Note

Viscoelastic Properties of Porcine Serum-Myosin Gel and the Sausage Fortified by Porcine Serum

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Heat-induced gels of charcoal-treated, freeze-dried porcine serum fortified by chicken myosin solution (2.5%, w/w), as well as pork sausage fortified by the serum, were analyzed in terms of their rheological properties by creep measurements at different protein concentrations. The creep curves of gels and sausages conformed to a six-element mechanical model consisting of one Hookean, two Voigt and one Newtonian component. The mixture of the serum and chicken myosin (5.6% total protein concentration) formed a harder gel upon heating than 6% serum alone. This indicated that interactions between porcine serum protein and myosin occurred with heating. The viscoelastic values of pork sausage increased with the addition of the serum. Moreover, pork sausage fortified by the serum had higher viscoelastic values than sausage fortified by egg white protein ($p < 0.05$). The serum may be a useful functional ingredient in meat products.

Keywords: charcoal treatment, porcine serum, egg white, gel, myosin, viscoelastic property

Proteins contribute to the functional behaviors and qualities of many traditional foods (Fox & Condon, 1981). An important technological property of protein is the ability of gel formation (Kinsella, 1983). The use of blood and its protein components in food processing has been studied in relation to its beneficial nutritional and functional properties. Blood serum or plasma is commonly used as a functional ingredient in meat products such as sausage and hamburger, mainly because of its gelling properties when exposed to heat (Hermansson & Lucisano, 1982). The gel structure contributes to food texture and acts as a matrix for the retention of water, as well as fat and other components.

The protein content of serum collected from blood is too low for use as a functional ingredient of meat products like pork sausage. Thus, concentration or dehydration by freeze-drying or spray-drying is necessary. However, an off-flavor develops in the dried serum protein during storage. Hemoglobin and polyunsaturated fatty acids responsible for the off-flavor development can be effectively removed by charcoal treatment at low pH (Hayakawa *et al.*, 1989). In a previous paper, the gelation of heat-induced porcine serum treated with charcoal was evaluated by gel strength determination (Ni & Hayakawa, 2001). We found that the charcoal treatment considerably enhanced the gel strength of heated porcine serum protein.

Viscoelastic properties of muscle myosin are predominantly responsible for gelation in meat systems (Ziegler & Acton, 1984; Gordon & Barbut, 1992; Wang & Smith, 1994). However, the viscoelasticity of charcoal-treated serum gel fortified by myosin has not previously been investigated. The purpose of this study was to evaluate the viscoelastic properties of heat-induced gels of charcoal-treated, freeze-dried porcine serum and serum-myosin

solutions. The creep phenomena of pork sausage fortified by the serum and egg white protein were also investigated.

Materials and Methods

Serum samples Serum from porcine blood was collected at a local slaughterhouse and treated with charcoal at low pH according to the method of Chen (1967), with slight modifications (Hayakawa *et al.*, 1989; Ni & Hayakawa, 2001). Serum was freeze-dried after dialysis in distilled water (two changes) for 3 days at 4°C. Protein content of freeze-dried serum was determined using the Kjeldahl method. The charcoal treated and freeze-dried porcine serum has been abbreviated as serum in this paper. The protein concentrations used for gel preparation for creep analysis were adjusted to between 6 and 12%, based on the method of Lowry *et al.* (1951).

Myosin preparation Myosin was prepared from chicken breast meat according to the method of Takashi *et al.* (1970), with minor modifications. Crude myosin was extracted with phosphate buffer (3.38 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 15.5 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; pH 7.5) containing 0.6 M NaCl, for 1 h at 4°C. The protein solution was clarified by centrifugation (10,000×g, 30 min) and the supernatant was dialyzed against 5 mM phosphate buffer (pH 7.0) containing 0.3 M NaCl for 3 days at 4°C. Dialysis solutions were changed twice. The concentration of myosin protein was adjusted to 2.5% based on the method of Lowry *et al.* (1951).

Preparation of egg white Homogenized chicken egg white was diluted with an equal volume of Milli Q pure water and the solution was adjusted to pH 6.0 with 0.1 N HCl. After removing precipitates by centrifugation at 3000×g, the solution was dialyzed against Milli Q pure water and freeze-dried.

Gel preparation The serum was dissolved in 2.5% myosin solution. The protein concentration of the mixtures was

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adjusted to between 5.6 and 11.0%, and salt concentration was adjusted to 0.3 M using solid NaCl. After degassing, about 100 ml of protein solution was sealed inside lengths of casing (30 cm×25 mm diameter) and heated at 80°C for 30 min in a water-bath. The gels were cooled in tap water for 20 min, then allowed to stand at room temperature for 30 min. The casing was removed and cut into 20 mm long cylindrical blocks for determination of creep characteristics.

Preparation of pork sausage One kilogram of lean pork meat and fat, combined at a ratio of 9:1, was mixed for 1 min with 250 ml iced water and 20 g NaCl, using a Food Processor (Stephen UM 5, Germany). Charcoal-treated, freeze-dried serum was added to the meat mixture to 5% of the weight of the meat mixture. After further mixing for 1 min with the food processor, the mixture was stuffed in pig intestine covered with plastic pipe (24 mm diameter) to keep the gel diameter constant. Pork sausage was also prepared at 70°C for 1 h where 20%, 50%, and all of the serum component was replaced by freeze-dried egg white.

Rheological measurements Creep behavior under compression was analyzed using a Rheoner II Creep Meter (RE2-3305, Yamaden, Co., Tokyo) interfaced with a computer for computation of creep phenomena. Prior to creep measurement, gel samples were placed on a stage for measurement of exact height using a Sample-Height Counter (HC2-3305, Yamaden, Co., Tokyo). The sample was tested on a stage moving upwards at a speed of 5 mm/s with a No. 2 probe (40 mm diameter). Creep behavior was tested under compression using constant strain (10–15% for porcine serum gels mixed with myosin, 18–25% for pork sausage), with creep and recovery each recorded for 1 min. The data reported are means of 8 (porcine serum gels) and 24 (porcine serum gels mixed with myosin, and pork sausage) replicates. The creep curves were analyzed with software developed for creep analysis (Ver. 2.1, Yamaden, Co.), where these gels conformed to a six-element mechanical model of one Hookean, two Voigt and one Newtonian component (Katsuta *et al.*, 1990). The spring and dashpot couples of the Voigt units start to move under the effects of constant applied stress. If the stress is sufficient to break the bonds in the structure, flow occurs and this is described in the model by the final dashpot which is expressed by η_N . This model can be described by the following equations (Peleg, 1980):

$$\varepsilon(t) = \frac{P_0}{E_0} + \frac{P_0}{E_1}(1 - e^{-\frac{t}{\tau_1}}) + \frac{P_0}{E_2}(1 - e^{-\frac{t}{\tau_2}}) + \frac{P_0}{\eta_N}t, \quad (1)$$

$$J(t) = \varepsilon(t)/P_0, \quad (2)$$

$$J(t) = J_0 + \sum_{i=1}^2 J_i(1 - e^{-\frac{t}{\tau_i}}) + t/\eta_N, \quad \tau_i = \eta_i \times J_i. \quad (3)$$

where $\varepsilon(t)$ =strain (dimensionless), P_0 =stress (N/m²), $J(t)$ =creep compliance (m²/N), J_0 =creep compliance from the elastic modulus of the Hookean body ($J_0=1/E_0$), J_i =creep compliance from the Voigt bodies, E_0 =elastic modulus of Hookean body (N/m²), η_N =viscosity of the Newtonian body (Pa·s), E_i =elastic modulus of Voigt bodies (N/m²), η_i =viscosity of Voigt bodies (Pa·s), τ_i =retardation time of Voigt body (s) and t =time (s). All measurements were performed within the same initial strain (in the linearity region, i.e. compliance was independent of applied stress).

Result and Discussion

Effect of protein concentration on the properties of the serum and serum-myosin gels The diameter and the area of all

Fig. 1. The creep and creep-recovery curves of the serum gels at different protein concentrations. Gelling conditions: heating at 80°C for 30 min, followed by cooling to room temperature by immersion in tap water; 5 mM phosphate buffer (pH 7.0); 0.3 M NaCl; protein 6–12%.

gel samples studied were kept strictly constant. Due to variability in the elasticity or viscosity of the gel samples measured under different conditions, the loads applied were altered to ensure that deformation was always between 10% and 15%. Stress is expressed as the force per unit area of sample, and it was difficult to compare the creep curves of various gels made with different protein concentrations on the same figure. However, when creep compliance [$J(t)$] was expressed as Equation (3), the data could be plotted so that the curves obtained for gels of different protein concentration were comparable. The creep compliance of gels with between 6.0% and 12.0% serum decreased with increasing protein concentration (Fig. 1). Because creep compliance is a reciprocal of the viscoelastic values [Eq (3)], the gels with higher compliance had lower viscoelastic values, indicating that the serum gels become more rigid and less fluid with increasing protein concentrations.

The effect on viscoelastic values was investigated for samples containing serum with different protein concentrations after heating at 80°C for 30 min. The viscoelastic values increased with increasing protein concentration (Fig. 2). Newtonian viscosity (η_N) may be attributed to the breakdown of protein network structure (Lynch & Mulvihill, 1994). The higher Newtonian viscosity of heat-induced gel of the serum suggests a greater resistance to flow for a longer period of time. This result suggested that the improvement of heat-induced gel strength of charcoal-treated porcine serum (Ni & Hayakawa, 2001) was due to an increased Newtonian viscosity.

Both the elastic modulus, which is an index of resistance to deformation, and the viscosity increased with rising protein concentration. In general, the relationship between viscoelastic values and protein concentrations can be expressed by the following equation:

$$E \text{ (or } \eta) = KC^\alpha \quad (4)$$

where C is the concentration of the gel, K is a proportionality constant and α is a power constant. Linear relationships were shown between log of viscoelastic constants (E or η) and log of protein concentrations of the serum gel (Fig. 2) as well as serum-myosin gel (Fig. 3). Although the protein concentration at which

Fig. 2. Changes in viscoelastic values, η_N (□), η_1 (Δ), η_2 (○), E_2 (●), E_1 (▲) and E_0 (■), of the serum gel at different protein concentrations. The constants are explained in the text (Equation 1). The correlation coefficients were within the range of $r=0.99-0.98$.

Fig. 3. Changes in viscoelastic values, η_N (□), η_1 (Δ), η_2 (○), E_2 (●), E_1 (▲) and E_0 (■), of serum-myosin gel at different protein concentrations. The constants are explained in the text (Equation 1). The correlation coefficients were within the range of $r=0.99-0.98$.

the viscoelastic properties of serum gel were measured were different from those of serum-myosin gel, it is possible to compare properties of gels using the slope, α value. The α value for the elastic modulus of the Hookean body (E_0) was 2.42, those for elastic moduli of the Voigt bodies (E_1 and E_2) were 2.50 and 2.55, and constants for viscosities of Voigt bodies (η_1 and η_2) and viscosity of Newtonian body (η_N) were 2.55 and 2.67, and 2.65, respectively.

Both covalent and non-covalent interactions are involved in network stabilization, and contribute proportionally with concentration to viscoelastic parameters. Viscoelastic values in this study increased with protein concentration (C) between powers of 2.42 and 2.67. Values in the same range (e.g., 2.0 for agar gels and 3.0 for egg white gels; Isozaki *et al.*, 1976) have been reported.

The effect on viscoelastic values of heat-induced serum gels fortified by myosin (constant myosin concentration of 2.5% w/

Table 1. Viscoelastic parameters of sausage-model gels fortified by freeze-dried egg white and CT-DPS.

Parameter	Control	A	B	C	E
$E_0(\times 10^5)$	1.46 ^{a)}	1.91 ^{b)}	1.91 ^{b)}	2.02 ^{c)}	1.82 ^{d)}
$E_1(\times 10^5)$	6.63	9.23	9.63	9.80	8.88
$E_2(\times 10^5)$	7.12	10.05	10.50	10.70	9.19
τ_1	9.49	9.49	9.63	9.56	9.53
τ_2	1.23	1.23	1.25	1.24	1.26
$\eta_1(\times 10^6)$	6.29	8.76	9.27	9.37	8.47
$\eta_2(\times 10^5)$	8.83	12.35	13.18	13.33	11.52
$\eta_N(\times 10^7)$	6.30 ^{a)}	8.00 ^{b,c)}	8.03 ^{b,c)}	8.35 ^{b)}	7.78 ^{c)}

A; 2.5% egg white and 2.5% CT-DPS, B; 1.0% egg white & 4.0% CT-DPS, C; 5.0% CT-DPS, E; 5.0% egg white. Values are means of 24 replicates. ^{a,b,c)} values in the same line with different superscripts are significantly different ($p<0.05$).

w) was investigated at 25°C for different total protein concentrations (Fig. 3). The compliance of the serum gel mixed with myosin at 5.6% protein concentration was lower than that of 6% serum alone. Although the protein concentration of serum-myosin gel was lower than that of serum, serum-myosin gel was more rigid than serum gel. This result showed that interactions between serum proteins and myosin may enhance the rigidity of heat-induced gels. The coefficients of the viscoelastic constants for the serum gel mixed with myosin were higher than those of serum gel (Fig. 2 and 3). The power constant, α for the elastic modulus of the Hookean body (E_0) was 2.60, those for the elastic moduli of Voigt bodies (E_1 and E_2) were 2.98 and 2.95, and constants for viscosities of the Voigt bodies (η_1 and η_2) and the Newtonian body (η_N) were 2.94 and 2.85, and 3.24, respectively.

The power constants for all components of the serum gels mixed with myosin were considerably higher than those of serum alone ($p<0.05$). The flexible denatured subunits in the mixture of myosin and serum protein were relatively free to rearrange between random cross-links, and did not reach an equilibrium between formation and dissociation. Thus, some physical entanglement between the macromolecular strands at junctions zones would occur. The entangled network formation could be one reason for the increase in the power constant with the presence of myosin.

Myosin was indispensable to the binding properties as well as the water-holding capacity of meat and meat products. Heat-induced gelation of myosin consists of two reactions: (1) aggregation of globular head segments of the myosin molecule, which is closely associated with the oxidation of sulfhydryl groups; and (2) network formation resulting from the unfolding of the helical tail segment. The heat proteins associate to form "super-junctions" which provide extra cross-linking to the gel network. With the exception of disulfide bonds, the myosin molecules are held together by a combination of weak intermolecular forces, i.e., hydrogen bonds, electrostatic forces and hydrophobic interactions. These cross linkages may not be point interactions but can involve extensive interactions between segments of two or more polymer molecules (Oakenfull, 1987).

Porcine serum albumin has 1 sulfhydryl group and 17 disulfide bonds per monomer (Spencer & King, 1971) and myosin has 43 SH groups (Lowey *et al.*, 1969). Intermolecular exchange reaction between sulfhydryl and disulfide bonds contributes to gel network formation (Smyth *et al.*, 1998). The sulfhydryl

groups of myosin could react with intramolecular disulfide bonds of porcine serum albumin leading to SH/SS exchange reactions. Wang and Damodaran (1990) reported that the function of SS bonds in gel matrix formation may be related to their ability to increase the average molecular weight of polypeptides in the network, which would increase the total number of stable non-covalent bonds, such as hydrophobic interactions and hydrogen bonds, between the polypeptides per unit cell of the gel. Thus, it is possible that physical constraints were responsible for the increase in gel strength (Smyth *et al.*, 1998).

Effect of addition of serum on sausage gel The effects on viscoelastic values of pork sausage fortified by serum and freeze-dried egg white after heating are shown in Table 1. It was observed that their addition remarkably improved the viscoelastic values of pork sausage gels ($p < 0.05$). Both the instantaneous elasticity (E_0) and the Newtonian viscosity (η_N) of these gels fortified by serum (5%) were higher than for gels amended with egg white ($p < 0.05$). When 50% or 80% of the egg white was substituted by serum, the instantaneous elasticity (E_0) of these gels was higher than that for control sausage without serum addition ($p < 0.05$), but no significant difference in Newtonian viscosity (η_N) of gels with and without the substitution of egg white was found. The greater instantaneous elasticity (E_0) of the pork sausage gels fortified by serum (5%) indicated that it was more rigid than control, and the greater Newtonian viscosity of the fortified gels suggests a greater resistance to flow for longer periods. Each Voigt element of the pork sausage gels fortified by serum (5%) exhibited a greater retarded elasticity (E_1 and E_2) and a greater retarded viscosity (η_1 and η_2) than the control. Thus, control could be described as soft and less viscoelastic than the pork sausage gels fortified by serum. These results showed that protein matrix of the gels fortified by serum differed from control sausage.

Pronounced differences were not observed in the means of appearance, flavor, texture and acceptability of the test samples compared to control samples. From this study, it is believed that the addition of 5% serum to meat products does not affect the sensory properties of the final product. This result suggests that dried porcine serum could be incorporated into sausage to improve its viscoelastic properties.

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