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Thermal denaturation and aggregation of egg proteins [1]

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Abstract

The thermal denaturation of egg white and egg yolk proteins has been investigated by means of differential scanning calorimetry in natural, spontaneously aged samples. pH and protein content change with ageing.

The overall endothermic signal from fresh egg white has been deconvoluted into three main gaussian components relevant to the denaturation of conalbumin, lysozyme and ovoalbumin. The main modifications of the denaturation signal in aged egg white are attributed to progressive conversion of ovoalbumin to intermediate and S-ovoalbumin conformations denatured at slightly higher temperatures. Aggregation of denatured proteins gives an exothermic signal that partially overlaps the denaturation peak. This implies some uncertainty in evaluation of each thermal effect, especially for non-aged samples. Nonetheless, the results obtained are comparable with the latest literature data on aqueous solutions of individual proteins.

No deconvolution has been attempted for the endothermic DSC signal from egg yolk samples in the absence of literature on the DSC investigation of individual yolk proteins.

INTRODUCTION

Egg proteins have been extensively studied by food scientists [1–3] because of their peculiar role in the overall behaviour of egg derivatives used as ingredients and technological coadjuvants in industrial processes. Pasteurization of these products is usually carried out at a mild temperature to preserve protein native state and avoid the formation of aggregated phases. They are then stored at low temperatures or dried.

Aggregates are formed by thermal denaturation of proteins and may appear from the beginning of the process. Denaturation therefore proceeds within a growing aggregate and in conditions of increasing viscosity, because of the extension of a cross-linked network. The overall process involves superposition of two irreversible phenomena, namely endothermic denaturation and exothermic aggregation.

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Aggregation therefore affects calorimetric findings, especially at high protein concentrations, or when the pH of the protein solution encourages it (i.e. pH $\leq$ 5). Ionic strength, too, affects aggregation. Salt-free ovoalbumin solutions, for example, aggregate at temperatures significantly lower than salt-loaded solutions [4]. Appropriately chosen ionic strength, protein concentration, and pH values result in minor interferences between denaturation and aggregation, and are therefore more adequate for calorimetric investigations. These conditions are those set in the study of protein denaturation in diluted and buffered aqueous solutions. This approach [1–3] gives useful information about the thermal stability of single proteins and can be considered preliminary to actual characterization of the natural product, where the protein concentration may be high (in egg white it is about 12.5%) and the pH may change with ageing.

This paper describes calorimetric investigations of egg white and egg yolk in their natural state, i.e. without chemical manipulation. Protein content and pH were checked in each sample in the course of natural egg ageing.

MATERIALS AND METHODS

Fresh hen eggs of known laying date were used. Their ageing took place in a thermostat set at 25°C.

Protein content and pH were determined in white and yolk samples homogenized for 30 s with a Sorvall Omni-mixer blender at 4000 rev min^{-1}. Protein content was assessed as total nitrogen (with 6.62 as a conversion factor) with the Kjeldahl method. Natural egg white contains minor quantities of other components, such as salts and sugars, which are completely dissolved in the aqueous medium, and traces of emulsified lipids in the liquid state. Our results therefore apply to samples where many possible interactions between these and proteins can take place. The same is true of egg yolk, whose composition is even more complex [5] because of the presence of large amounts of liquid lipids within granules and vesicles grossly dispersed in the aqueous medium.

Aqueous solutions (7.7%, w/v) of commercial salt-free ovoalbumin (Sigma, St Louis, MO) in 0.2 M phosphate buffer at pH 6.8 were investigated separately.

A Mettler DSC 20 connected to an on-line processor was employed at 2°C/min^{-1} heating rate in the range 30–120°C. Samples of about 100 mg were placed in mechanically sealed aluminium pans (160 μl capacity) and tested versus distilled water as reference material. The traces were reproducible within 10–15% error for 3–5 scans per type of sample. Calorimetric data received as an ASCII file from the on-line processor were assembled in a LOTUS 4 worksheet. Peak deconvolution was carried out after tentatively assessing the baseline.

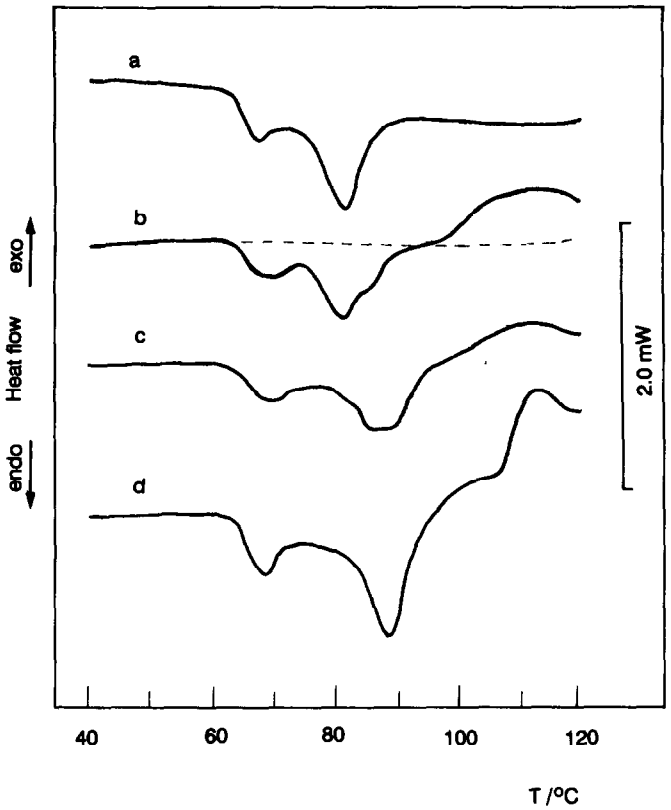

Fig. 1. DSC traces (2°C/min^{-1} heating rate) of egg white: (a) fresh, (b) 4 days, (c) 19 days, (d) 34 days. Typical sample mass ranged about 100 mg. The baseline used for integration and deconvolution was the trace obtained in the immediately subsequent scan of the same sample (see the dotted line drawn through trace (b)).

Inspection of the traces suggested that direct evaluation of heat capacity change (i.e. shift of the baseline across the signal) would not be reliable, because the apparent peak overlapping implied denaturation of some native proteins in the presence of others previously denatured. Accordingly, the baseline change for each C_p drop across denaturation, and aggregation of a single protein would be disturbed by the immediately ensuing denaturation of more thermolabile proteins. The overall C_p drop might be apparent in DSC traces from fresh egg white (see Fig. 1), though here the signal certainly includes the exothermic contribution of the aggregation expected (because of the pH conditions; see below) to occur within the same temperature range as denaturation. Finally, even the simplest approach, i.e. drawing a straight line through onset and end points of the endothermic signal, was not always possible, because the onset of the exothermic peak of aggregation made the choice of the end point of the endothermic signal rather arbitrary.

Because the C_p drop produces an endothermic shift of the baseline, any concomitant exothermic effect, such as that due to aggregation, plays an opposite role. Accordingly, a reasonable compromise was to treat the overall endothermic signal as overlying a flat baseline tentatively identified with the flat trace of a subsequent scan of the same sample (hence already denatured).

This crude approximation appeared to be fortuitously reliable, since the baseline generally gave acceptable onset and end points, and, as expected (see below), permitted recognition that the exothermic effects after the denaturation signal of aged samples increased with ageing.

The trace was reconstructed as two worksheet columns, for temperature and scaled signal respectively, and inserted in a STATGRAFICS regression subroutine operating with a sum of distinct gaussian functions (no more than three at a time). Regression was accelerated by assuming that the maxima of each gaussian lay in the position of the relative maxima of the trace. The denaturation temperature T_d of each protein was assumed to be that of the maximum of the related endothermic peak.

RESULTS AND DISCUSSION

The calorimetric signals for egg yolk or white could be considered as a series of partially overlapped endothermic peaks. These were recognized after deconvolution of the signal into gaussian components, whose number was suggested by extra information, such as chemical identification of the main components in the material, and calorimetric traces from aqueous solution of each compound. The literature data [1] were confirmed for egg white, whereas no information was found for egg yolk components. The present discussion therefore mainly concerns egg white.

Previous studies [1,2] had shown that traces from suitably treated fresh egg white can be interpreted as the convolution of three main endothermic peaks attributed to the denaturation of conalbumin, lysozyme, and ovoalbumin (which account for 16.8%, 3.7% and 64.5% of egg white proteins respectively), within the temperature range 60–100°C. It was claimed that the other recognized proteins, namely ovomucoid and globulins (11.3% and 3.7%), unfold within a wider temperature range encompassing that of the main components, and therefore give no distinct signal. Their contribution to the overall thermal effect was small. It was eliminated [1,2] by taking the straight line through onset and end point of the overall endothermic signal as the baseline, since the low protein concentration enabled any thermal effect due to aggregation to be ignored. These observations were taken into account to deconvolve our calorimetric traces.

In agreement with Donovan and Mapes [2], the signal attributable to ovoalbumin denaturation showed modifications and splitting in samples of different age. These modifications are thought to be due to changes in the

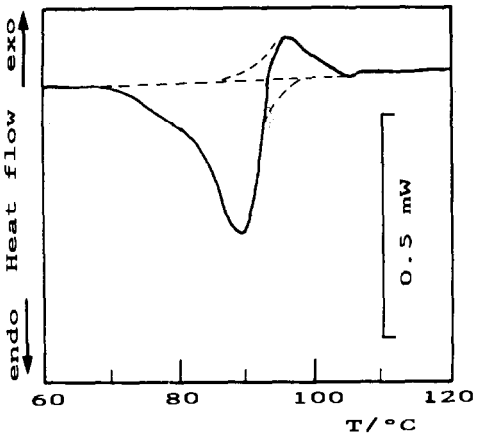

Fig. 2. DSC trace of commercial ovoalbumin in phosphate buffer (pH 6.8). The deconvolution into exo- and endothermic effects is rather arbitrary, but leads to a reasonable value of the heat of denaturation (see text). The exothermic contribution depends on aggregation following denaturation.

protein molecule resulting in a more stable conformation, the so called S-ovoalbumin, through an intermediate relatively stable state [2].

Figure 1 reports traces from eggs of different age. The lower temperature region, where conalbumin and lysozyme undergo denaturation, displayed minor modifications, whereas that corresponding to ovoalbumin changed significantly with ageing. T_d and splitting of the endothermic peak, and the presence and shape of the exothermic signal, were primarily involved.

The oldest samples showed a better defined ovoalbumin peak: deconvolution (see below) supported the conclusion that the native ovoalbumin was completely converted to intermediate and S-modifications.

The multicomponent character of this signal was confirmed in preliminary tests on buffered aqueous solutions of commercial ovoalbumin, which gave a rather broad peak with an apparent exothermic component at the high temperature end. This was assumed to reflect protein aggregation proceeding from denaturation.

Since the software employed did not allow summation of gaussians of opposite trend, the good pre- and post-signal alignment of the baselines was relied upon for a tentative manual deconvolution (see Fig. 2), by assuming the exothermic component to be symmetrical.

The area of the endothermic component corresponded to a thermal effect of 13.14 J g^{-1} (referred to pure protein), which falls between the literature data for similar pH conditions: 15.22 J g^{-1} [1] and 9.61 J g^{-1} [6]. The observable part of the exothermic effect of aggregation in commercial ovoalbumin (Fig. 2) did not exceed 15% of the overall signal, though a significant underlying exothermic contribution could not be ruled out. The

TABLE 1

Protein content and pH of egg white and yolk during ageing

Ageing (days)	Egg white		Egg yolk	
	Protein (%)	pH	Protein (%)	pH
1	12.13	8.58	16.93	6.25
4		9.15		6.50
19	10.50	9.31	18.30	6.34
34	11.23	9.37	16.70	6.70

actual denaturation heat may thus be greater and reconcilable with the value of Donovan et al. [1].

When this peak was superimposed on the egg white traces, it did not fit the ovoalbumin signal from fresh samples, but was shifted to higher temperature and significantly broader. On the basis of Donovan and Mapes' interpretation [2], it was concluded that the fresh egg ovoalbumin was mainly native, whereas that in the commercial sample had been mainly converted to the intermediate state.

Deconvolution required prior evaluation of the contributions due to protein aggregation. As mentioned above, this is an exothermic process occurring at pH-dependent temperature [4]. The pH of egg white increases with ageing [7] (from 8.6 fresh to 9.4 at 34 days; see Table 1). A tentative explanation was therefore put forward for the observation that aged samples display an exothermic signal at temperatures above those of protein denaturation, whereas this effect was absent or minor in fresh and moderately aged samples respectively. In the latter case, the trace may express exo- and endothermic overlapping. Deconvolution, therefore, could not reveal the aggregation signal, since it would be spread over the denaturation temperature range.

The exothermic effect seemed to increase with age (i.e. with pH), possibly as a result of the shift of protein aggregation to higher temperatures. No quantitative assessment was made of the contributions of each protein species.

The endothermic region was deconvolved into three main signals attributed to conalbumin, lysozyme and ovoalbumin in order of increasing thermal stability. The third signal was separately deconvoluted into gaussian components to identify the contributions of native, intermediate and S-ovoalbumin and obtain traces with more than three peaks, despite the software limitations.

Figures 3–5 show traces for different ages; since the main changes concerned ovoalbumin, the traces in Figs. 4 and 5 were limited to deconvolution of its region. Ovoalbumin modifications were simultaneously present in the 19-day sample (Fig. 4); whereas only intermediate and S-ovoalbumin were found after 34 days ageing (Fig. 5).

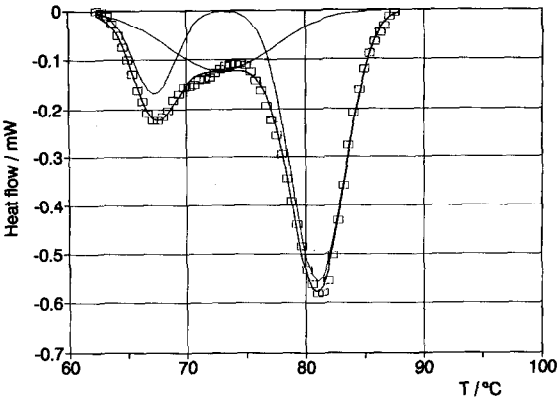

Fig. 3. Deconvoluted DSC trace of fresh egg white. Three gaussian components were ascribed to denaturation of conalbumin (left), lysozyme (middle) and ovoalbumin respectively. Symbols reproduce the experimental trend.

The temperatures of the peak maxima T_d were 65.7°C, 73.4°C, 81.1°C and 97.9°C for conalbumin, lysozyme, native, intermediate and S-ovoalbumin, respectively. Due to the lower heating rate employed in the present investigations, these temperatures were 1–2 degrees lower than those in the literature [1]. The lysozyme peak decreased with ageing as expected [7].

The overall thermal effect observed, 2.58 J g^{-1}, is equivalent to 21.0 J g^{-1} when referred to pure proteins, i.e. 30% more than the value of Donovan et al. (15.5 ∓ 1.1) J g^{-1} [1], but in agreement with recent results (19.9 J g^{-1}) [8]; the differences may depend on choice of the baseline, heating rate, pH, and particularly the fact that protein aggregation was taken into account.

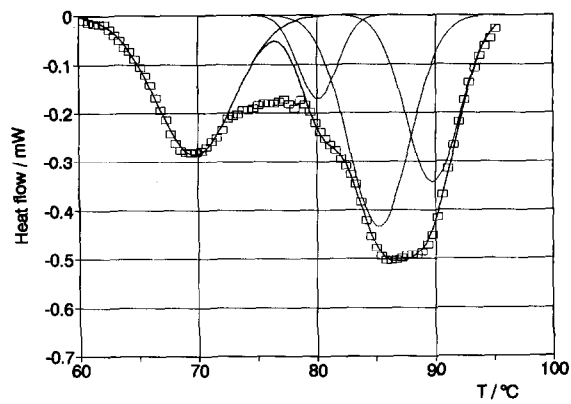

Fig. 4. Deconvoluted DSC trace of 19-day egg white. The traces concern the temperature range of ovoalbumin denaturation; the peak (T_d = 81.1°C) is split into three components: from left to right, native, intermediate and S-ovoalbumin.

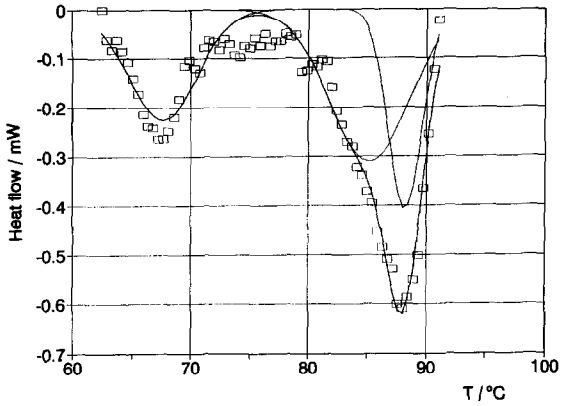

Fig. 5. As Fig. 4 for 34-day egg white.

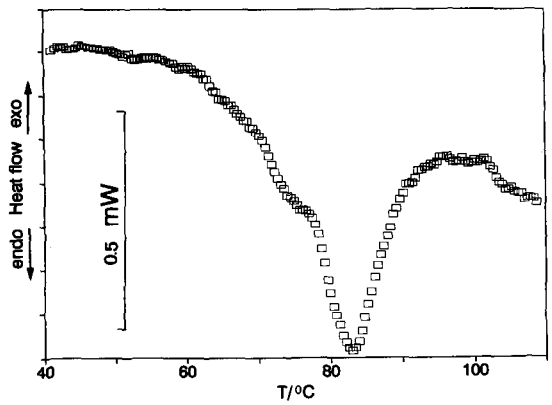

Fig. 6. DSC trace (2°C min^{-1} heating rate) of fresh egg yolk: sample mass 100 mg.

Traces from egg yolk (Fig. 6) showed a broad peak with an evident shoulder at the low T side. Its onset was close to 64°C, and its maximum occurred at about 84°C. Since the total egg yolk protein was close to 16.9% (w/w), the overall thermal effect (2.0 J g^{-1}) was equivalent to 11.8 J per gram of proteins. Since no calorimetric investigations of egg yolk and its proteins were found in the literature, deconvolution is now being undertaken to test individual proteins separately.

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