seguida pela precipitação das proteínas pelo ajuste do pH no ponto isoelétrico (Gnanasambandam, 1997; Bizzotto, 2006).

A concentração de proteína no arroz pode variar entre 4,3 e 18,2% (Lumen & Chow, 1995). Essa variação deve-se principalmente às características genéticas, de adubação nitrogenada, radiação solar e temperatura durante o desenvolvimento do grão. As proteínas de arroz são categorizadas de acordo com a sua solubilidade, conforme classificação descrita por Osborne (1924), em albumina (solúvel em água), globulina (solúvel em sal), glutelina (alcalino / solúvel em ácido) e prolamina (solúvel em álcool).

Poucos estudos relatam a utilização de arroz como matéria-prima adequada para a preparação de filmes e revestimentos, dentre eles: farelo (Gnanasambandam, 1997), amido (Dias, 2011; Das, 2013), farinha (Dias, 2010; Dias 2011) e proteína do farelo (Adebiyi, 2008). O uso de óleo de farelo de arroz também foi estudado e seu efeito foi positivo na preservação da qualidade interna de ovos crus (Nongtaodum et al., 2013). Entretanto, não foram encontrados registros de estudos avaliando a utilização de revestimentos comestíveis à base de proteína concentrada de arroz para cobertura de ovos.

## 2.6. Uso de revestimentos em ovos

A casca do ovo apresenta um total variável de 7 a 17 mil poros que permitem o movimento de umidade e carbono dióxido para o exterior. Esses poros possuem diâmetros variados, podendo ser encontrados poros de 0,22 até 0,054 mm (Oliveira & Oliveira, 2013). Essa porosidade encurta o período de vida útil do produto em decorrência da perda de água e degradação de proteínas. Assim, os poros na casca do ovo precisam ser selados não só para impedir a evaporação de dióxido de carbono (Caner, 2005), mas também para aumentar sua resistência (Wong, 1996; Caner, 2005).

A penetração de microrganismos através da casca depende de vários fatores, como a qualidade e integridade da casca e da cutícula, além das condições e da duração do armazenamento (EMBRAPA, 2004). A lavagem dos ovos pode acarretar na remoção da cutícula que protege os poros da casca, permitindo assim a entrada de microrganismos e consequentemente contaminação e deterioração do produto. A higienização é um assunto ainda polêmico em se tratando de qualidade de ovos, pois alguns autores questionam o seu efeito e a ação dos desinfetantes sobre a casca do ovo, que se torna mais frágil e susceptível à recontaminação após esta etapa (Almeida, 2013).

A Portaria N° 01 de 21 de fevereiro de 1990 do Ministério da Agricultura Pecuária e Desenvolvimento recomenda a lavagem dos ovos previamente à quebra e adverte que a lavagem e secagem devem ser feitas

por meios mecânicos com procedimentos que impeçam a penetração microbiana para o interior do ovo (MAPA, 1990). Os Estados Unidos da América, o Japão e a Austrália também adotam procedimentos de lavagem de ovos, enquanto muitos países - incluindo o Reino Unido e a União Europeia - têm resistido à prática (Jones, 2018). Liu et al. (2016) observaram através do método de microscopia eletrônica de varredura, que as cascas de ovos submetidos ao processo de lavagem e desinfecção sofreram alterações significativas em sua estrutura, como a remoção da cutícula protetora que envolve a casca do ovo.

Diversas pesquisas têm sido conduzidas avaliando técnicas que permitam prolongar o tempo de prateleira de ovos (Almeida, 2013). O uso de revestimentos em alimentos evita a perda de compostos voláteis e retarda a taxa de deterioração por controlar a transferência de umidade e oxigênio (Maia et al., 2000), o que pode causar mudanças indesejáveis no alimento.

Estudos anteriores relataram melhorias na manutenção da qualidade interior e também a redução de quebra da casca do ovo após a aplicação revestimentos (Xie, 2002; Caner, 2005; Câncer & Cansiz, 2008). Reduzir a quebra de ovos é um importante fator, uma vez que o aumento da resistência da casca irá diminuir potencialmente o número de ovos rachados e resultar em economia significativa para a indústria (Caner, 2015).

Revestimentos como óleo mineral (Jirangrat et al., 2010), proteína isolada de soja (Biladeau & Keener, 2009), proteína isolada ou concentrada do soro de leite (Caner, 2005; Almeida, 2016), quitosana (Caner & Cansiz, 2007) e zeína (Caner & Yuceer, 2015) podem auxiliar na manutenção da qualidade interna de ovos durante o armazenamento por longos períodos. Apesar da diversidade de matérias-primas já disponíveis, o desenvolvimento de revestimentos a partir de subprodutos é uma alternativa economicamente interessante para a indústria.

Outros produtos podem ser incluídos nos revestimentos para agregar características adicionais de proteção. Uma das substâncias com uso potencial nos revestimentos é a própolis, um material resinoso que contém uma mistura complexa de substâncias, produzida pelas abelhas, que resulta da coleta de substâncias secretadas por diferentes plantas. Durante a coleta de própolis, as abelhas misturam a cera e a própolis coletada com sua saliva (Park et al., 1998). As abelhas usam a própolis para proteger a colônia da chuva e para fornecer isolamento térmico, bem como para reforçar a estabilidade estrutural da colméia (Costa et al., 2011). A própolis também possui várias propriedades, como atividades antibacterianas (Silici e Kutluca, 2005), antifúngicas (Seven et al., 2011), antiprotozoárias e antivirais (Schhnitzler et al., 2010). Os efeitos observados são complexos, devido à grande variedade de componentes em sua composição química, pois pode conter mais de 300 substâncias, incluindo flavonóides, ácido fenólico, ésteres, terpenos e açúcares (Aygun, 2016).

O Brasil é um grande produtor e exportador de própolis de Apis mellifera e a própolis brasileira é caracterizada pela presença de ácido hidroxicinâmico (Oldoni et al., 2015). No entanto, a composição e a atividade biológica da própolis brasileira variam significativamente, dependendo do tipo de amostra e da área geográfica de coleta (Machado et al., 2016). O extrato de própolis apresentou resultados satisfatórios quando utilizado em ovos produzidos no sistema convencional (Carvalho, 2013) e em ovos de codornas

(Aygun, 2013; Akpinar, 2015). A técnica de recobrimento de ovos com própolis é uma alternativa de fácil execução que pode ser viável para a maioria dos pequenos produtores, especialmente em sistemas alternativos.

Os óleos essenciais são metabólitos secundários de plantas aromáticas, e possuem uma ampla gama de atividades biológicas (Abd-Elsalam e Khokhlov, 2015). Vários óleos essenciais estão disponíveis e alguns merecem destaque. O *tea tree* é um óleo essencial de *Melaleuca alternifolia* e é uma mistura complexa de hidrocarbonetos terpênicos e álcoois terciários. Seus principais componentes são o terpinen-4-ol e o 1,8-cineole (Jamróz, 2018). A copaíba (*Copaifera langsdorffii*) apresenta diferentes quantidades de substâncias na composição do óleo. Cerca de 80% são sesquiterpenos, uma classe de terpenos, e 20% são diterpenos. Entre os sesquiterpenos, cerca de 50% da composição é β-cariofileno, seguido por α-humuleno, α-copaeno, α-bergamoteno e δ-cadineno (Tobouti, 2017). Já o tomilho (*Thymus vulgaris*) contém altas concentrações de compostos fenólicos, incluindo carvacrol, timol, p-cimeno e ɣ-terpineno (Marino et al., 1999). Devido à presença dessas substâncias, os óleos essenciais podem ser utilizados em diferentes aplicações, como antimicrobianos e antioxidantes.

Os revestimentos comestíveis com óleos essenciais são considerados um método eficaz e inovador na manutenção da qualidade dos alimentos, aumentando sua distribuição nas áreas onde os microrganismos crescem e proliferam, bem como aumentando sua atividade antimicrobiana. Revestimentos com óleos essenciais são uma mistura de óleos essenciais e biopolímeros, que são capazes de transportar óleo (proteína, goma natural, amido modificado, lipídios, etc.). Ele não só pode impedir a troca de oxigênio, água e dióxido de carbono, mas também pode retardar a deterioração dos alimentos, de modo a desempenhar um papel em sua preservação (Ju et al., 2018). Upadhyaya et al. (2016) relataram que os fitoquímicos, especialmente carvacrol e eugenol, quando aplicados em revestimento à base de pectina e goma arábica, foram eficazes na redução de *Salmonella Enteritidis* em ovos de casca.

Apesar das boas perspectivas de uso, não foram encontrados relatos na literatura sobre a utilização de revestimentos à base de arroz com ou sem a incorporação de extrato de própolis ou óleos essenciais em revestimentos para ovos convencionais ou orgânicos com o objetivo de aumentar a vida útil do produto durante o período de estocagem. Os projetos descritos neste documento foram desenvolvidos, portanto, para cobrir esta lacuna de informação.

### 2.7 Desenvolvimento do projeto de pesquisa

A idéia inicial do projeto era o desenvolvimento de um revestimento a base de farinha de arroz, devido ao baixo custo da matéria prima. Entretanto, devido ao grande teor de amido presente na farinha, os revestimentos produzidos eram extremamente densos, o que dificultava a sua aplicação nos ovos e favorecia a contaminação fúngica, sendo essa observada poucos dias após o revestimento dos ovos. A partir disso, optou-se pela utilização da proteína concentrada de arroz, que apesar de ser uma matéria prima de custo mais elevado, formava um revestimento líquido e de fácil aplicação nos ovos. O revestimento à base de proteína de arroz não altera o aspecto nos ovos e não deixa nenhum odor. Neste estudo, foi utilizado o método de imersão para o

revestimento dos ovos, já que o método de aspersão não trouxe resultados satisfatórios para a cobertura dos ovos, devido a densidade dos revestimentos. No primeiro experimento foram testadas três concentrações da proteína do arroz (5, 10 e 15%). Esses revestimentos foram testados tanto em ovos produzidos no sistema convencional, quanto em ovos produzidos em sistema orgânico. Devido as diferenças encontradas nos parâmetros de qualidade interna dos ovos, oriundos dos diferentes sistemas de produção, no dia zero de armazenamento, optou-se por analisar os resultados de forma separada dando origem aos artigos 1 e 2. Posteriormente, optou-se pela utilização de revestimento com 8% de proteína de arroz para a inclusão das outras matérias primas como a própolis ou os diferentes óleos essenciais (artigo 3 e 4). No artigo 5 foram utilizados três tipos de plastificantes para avaliar qual teria melhor aderência quando incorporado ao revestimento. No primeiro teste os ovos foram armazenados por 60 dias. Entretanto, devido ao aspecto dos ovos no final do período de armazenamento, optou-se por um período de armazenamento de 42 dias no teste seguinte.

# 3. HIPÓTESES E OBJETIVOS

O objetivo central da pesquisa foi o desenvolvimento de um revestimento à base de proteína concentrada de arroz e a incorporação de extrato de própolis ou óleos essenciais (copaíba, melaleuca ou tomilho), além de diferentes tipos de plastificantes (glicerol, propileno glicol ou sorbitol) para verificar a sua eficiência na manutenção da qualidade interna de ovos convencionais ou orgânicos armazenados em temperatura ambiente (20 °C) por até 42 ou 60 dias. A hipótese principal é de que estes revestimentos podem prolongar a vida de prateleira deste produto.

Os objetivos específicos foram:

Desenvolver um revestimento para ovos à base de proteína concentrada de arroz em diferentes concentrações (5, 10 ou 15%).

Desenvolver um revestimento para ovos a base de proteína concentrada de arroz com a incorporação de extrato de própolis (5 ou 10%).

Desenvolver um revestimento para ovos à base de proteína concentrada de arroz com a incorporação de 1% de óleos essenciais (copaíba, melaleuca ou tomilho).

Desenvolver um revestimento para ovos à base de proteína concentrada de arroz com diferentes plastificantes (glicerol, propileno glicol ou sorbital).

Avaliar a qualidade interna de ovos convencionais e orgânicos armazenados por até 42 ou 60 dias após a aplicação dos diferentes revestimentos.

Avaliar as alterações morfologicas da casca de ovos convencionais após a aplicação dos diferentes revestimentos.

## CAPÍTULO II

Rice protein coating in extending the shelf-life of conventional eggs

Este capítulo é apresentado de acordo com as normas de publicação da **Poultry Science**.

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# Rice protein coating in extending the shelf-life of conventional eggs

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**ABSTRACT**

The effectiveness of rice protein coatings or mineral oil on maintaining interior quality and eggshell breaking strength of fresh eggs was evaluated during storage at 20 ºC for 8 weeks. Egg quality was assessed by weight loss, Haugh unit (HU), albumen pH, yolk index (YI), shell strength and scanning electron microscopy in uncoated eggs (control treatment) and eggs coated with mineral oil or rice protein concentrate at 5, 10, or 15%. The HU and YI were higher in coated eggs ($P < 0.001$). Weight loss increased ($P < 0.001$) during long-term storage. Uncoated eggs showed the highest weight loss (8.28%), while mineral oil (0.87%) and rice protein at 5% (5.60%), 10% (5.45%), 15% (5.54%) solutions were effective in preventing weight lost ($P < 0.001$). The use of the coatings preserved the internal quality of the eggs for up to 4 weeks longer than uncoated eggs (HU, YI, and pH). Uncoated eggs had the worst ($P < 0.001$) HU (54.45), albumen pH (9.18), and YI (0.28) after 8 weeks of storage. Among the coated eggs, the mineral oil had the best values of HU (70.54), pH (8.48) and YI (0.35) after storage. The eggs coated with 5, 10 and 15% of rice protein presented results with similar intern quality between them and intermediary quality in relation to the others treatments during all the storage period. Scanning electron microscopy demonstrated a lower surface porosity in coated eggshell, indicating that the use of the coating may provide a protective barrier against the transfer of gases and moisture. In conclusion, the use of coatings based on rice protein concentrate or mineral oil influences the internal quality of eggs during storage and may be an effective alternative for increasing the shelf-life of commercial eggs.

**Key words:** eggshells, mineral oil, protein coatings, storage, yolk index

# INTRODUCTION

Eggs are an excellent protein source and are among the most nutritious foods consumed on a daily basis (Yuceer and Caner, 2014) because they are rich in vitamins, minerals, fatty acids, and proteins of excellent biological value (Rêgo et al., 2014). However, eggs are a perishable product and should be kept refrigerated from production to consumption. In 2016, the world egg production reached approximately 96 million dozen or the equivalent 1,5 billion pounds (USDA, 2018), from which approximately 39 billion units were produced in Brazil. The per capita consumption of eggs by the Brazilian population increased from 182 eggs in 2014 to 190 eggs in 2016 (ABPA, 2017). In Brazil, 92% of the eggs marketed *in natura* are exposed to room temperature and cooled only in the consumer's home (Oliveira and Oliveira, 2013). Therefore, the shelf-life of eggs in relation to their physical-chemical and microbiological quality will depend on several factors such as environmental conditions of production, storage, handling, and processing.

Washing eggs is a controversial subject when it comes to egg quality, since some authors question its effect and the action of disinfectants on eggshell, which becomes more fragile and susceptible to contamination after this procedure (Favier et al., 2000; Liu et al., 2016). The washing process can deplete the outer cuticle of eggshell as demonstrated by Jones et al. (2018) and Kulshreshtha et al. (2018). In Brazil, washing the eggs before breaking is a recommended process that must be done by mechanical devices with procedures that prevent the microbial penetration into the egg (Brazil, 1990). The United States of America, Japan, and Australia also adopt egg-washing procedures, while many countries - including the United Kingdom and EU - have resisted the practice (Jones, 2018).

Previous studies have shown that the use of protein coatings after egg washing can help maintain internal egg quality during storage for long periods (Biladeau and Keener, 2009; Caner and Yuceer, 2015). Despite the diversity of feedstock already available, the development of coatings from by-products is an economically interesting alternative for the industry. In this context, the rice by-products deserves highlight due to its availability in many countries, such as Brazil, where rice harvested from February to August (2017), has been estimated at a bumper level of 12.3 million tonnes (FAO, 2017). Studies describe the use of rice protein as a feedstock for the preparation of an edible coating for food products (Dias, 2010; Das, 2013). The use of rice bran oil was also studied and previous research suggests its effect in preserving the internal quality of raw eggs (Nongtaodum et al., 2013). However, no reports of the use of rice protein-based coatings for eggs were found. The aim of the study was, therefore, to evaluate the internal quality, resistance and morphological changes of eggshell after application of rice protein coating of different concentrations in eggs after 8 weeks of storage at ambient conditions (20 °C).

## MATERIAL AND METHODS

Four hundred and thirty-two non-fertile eggs, freshly laid (one-day-old) from ISA Brown hens, were supplied by a commercial farm (Rio Grande do Sul, Brazil) and used in the present study. All eggs were obtained from birds of the same age, maintained under a similar environment, handling and feeding conditions.

The eggs were randomly divided into five treatments. Uncoated eggs were used as a control treatment. Mineral oil (Dynamics, São Paulo, Brazil) was used as a coating in another treatment to characterize the industry standards in Brazil. The other treatments consisted of coatings based on rice protein concentrate (RPC). The coatings were prepared at 5, 10, or 15% (w/w protein) using RPC (MidWay Labs, FL, USA).

### *Preparation of Coating Solutions and Coating of Shell Eggs*

Glycerol (Neon, São Paulo, Brazil) was then added to give a protein : plasticizer ratio of 2:1 w/w. The solutions were kept on a magnetic stirrer for five minutes and after heated in a water bath (90 °C) for 30 minutes (Antunes, 2003). Then, the temperature was reduced to 25 °C and the pH adjusted to 10 with 1N NaOH solution, for the dissolution of the proteins in the film-forming solution.

All eggs were washed with water at 42 °C and chlorine (50 ppm) was used as a sanitizer (Brazil, 1990). The eggs were immersed for 1 min each followed by drying time of 5 min. The clean eggs were individually submerged in the coating solutions at 24ºC for 1 min, so that the coating visibly covered the entire shell surface. The eggs were then dried (Caner and Cansız, 2008) and stored at a controlled ambient temperature (20 °C) for up to 8 weeks in plastic trays specific for eggs. The uncoated washed eggs served as a control treatment.

Twelve eggs were immediately submitted to the quality analysis to represent the characteristics of fresh eggs (zero days of storage). Weekly during the study, 12 eggs from each group were randomly separated for quality evaluation (weight loss, Haugh unit, yolk index and albumen pH) at each storage interval (one to six weeks - with an extra evaluation in the eighth week). Breaking strength measurements (twelve eggs per treatment) and scanning electron microscopy of the shells (three eggs per treatment) were performed at the end of the experiment.

#### *Weight loss*

The eggs were weighed individually using a digital precision (±0.001 g) scale (Bel, Mark M 214A, Milano, Italy). Weight loss (%) during storage was calculated as described by Caner and Cansız (2008), using the following equation:

$$Weight\ loss, \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} x\ 100$$

Twelve eggs for each treatment were taken at weekly intervals for determination of weight loss. The weight loss was calculated weekly in relation to the respective egg weight at the beginning of the trial.

***Haugh Unit (HU)***

The albumen height was measured with a digital caliper (TMX PD - 150, China) at a distance of 10 mm from the yolk. After, the HU was obtained through the equation proposed by Haugh (1937):

$$HU = 100\ log\ [h - \frac{\sqrt{(30W^{0.37} - 100)}}{100} + 1.19$$

where *h* is the thickness of albumen (mm) and *W* is the mass of the entire egg (g).

Based on the HU results, the eggs were graded as: Class AA, when HU was higher than72; Class A, eggs with HU from 71 to 60; Class B, eggs with HU from 59 to 31; or Class C, when HU was lower than 30 (Yuceer and Caner, 2014).

***Yolk Index***

The width and height of the yolk (mm) were measured with a digital caliper (TMX PD - 150, China). After, the yolk index was calculated through the equation (Sharp and Powell, 1930):

$$Yolk\ Index = \frac{(Yolk\ height)}{(Yolk\ width)}$$

### *pH Measurements*

After separation of the yolk and albumen, the dense and the fluid albumen were homogenized for 20 seconds, and then the pH was determined using a digital pHmeter (Kasvi model k39-2014B, Paraná, Brazil) previously calibrated with buffer solutions of pH 7 and 10 (Brazil, 1999).

### *Eggshell Breaking Strength*

Eggshell breaking strength (puncture strength) was determined at the end of the 8 week storage period using a texture analyzer (TA.XT Texture Analyzer, Stable Micro Systems, Surrey, England) with a 5-kg load cell. Each egg was mounted on a texture analyzer platform and the eggshell was punctured at the top (small end) using a 3 mm die probe at 5 $mms^{-1}$ constant speed and a distance of 6 mm. The trigger force used was 3 g, following the method described by Oliveira (2006). The force (N) required to puncture the shell was recorded as the eggshell breaking strength (Yuceer and Caner, 2014).

### *Ultrastructural Assessment*

Three eggs from each treatment were randomly selected and lightly broken. After, their eggshells were segmented with scissors in three parts corresponding to the apical, equatorial and basal regions. Residual albumen was removed. Then, fragments of approximately 0.5 $cm^2$ were removed from each egg region. The samples were mounted on a stub, coated with gold-palladium of 35 nm for 3 minutes (Sputter Coater - SCD 050 Balzers, Germany) and analyzed through a scanning electron microscope (JEOL 6060, Japan) at a standard magnification of 500×.

### *Statistical Analysis*

Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). The normality of the data was verified using the Shapiro-Wilks test through the UNIVARIATE procedure. Afterward, the data were submitted to analysis of variance using PROC GLM, considering each egg an experimental unit. Statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods); except for eggshell breaking strength, which was evaluated only once at the end of the project and was analyzed considering only the treatment effect. Eventual differences ($P < 0.05$) were assessed with a Tukey multiple comparison test.

## RESULTS AND DISCUSSION

The eggs evaluated at day zero presented mean HU values of 81.54, assuring their excellent quality (AA) standard according to the USDA (2000) recommendation. The other quality parameters evaluated in the beginning of the trial were also in accordance with the Brazilian legislation (Brazil, 1997), which determine minimum internal quality conditions for yolk (translucent, firm, consistent, and without germ) and albumen (transparent, consistent, limpid, no stain, and intact chalaza).

### *Weight Loss*

Egg size and weight are measures that will influence other variables such as HU and shell thickness, consequently the resistance of the shell is affected by the size of the eggs (Oliveira and Oliveira, 2013). However, in this study, the egg weight did not differ ($P > 0.05$) between uncoated eggs (63 g) and eggs coated with mineral oil (63.09 g), neither those coated with 5% (64.56 g), 10% (64.71) and 15% (63.06). The accumulated

weight loss of the eggs during the 8 weeks of storage is shown in Table 1. Weight loss increased ($P < 0.001$) with storage time, which was already reported in previous studies (Kim, 2006; Caner and Yuceer, 2015; Jones, 2018). Weight loss during storage provides an important information when monitoring fresh egg quality because weight loss occurs mainly due to the transfer of moisture from the albumen to the external environment through the shell (Scott and Silversides, 2000). Egg weight reduction may also be caused by the probable loss of ammonia, nitrogen, and hydrogen sulfide that are products of the chemical degradation of their organic constituents (Solomon, 1997).

Treatment by time interaction ($P < 0.001$) was found for weight loss, with differences ($P < 0.001$) among treatments observed in all studied periods. Eggs from the control group (uncoated) had the highest weight loss (8.28%). Eggs coated with 5, 10, and 15% RPC showed similar weight loss among each other and intermediate values in relation to the other treatments throughout the experiment (5.60, 5.45 and 5.54%, respectively). Oil-coated eggs showed the lowest weight loss in comparison to the other treatments during the entire trial (0.87%).

Various studies have shown the enhancement effects of using coatings on the moisture loss of the eggs during storage. These effects were associated with the use of protein-based coatings, such as soy protein isolate (Biladeau and Keener, 2009), whey protein isolate or concentrate (Caner, 2005; Caner and Yuceer, 2015), and zein (Caner and Yuceer, 2015).

In this study, the mineral oil demonstrated excellent sealing properties, avoiding the evaporation of moisture and gases. These results are in accordance with Jirangrat et al. (2010) and Jones et al. (2018), that reported reduced weight loss in eggs coated with mineral oil even after 15 weeks of storage. Eggshells coated with mineral oil or RPC showed a lower surface porosity in the ultrastructural assessment (Figure 1), which may

have contributed to a lower weight loss during storage. This demonstrates that the use of coatings may provide a protective barrier against the transfer of gases and moisture through the eggshell (Lee et al., 1996; Kim et al., 2006).

***Haugh Unit***

Haugh unit (HU) results of uncoated and coated eggs are shown in Table 2. The HU decreased ($P < 0.001$) over the storage period. However, treatment by time interaction ($P < 0.001$) was observed, and the decrease in HU occurred more slowly in eggs coated with mineral oil or RPC compared to the uncoated ones. The fluidization and loss of viscosity of the dense albumen is a consequence of the hydrolysis of the amino acid chains, which release the water when degraded (Moreng and Avens, 1990). The liquefaction of the dense albumen is evidenced by the reduction of HU values. These results are in agreement with Biladeau and Keener (2009), Wardy et al. (2011), and Caner and Yuceer (2015) that also demonstrated the benefits of using different coatings on the maintenance of albumen quality.

The HU values indicated that uncoated eggs changed in quality from grade "AA" to "A" after 2 weeks, and to grade "B" after 6 weeks. Meanwhile, eggs coated with 5% RPC changed from "AA" to "A" after 5 weeks of storage and eggs coated with mineral oil or 15% of RPC changed from "AA" to "A" only after 6 weeks of storage. In this assessment, the best results were observed for eggs coated with 10% RPC, which maintained grade "AA" up to 8 weeks of storage at 20 °C. This demonstrated that the use of coatings can preserve the internal egg quality (grade A maintenance) for 3 to 4 weeks longer compared to uncoated eggs. Similar advantages of coatings (grade A maintenance for 4 to 5 weeks) were already reported by Wardy et al. (2011) and Nongtaodum et al. (2013) for eggs stored at 25 °C.

***Yolk Index***

The yolk index of uncoated and coated eggs decreased ($P < 0.001$) throughout the storage (Table 3), as already reported in previous studies (Caner, 2005; Caner and Yuceer, 2015). After 8 weeks of storage, the YI of the uncoated eggs decreased from 0.43 to 0.28, while eggs coated with mineral oil and 5, 10, or 15% of rice protein coated showed YI values of 0.35, 0.31, 0.32, and 0.32, respectively, at the end of the project. A fresh egg of good quality has a YI of around 0.45, while an older egg will have a lower YI. The higher the YI, the better is the quality of the yolk (Yuceer and Caner, 2014). In this study, means lower than 0.3 were reported for uncoated eggs at the fifth week of storage. Coatings seems to be efficient to reduce the mass transfer rate (water and $CO_2$ loss) from the albumen through the eggshell during long-term storage. This process inhibits albumen liquefaction and water absorption by the yolk and minimizes a reduction in yolk quality (Caner and Yuceer, 2015), which could explain the advantages for coated eggs in the present research.

Despite the interaction observed between storage time and different treatments ($P < 0.001$), the YI did not differ among treatments after 8 weeks of storage. Current results are in agreement with previous studies (Caner, 2005; Yuceer and Caner, 2014; Caner and Yuccer, 2015), which demonstrated that the use of coating was able to preserve the YI for a longer time than uncoated eggs, but only for a period shorter than 8 weeks.

***pH measurement in albumen***

Albumen pH can also be used as a quality index in addition to the previously presented ones. As it is not affected by the age or strain of hen, it can be used to measure the freshness of an egg without this bias (Scott and Silversides, 2000). Moisture and carbon dioxide in the albumen evaporate through the pores, allowing more

air to penetrate the shell (Caner and Yuccer, 2015). During storage, $CO_2$ escapes through the eggshell pores. The increase in albumen pH over time is may be due to the the loss of CO2 and ⁄ or a change in the bicarbonate buffer system (Biladeau and Keener, 2009),

The albumen pH varies between 7.5 and 8.5 immediately after oviposition and may rise to 9 during storage time (Scott and Silversides, 2000; Yuceer and Caner, 2014). In this study, the albumen pH varied ($P < 0.001$) over the storage period (Table 4) with treatment by time interaction ($P < 0.001$). After 8 weeks of storage, the pH of the uncoated eggs decreased from 8.53 to 9.18, while eggs coated with mineral oil and 5, 10, or 15% of rice protein coated showed pH values of 8.48, 9.11, 9.10, and 9.14, respectively. At the end of the second week of storage, the albumen of eggs coated with mineral oil or 15% of RPC presented lower ($P < 0.001$) pH values than uncoated eggs. In the third week, different results ($P < 0.001$) from the control treatment were observed in eggs coated with mineral oil, 10, or 15% of RPC. In the fourth week, only eggs coated with mineral oil and 10% of RPC showed lower ($P < 0.001$) albumen pH. However, after the fifth week of storage, only mineral oil was able to maintain albumen pH lower than the values observed in uncoated eggs.

The increase in albumen pH causes a decrease in egg quality. This implies that the use of mineral oil as a coating can delay the loss of $CO_2$ through the pores of the eggshell, acting as a barrier. Torrico et al. (2010) already observed that albumen pH in egg coated with mineral oil was lower than uncoated eggs up to five weeks. Results are also in agreement with previous studies that used protein coatings (Caner, 2005; Biladeau and Keener, 2009; Caner and Yuceer, 2015).

### *Eggshell Breaking Strength*

The shell is responsible to protect the egg from mechanical impact and allows a controlled exchange of fluid and gas through the pores, besides providing a protection against microbial contamination. Improving shell quality is important in the poultry industry because it may be related to a reduction on egg breaking. The use of coatings may be an edible tool to improve the shell quality (Caner and Yuceer, 2015), mainly because they can increase the eggshell thickness and, consequently, the eggshell breaking strength. However, in this study, eggshell breaking strength did not differ ($P > 0.05$) between uncoated eggs (4.51 kg force) and eggs coated with mineral oil (3.66 kg force), neither compared to those coated with 5% (4.04 kg force), 10% (3.67 kg force), and 15% (3.38 kg force) RPC after 8 weeks of storage. Although previous studies have described improvements to shell quality and reduction of eggshell breakage after coating application (Xie, 2002; Caner and Cansız 2008; Caner and Yuceer, 2015), this characteristic seems to be associated with specific properties of the coatings used and was not observed in this trial.

## CONCLUSIONS

Rice protein coating can be used for extending the shelf life of eggs in the storage period. Coating with different percentages of RPC in solution, especially 10 and 15% RPC, is a effective way to preserve the interior quality of eggs in the room temperature. These facts may help egg industry in decreasing economic losses during storage. The loss of albumen and yolk quality is influenced by the capacity of the coating to block the pores on the surface of the shell. In general, the effects of coatings on albumen and yolks are favorable, indicating that the use of RPC-based coating may be a viable alternative to maintain functional properties (HU, YI, pH) of the eggs, which

are adversely affected by storage period. Future studies are needed to verify the use of RPC-based coatings associated with the presence of active antimicrobial agents in order to minimize contamination by microorganisms.

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**Table 2.1.** Effect of rice protein concentrate (RPC) coatings on cumulative weight loss (% in relation to week 0) of eggs during 8 weeks of storage at 20 ºC[1].

| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
|---|---|---|---|---|---|
| 1 | 1.37±0.20$^{Fa}$ | 0.08±0.08$^{Cc}$ | 0.83±0.03$^{Fb}$ | 0.77±0.06$^{Fb}$ | 0.76±0.03$^{Eb}$ |
| 2 | 2.02±0.17$^{Fa}$ | 0.29±0.25$^{BCc}$ | 1.21±0.06$^{Fb}$ | 1.28±0.18$^{Eb}$ | 1.15±0.12$^{Eb}$ |
| 3 | 3.06±0.23$^{Ea}$ | 0.30±0.16$^{BCc}$ | 2.22±0.09$^{Eb}$ | 2.31±0.16$^{Db}$ | 2.23±0.16$^{Eb}$ |
| 4 | 4.05±0.36$^{Da}$ | 0.36±0.18$^{BCc}$ | 2.87±0.17$^{Db}$ | 2.54±0.26$^{Db}$ | 2.71±0.20$^{Db}$ |
| 5 | 4.85±0.29$^{Ca}$ | 0.42±0.27$^{BCc}$ | 3.49±0.29$^{Cb}$ | 3.50±0.26$^{Cb}$ | 4.35±0.19$^{Cb}$ |
| 6 | 6.04±0.47$^{Ba}$ | 0.64±0.28$^{ABc}$ | 4.48±0.31$^{Bb}$ | 4.56±0.25$^{Bb}$ | 4.44±0.26$^{Bb}$ |
| 8 | 8.28±0.78$^{Aa}$ | 0.87±0.33$^{Ac}$ | 5.60±0.30$^{Ab}$ | 5.45±0.21$^{Ab}$ | 5.54±0.23$^{Ab}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-F] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 2.2.** Effect of rice protein concentrate (RPC) coatings on Haugh unit (HU) and egg grade[1] (designated after each mean, in the parenthesis) during 8 weeks of storage at 20 ºC[2].

| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
|---|---|---|---|---|---|
| 0 | 81.54(AA)±3.84$^{Aa}$ | 81.54(AA)±3.84$^{Aa}$ | 81.54(AA)±3.84$^{Aa}$ | 81.54(AA)±3.84$^{Aa}$ | 81.54(AA)±3.84$^{Aa}$ |
| 1 | 75.56(AA)±2.60$^{Ba}$ | 78.31(AA)±2.43$^{ABa}$ | 77.62(AA)±4.24$^{ABa}$ | 80.90(AA)±3.09$^{Aba}$ | 80.45(AA)±1.76$^{ABa}$ |
| 2 | 69.71(A)±2.57$^{Cb}$ | 76.75(AA)±2.09$^{ABCa}$ | 76.97(AA)±1.69$^{ABa}$ | 78.07(AA)±3.56$^{ABCa}$ | 78.32(AA)±2.63$^{ABa}$ |
| 3 | 68.41(A)±2.26$^{Cb}$ | 75.75(AA)±2.20$^{ABCa}$ | 75.92(AA)±3.57$^{BCa}$ | 76.75(AA)±3.91$^{ABCa}$ | 76.63(AA)±2.63$^{BCa}$ |
| 4 | 61.72(A)±3.19$^{Db}$ | 74.74(AA)±2.98$^{ABCa}$ | 73.11(AA)±2.00$^{BCDa}$ | 76.81(AA)±3.19$^{ABCa}$ | 75.98(AA)±2.40$^{BCa}$ |
| 5 | 60.92(A)±3.70$^{Db}$ | 74.25(AA)±2.64$^{BCa}$ | 71.81(A)±2.07$^{CDa}$ | 75.89(AA)±2.47$^{BCa}$ | 73.01(AA)±3.18$^{CDa}$ |
| 6 | 56.15(B)±1.58$^{Dc}$ | 71.17(A)±3.82$^{Cb}$ | 68.70(A)±3.63$^{Db}$ | 73.35(AA)±3.74$^{CDa}$ | 71.74(A)±3.28$^{CDab}$ |
| 8 | 54.45(B)±1.92$^{Db}$ | 70.54(A)±2.81$^{Ca}$ | 67.38(A)±1.57$^{Da}$ | 67.86(A)±1.70$^{Da}$ | 67.54(A)±3.36$^{Da}$ |

[1] Egg grades: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

[2] Data are expressed as means (egg grades) ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-D] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 2.3**. Effect of rice protein concentrate (RPC) coatings on yolk index during 8 weeks of storage at 20 ºC[1].

| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
|---|---|---|---|---|---|
| 0 | $0.43\pm0.01^{Aa}$ | $0.43\pm0.01^{Aa}$ | $0.43\pm0.01^{Aa}$ | $0.43\pm0.01^{Aa}$ | $0.43\pm0.01^{Aa}$ |
| 1 | $0.41\pm0.01^{ABa}$ | $0.43\pm0.01^{Aba}$ | $0.38\pm0.02^{Aa}$ | $0.38\pm0.02^{Aa}$ | $0.40\pm0.02^{Aa}$ |
| 2 | $0.39\pm0.02^{Ba}$ | $0.42\pm0.02^{ABCa}$ | $0.37\pm0.02^{Aa}$ | $0.38\pm0.02^{Aa}$ | $0.37\pm0.02^{Aa}$ |
| 3 | $0.35\pm0.02^{Ca}$ | $0.38\pm0.02^{ABCa}$ | $0.36\pm0.07^{Aa}$ | $0.38\pm0.03^{Aba}$ | $0.38\pm0.02^{Aa}$ |
| 4 | $0.33\pm0.02^{Cb}$ | $0.37\pm0.01^{ABCa}$ | $0.37\pm0.01^{Aa}$ | $0.37\pm0.03^{Aa}$ | $0.37\pm0.01^{Aa}$ |
| 5 | $0.29\pm0.02^{Dc}$ | $0.36\pm0.03^{BCa}$ | $0.33\pm0.01^{Bb}$ | $0.35\pm0.01^{ABb}$ | $0.33\pm0.01^{BCb}$ |
| 6 | $0.28\pm0.02^{Db}$ | $0.35\pm0.02^{Ca}$ | $0.31\pm0.02^{Bb}$ | $0.35\pm0.02^{Ba}$ | $0.32\pm0.01^{BCb}$ |
| 8 | $0.28\pm0.02^{Db}$ | $0.35\pm0.01^{Ca}$ | $0.31\pm0.01^{Bb}$ | $0.32\pm0.02^{Bb}$ | $0.32\pm0.02^{BCb}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 2.4**. Effect of rice protein concentrate (RPC) coatings on albumen pH during 8 weeks of storage at 20 ºC[1].

| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
|---|---|---|---|---|---|
| 0 | $8.53\pm0.10^{Ca}$ | $8.53\pm0.10^{BCa}$ | $8.53\pm0.10^{Ba}$ | $8.53\pm0.10^{Ca}$ | $8.53\pm0.10^{Ba}$ |
| 1 | $8.90\pm0.12^{Ba}$ | $8.53\pm0.13^{BCa}$ | $8.73\pm0.22^{Ba}$ | $8.82\pm0.15^{BCa}$ | $8.56\pm0.18^{Ba}$ |
| 2 | $9.22\pm0.07^{Aa}$ | $8.84\pm0.13^{Ab}$ | $9.10\pm0.05^{Aab}$ | $9.01\pm0.10^{ABab}$ | $9.04\pm0.10^{Ab}$ |
| 3 | $9.24\pm0.02^{Aa}$ | $8.72\pm0.10^{ABc}$ | $9.11\pm0.10^{Aab}$ | $9.03\pm0.23^{ABb}$ | $8.96\pm0.10^{ABbc}$ |
| 4 | $9.16\pm0.03^{Aa}$ | $8.62\pm0.13^{BCc}$ | $9.06\pm0.05^{Aab}$ | $8.91\pm0.08^{ABCbc}$ | $9.07\pm0.03^{Aab}$ |
| 5 | $9.22\pm0.03^{Aa}$ | $8.63\pm0.07^{BCb}$ | $9.11\pm0.05^{Aa}$ | $9.07\pm0.05^{Aba}$ | $9.08\pm0.12^{Aa}$ |
| 6 | $9.17\pm0.04^{Aa}$ | $8.63\pm0.12^{ABCb}$ | $9.13\pm0.04^{Aa}$ | $9.13\pm0.07^{Aa}$ | $9.11\pm0.14^{Aa}$ |
| 8 | $9.18\pm0.10^{Aa}$ | $8.41\pm0.10^{Cb}$ | $9.11\pm0.03^{Aa}$ | $9.10\pm0.03^{Aa}$ | $9.14\pm0.02^{Aa}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($P < 0.001$).

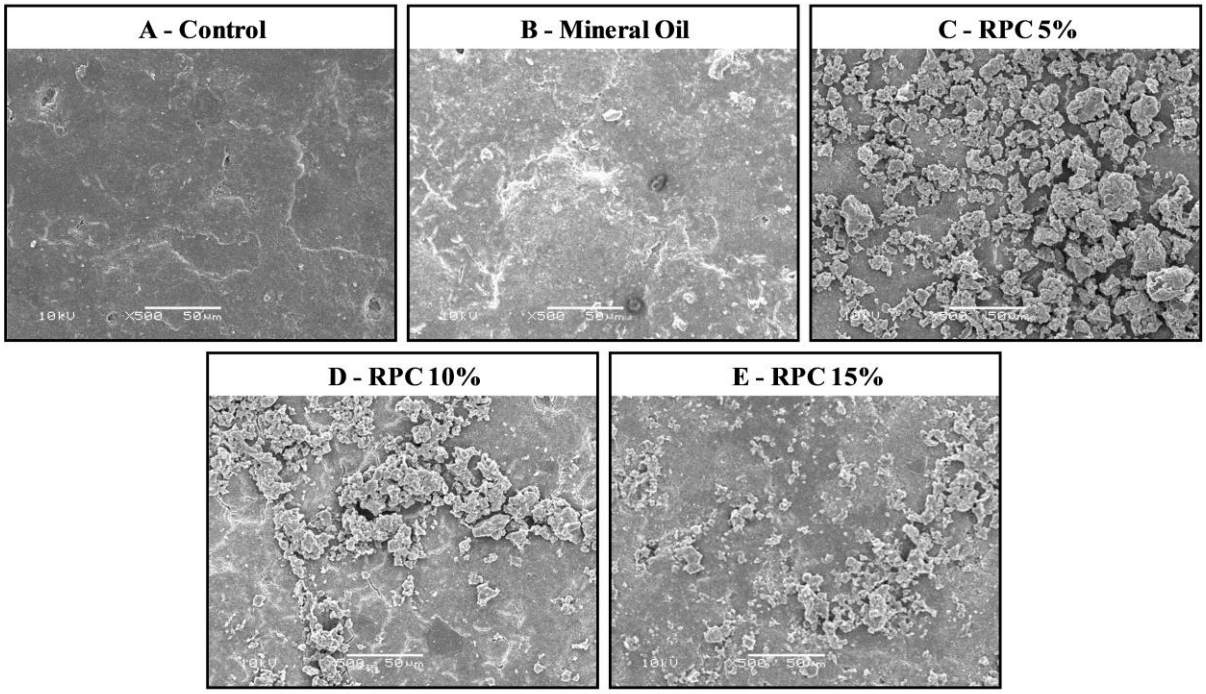


**Figure 2.2** Scanning electron microscopy (×500) of uncoated eggshell (A) and coated eggs (B to E) after 8 wk of storage. MO: mineral oil; RPC: rice protein concentrate coating.

## CAPÍTULO III

Efficacy of rice protein coatings on improving the shelf life of organic eggs

Este capítulo é apresentado de acordo com as normas de publicação da **The Journal of Applied Poultry Research**

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**Efficacy of rice protein coatings on improving the shelf life of organic eggs**

**Primary Audience:** Poultry Researchs

## SUMMARY

The effect of using rice protein coatings at 5, 10 or 15% or mineral oil on the quality of organic eggs was evaluated during eight weeks at 20 ºC. Egg quality was assessed by weight loss, Haugh unit (HU), albumen pH, yolk index (YI) and shell strength in uncoated eggs and eggs coated with mineral oil or rice protein concentrate at 5, 10, or 15%. Coating with mineral oil (1.27% of weight loss) or rice protein at 5% (5.54%), 10% (5.77%), and 15% (5.70%) were effective ($P < 0.001$) in preventing weight lost compared to uncoated eggs (9.14%) even after eight weeks. Uncoated eggs had the worst ($P < 0.001$) HU (53.22), albumen pH (9.18), and YI (0.28) after 8 weeks of storage. Among the coated eggs, the mineral oil had the best values of pH (8.48) and YI (0.35) after storage. The eggs coated with rice protein presented results with similar quality between them and intermediary quality in relation to the eggs coated with rice protein and propolis during the storage period. In conclusion, coatings based on rice protein or mineral oil influence the internal quality of organic eggs during storage and can preserve the eggs for three weeks longer than uncoated eggs.

**Key-words:** egg quality, eggshells, mineral oil, storage, yolk index

## DESCRIPTION OF PROBLEM

Eggs are considered to be nature's perfect food. The egg albumen is an excellent source of high quality protein, which is rich in essential amino acids; while the yolk is a source of antioxidants, aromatic amino acids, carotenoids, vitamins, phospholipids, and proteins, which not only provide nutritional value but also act as pro-health substances [1].

The demand for food from more extensive production systems, such as free-range egg production, increased because of consumers concerns about the use of the cage environment [2]. Several studies have demonstrated the effects of alternative production systems on egg quality and chemical composition [3,4]. Best shell quality was observed in eggs from the conventional system [5]. In addition, lower shell thickness was observed in eggs produced in the outdoors when compared to eggs produced in cages [6]. The eggshell is essential to maintain the integrity of the internal egg components.

Eggs are perishable products and lose quality if they are not handled and stored properly. From the oviposition, the egg is subject to physical and chemical changes in the albumen and yolk that could result in changes in the flavor, freshness and palatability. The longer is the storage time, the greater is the deterioration of internal quality and the higher is the carbon dioxide movement through the shell, especially at room temperature [7]. In this context, greater loss of quality could be expected in products with narrower market, such as organic eggs, which tend to remain for a longer period on supermarket shelves [3].

Currently, there is growing interest in developing effective methods to maintain internal egg quality and reduce the percentage of shell breaking [8,9]. The use of edible

coatings is a simple technology and already demonstrates favorable results. Previous studies have shown that the use of protein coatings after egg washing can help maintain internal egg quality during storage for long periods [10,11]. Despite the diversity of feedstock already available, the development of coatings from by-products is an economically interesting alternative for the industry. In this context, the rice by-products probably deserves highlight due to its availability in many regions, such as Asia and Brazil. Studies describe the use of rice as a feedstock for the preparation of edible coating [12,13]. However, very few information is available on the use of rice protein coating for eggs, specially on the organic segment.

Thus, it is worthy of interest to formulate a novel coating based on vegetable product, such as rice protein, for organics eggs. So, after reviewing recent researches on the effects of storage time and the use of protein coatings for eggs, the effects of the use of the rice protein coating was evaluated on egg quality parameters during storage at room temperature (20 °C). The information provided by this study are likely to be of great interest to the researchers in organics products areas and may also be helpful for the egg production industry, especially in places where egg refrigeration is not required.

## MATERIAL AND METHODS

Four hundred and thirty-two non-fertile eggs, freshly laid (one-day-old) from ISA Brown hens were used in the present study. These eggs were supplied by a commercial farm (Rio Grande do Sul, Brazil), which has organic production certification, in accordance with current standards and governmental instructions [14,15]. All eggs were obtained from birds of the same age, maintained under similar environment, handling, and feeding conditions.

The eggs were randomly divided into five treatments. Uncoated eggs were used as a control treatment. Mineral oil (Dynamics, São Paulo, Brazil) was used as a coating in another treatment to characterize the industry standards in Brazil. The other treatments consisted of coatings based on rice protein concentrate (RPC) in different dilutions (5, 10 or 15%).

### *Preparation of coating solutions and coating of shell eggs*

Rice protein films were prepared at 5, 10 or 15% concentrations (w/w protein) using RPC (MidWay Labs, FL, USA). Glycerol (Neon, São Paulo, Brazil) was then added to give a protein:plasticizer ratio of 2:1 w/w. The solutions were kept on a magnetic stirrer for five minutes and then heated in a water bath (90 °C) for 30 min. (Antunes, 2003). Then, the temperature was reduced to 25 °C and the pH adjusted to 10 with 1N NaOH solution, for the dissolution of the proteins in the film-forming solution.

All eggs were washed with water at 42 °C and chlorine (50 ppm) was used as a sanitizer [16]. The eggs were immersed for 1 min each followed by a drying time of 5 min. The clean eggs were individually submerged in the coating solutions at 24 ºC for 1 min, so that the coating visibly covered the entire shell surface. The eggs were then dried [17] and stored at a controlled ambient temperature (20 °C) for up to 8 weeks in plastic trays specific for eggs. Twelve eggs were immediately submitted to the quality analysis to represent the characteristics of fresh eggs (zero days of storage). Weekly during the study, eggs from each group were randomly separated for quality evaluation (weight loss, Haugh unit, yolk index and albumen pH) at each storage interval (1 to 6 weeks - with an extra evaluation in the eighth week). Breaking strength measurements (12 eggs per treatment) were performed at the end of the experiment.

***Egg quality analyses***

***Weight loss***

The eggs were weighed individually using a digital precision (± 0.001 g) scale (Bel, Mark M 214A, Milano, Italy). Weight loss (%) during storage was calculated as described by Caner [17], using the following equation:

$$Weight\ loss, \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} x\ 100$$

The weight loss was calculated weekly in relation to the respective egg weight at the beginning of the trial.

***Haugh unit (HU)***

The albumen height was measured with a digital caliper (TMX PD - 150, China) at a distance of 10 mm from the yolk. After, the HU was obtained through the equation proposed by Haugh [18]:

$$HU = 100\ log\ [h - \frac{\sqrt{(30W^{0.37} - 100)}}{100} + 1.19$$

where *h* is the thickness of albumen (mm) and *W* is the mass of the entire egg (g).

Based on the HU results, the eggs were graded as: class AA, when HU was higher than 72; class A, eggs with HU from 71 to 60; class B, eggs with HU from 59 to 31; or class C, when HU was lower than 30 [19].

***Yolk index***

The width and height of the yolk (mm) were measured with a digital caliper (TMX PD - 150, China). After, the yolk index was calculated through the equation [20]:

$$Yolk\ Index = \frac{(Yolk\ height)}{(Yolk\ width)}$$

***pH measurements***

After separation of the yolk and albumin, the dense albumen and the fluid were homogenized for 20 seconds, and then the pH was determined using a digital pHmeter (Kasvi model k39-2014B, Paraná, Brazil) previously calibrated with buffer solutions of pH 7 and 10 [21].

***Eggshell breaking strength***

Eggshell breaking strength (puncture strength) was determined at the end of the 8 weeks storage period using a texture analyzer (TAXT Texture Analyzer, Stable Micro Systems, Surrey, England) with a 5kg load cell. Each egg was mounted on a texture analyzer platform and the eggshell was punctured at the top (small end) using a 3 mm die probe at 5 mm $s^{-1}$ constant speed and a distance of 6 mm. The trigger force used was 3 g, following the method described by Oliveira [22]. The force (N) required to puncture the shell was recorded as the eggshell breaking strength [19].

***Statistical analysis***

Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). The normality of the data was verified using the Shapiro-Wilks test through the UNIVARIATE procedure. Afterward, the data were

submitted to analysis of variance using PROC GLM, considering each egg as an experimental unit. Statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods); except for eggshell breaking strength, which was evaluated only once at the end of the project and was analyzed considering only the treatment effect. Eventual differences ($P < 0.05$) were assessed with a Tukey multiple comparison test.

## RESULTS AND DISCUSSION

The eggs evaluated at day zero presented mean UH values of 74.04 assuring their excellent quality (AA) standard according USDA [23]. The other quality parameters evaluated in the beginning of the trial were also in accordance with the Brazilian legislation [21], which determine minimum internal quality conditions for yolk (translucent, firm and consistent) and albumen (transparent, consistent, limpid, no stain and with intact chalaza). The loss of internal quality of organic eggs presented a similar pattern to that described by Pires et al. [24] in conventional eggs stored at room temperature (20 °C) for up to 8 weeks, with interms of weight loss (8.28%), HU (54.45), YI (0.28) and pH (9.18).

### *Weight loss*

The initial egg weight did not differ ($P > 0.05$) between uncoated eggs (66.8 g) and eggs coated with mineral oil (66.9 g), or with 5% (67.3 g), 10% (66.5 g) and 15% (65.5 g) of RPC. This similarity among treatments is important because the egg weight are measures that will influence other variables, such as HU and shell thickness. Weight

loss of eggs is one of the most important measurements when monitoring the change in quality of fresh eggs during storage [25].

The decrease in egg weight occurs due to the transfer of moisture from the albumen to the external environment through the shell [26]. The cumulative weight loss of the eggs during the 8 weeks of storage is shown in Table 1. Weight loss increased ($P < 0.001$) with storage time, which was already reported in several studies using eggs from conventional production systems [11,27]. Despite the treatment, organic eggs lost on average 5% of their initial weight after 8 weeks in this current study, which is similar to the values previously reported for the same storage time in conventional eggs [24].

There was interaction between the storage time and the different treatments ($P < 0.001$) in the assessment of weight loss. Weight variation was also influenced ($P < 0.001$) by treatments. The uncoated eggs showed the highest weight loss compared to the other treatments from the first week to the end of the study, except for the fifty weeks. Eggs coated with 5, 10, and 15% of RPC showed similar weight loss among each other and intermediate to the other treatments throughout the experiment. An exception was observed in the fifty weeks when RPC coated eggs produced similar results than uncoated eggs. Oil-coated eggs showed the lowest weight loss among the treatments from the first week of storage.

In this study, the mineral oil demonstrated excellent renders and sealing properties, avoiding the evaporation of moisture and gases, which resulted in the lowest weight loss during the eight weeks of storage. It confirms earlier results of Jones [27] that reported lower weight loss after 15 weeks of storage in eggs coated with mineral oil in combination with refrigeration compared to uncoated eggs. The positive effects of

mineral oil use were also described in several previous studies Biladeau and Kenner [10] and Torrico et al. [28].

The RPC coating exhibited sufficient hydrophobicity and sealing properties required to effectively retard water loss during the storage at room temperature. Previous study has shown the enhancement effects of the use of similar coatings on the moisture loss of the eggs during storage [24]. Positive effects associated with the use other protein-based coatings were also reported for up to 6 weeks of storage [10, 11, 29].

***Haugh unit***

Changes in the HU of uncoated and coated eggs are shown in Table 2. Overall, HU decreased ($P < 0.001$) over the storage period. However, there was interaction between storage time and treatments ($P < 0.001$), as the decrease in HU occurred more slowly in eggs coated with mineral oil or RPC than in uncoated eggs. Haugh unit values rapidly decreased during storage, in agreement with previous investigations Caner and Yuceer [11] and Jones [27]. In the uncoated eggs, only one week of storage was enough to produce changes in the HU and also in the egg grade.

The fluidization and loss of viscosity of the dense albumen occurs as a consequence of the hydrolysis of the amino acid chains that, when degraded, release the water bound to large protein molecules [30]. The liquefaction of the dense albumen is evidenced by the reduction of HU values. These results were in agreement with Caner and Yuceer [11], who demonstrated that the use of different coatings may assist in the maintenance of albumen quality.

Uncoated eggs changed in quality grade from “AA” to “A” after 1 week, and from "A" and "B" after 5 weeks. On the other hand, eggs coated with 5% RPC changed from "AA" to "A" after 4 weeks of storage, while eggs coated with mineral oil changed from "AA" to "A" after 2 weeks of storage. Eggs coated with 10 or 15% of RPC changed the grade "AA" after 5 weeks of storage at 20 °C. This study demonstrated that the use of RPC coatings can preserve optimal internal egg quality (grade A maintenance) for 4 weeks longer than uncoated eggs. Yuceer and Caner [19] reported that the albumen quality was extended by at least three weeks by the use of the coating. In addition Pires et al. [24] reported that use of rice protein coating was effective in extending shelf life of conventional eggs.

***Yolk index (YI)***

The yolk index is a criterion used to determine the firmness of this structure, calculated based on its width and height Sharp and Powell [20]. The higher the YI, the better is the quality of the yolk [19]. As expected, the yolk index of uncoated and coated eggs decreased ($P < 0.001$) throughout the storage (Table 3), which is in agreement with previous studies developed with conventional eggs [11]. Interaction was observed between storage time and treatments ($P < 0.001$).

The effect of the coating was observed from the second week of storage, when all RPC coatings had a higher YI ($P < 0.001$) compared to control treatment. Eggs coated with RPC concentration presented better YI results up to the fourth week of storage when compared to uncoated treatments. The mineral oil coating presented superior YI ($P < 0.001$) to the other treatments even after the 8 weeks of storage. This study demonstrated that the use of coating was able to preserve the yolk quality for a

longer time in than uncoated eggs. These results are in agreement with previous studies by Caner and Yuccer, [11] and Pires et al. [24].

***pH measurement in albumen***

The albumen pH increases with to the increase in the storage period of the egg and can reach 9.5 [31]. This increase over time occurs due to the dissociation of carbonic acid ($H_2CO_3$), a reaction which forms water and carbon dioxide [32]. The pH of the albumen is a suitable measure to evaluate the freshness of the eggs, since there is little influence of the strain and age of the bird on is variable [33].

In this study, the albumen pH varied ($P < 0.001$) over the storage period (Table 4). The average initial albumen pH of the eggs was 8.75 and this value increased to 9.18 at the end of the trial in the eggs uncoated eggs. At the third week of storage, the albumen of eggs coated with mineral oil or RPC at 10 and 15% concentration presented lower ($P<0.001$) pH values than uncoated eggs. In the fourth week, only eggs coated with mineral oil and 10% of RPC showed lower ($P < 0.001$) pH values of the albumen compared to the control treatment. However, only mineral oil was able to maintain pH values lower than uncoated eggs after the fifth week of storage.

The use of mineral oil as a coating can delay the loss of carbon dioxide through the pores of the eggshell, acting as a barrier. Torrico et al. [28] reported that all albumen pH values of egg coated with mineral oil were lower than uncoated eggs for 5 weeks. Results are also in agreement with previous studies that used protein coatings [11, 34].

***Eggshell breaking strength***

The shell is responsible to protect the egg from mechanical impact and allows a controlled exchange of fluid and gas through the pores, besides providing a protection against microbial contamination. Improving shell quality is important in the poultry industry because it may be related to a reduction on egg breaking. The use of coatings may be an edible tool to improve the shell quality [11], mainly because they can increase the eggshell thickness and, consequently, the eggshell breaking strength. However, data obtained in the current trial showed that eggshell breaking strength did not differ ($P > 0.05$) between uncoated eggs (4.94 kgf) and eggs coated with mineral oil (3.78 kgf) or 5% (4.44 kgf), 10% (3.15 kgf) or 15% (3.98 kgf) of RPC after eight weeks of storage. Previous studies have described improvements to shell quality and reduction of egg shell breakage after application coatings [11, 17]. However, this characteristic seems to be associated with specific properties of the coatings used.

## CONCLUSIONS AND APPLICATIONS

1. The use of a coatings based on a vegetable protein, originated from a renewable source and produced on a large scale (as rice) is presented as an alternative to reduce the environmental impact by not using feedstock of fossil origin (such as petroleum products). This fact is even more interesting from the commercial standpoint, since consumers of organic eggs are likely to be more concerned about food safety, environment sustainability and on residue-free products. The use of coatings with different concentrations of RPC, especially 10 and 15%, are effective in preserving the internal quality of organics eggs stored at room temperature (20 °C).

2. The loss of albumen and yolk quality can be influenced by the capacity of the coating to block the pores on the surface of the shell. In general, the effects of coatings on albumen and yolks are favorable, indicating that the use of RPC-based coatings may be a viable alternative to maintain functional properties (HU, YI and pH) of the eggs, which are adversely affected by storage period.
3. Future studies are needed to verify the use of RPC-based coatings associated with the presence of active antimicrobial agents in order to minimize contamination by microorganisms.
4. The use of the coatings was not able to increase the resistance of the shell to breaking. Improving shell quality is important in the poultry industry because

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**Table 3.1.** Effect of rice protein concentrate (RPC) coatings on cumulative weight loss (% in relation to initial weight) of organic eggs during eight weeks of storage at 20º C[1].

| | Treatments | | | | |
|---|---|---|---|---|---|
| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
| 1 | $1.40\pm0.34^{Fa}$ | $0.09\pm0.09^{Cc}$ | $1.05\pm0.01^{Fb}$ | $1.05\pm0.03^{Gb}$ | $1.06\pm0.02^{Fb}$ |
| 2 | $2.30\pm0.50^{EFa}$ | $0.10\pm0.12^{Cc}$ | $1.53\pm0.34^{Eb}$ | $1.90\pm0.25^{Fab}$ | $1.54\pm0.23^{Eb}$ |
| 3 | $3.35\pm0.52^{DEa}$ | $0.38\pm0.29^{BCc}$ | $2.47\pm0.18^{Db}$ | $2.56\pm0.18^{Eb}$ | $2.48\pm0.22^{Db}$ |
| 4 | $4.24\pm0.63^{CDa}$ | $0.45\pm0.29^{BCc}$ | $3.39\pm0.28^{Cb}$ | $3.43\pm0.24^{Db}$ | $3.49\pm0.24^{Cb}$ |
| 5 | $5.45\pm0.89^{BCa}$ | $0.59\pm0.47^{BCc}$ | $4.44\pm0.25^{Ba}$ | $4.52\pm0.30^{Ca}$ | $3.50\pm0.27^{Ca}$ |
| 6 | $6.60\pm0.99^{Ba}$ | $0.94\pm0.46^{Abc}$ | $5.41\pm0.14^{Ab}$ | $5.56\pm0.19^{Bb}$ | $4.60\pm0.24^{Bb}$ |
| 8 | $9.14\pm1.77^{Aa}$ | $1.27\pm0.64^{Ac}$ | $5.54\pm0.52^{Ab}$ | $5.77\pm0.26^{Ab}$ | $5.70\pm0.40^{Ab}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[YA-F] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 3.2.** Effect of rice protein concentrate (RPC) coatings on Haugh unit (HU) and egg grade[1] (designated after each mean, in the parenthesis) during eight weeks of storage at 20 ºC[2].

| | Treatments | | | | |
|---|---|---|---|---|---|
| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
| 0 | 74.04(AA)±2.40$^{Aa}$ | 74.04(AA)±2.40$^{Aa}$ | 74.04(AA)±2.40$^{Aa}$ | 74.04(AA)±2.40$^{Aa}$ | 74.04(AA)±2.40$^{Aa}$ |
| 1 | 67.93(A)±2.77$^{Bb}$ | 74.18(AA)±1.29$^{Aa}$ | 73.08(AA)±1.65$^{Aa}$ | 73.67(AA)±1.48$^{Aa}$ | 73.61(AA)±1.80$^{Aa}$ |
| 2 | 63.27(A) ±2.92$^{BCc}$ | 67.61(A) ±3.56$^{Bb}$ | 73.64(AA) ±1.67$^{Aa}$ | 73.08(AA) ±3.31$^{Aa}$ | 73.80(AA) ±2.14$^{Aa}$ |
| 3 | 61.82(A) ±2.25$^{Cc}$ | 67.53(A) ±4.20$^{Bb}$ | 73.17(AA) ±3.56$^{Aa}$ | 72.46(AA) ±3.91$^{Aa}$ | 72.65(AA) ±2.63$^{ABa}$ |
| 4 | 60.37(A) ±3.48$^{CDc}$ | 65.07(A) ±3.95$^{Bb}$ | 70.18(A) ±2.54$^{ABa}$ | 72.81(AA) ±1.94$^{Aa}$ | 72.62(AA) ±1.27$^{ABa}$ |
| 5 | 55.93(B) ±3.53$^{DEb}$ | 64.69(A) ±3.95$^{Ba}$ | 69.85(A) ±2.46$^{ABa}$ | 69.27(A) ±4.01$^{Aba}$ | 70.08(A) ±3.21$^{ABCa}$ |
| 6 | 54.71(B) ±2.11$^{Eb}$ | 63.92(A) ±4.18$^{Ba}$ | 65.35(A) ±4.39$^{Ba}$ | 66.14(A) ±2.85$^{Ba}$ | 68.50(A) ±3.09$^{BCa}$ |
| 8 | 53.22(B) ±4.21$^{Ea}$ | 61.70(A) ±3.37$^{Ba}$ | 64.42(A) ±4.15$^{Ba}$ | 64.32(A) ±4.37$^{Ba}$ | 64.97(A) ±2.61$^{Ca}$ |

[1] Egg grades: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

[2] Data are expressed as means (egg grades) ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-D] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 3.3.** Effect of rice protein concentrate (RPC) coatings on yolk index during eight weeks of storage at 20º C[1].

| | Treatments | | | | |
|---|---|---|---|---|---|
| Week | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
| 0 | $0.40\pm0.02^{Aa}$ | $0.40\pm0.02^{Aa}$ | $0.40\pm0.02^{Aa}$ | $0.40\pm0.02^{Aa}$ | $0.40\pm0.02^{Aa}$ |
| 1 | $0.39\pm0.02^{Ba}$ | $0.39\pm0.01^{ABa}$ | $0.38\pm0.01^{Aa}$ | $0.38\pm0.02^{Aa}$ | $0.40\pm0.02^{Aa}$ |
| 2 | $0.35\pm0.02^{Bb}$ | $0.37\pm0.02^{ABCab}$ | $0.38\pm0.02^{Aa}$ | $0.38\pm0.04^{Aa}$ | $0.37\pm0.03^{Aab}$ |
| 3 | $0.35\pm0.02^{Bb}$ | $0.38\pm0.02^{ABCa}$ | $0.37\pm0.02^{Aab}$ | $0.38\pm0.02^{ABa}$ | $0.38\pm0.01^{Aba}$ |
| 4 | $0.33\pm0.02^{Bb}$ | $0.37\pm0.02^{ABCa}$ | $0.37\pm0.02^{Aa}$ | $0.37\pm0.02^{Aa}$ | $0.37\pm0.01^{Aa}$ |
| 5 | $0.29\pm0.02^{Cc}$ | $0.36\pm0.01^{BCa}$ | $0.33\pm0.01^{Bc}$ | $0.35\pm0.07^{ABab}$ | $0.34\pm0.01^{BCbc}$ |
| 6 | $0.29\pm0.02^{Cc}$ | $0.35\pm0.02^{Ca}$ | $0.31\pm0.02^{Bc}$ | $0.32\pm0.01^{Bb}$ | $0.32\pm0.02^{Cc}$ |
| 8 | $0.28\pm0.02^{Cc}$ | $0.35\pm0.01^{BCa}$ | $0.31\pm0.03^{Bc}$ | $0.32\pm0.01^{Bb}$ | $0.32\pm0.01^{Cc}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 3.4.** Effect of rice protein concentrate (RPC) coatings on albumen pH during eight weeks of storage at 20 ºC[1].

| Week | Treatments | | | | |
|---|---|---|---|---|---|
| | Control | Mineral oil | RPC 5% | RPC 10% | RPC 15% |
| 0 | $8.75\pm0.10^{Ba}$ | $8.75\pm0.10^{ABa}$ | $8.75\pm0.10^{Ba}$ | $8.75\pm0.10^{Ca}$ | $8.75\pm0.10^{BCa}$ |
| 1 | $8.88\pm0.08^{Ba}$ | $8.68\pm0.16^{ABa}$ | $8.73\pm0.15^{Ba}$ | $8.82\pm0.13^{BCa}$ | $8.66\pm0.19^{Ca}$ |
| 2 | $9.22\pm0.08^{Aa}$ | $8.94\pm0.21^{Ab}$ | $9.10\pm0.10^{Aab}$ | $9.01\pm0.16^{ABab}$ | $9.04\pm0.14^{Aab}$ |
| 3 | $9.24\pm0.02^{Aa}$ | $8.86\pm0.10^{Ac}$ | $9.11\pm0.10^{Aab}$ | $9.03\pm0.23^{ABb}$ | $8.96\pm0.10^{ABbc}$ |
| 4 | $9.16\pm0.04^{Aa}$ | $8.75\pm0.2^{ABc}$ | $9.06\pm0.07^{Aab}$ | $8.91\pm0.24^{ABCbc}$ | $9.07\pm0.07^{Aab}$ |
| 5 | $9.22\pm0.04^{Aa}$ | $8.68\pm0.18^{ABb}$ | $9.11\pm0.11^{Aa}$ | $9.07\pm0.07^{Aba}$ | $9.08\pm0.08^{Aa}$ |
| 6 | $9.17\pm0.05^{Aa}$ | $8.70\pm0.15^{ABb}$ | $9.13\pm0.07^{Aa}$ | $9.13\pm0.08^{Aa}$ | $9.11\pm0.06^{Aa}$ |
| 8 | $9.18\pm0.02^{Aa}$ | $8.48\pm0.06^{Bb}$ | $9.11\pm0.08^{Aa}$ | $9.10\pm0.07^{Aba}$ | $9.14\pm0.05^{Aa}$ |

Data are expressed as means ± standard deviations.

[a-d]Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C]Means in the same column with different capital letters are significantly different ($P < 0.001$).

[1]Interaction between storage time and coating type ($P < 0.001$).

## CAPÍTULO IV

**Effects of rice protein coatings combined or not with propolis on shelf life of eggs**

Este capítulo é apresentado de acordo com as normas de publicação da **Poultry Science.**

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# Effects of rice protein coatings combined or not with propolis on shelf life of eggs

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## ABSTRACT

Although eggs are an excellent protein source, they are a perishable product. Many methods exist to extend shelf life of food and one of them is the use of protein coatings that may be combined with antimicrobial substances, as propolis. The effectiveness of rice protein coatings plus propolis on maintaining interior quality and eggshell breaking strength of fresh eggs was evaluated during storage at 20 ºC for 6 weeks. Egg quality was assessed by weight loss, Haugh unit (HU), albumen pH, yolk index (YI), shell strength and scanning electron microscopy in uncoated eggs (control treatment) and eggs coated with rice protein concentrate and propolis at 5 or 10%. The HU and YI were higher in coated eggs ($P < 0.001$). Weight loss increased ($P < 0.001$) during long-term storage. Uncoated eggs showed the highest weight loss (5.39%), while rice protein (4.27%) and rice protein plus propolis at 5% (4.11%) and 10% (4.40%) solutions were effective in preventing weight lost ($P < 0.001$). Uncoated eggs had the worst ($P < 0.001$) HU (58.47), albumen pH (9.48), and YI (0.33) after 6 weeks of storage. The eggs coated of rice protein and rice protein plus propolis presented results with similar intern quality between them during all the storage period. Scanning electron microscopy demonstrated a lower surface porosity in coated eggshell, indicating that the use of the coating may provide a protective barrier against the transfer of gases and moisture. In conclusion rice protein and propolis treatments helped to maintain egg quality for a longer time compared to uncoated eggs. These could be a viable alternative for maintaining the internal quality of fresh eggs during long-term storage in room temperature.


**Key words:** egg quality, eggshell, natural antimicrobial, protein coating, storage time

## INTRODUCTION

Eggs are an excellent natural source of high-quality protein, antioxidants, carotenoids, vitamins and phospholipids (Lesnierowski and Stangierski, 2018). Immediately after they are laid, aging processes begin in shell eggs, altering their chemical, physical, and functional characteristics (Lucisano et al., 1996). The porosity of the egg shell allows gas exchange with the external environment, facilitating the loss of water and $CO_2$. The longer the storage time, the greater is the deterioration of the internal quality, due to the greater $CO_2$ movement through the shell (Oliveira and Oliveira, 2013). According to the Brazilian legislation (Brasil, 1990), an egg is fresh up to 28 days after being laid and the refrigeration of the eggs in points of sale is optional and, therefore, does not occur in practical conditions. Storage technologies have been developed to extend the shelf life of eggs. For example, promising results have been obtained in the coating of eggshells, with natural products such whey protein, zein (Caner and Yüceer, 2015), rice protein (Pires et al., 2018) and propolis (Copur et al., 2008, Akpinar et al., 2015).

Propolis is a resin containing a complex mixture of substances, produced by honey bees, that results from the collection of substances secreted by different plants. During propolis collection, bees mix the beeswax and the collected propolis with their saliva (Park et al., 1998). Bees use the propolis to protect the colony from rain and to provide thermal insulation, as well as to reinforce the structural stability to the hive (Costa et al.,2011). Propolis also has several properties, such as antibacterial (Silici and Kutluca, 2005), antifungal (Seven et al., 2011), antiprotozoan, and antiviral activities (Schhnitzler et al., 2010). The effects observed are complex, due to the wide array of components in its chemical composition, as it may contain more than 300 substances including flavonoids, phenolic acid, esters, terpenes, and sugars (Aygun, 2016). Brazil is a great producer and exporter of propolis of *Apis mellifera* and the Brazilian propolis is characterized by the presence of hydroxycinnamic acid (Oldoni et al., 2015). However, the composition and biological activity of the Brazilian propolis vary

significantly, depending on the type of sample and geographical area of collection (Machado et al., 2016).

Rice (*Oryza sativa* L.) is a major food crop, with global annual production estimated at about 480 million metric tons (expressed on a milled rice basis) (USDA, 2015). Rice bran is the major by-product generated during milling and the defatted residues of bran contain ranges from 10 to 16% of protein (Cao et al., 2009, Faria et al., 2012). Rice proteins are generally regarded as hypoallergenic (Fiocchi et al., 2006), antioxidant (Faria et al., 2012), and are considered an emulsifier, also showing the ability of blinding oil and water (Chandi et al., 2007). Those properties make rice protein suitable for a broad range of industrial food applications.

Previous studies already described the use of rice by-products and propolis as feedstocks for the preparation of edible coating (Park et al., 1998, Dias et al., 2010, Das et al., 2013, Akpinar et al., 2015). However, information available on the combined effects of these products is very limited, particularly in eggs. Thus, the aim of the study was to evaluate the internal quality and the resistance of eggshell after application of rice protein coating combined with propolis in eggs after 6 weeks of storage

## MATERIALS AND METHODS

Three hundred table eggs, freshly laid (one-day-old) from ISA Brown hens, were supplied by a commercial farm (Rio Grande do Sul, Brazil) and used in the present study. All eggs were obtained from birds of the same age, maintained under similar environment, handling, and feeding conditions. The eggs were randomly divided into four treatments. Uncoated eggs were used as a control treatment. The other treatments consisted of coatings based on rice protein concentrate (**RPC**) with different inclusions of *Apis mellifera* propolis (0, 5, or 10%) according Aygun et al. (2012).

***Preparation of coating solutions and coating of shell eggs***

Rice protein film-forming solution was prepared by dissolving 8% (w/w) RPC (MidWay Labs, FL, USA) in distilled water, and adding 20% (w/w) glycerol (Neon, São Paulo, Brazil) as plasticizer. Propolis solution was prepared by dissolving 5 or 10% of dry extract of propolis (Apis Flora, São Paulo, Brazil) in distilled water. The propolis solution was then mixed into the rice protein solution at concentrations of 0, 5, and 10%. The solutions were kept on a magnetic stirrer for five minutes and after heated in a water bath (90 °C) for 30 minutes, following the procedures described by Antunes (Antunes, 2003). Then, the temperature was reduced to 25 °C and the pH adjusted to 10 with 1N NaOH solution, in order to proceed the dissolution of the proteins in the film-forming coating.

All eggs were washed with water at 42 °C and chlorine (50 ppm) was used as a sanitizer following the standard practices recommended by Brazilin legislation (BRASIL, 1990). Eggs were divided into four treatments: a control uncoated group, a rice protein-coated group, and two rice protein-coated groups that were combined with propolis at 5 and 10% solutions. The clean eggs were individually submerged in the coating solutions at 24 ºC for 1 min, so that the coating visibly covered the entire shell surface. The eggs were then dried for 5 min (Caner and Cansiz, 2008) and stored at a controlled ambient temperature (20 °C) and humidity (±65 %) for up to 6 weeks in plastic trays specific for eggs. The uncoated washed eggs served as a control treatment.

Twelve eggs were immediately submitted to the quality analysis to represent the characteristics of fresh eggs (zero days of storage). Weekly during the study, twelve eggs from each group were randomly separated for quality evaluation (weight loss, Haugh unit, yolk index, and albumen pH). Breaking strength (twelve eggs per treatment), color (six eggs per treatment), and electron microscopic structure of the shells (three eggs per treatment) were evaluated at the end of the experiment.

***Weight Loss***

The eggs were weighed individually using a digital precision (±0.001 g) scale (Bel, Mark M 214A, Milano, Italy). Weight loss (%) during storage was calculated as described by Caner and Cansız (2008), using the following equation:

$$Weight\ loss, \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} x\ 100$$

***Haugh unit (HU)***

The albumen height was measured with a digital caliper (TMX PD - 150, China) at a distance of 10 mm from the yolk. After, the HU was obtained through the equation proposed by Haugh (1937):

$$HU\ =\ 100\ log\ [h - \frac{\sqrt{(30W^{0.37} - 100)}}{100} + \ 1.19$$

where *h* is the thickness of albumen (mm) and *W* is the mass of the entire egg (g).

Based on the HU results, the eggs were graded as: Class AA, when HU was higher than 72; Class A, eggs with HU from 71 to 60; Class B, eggs with HU from 59 to 31; or Class C, when HU was lower than 30 (Yuceer and Caner, 2014)

***Yolk index (YI)***

The width and height of the yolk (mm) were measured with a digital caliper (TMX PD - 150, China). After, the yolk index was calculated through the equation (Sharp and Powell, 1930):

$$Yolk\ Index = \frac{(Yolk\ height)}{(Yolk\ width)}$$

***pH measurements***

After separation of the yolk and albumin, the dense and the fluid albumen were homogenized for 20 seconds, and then the pH was determined using a digital pHmeter (Kasvi model k39-2014B, Paraná, Brazil) previously calibrated with buffer solutions of pH 7 and 10 (Brasil, 1999).

***Eggshell color***

Six eggs from each treatment were evaluated for color using the Colorimeter Konica Minolta Chroma Meter CR-410, (Osaka, Japão) The L* (lightness), a* (greenness), and b* (yellowness) values were obtoined after the storage period and values were taken at 3 random locations on each egg. At least 1 value was taken at the blunt or round tip for every egg (Biladeau and Keener, 2009).

***Eggshell breaking strength***

Eggshell breaking strength (puncture strength) was determined at the end of the 6 week storage period using a texture analyzer (TAXT Texture Analyzer, Stable Micro Systems, Surrey, England). Each egg was mounted on a texture analyzer platform and the egg shell was punctured at the top (small end) using a 3 mm die probe at 5 $mms^{-1}$ constant speed and a distance of 6 mm. The trigger force used was 3 g, following the method described by Oliveira (Oliveira, 2006). The force (N) required to puncture the shell was recorded as the eggshell breaking strength (Yuceer and Caner, 2014).

***Ultrastructural assessment***

At the end of the project, three eggs from each treatment were randomly selected and lightly broken. After, their eggshells were segmented with scissors in three parts corresponding

to the apical, equatorial and basal regions. Residual albumen was removed. Then, fragments of approximately 0.5 cm² were removed from each egg region. The samples were mounted on a stub, coated with gold–palladium of 35 nm for 3 minutes (Sputter Coater - SCD 050 Balzers, Germany) and analyzed through a scanning electron microscope (JEOL 6060, Japan) at a standard magnification of 250×.

### *Statistical analysis*

Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). The normality of the data was verified using the Shapiro-Wilks test through the UNIVARIATE procedure. Afterward, the data were submitted to analysis of variance using PROC GLM, considering each egg an experimental unit. Statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods); except for eggshell color and breaking strength, which was evaluated only once at the end of the project and was analyzed considering only the treatment effect. Eventual differences ($P < 0.05$) were assessed with a Tukey multiple comparison test.

## RESULTS AND DISCUSSION

The eggs evaluated at day zero presented mean HU value of 82.02, assuring their excellent quality (AA) standard according to the USDA (USDA, 2000) recommendation. The other quality parameters evaluated in the beginning of the trial were also in accordance with the Brazilian legislation (Brasil, 1997), which determine minimum internal quality conditions for yolk (translucent, firm, consistent, and without germ) and albumen (transparent, consistent, limpid, no stain, and intact chalaza).

***Weight loss***

The initial egg weight did not differ ($P > 0.05$) between uncoated eggs (68 g) and eggs coated with RPC (69 g), neither those coated with RPC + 5% (68 g) or RPC + 10% (69 g) of propolis. The weight loss ($P < 0.001$) increased with increasing storage periods, ranging from 4.40% to 5.39% after 6 weeks (Table 1). Weight loss during storage has already been reported (Kim et al., 2006, Jones et al., 2018) and is caused primarily by evaporation of water and loss of carbon dioxide trough the pores of shells. This is one of the important measurements to monitor the changes in quality of fresh shell eggs during storage (Caner, 2005). Eggs may be classified by weight. In this case, more profit could be achieved by reducing water loss (Biladeau and Keener, 2009).

Treatment by time interaction ($P < 0.001$) was found for weight loss, with differences ($P < 0.001$) among treatments observed in all studied periods of Control group (uncoated) eggs had the highest weight loss during the entire project reaching 5.39% weight loss at the end of the study. Eggs coated with RPC and 5 or 10% of propolis showed weight loss of 4.27, 4.11 and 4.40 %, respectively, throughout the experiment.

According to FAO (2003), 2–3% loss of egg weight during storage is acceptable. In this study, the egg coating kept the weight loss within the acceptable range up to 4 weeks of storage, which was not observed in the uncoated eggs (3.45% at this same time).

Various studies have shown the enhancement effects of using coatings on the moisture loss of the eggs during storage. These effects were associated with the use of protein-based coatings (Caner and Yuceer, 2015, Almeida et al., 2016, Xu et al., 2017) and propolis (Copur et al., 2008, Akpinar et al., 2015). Variations in egg weight loss between studies may be due to different storage times, storage temperatures, egg sizes, or shell porosities (Akpinar et al., 2015). In the current study, eggshells coated with RPC, alone or in combination with propolis, showed a lower surface porosity in the ultra structural assessment (Figure 1), which may have contributed

to a lower weight loss during storage. This demonstrates that the use of coatings may provide a protective barrier against the transfer of gases and moisture through the eggshell (Lee et al., 1996, Kim et al., 2006). Previous study (Wong et al.,1996) also indicated a more porous structure of the uncoated shells, which was evident in the thicker and stronger shells and lower weight loss for the coated eggs compared to the uncoated eggs.

***Haugh unit***

The liquefaction of the dense albumen is evidenced by the reduction of HU values. Haugh unit results of uncoated and coated eggs are shown in Table 2. The initial HU value (82.02) decreased with increasing storage time ($P < 0.001$). The reduction of HU value can be attributed to ovomucine proteolysis, cleavage of disulfide bridges or by the interaction between α and β ovomucines (Oliveira and Oliveira, 2013). During the storage, the enzymes present in the albumen hydrolyse the amino acid chains and, by destroying the protein structure, release the water that was bound to the large protein molecules, which leads to fluidization of the albumen and loss of the viscosity of the denser albumen (Brasil, 1990).

Interaction was observed between storage time and different treatments ($P < 0.001$). At the end of the tested storage time (week 6), the HU means ranged from 58.47 (uncoated eggs) to 62.72 (RPC combined with propolis). The HU of the uncoated eggs decreased more rapidly than in the coated eggs, with the differences among treatments observed early as the first week and maintained up to the end of the project. These results support previous observations that different protein coatings (Caner and Yuceer, 2015, Xu et al., 2017, Pires et al., 2018) were effective in preserving the albumen quality of eggs. Advantages of using coatings containing propolis were observed ate the and of the project, when treatments with this substance at 5 and 10% showed better results compared to the treatment that used RPC alone. These results agree with previous observations (Copur et al., 2008, Akpinar et al., 2015)

The HU values indicated that uncoated eggs changed in quality from grade "AA" to "A" after 3 weeks, and to grade "B" after 6 weeks. Meanwhile, eggs coated with RPC changed from "AA" to "A" after 4 weeks of storage and eggs coated with RPC combined with propolis changed from "AA" to "A" only after 5 weeks of storage at 20 °C. This demonstrated that the use of coatings can preserve the internal egg quality (grade maintenance) for 1 to 2 weeks longer compared to uncoated eggs. Advantages of coatings (grade maintenance) were already reported (Caner and Yuceer, 2015, Pires et al., 2018) for stored eggs.

***Yolk index***

The yolk index of uncoated and coated eggs decreased ($P < 0.001$) throughout the storage (Table 3), as already reported in previous studies (Akpinar,2015, Almeida et al., 2016, Xu et al. 2017). During storage, water is transferred from the albumen to the yolk, which increases his weight and make the yolk membrane less elastic and more susceptible to rupture (Oliveira and Oliveira, 2013). A fresh egg of good quality has a yolk index of around 0.45, while an older egg will have a lower yolk index. The higher the YI the better is the quality of the yolk (Yuccer and Caner, 2014).

Interaction was observed between storage time and different treatments ($P < 0.001$). The effect of the coating was observed from the first week of storage, when all coatings tested had a higher yolk index ($P < 0.001$) compared to the control treatment. At the end of the project, the best yolk index mean was observed in the treatment that combined RPC and propolis at 10% solution, followed by the other coated treatments. This study demonstrated that the use of coating was able to preserve the yolk quality for a longer time than uncoated eggs, which agree with previous studies (Torrico et al., 2010, Caner and Yuceer, 2015, Pires et al., 2018).

***pH measurement in albumen and yolk***

The albumen pH varied ($P < 0.001$) over the storage period (Table 4). The average initial albumen pH of the eggs was 8.05 and this value increased to 9.40 at the end of the 6 weeks in the uncoated eggs. Coated eggs differed ($P < 0.001$) from uncoated treatments in terms of albumen pH from the first week up to the end of the project. The results agree with previous studies (Caner and Yuceer,2015, Biladeau and Keener, 2009), which reported that different coatings were able to extend the shelf-life of eggs in relation to albumen pH. This implies that the use of rice protein and propolis coatings act as barrier and help diffuse gases less rapidly through the shell.

No differences among the treatments in terms of yolk pH were observed up to the second week (Table 5). From week 3 to 5, the pH of the yolk in coated eggs was lower than of the uncoated eggs. However, at week 6, there was no difference among the pH of the yolk in control and any coated eggs. The pH of the yolk in uncoated eggs increased ($P < 0.001$) from pH 6.24 at week 0 to pH 7.00 at week 6. Few variations in pH of egg yolk was expected because the pH of the albumen increases during storage due to $CO_2$ loss and migrations of water from the albumen into the yolk during storage (Biladeau and Keener, 2009).

***Eggshell color***

The coloration is an important shell quality parameter and has a positive influence on consumer preference (Samiullah et al.,2015). Discoloration of products may lead to dissatisfaction for consumers (Caner, 2005). The L∗ values, an indication of lightness or brightness of the shell, ranged from 80.68 to 85.10, indicating light-colored shells (Table 6). Eggs coated with propolis had the lowest L* values, which could be explained by the presence of the yellow pigment, probably present in the propolis. Similar relationship was described (Wong et al.,1996) for eggs coated with corn zein, which could be explained by the presence of the

yellow pigment (xanthophyll) in the coating. In addition, other study (Biladeau and Keener, 2009) found that wax-coated eggs had a decreasing in L* over time and soy protein isolate had a yellowing effect oven time. In this project, eggs coated with a whey protein isolate, soy protein isolate and wax-coated eggs were darker (less glossy) than the uncoated (lower L*).

There was no difference for a* values among treatments. However, the uncoated eggs showed higher b* values than RPC-coated eggs, while the propolis-coated eggs were more yellow than the control and RPC. Other studies (Biladeau and Keener, 2009, Caner, 2005) reported that there was no difference in yellow color between protein-based coating eggs and uncoated ones.

The coatings altered the egg's visual appearance. Color values such as L*, a*, and b* provide an objective evaluation of the appearance of coated shell eggs. Even though all proteins will not serve as consumer-acceptable coatings, processors may still be willing to purchase colored shell eggs because they have enhanced mechanical and barrier properties (Wong et al., 1996).

### *Eggshell breaking strength*

Reducing egg breaking is important in the poultry industry. Thus, improving shell resistance would result in economic savings due to the reduced incidence of breakage or downgraded eggs (cracks) during handling and storage (Caner and Yuceer, 2015). However, in this study, eggshell breaking strength did not differ ($P > 0.05$) among uncoated eggs (4.22 kgf) and eggs coated with RPC alone (4.55 kgf), or in combination with 5% (4.64 kgf) or 10% (4.79 kgf) of propolis after 6 weeks of storage. Although previous studies have described improvements to shell quality and reduction of eggshell breakage after coating application (Caner and Yuceer, 2015, Biladeau and Keener, 2009) this characteristic seems to be associated with specific properties of the coatings used in the studies and was not observed in this trial.

### *Coating Effects*

The RPC coating exhibited sufficient hydrophobicity and sealing properties required to effectively retard water loss during the storage at room temperature for up to 6 weeks. Propolis is a hydrophobic compound that contributes to improve some properties of coatings, such as the water vapor barrier that reduces the loss of mass by transpiration, which naturally occurs in foods during storage (Pastor et al., 2010). The loss of albumen and yolk quality can be influenced by the capacity of the coating to block the pores on the surface of the shell. In general, the effects of the tested coatings on albumen and yolks are favorable, indicating that the use of RPC-based coating may be a viable alternative to maintain functional properties (Haugh unit, yolk index, pH) of the eggs, which are adversely affected by storage period.

## CONCLUSIONS

Coating base on rice protein and propolis coating has been successfully used for extending shelf life of the egg when stored. These properties may help egg industry in decreasing economic losses during storage at room temperature. Future studies are needed to verify if the use of rice protein coatings associated with propolis can also minimize the contamination of the shell by microorganisms.

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**Table 4.2.** Effect of rice protein concentrate and propolis coatings[1] on cumulative weight loss (% in relation to week 0) of egg during 6 weeks of storage at 20 ºC[1].

| Coating | 1 wk | 2 wk | 3 wk | 4 wk | 5 wk | 6 wk | P-value |
|---|---|---|---|---|---|---|---|
| Control | 1.05±0.02$^{Fa}$ | 1.32±0.09$^{Ea}$ | 2.61±0.11$^{Da}$ | 3.45±0.12$^{Ca}$ | 4.55±0.17$^{Ba}$ | 5.39±0.17$^{Aa}$ | 0.0001 |
| RPC | 0.79±0.03$^{Fb}$ | 1.04±0.07$^{Eb}$ | 1.78±0.10$^{Db}$ | 2.40±0.16$^{Cb}$ | 3.56±0.14$^{Bb}$ | 4.27±0.19$^{Abc}$ | 0.0001 |
| RPC+P5 | 0.73±0.05$^{Fc}$ | 1.06±0.07$^{Eb}$ | 1.66±0.09$^{Dbc}$ | 2.24±0.12$^{Cb}$ | 3.43±0.15$^{Bb}$ | 4.11±0.07$^{Ac}$ | 0.0001 |
| RPC+P10 | 0.57±0.04$^{Ed}$ | 1.07±0.03$^{Db}$ | 1.59±0.14$^{Cc}$ | 1.71±0.12$^{Cc}$ | 3.43±0.17$^{Bb}$ | 4.40±0.18$^{Ab}$ | 0.0001 |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

[A-F] Means in the same row with different capital letters are significantly different ($P < 0.001$).

RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

**Table 4.2.** Effect of rice protein concentrate and propolis coatings on Haugh unit (HU) and egg grade[1] (designated after each mean, in the parenthesis) during up to 6 weeks of storage at 20 ºC[2].

| Coating | 0 wk | 1 wk | 2 wk | 3 wk | 4 wk | 5 wk | 6 wk | P-value |
|---|---|---|---|---|---|---|---|---|
| Control | 82.02±0.39(AA)$^{Aa}$ | 79.65±0.45(AA)$^{Bb}$ | 75.32±0.34(AA)$^{Cc}$ | 70.90±0.55(A)$^{Db}$ | 66.49±0.53(A)$^{Eb}$ | 63.23±0.49(A)$^{Fb}$ | 58.47±0.52(B)$^{Gc}$ | 0.0001 |
| RPC | 82.02±0.39(AA)$^{Aa}$ | 81.14±0.52(AA)$^{Ba}$ | 78.40±0.32(AA)$^{Cb}$ | 76.96±0.36(AA)$^{Da}$ | 71.81±0.40(A)$^{Ea}$ | 68.60±0.48(A)$^{Fa}$ | 61.55±0.62(A)$^{Gb}$ | 0.0001 |
| RPC+P5 | 82.02±0.39(AA)$^{Aa}$ | 81.17±0.23(AA)$^{Ba}$ | 78.89±0.26(AA)$^{Cab}$ | 77.19±0.26(AA)$^{Da}$ | 72.37±0.55(AA)$^{Ea}$ | 68.68±0.26(A)$^{Fa}$ | 62.67±0.35(A)$^{Ga}$ | 0.0001 |
| RPC+P10 | 82.02±0.39(AA)$^{Aa}$ | 81.46±0.32(AA)$^{Aa}$ | 79.09±0.57(AA)$^{Ba}$ | 77.35±0.39(AA)$^{Ca}$ | 72.31±0.55(AA)$^{Da}$ | 69.10±0.34(A)$^{Ea}$ | 62.72±0.38(A)$^{Fa}$ | 0.0001 |

[1] Egg grades: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

[2] Data are expressed as means (egg grades) ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

[A-D] Means in the same row with different capital letters are significantly different ($P < 0.001$).

RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

**Table 4.3**. Effect of rice protein concentrate (RPC) and propolis (P) coatings on yolk index during up to 6 weeks of storage at 20 ºC[1].

| Coating | 0 wk | 1 wk | 2 wk | 3 wk | 4 wk | 5 wk | 6 wk | P-value |
|---|---|---|---|---|---|---|---|---|
| Control | $0.49\pm0.01^{Aa}$ | $0.45\pm0.01^{Bb}$ | $0.40\pm0.01^{Cc}$ | $0.38\pm0.01^{Db}$ | $0.36\pm0.01^{Ec}$ | $0.36\pm0.01^{Fb}$ | $0.33\pm0.01^{Gc}$ | 0.0001 |
| RPC | $0.49\pm0.01^{Aa}$ | $0.46\pm0.01^{Ba}$ | $0.42\pm0.01^{Cb}$ | $0.40\pm0.01^{Da}$ | $0.38\pm0.01^{Eb}$ | $0.37\pm0.01^{EFa}$ | $0.36\pm0.01^{Fb}$ | 0.0001 |
| RPC+P5 | $0.49\pm0.01^{Aa}$ | $0.46\pm0.01^{Ba}$ | $0.42\pm0.01^{Cb}$ | $0.41\pm0.01^{Da}$ | $0.39\pm0.01^{Ea}$ | $0.37\pm0.01^{Fa}$ | $0.36\pm0.01^{Fb}$ | 0.0001 |
| RPC+P10 | $0.49\pm0.01^{Aa}$ | $0.46\pm0.01^{Ba}$ | $0.43\pm0.01^{Ca}$ | $0.41\pm0.01^{Da}$ | $0.40\pm0.01^{Ea}$ | $0.38\pm0.01^{Fa}$ | $0.37\pm0.01^{Fa}$ | 0.0001 |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same row with different capital letters are significantly different ($P < 0.001$).

RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

**Table 4.4**. Effect of rice protein concentrate and propolis coatings on albumen pH during up to 6 weeks of storage at 20 ºC[1].

| Coating | 0 wk | 1 wk | 2 wk | 3 wk | 4 wk | 5 wk | 6 wk | P-value |
|---|---|---|---|---|---|---|---|---|
| Control | 8.05±0.02$^{Ea}$ | 8.35±0.02$^{Da}$ | 8.70±0.05$^{Ca}$ | 9.08±0.04$^{Ba}$ | 9.21±0.06$^{Ba}$ | 9.46±0.16$^{Aa}$ | 9.48±0.11$^{A}$ | 0.0001 |
| RPC | 8.05±0.02$^{Ea}$ | 8.14±0.05$^{Eb}$ | 8.37±0.06$^{Db}$ | 8.49±0.10$^{Cb}$ | 9.09±0.07$^{Bb}$ | 9.17±0.06$^{ABb}$ | 9.20±0.04$^{Ab}$ | 0.0001 |
| RPC+P5 | 8.05±0.02$^{Da}$ | 8.10±0.04$^{Db}$ | 8.28±0.07$^{Cc}$ | 8.41±0.10$^{Bb}$ | 9.10±0.08$^{Ab}$ | 9.12±0.05$^{Ab}$ | 9.19±0.10$^{Ab}$ | 0.0001 |
| RPC+P10 | 8.05±0.02$^{Da}$ | 8.09±0.05$^{CDb}$ | 8.19±0.08$^{Cc}$ | 8.46±0.08$^{Bb}$ | 9.08±0.07$^{Ab}$ | 9.11±0.05$^{Ab}$ | 9.13±0.06$^{Ab}$ | 0.0001 |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment.

Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same row with different capital letters are significantly different ($P < 0.001$).

RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

**Table 4.5**. Effect of rice protein concentrate and propolis coatings on yolk pH during up to 6 weeks of storage at 20 ºC[1].

| Coating | 0 | 1 | 2 | 3 | 4 | 5 | 6 | P-value |
|---|---|---|---|---|---|---|---|---|
| Control | 6.24±0.15$^{Ca}$ | 6.45±0.14$^{Ba}$ | 6.58±0.17$^{Ba}$ | 6.92±0.45$^{Aa}$ | 6.95±0.04$^{Aa}$ | 6.97±0.03$^{Aa}$ | 7.00±0.04$^{Aa}$ | 0.0001 |
| RPC | 6.24±0.15$^{Ca}$ | 6.30±0.12$^{Ca}$ | 6.50±0.19$^{BCa}$ | 6.46±0.23$^{Cb}$ | 6.45±0.10$^{Cb}$ | 6.73±0.21$^{ABb}$ | 6.79±0.06$^{Aa}$ | 0.0001 |
| RPC+P5 | 6.24±0.15$^{Ca}$ | 6.28±0.16$^{Ca}$ | 6.41±0.23$^{Ca}$ | 6.48±0.19$^{BCb}$ | 6.46±0.11$^{BCb}$ | 6.68±0.13$^{ABb}$ | 6.84±0.10$^{Aa}$ | 0.0001 |
| RPC+P10 | 6.24±0.15$^{Ba}$ | 6.28±0.11$^{Ba}$ | 6.46±0.19$^{ABa}$ | 6.48±0.21$^{ABb}$ | 6.51±0.14$^{ABb}$ | 6.68±0.19$^{Bb}$ | 6.72±0.39$^{Aa}$ | 0.0001 |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment.

Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same row with different capital letters are significantly different ($P < 0.001$).

RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

**Table 4.6.** Effect of rice protein concentrate and propolis oh the lightness (L*), greenness (a*), yellowness (b*) values of eggshell after 6 weeks of storage at 20 ºC.

| | L* value | a* value | b* value |
|---|---|---|---|
| Control | 85.10±3.59[a] | 0.44±0.02 | 1.69±0.06[c] |
| RPC | 83.86±2.87[ab] | 0.41±0.01 | 1.82±0.10[b] |
| RPC+P5 | 81.43±2.38[b] | 0.39±0.02 | 1.93±0.09[a] |
| RPC+P10 | 80.68±3.36[b] | 0.42±0.02 | 2.00±0.05[a] |
| P-value | 0.0001 | 0.0880 | 0.0001 |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment.

[a-d] Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

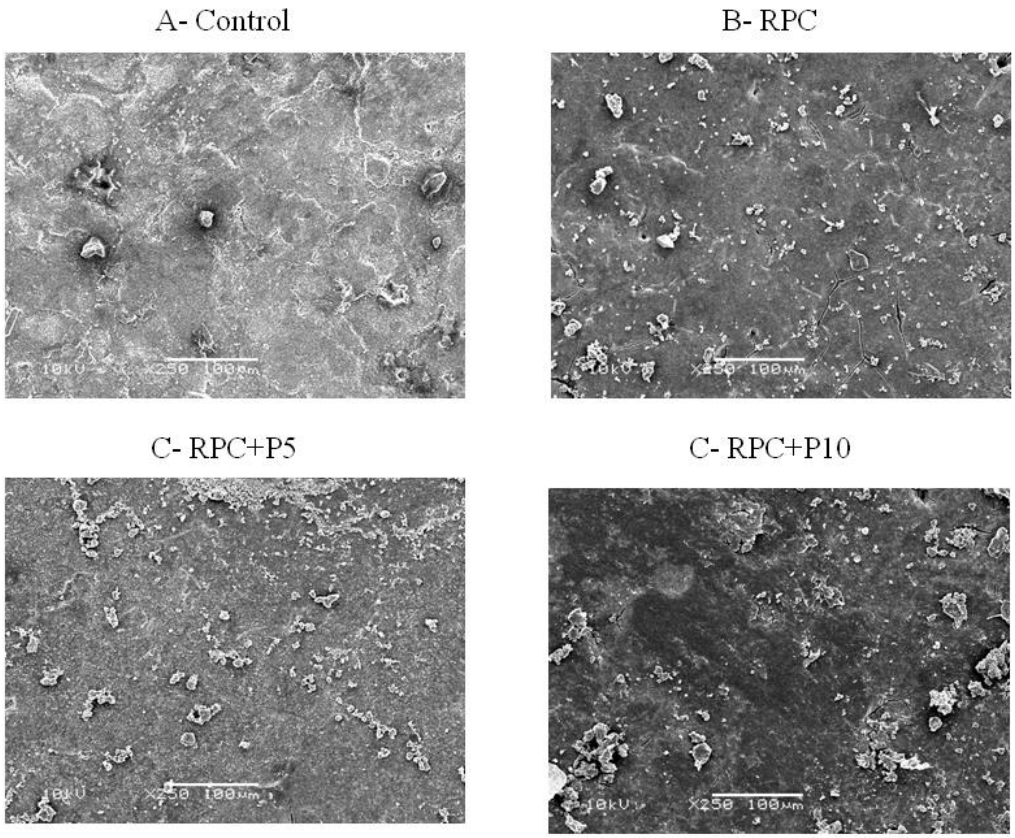


**Figure 4.1.** Scanning electron microscopy (×250) of uncoated eggshell (picture a) and coated eggs (pictures b to d) after 6 weeks of storage. RPC: rice protein concentrate coating.

## CAPÍTULO V

Effects of rice protein coating enriched with essential oils on internal quality and shelf-life of eggs during room temperature storage

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# Effects of rice protein coating enriched with essential oils on internal quality and shelf-life of eggs during room temperature storage

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## ABSTRACT

The effectiveness of rice protein coatings enriched with essential oils on maintaining interior quality of fresh eggs was evaluated during storage at 20 ºC for 6 weeks. Egg quality was assessed by weight loss, Haugh unit (HU), albumen pH, and yolk index (YI) in uncoated eggs (control treatment) and eggs coated with rice protein concentrate at 8% enriched or not with different essential oils (1%): tea tree **(***Melaleuca alternifolia*), copaíba (*Copaifera langsdorffii*), or thymo (*Thymus vulgaris*). The HU and YI were higher in coated eggs ($P < 0.001$). Data were submitted to variance analysis and the statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods). Weight loss increased ($P < 0.001$) during long-term storage. Uncoated eggs showed the highest weight loss (5.43%), while coatings of rice protein alone (4.23%) or enriched with tea tree (4.10%), copaíba (3.90%), and thymo (4.08%) solutions were effective in preventing weight lost ($P < 0.001$). The coating use preserved the internal quality of the eggs for up to 3 weeks longer than uncoated eggs in terms of HU, YI, and pH. Uncoated eggs had the worst ($P < 0.001$) HU (58.46), albumen pH (9.48), and YI (0.33) after 6 weeks of storage. In conclusion, the use of coatings based on rice protein concentrate enriched with different essential oils influences the internal quality of eggs during storage and may be an effective alternative for increasing the shelf-life of commercial eggs.


**Key words:** copaíba, phytochemicals, storage, tea tree, thymo

## INTRODUCTION

Eggs are perishable products and lose quality if they are not handled and stored properly. From the oviposition, the egg is subject to physical and chemical changes in the albumen and yolk that could result in changes in the flavor, freshness, and palatability. The longer is the storage time, the greater is the deterioration of internal quality and the higher is the carbon dioxide movement through the shell, especially at room temperature (Oliveira and Oliveira, 2013). In Brazil, washing the eggs before breaking is a recommended process that must be done by mechanical devices with procedures that prevent the microbial penetration into the egg (Brazil, 1990). The United States of America, Japan, and Australia also adopt egg-washing procedures, while many countries - including the United Kingdom and EU - have resisted to the practice (Jones, 2018). Previous studies have shown that the use of protein coatings after egg washing can help maintain internal egg quality during storage for long periods (Biladeau and Keener, 2009; Caner and Yuceer, 2015).

Despite the diversity of feedstock already available, the development of coatings from by-products is an economically interesting alternative for the industry. In this context, the rice by-products probably deserves highlight due to its availability in many regions, such as Asia and Brazil. Studies had described the use of rice as a feedstock for the preparation of edible coating (Dias et al., 2010; Das et al., 2013).Rice protein coating was also studied and previous research suggests its effect in preserving the internal quality of raw eggs (Pires et al., 2018).

Essential oils are the secondary metabolite of aromatic plants, which has a wide range of biological activity (Abd-Elsalamand, 2015). Tea tree is essential oil of *Melaleuca alternifolia* and it is a complex mixture of terpen hydrocarbons and tertiary alcohols. Its main components are terpinen-4-ol and 1.8-cineole (Jamróz, 2018). The

copaíba present different amounts of substances in the oil composition. About 80% are sesquiterpenes, a class of terpenes, and 20% are diterpenes. Among the sesquiterpenes, about 50% of the composition is β-caryophyllene, followed by α-humulene, α-copaene, α-bergamotene, and δ-cadinene (Tobouti, 2017). Thyme contains high concentrations of phenolic compounds including carvacrol, thymol, p-cymene, and ɣ-terpinene (Marino et al., 1999). Due to the presence of these substances, essential oils can be used in different applications, such as antimicrobials and antioxidants.

Essential oil-edible coatings are considered as an effective and innovative method in maintaining food quality by increasing their distribution in the food areas where microorganisms grow and proliferate, as well as by enhancing their antimicrobial activity. Coatings with essential oils are a layer of the mixture of essential oils and biological polymers, which are able to carry oil (protein, natural gum, modified starch, lipids, etc.). It can not only prevent the exchange of oxygen, water, and carbon dioxide, but also can delay the deterioration of food, so as toplay a role in preservation (Ju et al., 2018). Upadhyaya et al. (2016) reported that the phytochemicals, especially carvacrol and eugenol, when applied in pectin and gum arabic based coating were effective in reducing *Salmonella Enteritidis* on shell eggs. However, there are no previous reports on the use of coating enriched with phytochemicals to maintain egg quality. Thus, the aim of the study was, therefore, to evaluate the internal quality eggs after application of rice protein coating enriched with different essential oils in eggs during 6 weeks of storage at room temperature (20 °C).

## MATERIAL AND METHODS

Three hundred and seventy two non-fertile eggs, freshly laid (one-day-old) from ISA Brown hens, were supplied by a commercial farm (Rio Grande do Sul, Brazil). All eggs were obtained from birds of the same age, maintained under similar environment, handling and feeding conditions. The eggs were randomly divided into five treatments. Uncoated eggs were used as a control treatment. The other treatments consisted of coatings based on rice protein concentrate (RPC). The coatings were prepared at 8% (w/w protein) using RPC (MidWay Labs, FL, USA) enriched or not with different essential oils at 1%: tea tree (*Melaleuca alternifolia*), copaíba (*Copaifera langsdorffii*), or thymo (*Thymus vulgaris*). All essential oils were commercial available products (Phytoterápica, São Paulo, Brazil). The tea tree and thyme oils were extracted by steam distillation method. Copaiba oil is produced by extraction of the trunk of the trees belonging to the genera Copaifera. The essential oil can also be extracted through fractional distillation method.

### *Preparation of Coating Solutions and Coating of Shell Eggs*

Glycerol (Neon, São Paulo, Brazil) was added to give a protein : plasticizer ratio of 2:1 w/w. The solutions were kept on a magnetic stirrer for five minutes and after heated in a water bath (90 °C) for 30 minutes (Antunes, 2003). Then, the temperature was reduced to 25 °C and the pH adjusted to 10 with 1N NaOH solution, for the dissolution of the proteins in the film-forming solution.

All eggs were washed with water at 42 °C and chlorine (50 ppm) was used as a sanitizer (Brazil, 1990). The eggs were immersed for 1 min each followed by a drying time of 5 min. The clean eggs were individually submerged in the coating solutions at 24ºC for 1 min, so that the coating visibly covered the entire shell surface. The eggs

were then dried (Caner and Cansız, 2008) and stored at a controlled ambient temperature (20 °C) for up to 6 weeks in plastic trays specific for eggs. The uncoated washed eggs served as a control treatment.

Twelve eggs were immediately submitted to the quality analysis to represent the characteristics of fresh eggs (zero days of storage). Weekly during the study, 12 eggs from each group were randomly separated for quality evaluation (weight loss, Haugh unit, yolk index, and albumen pH) at each storage interval (one to 6 weeks).

***Weight loss***

The eggs were weighed individually using a digital precision (± 0.001 g) scale (Bel, Mark M 214A, Milano, Italy). Weight loss (%) during storage was calculated weekly in relation to the respective egg weight at the beginning of the trial,as described by Caner and Cansız (2008), using the following equation:

$$Weight\ loss, \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} x\ 100$$

***Haugh Unit (HU)***

The albumen height was measured with a digital caliper (TMX PD - 150, China) at a distance of 10 mm from the yolk. After, the HU was obtained through the equation proposed by Haugh (1937):

$$HU\ =\ 100\ log\ [h - \frac{\sqrt{(30W^{0.37} - 100)}}{100} + 1.19$$

where *h* is the thickness of albumen (mm) and *W* is the mass of the entire egg (g).

Based on the HU results, the eggs were graded as: Class AA, when HU was higher than72; Class A, eggs with HU from 71 to 60; Class B, eggs with HU from 59 to 31; or Class C, when HU was lower than 30 (Yuceer and Caner, 2014).

***Yolk Index***

The width and height of the yolk were measured with a digital caliper (TMX PD - 150, China). After, the yolk index was calculated through the equation (Sharp and Powell, 1930):

$$Yolk\ Index = \frac{(Yolk\ height)}{(Yolk\ width)}$$

***pH Measurements***

After the separation of yolk and albumen, the dense and the fluid albumen were homogenized for 20 seconds, and then the pH was determined using a digital pHmeter (Kasvi model k39-2014B, Paraná, Brazil) previously calibrated with buffer solutions of pH 7 and 10 (Brazil, 1999).

***Ultrastructural assessment***

At the end of the project, three eggs from each treatment were randomly selected and lightly broken. After, their eggshells were segmented with scissors in three parts corresponding to the apical, equatorial and basal regions. Residual albumen was removed. Then, fragments of approximately 0.5 $cm^2$ were removed from each egg region. The samples were mounted on a stub, coated with gold–palladium of 35 nm for 3 minutes (Sputter Coater - SCD 050 Balzers, Germany) and analyzed through a scanning electron microscope (JEOL 6060, Japan) at a standard magnification of 250×.

### *Statistical Analysis*

Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). The normality of the data was verified using the Shapiro-Wilks test through the UNIVARIATE procedure. Afterward, the data were submitted to analysis of variance using PROC GLM, considering each egg an experimental unit. Statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods). Eventual differences ($P<0.05$) were assessed with a Tukey multiple comparison test.

## RESULTS AND DISCUSSION

The eggs evaluated at day zero presented mean HU values of 81.99, assuring their excellent quality (AA grade) standard according to the USDA (2000) recommendation. The other quality parameters evaluated atthe beginning of the trial were also in accordance with the Brazilian legislation (Brazil, 1997), which determine minimum internal quality conditions for yolk (translucent, firm, consistent, and without germ) and albumen (transparent, consistent, limpid, no stain, and intact chalaza).

### *Weight Loss*

Egg size and weight are measures that will influence other variables such as HU and shell thickness, consequently the resistance of the shell is affected by the size of the eggs (Oliveira and Oliveira, 2013). However, in this study, the egg weight did not differ ($P> 0.05$) between uncoated eggs (67.93 g) and eggs coated with RPC (69.25 g), neither those coated with RPC combined with tea tree (69 g), copaíba (68.72g) and thymo (69.08 g) at the beginning of the study.

Weight loss of eggs is one of the most important measurements in monitoring the change in quality of fresh eggs during storage (Suppakul et al.,2010). The accumulated weight loss of the eggs during the 6 weeks of storage is shown in Table 1. Weight loss increased ($P$ <0.001) with storage time, which was already reported in previous studies (Biladeau and Keener, 2009; Yuceer and Caner, 2014) and is caused primarily by evaporation of water and loss of carbon dioxide trough the pores of shells (Scott and Silversides, 2000).Water loss depends on the temperature, airflow, and relative humidity during storage. The longer the storage period, the more critical these factors become, especially under room temperature (Feddern, et al., 2017).

Treatment by time interaction ($P$ < 0.001) was found for weight loss, with differences ($P$ < 0.001) among treatments observed in all studied periods. Eggs from the control group (uncoated) had the highest weight loss during the entire when compared to eggs coated with RPC and RPC plus tea tree, copaíba, or thymo. According to FAO (2003), a 2–3% loss of egg weight during storage is acceptable. In this study, the eggs coating kept the weight loss within the acceptable range up to 4 weeks of storage, which was not observed in uncoated eggs (3.46% at this same time). Various studies have shown the enhancement effects of using coatings on the moisture loss of the eggs during storage. These effects were associated mainly with the use of protein-based coatings (Biladeau and Keener, 2009; Caner and Yuceer, 2015; Xu et al., 2017; Pires et al., 2018).

Eggshells coated with RPC and RPC plus tea tree, copaíba, or thymo showed a lower surface porosity in the ultrastructural assessment (Figure 1), which may have contributed to thelower weight loss during storage. Essential oils can provide a barrier to loss mass and oxygen due to characteristic lipophilic.

***Haugh Unit***

The liquefaction of the dense albumen is evidenced by the reduction of HU values. Haugh unit results of uncoated and coated eggs are shown in Table 2. The initial HU value (81.99) decreased throughout the storage time ($P < 0.001$). The reduction of HU value can be attributed to ovomucine proteolysis, cleavage of disulfide bridges, or by the interaction between α and β ovomucines. During the storage, the enzymes present in the albumen hydrolyse the amino acid chains and, by destroying the protein structure, release the water that was bound to the large protein molecules occurs, which leads tothe fluidization of the albumen and the lost of the viscosity of the denser albumen (Oliveira and Oliveira, 2013).The liquefaction of the dense albumen is evidenced by the reduction of HU values. These results were in agreement with Biladeau and Keener (2009) and Pires et al. (2018), who demonstrated that the HU decreased during storage.

At the end of the tested storage time (week 6), the HU means ranged from 58.46 (uncoated eggs) to 61.47 (RPC). The HU of the uncoated eggs decreased more rapidly than coated eggs, with the differences between treatments observed early as the first week and maintained up to the end of the project. These results support previous observations that different protein coatings (Biladeau and Keener, 2009; Canner and Yuccer, 2015), may be effective in preserving the albumen quality of eggs.

The HU values indicated that uncoated eggs changed in quality from grade "AA" to "A" after 3 weeks, and to grade "B" after 6 weeks. Meanwhile, eggs coated with RPC changed from "AA" to "A" after 5 weeks of storage and eggs coated with RPC combined with essential oil changed from "AA" to "A" only after 6 weeks of storage at 20 °C. This demonstrated that the use of coatings allowed preserving the internal egg quality (grade A maintenance) for 2 to 3 weeks longer compared to uncoated eggs.

Advantages of coatings (grade A maintenance) were already reportedby Wardy et al. (2011) and Nongtaodum et al. (2013) for stored eggs.

### *Yolk Index*

The yolk index of uncoated and coated eggs also decreased ($P < 0.001$) throughout the storage (Table 3), as already reported in previous studies (Canner and Yuccer, 2015; Xu et al., 2017; Drabik et al., 2018). In the current study, interaction was observed between storage time and different treatments ($P < 0.001$). The effect of the coating was observed from the second week of storage, when all coatings tested had a higher yolk index ($P < 0.001$) compared to control treatment. The higher the yolk index, the better is the quality of the yolk (Yuceer and Caner, 2014). At the end of the project, the best yolk index was observed in the treatment that combined RPC and essential oils, followed by the eggscoated with RPC without other substances. This study demonstrated that the use of coating was able to preserve the yolk quality for a longer time than uncoated eggs, which are in agreement with previous studies (Caner and Yuccer, 2015; Xu et al., 2017). These results also indicated a positive effect of essential oils in preserving the yolk quality.

Coatings seems to be efficient to reduce the mass transfer rate (water and $CO_2$ loss) from the albumen through the eggshell during long-term storage. The increasing the width of the yolk is a process that caused by the diffusion of water through the vitelline membrane (from albumen to yolk). This process inhibits albumen liquefaction and water absorption by the yolk and minimizes a reduction in yolk quality (Caner and Yuceer, 2015), which could explain the advantages for coated eggs in the present research.

***pH Measurement in Albumen and Yolk***

The increase in albumin pH occurs due to the dissociation of carbonic acid ($H_2CO_3$), forming water and carbon dioxide (Figueiredo et al., 2013). The pH determination of the albumen is a suitable measure to evaluate the freshness of the eggs, since there is less influence of the strain and age of the bird on the pH compared with other quality measurements (Silversides and Scott, 2001). The albumen pH of the freshly laid egg usually ranges from 7.6 to 7.9. However, the albumen pH increases with the storage period of the egg and can reach 9.5 (Alleoni and Antunes, 2001). On the other hand, the increase in yolk pH (6.0) has little variation (6.4 to 6.9) even after long storage periods (Oliveira; Oliveira, 2013). During storage, $CO_2$ escapes through the eggshell pores. So, the increase in albumen pH over time may be due to the loss of $CO_2$ and⁄or a change in the bicarbonate buffer system (Biladeau and Keener, 2009).

In the current study, the albumen pH varied ($P < 0.001$) throughout the storage period (Table 4). The average initial albumen pH of the eggs was 8.05 and this value increased to 9.48 after 6weeks in the uncoated eggs. Coated and uncoated treatments differed ($P < 0.001$) in terms of albumen pH early from the first week up to the end of the project. The results agree with previous studies (Caner and Yuceer, 2015; Pires et al. 2018) which reported that different coatings were able to extend the shelf-life of eggs in relation to albumen pH. The treatments with essential oils showed similar albumen pH at the end of the project when compared to the eggs coated with RPC alone.

The yolk pH varied ($P < 0.001$) over the storage period (Table 5). After 6 weeks of storage, the pH of the uncoated eggs decreased from 6.22 to 7.05. Previous research has documented a maximum increase in yolk pH of 6.0 to 6.27 (Biladeau and Keener, 2009). From week 3 to 5, the pH of the yolk in coated eggs was lower than of the uncoated eggs. However, at week 6, there was no difference among the pH of the yolk

in control and any coated eggs. Few variation in pH of egg yolk was expected because the pH of the albumen increases during storage due to $CO_2$ loss and migrations of water from the albumen into the yolk during storage (Biladeau and Keener, 2009).

### *Coating effects*

The rice protein coating exhibited sufficient hydrophobicity and sealing properties required to effectively retard water loss during the storage at room temperature for up to 6 weeks. The addition of materials lipids to the coating may, improve the barrier properties of moisture. Essential oils can provide a barrier to loss mass and oxygen due to characteristic lipophilic. The loss of albumen and yolk quality can be influenced by the capacity of the coating to block the pores on the surface of the shell. In general, the effects of coatings on albumen and yolks are favorable, indicating that the use of RPC-based coating plus essential oil may be a viable alternative to maintain functional properties (HU, yolk index, pH) of the eggs, which are adversely affected by storage period.

## CONCLUSIONS

Rice protein coating can be used for extending the shelf life of eggs. The use of coatings with RPC and essential oils is a effective way to preserve the interior quality of eggs during the storage in room temperature. These positive effects may help egg industry in decreasing economic losses during storage. In general, the effects of coatings on albumen and yolks are favorable, indicating that the use of RPC-based coating may be a viable alternative to maintain to maintain the quality of eggs. Future studies are needed to verify the use of RPC-based coatings associated with essential oil in this concentration are efficient to minimize contamination by microorganisms.

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**Table 5.1.** Effect of rice protein concentrate (RPC) coatings enriched with essential oils tea tree (TEA), copaiba (COP), or thymo (THY) on cumulative weight loss (% in relation to week 0) of eggs during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC | RPC+TEA | RPC+COP | RPC+THY |
|---|---|---|---|---|---|
| 1 | $1.05\pm0.02^{Fa}$ | $0.79\pm0.03^{Fb}$ | $0.74\pm0.03^{Fc}$ | $0.74\pm0.04^{Fc}$ | $0.76\pm0.03^{Fbc}$ |
| 2 | $1.32\pm0.09^{Ea}$ | $1.04\pm0.07^{Ec}$ | $1.04\pm0.05^{Ec}$ | $1.06\pm0.08^{Ec}$ | $1.16\pm0.11^{Ebc}$ |
| 3 | $2.62\pm0.11^{Da}$ | $1.77\pm0.10^{Db}$ | $1.49\pm0.05^{Dc}$ | $1.35\pm0.11^{Dc}$ | $1.51\pm0.12^{Dd}$ |
| 4 | $3.46\pm0.12^{Ca}$ | $2.42\pm0.16^{Cb}$ | $1.99\pm0.10^{Cc}$ | $2.32\pm0.11^{Cd}$ | $2.57\pm0.21^{Cc}$ |
| 5 | $4.56\pm0.17^{Ba}$ | $3.59\pm0.13^{Bb}$ | $3.21\pm0.17^{Bc}$ | $3.42\pm0.19^{Bbc}$ | $3.43\pm0.23^{Bbc}$ |
| 6 | $5.43\pm0.17^{Aa}$ | $4.23\pm0.19^{Ab}$ | $4.10\pm0.18^{Abc}$ | $3.90\pm0.18^{Ac}$ | $4.08\pm0.22^{Abc}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-F] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 5.2.** Effect of rice protein concentrate (RPC) coatings enriched with essential oils tea tree (TEA), copaiba (COP), or thymo (THY)on Haugh unit (HU) and egg grade[1] (designated after each mean. in the parenthesis) during 6 weeks of storage at 20 ºC[2].

| Week | Control | RPC | RPC+TEA | RPC+COP | RPC+THY |
|---|---|---|---|---|---|
| 0 | 81.99(AA)±0.39$^{Aa}$ | 81.99(AA)± 0.39$^{Aa}$ | 81.99(AA)± 0.39$^{Aa}$ | 81.99(AA)± 0.39$^{Aa}$ | 81.99(AA)± 0.39$^{Aa}$ |
| 1 | 79.62(AA)±0.45$^{Ba}$ | 81.10(AA)±0.52$^{Bb}$ | 81.36(AA)±0.36$^{Ab}$ | 81.19(AA)±0.61$^{ABb}$ | 81.23(AA)±0.63$^{ABb}$ |
| 2 | 75.28(AA)±0.34$^{Cc}$ | 78.43(AA)±0.32$^{Cb}$ | 80.23(AA)±1.13$^{Ba}$ | 80.52(AA)±1.44$^{Ba}$ | 80.23(AA)±0.84$^{Ba}$ |
| 3 | 70.92(A)±0.55$^{Db}$ | 76.92(AA)±0.36$^{Da}$ | 77.21(AA)±1.14$^{Ca}$ | 77.60(AA)±0.67$^{Ca}$ | 77.50(AA)±0.92$^{Ca}$ |
| 4 | 66.47(A)±0.53$^{Ec}$ | 71.90(A)±0.40$^{Eb}$ | 77.17(AA)±0.58$^{Ca}$ | 77.07(AA)±0.40$^{Ca}$ | 77.23(AA)±0.76$^{Ca}$ |
| 5 | 63.29(A)±0.49$^{Fc}$ | 68.58(A)±0.48$^{Fb}$ | 74.06(AA)±0.58$^{Da}$ | 72.12(AA)±2.40$^{Da}$ | 73.90(AA)±1.16$^{Da}$ |
| 6 | 58.46(B)±0.52$^{Gc}$ | 61.47(A)±0.62$^{Gb}$ | 71.53(A)±0.75$^{Ea}$ | 71.67(A)±2.90$^{Da}$ | 72.76(AA)±1.30$^{Da}$ |

[1] Egg grades: AA. HU >72; A. HU = 71–60; B. HU = 59–31; C. HU <30.

[2] Data are expressed as means (egg grades) ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments

($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods,$P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-D] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 5.3.** Effect of rice protein concentrate (RPC) coatings enriched with essential oils tea tree (TEA), copaiba (COP), or thymo (THY) on yolk index during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC | RPC+TEA | RPC+COP | RPC+THY |
|---|---|---|---|---|---|
| 0 | $0.49\pm0.01^{Aa}$ | $0.49\pm0.01^{Aa}$ | $0.49\pm0.01^{Aa}$ | $0.49\pm0.01^{Aa}$ | $0.49\pm0.01^{Aa}$ |
| 1 | $0.44\pm0.04^{Bab}$ | $0.46\pm0.03^{Ba}$ | $0.45\pm0.01^{Bab}$ | $0.45\pm0.01^{Bab}$ | $0.45\pm0.01^{Aab}$ |
| 2 | $0.40\pm0.04^{Cd}$ | $0.42\pm0.06^{Ccd}$ | $0.45\pm0.02^{Ba}$ | $0.43\pm0.01^{CDbc}$ | $0.44\pm0.01^{Bab}$ |
| 3 | $0.38\pm0.04^{Dd}$ | $0.40\pm0.08^{Dc}$ | $0.42\pm0.01^{Cb}$ | $0.44\pm0.01^{BCa}$ | $0.44\pm0.01^{Ba}$ |
| 4 | $0.36\pm0.05^{Ed}$ | $0.38\pm0.07^{Ec}$ | $0.41\pm0.01^{CDb}$ | $0.42\pm0.01^{Da}$ | $0.42\pm0.01^{Ca}$ |
| 5 | $0.36\pm0.04^{Fd}$ | $0.38\pm0.06^{EFcd}$ | $0.40\pm0.01^{Db}$ | $0.42\pm0.01^{Da}$ | $0.42\pm0.01^{Ca}$ |
| 6 | $0.33\pm0.03^{Gd}$ | $0.37\pm0.04^{Fc}$ | $0.38\pm0.01^{Ebc}$ | $0.39\pm0.02^{Eab}$ | $0.40\pm0.01^{Da}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 5.4.** Effect of rice protein concentrate (RPC) coatings enriched with essential oils tea tree (TEA), copaiba (COP), or thymo (THY) on pH during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC | RPC+TEA | RPC+COP | RPC+THY |
|---|---|---|---|---|---|
| 0 | $8.05\pm0.02^{Ea}$ | $8.05\pm0.02^{Fa}$ | $8.05\pm0.02^{Ca}$ | $8.05\pm0.02^{Da}$ | $8.05\pm0.02^{Ca}$ |
| 1 | $8.33\pm0.19^{Da}$ | $8.09\pm0.05^{Eb}$ | $8.14\pm0.16^{BCab}$ | $8.07\pm0.17^{CDab}$ | $8.07\pm0.07^{Cb}$ |
| 2 | $8.71\pm0.05^{Ca}$ | $8.37\pm0.06^{Db}$ | $8.24\pm0.09^{Ab}$ | $8.22\pm0.10^{Cb}$ | $8.26\pm0.07^{Ab}$ |
| 3 | $9.08\pm0.04^{Ba}$ | $8.48\pm0.10^{Cb}$ | $8.18\pm0.10^{Bb}$ | $8.13\pm0.08^{ABb}$ | $8.13\pm0.11^{Bb}$ |
| 4 | $9.21\pm0.06^{Ba}$ | $9.09\pm0.07^{Bc}$ | $9.23\pm0.12^{Aa}$ | $9.16\pm0.04^{Aab}$ | $9.16\pm0.07^{Aab}$ |
| 5 | $9.44\pm0.16^{Aa}$ | $9.16\pm0.06^{ABbc}$ | $9.24\pm0.09^{Ab}$ | $9.06\pm0.08^{Bc}$ | $9.16\pm0.08^{Abc}$ |
| 6 | $9.48\pm0.11^{Aa}$ | $9.20\pm0.04^{Ab}$ | $9.26\pm0.10^{Ab}$ | $9.16\pm0.08^{Ab}$ | $9.19\pm0.04^{Ab}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($P < 0.001$).

**Table 5.5.** Effect of rice protein concentrate (RPC) coatings enriched with essential oils tea tree (TEA), copaiba (COP), or thymo (THY) on yolk pH during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC | RPC+TEA | RPC+COP | RPC+THY |
|---|---|---|---|---|---|
| 0 | 6.22±0.15$^{Ca}$ | 6.24±0.15$^{Ca}$ | 6.24±0.15B$^{a}$ | 6.24±0.15$^{Ca}$ | 6.24±0.15$^{Ca}$ |
| 1 | 6.47±0.14$^{Ba}$ | 6.30±0.12$^{Cab}$ | 6.14±0.32$^{Bab}$ | 6.12±0.21$^{Cab}$ | 6.29±0.26$^{Bb}$ |
| 2 | 6.59±0.17$^{Ba}$ | 6.45±0.19$^{Ca}$ | 6.42±0.23$^{Ba}$ | 6.46±0.50$^{Ba}$ | 6.46±0.51$^{Aa}$ |
| 3 | 6.92±0.05$^{Aa}$ | 6.45±0.23$^{Cb}$ | 6.42±0.49$^{Bb}$ | 6.44±0.37$^{BB}$ | 6.53±0.31$^{Bb}$ |
| 4 | 6.96±0.04$^{Aa}$ | 6.50±0.10$^{BCb}$ | 6.50±0.47$^{Bb}$ | 6.52±0.29$^{Ab}$ | 6.50±0.43$^{Ab}$ |
| 5 | 6.97±0.03$^{Aa}$ | 6.75±0.20$^{ABb}$ | 6.59±0.80$^{Ab}$ | 6.66±0.30$^{Ab}$ | 6.56±0.22$^{Ab}$ |
| 6 | 7.05±0.04$^{Aab}$ | 6.81±0.06$^{Aa}$ | 6.58±0.36$^{Ab}$ | 6.60±0.41$^{Ab}$ | 6.64±0.22$^{Ab}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods,$P < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($P < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($P < 0.001$).

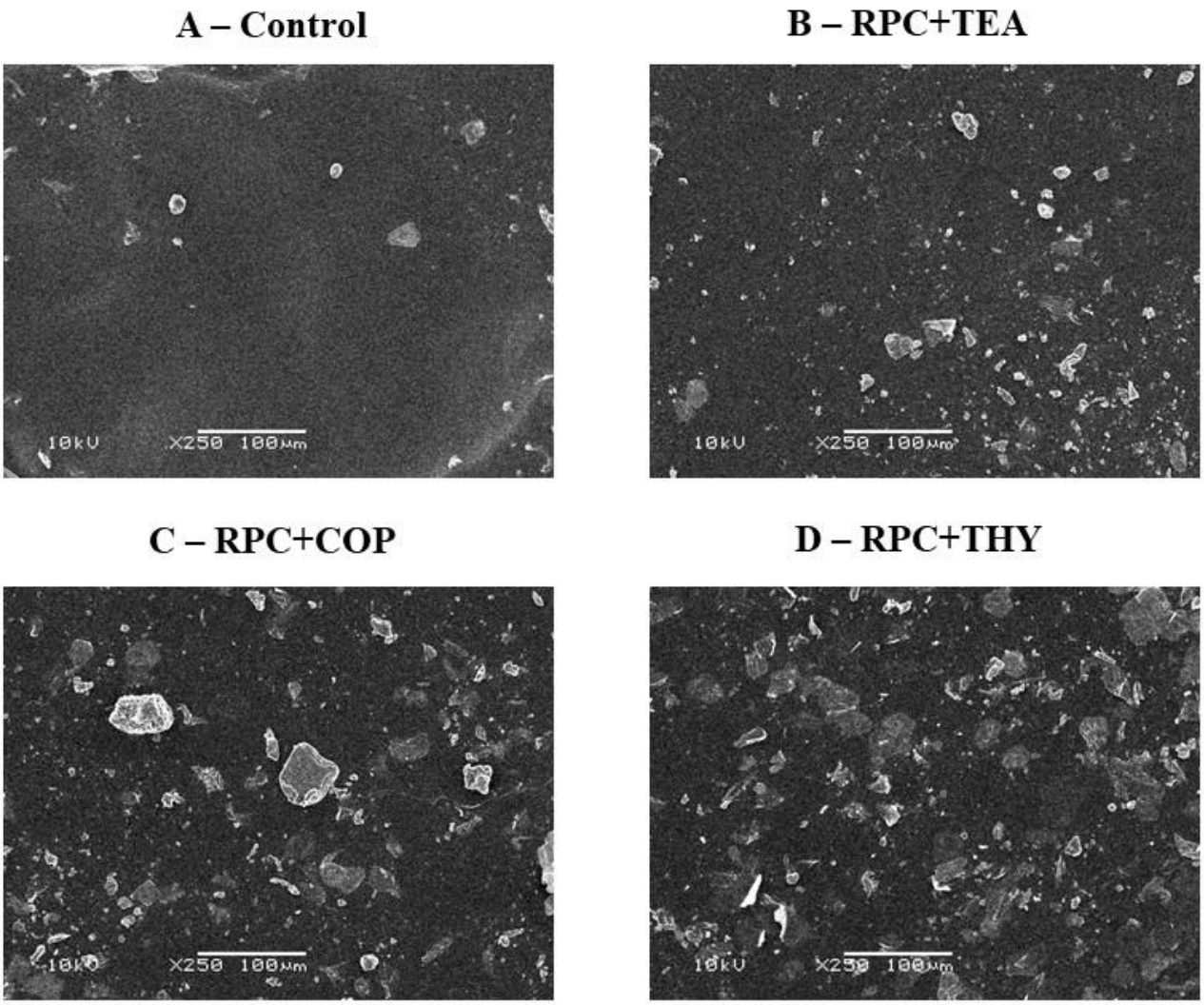


**Figure 5.1.** Scanning electron microscopy (×250) of uncoated eggshell (A) and coated eggs (B to D) after 6 wk of storage. RPC: rice protein concentrate coating; TEA: tea tree; COP: copaíba; THY: thymol.

## CAPÍTULO VI

Plasticizer types affect quality and shelf life of eggs coated with rice protein

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**Running title***:* Plasticizer types for storage of eggs

# Plasticizer types affect quality and shelf life of eggs coated with rice protein

**Summary:** This study was carried out to evaluate the effect of using rice protein coating with different plasticizers types on the quality of eggs storaged at 20 ºC for six weeks. In the experiment, 300 eggs were coated with rice protein at an 8% solution combined with glycerol, propylene glycol or sorbitol. Weight loss increased ($P < 0.001$) during long-term storage. Uncoated eggs showed the highest weight loss (5.31%), while rice protein with glycerol (4.29%) propylene glycol (4.13%) and sorbitol (4.07%) solutions were effective in preventing weight lost ($P < 0.001$). Uncoated eggs had the worst ($P < 0.001$) HU (58.40), albumen (9.52) and yolk (7.06) pH, and YI (0.33) after 6 weeks of storage. The eggs coated of rice protein with glycerol, propylene glycol and sorbitol presented results with similar intern quality between them during all the storage period. However, the use of sorbitol as a plasticizer in the coating is more efficient in maintaining control of the increase in albumen pH. Scanning electron microscopy demonstrated a lower surface porosity in coated eggshell, indicating that the use of the coating may provide a protective barrier against the transfer of gases and moisture. In conclusion, the best egg protection results in terms of egg quality are obtained in eggs coated with rice protein and sorbitol.

**Key words:** egg quality, glycerol, propylene glycol, protein coatings, sorbitol

## Introduction

Edible coatings can potentially extend the shelf life and improve the quality of food system by the control of mass transfer, moisture diffusion, gas permeability ($O_2$, $CO_2$), in addition to maintaining the mechanical and rheological characteristics (Guilbert et al., 1996). Proteins are commonly used as film-forming materials,mainly becaise the structures of proteins can be easily modified to achieve desirable film properties (Han, 2014). Proteins usually exhibite excellent oxygen, carbon dioxide, and lipid barrier properties, particularly at low relative humidities (Lacroix and Vu, 2014).

Plasticizers are required ingredients for preparing edible films and coatings, especially for proteins (Han, 2014). In the preparation of edible films or coatings, a plasticizer is often incorporated to induce flexibility (Wan et al. 2005). The plasticizers, such as glycerol, polyethylene glycol, or sorbitol, can also reduce film brittleness. Composite coatings have been developed to improve gas exchange, adherence to coated products, and moisture vapor permeability properties (Baldwin et al., 1995).

Eggs are a perishable product and earlier studies have demonstrated that protein coatings are effective in preserving the internal quality of eggs (Biladeau and Keener, 2009; Caner and Yuceer, 2015). Pires et al. (2018) reported that rice protein coating can be used for extending the shelf life of eggs. However, these studies have been conducted using glycerol as plasticizer. Kim et al. (2008) demonstrated that chitosan coating, despite of the plasticizer types (glycerol, sorbitol, and propylene glycol), reduced weight loss and preserved the albumen and yolk quality of eggs for almost 3 wk longer than observed for then on coated eggs during 5 wk of storage. However, very few information is available on the use of diferent plasticizer types in combination with other proteic products, such as rice protein. Thus, the objective of the present research

was to evaluate the effect of plasticizer types (glycerol, propylene glycol, or sorbitol) on the internal quality and morphological changes of eggshell of table eggs coated with rice protein during 6 weeks of storage at 20 ºC.

## Material and methods

Three hundred table eggs, freshly laid (one-day-old) from ISA Brown hens, were supplied by a commercial farm (Rio Grande do Sul, Brazil). All eggs were obtained from birds of the same age, maintained under similar environment, handling, and feeding conditions. The eggs were randomly divided into four treatments. Uncoated eggs were used as a control treatment. The other treatments consisted of coatings based on rice protein concentrate (RPC) combined with one of three different plasticizer types.

The coatings were prepared at 8% (w/w protein) using RPC (MidWay Labs, FL, USA) and the plasticizer types. Glycerol (Fontana, Brazil), propylene glycol (Ineos, France), or sorbitol (Ingredion, Brazil) were then added to give a protein:plasticizer ratio of 1:2 w/w. The solutions were kept on a magnetic stirrer for five minutes and after heated in a water bath (90 °C) for 30 minutes (Antunes, 2003). Then, the temperature was reduced to 25 °C and the pH adjusted to 10 with 1N NaOH solution, for the dissolution of the proteins in the film-forming solution.

All eggs were washed with water at 42 °C and chlorine (50 ppm) was used as a sanitizer (Brazil, 1990). The eggs were immersed for 1 min each followed by drying time of 5 min. The clean eggs were individually submerged in the coating solutions at 24 ºC for 1 min, so that the coating visibly covered the entire shell surface. The eggs were then dried (Caner and Cansız, 2008) and stored at a controlled ambient

temperature (20 °C) for up to six weeks in plastic trays specific for eggs. The uncoated washed eggs served as a control treatment.

Twelve eggs were immediately submitted to the quality analysis to represent the characteristics of fresh eggs (zero days of storage). Weekly during the study, 12 eggs from each group were randomly separated for quality evaluation (weight loss, Haugh unit, yolk index, and albumen pH) at each storage interval (one to six weeks).

***Weight loss***

The eggs were weighed individually using a digital precision (±0.001 g) scale (Bel, Mark M 214A, Milano, Italy). Weight loss (%) during storage was calculated as described by Caner and Cansız (2008), using the following equation:

$$Weight\ loss, \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} x\ 100$$

Twelve eggs for each treatment were taken at weekly intervals for determination of weight loss. The weight loss was calculated weekly in relation to the respective egg weight at the beginning of the trial.

***Haugh Unit (HU)***

The albumen height was measured with a digital caliper (TMX PD - 150, China) at a distance of 10 mm from the yolk. After, the HU was obtained through the equation proposed by Haugh (1937):

$$HU = 100\ log\ [h - \frac{\sqrt{(30W^{0.37} - 100)}}{100} + 1.19$$

where *h* is the thickness of albumen (mm) and *W* is the mass of the entire egg (g).

Based on the HU results, the eggs were graded as: Class AA, when HU was higher than72; Class A, eggs with HU from 71 to 60; Class B, eggs with HU from 59 to 31; or Class C, when HU was lower than 30 (Yuceer and Caner, 2014).

***Yolk Index***

The width and height of the yolk (mm) were measured with a digital caliper (TMX PD - 150, China). After, the yolk index was calculated through the equation (Sharp and Powell, 1930):

$$Yolk\ Index = \frac{(Yolk\ height)}{(Yolk\ width)}$$

***pH Measurements***

After separation of the yolk and albumen, the dense and the fluid albumen were homogenized for 20 seconds, and then the pH was determined using a digital pHmeter (Kasvi model k39-2014B, Paraná, Brazil) previously calibrated with buffer solutions of pH 7 and 10 (Brazil, 1999).

***Ultrastructural assessment***

At the end of the project, three eggs from each treatment were randomly selected and lightly broken. After, their eggshells were segmented with scissors in three parts corresponding to the apical, equatorial and basal regions. Residual albumen was

removed. Then, fragments of approximately 0.5 cm² were removed from each egg region. The samples were mounted on a stub, coated with gold–palladium of 35 nm for 3 minutes (Sputter Coater - SCD 050 Balzers, Germany) and analyzed through a scanning electron microscope (JEOL 6060, Japan) at a standard magnification of 500×.

### *Statistical Analysis*

Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). The normality of the data was verified using the Shapiro-Wilks test through the UNIVARIATE procedure. Afterward, the data were submitted to analysis of variance using PROC GLM, considering each egg an experimental unit. Statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods). Eventual differences ($p < 0.05$) were assessed with a Tukey multiple comparison test.

## Results

The accumulated weight loss of the eggs during the 6 wk of storage is shown in Table 1. For all eggs, the cumulative weight loss gradually increased with increased storage periods ($p < 0.001$). Eggs from the control group (uncoated) had the highest weight loss (reaching 5.31% at the end of the project), while eggs coated with RPC combined with glycerol, propylene glycol, and sorbitol showed less weight loss throughout the experiment. Coated eggshells showed a lower porosity in the ultrastructural assessment (Figure 1), which may have contributed to a lower weight loss during storage.

Haugh unit results of uncoated and coated eggs are shown in Table 2. The HU decreased ($p < 0.001$) over the storage period. All coated eggs had higher ($p < 0.001$) HU than uncoated eggs throughout the 6 wk of storage. The HU values indicated that uncoated eggs changed in quality from grade “AA” to “A” at the end of the 3th wk, reaching grade “B” at the end of the project (6 wk). Meanwhile, eggs coated with RPC and sorbitol changed from “AA” to “A” only at the end of the 5th wk of storage, while eggs coated with RPC and glycerol or propylene glycol changed from “AA” to “A” end of the 4th wk of storage. This demonstrated that the use of coatings can preserve the internal egg quality (grade AA maintenance) for up to 2 wk longer compared to uncoated eggs.

The YI of uncoated and coated eggs decreased ($p < 0.001$) throughout the storage (Table 3). After 6 wk of storage, the YI of the uncoated eggs decreased from 0.48 to 0.33, whereas eggs coated with RPC plus glycerol, propylene glycol, or sorbitol showed YI values of 0.37, 0.37, and 0.38, respectively, at the end of the project.

In this study, the albumen pH varied ($p < 0.001$) over the storage period (Table 4) with treatment by time interaction ($p < 0.001$). The average initial albumen pH of the eggs was 8.04 and this value increased to 9.52 after 6 wk in the uncoated eggs. Coated and uncoated treatments differed ($p < 0.001$) in terms of albumen pH early from the end of the first week up to the end of the project. Lower albumem pH was found in eggs coated with RPC plus sorbitol, followed by the treatments with glycerol and propylene glycol, which showed intermediate values to the control.

The yolk pH varied ($p < 0.001$) over the storage period (Table 5). After 6 weeks of storage, the pH of the uncoated eggs decreased from 6.72 to 7.05. From the first

week of storage, the pH of the yolk in coated eggs was lower ($p < 0.001$) than of the uncoated eggs, regardless the plasticizer.

## Discussion

The weight loss is one of the most important measurements when monitoring the change in quality of fresh eggs during storage (Suppakulet al., 2010). Egg starts losing water to the environment from the time it is laid. The porosity and shell structure allow exchanges with the external environment facilitating losses of water and $CO_2$, which consequently imply in loss of weight (Oliveira and Oliveira 2013; Feddern et al., 2017; Jones et al., 2018).The coats tested in the current study were able to prevent weight loss, which may represent an economical advantage as eggs may be classified by weight and more profit could be achieved by reducing its loss (Biladeau and Keener, 2009).

Various studies have also shown the preventing effects of using coatings on the moisture loss of the eggs during storage. These effects were associated with the use of protein-based coatings (Biladeau and Keener, 2009; Caner and Yuceer, 2015). The addition of plasticizers affects the mechanical properties and the resistance of coatings to permeation of vapors and gases (Sothornvit and Krochta, 2000, 2001). The plasticizers may also reduce coating brittleness. In addition, the use of composite coatings improves adherence to coated products (Baldwin et al., 1995).

Kim et al. (2008) used coatings based on chitosan combined with different types of plasticizers and did not observed difference in weight loss among treatments. However, the authors verified a statistical trend indicating that use of sorbitol rather than propylene glycol or glycerol as a plasticizer was more efficient in reducing weight

loss. In addition, Dias et al. (2010) observed that films with rice flour and sorbitol were less permeable to water and more rigid, while films with glycerol were more plasticized and have poorer water vapor barrier properties. Sorbitol may offer a protective barrier against the transfer of carbon dioxide and moisture through the eggshell, thus minimizing weight loss and extending the shelf life of eggs (Lee et al., 1996).

The decreasing HU values with increasing storage time were supported by previous studies (Feddern et al, 2017; Xu et al. 2017). The higher the albumen height, the better the egg quality. When there is an increase in time and temperature, there will be a decrease in albumen height and consequently in UH. This occurs due to the hydrolysis of the amino acid chains that destroy the protein structure and release the bound water to the protein molecules, with fluidification and loss of viscosity occurring in the denser part of the albumen (Oliveira and Oliveira, 2013). The liquefaction of dense albumen is evidenced by the reduction of UH values. Kim et al. (2008) observed a higher HU value in coated eggs with chitosan compared to uncoated eggs. Despite the types of plasticizers, the coatings preserved the albumen quality of the coated eggs for at least 3 wk more than in uncoated eggs. Pires et al. (2018) already demonstrated that the use of rice protein coatings with glycerol can preserve the internal egg quality (grade A maintenance) for 3 wk longer compared to uncoated eggs.

Over time, the yolk absorbs the water derived from the degradation of the albumen, becoming flatter. The YI eggs decreased throughout the storage as already reported in previous studies (Caner and Yuceer, 2015; Pires et al. 2018). According to Oliveira and Oliveira (2013), YI must be 0.39-0.45 in good quality eggs. The higher the YI, the better is the quality of the yolk (Yuceer and Caner, 2014). Caner and Yuccer, (2015), demonstrated that the use of coating was able to preserve the YI for a longer

time than uncoated eggs. Kim et al. (2008) observed that chitosan coating, irrespective of plasticizer types, helped preserve the yolk quality of coated eggs for almost 3 wk longer than observed for noncoated eggs.

The albumen pH measures primarily the freshness of the egg because this variable is not affected by the age or strain of hen (Scott and Silversides, 2000). The pH of albumin and yolk can be altered as a result of biochemical changes in albumen occurring during storage and transfer of water from albumen to yolk. The increase in albumen pH causes a decrease in egg quality. The albumen pH increases with to the increase in the storage period of the egg and can reach 9.5 (Alleoni and Antunes, 2001). Yuceer and Caner (2014) found that the albumen pH of uncoated eggs stored for 6 weeks ranged from 8.95 to 9.69. The use of coatings can delays the loss of $CO_2$ through the pores of the eggshell, acting as a physical barrier. Previous studies (Kim et al., 2008; Caner and Yuceer, 2015) reported that different coatings were able to extend the shelf-life of eggs in relation to albumen pH, as also observed in the current trial

Few variations in pH of egg yolk were expected because the pH of the albumen increases during storage due to $CO_2$ loss and migrations of water from the albumen into the yolk during storage (Biladeau and Keener, 2009). Previous research has documented a maximum increase in yolk pH of 6.01 to 6.27 (Biladeau and Keener, 2009) and 6.2 to 7.05 (Pires et al. 2018). These values are in agreement with results found in this study, where there was an increase in the pH of the yolk from 6.26 to 7.06 during storage.

In general, the effects of coatings on albumen and yolks are favorable, indicating that the use of RPC-based coatings may be a viable alternative to maintain functional properties (HU, YI and pH) of the eggs, which are adversely affected by storage period. The use of sorbitol as a plasticizer in the coating is more efficient in maintaining control

of the increase in albumen pH. However, for the other variables the plasticizers showed similar results, being in this case the use of glycerol due to its cost and ease of acquisition.

## Conclusion

Rice protein coating can be used for extending the shelf life of eggs. The use of coatings with RPC is an effective way to preserve the interior quality of eggs during the storage in room temperature. The protein coating may improve the barrier properties. The loss of albumen and yolk quality can be influenced by the capacity of the coating to block the pores on the surface of the shell. The effects of coatings on albumen and yolks are favorable, indicating that the use of RPC-based coating may be a viable alternative to maintain to maintain the quality of eggs. The use of sorbitol rather than glycerol and propylene glycol as plasticizer was generally shown to be better in a preserving the albumen pH of rice protein-coated eggs during storage periods.

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**Table 6.1 -** Effect of rice protein concentrate (RPC) combined with different plasticizer coatings on cumulative weight loss (% in relation to week 0) of eggs during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC+GLY | RPC+PRO | RPC+SOR |
|---|---|---|---|---|
| 1 | $1.04\pm0.02^{Fa}$ | $0.77\pm0.02^{Fb}$ | $0.76\pm0.02^{Fb}$ | $0.56\pm0.02^{Eb}$ |
| 2 | $1.32\pm0.03^{Ea}$ | $1.03\pm0.02^{Eb}$ | $1.07\pm0.03^{Eb}$ | $1.07\pm0.04^{Db}$ |
| 3 | $2.59\pm0.02^{Da}$ | $1.75\pm0.03^{Db}$ | $1.71\pm0.04^{Db}$ | $1.62\pm0.05^{Cb}$ |
| 4 | $3.50\pm0.05^{Ca}$ | $2.36\pm0.08^{Cb}$ | $2.25\pm0.11^{Cb}$ | $1.68\pm0.08^{Cc}$ |
| 5 | $4.57\pm0.07^{Ba}$ | $3.59\pm0.10^{Bb}$ | $3.49\pm0.12^{Bb}$ | $3.46\pm0.08^{Bb}$ |
| 6 | $5.31\pm0.17^{Aa}$ | $4.29\pm0.14^{Ab}$ | $4.13\pm0.11^{Ab}$ | $4.07\pm0.15^{Ab}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($p < 0.001$); Storage periods ($P < 0.001$), and interaction (treatments by storage periods ($p < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($p < 0.001$).

[A-F] Means in the same column with different capital letters are significantly different ($p < 0.001$).

RPC: Rice protein coating; GLY: Glycerol; PRO: Propylene glycol; SOR: Sorbitol.

**Table 6.2 -** Effect of rice protein concentrate (RPC) and different plasticizer coatings on Haugh unit (HU) and egg grade[1] (designated after each mean in the parenthesis) during 6 weeks of storage at 20 ºC[2].

| Week | Control | RPC+GLY | RPC+PRO | RPC+SOR |
|---|---|---|---|---|
| 0 | 81.98±0.36(AA)$^{Aa}$ | 81.98±0.36(AA)$^{Aa}$ | 81.98±0.36(AA)$^{Aa}$ | 81.98±0.36(AA)$^{Aa}$ |
| 1 | 79.82±0.25(AA)$^{Ba}$ | 81.21±0.22(AA)$^{Aa}$ | 81.16±0.36(AA)$^{Aa}$ | 81.47±0.29(AA)$^{Aa}$ |
| 2 | 75.30±0.19(AA)$^{Ca}$ | 78.25±0.31(AA)$^{Ba}$ | 79.83±0.26(AA)$^{Ba}$ | 79.98±0.18(AA)$^{Ba}$ |
| 3 | 70.83±0.26(A)$^{Db}$ | 76.93±0.22(AA)$^{Ba}$ | 77.16±0.35(AA)$^{Ca}$ | 77.31±0.24(AA)$^{Ca}$ |
| 4 | 66.33±0.33(A)$^{Eb}$ | 71.86±0.18(A)$^{Da}$ | 72.36±0.20(AA)$^{Da}$ | 72.32±0.21(AA)$^{Da}$ |
| 5 | 63.34±0.19(A)$^{Fb}$ | 68.61±0.18(A)$^{Ea}$ | 68.78±0.23(A)$^{Ea}$ | 69.06±0.22(A)$^{Ea}$ |
| 6 | 58.40±0.17(B)$^{Gb}$ | 61.52±0.25(A)$^{Fa}$ | 62.71±0.24(A)$^{Fa}$ | 62.73±0.22(A)$^{Fa}$ |

[1] Egg grades: AA. HU >72; A. HU = 71–60; B. HU = 59–31; C. HU <30.
[2] Data are expressed as means (egg grades) ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($p < 0.001$). storage periods ($p < 0.001$). and interaction (treatments by storage periods $p < 0.001$).
[a-d] Means in the same row with different lowercase letters are significantly different ($p < 0.001$).
[A-D] Means in the same column with different capital letters are significantly different ($p < 0.001$).
RPC: Rice protein coating; Glycerol; PRO: Propylene glycol; SOR: Sorbitol.

**Table 6.3** - Effect of rice protein concentrate (RPC) and different plasticizer coatings on yolk index during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC+GLY | RPC+PRO | RPC+SOR |
|---|---|---|---|---|
| 0 | $0.48\pm0.01^{Aa}$ | $0.48\pm0.01^{Aa}$ | $0.48\pm0.01^{Aa}$ | $0.48\pm0.01^{Aa}$ |
| 1 | $0.44\pm0.01^{Bb}$ | $0.46\pm0.02^{Aa}$ | $0.45\pm0.03^{Bb}$ | $0.46\pm0.01^{Bb}$ |
| 2 | $0.40\pm0.01^{Cc}$ | $0.42\pm0.01^{Bb}$ | $0.42\pm0.01^{Bb}$ | $0.44\pm0.04^{Ca}$ |
| 3 | $0.38\pm0.01^{Dc}$ | $0.40\pm0.02^{Cb}$ | $0.41\pm0.01^{Da}$ | $0.41\pm0.01^{Da}$ |
| 4 | $0.36\pm0.02^{Eb}$ | $0.38\pm0.01^{Da}$ | $0.39\pm0.01^{Ea}$ | $0.40\pm0.02^{Ea}$ |
| 5 | $0.36\pm0.01^{Eb}$ | $0.38\pm0.01^{DEa}$ | $0.37\pm0.02^{Fab}$ | $0.39\pm0.01^{EFa}$ |
| 6 | $0.33\pm0.01^{Fb}$ | $0.37\pm0.01^{Eab}$ | $0.37\pm0.01^{Fab}$ | $0.38\pm0.02^{Fa}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($p < 0.001$). storage periods ($p < 0.001$). and interaction (treatments by storage periods $p < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($p < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($p < 0.001$).

RPC: Rice protein coating; GLY: Glycerol; PRO: Propylene glycol; SOR: Sorbitol.

**Table 6.4** - Effect of rice protein concentrate (RPC) and different plasticizer coatings on albumen pH during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC+GLY | RPC+PRO | RPC+SOR |
|---|---|---|---|---|
| 0 | $8.04\pm0.01^{Fa}$ | $8.04\pm0.01^{Ea}$ | $8.04\pm0.01^{Ea}$ | $8.04\pm0.01^{Fa}$ |
| 1 | $8.38\pm0.01^{Ea}$ | $8.07\pm0.01^{Eb}$ | $8.01\pm0.03^{Ec}$ | $8.10\pm0.02^{Eb}$ |
| 2 | $8.69\pm0.02^{Da}$ | $8.35\pm0.03^{Db}$ | $8.27\pm0.01^{Dc}$ | $8.22\pm0.01^{Dc}$ |
| 3 | $9.12\pm0.02^{Ca}$ | $8.47\pm0.01^{Cb}$ | $8.41\pm0.02^{Cc}$ | $8.44\pm0.03^{Cbc}$ |
| 4 | $9.21\pm0.01^{Ba}$ | $9.10\pm0.04^{Bb}$ | $9.11\pm0.01^{Bb}$ | $9.07\pm0.04^{Bb}$ |
| 5 | $9.52\pm0.01^{Aa}$ | $9.16\pm0.01^{Ab}$ | $9.16\pm0.01^{Bb}$ | $9.11\pm0.03^{Ac}$ |
| 6 | $9.52\pm0.01^{Aa}$ | $9.20\pm0.02^{Ab}$ | $9.19\pm0.02^{Ab}$ | $9.13\pm0.01^{Ac}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($p < 0.001$). storage periods ($p < 0.001$). and interaction (treatments by storage periods. $p < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($p < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($p < 0.001$).

RPC: Rice protein coating; Glycerol; PRO: Propylene glycol; SOR: Sorbitol.

**Table 6.5** - Effect of rice protein concentrate (RPC) and different plasticizer coatings on yolk pH during 6 weeks of storage at 20 ºC[1].

| Week | Control | RPC+GLY | RPC+PRO | RPC+SOR |
|---|---|---|---|---|
| 0 | 6.26±0.01$^{Ea}$ | 6.26±0.01$^{Ea}$ | 6.26±0.01$^{Da}$ | 6.26±0.01$^{Da}$ |
| 1 | 6.41±0.01$^{Da}$ | 6.30±0.01$^{Db}$ | 6.29±0.01$^{Db}$ | 6.29±0.01$^{CDb}$ |
| 2 | 6.58±0.02$^{Ca}$ | 6.52±0.01$^{Cb}$ | 6.42±0.01$^{Cc}$ | 6.34±0.01$^{Cd}$ |
| 3 | 6.91±0.04$^{Ba}$ | 6.47±0.01$^{Cb}$ | 6.44±0.01$^{Cb}$ | 6.33±0.01$^{Cc}$ |
| 4 | 6.97±0.05$^{Aa}$ | 6.44±0.01$^{Cb}$ | 6.44±0.01$^{Cb}$ | 6.36±0.01$^{Cc}$ |
| 5 | 6.96±0.02$^{Aba}$ | 6.71±0.01$^{Bb}$ | 6.68±0.01$^{Bb}$ | 6.66±0.01$^{Bb}$ |
| 6 | 7.06±0.02$^{Aa}$ | 6.79±0.02$^{Ab}$ | 6.83±0.01$^{Ab}$ | 6.72±0.02$^{Ab}$ |

[1] Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $p < 0.001$).

[a-d] Means in the same row with different lowercase letters are significantly different ($p < 0.001$).

[A-C] Means in the same column with different capital letters are significantly different ($p < 0.001$).

RPC: Rice protein coating; GLY: Glycerol; PRO: Propylene glycol; SOR: Sorbitol.

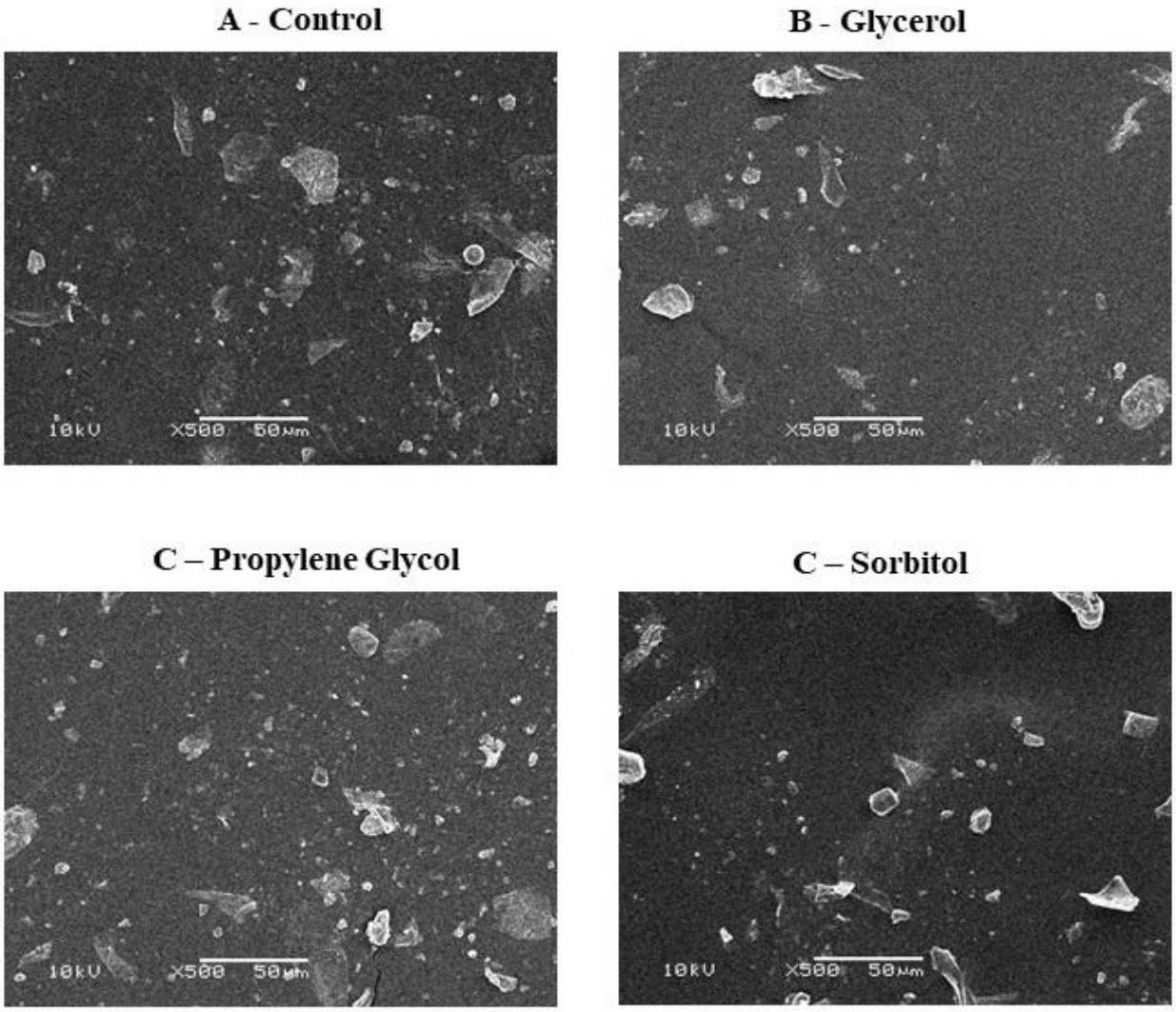


**Figure 6.1.** Scanning electron microscopy (×500) of uncoated eggshell (A) and coated eggs (B to D) after 6 wk of storage.

## 5. CONSIDERAÇÕES FINAIS

Os ovos são produtos de origem animal e perdem qualidade rapidamente logo após o momento da postura, sendo seu prazo de validade limitado de cerca de 3 a 4 semanas. Novas tecnologias de preservação são uma opção interessante para produzir produtos alimentícios de alta qualidade com uma vida útil prolongada em países onde a refrigeração não é obrigatória. Existe sim necessidade de desenvolver um método capaz de prolongar a qualidade interna dos ovos durante o armazenamento em temperatura ambiente. A vida útil dos ovos pode ser aumentada com o uso de revestimentos, o que beneficiaria a indústria, os produtores e os consumidores. Além disso, iniciativas como esta são oportunas em um cenário de demandas crescentes por alimentos acessíveis e de boa qualidade.

Os resultados encontrados neste estudo demonstram que os diferentes revestimentos à base de proteína concentrada de arroz com ou sem a incorporação de extrato de própolis ou diferentes óleos essenciais foram eficientes em preservar a qualidade de ovos armazenados em temperatura ambiente por até 42 ou 60 dias. O uso do sorbitol como plastificante na elaboração do revestimento se mostrou mais eficiente na manutenção do controle do aumento do pH do albúmen. Entretanto, para as demais variáveis, os plastificantes demonstraram resultados semelhantes, sendo neste caso indicado o uso do glicerol devido ao seu custo e facilidade de aquisição. Além das vantagens técnicas e produtivas, a utilização do arroz na preparação dos revestimentos é interessante pelo fato de que a produção do cereal predomina na região Sul do Brasil.

Futuros estudos podem focar na viabilidade do uso do revestimento em escala comercial e na análise do custo final para a sua produção. Por fim, a aplicabilidade real dos revestimentos desenvolvidos neste estudo será alcançada através de pesquisa em escala comercial.

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# APÊNDICES

## Apêndice 1: Produção bibliográfica e participações durante o curso

### Artigos publicados em periódico

ANDRETTA, I. ; HAUSCHILD, L. ; KIPPER, M. ; PIRES, P. G. S. ; POMAR, C. Environmental impacts of precision feeding programs applied in pig production. Animal, v. 11, p. 1-9, 2017.

FRANCESCHINA, C. S. ; PIRES, P. G. S. ; FRANCESCHI, C. H. ; MENDES, J.V . A utilização de fitase na dieta de poedeiras. Revista Eletrônica Nutritime, v. 13, p. 4529-4534, 2016.

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KIPPER, M. ; ANDRETTA, I. ; QUADROS, V. R. ; SCHROEDER, B. ; PIRES, P. G. S.; FRANCESCHINA, C. S. ; HICKMANN, F. ; FRANCA, I. . Performance responses of broilers and pigs fed diets with β-mannanase. Revista Brasileira de Zootecnia, 2019.

KIPPER, M ; ANDRETTA, I. ; RIBEIRO, A. M. L. ; PIRES, P. G. S. ; FRANCESCHINA, C. S. ; CARDINAL, K. M. ; MORAES, P. O ; SCHROEDER, B. . Assessing the implications of mycotoxins on productive efficiency of broilers and growing pigs. Scientia Agricola, 2018.

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ESPÍNDOLA, M. H. M.; ESPÍNDOLA, G.; DAHLKE, F.; HAUPTLI, L.; PIRES, P. G. S.; MORAES, P. O. Use of coating based on cassava starch on the quality of free-range eggs stored at room temperature. Ciência e Agrotecnologia, 2019.

### Textos em jornais de notícias/revistas

PIRES, P. G. S.; ANDRETTA, I. O uso de revestimentos a base de proteína de arroz na manutenção da qualidade de ovos armazenados. Revista do Ovo – Avisite. 2019.

PIRES, P. G. S.; ANDRETTA, I. . Estudo comprova que o revestimento de proteína de arroz e própolis pode ser alternativa para a manutenção da qualidade dos ovos. Revista ASGAV & SIPARGS, Porto Alegre, p. 30 - 31, 02 jan. 2019.

MELCHIOR, R.; PIRES, P. G. S. Arcabouço legal para a Postura Comercial e suas atualizações. A Revista do Ovo, v. 47, p. 12 - 13, 01 mar. 2018.

PIRES, P. G. S.. A necessidade de energia do gato aumenta no frio? Revista Pulo do Gato, p. 34 - 35, 01 set. 2015.

### Capitulo de livro

ANDRETTA, I. ; KIPPER, M ; PIRES, P. G. S. ; FRANCESCHINA, C. S. . Situação brasileira da ocorrência de micotoxinas em alimentos para suínos e meta-

análise do impacto produtivo. In: David E. Barcellos; Fernando P. Bortolozzo; Ivo Wentz; Mari Lourdes Bernardi; Ana Paula Mellagi; Rafael da Rosa Ulguim. (Org.). Avanços em sanidade, produção e reprodução de suínos. 1ed.Porto Alegre: UFRGS, 2017, v. , p. 103-113.

**Publicação de resumos em anais de eventos** (30 expandidos; 12 simples)

**Parecer para trabalho científicos:** Periódico: Revista Brasileira de Engenharia de Biossistemas; Revista de Ciências Agroveterinárias (UDESC); Revista Eletrônica Científica da UERGS; Periódico: Revista Brasileira de Saúde e Produção Animal.

**Participação em bancas:** (5 Trabalhos de conclusão de curso; 3 Eventos científicos)

**Premiações:** 2° Lugar - Talentos da Avicultura - Categoria Inovação e Pesquisa Avícola, Associação Gaúcha de Avicultura – ASGAV (2018);

Melhor trabalho científico na área de Marketing da carne suína, economia e extensão rural., VIII PorkExpo & Congresso Internacional de Suinocultura, PorkExpo (2016).

**Eventos:** (13 participações; 4 organização)

**Participação em projetos de pesquisa:**

Performance response of laying hens to digestible methionine intake;

Modelagem aplicada aos processos metabólicos e nutricionais de suínos e frangos de corte em diferentes contextos sanitários;

Avaliação do ciclo de vida da suinocultura brasileira: impactos ambientais de programas de alimentação convencional ou de precisão;

**Participação em projetos de extensão:**

Ações Para Capacitação E Formação Continuada Em Avicultura De Postura;

Grupo de Estudos em Suinocultura.

**Período de Doutorado Sanduíche na Université Laval, Quebec, Canadá (2018).**

# 7. VITA

Paula Gabriela da Silva Pires nasceu em Santana do Livramento, filha de Heron Vlademir da Rosa Pires e Leni da Silva Pires.

Em 2007 ingressou no curso de Medicina Veterinária da Universidade Federal de Pelotas – UFPel. Em 2012 e 2013 realizou Mestrado em Zootecnia na Universidade do Rio Grande do Sul, como bolsista CAPES. Durante o mestrado recebeu treinamento para executar a análise de inibidores de tripsina em Bogotá, Colômbia.

Em 2015, iniciou o doutorado em Zootecnia junto ao Programa de Pós-Graduação em Zootecnia da Universidade Federal do Rio Grande do Sul (UFRGS) na área de concentração Produção Animal, como bolsista CNPq. Em 2018, realizou estágio de doutorado na Université Laval, Quebec, Canadá, onde realizou o projeto Performance response of laying hens to digestible methionine intake.