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Research Article

Effect of Desalination on Physicochemical and Functional Properties of Duck (*Anas plotyrhyncus*) Egg Whites

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Abstract: Desalted Duck Egg Whites (DDEW) was prepared by electrodialysis desalination using Salted Duck Egg Whites (SDEW). DDEW and SDEW (used as control) were subjected to freeze drying process. Freeze Dried Desalted and Salted Duck Egg Whites (FDDEW and FSDEW, respectively) were assessed for functional properties (turbidity, foaming, emulsifying and gelation) and some physicochemical characteristics. Among the physicochemical parameters, the proximate composition, amino acid composition, pH, particle sizes, microstructure and color attributes were studied. The electrodialysis desalination process had significant effect on the physicochemical characteristics of FDDEW and FSDEW except for amino acids composition. Thus, the pH decreased from 8.07 to 7.40 while the NaCl content decreased from 3.76 to 0.18%. The same trend was observed for protein and ash contents. The functional properties were variable among the two samples. For instance, the gel characteristics decreased sharply after electrodialysis desalination treatment.

Keywords: Desalination, duck egg white, functional properties, physicochemical characteristics

INTRODUCTION

Egg is composed of three main parts; shell, albumen (egg white) and yolk. The yolk is surrounded by an albumen layer and this structure is covered by a hard eggshell (Yamamoto *et al*., 1996). The weight of an egg and the weight distribution of the three parts, differ considerably, depending on the kind of egg and their age (Okubo *et al*., 1997).

Eggs have been an important part of the human diet throughout the world. They have traditionally been used for breakfast, home meal preparation, baking and as an ingredient of many foods. Duck (*Anas plotyrhyncus*) eggs are listed among the most commonly eaten eggs and are highly nutritious. They supply a large amount of complete, high quality protein (which contains all essential amino acids for humans) and provide significant amounts of several vitamins and minerals (Gutierrez *et al*., 1996).

Traditionally, duck eggs are salted by pickling with laterite and salt or saline and are commonly consumed, especially with rice gruel for breakfast. The salted yolk is a preserved egg product which is very popular in Asia (Chi and Tseng, 1998). However, the salted egg white (containing 10% protein and 4-7% sodium chloride) is generally regarded as a useless by-product and discarded as waste leading to environmental pollution because the coagulated egg yolks are used for secondary processing in bakery products (Huang *et al*., 1999).

In general, egg whites have various functional properties such as foaming ability, emulsifying activity and gelation and have been used widely in food processing (Nakamura and Sato, 1964; Yang and Lin, 1990). However, dried salted duck egg white contains 30% sodium chloride and has hygroscopic properties, which makes it less suitable for food application. Therefore, the desalination (electrodialysis) of salted duck egg white is very important. Electrodialysis (ED) is a well known unit operation industrially used to separate or concentrate ions in solutions owing to their selective electro-migration through semi-permeable anion and cation-exchange membranes generated by a direct electric voltage applied to the electrodes (Lacey and Loeb, 1972; Ho and Sirkar, 1992; Fidaleo and Moresi, 2011). Its largest area of application is still in the production of potable water from brackish water (Ho and Sirkar, 1992; Audinos, 1992) and de-ashing of milk whey to obtain valuable raw materials for baby-foods (Batchelder, 1987). Several other ED applications have been extensively studied in the laboratory and pilot-plant scales and are basically directed to the removal of sodium chloride from highly concentrated solutions.

To date, few studied have been conducted on the physicochemical and functional properties of desalted

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duck egg white (Huang *et al*., 1999; Wang *et al*., 2013). Therefore, the purpose of this study was to investigate the effects of desalination on some physicochemical and functional properties (foaming ability, emulsifying activity, turbidity and gelation) of salted duck egg white.

MATERIALS AND METHODS

Materials: Salted duck eggs (*Anas plotyrhyncus*) with the weight range of 65 to 75 g used in this study were obtained from a local producer in Xiaognan City (Hubei, China). These eggs were washed using tap water, left to dry and separated into their respective egg yolks and egg whites. The egg whites were gently mixed and stored at 4°C within 3 days of purchase before experiments. All chemicals used in the experiments were of analytical grade.

Electrodialysis process: The electrodialysis was conducted according to Huang *et al*. (1999) with slight modifications. A laboratory Electrodialysis (ED) unit (model Micro Acilzer G3, Asahi Chemical Industry Co., Japan) was used. This unit was equipped with a membrane stack. The membrane type was AC-220-400, which provides 400 cm^2 effective membrane areas. The electrolyte was 500 mLot 0.5 M N_2SO_4, The flow velocity for salted duck egg white was 400-500 mL/min. The ED process was operated at 4°C. During the process, the salted duck egg white was re-circulated through the membrane stack until the desired conductivity (2 ms/cm) was reached.

At the end of the ED process, sample of salted and desalted duck egg whites were freeze dried. Freeze drying was conducted using a lab-scale freeze-dryer (Floor model Freeze Dryer, serial No. 050639219 A, Labconco Co., Kansas, USA) with vacuum collector at -52°C and absolute pressure at 0.035 mbar. Both salted and desalted dried duck egg white powders (Fig. 1A and C) were packed in polyethylene bags and stored in refrigerator at 5°C until use.

Egg whites gel preparation: Duck egg white gels were prepared as reported by Wang *et al*. (2013). Briefly, duck egg white solutions (10% w/w) were prepared by adding the freeze dried salted and desalted duck egg white (FSDEW and FDDEW, respectively) powders into distilled water followed by gentle stirring of the mixture at 20°C. Subsequently, 20 mL duck egg white solution was poured into a 25 mL beaker and heated in a water bath at 90°C for 30 min. Thereafter, the gel samples (SDEWG and DDEWG, respectively) were cooled, refrigerated at 4°C for 10 h (Fig. 1B and D) and finally cut into cylinders (diameter 20 mm, height 20 mm) using a thin-walled cylindrical stainless-steel tube for subsequent tests.

Proximate composition analysis and pH: The Salted and Desalted Duck Egg Whites (SDEW and DDEW, respectively) were analyzed for moisture, ash and

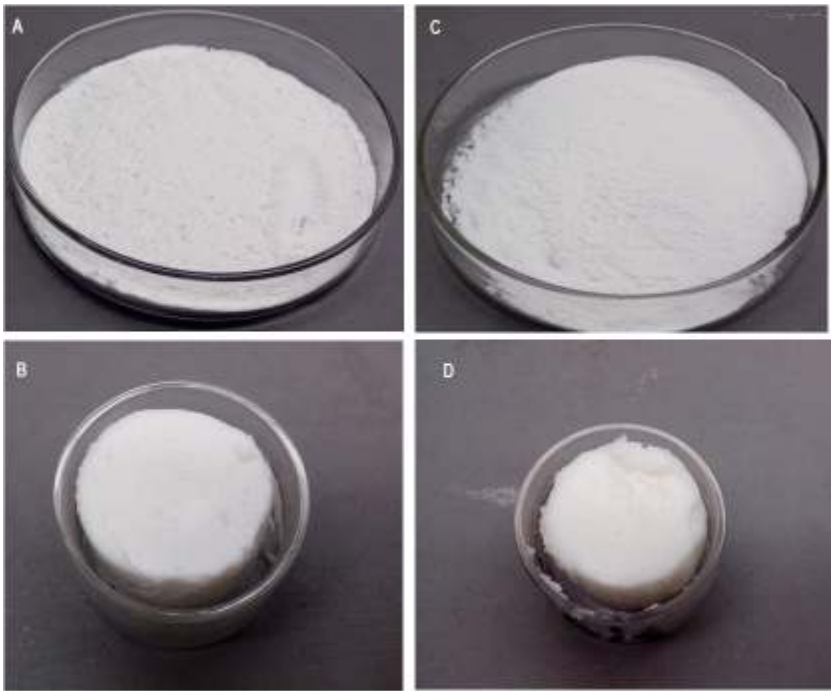


Fig. 1: Powder and gel pictures of duck egg whites, A) Freeze Dried Salted Duck Egg White (FSDEW), B) Salted Duck Egg White Gel (SDEWG), C) Freeze dried Desalted Duck Egg White (FDDEW), D) Desalted Duck Egg White Gel (DDEWG)

protein contents (AOAC, 2000). The pH values of SDEW and DDEW were determined by a pH meter (SP-2200, Suntex Instruments Co. Ltd., Taipei, Taiwan). Salt content in SDEW and DDEW was measured according to Kaewmanee *et al*. (2009). Sample (1 g) was treated with 20 mL of 0.1 N $AgNO_3$ and 10 mL of HNO_3. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was cooled using running water. Five mL of 5% ferric alum indicator ($FeNH_4$ $(SO_4)_2$.12 H_2O) were added. The mixture was titrated with the standardized 0.1 N KSCN until the solution became permanently light brown. The percentage of salt was then calculated as follows:

$$Salt\ (\%) = (5.8 \times [(V_1 \times N_1) - (V_2 \times N_2)]/W$$

where,
V_1 = The volume of $AgNO_3$ (mL)
N_1 = The concentration of $AgNO_3$ (N)
V_2 = The volume of KSCN (mL)
N_2 = The concentration of KSCN (N)
W = The weight of sample (g)

Determination of amino acid composition: The amino acid composition of FSDEW and FDDEW was determined as described earlier by Adeyeye (2009) with slight modifications. Thirty mg of sample was weighed into glass ampoules, 7 mL of 6 M HCl added and oxygen expelled by passing nitrogen into samples. The glass ampoules were sealed with a flame and heated at 105±5°C for 22 h. The ampoules were cooled, opened and the contents filtered. The filtrate was evaporated to dryness at 40°C under vacuum. The residue was dissolved with 5 mL acetate buffer (pH 2.0). Each sample (1 µL) was injected on a Zorbax 80 A C18 column (i.d., 4.6×180 mm, Agilent Technologies) at 40°C with detection at 338 nm. Mobile phase A was 7.35 mM/L sodium acetate/triethylamine/ tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM/L sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

Physical analysis: The color of FSDEW and FDDEW and their respective gels (SDEWG and DDEWG) was measured on 15 mm high packed and leveled samples in quadruplicates with a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) using the CIE (Commision Internationale de L' Enclairage) Lab scale (D65). The L*-, a*- and b*-values reflect lightness (0: black; 100: white), redness (-100: green; 100: red) and yellowness (-100: blue; 100: yellow), respectively (Hammershøj *et al*., 2006). Whiteness was calculated using the following equation: Whitness = 100- [(100 - $L^*)^2 + a^{*2} + b^{*2}$]1/2.

The particle sizes of the freeze dried FSDEW and FDDEW were estimated by laser particle size analyzer Microtrac S3500 and SDC Model Bluewave (Microtrac Inc-USA). An amount of 500 mg of powder of each sample was placed in the laser particle size analyzer for determination of its size distribution (Daou and Zhang, 2012). Representative pictures with 43-127 particles per sample were obtained in triplicate.

The images of microstructure of FSDEW and FDDEW were obtained using a scanning electron microscope (S-4800, Hitachi, Tokyo, Japan) at an accelerating voltage of 1.0 kV. Dried samples were coated using a gold-palladium alloy coater (Bal-Tec Co., Manchester, NH, USA) and the samples were observed at 600 and 2000 x magnification.

Determination of functional properties: The functional properties of salted and desalted duck egg whites were determined according to the following previously published methods: gelling properties (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience) by Bourne (1982); foaming capacity and foam stability, Wang *et al*. (2013); emulsifying properties, Guo and Mu (2011) and turbidity, Van der Plancken *et al*. (2006).

RESULTS AND DISCUSSION

Proximate composition: The proximate compositions and pH values of salted and desalted duck egg whites (SDEW and DDEW respectively) are presented in Table 1. The moisture, protein, salt and ash contents of SDEW are 83.54, 9.40, 3.76 and 3.04%, respectively; while those of DDEW appeared to be 85.75, 8.24, 0.18 and 0.12%, respectively. The pH values of SDEW and DDEW were 8.07 and 7.40, respectively. Electrodialysis reduced the sodium chloride content of SDEW to about 95%. As reported by Chi and Tseng (1998), during salting, water could migrate from egg yolk to egg white, then to the environment through the egg shell, as governed by pore sizes and structure of the shell. Thus, the inverse effect took place during desalination. Indeed, as the desalination proceeded, moisture content in DDEW increased, most likely due to the release of NaCl from egg white to the outside.

Amino acid profile: Seventeen amino acids namely aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine in FSDEW and FDDEW were analyzed (Table 2). All the essential amino acids (histidine, threonine, valine, methionine, phenylalanine, isoleucine, leucine and lysine) were present in the duck egg white samples investigated except tryptophan which was not determined. It is clear that FDDEW contains all the essential amino acids in good

Table 1: Proximate composition and pH of salted and desalted duck egg whites

Parameters	SDEW[a]	DDEW[b]
pH	8.07±0.01	7.40±0.010
Salt (%)	3.76±0.20	0.18±0.020
Protein (%)	9.40±0.01	8.24±0.030
Ash (%)	3.04±0.09	0.12±0.006
Moisture (%)	83.54±0.05	85.75±0.030

Means of three determinations±S.D.; [a]SDEW: Salted duck egg white; [b]DDEW: Desalted duck egg white

Table 2: Amino acid composition (g/100 g protein) of salted and desalted duck egg whites

			FAO/WHO/UNU[c]	
Amino acid	FSDEW[a]	FDDEW[b]	Child	Adult
Essential Amino Acid (EAA)				
Histidine	2.07	2.13	1.90	1.60
Threonine	5.78	5.86	3.40	0.90
Valine	6.62	6.59	3.50	1.30
Methionine	5.93	5.87	2.70	1.70
Phenylalanine	7.43	7.35	6.30	1.90
Isoleucine	4.43	4.28	2.80	1.30
Leucine	7.88	7.86	6.60	1.90
Lysine	6.59	6.93	5.80	1.60
Tryptophan	-	-	1.10	0.50
Non Essential Amino Acid (nEAA)				
Aspartic acid	8.88	8.98		
Glutamic acid	14.80	14.82		
Serine	7.10	7.19		
Glycine	3.40	3.44		
Arginine	4.46	4.43		
Alanine	4.63	4.57		
Tyrosine	4.03	4.01		
Cysteine-s	1.44	0.93		
Proline	4.47	4.01		

[a]FSDEW: Freeze dried salted duck egg white; [b]FDDEW: Freeze dried desalted duck egg white; [c]FAO/WHO/UNU: FAO (2007)

Table 3: Color characteristics of salted and desalted duck egg white powders and gels

Color attributes	SDEWG[a]	DDEWG[b]	FSDEW[c]	FDDEW[d]
L*	85.620±0.34	84.31±0.14	95.69±0.08	94.77±0.05
a*	-0.360±0.48	-1.08±0.05	-0.18±0.03	-0.73±0.02
b*	2.386±0.11	1.86±0.33	6.99±0.06	6.64±0.13
Whiteness	85.390±0.39	84.15±0.23	91.78±0.03	91.51±0.08

Means of three determinations±S.D.; [a]SDEWG: Salted duck egg white gel; [b]DDEWG: Desalted duck egg white gel; [c]FSDEW: Freeze dried salted duck egg white; [d]FDDEW: Freeze dried desalted duck egg white

proportion as compared to FSDEW. The results shown in Table 2 indicate that the amino acid composition of FDDEW closely resembles to that of FSDEW from which it was prepared. Moreover, all the essential amino acids in both samples were at a higher level than the Food and Agriculture Organization/World Health Organization reference pattern (FAO, 2007). And overall, FSDEW and FDDEW showed similar levels of all the amino acids. These results indicated that the desalination process did not have a considerable effect on the amino acid composition of the investigated samples.

Color: The color attributes of duck egg white gels (SDEWG, DDEWG) and powders (FSDEW, FDDEW) were analyzed and presented in Table 3 and Fig. 1. The heat induced gels (SDEWG, DDEWG) had significantly lower L-values than their respective powders (FSDEW, FDDEW) (Table 3). This might be due to the gelation process during which SDEWG and DDEWG were subject to high temperature (95°C for 30 min), leading to browning which ultimately results in color change deteriorations compared to FSDEW and FDDEW.

On the other hand, all the duck egg white products (gels and powders) produced negative a-values which indicate a greener color according to the Hunter scale (Hoppe, 2010). DDEWG had the lowest a-value at -1.08 followed by FDDEW (-0.73). With respect to b-values, gelation significantly decreased the values indicating a less yellow color. Although 'a' and b-values indicate the presence of other colors, all the samples were visually white.

Microstructure: The surface morphology or the surface micro-structural characteristic of FSDEW and FDDEW particles was examined in order to understand the effect of desalination treatment on the quality of FSDEW and FDDEW (Fig. 2). It can be observed from Fig. 2A that FDDEW possessed large particle (fragment) size while FSDEW (Fig. 2B) particles possess regular and uniform pore size and pore size distribution. The differences in the microstructure of FSDEW and FDDEW particles can be explained based on the alteration in composition and protein properties in FDDEW. Indeed, the desalination process leads to the formation of large protein agglomerates rather than uniform molecular dispersion as in FSDEW; where the high salt content reduces protein-protein interactions (electrostatic repulsion), thus producing a more uniform channel for vapor transfer within the material during the freeze drying process. A similar trend in microstructure differences was observed by Wang *et al*. (2013) concerning egg whites upon drying using different methods.

Particle size: Figure 3 showed the particle size distribution of FSDEW and FDDEW. Particle size measurements demonstrated that electrodialysis does have an impact. After desalination, FDDEW showed a narrow size distribution with particle up to 27 μm (d906.7 μm). A small shift to higher sizes was then visible for FSDEW. Some particles up to 500 μm (d9012.1 μm) were detectable. Particle size of duck egg white protein obviously increased in the presence of NaCl suggesting that NaCl played a role in modification of protein charge (Kaewmanee *et al*., 2011). This might govern the physicochemical changes of protein molecules, both native and denatured forms as evidenced by the increase in particle size of FSDEW.

Emulsion properties: Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI) are important parameters for the characterization of the quality of a dehydrated protein. Both the EAI and ESI are also

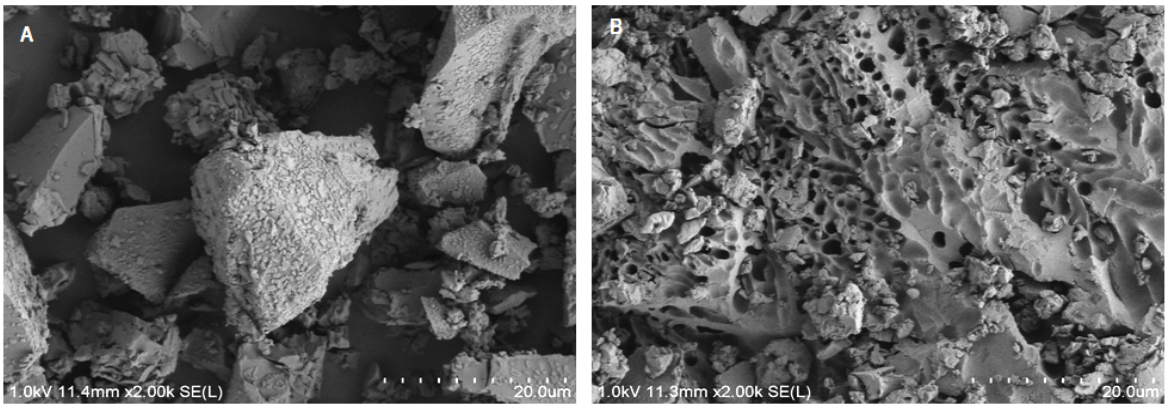


Fig. 2: Scanning electron micrographs of duck egg white powders, A) Freeze dried Desalted Duck Egg White (FDDEW), B) Freeze dried Salted Duck Egg White (FSDEW)

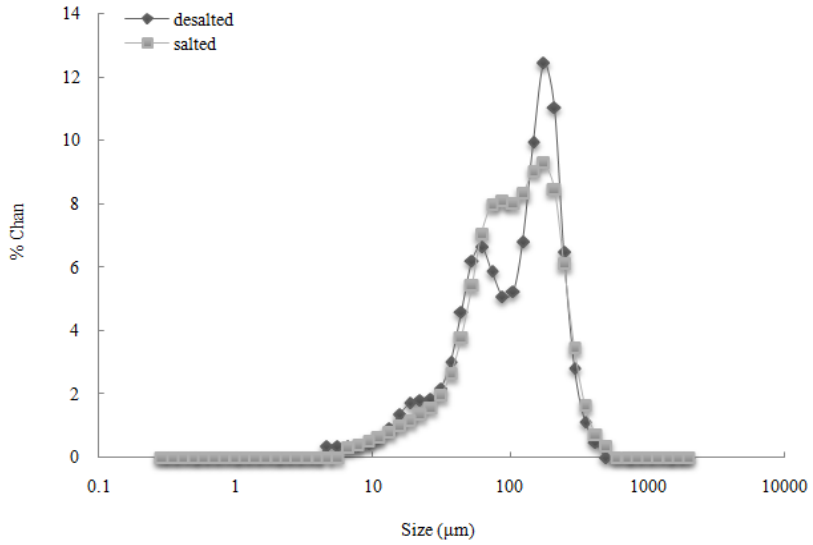


Fig. 3: Particle size distribution of Freeze dried Salted Duck Egg White (FSDEW) and Freeze dried Desalted Duck Egg White (FDDEW)

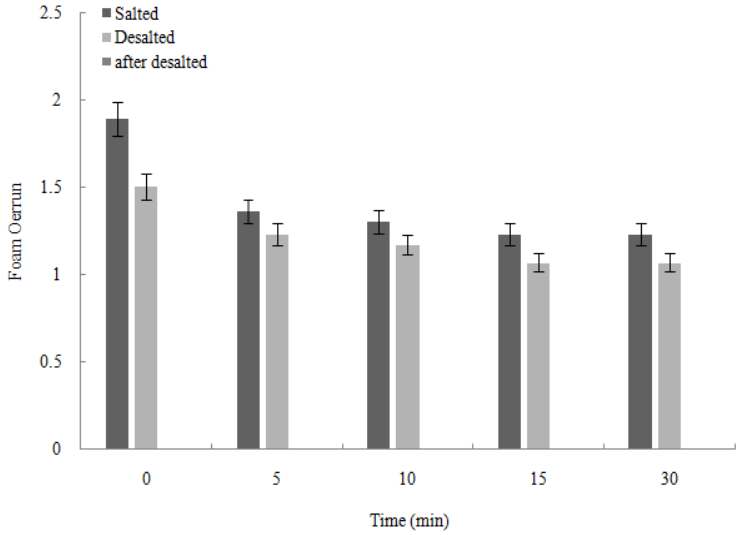
Table 4: Functional properties of salted and desalted duck egg white powders and gels

Gelling properties	SDEWG	DDEWG
Hardness	2113.27000	788.135300
Adhesiveness	-63.25700	-132.507000
Springiness	0.79600	0.595333
Cohesiveness	0.36967	0.276667
Gumminess	836.57800	197.811700
Chewiness	656.36500	111.464300
Resilience	0.16800	0.348000
Foaming and emulsification	FSDEW	FDDEW
Emulsion activity index	58.29	138.68
Emulsion stability index	14.64	12.04
Foam volume	56.80	45.00

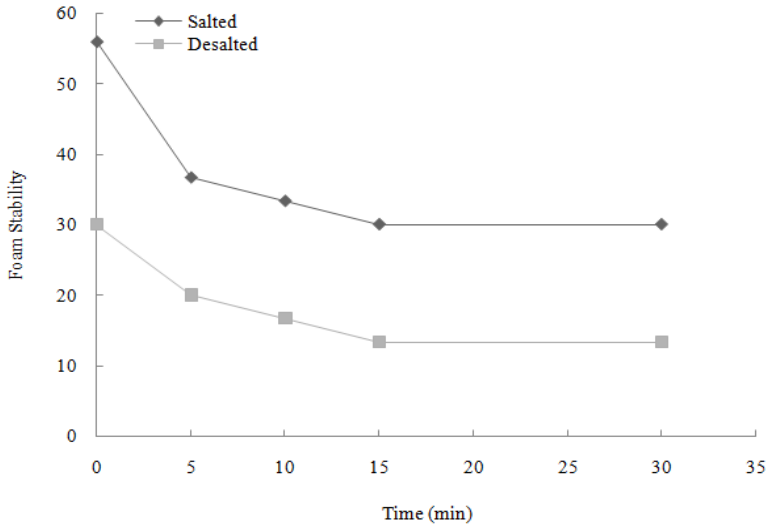
[a]SDEWG: Salted duck egg white gel; [b]DDEWG: Desalted duck egg white gel; [c]FSDEW: Freeze dried salted duck egg white; [d]FDDEW: Freeze dried desalted duck egg white

influenced by processing conditions, composition of the sample, the method of preparation of the sample and the extent of structural and chemical disruptions (Fennema, 1996; Wang *et al*., 2013). The emulsifying activity and emulsion stability of SDEW and DDEW are presented in Table 4. DDEW had a higher emulsifying activity index than SDEW. As reported by Paulson and Tung (1987) and Huang *et al*. (1999), this could be explained due to fact that DDEW had a higher soluble protein percentage which contributes to emulsifying activity. However, after electrodialysis treatment, the salted duck egg white slightly decreased in emulsifying stability.

Foam properties: Two of the most common measures of foaming properties of egg white include foam overrun and foam stability. Foam Overrun (OR) is defined as the foam volume measured against the initial liquid volume of the solution before foaming. Foam Stability (FS) is a measured by the amount of liquid drainage from the foam in relation to the initial liquid volume before foaming (Lomakina and Mikova, 2006; Hoppe, 2010). There are many factors that affect egg



(a)



(b)

Fig. 4: Foaming capacity (a) and stability (b) of Freeze dried Salted Duck Egg White (FSDEW) and Freeze dried Desalted Duck Egg White (FDDEW)

foam properties including but not limited to salt concentration, sugar content, pH and processing conditions. The presence of NaCl enhances foaming ability and increases foam overrun. The salt reduces protein-protein interactions (electrostatic repulsion) allowing them to unfold more readily and be incorporated in the air/water interface, thus increasing foaming capacity.

After electrodialysis treatment, FDDEW showed a decrease of foam-ability, suggesting that greater aggregation of protein had occurred (Fig. 4a) which affected protein unfolding when foaming and resulted in the lowered effectiveness of gas encapsulation by egg white protein. The foam stability decreased during setting times from 0 to 35 min in both FSDEW and FDDEW. However, FDDEW appeared to be more unstable (Fig. 4b). Indeed, the effect of salt on duck egg white was to reduce the electrostatic repulsive force between the protein molecules which caused the foam to collapse and lose its stability (Phillips *et al*., 1991).

Gelling: Gelation and gel structure of dried protein powders are widely used as quality indexes. According to Fennema (1996) and Joshi *et al*. (2011), physical and chemical changes can occur in the structure and composition of protein during gel formation.

From Table 4, it can be observed that after gelation with heat at 95°C, SDEWG had a hardness value of 2113.27 g, over twice the value (788.13 g) of DDEWG. Gel gumminess followed a similar pattern to

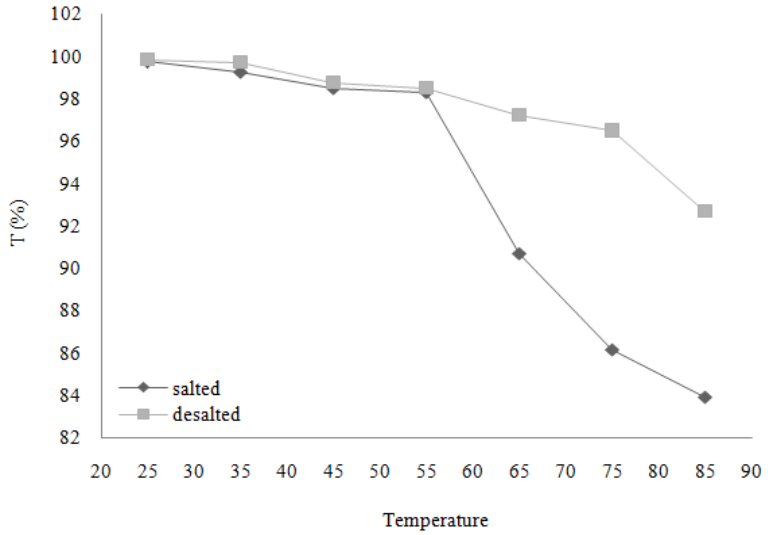


Fig. 5: Turbidity of Freeze dried Salted Duck Egg White (FSDEW) and Freeze dried Desalted Duck Egg White (FDDEW) measured at pH 7.6

gel hardness with SDEWG being gummier (Table 4). Indeed, according to Hoppe (2010) the textural properties of heat-induced gels tended to have greater measurement variation, particularly the hardness and gumminess properties. These variations may be explained by uneven heat transfer during treatment or insufficient cooking time (softer near the middle of sample). The same trend was observed for the other textural characteristic such as adhesiveness, springiness, cohesiveness and chewiness, except for resilience. As indicated in previous studies (Qingnong *et al*., 1995; Kitabatake *et al*., 1989), the removal of salt following electrodialysis process caused the protein aggregates in DDEWG to dissociate which resulted in an overall softer gel (Fig. 1D).

Turbidity: Turbidity development of duck egg white solutions (1 mg protein/mL, pH 7.6) heated from 20 to 90°C was monitored by the increase in transmittance as shown in Fig. 5. Turbidity is inversely proportional to the transmittance ($A_{650\ nm}$).

As the temperature is increasing from 20 to 90°C, the turbidity of FSDEW and FDDEW also increases. This phase could be considered as the phase of denaturation corresponding to the aggregation of the most resistant protein in FSDEW and FDDEW to heat such as ovalbumin, which represents 54% of proteins in egg white (Van der Plancken *et al*., 2005; Van der Plancken *et al*., 2006). At temperatures range between 45 and 55°C, the decrease in transmittance informs us on the speed of transition between the stage of denaturation and aggregation and could be explained by the denaturation and aggregation of the most sensitive proteins to heat such as the ovotransferrin (13% of proteins in egg white). And, at higher temperatures (≥65°C), the decrease in transmittance could be due to the denaturation and aggregation of the most heat-resistant proteins such as ovalbumin.

CONCLUSION

The results of the present study give a promising perspective for the utilization of desalted duck egg whites regarded as waste. After electrodialysis treatment, the NaCl content was reduced by 95% resulting in a slight decrease in foam overrun and an increase of foam stability. Simultaneously, the duck egg white gel characteristics decreased significantly suggesting that the interaction between protein molecules was weakened and the egg white gel became softer.

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