



## Albumen Foam Stability and S-Ovalbumin Contents in Eggs Coated with Whey Protein Concentrate<sup>(1)</sup>

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To Granja Bandeirantes, Comércio de Ovos e  
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### ABSTRACT

Food products such as breads, cakes, crackers, meringues, ice creams and several bakery items depend on air incorporation to maintain their texture and structure during or after processing. Proteins are utilized in the food industry since they improve texture attributes through their ability to encapsulate and retain air. The objectives of this work were to quantify s-ovalbumin contents in albumen and to determine alterations in egg white foam stability in fresh eggs, and in eggs coated and non-coated with a whey protein-based concentrate film (WPC), stored at 25°C for 28 days. The volume of drained liquid was higher in non-coated eggs than in coated eggs stored at 25°C at all storage periods. The difference on the third day of storage was in the order of 59% between coated and non-coated eggs, while on the twenty-eighth day it was 202%. During the storage period, an increase in pH and drainage volume was observed for non-coated eggs. After three days, the non-coated eggs showed a s-ovalbumin content 33% higher than coated eggs; this increase jumped to 205% at 28 days of storage. There was a positive correlation between s-ovalbumin content and the volume of drained liquid for coated and non-coated eggs; in other words, when the s-ovalbumin content increased, there was an increase in the volume of drained liquid and a decrease in foam stability. WPC coating maintain egg quality, since it is an effective barrier against the loss of CO<sub>2</sub>, avoiding changes in the pH of egg white.

### INTRODUCTION

Foams are used in the food industry in the manufacture of breads, cakes, crackers, meringues, ice creams, and several other bakery products (Pernell *et al.*, 2002). These food products depend on air incorporation to maintain their texture and structure during or after processing. Proteins encapsulate and retain air, improving desirable textural attributes (Phillips *et al.*, 1994). Proteins form and stabilize foam due to their amphiphilic behavior (polar/non-polar) (Du *et al.*, 2002).

Foaming properties of albumen are affected by several factors, such as protein concentration (Britten & Lavoie, 1992), composition (Johnson & Zabik, 1981), pH (Hammershøj *et al.*, 1999), ionic strength (Kato *et al.*, 1983), intermolecular interactions (Kinsella, 1984), heating, presence of salts, and composition of the liquid phase (Du *et al.*, 2002), which may change the configuration and the stability of protein molecules. These changes might affect the formation of the film and its properties on the interface, thus modifying foaming properties (Du *et al.*, 2002).

Egg white is a mixture of proteins that possess excellent foaming properties, since each of its components performs a specific function. Globulins facilitate foam formation, while the ovomucin-lysozyme complex (Cotterill & Winter, 1955) confers stability to the foam, and



both ovalbumin and conalbumin are heat-related (Yang & Baldwin, 1995). Conalbumin, lysozyme, ovomucin, and ovomucoid alone have little or no foaming ability, but the interaction between lysozyme and globulin is important to foam formation (Johnson & Zabik, 1981).

Ovalbumin is the largest protein in egg white and it contributes to foam formation properties in food systems where eggs are utilized as an ingredient. This phosphoglycoprotein represents 54% of the total protein in the albumen. S-ovalbumin is derived from ovalbumin, and was first reported by Smith (1964) and Smith & Back (1965). It is formed during the period of storage of shelled eggs and in ovalbumin solutions. There is no difference in amino acid composition between ovalbumin and s-ovalbumin. Ovalbumin deamidation may occur under alkali treatment or through egg white storage (Kato *et al.*, 1986). In most cases, the transformation of ovalbumin into s-ovalbumin brings about an increase in pH and temperature.

The constituents of heterogeneous protein systems, such as the egg white, have different isoelectric points and exhibit different charges. In the egg white, the electrostatic interactions contribute to the formation of an excellent heat-stable foam (Johnson & Zabik, 1981).

Coated eggs keep internal quality due to the reduction of the breakage of eggshell and egg microbial contamination (Wong *et al.*, 1995). Edible coating maintains the functional properties of foods by decreasing moisture loss and gas transport ( $O_2$  and  $CO_2$ ) (Donhowe & Fennema, 1994). Edible biopolymer coating made from whey proteins has attracted an increasing amount of research and industry attention, since it is an alternative of food packaging with reduced environmental and waste-disposal costs (Fang *et al.*, 2002).

The objective of this study was to evaluate changes in albumen pH, in egg white foam stability through drainage, and in s-ovalbumin contents in egg whites of chicken eggs coated with a whey protein concentrate, as a function of storage periods.

## MATERIAL AND METHODS

Eggs were collected right after laying in a poultry farm located in Sumaré – SP, Brazil. We utilized eggs from Hy-line w36 laying hens fed maize-based diet. The eggs were sanitized with 1% sodium hypochlorite for 30 s. A group of dried eggs were then immersed for 1 min in a solution of whey protein concentrate (WPC), and dried at ambient temperature for 24 h. A second group of eggs was not coated.

Eggs were stored in egg cartons, inside a BOD (Biological Oxygen Demand) incubator at 25°C. Determinations were made with three replicates per method and per analysis, at 3, 7, 10, 14, 21 and 28 days of storage. The experimental design was a 2x6 factorial arrangement, in which the main sources of variation were coating and time.

The coating solution was prepared according to the methodology described by Gennadios *et al.* (1993), containing 8% protein from the whey protein concentrate (WPC), 3.5% glycerol, and completed with 100g water (w/w). The solution was homogenized and submerged in water at 90°C for 30 min. After cooling to 25°C, pH was adjusted to 7.0 with 1.0 N NaOH.

The albumen pH was obtained with a Micronal B374 potentiometer. The egg white was separated from the yolk. After stirring lightly, pH was obtained. Foam stability was measured by the Mckellar & Stadelman (1955) method. Egg whites (28g) were placed in a 250-mL plastic beaker and kept in a double boiler until the temperature of the egg white reached 30°C. The egg whites were beaten for 120 s (value in the range recommended by Trziszka, 1993) at 2,200 rpm with a brand multimixer. The amount of drainage was measured with a graduated cylinder after the foam was allowed to rest for 30 min.

The s-ovalbumin content of the egg white was measured by the method of Smith & Nguyen (1984). Five grams of egg whites were placed in a 100-mL beaker, and 25 mL of 0.5 M phosphate buffer pH 7.5 were added; the mixture was agitated for 5 min with a magnetic stirrer. Afterwards, 5 mL of the suspension were placed in two test tubes; one of the tubes was heated at 75°C for 30 min. Upon cooling, 5 mL precipitating solution were added and the solution was transferred to centrifuge tubes, with the addition of another 5 mL precipitating solution. After resting for 10 min, the tubes were centrifuged at 10,000 rpm for 5 min and the supernatant was filtered. Two mL of the supernatant was placed in a test tube with 4mL Biuret solution. This supernatant aliquot was left to rest for 30 min, and the absorbance was read at 540 nm, in a Beckman DU-70 spectrophotometer.

Multiple comparisons of the means were performed for statistical analysis involving storage times and coating and non-coating process. An orthogonal contrast (treatments x fresh eggs) was applied. Means were compared by Tukey's test, when applicable.



## RESULTS AND DISCUSSION

The statistical analyses for volume of drained liquid from egg white foam and for s-ovalbumin content indicated that there were interactions between coating and storage time. Therefore, regressions were applied in both cases, and the results were interpreted within each storage period. Results from fresh egg (day 0) were used as reference.

### Foam Stability (expressed as drainage)

In all storage periods, the volume of drained liquid from the albumen foam was greater in the non-coated eggs (Table 1). As time passed, the films became progressively thinner and ruptured. Throughout the storage period, the fluid is lost by lamellar water drainage, resulting in a foam collapse (Phillips, 1981). During drainage, the van der Waals forces increase between films in adjacent bubbles (Phillips *et al.*, 1994).

On the 3<sup>rd</sup> day of storage, the difference in drainage volume was in the order of 59% between coated and non-coated eggs, while on the 28<sup>th</sup> day it was 202%. The results for volume of drained liquid were fitted to linear regressions with  $r^2 = 0.86$  for non-coated eggs, and  $r^2 = 0.84$  for coated eggs. The pH and the volume of drained liquid in coated eggs were 8.01 and 1.5 mL, respectively (Table 1). In non-coated eggs, the pH was 9.09, and the volume was 2.38 mL, in the same period. At 28 days of storage, the difference between coated and non-coated eggs was greater (Table 1), with a 13.3% increase between pH values, and a 202% increase between drainage values. Thus, the amount of liquid drained from the egg white foam of non-

coated eggs was three times greater than in coated eggs within this pH range.

The pH in the aqueous phase determines the magnitude and the nature of protein charges and therefore affects the foam (Kinsella & Phillips, 1989). The pH values in egg whites of coated eggs were always smaller than the values for non-coated eggs (Table 1). This could be due to the effect of coating on impairing albumen CO<sub>2</sub> loss through the shell, which would prevent expressive increases in pH.

During the entire storage period, the albumen pH of coated eggs remained around 8, while the variation in volume of drained liquid was 1.5 mL at three days and 3.3 mL at the end of the period. The foam stability behavior could be related to the pI values of most egg white proteins, which are between 4 and 6. Thus, proteins are negatively charged in the albumen. The positively charged lysozyme (pI=10.7) has a vital role in the formation and stability of the egg's albumen foam (Poole *et al.*, 1984). During foam formation, lysozyme and other negatively charged proteins may migrate to the air-liquid interface. On this interface, lysozyme interacts electrostatically with other negatively charged proteins, causing a reduction in the repulsive electrostatic interactions on the protein layer and in albumen foam stabilization (Poole *et al.*, 1984; Clark *et al.*, 1988; Damodaran, 1989; Damodaran, *et al.*, 1998).

Considering only the non-coated eggs, an increase in pH and drainage volume was observed during the storage period. The high drained liquid value is related to an increase in egg white pH, which interferes in the lamellar fluid viscosity, causing an approximation between the adjacent air bubbles. Consequently, bubble rupture and coalescence will occur, resulting in

**Table 1** - Volume of drained liquid, s-ovalbumin contents and pH of egg white foam stored at 25°C, as a function of storage period and coating with whey protein-based film (WPC).

Storage Period	Coating (WPC)	pH Albumen	Drained Liquid ML	S-ovalbumin%
Fresh egg	-	7.93 d ± 0.16	1.08 j ± 0.04	18.15 k ± 0.13
3 days	With <sup>1</sup>	8.01 d ± 0.13	1.50 i ± 0.03	18.52 j ± 0.06
	Without	9.09 b ± 0.17	2.38 h ± 0.04	24.68 g ± 0.31
7 days	With	8.03 d ± 0.10	2.36 h ± 0.16	23.33 h i ± 0.32
	Without	9.24 b ± 0.06	5.04 d ± 0.15	55.02 e ± 0.56
10 days	With	8.07 c d ± 0.15	2.59 g h ± 0.19	23.21 i ± 0.13
	Without	9.35 a b ± 0.06	6.28 c ± 0.08	61.99 d ± 0.27
14 days	With	8.21 c ± 0.10	2.89 f ± 0.05	22.95 i ± 0.25
	Without	9.47 a ± 0.10	8.52 b ± 0.23	65.34 c ± 0.44
21 days	With	8.23 c ± 0.14	2.89 f g ± 0.17	23.86 g h ± 0.55
	Without	9.46 a ± 0.08	9.86 a b ± 0.12	72.75 b ± 0.77
28 days	With	8.33 c ± 0.17	3.30 e ± 0.12	26.71 f ± 0.07
	Without	9.44 a ± 0.08	9.96 a ± 0.08	81.46 a ± 0.58

<sup>1</sup> - Means in the columns followed by same letter are not different (p > 0.05).



liquid drainage and foam destabilization (Phillips *et al.*, 1994).

Even though the positive effect of lysozyme on the properties of several acid proteins can be experimentally observed, there is no direct evidence of the formation of an electrostatic complex between lysozyme and the acid proteins in the film absorbed on the air-water interface (Damodaran *et al.*, 1998). However, several acid proteins form insoluble electrostatic complexes with lysozyme (Poole *et al.*, 1984; Damodaran & Kinsella, 1986), and insoluble complexes are usually not good foaming agents (Damodaran *et al.*, 1998).

### S-Ovalbumin

S-ovalbumin content was higher in non-coated eggs in all storage periods (Table 1). At three days, the non-coated eggs showed a content 33% higher in relation to coated eggs. At 28 days of storage, however, this difference was three times higher.

The pH in coated eggs stored at 25°C was around 8, and the s-ovalbumin content remained around 18%. Only at the 28th day its content reached 27%. Alleoni (1997) worked with periods and two chicken egg storage temperatures, and obtained similar values at the refrigeration temperature as those obtained for coated eggs in the present study. That author reported s-ovalbumin contents equal to 18.4%, 26.2% and 24.3% in eggs storage at 8°C for 7, 14, and 21 days, respectively. In the present study, the s-ovalbumin values of coated eggs were 23.3%, 22.9% and 23.9%, for the same storage periods. When the eggs were stored at 25°C, Alleoni (1997) obtained 55.7%, and 69.3%, for 7 and 14 days, respectively, while in the present work, contents were 55.0% and 65.3% in the same storage periods. Therefore, WPC coating had the same effect as the refrigeration temperature.

Ovalbumin can be adsorbed in aqueous solutions on the air-water interface and acts as a surfactant, reducing the surface tension and forming a cohesive film. The behavior of proteins on air-water interfaces influences the formation and stability of foam (Du *et al.*, 2002). As the pH increases, part of the egg white ovalbumin is transformed into s-ovalbumin. In view of this, the amount of ovalbumin in the egg white decreases, and this could interfere with the formation of a cohesive film on the air-water interface, causing a decrease in foam stability. S-ovalbumin is less hydrophobic than ovalbumin (Nakamura *et al.*, 1980; Schäfer *et al.*, 1999).

Hagolle *et al.* (2000) obtained the best foaming properties at 85°C for ovalbumin and at 75°C for

lysozyme; these temperatures are near the denaturation temperature of both proteins. As heat is applied, the tertiary and the secondary structures of the ovalbumin molecule become denatured, causing strong hydrophobic interactions between proteins, which lead to aggregation (Du *et al.*, 2002). It is possible to stabilize foams using denatured ovalbumin (Relkin *et al.*, 1999). Under acidic conditions, ovalbumin has greater flexibility and surface hydrophobicity.

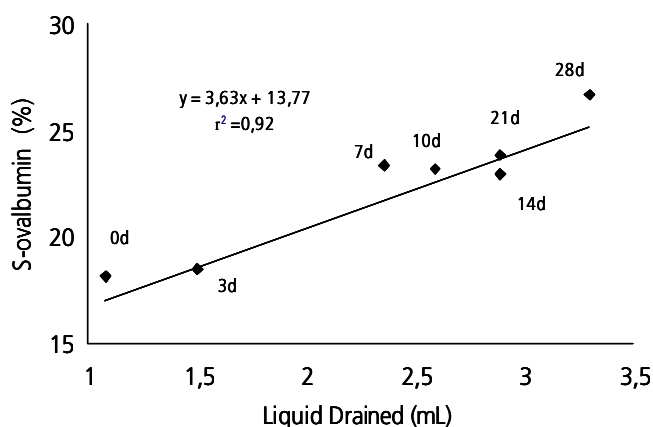
Ovalbumin plays an important role in egg white foam stability (Relkin *et al.*, 1999; Hagolle *et al.*, 2000; Du *et al.*, 2002). Considering the results obtained for non-coated eggs at 28 days of storage, in which the s-ovalbumin content reached 80 % and the amount of ovalbumin in the egg white decreased, a greater loss of egg white foam stability was observed in that storage period.

Schäfer *et al.* (1999) stored eggs for six weeks at 30°C and evaluated the composition of proteins in the egg white. No changes in lysozyme activity or in the amount of conalbumin were observed during storage. However, the ovalbumin pI decreased, which is in agreement with the formation of s-ovalbumin during storage. Changes were more influenced by temperature than storage period.

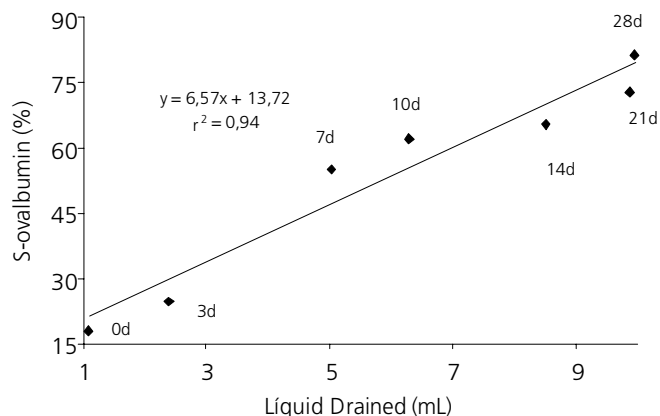
In eggs stored without coating, the activity of lysozyme does not decrease during storage, and the amount of conalbumin does not change (Schäfer *et al.*, 1999). Therefore, the s-ovalbumin contents increase in the same proportion as the volume of drained liquid. In the present work, the smallest values for volume of drained liquid and for s-ovalbumin contents were obtained with coated eggs. Under these conditions, the pH was maintained near 8. The smallest rate of conversion of ovalbumin into s-ovalbumin is verified at pH 8.5 (Smith & Nguyen, 1984).

In non-coated eggs, egg white pH varied from 9.1 to 9.4 during the storage period, while the volume of drained liquid ranged between 2.38 and 9.96 mL (Table 1), and the s-ovalbumin contents ranged between 24.7 and 81.5 % (Table 1). Thus, this protein could be related to a decrease in foam stability, despite the fact that the mechanisms involved are still unknown. The correlation between s-ovalbumin content and the volume of drained liquid were positive for both coated and non-coated eggs (Figures 1 and 2).

As s-ovalbumin content increased, the volume of drained liquid also increased, i.e., foam stability decreased. Therefore, it can be concluded that WPC coating maintain egg quality, since it is an effective barrier against the loss of CO<sub>2</sub>, avoiding changes in the pH of egg white.



**Figure 1** - Linear correlation between the volume of drained liquid and s-ovalbumin contents of coated eggs, during the storage period.



**Figure 2** - Linear correlation between the volume of the drained liquid and s-ovalbumin contents of non-coated eggs, during the storage period.

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