Molecular Forces Involved in Heat-Induced Porcine Blood Curd

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The contribution of the molecular forces of porcine plasma and hemoglobin to the texture of heat-induced porcine blood curd (Zisheokwai) was studied by chemical treatment. Not only do the hardness and penetration but the elasticity and water-holding capacity of hemoglobin and whole blood gels increase with propylene glycol (5–20%), whereas urea (2–10 M) weakens the texture of all three gels. Therefore, hydrophobic interaction and hydrogen bonding involved in the formation of porcine blood curd and hemoglobin play the primary role. The texture of whole blood and plasma gels decreases significantly with mercaptoethanol (25–100 mM), which reduces the disulfide group of gels. Furthermore, the decrease in the textural properties of gels made from various combinations of plasma and hemoglobin is attributed to the decrease in sulfhydryl groups from plasma. On the basis of these results, the intermolecular disulfide of plasma gel is necessary to stabilize the texture and structure of blood curd.

INTRODUCTION

Porcine blood curd (Zisheokwai) and porcine blood rice cake (Zisheokau) are both well-known Chinese porcine blood foods. The former is made from heat-induced coagulation of a mixture of fresh porcine blood with water, and the latter is made by steaming a blend of blood and water-soaked glutinous rice. In Chinese dietary culture, diced Zisheokwai is also referred to as blood tofu and is usually stir-fried with pickled mustard and edible porcine byproducts and seasoned with red pepper and fermented soy paste. Zisheokau is usually served by dipping in a soy sauce slurry and then adding chopped Chinese parsley and peanuts. The annual production of blood from slaughtered animals is about 40 mllion kg in Taiwan, and approximately 24% is utilized for human consumption. Nevertheless, this product has scarcely been studied. Recently, development and utilization of edible animal blood have been strongly encouraged. Not only the mechanisms but the effects of the formation of plasma and globin protein gels have been studied (Autio et al., 1985; Autio and Mietsch, 1990; Hermansson and Lucisano, 1982; O'Riordan et al., 1988). It is believed that gel stabilization might involve electrostatic interactions and disulfide bonding (O'Riordan et al., 1989). Hayakawa et al. (1983) suggested that hydrophobic interactions also contributed to the formation blood protein gels. On the other hand, the type of molecular force involved in the gel structure is also dependent upon protein (Utsumi and Kinsella, 1985; Shimada and Matsushita, 1980). However, the interaction between plasma and hemoglobin on the heat-induced gelation has not shown consistency.

The objective of this study was to assess the effects of various reagents on the gelation of porcine plasma, hemoglobin protein, and whole blood, respectively, to understand their contribution to the formation of porcine blood curd.

MATERIALS AND METHODS

Preparation of Porcine Blood and Blood Proteins. Fresh porcine blood was obtained from a local slaughterhouse and collected into a container (1 L) to which had been previously added 9 g of sodium chloride and 5 g of sodium citrate to prevent blood clotting; the blood was stored at 4 °C. Plasma and

hemoglobin were obtained by fractionation–centrifugation–washing replicatively from anticoagulated porcine blood with saline at 1000g for 15 min (Kubato KR-20000T centrifuge) as described by Tybor et al. (1975). The fractions were dialyzed against saline overnight at 4 °C and lyophilized (Labconco Freezer–Dryer 4.5).

Preparation of Porcine Blood Curd. One liter of blood sample was diluted 5:1 (v/v) with saline, and the pH was adjusted to pH 8.0 with 1 N NaOH or HCl and homogenized (Nissel non-bubble homogenizer) at low speed for 2 min. Aliquots (40 mL) of the blood were transferred into 50-mL glass beakers and covered with aluminum foil to prevent evaporation. The beakers were heated in a 80 °C water bath for 20 min and then removed and cooled in an ice-water bath for 30 min.

Preparation of Porcine Protein Gels. The stock solutions (28% w/v) of three porcine proteins were prepared by dispersing the freeze-dried proteins in a 0.08 M phosphate buffer (pH 8.0), respectively. To these solutions were added propylene glycol (5–20%), urea (2–10 M), and mercaptoethanol (25–100 mM), respectively. The preparation of gelling solution was accomplished using the method described by O'Riordan et al. (1989).

Measurement of Textural Properties. Texture analyses were carried out on a gel section (5 cm diameter × 2.5 cm high) at room temperature using a Foudon rheometer (NRM-2010J, Foudon Co.). Hardness and penetration were determined by using a circular pressure plate (20 mm in diameter) and a flat circular punch, respectively, at a speed of 5 mm s⁻¹. Gel hardness and penetration were defined as force in grams exerted by the gel instantly after 4-mm compression as F_0. The gel elasticity was taken as $1 - (F_0 - F_5)/F_0$. F_5 was measured again after 5 min. The measurement was carried out with nine gels for each treatment, and the averages were calculated.

Determination of Water-Holding Capacity (WHC). The gel was placed in a 50-mL plastic centrifuge tube. Centrifugation was carried out with a fixed-angle rotor at 3000g for 15 min. WHC was expressed as the percentage of initial gel water remaining in the gel after centrifugation. The procedure followed was that of Hegg (1982).

Determination of Sulfhydryl Groups. *Measurement of Total Sulfhydryl Group.* The total sulfhydryl group was determined according to the procedures of Ellman (1959), Butterworth et al. (1967), and Toro-Vazouez and Regenstein (1989). The gels and blood proteins were solubilized in 0.08 M phosphate buffer (pH 8.0) containing 0.05% EDTA, 2% SDS, and 8 M urea and homogenized for 1 min. To the solution was added 0.5 mM mercaptoethanol, and the mixture was incubated at room temperature for 1 h. After incubation, 2 g of (NH₄)₂SO₄ was added to the solution and incubation was continued for 1 h. Finally, the solution wsa centrifuged at 6000g for 10 min, the supernatants were collected, and the precipitates were washed

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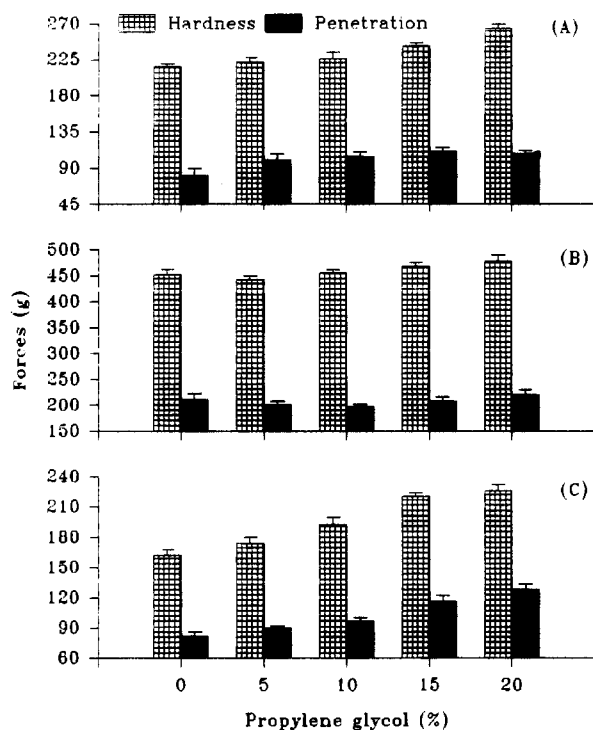1086 *J. Agric. Food Chem.*, Vol. 42, No. 5, 1994

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Figure 1. Effect of hardness and penetration of heat-induced porcine blood gels on propylene glycol: (A) porcine blood curd; (B) porcine plasma; (C) porcine hemoglobin.

with 0.08 M phosphate buffer (pH 8.0), replicatively, and then filtered with Toyo No. 5C filter paper. To the combination of supernatant and filtrate was added 0.5 mL of color reagent, and the mixture was allowed to stand for 15 min. Finally, 0.05 mL of 30% H_2O_2 was added to the sample solutions which were then aliquoted (100 mL) in a volumetric flask with phosphate buffer (pH 8.0). The color reagent was prepared with 200 mg of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma Chemical Co.) and suspended in 100 mL of 0.08 M phosphate buffer (pH 8.0). The absorbances of sample solution were recorded at 412 nm (Milton Roy 601 Spectronic) against DTNB reagent blank. A molar extinction coefficient of 1.36 × 10⁴ M^{-1} cm^{-1} was used for calculating micromoles of SH per gram of protein solubilized in the phosphate buffer (pH 8.0) containing 0.05% EDTA, 2% SDS, and 8 M urea.

Free Sulfhydryl Group. To solubilized gel and protein solutions was added 2 g of $(NH_4)_2SO_4$; the remainder of the procedure from centrifugation at 6000g for 10 min to determining the absorbance of the sample solution at 412 nm was the same as mentioned above.

Protein Concentration. A modification of the method of biuret assay (Plummer, 1987) was used. To reduce heme group blood proteins from interfering with spectrophotometry, a mixture of 0.1 mL of solubilized gel and protein solutions, 9.9 mL of distilled water, and 0.01 mL of 30% H_2O_2 was prepared. To 2 mL of this mixture was added 3 mL of biuret reagent (Sigma), and the mixture was incubated in a 37 °C water bath for 10 min. The absorbance of sample solutions was read at 540 nm, and protein concentration was calibrated with 0.2–5 mg of bovine serum albumin (Sigma).

Statistical Analysis. Data were analyzed by repeated-measure analysis of variance (Gill and Hafs, 1971) with the General Linear Model procedure of the SAS package (SAS Institute, 1988). Comparison of treatment means was based on Duncan's multiple-range test (Montgomery, 1991).

RESULTS AND DISCUSSION

The effect of propylene glycol on the hardness and penetration of three porcine blood gels is shown in Figure 1; both hardness and penetration of porcine blood curd and hemoglobin gels (Figure 1A,C) increased significantly ($p < 0.05$) with propylene glycol. In plasma gel (Figure 1B), no significant improvement was observed until the propylene glycol was above 15%. The alcoholic compound enhanced protein electrostatic interaction (Reynolds et al., 1968) by lowering the intermolecular energy barrier (Mulvihill et al., 1991) or dielectric constant (Utsumi and Kinsella, 1985) and prompted hydrogen bonding (Kella and Rao, 1985). In the presence of 20% propylene glycol, water-holding capacity and elasticity of blood curd and hemoglobin gel were also improved significantly ($p < 0.05$) as shown in Table 1. The hydrodynamic size of the protein might increase because of the elevating level of hydrogen bonding and hydrophobic interaction of protein (Labuza, 1977). It is a desirable effect on the protein–protein interaction and protein–water hydration and resulted in less synersis of the blood curd and hemoglobin gel than the control after centrifugation. In addition, the more cross-linking of hydrophobicity of unfolding proteins (Voutsinas et al., 1983), the more the elasticity of the protein gel was enhanced. However, neither significant improvement of water-holding capacity nor elasticity of plasma gels was observed in the presence of 20% propylene glycol. We suggest that the hydrogen bonding and hydrophobic interaction of porcine blood curd were enhanced by propylene glycol and hemoglobin contributed to this effect more significantly than did plasma.

In the presence of urea, the hardness and penetration of three blood protein gels decreased markedly with concentration as shown in Figure 2. Urea was an effective protein denaturant due to the potential for cleavage of hydrogen bonds and hydrophobic interaction (Bohinski, 1987); therefore, the protein–protein hydrophobic interaction and hydrogen bonding were weakened. In hemoglobin gel liquefaction occurred with urea concentration and it was difficult to section the gel for measurement (Table 1). As Tanford and Nazaki (1970) indicated, in a high level of urea, certain functional groups of hemoglobin polarize and destroy the balance between hydrophilic groups and hydrophobic groups in proteins. Hence, the decrease in hardness and penetration of the plasma gel might be due to the decreasing level of hydrogen bonding and hydrophobic interaction. However, the hardness and penetration of plasma gel and blood curd were still detectable even with urea >8 M. As Kella and Kinsella (1988) described, urea might expose the free thiol in protein and then induce the formation of a weak gel structure after heating. The effect would suggest that disulfide linkage was also responsible for the formation of blood curd structure in a high level of urea. In other words, both hydrophobic group interaction and hydrogen bonding of plasma and hemoglobin gel certainly had significant importance to the formation of the porcine blood curd structure. However, we should not neglect the contribution of the intermolecular disulfide to the texture of blood curd structure as results show in Figure 2A,C.

The effect of mercaptoethanol on the hardness and penetration of blood curd, plasma, and hemoglobin gel is shown in Figure 3. The hardness and penetration decreased progressively with mercaptoethanol (0–100 mM); however, no marked change was observed in hemoglobin gel (Figure 3C). Disulfide bonding of protein was easily reduced to free sulfhydryl by mercaptoethanol, inhibiting the intermolecular sulfhydryl–disulfide group interchange (Wang and Damodaran, 1990) and hence decreasing the texture, especially in plasma gels. That the disulfide bond played an important role in the covalent stabilization of gel structures has already been found (Shimada and Cheftel, 1989). However, the effect of disulfide bonding on the formation of gel structures depended upon various

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Table 1. Effect of Various Reagents on the Elasticity and Water-Holding Capacity of Heat-Induced Gels from Porcine Plasma, Hemoglobin, and Whole Blood[a]

	plasma		hemoglobin		blood[b]	
	elasticity	WHC	elasticity	WHC	elasticity	WHC
control	93.2 ± 2.1	87.2 ± 1.6	72.1 ± 3.4	64.7 ± 2.8	85.8 ± 1.1	78.8 ± 1.6
PG[c] (20%)	96.8 ± 1.4	91.8 ± 1.8	79.4 ± 2.8	72.4 ± 3.9	89.7 ± 2.5	84.6 ± 2.7
urea (8 M)	20.1 ± 4.9	ND[d]	ND	ND	ND	ND
2-ME[c] (0.1 M)	48.8 ± 3.2	54.2 ± 2.4	78.7 ± 3.4	65.2 ± 4.2	57.2 ± 2.9	61.5 ± 3.3

[a] Mean ± standard, error, n = 9. [b] Porcine blood proteins content: 14%. [c] PG, propylene glycol; 2-ME, 2-mercaptoethanol. [d] ND, not detectable.

Table 2. Physicochemical Properties of Porcine Blood Gels Made from Various Combinations of Plasma and Hemoglobin Proteins[a]

	plasma (%) + hemoglobin (%)				
property	14.0 + 0.0	10.5 + 3.5	7.0 + 7.0	3.5 + 10.5	0.0 + 14.0
hardness (g)	432 ± 12	328 ± 24	242 ± 18	178 ± 11	130 ± 16
penetration (g)	216 ± 8	184 ± 10	105 ± 10	86 ± 7	63 ± 8
elasticity (%)	94.6 ± 1.7	92.1 ± 0.8	87.5 ± 1.3	84.2 ± 2.5	73.7 ± 1.3
WHC (%)	88.1 ± 1.2	86.7 ± 0.8	84.9 ± 0.3	72.1 ± 0.5	62.4 ± 0.7
total SH[b] group	96.2 ± 6.8	74.3 ± 5.7	51.1 ± 3.3	30.4 ± 5.9	4.6 ± 2.3
free SH group	48.4 ± 3.9	36.1 ± 7.1	26.3 ± 3.0	13.3 ± 1.5	<1

[a] Mean ± standard error, n = 9. [b] SH group, sulfhydryl group (μmol/g of protein).
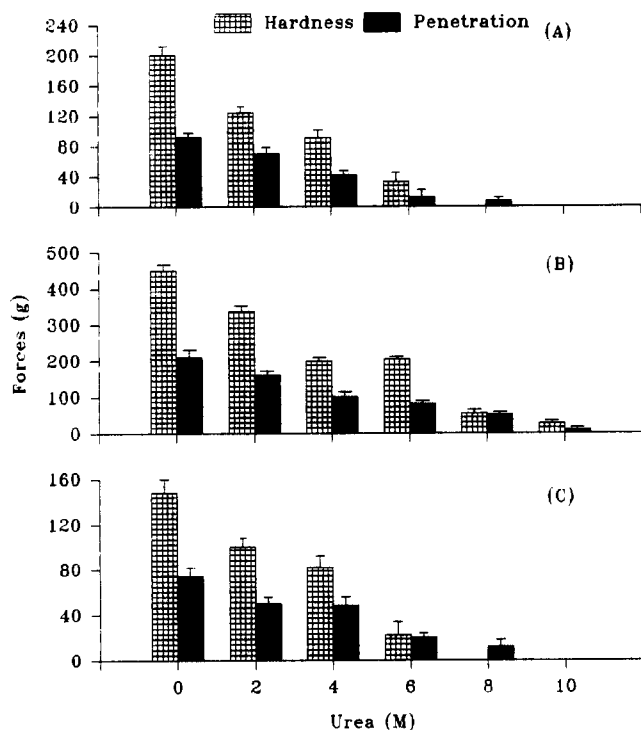

Figure 2. Effect of hardness and penetration of heat-induced porcine blood gels on urea: (A) porcine blood curd; (B) porcine plasma; (C) porcine hemoglobin.

Figure 3. Effect of hardness and penetration of heat-induced porcine blood gels on mercaptoethanol: (A) porcine blood curd; (B) porcine plasma; (C) porcine hemoglobin.

proteins (Wang and Damodaran, 1991) and the factors and conditions of thermal coagulation (Hayakawa and Nakai, 1985; Hegg, 1982). Evidence indicated the texture of hemoglobin gels was not affected by mercaptoethanol. Therefore, the disulfide group might not be involved in heat-induced coagulation of hemoglobin gels. On the contrary, the disulfide interaction of plasma gels might be important to the covalent stabilization of blood curd. The effect of mercaptoethanol on texture of gels was consistent with the findings of Hirose et al. (1980) and Mulvihill et al. (1991).

To ascertain the role of plasma and hemoglobin in the physicochemical properties of porcine blood curd, the physicochemical properties of gels made from various mixtures of plasma and hemoglobin were measured; the results are given in Table 2. In preliminary tests, the
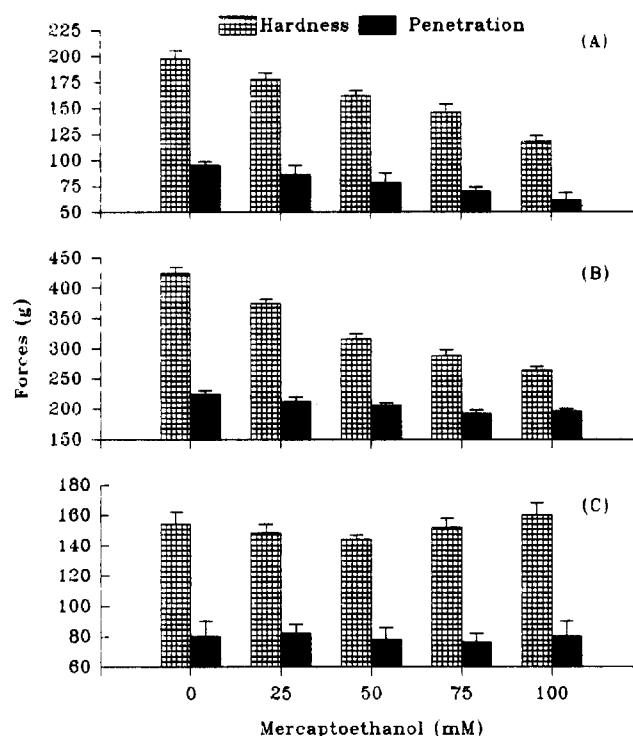
protein concentration of commercial porcine blood curd was about 14%; therefore, the concentration of all of the mixtures was adjusted. The hardness, penetration, water-holding capacity, and elasticity of mixture gel decreased significantly ($p < 0.05$) in the presence of hemoglobin. As Hayakawa et al. (1983) described, the coagulation of globin molecules was due to hydrophobic interaction. Friedman (1972) also indicated that hemoglobin possessed no intermolecular disulfide bond and only one sulfhydryl group in β-globin. However, blood serum albumin, the principal plasma protein, contained 17 intermolecular disulfide bonds, and fibrinogen and globulin also contained disulfide (Peters, 1985). These results, together with the decreased texture of the gel made from a mixture of plasma and hemoglobin, suggest a reduction in the number of intermolecular disulfide bonds in the gel. Similar results

were described by Catsimpoolas and Meyer (1970) in soy protein gels. Therefore, we concluded that plasma intermolecular disulfide bonds play an important role in the stabilization of the porcine blood curd and the degree of sulfhydryl–disulfide interchange decreases in the presence of hemoglobin.

In conclusion, hydrogen bonding, hydrophobic interacton, and intermolecular disulfide bonds are involved in the heat-induced porcine blood curd. We suggest that the hydrogen bonding and hydrophobic interaction are mainly derived from hemoglobin and that intermolecular disulfide bonds from plasma play a more important role in the stabilization of porcine blood curd than hemoglobin.

ACKNOWLEDGMENT

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