

## Heat stability and emulsifying ability of whole egg and egg yolk as related to heat treatment

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### Abstract

Egg proteins are extensively utilised in many food products due to their unique functional properties. Some of the egg proteins are particularly sensitive to heat treatment and that can be a limitation to the processing of egg containing products. Suspensions of whole egg and egg yolk were heated at various temperatures in the presence of variable concentrations of sugar and salt. It was shown that egg proteins can withstand severe heat treatments when sugar and salt are present during the process. Depending on the sugar and salt concentrations, whole egg and egg yolk suspensions can be heated at temperatures as high as 80 °C (2 min). Sugar and to a higher extent salt had an impact in delaying the denaturation of egg proteins and thus, increased heat stability. The effects of this heat treatment on the emulsifying properties of egg proteins were investigated for a range of protein content levels. Our results indicate that despite the severe heat treatment the egg proteins heated in the presence of sugar and salt were still capable of forming and stabilising emulsions. The sugar and salt concentrations present during the heating process, under specific temperature–time conditions, are correlated with the ionic strength of the solution and the degree of denaturation of the egg proteins, which in turn determine the adsorption capacities of the latter to the interfacial film.

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*Keywords:* Oil-in-water emulsion; Heat stability; Egg proteins; Emulsifying ability; Sugar and salt concentrations

### 1. Introduction

Hen's egg either as whole or its constituents (egg yolk, egg white) is a key ingredient in many food products. Egg proteins add a high nutritional value to egg containing foods and they are also associated with several functional properties for industrial applications (Kato, Minaki, & Kobayashi, 1993). These include gel formation, foaming capacity and emulsifying ability among others.

To ensure that egg products are free from pathogenic bacteria and particularly *Salmonella* whole egg, egg yolk and egg white need to be pasteurised under certain conditions. The pasteurisation processes are determined by regulations and they sometimes vary from one country to another. Egg processors are concerned about these time–temperature treatments because the products should not only be microbiologically safe but should also perform satisfactorily (Cunningham, 1995). Severe heat treatments

can further ensure microbial safety and increase shelf-life of egg products but can have detrimental effects on the functional properties of egg proteins resulting in commercially undesirable finished products (Le Denmat, Anton, & Gandemer, 1999). Therefore several attempts have been made by researchers to identify methods which would allow eggs to withstand severe heat treatments without altering or at least damaging their physical and functional properties (Aoki et al., 1999; Dutilh & Groger, 1981; Handa & Kuroda, 1999; Kato, Aoki, Kato, Nakamura, & Matsuda, 1995).

It has been reported that the addition of salt to egg suspensions promotes protein aggregation which may result in network formation (Yang & Baldwin, 1995). Salt affects hydrogen bonds and inhibits interactions between water molecules and the hydrophilic groups in the protein backbone, promoting the formation of coagulum as a result of increased hydrophobicity. Moreover, sucrose is known to elevate the temperature required to cause coagulation of egg proteins in proportion to the amount added (Yang & Baldwin, 1995).

Our objective was to apply a novel approach (Campbell, 2002) for heat treatment of whole egg and egg yolk

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and study the effect on heat stability. The aim of this study was to investigate the combined effect of NaCl and sucrose on the heat-induced aggregation of whole egg and egg yolk proteins. Up to date, there has not been a study of the combined effect of NaCl and sucrose on heat stability of whole egg and egg yolk proteins. Moreover, adding high amounts of salt or sugar, in order to increase the heat stability of the samples, could result to undesirable products from a commercial point of view. Therefore, the addition of both ingredients at relatively low concentrations would be more suitable for potential industrial applications of the egg samples. We varied the conditions involved in the novel heat treatment in order to study how these affect the stability of the egg samples. Furthermore, the effect of the above heat treatment under differing conditions on protein denaturation and the emulsifying ability of whole egg and egg yolk was investigated. The emulsifying ability of the so-treated egg samples was also compared to that resulting from a commercial heat treatment.

## 2. Experimental

### 2.1. Materials

Fresh eggs and sunflower oil were purchased from Safeway's supermarket, UK. Eggs were manually broken and yolk was separated from albumen. Sodium chloride and sucrose were obtained from Sigma Chemicals Co. Acetic acid was obtained from Fisons (analytical grade).

### 2.2. Emulsion preparation

Suspensions of whole egg and egg yolk were subjected to heat treatment in a CM4 mashing water bath (Canongate Technology Ltd). The egg samples were heated in the presence of NaCl and sucrose. The concentrations of NaCl and sucrose were varied. Egg suspensions were placed in the water bath during the whole warming up period to ensure they were treated at the required temperature for the given period of time. The samples were placed on ice immediately after heating to prevent any further aggregation of the egg proteins. Emulsions were prepared using a standard (65% w/w sunflower oil, 10% w/w acetic acid (10% v/v), 1.2% w/w NaCl, 1.2% w/w sucrose) recipe. The egg content varied from 8.93 to 0.45% w/w. The recipe was adjusted by adding the appropriate amount of purified water depending on the egg content used. The appropriate amounts of sugar and salt were also added in order to have a final concentration of 1.2% w/w, taking into account the quantities present during the heating process. The egg samples were emulsified with sunflower oil using a Braun mixer at speed 4. The sunflower oil was added at a steady rate (about 2 min) and after all the oil was added, the emulsion was further mixed using

the Braun mixer at speed 5 for 30 s. The emulsions were then homogenised in an APV homogeniser (APV Systems, Albertslund, Denmark). A pressure of 3–4 MPa was used for homogenisation and the process was carried out at room temperature.

### 2.3. Turbidimetric assay for protein aggregation

Turbidity of the heated egg samples was measured using a HACH 2100N Turbidity meter (CAMLAB). One hundred microliters of the samples were diluted 250-fold into aqueous solution (distilled water containing same concentration of NaCl and sucrose as the one used to heat the samples). The sample and the aqueous solution were mixed thoroughly for about 5 s using a vortex machine. Blank measurements were recorded using aqueous solution. Turbidity was measured using the single 90° detector which receives the light scattered by the particles. Three replicate measurements were recorded and the results presented are the mean of these three replicates.

### 2.4. Emulsifying activity

The droplet size distribution ( $D_{3,2}$ , surface weighted mean) was estimated by laser light diffraction using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). The refractive index (RI) of particles was set at 1470 and the laser obscuration was adjusted at about 10%. Triplicate measurements were carried out and the results presented are the mean of these three replicates.

### 2.5. Electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was carried out by following the method of Laemmli (1970) with minor modifications using an electrophoresis unit (XCell Surelock™ Mini Cell, Invitrogen life technologies). For native PAGE a 12% Tris–glycine precast separating gel was used. Samples were diluted 1/5 (v/v) in distilled water and were then dispersed in an equal volume of dissociation buffer (100 mM Tris HCl, 10% glycerol, 0.0025% bromophenol blue, pH 8.6). The migration buffer was a Tris Base 25 mM, glycine 192 mM, pH 8.3 solution. Electrophoretic migration was performed at 200 V (constant) for 3 h.

### 2.6. Statistical analysis

Three replicates were made for the following parameters: turbidity and average droplet size distribution. The *t*-test (Gosset, 1908) was used to detect significance of differences among means. Confidence levels were set at 95% ( $P < 0.05$ ).

### 3. Results

#### 3.1. Heat stability

##### 3.1.1. Turbidity measurements

Four whole egg suspensions containing variable sugar and salt concentrations were heated at the following temperatures for 2 min: 65, 70, 80 and 85 °C. The control was a plain whole egg suspension heated at 64 °C for 2 min. At 65 °C, the turbidity of all four samples indicates that the egg proteins were not denatured (Fig. 1). At 70 °C, the sample containing sugar/salt in a 12/0 (w/w) ratio was approximately twice as turbid (98.80 Neph) as compared to all other samples, suggesting that denaturation of whole egg proteins occurred to some extent. At 80 °C, most of the samples were too turbid and consequently no measurement could be recorded. However, the turbidity of the samples with the highest salt concentration (sugar/salt, 2/10 and 0/12, w/w) was still measurable. The turbidity of all samples heated at 65 and 70 °C was significantly lower as compared to the control. The control (heated at 64 °C) was approximately twice as turbid as the samples containing the highest salt concentration (sugar/salt, 2/10 and 0/12, w/w) and were heated at 80 °C. These data suggest that both sugar and salt have a significant effect on whole egg proteins and delay aggregation, which results in increased heat stability.

##### 3.1.2. Whole egg emulsions

The samples heated as described in Section 3.1.1 were used for preparing oil-in-water emulsions (65% oil, w/w). The emulsions formed were inspected visually and particle size measurements were carried out to investigate the emulsifying ability of the heat-treated proteins at temperatures that ranged from 65 to 85 °C (Fig. 2). All four samples formed fine emulsions when heated at 65 and 70 °C. The control heated at 64 °C formed a fine emulsion with an average droplet diameter slightly higher compared to the samples heated in the presence of sugar and salt at 65 °C. Heating the control at temperatures higher than 64 °C resulted in gelation of the egg proteins and as a result

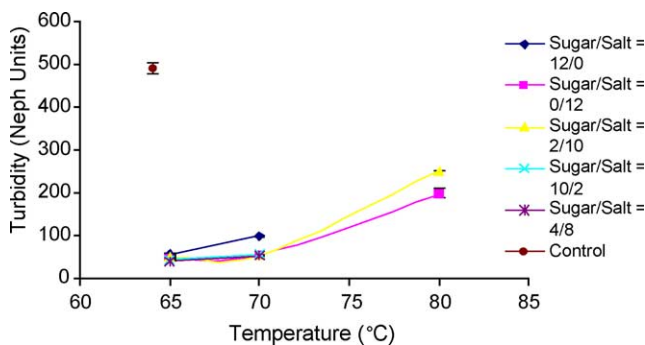


Fig. 1. Turbidity of heat-treated whole egg as a function of temperature. Sugar and salt ratios are expressed on a weight basis (w/w). Data are the mean of three measurements.

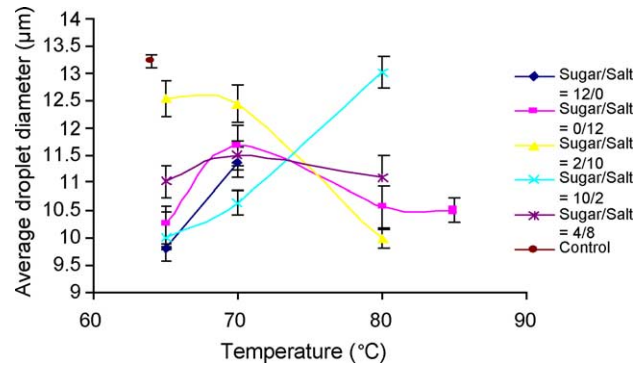


Fig. 2. Average droplet diameter ( $D_{3,2}$ ) of whole egg oil-in water-emulsions (65% oil, w/w) as a function of temperature. Sugar and salt ratios are expressed on a weight basis (w/w). Data are the mean of three measurements.

emulsions could not be prepared. At 80 °C the sample containing sugar and salt in a 12/0 weight ratio was gelled completely and therefore not usable. The sample with 10/2 (w/w) sugar and salt was still usable and formed an emulsion that exhibited higher average droplet diameter compared to the samples with higher salt concentrations. At 85 °C, the only sample that could be used to prepare an emulsion was the one with the highest salt concentration. The emulsion prepared was fine and the average droplet diameter was satisfactory (10.5  $\mu\text{m}$ ). All the samples that contained sugar and/or salt and were heated at a temperature range from 65 to 85 °C exhibited better emulsifying ability as compared to the control which was subjected to a less severe heat treatment. These data indicate that sugar and particularly salt have an impact in increasing heat stability of egg proteins given the fact that, under high salt conditions, their emulsifying ability is retained at temperatures as high as 85 °C.

#### 3.2. Emulsifying ability

##### 3.2.1. Egg content effect on average droplet diameter of whole egg emulsions

Whole egg was heated at 77 °C for 2 min. The egg sample contained sugar and salt in the following ratio (w/w): egg/sucrose/NaCl, 100/4/8. The average droplet diameter ( $D_{3,2}$ ) varied from 11.4 to 38.9  $\mu\text{m}$  (Fig. 3). As the egg protein content used to prepare the emulsion decreases, the average droplet distribution increases significantly. The average droplet size shifts to higher values dramatically when less than 0.89% (w/w) whole egg is used for emulsion formation.

##### 3.2.2. Sugar and salt concentration effect on average droplet diameter of whole egg emulsions

Suspensions of whole egg containing differing levels of sugar and salt were heated at 77 °C for 2 min. The control contained plain whole egg that was treated according to the conventional pasteurisation conditions (64 °C, 2 min).

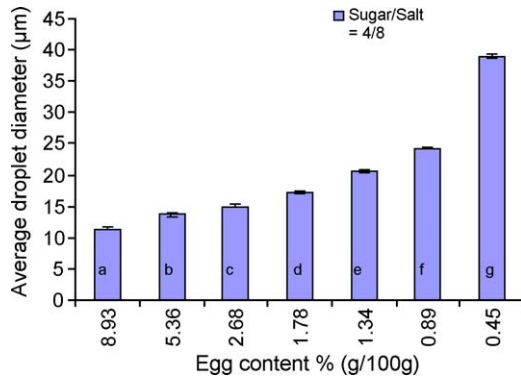


Fig. 3. Average droplet diameter of whole egg oil-in-water emulsions (65% oil, w/w) as a function of egg content. Sugar and salt ratios are expressed on a weight basis (w/w). Data are the mean of three measurements. Means with different superscripts differ significantly ( $P < 0.05$ ).

The average diameter of droplets ( $D_{3,2}$ ) was measured for emulsions containing variable egg protein content (Fig. 4). The average droplet diameter of the samples heated at 77 °C was, in most of the cases, lower compared to the control. The sample containing sugar and salt in a 4/8 (w/w) ratio exhibited significantly lower average droplet diameter compared to the control at all egg content levels. It is worth mentioning that the egg content affects more the average droplet distribution of the emulsions formed compared to the differing levels of sugar and salt present in the whole egg samples during the heating process.

### 3.2.3. Sugar and salt concentration effect on average droplet diameter of egg yolk emulsions

Suspensions of egg yolk containing different sugar and salt concentrations during the heating process (77 °C, 2 min) were compared in terms of the average droplet diameter of the emulsions they formed. The control contained plain egg yolk that was treated according to the conventional

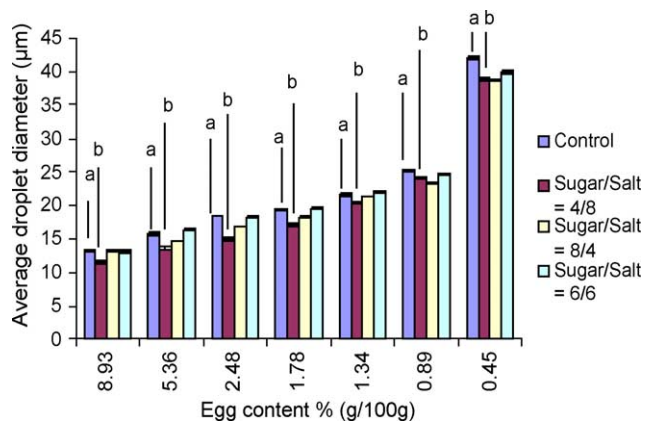


Fig. 4. Influence of sugar and salt concentration contained during the heating process on the average droplet diameter of whole egg oil-in-water emulsions (65% oil, w/w). Sugar and salt ratios are expressed on a weight basis (w/w). Data are the mean of three measurements. Means with different superscripts (for each content level) differ significantly ( $P < 0.05$ ).

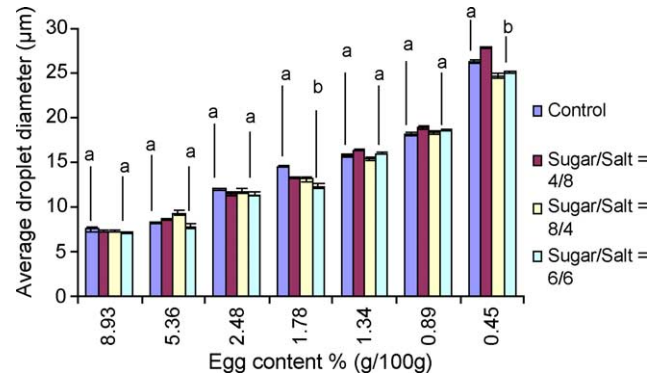


Fig. 5. Influence of sugar and salt concentration on the average droplet diameter of egg yolk oil-in-water emulsions (65% oil, w/w). Sugar and salt ratios are expressed on a weight basis (w/w). Data are the mean of three measurements. Means with different superscripts (for each content level) differ significantly ( $P < 0.05$ ).

pasteurisation conditions (64 °C, 2 min). Again, the average diameter of droplets ( $D_{3,2}$ ) was measured for emulsions containing variable egg protein content (Fig. 5). The average droplet diameter of the samples heated at 77 °C was, in most of the cases, not significantly different from the control. Nevertheless, the sample containing sugar/salt in a 6/6 (w/w) ratio appears to give the lowest average droplet diameter among all samples for the emulsions with an egg content from 8.93 to 1.78% (w/w). Again, the amount of egg yolk used to prepare the emulsions has a more profound effect on the average droplet distribution than the sugar and salt concentrations present during the heating process.

### 3.3. Protein denaturation

Fig. 6 shows the electrophoretic profiles of whole egg proteins under non-denaturing conditions (native PAGE). The band of 45,000 Da corresponds to ovalbumin whereas the dense band of about 130,000 Da possibly represents an aggregate made up of both LDL and granular proteins, which dominate the protein fraction of yolk (Le Denmat, Anton, & Beaumel, 2000). The possibility of the dense band corresponding to an HDL–phosvitin complex through phosphocalcic bridges between seryl residues of HDL and phosvitin (Anton, Beaumel, & Gandemer, 2000) is low at the given ionic strength conditions ( $< 0.17$  M). Four other bands corresponding to proteins ranging from 50,000 to 70,000 Da can be clearly seen. However, it was difficult to assign these bands due to the fact that they were not clearly separated and several egg proteins coincide within this molecular weight range. Nevertheless, the electrophoretic pattern of those bands suggests that the specific proteins are affected by temperature. As temperature increases from 73 to 75 °C, the band intensity progressively decreases. At 77 °C the band corresponding to a protein of 55,000 Da (possibly HDL) disappears, indicating that possibly higher molecular weight aggregates are formed.



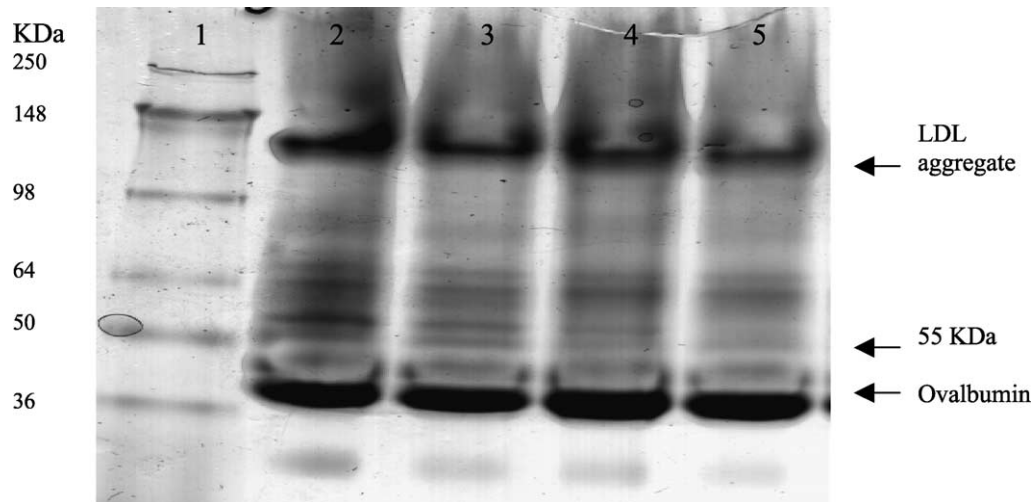


Fig. 6. Native (PAGE) electrophoresis of unheated (control) and heated (73, 75, 77 °C, 2 min) whole egg proteins. From left to right, lane 1: molecular standards, lane 2: control (unheated whole egg), lane 3: heated at 73 °C, lane 4: heated at 75 °C, lane 5: heated at 77 °C. A 12% Tris–glycine precast separating gel was used. Samples were heated in the presence of sugar and salt in a weight ratio 4/8.

## 4. Discussion

### 4.1. Heat stability of egg proteins in the presence of sugar and salt

When whole egg is pasteurised the proteins are subject to structural changes. Depending on the temperature–time conditions of the pasteurisation process these changes may involve denaturation of the proteins with subsequent aggregation and possibly gel formation (Gossal & Ross-Murphy, 2000). A turbidimetric assay system has been developed by Kim, Lee, and Corry (1992) for quantitation of heat induced protein aggregation as a result of protein denaturation. By using this method it has been shown (unpublished results) that there is a linear correlation between increase in turbidity and denaturation of egg white proteins. We employed this turbidimetric method with minor modifications to study the denaturation caused in egg proteins by heat treatment in the presence of sugar and salt.

Our results clearly show that sugar and particularly salt protect to a certain extent the egg proteins from thermal denaturation. When egg proteins are heated in the presence of sugar and salt, denaturation is delayed and the formation of aggregates occurs at much higher temperatures compared to those if plain egg was heated (~64 °C). At 70 °C most of the egg samples (apart from the one containing no salt) appear to be completely unaffected in terms of denaturation by the heat treatment. These findings are in agreement with the results of other researchers who demonstrated that either salt (Boye, Alli, Ismail, Gibbs, & Konishi, 1995) or sugar (Jou & Harper, 1996), when present during the heating process, can increase the denaturation temperature ( $T_D$ ) of whey protein solutions. However, there has not been a study yet on the combined effect of sugar and salt on the denaturation of egg proteins. In this investigation the combined effect of sugar and salt on egg protein

heat-induced denaturation (according to the novel method) was studied. Under high salt conditions, whole egg can be heated as high as 85 °C (2 min) without complete gelation of the proteins. This increased heat stability exhibited by the egg proteins when heated in the presence of sugar and salt can facilitate the use of severe heat processing methods for egg containing products that lack microbial safety.

However, the objective in food processing is not only to increase the heat stability of various components in order to ensure microbial safety but also to assure that these components retain, if not improve, their functional properties. Whole egg (or egg yolk) is used in the food industry as an emulsifier for the production of mayonnaise. Upon pasteurisation of whole egg in the absence of sugar or salt at temperatures higher than 64 °C (2 min), denaturation of egg proteins could occur, which would result in its precipitation upon acidification (acidification is a necessary step in mayonnaise production), thereby resulting in decreased emulsion stability (Campbell, Raikos, & Euston, 2003). Our results indicate that despite the severe heat treatment the egg proteins heated in the presence of sugar and salt were still able to form and stabilise emulsions. All egg samples heated up to 70 °C formed fine emulsions with satisfactory average droplet diameter ( $D_{3,2}$ , 9.8–12.4 μm). At 80 °C, the sample that contained no salt during the heating process was gelled and consequently not able to form an emulsion. The sample with low salt concentration (sugar/salt, 10/2, w/w) formed an emulsion that exhibited higher average droplet diameter compared to the rest of the samples. This may be attributed to extensive denaturation of the most thermolabile egg proteins, which precipitated upon heating and were thus unavailable for stabilising the emulsion. Interestingly, the sample containing the highest salt concentration (sugar/salt, 0/12, w/w) withstood the heat treatment at 85 °C (2 min) and formed an emulsion with fine average droplet diameter ( $D_{3,2}$ , 10.5 μm).

#### 4.2. Emulsifying properties of whole egg and egg yolk proteins heated in the presence of sugar and salt

The effect on average droplet diameter ( $D_{3,2}$ ) of whole egg emulsions as a function of protein concentration is shown in Fig. 3. It is evident that there is a decrease in average droplet diameter with increase in the amount of protein used to prepare the emulsion. By increasing the concentration of proteins, a reduction in the interfacial tension of the droplets is facilitated, which results in their breakdown into smaller droplets (Parker, 1987). Similar results have been reported for egg yolk granule proteins by other researchers (Aluko & Mine, 1998). As shown from Fig. 3 stable emulsions may be prepared with less than maximal amounts of protein covering the oil droplet surface. When emulsions are made with less than saturating amounts of protein, the latter spreads across the surface of the emulsion droplets to cover as much of the surface as possible. This mechanism leads to the formation of a stable emulsion with gaps between the adsorbed protein molecules (Dalgleish, 1999).

It is evident that different proteins have different emulsifying capacities. That may be attributed to particular properties of the proteins, which affect their adsorption capacities at the oil-in-water interface. It has been stated that yolk proteins and particularly lipoproteins have a higher adsorption capacity, compared to globular proteins, due to their flexible molecular structure and a greater surface hydrophobicity (Mine, 1998).

Nevertheless, the efficiency of a protein as an emulsifier depends not only on the type of protein but pH of solution, presence of other emulsifiers, ionic strength and type of oil added (Hermansson, 1979). That means that making an emulsion with proteins that vary in emulsifying capacities, does not ensure that the most surface active proteins end up dominating the interface (Dalgleish, 1999). Consequently, the composition of the interfacial film depends on the conditions under which an emulsion is formed as well as on the type of proteins used (Anton & Gandemer, 1999). Fig. 6 suggests that the conditions under which the egg proteins are heated prior to the emulsification process affect the average droplet diameter ( $D_{3,2}$ ) of the emulsions formed. For whole egg, the sample heated in the presence of sugar and salt in a 4/8 weight ratio gives the lowest average droplet diameter for most egg content levels. That may be associated with the degree of denaturation of the proteins and the ionic strength of the solution. In other words, the degree of unfolding and salt conditions may cause the most surface active proteins to dominate the interface and/or the less active ones to remain unadsorbed or aggregated (Fig. 6). For egg yolk, the sample heated in the presence of sugar and salt in a 6/6 weight ratio appears to give the lowest average droplet diameter for most egg content levels. Again, the degree of denaturation of yolk proteins and the ionic strength of the solution may facilitate the adsorption

of the most surface active proteins (e.g. lipoproteins) at the oil and water interface.

The emulsifying ability of the whole egg and egg yolk samples heated in the presence of sugar and salt is, in most of the cases, slightly better compared to the control (treated according to commercial heat treatment). The disruption of the yolk granules by the addition of NaCl (Chang, Powrie, & Fennema, 1977) and the subsequent liberation of additional ingredients which adsorb to some extent at the oil and water interface could be a possible explanation (Anton et al., 2000). Despite the fact that the addition of sugar and salt prior to the heating process results in a slight improvement with respect to the emulsifying ability as compared to the control, the substantial increase in heat stability associated with the combination of the above ingredients could be beneficial for industrial applications.

## 5. Conclusions

Egg proteins can withstand severe heat treatments when sugar and salt are present during the process. This increased heat stability can be beneficial for processing of egg containing products that lack microbial safety. Salt had a more striking effect in protecting egg proteins from denaturation compared to sugar. Depending on the sugar and salt concentrations, whole egg and egg yolk solutions can be heated at temperatures as high as 80 °C (2 min) without any negative impact on their emulsifying properties. The emulsifying properties of the so-treated egg proteins remain unaffected and in some cases improved compared to the ones exhibited by the egg proteins processed according to conventional heating conditions. The ionic strength of the egg solution and the degree of denaturation of the proteins at given heating conditions (temperature–time) are speculated to affect, sometimes significantly, the average droplet diameter ( $D_{3,2}$ ) of the emulsions formed and are determined by the amounts of sugar and salt added during the heating process.

Further investigations on the interfacial film composition are required to verify these findings. Additional research is required to ensure that such practice does not have a negative impact on emulsion stability. The effect of the specific treatment to the egg samples on properties such as water binding ability, which sometimes correlates with the viscosity of the emulsions need to be investigated.

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