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Internal quality of commercial eggs subjected to different shell treatments and storage times

Qualidade interna de ovos comerciais submetidos a diferentes tratamentos de casca e tempos de armazenamento

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Highlights

Albumen pH is not altered by cleaning the eggshell.
Storage time increases albumen pH.
Coverage of whey concentrate reduces pH in stored eggs.
Lipid oxidation is not affected by cleaning and coverage with whey protein concentrate.
Storage time increases lipid oxidation of egg yolks.

Abstract

Five hundred sixty eggs from commercial layers aged 74 weeks were subjected to sanitization and shell-covering procedures. The treatments consisted of two egg sanitization methods, sanitized (S) and not sanitized (NS), and two methods of shell covering, not covered (NC) and covered (C) with whey protein concentrate solution, arranged in a completely randomized design in a 2 × 2 factorial scheme (sanitization × shell coating), with five replications obtained from the average of four eggs evaluated in seven storage periods (1, 7, 14, 21, 28, 35, and 42 days). Albumen pH and yolk lipid oxidation using the TBARS test were evaluated. The averages for each period were submitted to analysis of variance, any differences observed were submitted to the Tukey test (5%), and regression analysis was performed between periods for each method. Using SDS-PAGE, the protein profile of albumen at 1 and 42 days was evaluated descriptively. Using methods S and NS, the pH and TBARS of eggs did not change on any of the days evaluated (P>0.05). Methods C and NC had similar pH values (P>0.05) only when using fresh eggs; however, the pH was higher when using eggs from Method NC (P<0.0001) compared with Method C. For all techniques, the analysis of pH regression between periods revealed quadratic behavior. While regression analysis demonstrated an increasing linear behavior for all methods, the TBARS analysis

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results for C and NC on all evaluated days were comparable (P>0.05). Whey protein concentrate does not affect the pH of sanitized eggs. The protein profile of the albumen and TBARS values of egg yolks stored at room temperature are unaffected by sanitation and whey protein concentrate covering, and TBARS values increase over storage days in all cases.
Key words: Albumen. Egg sanitization. Egg yolk. Protein-concentrated whey.

Resumo

Avaliou-se 560 ovos de poedeiras comerciais com 74 semanas submetidos a procedimentos de sanitização e recobrimento da casca. Os tratamentos utilizados consistiram de dois métodos de sanitização de ovos, sanitizados (S) e não sanitizados (NS), e dois métodos de cobertura da casca, não coberta (NC) e coberta (C) com solução concentrada de proteína de soro de leite, dispostos em delineamento inteiramente casualizado em esquema fatorial 2x2 (sanitização x recobrimento da casca), com cinco repetições obtidas da média de quatro ovos avaliados em sete períodos de armazenamento (um, sete, 14, 21, 28, 35 e 42 dias). Foram avaliados o pH do albúmen e a oxidação lipídica da gema pelo teste TBARS. Os resultados das médias de cada período foram submetidos à análise de variância e quando observado diferenças foram submetidas ao teste de Tukey (5%) e foi realizada análise de regressão entre períodos para cada método. Utilizando eletroforese em gel de poliacrilamida (SDS-PAGE), o perfil proteico do albúmen em um e 42 dias foi avaliado descritivamente. Utilizando os métodos S e NS, o pH e TBARS dos ovos não se alteraram (P>0,05) em nenhum dos dias avaliados. Os métodos C e NC apresentaram valores de pH semelhantes (P>0,05) apenas quando utilizaram ovos frescos; entretanto, ao utilizar ovos do Método NC, o pH foi superior (P<0,0001) ao do Método C. Para todas as técnicas, a análise de regressão do pH entre os períodos revelou comportamento quadrático. Embora a análise de regressão tenha demonstrado comportamento linear crescente para todos os métodos, o resultado da análise TBARS para C e NC em todos os dias avaliados foi semelhante (P>0,05). O concentrado protéico de soro de leite não afeta o pH dos ovos higienizados. O perfil proteico do albúmen e os valores de TBARS das gemas armazenadas em temperatura ambiente não são afetados pela higienização e pela cobertura do concentrado protéico de soro de leite, e os valores de TBARS aumentam ao longo dos dias de armazenamento em todos os casos.
Palavras-chave: Albúmen. Concentrado proteico. Gema de ovo. Sanitização de ovos.

Introduction

Several internal quality characteristics of eggs diminish over time (Barbosa et al., 2008; Uysal et al., 2017), and the rate of change in albumen and yolk is influenced by temperature and the movement of carbon dioxide through the shell (Almeida et al., 2016). If the shell is made impermeable to the loss of carbon dioxide, the internal quality of eggs stored at room temperature or in the refrigerator can be preserved for longer (Lana et al., 2017).

As the egg ages, chemical reactions inside the egg transform the dense albumen into a liquid, reducing its height. Carbonic acid (H_2CO_3), a component of the albumen's buffer system, may play a role in these reactions.

When carbonic acid decomposes, it emits water and carbon dioxide, which, under normal circumstances, pass through the shell and are lost to the environment (Almeida et al., 2016). Due to this H_2CO_3 release, the pH of the albumen rises, resulting in the chemical dissociation of the protein complex (Ordóñéz et al., 2005). The double bonds of unsaturated fatty acids present in the yolk are especially susceptible to oxidative degradation and may be responsible for the formation of peroxides and changes in odor, taste, texture, and color, as well as the loss of nutrients and the production of toxic compounds (Franchini et al., 2002).

The egg sanitization procedure enhances the appearance of commercialization (Almeida et al., 2016), thereby reducing the likelihood of contamination and the risk to food safety (Stringhini et al., 2009). Brazilian law (Ministério da Agricultura Pecuária e Abastecimento [MAPA], 1990) recommends that eggs be sanitized before breaking. However, the sanitization process is controversial due to the possibility of physical damage to the product if the protective cuticle covering the shell is damaged or removed. As a result, eggs are more susceptible to the exchange of gases, moisture, and microorganisms through the shell's pores, which hastens their decomposition (Stringhini et al., 2009).

When properly processed, whey proteins produce flexible, transparent, and odorless coverings that can close eggshell pores, reduce moisture loss, and transport gases, thereby extending the storage time (Almeida et al., 2016). As a result, the analysis of proteins still constitutes one of the most challenging tasks due to the heterogeneity of the protein fraction (Uysal et al., 2017).

The typical method used to assess lipid oxidation in fatty-acid-rich foods is the thiobarbituric acid (TBA) test because it is simple and fast. This test quantifies the level of malonaldehyde (MDA), which is one of the main products of the breakdown of hydroperoxides produced during the oxidation of polyunsaturated fatty acids (Giampietro-Ganeco et al. (2012). Based on the SDS-PAGE technique, the approach was proposed to determine the yolk:white ratio of liquid egg based on its protein components, which reveals changes in egg composition and enables the detection of liquid egg adulteration (Uysal et al., 2020).

The objective of this study was to evaluate the pH and protein profile of the albumen and the lipid oxidation of the yolk of commercial eggs subjected to sanitization and immersion in a solution of whey protein concentrate as a function of storage at room temperature.

Material and Methods

A total of 560 brown eggs, extra-type, originating from 74-week-old Hissex Brown, laid on a commercial farm, were used. After oviposition, 280 eggs were washed using a commercial Yamasa machine (model: LCHS-108.000) with water at room temperature and sodium hypochlorite solution (2 ppm), while the remaining 280 eggs were left unwashed. One of these two groups received a coating of whey protein concentrate solution (WPCS), while the other did not. The eggs were coated by submerging them for one minute in the WPCS solution and then drying them at room temperature. A method adapted from Antunes (2003) was

used to prepare the coating solution, which contained 539 g of WPCS (80% protein), 17.5 g of glycerol, and 500 g of water (w/w). After being slowly homogenized on a magnetic stirrer until complete dissolution, the mixture was immersed for 30 minutes in water at 90 °C. It was then cooled to 25 ºC, and the pH was adjusted to 7.0 with 1.0 N NaOH. All eggs were stored in previously sanitized plastic trays at room temperatures averaging 20.2 °C and humidity levels averaging 68%, as measured daily by an Incoterm brand digital thermo-hygrometer.

Thus, the treatments used consisted of two egg sanitization methods, sanitized (S) and not sanitized (NS), and two methods of shell covering, covered (C) and not covered (NC) with WPCS, evaluated in seven periods of storage (1, 7, 14, 21, 28, 35, and 42 days), arranged in a completely randomized design in a 2 × 2 factorial arrangement (sanitization × shell covering), with five replications obtained from the average of four eggs in each period.

After separation of the egg yolks, the albumens were placed in plastic flasks with lids, and the pH was measured using a digital pH meter with the electrode inserted directly into the albumen. Each evaluation period began with calibration of the device.

Using the TBARS values, lipid oxidation in the egg yolks was evaluated following the methods described by Vyncke (1970) and Ramanathan and Das (1992), with some modifications. The assay contained 100 L of the sample (raw egg yolk), 600 L of 7.5% acetic acid, 280 L of 0.02M TBA (thiobarbituric acid), and 20 L of BHT in a volume of 1 mL. After one minute of vortexing, the samples were heated in a water bath at 90 ºC for 40 minutes. After removal of the supernatant and centrifugation of the samples, the absorbance was measured with a digital spectrophotometer. Malonaldehyde was quantified using calibration curves created with known concentrations of malonaldehyde. In an acidic test environment, the reference material tetraethoxypropane (TEP) was hydrolyzed and released malonaldehyde. The initial pH and TBARS measurements were performed 12 hours after oviposition, and subsequent measurements were performed every seven days, always at the same time.

The samples were stirred continuously for 30 minutes on a magnetic stirrer to determine the protein profile of albumen. Following the modified procedure described by Stephan and Nascimento (2004), acetone was used as a solvent for the preparation and homogenization of the protein material. The mixture of 100 L of albumen, 400 L of acetone, and 5 L of PMSF (a protease inhibitor) was placed in a freezer for two hours. To suspend the proteins, the samples were centrifuged for 15 minutes at 13,000 × g in a chilled centrifuge. The sample was rehydrated with 1.0 mL of distilled water, 0.0028 g of DTT (dithiothreitol), 0.42 g of 7 M urea, 0.152 g of 2 M thiourea, and 0.02 g of CHAPS (2%). The protein concentration of the samples was then determined using a calibration curve, and the sample volume required to produce 30 g of protein was calculated. The sample was prepared by adding 3.0 mL of distilled water, 1.0 mL of Tris-HCl solution (pH 6.7), 1.6 mL of 100% glycerol, 1.6 mL of SDS 10%, 0.4 mL of 14 M-mercaptoethanol, and 0.4 mL of 0.5% bromophenol blue solution to a sample buffer. The sample and buffer were combined in a ratio of 2:1 (v/v) and boiled for five minutes. The samples were then subjected to SDS-PAGE using the Bio-

Rad electrophoresis system with a 12% running gel, a 4% packing gel, and a Bio-Rad molecular weight marker. The gels were run for approximately two hours at a voltage of 150 V with 40 mA of current and then stained overnight with "Coomassie Brilliant Blue R-250" (0.5 g of Coomassie Brilliant Blue R-250 in 500 mL of a solution containing 20% methanol, 8% acetic acid, and 76% distilled water). To decolorize the gels, a mixture of 20% methanol, 8% acetic acid, and 76% distilled water was applied overnight. The protein profile was determined by analyzing pools of five eggs from each treatment at 1 and 42 days of storage.

When the results for albumen pH and TBARS differed between methods, the means were compared using Duncan's test at a significance level of 5% (Statistical Analysis System [SAS], 2012). The results of the methods were subjected to regression analysis across time intervals. Through descriptive analysis of the results, the protein profile was evaluated.

Results and Discussion

The albumen pH (Table 1) was not altered by the S or NS methods ($P>0.05$); however, the albumen pH was similar between the C and NC methods only for fresh eggs ($P>0.05$); on the other days, eggs submitted to treatment C had a lower pH than NC ($P<0.0001$). The methods of disinfection and covering did not interact ($P>0.05$). The analysis of pH regression between days of storage for all methods (S, NS, C, and NC) revealed a quadratic behavior. However, each method's regression analysis revealed an increasing linear relationship between ages. The TBARS analysis of the yolks (Table 2) revealed no variation on any of the days evaluated ($P>0.05$), and there was no interaction ($P>0.05$) between the sanitation and covering methods.

According to all methods, the pH of albumen reached its peak after approximately 14 days of storage and then began to decline (Table 1). Giampietro-Ganeco et al. (2012) also discovered that the pH of the egg albumen tends to increase with storage in refrigerated eggs and then decreases, approaching that of a fresh egg after 56 days.

Except for the first day, unprotected eggs had higher pH levels. The rate of change in the pH of an egg during storage is influenced by the storage temperature and the flow of carbon dioxide through its shell. Carbonic acid (H_2CO_3), one of the elements of the albumen's buffer system, dissociates in these reactions to produce water and carbon dioxide, which diffuse through the shell and are expelled into the environment (Almeida et al., 2016). This release chemically dissociates the protein complex, increasing the pH of the albumen (Lana et al., 2017; Souza et al., 2020). Consequently, the WPCS-coated eggs maintained a lower pH than the uncoated eggs because, as stated previously, the covering can block the pores in the eggshell, reducing the transport of gases through the shell and the subsequent release of carbon dioxide. Although there were no differences between the methods on any of the days evaluated, the TBARS values steadily increased during storage. Consequently, there was a correlation between the amount of TBARS in the egg yolks and the storage time. The longer the storage time and exposure to oxidizing agents, the greater the formation of

malonaldehyde, one of the principal products of the decomposition of the hydroperoxides of polyunsaturated fatty acids formed during the oxidative process, and consequently, the greater the product degradation. Lipid oxidation, also known as rancidity, is the most significant deterioration that affects this kind of product and determines its shelf life. Gray (1978) found that lipid oxidation generates undesirable sensory products and destroys essential fatty acids and fat-soluble vitamins. This study supports the findings of Giampietro-Ganeco et al. (2012), who examined the effect of storage time on the oxidation of lipids in lyophilized yolks from commercial eggs. They discovered that TBARS values increased with egg age and that the oxidation of lipids in the yolks worsened with egg age.

Table 1
Albumen pH of eggs submitted to different methods of shell sanitization and covering with whey concentrate on different days at room temperature

Treatment	Storage day							Equation	R^2
	1	7	14	21	28	35	42		
Sanitized	8.80	9.06	9.61	9.51	8.89	9.17	9.11	$-0.001x^2 + 0.045x + 8.86$	0.36
Non-sanitized	8.76	9.00	9.63	9.51	8.86	9.16	9.10	$-0.001x^2 + 0.050x + 8.81$	0.34
Covered	8.73	8.86b	9.54b	9.47b	8.87b	9.13b	9.03b	$-0.001x^2 + 0.054x + 8.71$	0.41
Uncovered	8.82	9.20a	9.80a	9.54a	8.89a	9.19a	9.19a	$-0.0009x^2 + 0.043x + 8.94$	0.29
CV (%)	0.92	0.75	0.50	0.22	0.22	0.20	0.36		
			ANOVA						
Sanitization (S)	NS	NS	NS	NS	NS	NS	NS		
Coverage (C)	NS	<0.001	<0.001	<0.001	0.012	<0.001	<0.001		
S X C	NS	NS	NS	NS	NS	NS	NS		

Means followed by unequal letters between methods in the columns differ statistically (P<0.05)
Equations: H
R^2: coefficient of determination.

Figure 1 demonstrates that the sanitization and covering procedures, as well as storage times ranging from 1 to 42 days, did not affect the identity pattern of albumen proteins as determined by the SDS-PAGE technique.

Table 2
Analysis of TBARS (× 10^{-3} mg TEP kg^{-1}) of egg yolks submitted to different methods of sanitization and shell covering with whey concentrate on different days of storage at room temperature

Treatment	Storage day							Equation	R^2
	1	7	14	21	28	35	42		
Sanitized	0.424	0.502	0.537	0.581	0.629	0.678	0.851	0.0089x + 0.411	0.62
Non-sanitized	0.408	0.478	0.518	0.567	0.614	0.664	0.817	0.0088x + 0.394	0.56
Covered	0.420	0.483	0.514	0.576	0.607	0.674	0.803	0.0084x + 0.044	0.56
Uncovered	0.412	0.497	0.541	0.572	0.636	0.669	0.865	0.0093x + 0.402	0.56
CV (%)	24.3	15.5	15.0	10.7	18.9	15.3	20.4		
ANOVA									
Sanitization (S)	0.718	0.483	0.604	0.615	0.776	0.754	0.663		
Coverage (C)	0.868	0.679	0.446	0.891	0.594	0.917	0.428		
S X C	0.770	0.577	0.933	0.662	0.943	0.808	0.884		

Means followed by different letters between methods in the columns differ statistically (P<0.05)
R^2: Coefficient of determination.

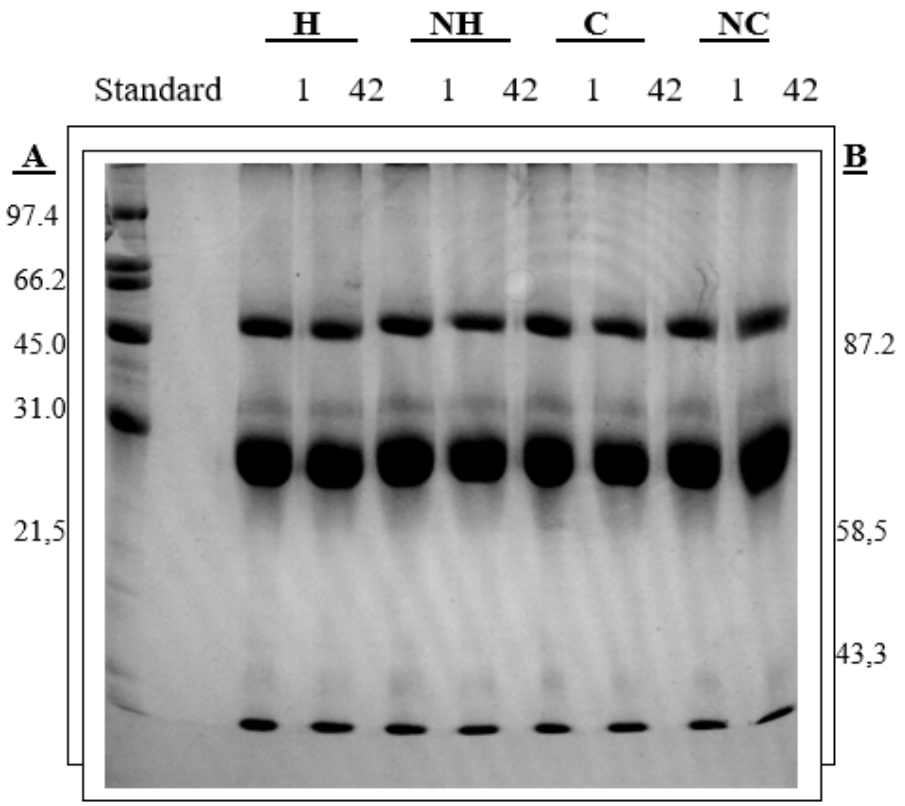

Figure 1. Identity pattern in samples of albumen protein obtained by polyacrylamide gel electrophoresis (SDS-PAGE), on the first (1) and 42nd (42) days of storage of sanitized (S), non-sanitized (NS), covered (C), and uncovered (NC) eggs.

Uysal et al. (2020) concluded that SDS-PAGE analysis is an excellent technique for assessing egg quality. In addition to the standard protein molecular weight markers (Bio-Rad, High Range), the migration profile of albumen proteins can be observed by the presence of three strongly stained bands with estimated molecular weights of 76.6 kDa, 45.0 kDa, and 14.4 kDa when subjected to the electric current of electrophoresis. All of the estimated molecular weights of the proteins in the gel, however, have been previously reported in the scientific literature (Sgarbieri, 1996) and correspond to those previously reported for ovotransferrin, ovalbumin, and lysozyme. This is because a marker with a high molecular weight was utilized, preventing the patterns of low-weight proteins from being visible on the gel, which only permits resolutions between 200 and 45 kDa. A weakly stained band with a molecular weight of approximately 58.0 kDa was also observed. This band was first described by Stephan and Nascimento (2004) when they examined the electrophoretic profile of organic chicken eggs. However, no description of a band with a comparable molecular weight as a crucial component of the protein pool that makes up egg albumen has been found in the scientific literature. According to the results of the albumen samples compared to the standard, 42-day-old eggs have the same electrophoretic profile as fresh eggs, indicating that the storage time does not affect the protein profile. In terms of both quality and quantity, the electrophoretic profiles of the coating techniques were comparable. This demonstrates that the 1-day and 42-day WPCS sanitizing and covering methods do not lead to alterations in the identity pattern of albumen protein during storage.

Conclusion

Whey protein concentrate does not affect the pH of sanitized eggs. The protein profile of the albumen and TBARS values of egg yolks stored at room temperature are unaffected by sanitation and whey protein concentrate covering, and TBARS values increase over storage days in all cases.

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References

Almeida, D. S., Schneider, A. F., Yuri, F. M., Machado, B. D., & Gewehr, C. E. (2016). Egg shell treatment methods effect on comercial eggs quality. *Ciência Rural, 46*(2), 336-341. doi: 10.1590/0103-8478cr2014 0904

Antunes, A. J. (2003). *Funcionalidade de proteínas do soro de leite bovino.* Manole.

Barbosa, N. A. A., Sakomura, N. K., Mendonça, M. O., Freitas, E., & Fernandes, J. B. K. (2008). Qualidade de ovos comerciais provenientes de poedeiras comerciais armazenados sob diferentes tempos e condições de ambientes. *ARS Veterinária, 24*(2), 127-133.

Franchini, A., Sirri, F., Tallarico, N., Minelli, G., Iaffaldano, N., & Meluzzi, A. (2002). Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamins E and C. *Poultry Science, 81*(11), 1744-1750. doi: 10.1093/ps/81.11.1744

Giampietro-Ganeco, A., Scatolini-Silva, A. M., Borba, H., Boiago, M. M., Lima, T. M. A., & Souza, P. A. (2012). Estudo comparativo das características qualitativas de ovos armazenados em refrigeradores domésticos. *Ars Veterinária, 28*(2), 100-104.

Gray, J. I. (1978). Measurement of lipid oxidation: a review. *Journal of the American Oil Chemists' Society, 55*(6), 539-546. doi: 10.1007/BF02668066

Lana, S. R. V., Lana, G. R. Q., Salvador, E. L., Lana, Â. M. Q., Cunha, F. S. A., & Marinho, A. L. (2017). Qualidade de ovos de poedeiras comerciais armazenados em diferentes temperaturas e períodos de estocagem. *Revista Brasileira de Saúde de Produção Animal, 18*(1), 140-151. doi: 10.1590/S1519-99402017000100013

Ministério da Agricultura Pecuária e Abastecimento (1990). Secretaria de Inspeção de Produto de Origem Animal. Portaria nº 01 de 21 de fevereiro de 1990. Normas Gerais de Inspeção de Ovos e Derivados. *Diário Oficial [da] República Federativa do Brasil.* Brasília, DF. http://sistemasweb.agricultura.gov.br/ sislegis

Ordóñéz, J. A., Rodrigues, M. I. C., Álvarez, L. F., Sanz, M. L. G., Minguillón, G. D. G. F., Perales, L. H., & Cortecerco, M. D. S. (2005). *Tecnologia de Alimentos: alimentos de origem animal.* Artmed.

Ramanathan, L., & Das, N. P. (1992). Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. *Journal of Agriculture and Food Chemistry, 40*(1), 17-21. https://scholarbank.nus.edu.sg/handle/10635/38079

Sgarbieri, V. C. (1996). *Proteínas em alimentos proteicos: propriedades, degradações, modificações.* Editora-Livraria Varela.

Souza, T., Lopes, C. C., Vieira, R. B., Santos, G. C., Silva, R. F., Calixto, E. S., & Azevedo, J. A. (2020). Quality of brown-shelled eggs marketed in different commercial establishments. *Acta Scientiarum. Animal Sciences, 42,* e46552. doi: 10.4025/actascianimsci.v42i1.46552

Statistical Analysis System (2012). *SAS users guide: statistics.* SAS Institute Inc.

Stephan, M. P. M., & Nascimento, G. F. do. (2004). *Desenvolvimento de método de preparação de amostras para a solubilização de proteínas de clara de ovo orgânico para aplicação em eletroforese.* (Comunicado Técnico). Centro Nacional de Pesquisa de Tecnologia Agroindustrial de Alimentos.

Stringhini, M. L. F., Andrade, M. A., Mesquita, A. J., Rocha, T. R., Rezende, P. M., & Leandro, N. S. M. (2009). Características bacteriológicas de ovos lavados e não lavados de granjas de produção comercial. *Ciência Animal Brasileira, 10*(4), 1317-1327. doi: 10.5216/CAB.V10I4.4209

Uysal, R. S., Boyaci, I. H., Soykut, E. A., & Ertas, E. A. (2017). Effects of heat treatment parameters on liquid whole egg proteins. *Food Chemistry, 216*(12), 201-208. doi: 10.1016/j.foodchem.2016.08.050

Uysal, R. S., Soykut, E. A., & Boyaci, I. H. (2020). Determination of yolk:white ratio of egg using SDS-PAGE. *Food Science and Biotechnology, 29*(2), 179-186. doi: 10.1007/s10068-019-00650-4

Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. *Fette-Seifen Anstrichmittel, 72*(12), 1084-1087. doi: 10.1002/lipi.19700721218