Vol. 07, e3022020JBFS, 2020
https://dx.doi.org/10.18067/jbfs.v7i3.302
ISSN 2359-2710 Online Edition

Determination of emulsifying properties of chicken egg white and dehydrated egg white in different vegetable oils and ion concentration

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Received: 2020.05.04; **Revised:** 2020.06.22; **Accepted:** 2020.06.22; **Published:** 2020.07.01

Highlights: The paper addresses the "Emulsion and Emulsification Technology" and is important because "it evaluates the effects of WO emulsions using egg white protein depending on the concentration, type of oil and salt concentration, aiming at application in product development and improvements foodstuffs.

Section: This paper was submitted in Food Science and Technology, a section of the *J. Bioen Food Sci.*

Competing interests: There is not conflict of interest in the research conducted.

Funding: The authors received no specific funding for this work.

Citation as (APA): Silva, G.C.R. (2020). Determination of emulsifying properties of chicken egg white and dehydrated egg white in different vegetable oils and ion concentration, *Journal of Bioenergy and Food Science, 7*(3), e3022020JBFS. http://doi.org/10.18067/jbfs.v7i3.302.

Edited by Dr. Harvey Alexander Villa Velez – Federal University of Maranhão, Brazil.

Review processes: 3022020R01 (Brazil) | 3022020R02 (Brazil)

ABSTRACT - Emulsifying properties of oil in water emulsions using chicken egg white and dried egg white as an emulsifying agent were investigated using the conductivity technique. Changes in emulsion conductivity were recorded during and after homogenization and interpreted in terms of properties related to the emulsifying activity (EA) and emulsion stability (ES). The effect of NaCl concentration (0.0, 0.1, 0.5 and 1.0 % w/w), chicken egg white and dried egg white concentration (1.0, 2.5 and 5.0 % w/w) was studied using two vegetable oils, namely corn and canola. In general, it was observed that the EA and ES increase with increasing protein content and salt concentration, in a manner consistent with past research.

Keywords: Conductivity, Emulsifying activity, Emulsion stability, Proteins; Salt; Canola, Corn.

INTRODUCTION

Egg white is an important ingredient in food processing because of its variety of functional properties such as gel formation, water-holding capacity, foaming capacity, flavor, and emulsifying ability (Abbaszaadeh, Ghobadian, Omidkhah, & Najafi, 2012; Ahmadi, Asaadian, Kord, & Khadivi, 2019; Kazemzadeh, Ismail, Rezvani, Sharifi, & Riazi, 2019; Kuhn & Cunha, 2012; Paulo, Alvim, Reineccius, & Prata, 2020; Souza & Rojas, 2012).

Food emulsions are preparation of one immiscible liquid distributed as small droplets throughout the matrix of a second liquid by means of an emulsifying agent. The dispersed liquids the discontinuous phase and the dispersion medium is the continuous phase (Ding, Shi, & Dong, 2020; M. Ding et al., 2020; Salerni et al., 2019; Sukhotu et al., 2016). Emulsions are characterized by the presence of at least one polar hydrophilic liquid and at least one lipophilic liquid (Cabrera-Trujillo, Filomena-Ambrosio, Quintanilla-Carvajal, & Sotelo-Díaz, 2018; Mohammadzadeh, Koocheki, Kadkhodaee, & Razavi, 2013). The two liquids are water and oil. When water is the dispersed phase it is known as water in oil (w/o) emulsion, whereas when oil is the dispersed phase it is known as oil in water (o/w).

Main property of emulsions is also characterized by having a large interfacial area between the liquid phases, allowing a faster exchange process or chemical reaction to take place at the interface. So, make emulsions useful in foodstuff, pharmaceuticals, cosmetics, laundry and cleaning agents, and lubricants, as well as in agents for crop production (Kazemzadeh et al., 2019; Kuhn & Cunha, 2012; Ozturk & McClements, 2016; Sartomo, Santoso, Ubaidillah, & Muraza, 2020).

Emulsifying properties are often discussed in terms of emulsifying activity and emulsion stability (Ahmadi et al., 2019). Kato, Fujishige, Matsudomi, and Kobayashi (1985) developed a method to measure emulsifying properties, by the conductivity technique. They defined the emulsifying activity (EA) of a given protein as the difference between the conductivity of the protein solution before homogenization begins (C_0) and the minimum conductivity achieved during homogenization from 3 min (C_e) as given by Eq. (1):

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

The EA of protein depends on the area of interface-stabilized, dispersed oil droplets, and would be a function of the oil volume fraction and protein concentration in the system (Souza & Rojas, 2012).

In addition, Kato et al. (1985) related ES to the emulsion activity and the initial slope of the conductivity curve just after the homogenization step is completed, as given by Eq. (2).

$$ES = EA/(dC/dT) \quad (2)$$

where *(dC/dt)* is the initial slope of the conductivity curve (just after the homogenization step is completed).

Later, M. O. J. Azzam and Omari (2002), suggested a new ES index. For ES calculations, the conductivity curves are analyzed after stopping the homogenizer and is calculated by the Eq. (3):

$$ES = EA \times \left\{\frac{\tau_{50}}{[(C_T - C_B) \times S_{\tau_{50}}]}\right\}^{1/2} \quad (3)$$

where EA is the emulsifying activity, C_T is the maximum conductivity after homogenizer is stopped, C_B is the minimum conductivity after homogenizer is stopped, τ_{50} is the time for conductivity to reach the value of $[C_B + 0.5 \times (C_T - C_B)]$ and $S_{\tau_{50}}$ is the parameter which describes the slope of the curve at τ_{50}.

In general, oil volume fraction, oil type, temperature, pH, type and amount of emulsifier, and the presence of counter ions and legends (salts, sugars, and carbohydrates) change emulsions proprieties by M. O. J. Azzam and Omari (2002).

Several authors have studied the emulsifying properties of eggs (Mohammed O. J. Azzam, Al-Malah, & Omari, 2012; M. O. J. Azzam & Omari, 2002; Souza & Rojas, 2012). However, such researched are limited because have not been investigated the influence of the conditions as type of oil, protein concentration, salt concentration on the emulsifying properties of egg white and dried egg white.

This study is a preliminary investigation of the emulsifying properties of chicken egg white and dried egg white. The effect of emulsifier concentration, salt concentration, and oil type on EA and ES was investigated. The emulsifying properties were determined by the conductivity curve of the emulsion versus time after the homogenization of oil in protein aqueous solution.

MATERIAL AND METHODS

a) Material

Fresh eggs were purchased from the local market in Divinópolis – MG (Brazil). Egg white was separated manually from the yolk. The dried egg white was purchased Food Mizumoto Ltda (Guarapirama, Brazil). All chemicals were of analytical grade and were readily available commercial products. Deionized water for all the experiments was obtained from a Milli-Q system (Millipore Inc., MA, USA). Corn and canola oil were obtained from Bunge Alimentos (SP, Brazil).

b) Methodology

Protein quantification

The protein content was determined by measuring the absorption at 540 nm (Spectrophotometer Cary 50, Varian, Australia), according to the method of Biuret (Gornall, 1949). The analytical curve was obtained using ovalbumin (Sigma-Aldrich) as standard, at increasing concentration of white egg solutions (0.1 to 0.8 mg/mL).

Measurement of conductivity of emulsion

The initial conductivity of the chicken egg white (HEW) or dried egg white (DEW) solution was recorded using an electrode fixed in the bottom of a glass recipient (volume 600 mL, 70 mm x 150 mm) connecting to a conductivity meter (Orion 145 A+, USA). The emulsions were prepared mixed and homogenized (Braun, MR400, USA) 200 mL protein solution at pH 7.5 and 50 mL oil (corn or canola) for 3 min at room temperature (25°C) at the speed of 10.000 rpm. Emulsion conductivity (mS/cm) was measured continuously during the homogenization period, and for 32 min after stopping the homogenizer. The selected methods were adapted from Kato et al. (1985).

Emulsifying properties determination

Following the conductivity technique, activity and stability of emulsions were obtained by using, respectively, Eq. (1) and Eq. (3). The conductivity of the emulsion decreased rapidly with the progress of homogenization, due to the addition of non-conductor oil into the conductor protein solution. After emulsion homogenization, the two phases form oil in water emulsion, and the conductivity increases. The conductivity increase is dependent on the protein.

The conductivity curves observed by Kato et al. (1985) had the general shape shown in Figure 1.

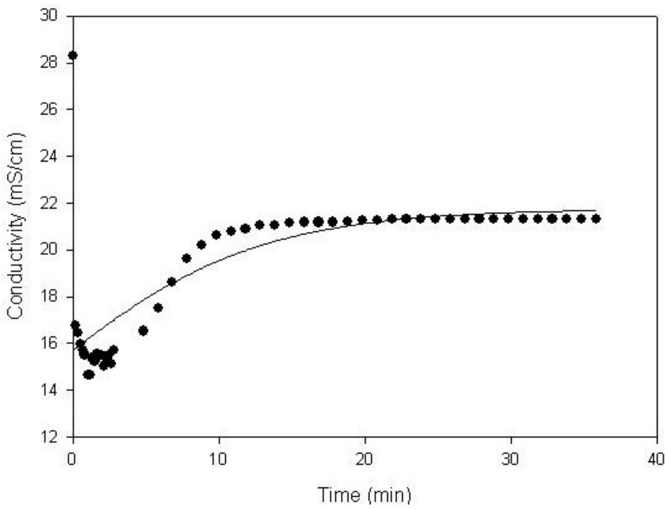
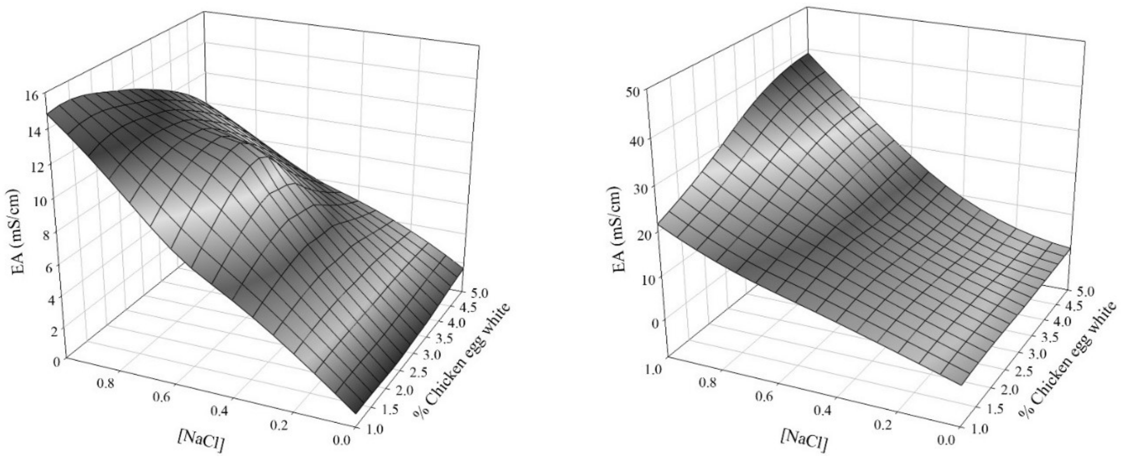


Figure 1 - Sample conductivity curves for canola oil/water emulsions with dried white egg concentration of 0,5% (w/w), at 0,5% (w/w) NaCl. Solid lines represent sigmoid function fit to experimental data of our results (shown as points).

RESULTS AND DISCUSSION

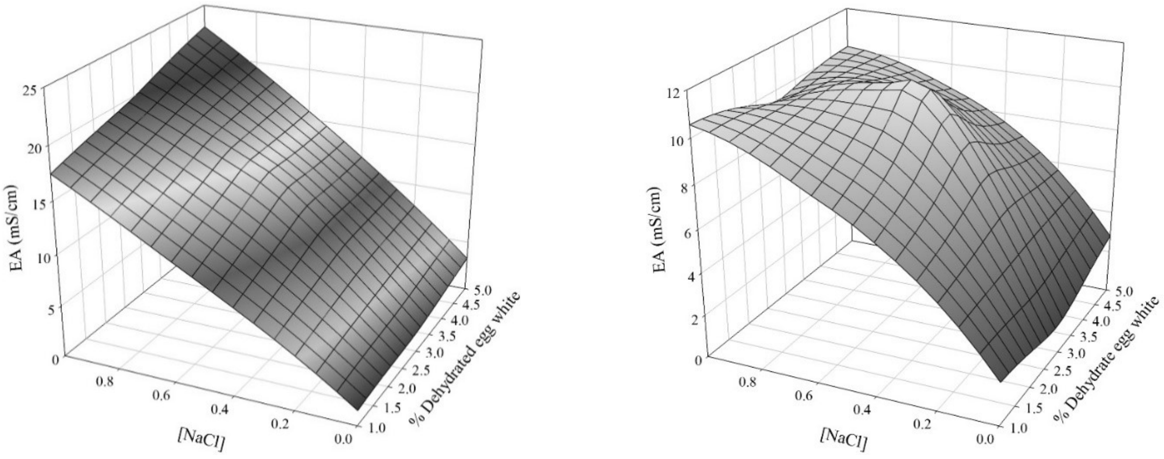
a) Effect of protein and salt concentration on EA

In Figures 2 and 3, show a correlation between EA and protein concentration in (a) canola and (b) corn oils. In general, EA increases with increasing HEW and DEW concentration. This behavior may be attributed to the increase in the protein surface coverage of the at a newly formed oil-water interface (during homogenization) (Mohammed O. J. Azzam et al., 2012). Similar results were found by Mohammed O. J. Azzam et al. (2012). These authors studied the emulsifying properties of α-lactalbumin, β-lactoglobulin, and bovine serum albumin (BSA) in corn oil/water systems using the conductivity technique.



(a) (b)

Figure 2. EA as a function of salt concentration using different concentrations of chicken egg white for: a) canola oil and b) corn oil.



(a) (b)

Figure 3. EA as a function of salt concentration using different concentrations of the dehydrated white egg for: a) canola oil and b) corn oil.

Kato et al. (1985) measured EA of k-casein, 11S globulin, and ovalbumin at concentrations ranging from 1 to 10 mg/mL and found that EA increased with concentration. They suggested that this phenomenon was compatible with the theory that the EA of proteins is a function of protein concentration.

According to Souza and Rojas (2012), the proteins arrive at an oil-water interface at a rate proportional to their concentration. Higher concentrations provide more protein molecules to mediate the formation of emulsions, binding both water, and oil molecules to form thick barriers that help prevent the oil particles from coalescing (Costa et al., 2019; Gulão, Souza, Costa, Rocha-Leão, & Garcia-Rojas, 2018). This is thought to occur due to the presence of amphipathic phospholipids in egg white decreasing interfacial tension among formed oil drops (Costa et al., 2019).

The addition of NaCl also affected the EA of both egg white and dried egg white. It was verified a tendency of EA increase with the increase of salt concentration. This fact is probably since the addition of NaCl improved solubility of the protein and, accordingly, the EA (Sousa et al., 2007).

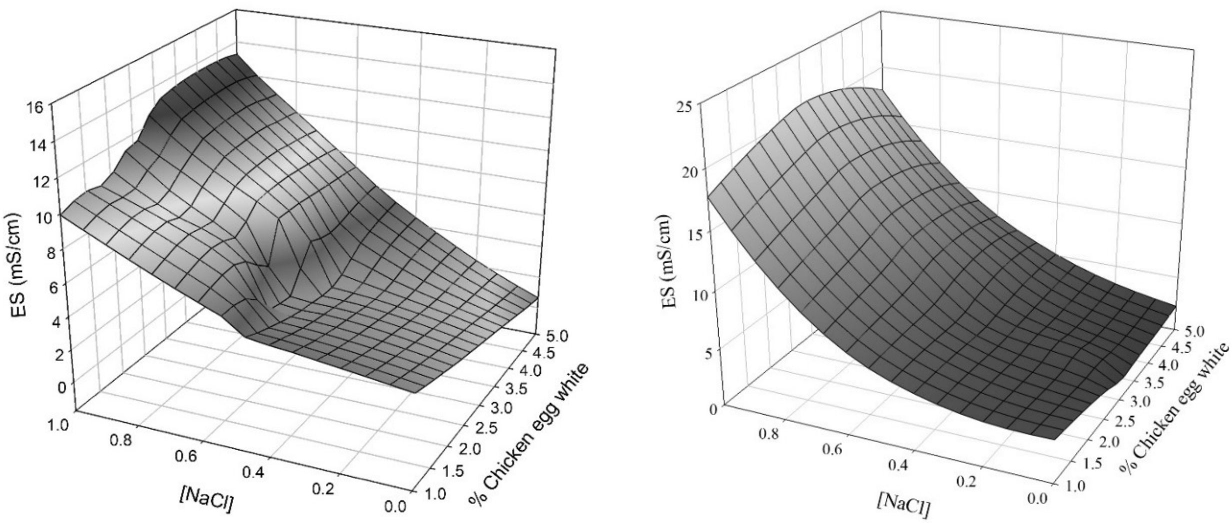
When the effect of oil type on EA is investigated, corn resulted in smaller EA values (< 11.1 mS/cm) than canola oil. The mechanism of this phenomenon is poorly understood. Monounsaturated fatty acid, specifically oleic acid, further facilitates interactions between hydrophobic protein groups and oil droplets (Khadem & Sheibat-Othman, 2017; Souza & Rojas, 2012). This hypothesis is supported by the oleic acid content in canola oil 62.41% being greater than corn oil 24.23-25.54% (Zambiazi, Przybylski, Zambiazi, & Mendonça, 2007).

b) Effect of protein and salt concentration on ES

Figures 4 and 5 shows the ES values as a function of salt concentration in different concentration of hen egg white or dried egg white increase with increasing concentration egg white. This behavior may be attributed to the increase in the egg white surface coverage of the newly formed interfacial area (Delahaije, Lech, & Wierenga, 2019).

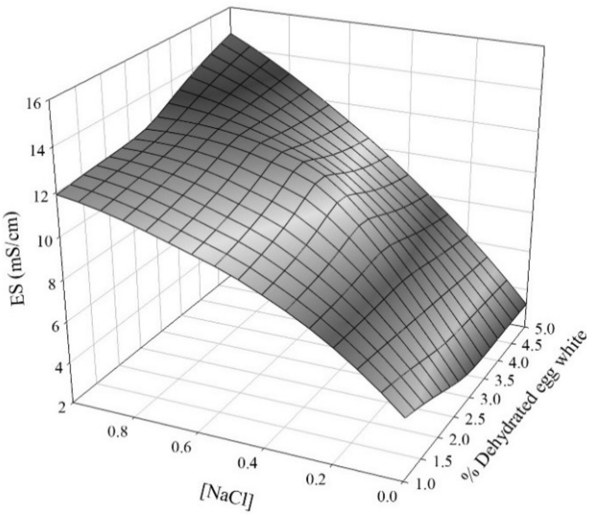
These results are like the ones of Al-Malah, Azzam, and Omari (2000) that investigated the emulsifying properties of BSA in different vegetable oil emulsions using the conductivity technique. M. O. J. Azzam and Omari (2002) studied the stability of egg white in different edible oils using the conductivity technique. These authors found that the ES increases with protein concentration increases.

The increasing salt concentration affected the ES positively. The salt addition can decrease the ES of oil/water emulsion, in which the emulsifying agent is a protein. In these emulsions, oil droplets showed a resultant electric load of the ionized protein groups, as also of ions proceeding from the water. In this way, ions deriving its salt form in a double layer, because the system needs to be electrically neutral. As the load density decreases exponentially within the distance of the center of this system, the addition of the increasing amount of salt promotes a reduction of the repulsion forces between oil droplets that, then, leading to the flocculation and the loss of the stability. Thus, the amount of salt added in the present study probably was not enough to produce this negative effect in the ES of the protein (Ahmadi et al., 2019; Kazemzadeh et al., 2019; Sapei, 2020).

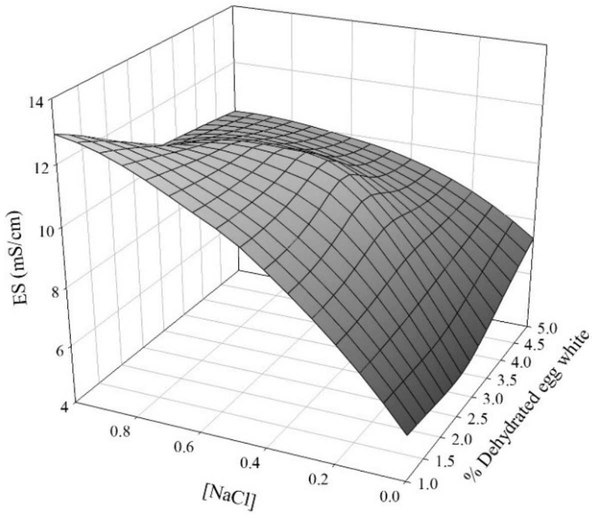


(a) (b)

Figure 4. ES as a function of salt concentration using different concentrations of chicken egg white for: a) canola oil and b) corn oil.



(a)



(b)

Figure 5. ES as a function of salt concentration using different concentrations of the dried white egg for: a) canola oil and b) corn oil.

Polynomial model for the EA and ES as a function of the mass of chicken egg white, dehydrated egg white and NaCl, was fitted to the experimental data. The general quadratic model (Equation 4) was first analyzed and no significant parameters were eliminated based on the t-test with p>0.05. Table 1 shows the predictive models based on the equation model (4) for each used oil.

$$x = A + BW_1 + CW_2 + DW_1{}^2 + EW_2{}^2 + FW_1W_2 \qquad (4)$$

where x is the emulsion activity or stability emulsion, W_1 is the mass of egg white protein, W_2 is the mass of NaCl and A, B, C, D, E, and F were obtained by nonlinear regression. The mathematical polynomial model was well adjusted to the experimental data, as observed in Table 1. This is indicative of a good fit to the experimental data.

Table 1- – Models to calculate EA and ES of the emulsions for canola and corn oil, (p>0.05).

Cod	Type of protein	Oil	A	B	C	D	E	F	R^2
EA	Chicken	Canola	-1.26	1.86	32.03	-0.244	-17.63	1.231	97.72%
		Corn	1.79	0.67	-3.68	-0.243	15.28	6.15	97.20%
	Dehydrated	Canola	-4.26	3.97	29.7	-0.599	-17.3	-1.4	54.46%
		Corn	-1.05	1.08	43.82	-0.131	-34.54	-0.364	97.28%
ES	Chicken	Canola	4.2	-0.44	31.04	-0.023	-26.1	1.55	86.59%
		Corn	2.87	0.79	-2.7	-0.187	16.34	0	86.05%
	Dehydrated	Canola	6.95	-4.21	20.21	0.672	-2.86	-1.41	63.00%
		Corn	3.95	-0.02	33.84	0.0097	-24.35	-1.45	74.50%

Statistical analyzes revealed that the increase in the concentration of salt (NaCl) generated emulsifications with better activities and stability in the different oils. Salts can also affect the electrostatic interactions between macromolecules and increase protein solubility at concentrations of 0.1 to 1.0 mol/L. However, this behavior depends on the concentration and type of salt present in the medium and protein structure. Salt ions interact with opposing charge groups on the protein to form a double layer of ionic groups, which diminish the electrostatic interaction between the molecules causing more protein solvation, thus increasing solubility. Solubility decreases when there is greater competition between proteins and salt ions for water (Santos et al., 2015). There was no significant difference between the proteins obtained from fresh and dehydrated eggs.

CONCLUSION

In this work four parameters were studied, namely chicken egg white or dehydrated egg white concentration, oil type, and salt concentration. The conductivity curves were used to calculate emulsifying activity (EA) and emulsion stability (ES). The pattern of conductivity was fit to the sigmoid function, which revealed the model parameters that were used later to quantify ES. In general, AE and ES showed an increase with increasing protein and salt concentration. The emulsifying properties of egg white were higher than in the salt absence. However, the addition of salt in the systems changed their emulsifying properties. It wasn't observed a systematic behavior for EA and ES as related to oil type. It was observed that oil with higher concentrations in monounsaturated fatty acids are more efficient in the formation of emulsions and facilitate the emulsification process and that the combination of low protein concentrations with low salt concentrations resulted in unstable emulsions. The polynomial model used to predict the EA and ES of emulsions had a good fit to the experimental data and holds promise for further studies to assess the functional properties of this new emulsion.

Declaration of Conflicting Interests

The authors have declared that there is no conflict of interest.

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