EFFECT OF PH, SALT CONCENTRATION AND TIME AGITATED ON THE SOLUBILITY OF QUAIL (*COTURNIX COTURNIX JAPONICA)* EGG WHITE PROTEIN

Daniela O. dos Santos. danielaengenharia@hotmail.com
Jane Sélia R. Coimbra, jcoimbra@ufv.br
Carolina Reis Teixeira, carolzinharei@hotmail.com
Fabíola Cristina de Oliveira, fabicrisoliv@yahoo.com.br
Rita C. Superbi de Souza, ritacsuperbi@yahoo.com.br
Rafaela de Araújo Lira, rafaelira@gmail.com

Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Av. P.H. Rolfs s/n, 36570-000, Viçosa, MG, Brazil.

Abstract. *This work evaluated the influence of different values of pH (3.0, 6.2, and 10.0) at salt concentrations (0.05, 0.3, 0.5, 0.8 and 1.0) mol/L and different agitation time (0.5, 1.0, 1.5, and 2.0) on the solubility of quail (Coturnix coturnix japonica) egg white proteins for of salts (NaCl), at temperature 25 ºC. The experiment was carried out in a completely randomized design, using five pH values, five salt concentration levels, four agitation time and two repetitions. The variations in pH, salt concentration values had a significant effect on the protein solubility data. A 2th-order polynomial model fitted the solubility data with R^2 values higher than 0.89. The variations in pH, salt concentration and agitation time values had a significant effect on the protein solubility data. The highest solubility value (98.92 g/100 g) was obtained in the system containing 0.05 mol/L (NaCl), at pH 10.0 and one hour of agitation. The lowest solubility value (68,35 g/100 g) was obtained in the system containing 1.0 mol/L (NaCl), at pH 3.0 and two hour of agitation. Under the tested conditions, the aqueous solutions at 1.0 mol/L of NaCl and pH 3.0 and two hour of agitation might lead to a higher protein extraction.*

Keywords: *solubility, pH, salt concentration.*

1. INTRODUCTION

Owing to the fact that quail egg white protein has a high biological value, the egg is considered an important food for human diet, containing practically all the essential nutrients indispensable to human life. The protein content of quail egg accounts for 6.3% of the total egg weight, with around 91% of the proteins being found in the albumen (Stadelman and Cotterill 1995; Albino 2003).

In general, proteins used for functionality are required to hove high solubility, in order to provide good emulsion, foam, gelation and whipping properties (Vojdani, 1996). Solubility is affected by many factors such as amino acid composition, protein structure (native or denatured) and medium factors, such as pH, temperature, pressure, type and content of salts and protein concentration. Overall, proteins remain in solution until reaching a maximum quantity, after which they precipitate (Vojdani, 1996).

The way proteins interact with a solvent is a manifestation of the physicochemical properties of proteins under given conditions. A wide range of protein–protein and protein–solvent interactions are involved. Many important functional food properties, including solubility, may be described through these interactions (Kakalis and Regenstein, 1986). The pH affects the nature and distribution charges of a protein. In general, proteins are more soluble in low (acid) or high (alkaline) pH values, due to excess of charges of the same signal, producing repulsion among the molecules and, consequently, contributing to their higher solubility (Pelegrine and Gasparetto, 2005).

Proteins at isoelectric point (pI) generally present minimum solubility. The protein–protein interaction increases, at pI, because the electrostatic forces between the protein molecules and water molecules are minimum, which reduces the number of water molecules that interact with protein molecules. This is a favorable condition for the approximation and aggregation of protein molecules, leading to their precipitation (Vojdani, 1996).

Salts can also affect the electrostatic interactions among the macromolecules, contributing through the ionic force (Fennema, 1993). Salting-in and salting-out effects, related with the surface characteristics, also effect protein solubility, which influences thickening, foaming, emulsification and gelation (Damodaran, 1997).

Determination of protein solubility data are necessary to define the processing conditions of foods containing different protein sources, to obtain protein concentrates, for the development of the extraction and purification processes of proteins with high aggregate value, such as quail egg white proteins, and to use in the acquirement of other functional properties of foods. In view of the lack of solubility data of quail egg white, this work evaluated the solubility of quail egg lyophilized white proteins, as a function of agitation time, pH and salt concentration (NaCl), at temperature 25 ºC.

2. MATERIALS AND METHODS

2.1. Materials

Laying quails eggs (*Coturnix coturnix japonica*) were obtained of Experimental Aviary of Zootecny of Federal University of Viçosa (Brazil). Egg white was manually separated from the frozen and lyophilized (Edwards L5KR, USA). All chemicals were of analytical grade and the water was deionized using a Milli-Q device (0.22 mm membrane, Millipore Inc., USA). The analytical curve was obtained using ovalbumin as standard with 98% purity (Sigma Chemicals, St. Louis, MO).

2.2. Protein quantification

Protein content was quantified by the Biureto reaction (Gornall *et al*. 1949), using an analytical curve built by varying ovalbumin (Sigma Chemicals, USA) concentration in aqueous solutions from 1.0 to 10.0 mg/ml. Absorbance determination was performed at 540 nm (spectrophotometer Cary 50, Varian, Australia), followed by determination of the protein amount (mg) in each sample, using the analytical curve.

2.3. Solubility determination

Solubility data were obtained according to Kakalis and Regenstein (1986), by adding 120 mg of lyophilized egg white into 20 ml of a buffer solution, corresponding to a concentration of 6 mg of proteins/mL. The systems were prepared in buffer solutions with predefined pH values (3.0, 6.2 or 10.0) containing salt to be evaluated (NaCl) at different salt concentrations (0.05, 0.3, 0.5, 0.8 or 1.0 mol/L). The agitation time was also evaluated (0.5, 1.0, 1.5 and 2.0 h). The buffers (0.1 mol/L) added to achieve the desired pH were glycine–HCl (pH 3.0), citrate–citric acid (pH 6.2) and carbonate (pH 10.0) (Mohan 1995). The suspensions were mixed, using the device simulating an agitated tank MASTERFLEX L/S ™ (Cole-Parmer Instrument Company) with 14-rpm rotation at 25 ºC. Immediately after homogenization, the systems were centrifuged at 20,000 x *g* for 20 min at 4 ºC (Beckman centrifuge, model J2-MC, USA). After centrifugation, an aliquot of 1 mL was collected from the supernatant for further analysis. The soluble proteins in the aliquot were then quantified by the Biureto reaction at 540 nm (spectrophotometer Cary 50, Varian). The protein solubility, *P.S* (g/100 g), or the soluble protein content in the sample was calculated by Eq. (1):

$$P.S = \left[\frac{A}{W * S / 100} \right].100 \tag{1}$$

where, *A* is the amount of soluble protein in the supernatant (g), *W* is the total amount of protein in the sample before centrifugation (g) and S is the concentration amount of protein (%).

The experiment was carried out in a completely randomized factorial scheme with three pH values, five salt concentrations and four agitation time, consisting of 60 treatments and two repetitions. Each experimental unit was represented by 120 mg of lyophilized quail egg white. The solubility data were analyzed statically using the PROC GLM of the statistical software SAS (SAS version 8.0, Cary, NC; SAS Institute, Inc. 1998).

3. RESULTS AND DISCUSSION

It can be observed in “Tab. 1” and Fig. 1” the solubility of quail egg white proteins varies according to concentration of salt present in the medium for a respective pH and agitation time.

In the pH values of 3.0, it was verified the solubility of the proteins decreased with the increase of the salt concentration for all the agitation time evaluated. Similar behavior was verified by Kakalis and Regenstein (1986) at low pH values, evaluating the influence of pH and salt concentration on the solubility of egg white proteins. Such behaviour can be attributed that a low pH value associated to the increasing of the salt concentration increases the hydrophobic interactions (protein–protein) thus matching the aggregation of protein molecules, followed by precipitation (Kiosseglou, 1989; Fennema, 1993). In this pH, the protein solubility decreased with the increases on the agitation time, as observed in the “Fig. 1a".

In the pH values of 6.2 protein solubility increased the salt concentration up to 0.3mol/L, as a result of the salting-in effect, and decreased of increased the salt concentration as a result of the salting-out effect. Similarly, Anton and Gandemer (1997) verified changes in the solubility data behavior of chicken egg whites, with variation of the type of salt studied, likely caused by preferential hydration of each protein for each type of salt present in the medium. The “Fig. 1b” shows that up to 1,5 hours of agitation the solubility increases. The increase over the time of unrest causes changes in the conformation of the protein (secondary, tertiary or quarternária), reducing the solubility.

Table 1. Quail egg white protein (g/100 g) solubility as a function of pH, salt concentration and time agitated.

pH 3.0				
NaCl (mol/L)	Time 5.0 h	Time 1.0 h	Time 1.5 h	Time 2.0 h
0.05	97.78 ± 0.62	95.59 ± 0.13	89.51 ± 0.14	88.51 ± 0.19
0.30	95.99 ± 0.02	90.19 ± 1.20	84.92 ± 0.87	84.44 ± 0.05
0.50	90.03 ± 0.18	81.77 ± 0.76	82.79 ± 0.21	80.55 ± 0.35
0.80	82.6 ± 0.42	79.32 ± 0.68	74.48 ± 0.21	71.95 ± 1.20
1.0	79.88 ± 1.15	76.93 ± 0.78	72.27 ± 0.80	68.35 ± 0.40

pH 6.2								
NaCl (mol/L)	Time 5.0 h		Time 1.0 h		Time 1.5 h		Time 2.0 h	
0.05	81.69	±0.17	88.4	±1.28	95.75	±0.62	84.07	±0.00
0.30	88.69	±0.92	92.83	±0.49	96.38	±0.10	86.56	±0.07
0.50	88.51	±0.61	93.93	±0.46	95.46	±0.31	85.96	±0.59
0.80	88.79	±0.45	88.85	±0.08	95.07	±0.49	86.54	±0.14
1.0	87.34	±0.38	86.5	±0.13	94.43	±0.62	83.72	±0.21

pH 10.0								
NaCl (mol/L)	Time 5.0 h		Time 1.0 h		Time 1.5 h		Time 2.0 h	
0.05	88.49	±0.26	98.92	±0.16	97.85	±0.21	87.04	±0.33
0.30	88.34	±0.52	97.67	±0.05	97.01	±0.36	86.49	±0.07
0.50	88.21	±0.05	97.27	±0.26	95.53	±0.42	86.01	±0.09
0.80	87.31	±0.47	96.42	±0.05	95.39	±0.10	85.81	±0.14
1.0	87.24	±0.05	95.64	±0.10	94.83	±0.10	85.52	±0.31

In the pH values of 10.0, it was verified the solubility of the proteins decreased with the increase of the salt concentration for all the times evaluated, as a result of the salting-out effect. . In this case, the competition between protein and the salt ions for the water molecule is observed, leading to the removal of the protein hydration water, and to a larger number of hydrophobic interactions (protein–protein) than protein–water interactions, thus matching the aggregation of protein molecules, followed by precipitation (Fennema, 1993; Pharmacia Biotech, 1993). The higher solubility value for the pH 10,0, was verified with 1 hour of agitation ("Fig. 1c"). In this condition, the agitation time of unrest favors the increase of water-protein interactions, the electrostatic forces between molecules of protein are greater and greater quantity of water molecules interact with the molecules of protein.

Table 1 shows the lowest solubility value was obtained at 1 mol/L NaCl for the three pH values tested. Similar results were reported by Kakalis and Regenstein (1986). According to these authors in high salt concentrations, the most of the water molecules is strongly bond to salt ions, while there is some reorganization of the water molecules around the proteins. The protein–protein interaction increases with subsequent molecules precipitation.

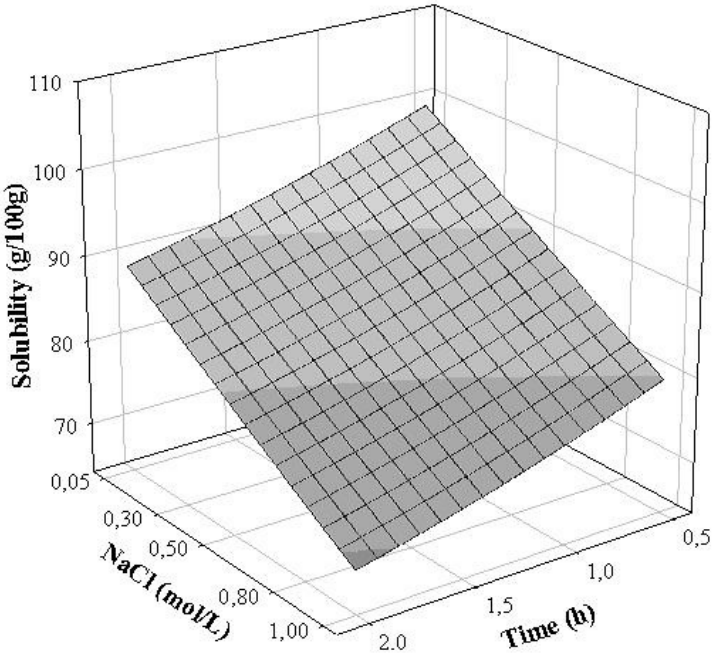

Figure 1a. Solubility of quail egg white proteins in pH 3.0 as a function of time agitated and salt concentration, for NaCl.

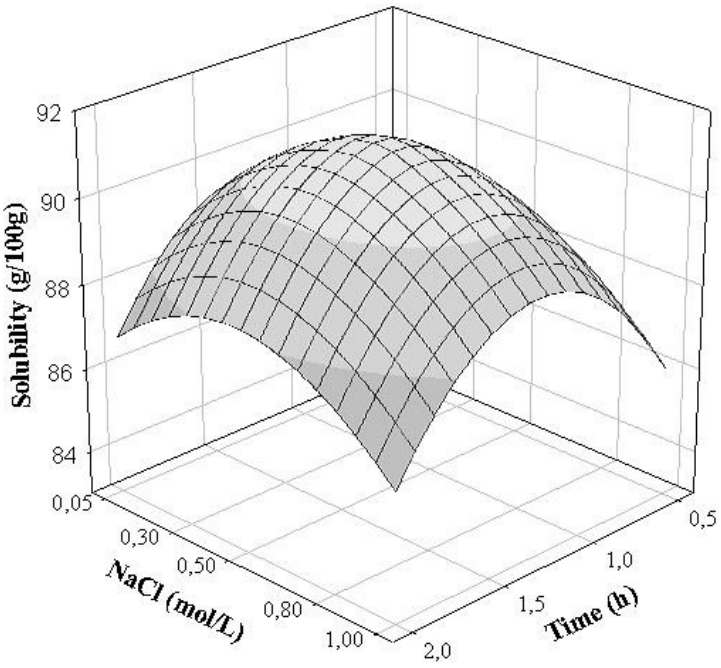

Figure 1b. Solubility of quail egg white proteins in pH 6.2 as a function of time agitated and salt concentration, for NaCl.

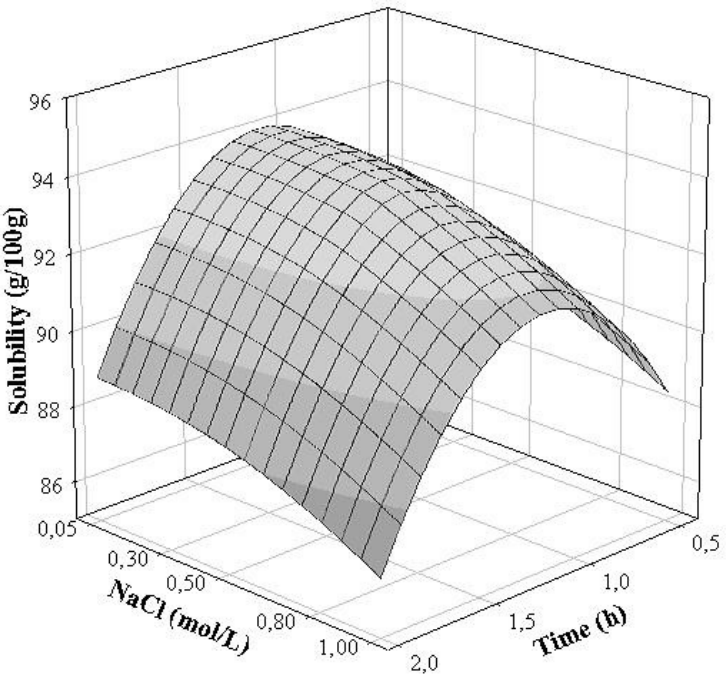

Figure 1c. Solubility of quail egg white proteins in pH 10.0 as a function of time agitated and salt concentration, for NaCl.

A 2th-order polynomial model (Eq. (3)) was used to fit the solubility of quail egg white proteins data:

$$S = a + b\,t + c\,Cs + d\,t^2 + e\,pH^2 + f\,t\,pH + g\,Cs\,pH \qquad (2)$$

Where, t is the agitation time (hours), Cs is the salt concentration (mol/L) and a–g are the parameters fitted by regression for each type of salt.

Table 2. Coefficients of Eq. (2)

Parâmetro	*a*	*b*	*c*	*d*	*e*	*f*	*g*	R^2	Pr
NaCl	88,22	16,43	-24,16	-9,97	-0,1	0.89	2,57	0.99	*

* $p<0.05$.

The result of the variance analysis of the regression indicates that the fourth order model Eq. (2) is significant at a level of 0.05 of probability [(P_{model}>F) = 0.0001]. The model fitted the experimental data with a R^2 above 0.89. Table 2 shows the estimates of each parameter, the t-value of the Student test and the probability (Pr) value of each parameter. All parameters were significant at a level of 0.05. According to Box, Hunter and Hunter (1978), high t-values and low Pr values lead to a higher significance of the studied parameters.

4. CONCLUSIONS

The solubility is a functional property used as a critical pre-requisite for potential applications of protein ingredients in foods and can influence other functional protein properties. The solubility of quail egg white proteins was influenced by the pH variations, agitation time and salt concentrations, with a differentiated behavior being exhibited for each condition. The highest solubility value (98.92 g/100 g) was obtained in the system containing 0.05 mol/L (NaCl) at pH 10.0 with two hours of agitation. The lowest solubility value (68.35 g/100 g) was obtained in the system containing 1.0 mol/L (NaCl), at pH 3.0 with two hours of agitation. Under the tested conditions, the aqueous solutions at 1.0 mol/L of NaCl and pH 3.0 might lead to a higher protein extraction.

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