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Emulsifying properties of quail egg white proteins in different vegetable oil emulsions

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ABSTRACT. Emulsifying properties of oil in water emulsions using quail egg white protein (*Coturnix coturnix japonica*) as an emulsifying agent were investigated using the conductivity technique. Changes in emulsion conductivity were recorded during and after homogenization. The results were interpreted in terms of properties related to the emulsifying activity and emulsion stability. The effect of salt concentrations (NaCl) of 0.0, 0.29, 0.59, 1.17, 1.76, and 2.34% (w v^{-1}) when mixed with egg white concentrations of 0.50, 1.00 and 1.50% (w v^{-1}) were studied using corn and soybean vegetable oils. Globally, one observed that emulsifying activity and emulsion stability increase with the enhancement of salt concentration. However, the increase of the egg white concentration did not present a significant influence on emulsifying activity, causing an increment only in emulsion stability.

Keywords: functional properties; protein concentration; salt concentration; conductivity; emulsifying activity; emulsion stability.

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Introduction

The Japanese quail culture has little initial investment and fast return of capital. This has created a significant impact in recent years, and many quail farms have been established in different countries both for egg and meat production. About 10 quails require the same amount of space needed for one chicken. Furthermore, the quail is efficient for egg-laying. Quails can begin to lay eggs when they reach an age of 35 to 45 days. They can lay an average of up to 300 eggs per year, and have a life span of 2.5 to 3 years meaning that quails can produce up to 900 eggs of 10 g in their lifetime. The birds require smaller housing for rearing, which results in increased labor efficiency, and better utilization of land space. Therefore, quails have been farmed in many parts of the world and used for different purposes (Priti & Satish, 2014).

Eggs and egg products are used in the food industry because of their nutritional value and functional properties (Stadelman, Newkirk, & Newby, 2017). As for the nutritional criteria, the quail eggs are far better when compared to chicken eggs, and they have low cholesterol percentage as well. Egg proteins are mainly located in the egg white; however, a small number of proteins in the egg yolk are conjugated with carbohydrates or lipids to form glycoproteins or lipoproteins (Yu, Yin, Zhao, Chen, & Liu, 2014). The proteins found in quail eggs present high biological value. Therefore this egg is considered an excellent food in the composition of the human diet, containing practically all essential nutrients for human life (Jeke, Phiri, Chitiindingu, & Taru, 2018). Besides, the high concentration of protein present in the quail egg white favors the application of its functional properties in different food processes (Segura-Campos et al., 2013).

One of these functional properties is the ability of emulsification (Mine & Zhang, 2013). It is a relevant functional property in many food processes and is commonly discussed in terms of emulsion stability and emulsifying activity (Kudre, Bejjanki, Kanwate, & Sakhare, 2018). Emulsifying activity (EA) can be defined as the ability of the active surface of the molecules (proteins or phospholipids) to cover the oil/water interface created by the mechanical homogenization (Aryee, Agyei, & Udenigwe, 2018). Emulsion stability (ES) measures the capacity of the proteins to maintain the homogeneous mixture when it is submitted to an external force or heat. Concentration and solubility, the pH of the medium, and the contents of sucrose and salts affect the protein emulsifying properties for most of the proteins (Lam & Nickerson, 2013; Santos et al., 2015; Taha et al., 2019; Zhang, Zhou, Zhong, Tan, & Liu, 2019). The proteins adsorbed in emulsions interface are usually electrically charged, especially at pH values far from the isoelectric point. As a consequence, the main mechanism preventing droplet flocculation and phase separation in protein-stabilized emulsions is electrostatic repulsion, rather than steric repulsion (Gulão, Souza, Costa, Rocha-Leão, & Garcia-Rojas, 2018). In the presence of salts, emulsions stabilized by proteins tend to flocculate when ionic strength reaches high levels. Thus, the electrostatic repulsion is not strong enough to overcome the attractive interactions, like van der Waals, hydrophobic, or depletion (Dickinson, 2019). Other factors such as temperature, equipment design, and nature of the proteins also have been investigated as influencers on the formation and stability of emulsions (Amine, Dreher, Helgason, & Tadros, 2014; Lam & Nickerson, 2015; Li & Xiang, 2019; Zhao et al., 2019).

Several authors have studied the emulsifying properties of chicken egg white (Abeyrathne, Lee, & Ahn, 2013; Iqbal, Batool, Ajaz, Ambreen, & Akhlaq, 2017; Huda, Tze, Dewi, & Hashim, 2018; Wang, Horimoto, Nau, & Hatta, 2018). Despite studies showing that the functional properties of quail egg proteins are superior to chicken egg proteins (Kudre et al., 2018), studies on physicochemical and functional properties of Japanese quail egg proteins are still limited, and very few studies evaluating its emulsifying properties have been found in the current literature (Segura-Campos et al., 2013; Kudre et al., 2018; Velioglu, 2019). These studies focused on the evaluation of the emulsifying activity and emulsion stability concerning the protein concentration, different pH values, and different drying treatments to obtain the quail egg powder. Information on the effect of the emulsifying properties of quail egg proteins has not yet been addressed.

Therefore, the purposes of this work were to study the emulsifying properties of quail egg white and also the effects of egg white concentration, salt concentration, and oil type (corn and soybean) on EA and ES. Emulsifying properties were determined using the conductivity curve of the emulsion versus the time after the homogenization of oil in the aqueous protein solution.

Material and methods

Material

The eggs used in the experiment were donated by the Experimental Poultry House of the Animal Science Department of the *Universidade Federal de Viçosa* (UFV), where Japanese quails (*Coturnix coturnix japonica*) were bred. Egg white was separated manually, frozen, and lyophilized (Edwards L5KR, USA) under the following conditions: the condenser temperature was -40°C, the tray temperature was 0°C. The vacuum pressure supplied by the pump was of 10 μHg. All the experiments were performed using distilled water and analytical grade reagents. The soybean (Cocinero, Brazil) and corn (Velero, Brazil) oils were obtained from a local market. Both oils have gone through similar processes, direct extraction using organic solvents. Experiments were performed in a factorial scheme with a completely randomized design, comprising 36 treatments and 2 repetitions.

Conductivity measurement of the emulsion

Emulsifying properties were determined by the specific electrical conductivity of the emulsion, described by Kato, Fujishige, Matsudomi, and Kobayashi (1985) with some modifications (Kato et al., 1985). Two hundred millimeters of egg white solution in different concentrations (0.50, 1.00 and 1.50% w v^{-1}) and 50 mL of the oil (corn or soybean) were homogenized at 12500 rpm (Mix Walita, HL 3124, Brazil) for 1.5 min. at (25 ± 2°C). Solutions were prepared with distilled water adjusted to a pH of 7.0 by the addition of a sodium phosphate buffer. In order to analyze the effect of the salt concentration, 0.00, 0.58, 1.16, 2.30, 3.50, and 4.60 g of NaCl were added to the protein aqueous solutions to reach saline concentrations of 0.00, 0.05, 0.10, 0.20, 0.30, and 0.40 mol L^{-1}, respectively. The conductivity of the emulsion was measured continuously during homogenization and 13.5 min. after the completion of homogenization. The apparatus used was similar to that developed by Al-Malah, Azzam, and Omari (2000). An electrode was fixed on the bottom of a beaker containing the emulsion (Figure 1), connected to a conductivity meter (Orion 145 A+, USA). The methods selected for calculation of EA and ES were also adapted from Azzam and Omari (Azzam & Omari, 2002).

Determination of emulsifying properties

Emulsifying activity data were obtained from Equation 1.

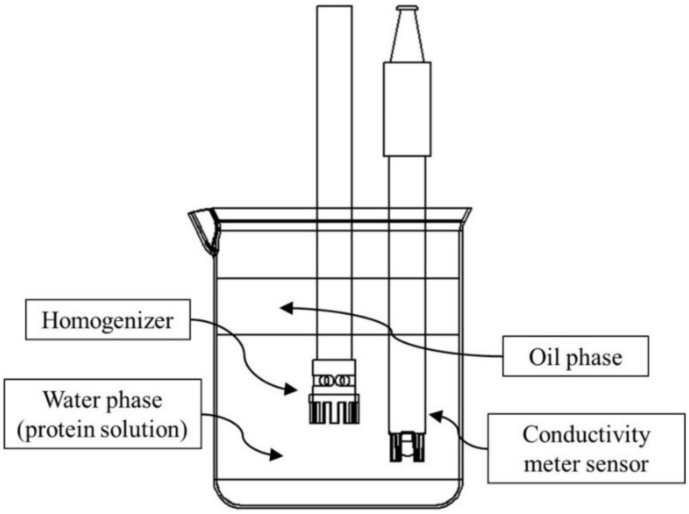

Figure 1. Schematic diagram of the experimental setup for conductivity measurements of quail egg white stabilized emulsions.

$$EA = C_0 - C_e(1) \quad (1)$$

where: C_0 (mS·cm^{-1}) is the conductivity of the protein solution before the homogenization, and C_e (mS·cm^{-1}) is the minimal conductivity reached during 3 min. of homogenization.

Emulsifying stability data were obtained using Equation 2.

$$ES = {}^{\text{EA x }\tau 50}/_{[(\text{CT} - \text{CB}) \text{ x S}\tau 50]} \quad (2)$$

where: CT (mS·cm^{-1}) is the maximal conductivity after stopping the homogenizer, CB (mS·cm^{-1}) is the minimal conductivity after the homogenizer was stopped, τ50 (min.) is the time for the conductivity to reach the value of [(CT - CB) x Sτ50], and Sτ50 (min.·cm·mS^{-1}) is the parameter which describes the declivity of the curve in τ50. Values of τ_{50} and $S_{\tau 50}$ were estimated in each case according to the sigmoidal function.

Statistical analysis

Experimental data of solubility are analyzed using the SAS® version 9.0 statistical package and REG procedure (Statistical Analysis System [SAS], 2004).

Results and discussion

Emulsion conductivity

Figure 2 shows representative conductivity curves for the emulsion of soybean oil and quail egg white at three different concentrations. Curves for both oils and different salt concentrations presented similar shapes that were close to that of a sigmoidal function. Before homogenization, high conductivity of the protein solution was observed due to the presence of conductive particles, such as charged proteins and ions. The Rotor-Stator Mixer homogenizer was used, which in addition to providing agitation and mixing, increases the solution's shear rate (Håkansson, 2019). Consequently, there is a decrease in the diameter of the oil drops, which favors the oil-protein interaction by increasing the surface area of the oil. The interaction between oil, protein, and ions solvated generates particles with a larger size than the particles present in the initial solution. The larger the size of the particles, the lower their mobility, and therefore, the lower the electrical conductivity of the solution (Palaniappan & Sastry, 1991).

An increase in conductivity after homogenization is an indication of phase separation, suggesting that the dispersed oil droplets coalesced to form a top phase (Kato et al., 1985). A greater number of hydrophobic amino acids is present in the white of a quail egg (Dey, 2012), and the exposure of hydrophobic regions in the surface of the protein is caused by the presence of salt (Jiang et al., 2015), making the hydrophobic interaction between the protein and the surface of the oil droplet stronger. Thus, the protein tends to remain absorbed at droplets interface after phase separation, while the ions might have remained in the bottom aqueous phase. Since these free ions present higher conductivity than the proteins, the final system conductivity showed greater value than the initial conductivity before the homogenization step. Regarding the effect of salt concentration, one can observe that the conductivity curves showed similar behaviors, although the magnitude of conductivity increased with increasing salt concentration (Figure 1, supplementary material). This increase in conductivity magnitude might be directly related to the increase of ions concentration, which facilitates the passage of electricity through the medium (Golnabi, Matloob, Bahar, & Sharifian, 2009).

Emulsifying activity

Figure 3 shows the EA as a function of egg white concentrations, NaCl concentrations for corn (Figure 3a), and soybean oil (Figure 3b). One found that emulsifying activity increased with higher NaCl concentrations for both evaluated oils. No systematic behavior for emulsion activity was observed regarding the oil type. Since soybean and corn oils show very similar physicochemical properties (Ayyildiz, Topkafa, Kara, & Sherazi, 2015), such as fatty acid profile, viscosity, and density, it was already expected that emulsifying activity regardless the oil used.

Before and during the homogenization step, it is likely that the proteins were solvated by counterions (ions of opposite net charge). It led to greater exposure of hydrophobic regions in the protein structure and resulted in higher adsorption of these molecules in the oil/water interface. Zayas (2012) reported that the protein surface loads of emulsions stabilized by β-lactoglobulin increased with increasing NaCl concentration (5-1000 mM, pH 6.2). This was probably due to electrostatic screening, which reduced electrostatic repulsion with increasing concentration of NaCl and facilitated the protein adsorption at the interface (Rangsansarid & Fukada, 2007; Taha et al., 2019). The protein adsorption in oil/water interface during homogenization decreases its mobility in the medium when compared to the initial solution. This might explain the decrease in emulsion conductivity during the homogenization step.

Regarding egg white protein concentration, one observed that the EA did not suffer significant variations with the changes in egg white concentrations for both soybean and corn oils. However, the emulsifying activity of the proteins varies according to its concentrations, and it depends on the interfacial area of the dispersed oil droplets (Sun & Gunasekaran, 2009). We suppose that the protein concentration range evaluated was enough to diffuse and adsorb in the oil/water interface. Since the homogenization was mild when compared to the conventional high energy processes, the droplets formed were quite big. Thus, the interfacial area available to protein adsorption was small, so the smallest protein concentration (0.50% w v^{-1}) was enough to saturate the interface.

Two first-order polynomial models were used to adjust the experimental EA data according to the NaCl concentration for corn oil (EA_c), Equation 3, and for soybean oil (EA_s), Equation 4:

$$EA_C = 2.60289 + 31.05965\, C_{salt} \quad (3)$$

$$EA_S = 1.94091 + 28.41860\, C_{salt} \quad (4)$$

where: C_{salt} is the salt concentration.

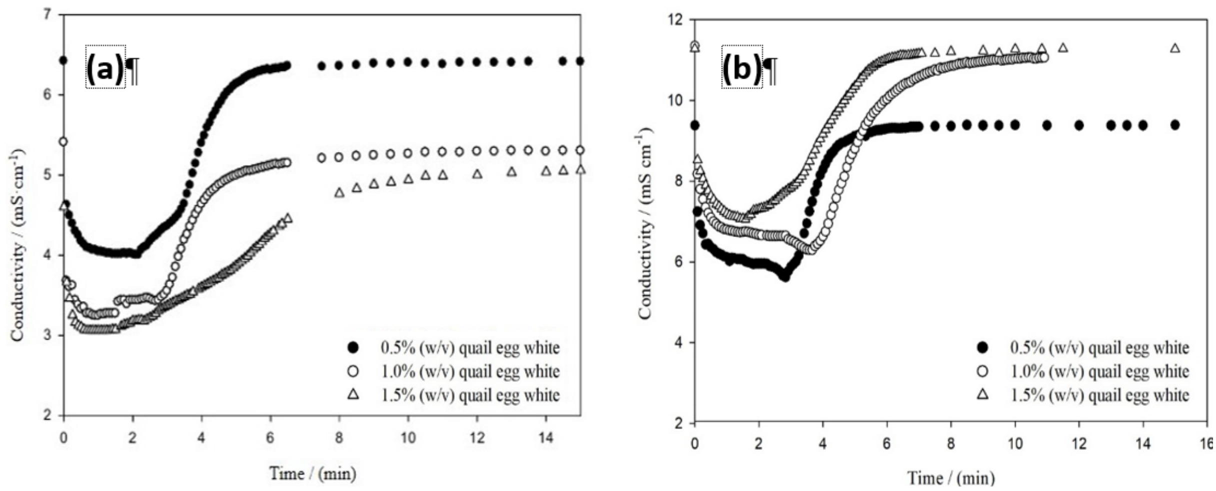

Figure 2. Conductivity curves for the three evaluated quail egg white concentrations during analysis time for (a) soybean and (b) corn oil emulsions and 0.0 mol L^{-1} of NaCl.

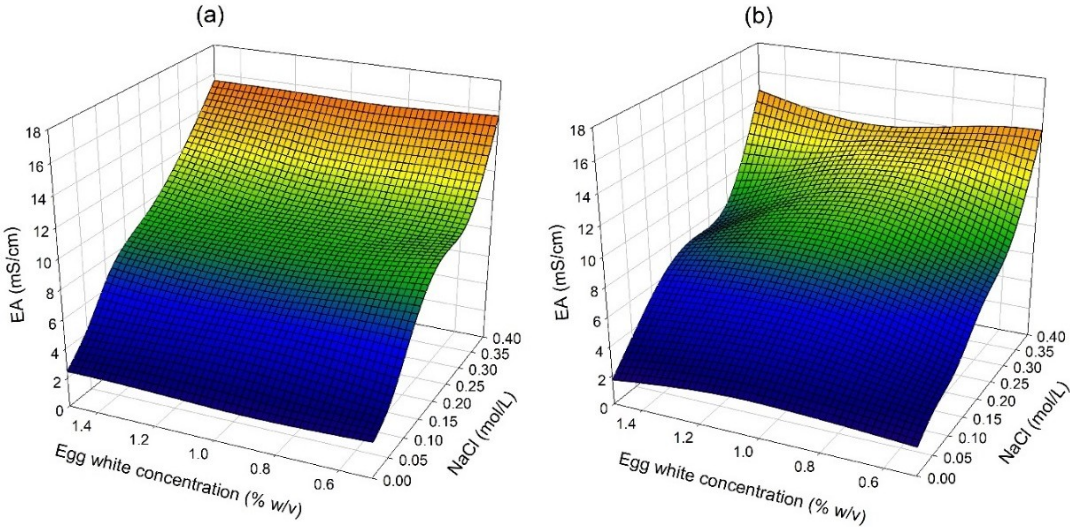

Figure 3. Emulsifying activity (EA) for (a) corn and (b) soybean oil emulsions according to the egg white and NaCl concentrations.

As the effect of the protein concentration was not significant on the emulsifying activity within the evaluated range, the predictive EA models were determined only concerning the saline concentration. The models presented satisfactory correlation coefficients ($R^2 > 0.9$).

Emulsion stability

The effect of salt and protein concentrations on ES of corn and soybean oils is shown in Figure 4. Regarding protein concentration, a systematic behavior of the egg white effect on the ES is observed. Unlikely EA, the protein concentration directly influenced in ES. One observed an increment in the ES value when the egg white concentrations increased from 0.50 to 1.00% (w v^{-1}). This behavior can be attributed to the increase of the surface covered by the protein of the interface area recently formed. In smaller concentrations, the protein molecules rapidly diffuse to the droplet interface. Although these were the lowest evaluated concentrations, there were enough egg white protein molecules to ensure an efficient covering of oil droplets, favoring the emulsion formation and protecting them against coalescence. These results are quite similar to those described by Al-Malah et al. (2000), who have investigated the emulsifying properties of BSA in different vegetable oil types using this conductivity technique. Iqbal et al. (2017), who studied the emulsifying properties of albumin from egg white on the stability of corn oil-in-water emulsion, also observed higher emulsifying stability for higher protein concentrations.

On the other hand, one observed that ES value decreased when the egg white protein concentration increased from 1.00 to 1.50% (w v^{-1}). Suttiprasit et al. (1993) also observed this behavior, claiming that it is systematic, but with no clear justification for this apparent anomaly. One suggestion is that at lower concentrations, there is more area available for adsorption of each protein molecule. Once adsorbed on the water-oil interface, the proteins can unwind and rearrange themselves and their tertiary structure. Furthermore, this allows them to remain adsorbed for more extended periods of time. Also, it is reasonable to assume that the capacity of the protein to make several relatively high-efficiency hydrophobic interactions with the oil better stabilizes the emulsion. If relatively high protein concentrations, such as 1.50% (w v^{-1}) of egg white is concerned, the surface of the oil droplet may remain compressed, and there is less area available for the molecule to rearrange, causing lower emulsion stability. Other than that, the accumulation of proteins in the aqueous phase might decrease ES of quail egg white, since this free protein can compete with the ions in the medium, decreasing the electrostatic repulsion among the newly formed emulsion droplets.

Regarding the presence of NaCl, one observed that ES increased with the increase in salt concentration. The relatively high concentration of sodium ions would allow their continuous interaction with the hydrophilic regions of the proteins, which may increase electrostatic repulsion among their molecules. The enhancement of electrostatic repulsive interactions, thereby increases the apparent protein dispersibility, causing a ‘salting-in’ effect (Xu, Liu, & Zhang, 2015). Also, this result suggests that the higher the concentration of ions, the greater the exposure of hydrophobic regions on the protein surface, leading to stronger interactions between these molecules and the oil drops. As a consequence, good electrostatic repulsion among the droplets was achieved, increasing the emulsion stability (Taha et al., 2019).

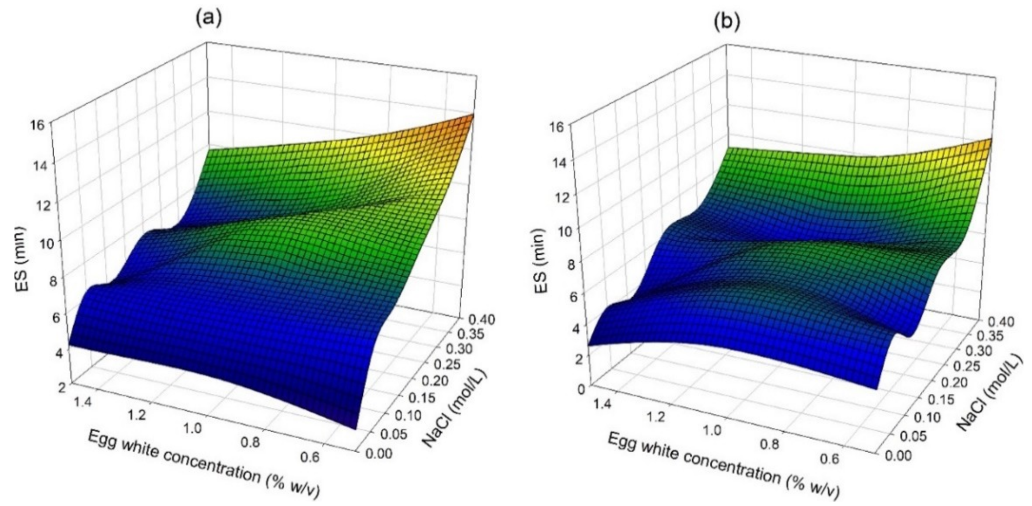

Figure 4. Emulsifying stability (ES) for (a) corn and (b) soybean oil emulsions according to the egg white and NaCl concentrations.

Two first-order polynomial models were used to adjust the experimental ES data according to the NaCl and egg white concentrations for corn oil (ES_c), Equation 5, and for soybean oil (ES_s), Equation 6:

$$ES_C = 6.76176 + 16.10421\, C_{salt} - 1.88917 C_{white} \quad (5)$$

$$ES_S = 5.63317 + 13.18982\, C_{salt} - 1.64500 C_{white} \quad (6)$$

where: C_{white} is the egg white protein, and C_{salt} is the NaCl concentration.

In summary, we could observe that egg white proteins play a different role regarding emulsions activity and stability. It is important to make sure that there is sufficient protein for the complete coverage of oil droplets. The lowest protein concentration (0.50% w v^{-1}) was sufficient to saturate the interface, so this parameter depends exclusively on the salt concentration. On the other hand, the ES was strongly linked to the protein concentration. To some extent, the increase in protein concentration is advantageous for the stabilization of the emulsion. For higher values, however, the excess of protein begins to impair stability, since the protein adsorbed to the drop cannot be unfolded by the whole, performing fewer interactions with the interface. The free protein in the medium can also compete with the ions and counterions, decreasing the electrostatic repulsion and, consequently, increasing the droplet-droplet interactions, favoring the coalescence and the emulsion break-up.

Conclusion

Emulsions are the base of a large group of food products established by proteins. One of the purposes of this work was to further understand the influence of quail egg white, salt concentration, and oil type on emulsifying properties of proteins from the quail egg white. A better understanding of these effects rationally and systematically facilitates the projection of products that attend consumer demands for natural and healthy products. One could observe that EA increased as salt concentration was elevated. However, the EA underwent no significant variations with changes in egg white concentration. The ES increased with the augment in salt concentration. The effect of egg white concentration caused an increase in the ES when varying from 0.50 to 1.00% (w v^{-1}) and a decrease in the ES when egg white concentration increased from 1.00 to 1.50% (w v^{-1}).

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