Characterisation of Egg Albumen Gels as a Function of Dry-Powder Pasteurisation Conditions

Marianne Hammershøj[1] and Lars Valentin Peters[2]

[1]Department of Animal Product Quality, Danish Institute of Agricultural Sciences, P.O.Box 50, DK-8830 Tjele, Denmark
[2]Sanovo Foods A/S, P.O.Box 139, DK-5100 Odense C, Denmark

ABSTRACT

Dry-heating temperature and time of egg albumen powder lead to increase in the textural properties stress and strain at fracture of egg albumen gels. Higher WHC and protein binding of gels were obtained under prolonged pasteurisation time. Furthermore, surface pressure in liquid samples correlated with dry-heating time. The surface hydrophobicity increased at short dry-heating periods, after which it stagnated. The molecular changes resulting in higher hydrophobicity and improved gel properties was mainly induced by heating time.

INTRODUCTION

One way to ensure egg albumen powder a microbiologically long shelf life is through thermal treatment, which at a given temperature has to meet a minimum holding time. The beneficial effects of pasteurisation in the dry state of egg albumen (EA) powder on the technological properties gelation, foaming and emulsification were first reported by Kato et al.[1], who applied a 80°C treatment for 10 days. Since then, dry-heat pasteurisation has been widely implemented in the egg product industry. However, many various combinations of heating time and temperature find use.

In order to optimise the use of storage capacity and energy, it would be beneficial if similar positive effects are obtainable during shorter pasteurisation time. The mechanisms involved in improving the subsequent gel strength have previously been suggested to be intermolecular hydrophobic interactions and disulfide bonding resulting in protein aggregation favouring the gelling properties of EA[2]. However, the extent and exact role of different interactions during dry-heating is not fully understood.

The aim of the present work was to study the effect of dry-heat pasteurisation time and temperature of EA powder on the protein surface activity, surface hydrophobicity and resulting gel texture, colour and water-holding capacity.

MATERIALS AND METHODS

Materials

One batch of spray-dried EA powder (Sanovo Foods A/S, Odense, Denmark) was subjected to dry-heat pasteurisation in an air-heating cabinet. Treatment combinations were temperatures of 80, 85, 90 or 95°C and time periods of 3, 5, 7, 16, 24 or 30 h. A spray-dried non-pasteurised sample was used as control (time = 0 h).

pH and conductivity

The EA powder was reconstituted in demineralised water to a concentration of 100 g/L. The pH and conductivity of the liquid samples were measured (Radiometer, Copenhagen, Denmark).

Surface tension

The surface tension γ (mN/m) of EA powder samples reconstituted to 1 g/L in demineralised water was measured in a Sigma 701 Tensiometer (KSV Instruments Ltd., Helsinki, Finland) with a platinum Wilhelmy plate. The γ was recorded as function of time up to 1500 s after sample loading. The surface pressure π was calculated as the difference between γ_0 (of pure water) and the γ of EA samples.

Surface hydrophobicity

The hydrophobicity of 0.1-1 g/L EA samples in 10 mM phosphate buffer pH 7.4 was analysed by a fluorescence method using *cis*-parinaric acid (Molecular Probes Inc., Eugene, OR, USA) as probe [3, 4]. The samples were excited at 325 nm, and the emission at 413 nm was recorded. Surface hydrophobicity S_0 was calculated as the fluorescence intensity/protein concentration.

Gel preparation

EA gels were prepared according to Hammershoj et al.[5] by heating 30 mL of EA c=100 g/L for 20 min in nylon tubes at 90°C in a water bath. The resulting gels were cooled at 4°C for 18 h.

Gel colour

Colour of 0.015 m thick gels was measured in quadruplicate on a Minolta Chroma Meter CR-300 (Minolta Co. Ltd., Osaka, Japan) using the CIE Lab scale (D65). The L*-value represents lightness, a*-value represents redness and b*-value measures yellowness of the samples.

Gel texture

Gel textural properties were analysed at 20°C on gel cylinders of the dimensions Ø = 0.015 m and H = 0.015 m by uniaxial compression until fracture by a TA-HD*i* Texture Analyzer (Stable Micro Systems Ltd., Surrey, England). Force-displacement recordings were converted into axial stress σ (Eq.1) and Hencky strain ε (Eq. 2) with σ_f as stress at fracture and ε_f as strain at fracture. The equations include A = initial end area (m^2), H_i = initial height (m), H = height (m), and F = force (N).

$$\sigma = \frac{F}{A}\frac{H}{H_i} \quad [\text{Pa}] \qquad (1)$$

$$\varepsilon = -\ln\frac{H}{H_i} \quad [-] \qquad (2)$$

Water-holding capacity (WHC) and protein exclusion

The WHC of gels (Ø = 0.008 m and H = 0.015 m) was measured according to the method of Handa et al.[6]. The gel cylinders were transferred to centrifuge tubes and centrifuged (Biofuge Pico, Hereaus Instruments, Inc., South Plainfield, NJ, USA) for 30 min at 10,000 *x g*. The WHC was determined by eq.3, where W_0 = the weight of the EA solution and W_1 = the weight of the centrifuged gel. The protein content in the syneresis liquid as result of

$$\text{WHC} = \frac{W_1}{W_0} \times 100 \ (\%) \qquad (3)$$

centrifugation was measured as UV-absorbance at 280 nm.

Statistical analysis

All data were subjected to statistical analysis by the Generalised Linear Model procedure of the SAS 8.01 (SAS Institute Inc., Cary, NC, USA). Variance inhomogeneity was analysed and found insignificant. No data transformation was done as data distribution fitted the normality function. The model of analysis (Eq.4) includes a = temperature i (80, 85, 90, 95°C), b = time j (0, 3, 5, 7, 16, 24, 30 h), and e = replicate l (1, 2).

$$Y_{ijk} = a_i + b_j + e_{ijk} \qquad (4)$$

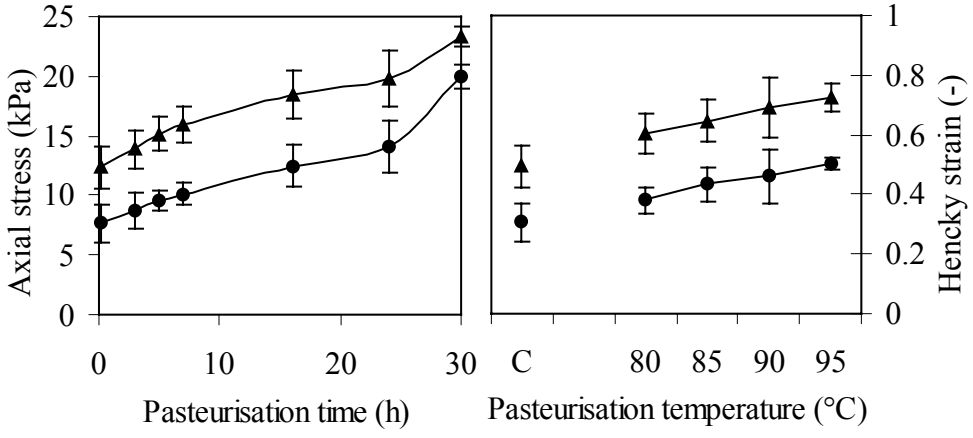

Figure 1. The textural properties σ_f (•) and ε_f (▲) of egg albumen gels at fracture as effect of dry-heat pasteurisation time and temperature. C = control sample, n=4

Interactive effects were included in the model when significant. The LS-Means were calculated and differences regarded significant at minimum 95%-level (P<0.05). The Ryan-Einot-Gabriel-Welsch multiple range test was used for difference classification.

RESULTS AND DISCUSSION

The results in Table 1 show that increasing dry-heat pasteurisation time and temperature leads to significant pH decrease and conductivity increase of reconstituted EA powder

The gel texture analysed by uniaxial compression of cylinders until fracture confirmed previous observations of dry-heat pasteurisation as a promising approach for improving EA gel texture [2]. The calculated axial stress σ_f and Hencky strain ε_f at fracture both increased significantly (P<0.001) as function of dry-heat pasteurisation time and temperature, as given in Fig. 1.

The appearance of heat-induced gels was affected by temperature (P<0.001) towards lower L*-value (darker) and lower negative a*-value (more green), whereas the b*-value only showed minor changes. In addition, the effect of dry-heat pasteurisation time on the colour parameters was negative (P<0.001) as shown in Fig. 2.

In general, the appearance of EA gels prepared from shell eggs is white (L*-value ~ 89)[7] and opaque. The alteration towards darker gels (L*-values ~ 81-86, Fig.2) as a

Table 1. LS-Mean of 100 g/L EA powder pH and conductivity (Cond.) as function of pasteurisation time and temperature, n=2.

Time, h	pH	Cond., mS/cm
Control	6.68 a	5.23 cd
3	6.65 ab	5.33 bcd
5	6.65 ab	5.18 d
7	6.64 ab	5.49 bc
16	6.63 ab	5.52 bc
24	6.63 ab	5.59 b
30	6.60 b	6.12 a
F-test	*	***
Temp., °C	**pH**	**Cond., mS/cm**
Control	6.68 a	5.23 b
80	6.70 a	5.37 b
85	6.61 b	5.41 b
90	6.62 b	5.34 b
95	6.62 b	5.65 a
F-test	***	***

a,b,c means with different letters differ significantly

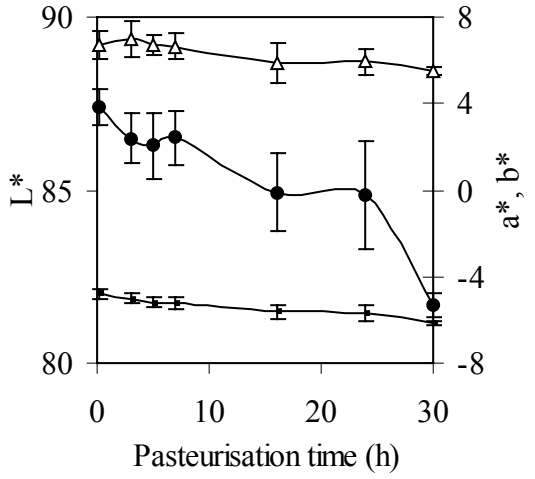

Figure 2. Effect of dry-heat pasteurisation time on egg albumen gel colour. L*=lightness (•), a*=redness (·), and b*=yellowness (△), n=4

result of pasteurisation of EA powder is unfavourable.

The water-holding capacity (WHC) of the formed gels was improved mainly as function of dry-heat pasteurisation time (P<0.001) and to a minor extent by temperature (P<0.05). In addition to stronger gels with higher WHC, the loss of protein (g/L) from the EA gels to the liquid during centrifugation decreased. The effect of dry-heat pasteurisation temperature on gel protein-binding capacity was, however, not unambiguous as shown in Fig. 3.

Previously, the protein composition analysed by SDS-PAGE indicated the formation of covalently linked protein polymers or aggregates during a commercial dry-heating at 90°C for 21 h[7]. Those covalent interactions were neither disulfide bonds nor dityrosine formations.

Results of surface activity analysis showed that the surface pressure of EA solutions increases as a logarithmic function of dry-heat pasteurisation time (Fig.4). The initial increase (within 40 s after sample loading) in π was 17.3 mN/m for the control and 21.2 mN/m for the 30 h samples. The accumulation of surfactant molecules at the air-water interface further increases the π after 1500 s to 26.9 and 29.6 mN/m, respectively. This corresponds to a higher affinity for the A-W interface, either due to unfolding of the molecules already during the dry-heat pasteurisation treatment and/or a higher quantity of hydrophobic residues at the interface, which may also contribute to extended hydrophobic interactions during gelation.

The analysis of surface hydrophobicity correlates to some extent with the surface pressure results, as the dry-heat

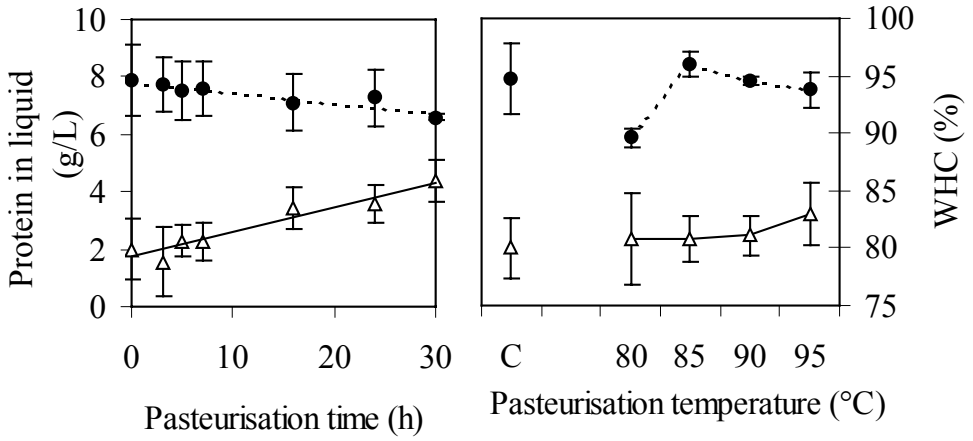

Figure 3. WHC (△) and protein in excluded liquid (•) from egg albumen gels as function of dry-heat pasteurisation time and temperature. C = control sample, n=3.

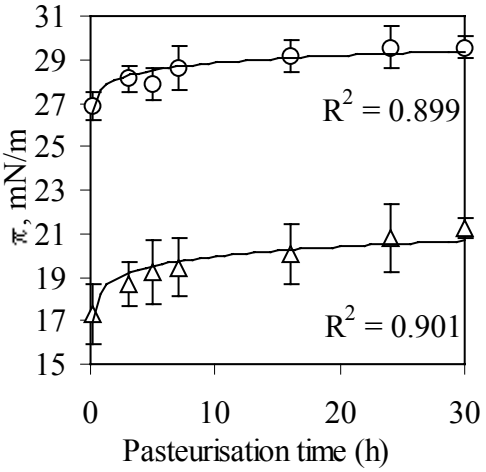

Figure 4. Surface pressure π of 1g/L egg albumen at 40s (△) and 1500s (O) after sample loading, n=4. Lines represent logarithmic functions of time.

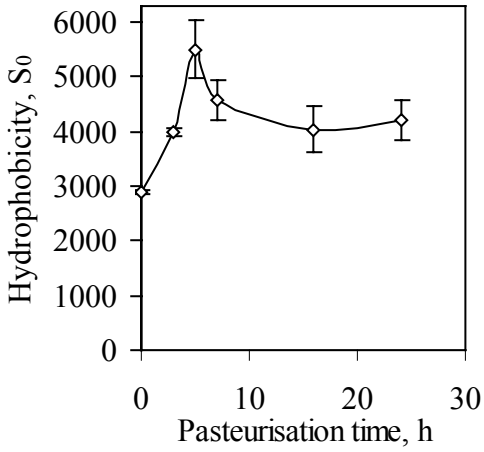

Figure 5. Surface hydrophobicity S_0 of egg albumen samples as function of dry-heat pasteurisation time, n=2.

pasteurisation time results in an increasing surface hydrophobicity compared with the control, as shown in Fig. 5. However, this increase is not uniform. After 5 h the hydrophobicity is increased 2-fold, but prolonged pasteurisation decreases the hydrophobicity to a level that is only 50% higher than the control sample. These results support the suggestion that the proteins unfold relatively fast during the dry-heating[2]. Present and previous[7] results indicate that increasing dry-heating time causes protein aggregation by means of e.g. hydrophobic interaction and covalent bonding. Prolonged heating may alter the conformation so fewer hydrophobic residues are exposed at the surface of these aggregates.

The spray-drying process induces a partial unfolding of the EA proteins, which is approximately 2.4-fold that of the native proteins of shell egg albumen, as illustrated in Fig. 6. There was no significant effect of pasteurisation temperature on the surface hydrophobicity.

Recently, a study of the protein legumin[8] lead to the conclusion that surface activity, i.e. surface tension, is governed more by molecular flexibility than by surface hydrophobicity. With this in mind, the steady increase in surface pressure (Fig. 4) with longer dry-heating time may also reflect the achievement of a higher flexibility of the EA proteins/protein aggregates together with the change in hydrophobicity, which was stagnating (Fig. 5).

CONCLUSIONS

In conclusion, dry-heating enhances the formation of a strong network in egg albumen protein gels. Additionally, the WHC and protein binding have increased, while the gel colour changes negatively with increasing dry-heating time. Surface activity analysed as surface pressure increases with dry-heating time, which is suggested to be caused by increased surface hydrophobicity and alteration of protein conformation and interactions.

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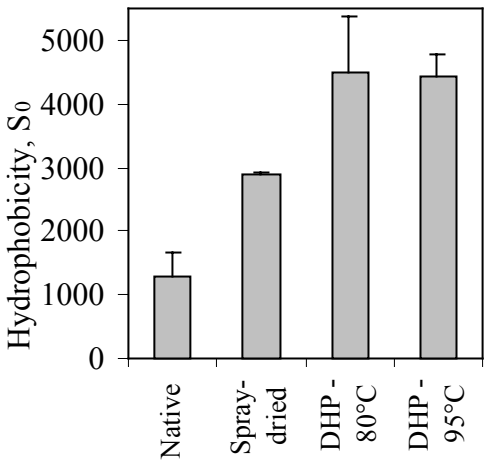

Figure 6. Surface hydrophobicity S_0 of egg albumen of shell egg (native), spray-dried and dry-heat pasteurised (DHP) nature, n=2.

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