Characteristics of Heat-Induced Networks for Mixtures of Ovalbumin and Lysozyme

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Proteins with opposite charges were heated together to assess the impact of this combination on the characteristics of the heat-induced protein networks produced. The thermal properties and dynamic rheological characteristics of heat-induced networks were evaluated for ovalbumin, lysozyme, and mixtures of the two at ratios ranging from that naturally found in egg white to 1:1. Ovalbumin formed stronger (high G' and G''), more well cross-linked networks (lower tan δ value) than lysozyme. Network formation was unaffected by mixing ovalbumin and lysozyme at pH 5.5, whereas at pH 7.0 and 8.5 network strength increased and cross-linking in the network decreased as the relative proportion of lysozyme was increased. Mixed protein systems can produce stronger, less structured networks than pure proteins. Removal of lysozyme from egg albumen can adversely affect its gel strength and hence reduce its contribution to the textural properties of food products.

INTRODUCTION

The production of a three-dimensional gel network is a determining factor in the appearance and texture of many cooked food products. Egg albumen is a key ingredient in many such products because of its ability to form a three-dimensional product. The coagulation of egg white, therefore, has been the subject of a number of investigations using both whole egg albumen (Shimada and Matsushita, 1980; Hickson et al., 1982; Gossett et al., 1983; Holt et al., 1984; Woodward and Cotterill, 1986; Kato et al., 1990; Margoshes, 1990) and isolated ovalbumin (Nakamura et al., 1978; Kitabatake et al., 1987; Arntfield et al., 1990a). Studies with the mixture of proteins found in albumen have been of a general nature involving changes with processing conditions, though the effects of electrostatic interactions (Gossett et al., 1983) and disulfide bond involvement (Margoshes, 1990) have received some attention. In work on pure ovalbumin, it has been shown that pH and, consequently, electrostatic interactions play a key role in both aggregation (Nakamura et al., 1978) and network formation (Arntfield et al., 1990a).

Few studies have looked specifically at the interactions between proteins and the impact of such interactions on network formation. In view of the role of electrostatic interactions on protein gelation, the interaction between proteins, particularly those whose net charge is opposite, could significantly impact the resulting networks. In studies looking at turbidity development due to protein aggregation between ovalbumin and lysozyme, it has been shown that aggregation results from electrostatic interactions and disulfide bond interchange (Matsudomi et al., 1986), with electrostatic interactions being the prominent factor if denatured ovalbumin was used (Matsudomi et al., 1987). In other words, the effect of combining a basic protein (lysozyme or clupeine) with an acidic protein (ovalbumin or bovine serum albumin) has been shown to enhance foaming properties at pH values between the isoelectric points of the two proteins (Poole et al., 1984).

Improved knowledge of network formation for protein combinations such as ovalbumin and lysozyme is important for two reasons. First, most food systems are heterogeneous with respect to proteins. This includes egg albumen or mixtures of proteins from plant and animal sources such that proteins with a variety of isoelectric points are present. Understanding the impact of interactions between these proteins on network formation should facilitate the prediction of network characteristics in protein mixtures. The second reason relates specifically to egg albumen, where technologies are now available to remove lysozyme and avidin (basic proteins) from egg albumen (Li-Chan et al., 1986; Durance and Nakai, 1988) and still have a marketable egg product. The impact of lysozyme removal on network characteristics will obviously relate to the value of the final product.

This paper describes the effect of mixing different ratios of ovalbumin and lysozyme on the rheological characteristics of heat-induced networks prepared from these mixtures. Thermal properties of the mixtures have also been monitored to see if they were in any way related to the rheological differences.

MATERIALS AND METHODS

Source of Material. Ovalbumin (Grade V) and lysozyme were obtained from Sigma Chemical Co. and used without further purification. All other chemicals were of reagent grade.

Sample Preparation. Samples were prepared at a concentration of 10% (w/w) protein in 150 mM NaCl. This protein concentration has been shown to be sufficiently high to produce well cross-linked networks for ovalbumin (Arntfield et al., 1990b). Samples containing both ovalbumin and lysozyme were mixed together in a dry state at the appropriate ratios prior to addition of 150 mM NaCl. Ovalbumin and lysozyme were mixed at ratios of 1:1, 4:1, 9:1, and 15:1; the 15:1 ratio was included to represent the proportions of ovalbumin and lysozyme naturally found in egg albumen. The pH was adjusted to the desired value with 1.0 M NaOH or 1.0 M HCl. To ensure the pH was maintained, samples were equilibrated for 30 min and the pH was rechecked prior to analysis. The pH values were 5.5, 7.0, and 8.5.

Calorimetry. The thermal properties of the ovalbumin, lysozyme, and ovalbumin/lysozyme preparations were determined using a Du Pont 9900 thermal analyzer with a 910 differential scanning calorimeter cell base. Thermal curves were obtained using 10–15 μL of sample and a heating rate of 2 °C/min with an empty pan as reference. Denaturation temperature (T_d), measured as the point of maximum heat flow, and enthalpy of denaturation (ΔH) were calculated using the General Analysis utility software (ver. 2.2) available for the instrument. For mixtures of ovalbumin and lysozyme, two endotherms were observed on the thermal curves; however, there was not baseline separation between the two endotherms. A typical example of this (ovalbumin to lysozyme at 4:1) is shown in Figure 1. While
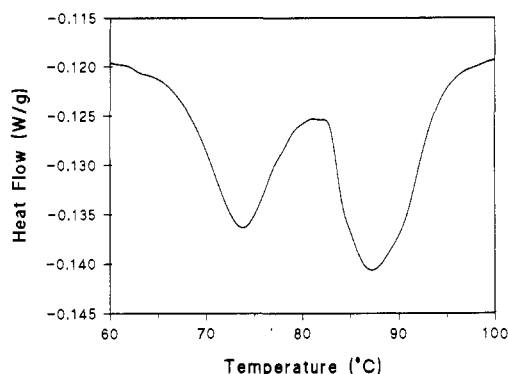
Figure 1. Thermogram from differential scanning calorimetry of an ovalbumin/lysozyme mixture (4:1). Sample contained 10% total solids and was run at a heating rate of 2 °C/min. Curve has been smoothed mathematically to minimize background noise at this heating rate.

the T_d values are easily detected in this system, the baseline must be set by the operator to calculate the ΔH value. For these mixed samples, the baseline was drawn to include both endotherms and a perpendicular dropped from the point of minimum heat flow between the two endotherms, so that ΔH values for both ovalbumin and lysozyme could be obtained from a single curve. The ΔH values reported have been reported as joules per gram of protein, where the values have been adjusted to account for the actual weight of ovalbumin or lysozyme present in a given sample.

Rheology. Heat-induced protein networks for rheological analysis were prepared in a Bohlin VOR rheometer (Bohlin Reologi, Inc., Edison, NJ) equipped with a programmable water bath. Samples (approximately 1 mL) at 25 °C were placed between parallel plates (30 mm) in the rheometer, and the gap between the two plates was adjusted to 1 mm, at which point the sample filled the gap between the plates.

Samples were heated to 95 °C at a rate of 2 °C/min, held there for 2 min, and then cooled to 25 °C at the same rate. To prevent drying, samples were surrounded by paraffin oil during the procedure. The rheological characteristics, G' (storage modulus) and G'' (loss modulus), were monitored during structure development using a frequency of 1 Hz. Input amplitude during this phase of the experiment was 0.2, a value found to be in the linear viscoelastic region in preliminary experimentation. The sensitivity of the measurement was determined by the torque bar calibrated to 93.2 g cm, attached to the upper plate of the rheometer. Due to the similarity of heating and cooling curves for all samples, these data have not been included in this paper. Dynamic properties of the networks formed from the thermal treatment were measured as a function of oscillatory frequency (ω) at 25 °C using the same strain amplitude and torque bar as for the thermal scans. In addition to G' and G'', the tan δ value (G''/G') was calculated. As there was a linear relationship between the log of G' and the log of ω, linear regression data were used to determine values of G', G'', and tan δ at a frequency of 1 Hz.

Statistical Analysis. All analyses were performed in duplicate on separate days with fresh sample. Statistical differences were determined using an analysis of variance in conjunction with Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS

Thermal Properties. Denaturation temperatures for ovalbumin, lysozyme, and mixtures thereof are given in Figure 2. The denaturation temperature of ovalbumin was unaffected by pH or the presence of lysozyme with an average value of 82.0 °C. This agrees with the data reported by Donovan et al. (1975) in that the T_d of isolated ovalbumin was unaffected by pH and was essentially the same when present in albumen. The T_d value in this study, however, is 2 °C lower than that reported by Donovan et al. (1975), probably because of the lower heating rate (2 °C/min compared to 10 °C/min) used in this study. For
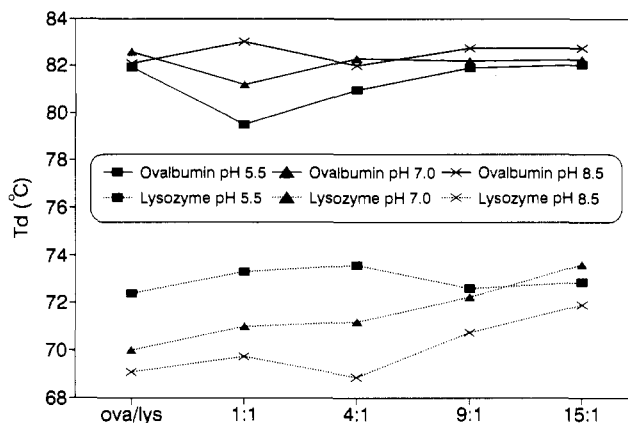

Figure 2. Changes in denaturation temperature (T_d) resulting from combining ovalbumin and lysozyme at different ratios at different pH values. Ova/lys indicates a pure sample of either lysozyme or ovalbumin depending on the curve, and ratios are in the format ovalbumin/lysozyme.
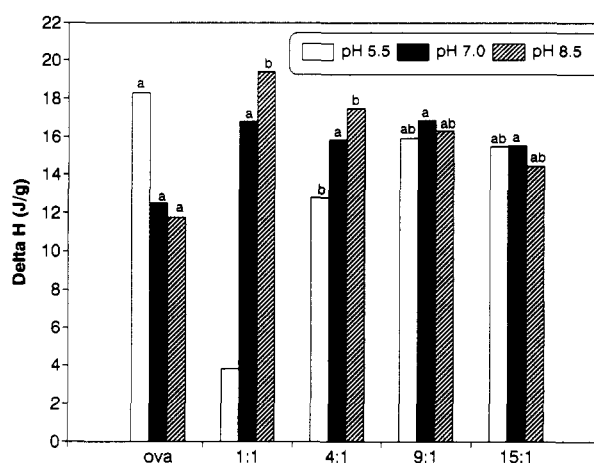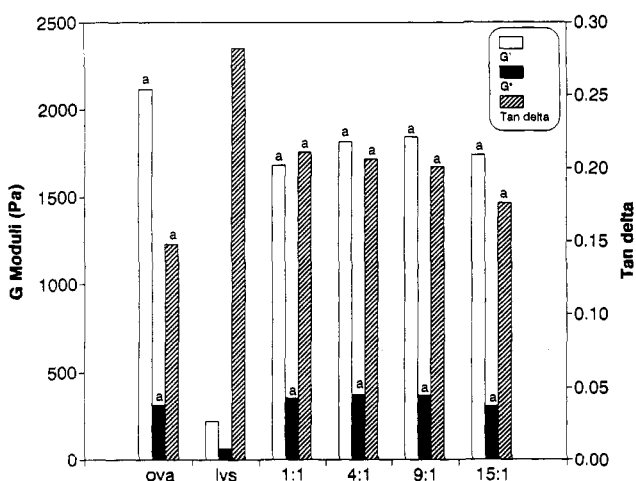

Figure 3. Changes in enthalpy of denaturation (ΔH) for the ovalbumin peak as a result of combining pure ovalbumin with different levels of lysozyme. Ratios are in the format ovalbumin/lysozyme. Bars at the same pH with similar letters are not statistically different ($P \leq 0.05$).

lysozyme, the dependence of T_d on pH was comparable to that reported previously (Donovan et al., 1975), only the T_d values were again slightly lower due to heating rate differences. The presence of ovalbumin had little impact on the T_d value for lysozyme. The only difference was at pH 7.0, where the value for lysozyme in the 15:1 mixture was significantly higher than that for the pure lysozyme. This is in contradiction to the results of Donovan et al. (1975), who demonstrated a higher T_d value in isolated lysozyme compared to that in egg albumen. Presumably, something other than ovalbumin was responsible for the lower T_d of lysozyme in the albumen.

The impact of mixing ovalbumin and lysozyme was more evident in the ΔH values (Figures 3 and 4) and was dependent on pH. For ovalbumin (Figure 3), at pH 5.5, the ΔH value was lower when there was a higher concentration of lysozyme present (e.g., 1:1 ratio). At pH 7.0, the ΔH values were unaffected by lysozyme addition, and at pH 8.5, a higher concentration of lysozyme increased the ΔH values for ovalbumin. With lysozyme, the inclusion of ovalbumin had no impact on the ΔH values at pH 8.5. At both pH 5.5 and 7.0, the presence of ovalbumin increased the ΔH values for lysozyme and the higher the level of ovalbumin, the greater the increase in the ΔH value. Clearly the presence of ovalbumin had a significant impact on the amount of energy required to denature lysozyme at these lower pH values.
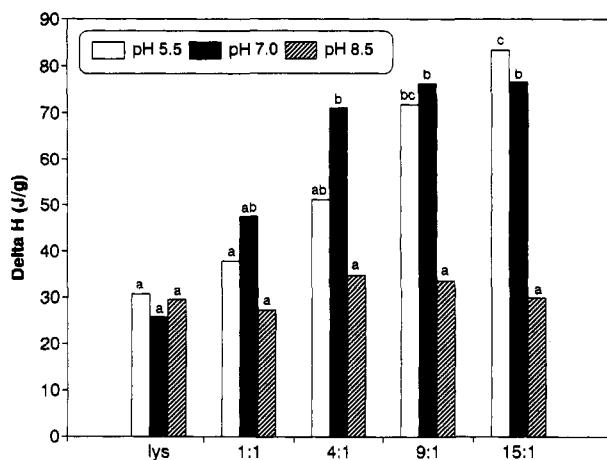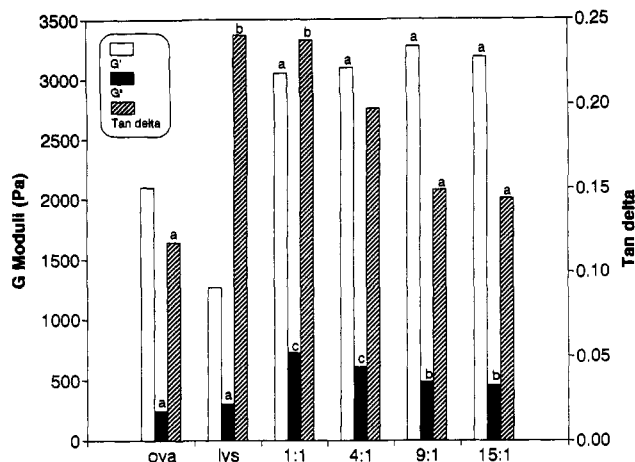
Figure 4. Changes in enthalpy of denaturation (ΔH) for the lysozyme peak as a result of combining pure lysozyme with different levels of ovalbumin. Ratios are in the format ovalbumin/lysozyme. Bars at the same pH with similar letters are not statistically different ($P \leq 0.05$).
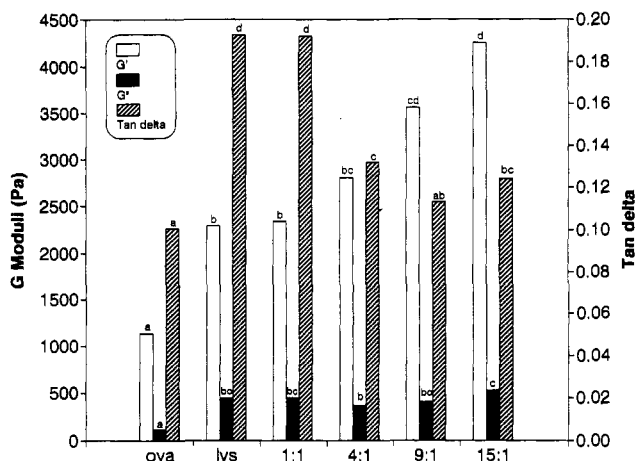

Figure 6. Effect of combining ovalbumin and lysozyme on the rheological properties of gels produced by heating at pH 7.0. Bars for the same rheological parameter with similar letters are not statistically different ($P \leq 0.05$).

Figure 5. Effect of combining ovalbumin and lysozyme on the rheological properties of gels produced by heating at pH 5.5. Bars for the same rheological parameter with similar letters are not statistically different ($P \leq 0.05$).

Figure 7. Effect of combining ovalbumin and lysozyme on the rheological properties of gels produced by heating at pH 8.5. Bars for the same rheological parameter with similar letters are not statistically different ($P \leq 0.05$).

Rheology. The effect of varying the lysozyme level added to ovalbumin was a function of pH. At pH 5.5, G', G'', and tan δ for the networks formed were not affected by the inclusion of lysozyme up to a proportion equal to ovalbumin (Figure 5). The only network that was significantly different was that from pure lysozyme, where G' and G'' were lower and tan δ higher, indicative of a weak network lacking rigid structure. This relationship between dynamic rheological data and the microstructure of the network has been demonstrated previously (Arntfield et al., 1990b).

At pH 7.0, there was a change in network characteristics as a result of lysozyme addition (Figure 6). While the G' values for the mixtures were not significantly different, they were all higher than the values for ovalbumin or lysozyme alone. The same observation was true for G'', except that there were also differences in the mixtures such that the G'' values increased with increasing lysozyme concentration. This increase in the G'' values for the mixtures was reflected in the tan δ values, which also increased with lysozyme concentration as the sample structure changed from the well cross-linked (low tan δ) ovalbumin gel to the more viscous structure obtained with pure lysozyme.

At pH 8.5, which is closer to the isoelectric point of lysozyme, the effect of lysozyme inclusion was again noticeable (Figure 7). At this pH, the G' values for pure lysozyme were higher than those for ovalbumin, unlike the situation at lower pH values. For the mixtures, those with lower lysozyme contents (e.g., 9:1 and 15:1) had the highest G' values. This was reflected to a certain extent in the G'' values, with that for ovalbumin being significantly lower than that for lysozyme, but the only differences in the mixtures was that the G'' value for the 15:1 mixture was significantly higher than the G'' value for the 4:1 mixture. As a result, the tan δ values were more dependent on the G' differences. The tan δ value for ovalbumin was low because both G' and G'' were low. The low G' values in the lysozyme and 1:1 mixture, coupled with higher G'' values, resulted in high tan δ values indicative of poor structure. The increase in G' values with decreasing lysozyme levels was responsible for the lower tan δ values for these networks.

DISCUSSION

The use of ovalbumin and lysozyme to evaluate network formation with protein mixtures provides an interesting system. As the isoelectric points for these two proteins are quite different (4.5 for ovalbumin and 9.0 for lysozyme), at any pH between the two, the net charge on the two

proteins will be opposite and the potential for interactions will exist. In addition, these two proteins occur naturally in the same source, egg albumen, and since there are technologies available for lysozyme removal (Li-Chan *et al.*, 1986; Durance and Nakai, 1988), the role of lysozyme in network formation in the mixed product can be significant, particularly in the evaluation of the performance of a lysozyme-free egg albumen.

The mixing of ovalbumin and lysozyme had little impact on the thermal stability of either protein, as T_d values were essentially the same and the conditions used for network formation were adequate to denature both proteins. While the ΔH values associated with the two proteins, especially lysozyme, were affected by the inclusion in the mixture of ovalbumin and lysozyme, there was no evident correlation between the change in ΔH and the network properties.

From the comparison of the gels for ovalbumin and lysozyme, it is clear by the higher tan δ values that lysozyme does not form well cross-linked networks regardless of the pH. Ovalbumin formed better networks at all pH values, although lower tan δ and G' values were observed at the higher pH. The impact of the presence of lysozyme on network characteristics was a function of pH. At pH 5.5, the network characteristics are dependent on ovalbumin, as the pH is closer to the isoelectric point of this protein. It would appear that this pH is sufficiently far from the isoelectric of lysozyme that this protein does not contribute to the network, as evidenced by the very low G' value for lysozyme at this pH. It has been demonstrated previously (Arntfield *et al.*, 1990a) that as the net charge on the protein is increased, the potential for interactions between proteins is decreased, and the protein remains soluble following denaturation. The fact that this high net positive charge on the lysozyme did not appear to interact with the negatively charged ovalbumin may reflect the low net charge on the ovalbumin at this pH and an extensive interaction between ovalbumin molecules which results.

At pH 7.0, the disparity between the networks for the two pure proteins was not as great, although ovalbumin gels still had higher G' and lower tan δ values, indicative of a stronger, better cross-linked network. Furthermore, the addition of lysozyme to the ovalbumin increased the overall strength of the networks formed as evidenced by increased G' and G'' values. As pH 7.0 is about midway between the isoelectric points of the two proteins, the potential for interaction in increased, thus promoting stronger gels. It should be noted that stronger gels are not necessarily better cross-linked and the higher tan δ values at higher lysozyme concentrations are indicative of this tendency toward aggregation rather than gelation. In fact, at a 1:1 ratio, the tan δ value for the mixture is equivalent to that for lysozyme. Of particular significance is the fact that the inclusion of lysozyme at the 15:1, ovalbumin to lysozyme, ratio results in an increase in gel strength with a significant change in the nature of the network formed (tan δ value). As this ratio represents that normally found in egg albumen, it is evident that the presence of lysozyme is important in supplying an egg product with high gel strength. Consequently, its removal could affect the quality of the egg product as gel strength can be used as an indicator of albumen quality.

At pH 8.5, the ability of lysozyme to interact when heated is evident by the high G' and G'' values compared to those from ovalbumin. The cross-linking is still better for ovalbumin as reflected by the lower tan δ values. As was the case at pH 7.0, the combination of lysozyme and ovalbumin results in higher G' and G'' values, but unlike

pH 7.0, higher G' values are associated with lower levels of lysozyme. It would appear that the presence of proteins with opposite charges increased the overall number of interactions and therefore strength in the system, but the organization of protein within the structure can be sacrificed if the levels of the two proteins are not balanced. The presence of proteins with the opposite net charge should promote protein–protein interactions. This situation is similar to that of a pure protein near the isoelectric point. While this increase in interactions can increase overall network strength, it does not necessarily produce a well cross-linked network. The impact of the removal of lysozyme from egg albumen is again evident in the G' value, indicating the gel strength would be decreased by lysozyme removal. There was, however, also a slight change in the tan δ value, indicating a better cross-linked network with ovalbumin alone.

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