

Protein Denaturation and Emulsifying Properties of Plasma and Granules of Egg Yolk as Related to Heat Treatment

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ABSTRACT

Solubility, electrophoresis, viscosity and emulsifying properties of heated solutions of yolk, plasma and granules were determined. Plasma and yolk were not affected when heated under 69°C. Above 69°C, protein solubility dropped sharply and apparent viscosity rose sharply because of aggregation of proteins. For granules, protein solubility and apparent viscosity were not modified up to 76°C. The constituents of granules were not denatured. Emulsifying activity of yolk and plasma decreased after heating at 72°C but remained steady for granules. Emulsion stabilization properties of yolk, plasma and granules were not influenced up to 76°C. Results suggested that intact granules withstood more severe heat treatments than egg yolk without lessening their emulsifying properties.

Key Words: egg yolk, plasma, granules, heat treatment, emulsions

INTRODUCTION

HEN EGG YOLK IS A KEY INGREDIENT IN MANY EMULSIONS SUCH as mayonnaise or salad dressings and it forms and stabilizes the emulsions. To ensure microbial safety, egg processors generally pasteurize yolk at 60 to 68°C for 3.5 to 4.5 min. These time-temperature treatments are designed to eradicate pathogenic microorganisms (such as *Salmonella*) without damaging yolk proteins and their functional properties (Powrie and Nakai, 1986; Kobayashi et al., 1997). However, they do not ensure complete eradication of microbial flora (Powrie and Nakai, 1986; Denis et al., 1995). Consequently, pasteurized yolk and food emulsions containing yolk, have limited shelf-life and must be kept at 4°C.

Some egg processors and users would prefer to apply stronger heat treatments to further ensure microbial safety of yolk and increase shelf life. However, such heat treatments would alter physical and functional properties of yolk. The viscosity of yolk sharply rises when heated above 65°C and yolk coagulates around 70°C. Several studies have indicated that some individual constituents of yolk are less sensitive to thermal denaturation than whole yolk. Low-density lipoproteins (LDL) solutions (4% w/v) start denaturing at 70°C and form gels at 75°C (Tsutsui, 1988). Phosvitins and some livetins are less sensitive to thermocoagulation than LDL (Dixon and Cotterill, 1981; Tsutsui and Obara, 1982; Woodward and Cotterill, 1987; Yang, 1987). Thus, some individual constituents of yolk may withstand stronger heat treatments than whole yolk without losing their emulsifying properties.

Individual constituents of yolk are difficult to prepare and only plasma (85 % LDL and 15 % livetin) and granules (70 % HDL, 16 % phosvitin and 12 % LDL) can be easily fractionated from yolk at an

industrial scale (McBee and Cotterill, 1979). The emulsifying properties of granules and plasma have been investigated (Dyer-Hurdon and Nnanna, 1993; Anton and Gandemer, 1997). However, there is no report on their sensitivity to heat treatment, or the effects of heat treatment on emulsifying properties of granules and plasma.

Our objective was to determine the effects of temperatures covering mostly those of pasteurization (55–76°C) on protein denaturation and emulsifying properties of plasma and granules compared to yolk. We measured protein solubility and apparent viscosity of heated solutions. We identified the denatured proteins by electrophoresis and evaluated emulsifying activity and emulsion stabilization properties of yolk, plasma and granules.

MATERIALS & METHODS

Preparation of yolk, plasma and granules

One-day-old eggs were collected from one flock of Isabrown hens which received the same standard diet and which were 39 to 46 wk old. The eggs were manually broken and yolks were separated from the albumen. Each yolk was carefully rolled on a filter paper (Whatman) to remove chalazae and traces of albumen adhering to the vitellin membrane. The vitellin membrane was then disrupted with a scalpel blade and yolk was collected in a beaker cooled in iced water.

Yolk was fractionated into plasma and granules according to the method described by McBee and Cotterill (1979). Yolk (~800g) was pooled and diluted with an equal weight of a 0.17M NaCl solution. The diluted yolk was stirred with a magnetic stirrer for 1 h before centrifugation at $10,000 \times g$ for 45 min at 10°C. The supernatant (plasma) was separated from the sediment (granules). It was centrifuged again to avoid contamination with granules. The granules were washed with a 0.17M NaCl solution and collected. Yolk, plasma and granules contained 52.0%, 24.0% and 44.0% dry matter respectively.

Yolk and granules were diluted in a 0.17M NaCl solution to adjust dry matter content to that of plasma (24.0%). After dilution, their ionic strength was close to that of yolk before dilution. The pH of yolk, plasma and granules was adjusted to 6.1 with 1M HCl or 1M NaOH solutions. The standardized preparations of yolk, plasma and granules were kept at 4°C before being heated.

Heat treatment

Six temperatures (°C) were tested : ambient (20–22), 55, 62, 69, 72 and 76. Yolk, plasma and granules preparations were divided into 25 mL samples which were poured into 50 mL test tubes (i.d. 20 mm). The tubes were plunged into a water bath over a multipost magnetic stirrer for 320s. A thermal probe indicated that the temperature in samples reached that of the bath in 170s. The samples were kept at the selected temperatures for 150s. Then, the tubes were immediately plunged into iced water to cool samples as fast as possible. The samples were then stored at 4°C until they were analyzed.

Apparent viscosity

Samples of yolk, plasma and granules were analyzed 48h after heating. Flow curves of the samples (4 mL) were established at 25°C on a stress-controlled rheometer CARRIMED CSL50 (T.A. Instru-

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ments, New Castle, DE) in a cone-plate geometry (diameter : 6 cm, angle : 3°57', truncation : 204 μm). This rheometer enabled accurate determination of the flow behavior of samples ranging from Newtonian fluid (low viscosity) to materials exhibiting plastic behavior. Trials were required to settle the stress conditions which gave comparable ranges of shear rate in the different samples. An apparent viscosity (μ_{40}) was calculated at a shear rate of 40 s^{-1} and expressed in Pa·s.

Protein solubility

Heated samples of yolk, plasma and granules were diluted in a 0.17M NaCl solution to an approximate protein concentration of 1 mg/mL. The diluted samples (24 mL) were equilibrated for 1 h at 20–22°C with a magnetic stirrer. A sample (4 mL) was taken for the determination of initial protein content. The remaining 20 mL were centrifuged at $10,000 \times g$ for 20 min at 10°C and 4 mL of the supernatant were collected. Protein content was determined by the procedure of Markwell et al. (1978). Calibration curve was established with bovine serum albumin. The protein solubility was calculated as mg protein in supernatant/mg protein in initial dilution $\times 100$.

Emulsifying activity

Heated samples of yolk, plasma and granules were diluted in 12 mL 0.5M NaCl solution to a final concentration of 0.5 % (w/w). The pH was adjusted to 6.5. A commercial sunflower oil (28 mL) was added to prepare oil in water emulsions (oil volume fraction=0.70). The mixture was homogenized at 20,000 rpm for 120s with a shear agitator (Polytron PT 3000, Kinematica, Littau, Switzerland) equipped with a 12 mm diameter head. Immediately after homogenization, 2 mL of emulsion were removed and diluted (1/25) with a 0.05M Tris-HCl, 1% sodium dodecyl sulfate, pH 8.0 solution. The size distribution of droplets was determined on the diluted emulsions by laser light diffraction using a Mastersizer 3600 (Malvern Instruments Ltd, Malvern, UK) in the Fourier conformation (focus: 100 mm). The median diameter of the droplets (d_{50} expressed in μm) was calculated from the data (the smaller the median diameter, the higher the emulsifying activity).

Emulsion stability

Heated samples of yolk, plasma and granules were diluted in 25 mL 0.5M NaCl solution to a final concentration of 0.5 % (w/w). A commercial sunflower oil (15 mL) was used to prepare oil in water emulsions (oil volume fraction=0.375). Emulsification was performed with a shear agitator (Polytron PT 3000, Kinematica, Littau, Switzerland) at 20,000 rpm for 30s at 23°C equipped with a 12 mm diameter head.

Emulsion stability was determined as described by Anton and Gandemer (1997). The kinetics of oil volume fraction in the creamed phase (Φ) were determined by electrical conductivity of emulsions during 4.5h. Φ was plotted vs time and two values were calculated : the creaming rate using the initial slope of the curve (between 300s and 1200s after emulsification) and the final Φ value (15800s to 16200s after emulsification).

Evaluation of protein denaturation

Polyacrylamide gel electrophoresis was performed under non denaturing conditions. Two gels were prepared: one with samples of unheated yolk, plasma and granules, and the second with heated samples of yolk. Samples were diluted 1/10 in a 0.5M NaCl solution to assure complete solubilization of proteins. The diluted samples were dispersed in an equal volume of dissociation buffer (0.5M Tris HCl pH 6.8, 0.05% bromophenol blue, 10% glycerol, 50% H_2O). The stacking and running gels were 3.5% and 6% polyacrylamide, respectively. The migration buffer was a Tris HCl 0.05M, glycine 0.17M, pH 8.8 solution. About 30 μg protein sample was placed on the gel. Electrophoretic migration was performed at 25 mA for 1.5h. The proteins were stained with a Coomassie blue (0.05%)/ethanol (25%)/acetic acid

(10%)/triton (1%)/aluminium nitrate (0.1M) solution. Gels were destained in acetic acid (7%)/ethanol (40%)/water (53%).

Statistical analysis

Three replicates were made for the following parameters: apparent viscosity, protein solubility, emulsifying activity and emulsion stability. For each fraction, results were subjected to a 1-way analysis of variance according to the GLM procedure with least-square means effects using STATGRAPHICS (Statistical Graphics Corporation, Rockville, MD) software. The temperature was the experimental factor with 6 levels: ambient (20–22°C), 55°C, 62°C, 69°C, 72°C and 76°C. Confidence intervals were set at 95% ($p < 0.05$).

RESULTS

Apparent viscosity and protein solubility

For unheated yolk and plasma, the flow curves were characteristic of Newtonian liquids with low viscosity (5×10^{-3} Pa·s). In contrast, unheated granules had a much higher apparent viscosity (1.8×10^{-2} Pa·s) and exhibited a low yield stress with flow behavior (Fig. 1). Solubility of granules proteins was low compared to those of yolk and plasma: 24% vs 71% and >90%, respectively (Fig. 2).

When the temperature was increased from 55 to 76°C, the apparent viscosity of solutions and the protein solubility curves showed

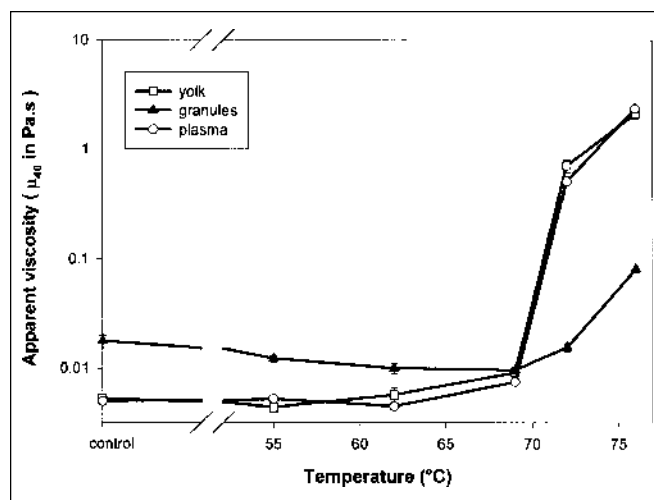


Fig. 1—Changes in apparent viscosity of yolk, plasma and granules as related to temperature. 24.0 $\pm$ 0.5 % dry matter ; viscosity measurement: shear rate = 40 s^{-1} at 25°C.

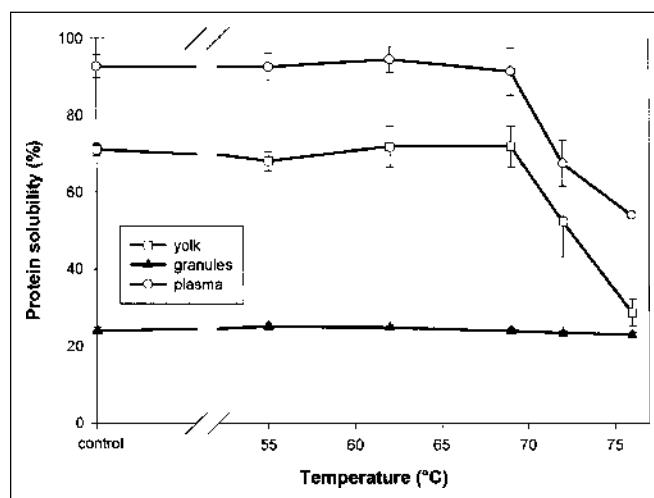


Fig. 2—Changes in protein solubility of yolk, plasma and granules as related to temperature. pH 6.1; 0.17M NaCl.

similar patterns for yolk and plasma (Fig. 1, Fig. 2). Below 69°C, the apparent viscosity and protein solubility were not changed ($p < 0.05$). The apparent viscosity increased sharply between 69°C and 72°C (2.0 log units) and at a slower rate between 72°C and 76°C (0.3 log unit). Concurrently, the protein solubility dropped by 40% and 50%, respectively, between 69 and 76°C. Yolk and plasma heated at 72°C and 76°C were gellified as observed visually.

The apparent viscosity and protein solubility of granules were less influenced by temperature than those of yolk and plasma (Fig. 1, Fig. 2). Between 55°C and 69°C, a slight decrease of apparent viscosity was observed with no change in protein solubility. Between 69°C and 76°C, heating caused a slight increase of the apparent viscosity (0.7 log unit) but the solutions did not gel. Concurrently, protein solubility was not significantly affected (3% of loss).

Protein denaturation

The electrophoresis of unheated yolk performed in non-denaturing conditions showed 11 bands which were identified from results previously published (Dixon and Cotterill, 1981 ; Abe et al., 1982 ;

Yang and Cotterill, 1989 ; Causeret et al., 1991) (Fig. 3a). Band 1 (immobile materials) was assumed to be LDL, 2 and 4 were identified as β - and α -HDL, 3 was γ -livetins, 5 to 8 were β -livetins, 9 was α -livetins and 10 and 11 were, respectively, β - and α -phosvitins. Plasma contained LDL and livetins, whereas granules contained HDL and phosvitins.

The LDL band progressively disappeared between 72°C and 76°C (Fig. 3b). The intensity of γ -livetins band decreased after heating at 62°C and the band was not detectable above 69°C. The intensity of β -livetins bands was not affected by temperature. The intensity of α -livetins band decreased when samples were heated at 72°C and the band disappeared after heating at 76°C. The intensity of β -HDL band was similar at all temperatures whereas that of α -HDL band decreased after heating at 72°C and further decreased after heating at 76°C. α - and β -Phosvitins bands were not affected by temperature (Fig. 3b). Consequently, we assumed that proteins and lipoproteins of plasma (livetins and LDL) were more affected by heat treatment than proteins and lipoproteins of granules (phosvitins and HDL).

Emulsifying activity

Unheated granules had a better emulsifying activity than yolk and plasma on an equal dry matter basis. The median diameter of oil droplets in emulsions prepared with granules was significantly lower than with yolk and plasma (25.7 μm vs 29.9 μm and 40.4 μm for yolk and plasma respectively) (Fig. 4).

When the samples were heated, changes in the median diameter of oil droplets in emulsions prepared with yolk and plasma were similar (Fig. 4). Between 55°C and 69°C, the median diameter of the oil droplets remained constant. Between 69°C and 76°C, it rose significantly, increasing by 15 μm and 21 μm for emulsion prepared with yolk and plasma heated at 76°C (compared with unheated preparation). In contrast to yolk and plasma, the median diameter of emulsions made with granules was similar at all temperatures (Fig. 4).

Emulsion stability

For unheated samples, creaming rates (Fig. 5) and final oil volume fraction (Φ) (Fig. 6) were lower when emulsions were prepared with granules compared to yolk and plasma ($0.43 \cdot 10^{-5} \text{ s}^{-1}$ vs $2.5 \cdot 10^{-5} \text{ s}^{-1}$ and $3.3 \cdot 10^{-5} \text{ s}^{-1}$ and 0.49 vs 0.53, 0.52, respectively). These two measurements showed that granules provided better emulsion stability than yolk and plasma. When samples were heated at 55°C to 76°C, no change was observed for any fraction (yolk, plasma or granules) for creaming rate or final oil volume fraction, suggesting that emulsion stability was not affected by temperature.

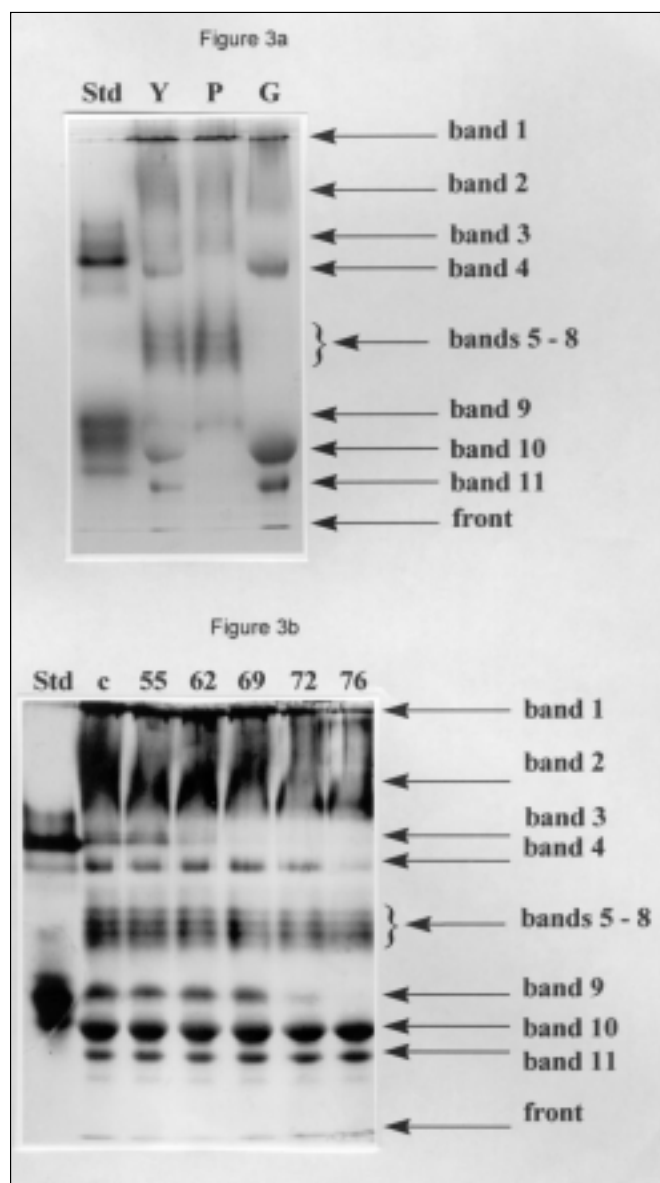


Fig. 3—(a) Polyacrylamide gel electrophoresis of unheated solutions of yolk, plasma and granules. Std=molecular standards, Y=yolk, P=plasma, G=granules. (b) Polyacrylamide gel electrophoresis of yolk solution heated at different temperatures (55, 62, 69, 72, and 76°C). Std= molecular standards; C=unheated yolk solution; stacking and running gels: 3.5 % and 6 % polyacrylamide, respectively.

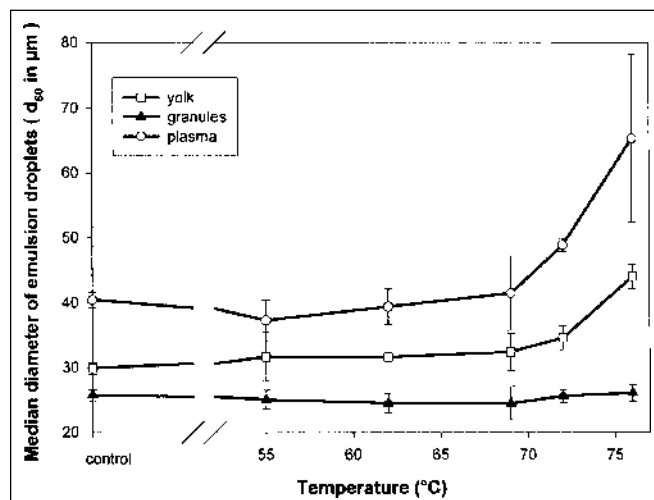


Fig. 4—Changes in median diameter of droplets in emulsions prepared from yolk, plasma and granules as related to temperature. Emulsions O/W 70 : 30 ; 0.5 % dry matter in the aqueous phase; pH 6.5; 0.50M NaCl.

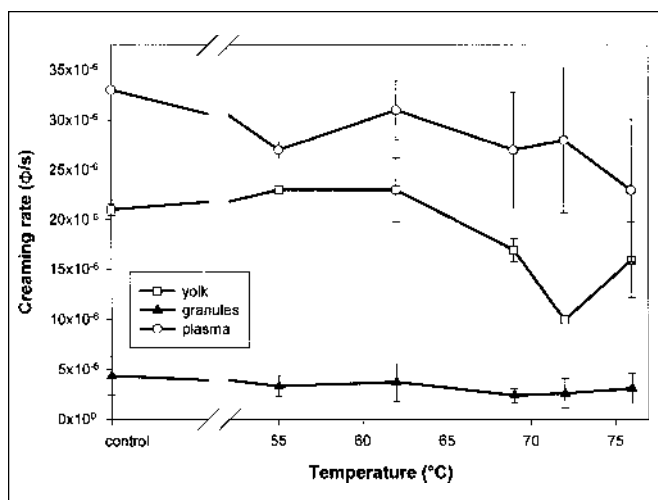


Fig. 5—Changes in creaming rate of emulsions prepared from yolk, plasma and granules as related to temperature. Emulsions O/W 37.5:62.5; 0.5% dry matter in the aqueous phase; pH 6.5; 0.50M NaCl.

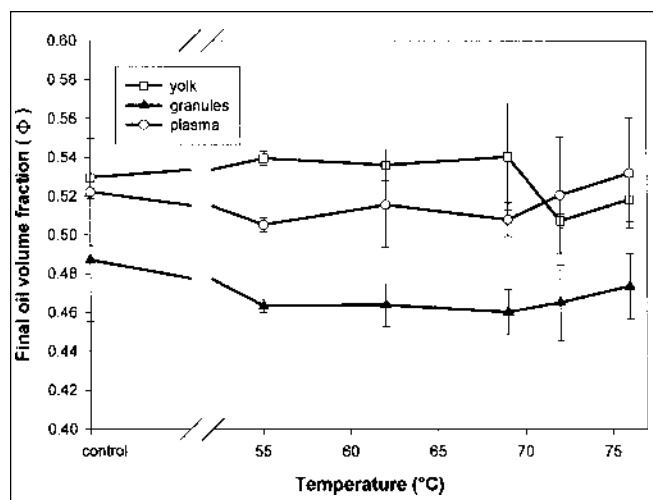


Fig. 6—Changes in the final oil volume fraction (Φ) in the creamed phase of emulsions prepared from yolk, plasma and granules as related to temperature. Emulsions O/W 37.5:62.5; 0.5% dry matter in the aqueous phase; pH 6.5; 0.50M NaCl.

DISCUSSION

Granules were resistant to heat treatment

Results showed that granules were less sensitive to heat than plasma and yolk. In our experimental conditions (pH 6.1 and NaCl 0.17M), the constituents of granules: HDL and phospholipids, were bound together by phosphocalcic bridges between their seryl residues to form nonsoluble complexes (Causeret et al., 1991). These complexes could protect the proteins against thermal denaturation. Further studies in experimental conditions where phosphocalcic bridges are totally disrupted (high concentration of monovalent cations and/or strongly acidic or basic pH) are needed. This would clarify whether granules structure protects proteins against heat denaturation or whether the granules proteins were intrinsically more resistant to heat denaturation.

Plasma was very sensitive to heat treatment

Results showed that plasma was as sensitive to heat as yolk and more sensitive than granules. These results were related to the denaturation of plasma proteins: γ -livetins, α -livetins and LDL. The LDL must have contributed to both the decrease in protein solubility and the increase in apparent viscosity. They have been shown to form strong cohesive gels when heated at 75°C in conditions close to those we used (Nakamura et al., 1982; Kojima and Nakamura, 1985; Tsutsui, 1988). Both γ -livetins and α -livetins were also denatured at low temperatures (60°C and 69°C, respectively) (Dixon et Cotterill, 1981). However, their effects on gelation of plasma and yolk have not been established. Consequently, LDL are likely to be the primary contributors to plasma heat-induced gelation.

Effects of heat on emulsifying properties of plasma and granules

The emulsion stabilization properties of plasma and granules were not affected by heat. The emulsifying activities of both fractions were not affected by heat treatment except for plasma heated above 72°C. These results confirmed those of Varadarajulu and Cunningham (1972), Cotterill et al. (1976) and Lefebvre (1993) that pasteurization process had a very slight effect on emulsifying properties of yolk.

For granules, these results were consistent with the lack of changes in protein solubility and apparent viscosity of solutions. The same explanation could be given for plasma heated below 72°C. At 72°C and above, the decrease in emulsifying activity of plasma is correlated with that of protein solubility ($r^2=0.93$ for yolk and 0.91 for

plasma). Thus, the proteins could not adsorb at the interface when they were unsolubilized and aggregated.

CONCLUSION

AT LOW NaCl (0.17M) CONCENTRATION AND AT PH 6.1, GRANULES were less sensitive to heat treatments (55–76°C) than plasma and yolk. Granules could be subjected to greater heat treatment than plasma or yolk without lowering their emulsifying properties. Yolk showed the same pattern as plasma regarding its thermal sensitivity. Therefore, some technological problems caused by heat treatment of yolk could be overcome in part by substituting granules for yolk in food emulsions.

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