Studies on the Processing Properties and Interactions Between Porcine Blood Proteins and Waxy Rice Starch During Making Porcine Blood Cake

Chin-Wen Lin*, Jeng-Huh Yang, Hsien-Pin Chu, Bo-Ping Su, Hsiao-Ling Chen and Chia-Cheng Huang
Graduate Institute of Animal Science, National Taiwan University, Taipei, Taiwan, ROC

ABSTRACT : The physiochemical properties and interactions between porcine blood and waxy rice were determined. Addition of calcium chloride (0.15%) improved acceptability of blood cake and increased the gelatinization degree of waxy rice. The water-holding capacity of porcine blood gel (blood/water=60/40, v/v), extent of absorption and gelatinization of waxy rice, and scanning electron microscopy showed that blood protein matrix and waxy rice are competitors for holding water in the cooking procedure. Non-haem iron content increased linearly (R=0.95) when heating temperature rose. The presence of blood proteins caused increasing of peak temperature (Tp) of gelatinization in differential scanning calorimetric thermal gram. The microstructure of plasma proteins and haemoglobin appeared continuous changes, and interacted with surface of waxy rice flour in terms of network and mosaic form, respectively. The electrophoretic patterns revealed an interaction between plasma proteins and waxy rice glutelin and haemoglobin when heated could be found at temperatures above 60℃. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 3 : 358-364*)

Key Words : Porcine Blood Cake, Waxy Rice, Gelatinization, Differential Scanning Calorimetry, Microstructure, Electrophoresis

INTRODUCTION

Edible porcine blood cake is a specialized and traditional product, in which waxy rice and blood are the two major ingredients. Generally, waxy rice is evenly spread with porcine blood, then they are steam-cooked (Lieu, 1991). Porcine blood cake has a brownish appearance and special texture with cooked rice and heat-denatured blood protein, and it is a common snack in road side vendors or night markets in Taiwan. Recently, this product has been improved to a retailed package, and chilled for selling, but its appearance and texture are not fully accepted by local consumers (Lieu and Chen, 1991).

If the processing of this product was improved, consumption should increase and would also solve the disposal problem of animal blood. The purpose of this study was to survey the manufacture of this product, analyze its composition, determine the effect of heating on non-haem iron in blood cake, observe any interactions between waxy rice and blood proteins by scanning electron microscopy (SEM), and determine the heat denaturation with differential scanning calorimetry (DSC) and electrophoresis.

MATERIALS AND METHODS

Materials

Porcine blood that passed safety inspection by a veterinarian was collected from a slaughterhouse in Taoyuan, Taiwan. Sodium chloride 0.9% and sodium citrate 0.5% were added as anticoagulants. All samples were stored under refrigeration (4℃). The various analyses described below were then performed.

The procedure for making porcine blood cake is shown in figure 1. In addition some commercial porcine blood cakes were bought from a local supermarket.

Composition

Commercial and self-produced porcine blood cakes were analysed for their moisture, crude protein, crude fat and ash contents according to AOAC (1984).

Sodium chloride content

Sodium chloride content was determined with silver nitrate standard solution according to Sebranek (1978).

Water-holding capacity

Porcine blood treated with 0.9% sodium chloride and 0.5% sodium citrate, was diluted to 60% of the original concentration with deionized water. Forty ml of diluted blood was decanted into a 50 ml beaker, one ml of 20% calcium chloride was added, and when the blood gelled the beaker was covered with a piece of aluminum foil and set in a water bath (Kwang-shen constant temperature bath, Taiwan) for heating at 60, 75, or 90℃ for 30 min, or at 105 or 120℃ for 30 min in an autoclave (Speedy autoclave TM 228, Tomin Medical Equipment Co., Ltd., Taiwan).

A portion of 200-500 mg gelled blood cube was cut and put on 5 sheets of filter paper (Toyo No.1, 9 cm dia.), and another 5 sheets of paper were put on the blood cube. Filter paper and blood cube were

* Address reprint request to Chin-Wen Lin. Laboratory of Animal Products Chemistry, Department of Animal Science, National Taiwan University, No. 50, Lane 155, Keelung Road Sec. 3, Taipei, Taiwan, ROC. Tel: +886-5-2717538, Fax: +886-5-2782479, E-mail: jhyang@mail.ncyu.edu.tw.
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weighed, then pressed at 5 kg/cm^2 for 30 sec with a Carver press apparatus (JC 468, Saitama Keiki Seisakusho Co., Ltd., Tokyo, Japan). After pressing, the crushed blood cube was carefully removed and the water-absorbed filter paper was weighed; the water-holding capacity of the blood sample was calculated as follows:

$$\text{Water-holding capacity (\%)} = \left|1-\frac{(A3-A1)}{(A2-A1)}\times M\right|\times 100$$

where A1: dry filter paper weight (g), A2: sample weight plus filter paper weight (g), A3: water-absorbed filter paper weight (g), M: moisture content of sample (%)

Total iron content

With in-cineration (Thermolyne 1400, Thermolyne Corporation, USA) and atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, Perkin-Elmer 3100, USA), total iron content was determined according to Schricker et al. (1982).

Non-haem iron content

The non-haem iron content in blood solution or gel was determined by the method of Schricker et al. (1983) and Wang (1992).

Haem iron content

Haem iron content (μg Fe/g wet tissue) was calculated by deducting non-haem iron content from total iron content.

The degree of gelatinization

The degree of gelatinization of the starch of the waxy rice was determined by the method of Chiang and Johnson (1977) with slight modification. Forty milligrams of waxy rice (brought from a local supermarket, Taipei) was weighed, 5 ml deionized water was added to make a suspension and then heated at 60, 75, 90, 105 or 120℃ for 30 min, with unheated as control. After heating, the samples were immersed in water bath at 40℃. Another portion of 20 mg waxy rice was weighed, and 3 ml deionized water was added to make a suspension, 1 ml 1 N NaOH was added and vigorously shaken for 5 min, then 1 ml 1 N HCl was added to neutralize the pH; this treatment was regarded as complete gelatinization.

To the samples treated by different heating temperatures were added 25 ml glucoamylase preparation: 2 g Rhizopus glucoamylase (Sigma Chem. Co., MO, USA) was dissolved in 250 ml sodium acetate buffer (pH 4.5) and filtered through a glass fiber filter; it was used within 2 h. After being held for 30 min at 40℃, 2 ml 25% trichloroacetic acid was added to terminate the hydrolysis. The supernatant was obtained by centrifugation at 16,000 × g for 5 min (Kubota KR-20000 T, Kubota Corporation, Tokyo, Japan). A 0.5 ml portion of supernatant was poured into a test tube, 4.5 ml colourant (1.5 g thiourea dissolved in 940 ml glacial acetic acid plus 60 ml o-toluidine) was added, and then the test tube was heated in a boiling water bath for 10 min. When cooled, 5 ml glacial acetic acid was added. The solution was sampled and the absorbance at 630 nm wavelength was determined (Shimadzu UV-160A, Shimadzu Corporation, Tokyo, Japan).

Waxy rice (glutinous rice)
↓
Washing and soaking for 1 h
↓
Draining (dripping off water)
↓
Mixing blood with rice
(Wet rice/blood=5/3 (w/w))
↓
0.15% $CaCl_2$ added to the mixture
↓
Stirring and standing for coagulation *ca.* 30 min
↓
Cooking (120℃, 30 min)
↓
Cooling
↓
Porcine blood cake

Figure 1. The manufacture of porcine blood cake

Moisture absorption

The degree of moisture absorption of waxy rice during heating was determined. Waxy rice was washed and soaked in water for 1 h. Five gram of wet rice was weighed into a bottle, 8 ml deionized water was added and weighed again, then heated at 60, 75, 90, 105 or 120℃ for 30 min, and cooled at ambient temperature; free water was centrifuged off at 1,000 × g for 1 min.

$$\text{Moisture absorption (\%)} = \frac{\text{Rice weighed after heating} - \text{wet rice weighed before heating}}{\text{Wet rice weighed before heating}}\times 100$$

Differential scanning calorimetry (DSC)

To whole porcine blood was added 40% v/v of deionized water; to waxy rice powder was added 60 % of deionized water; a mixture of porcine blood and waxy rice powder was made with a 5:3 ratio. Ten milligram quantities were weighed and put in an aluminum disc, and carefully sealed with a clamp. The DSC method was used (Thermal Analyzer, General V41C, Dupont 2200, Dupont Corporation, USA). The

samples were heated from 20 to 120℃ at a rate of 10℃/min. The enthalpy, initial gelatinized temperature (To), complete gelatinized temperature (Tc), peak temperature of gelatizination (Tp) and total absorbed energy of the process of heating (J/g) were determined.

Scanning electron microscopy methodology (SEM)

The SEM of the mixture of waxy rice and porcine blood was observed by the method of Woodward and Cotterill (1986) with slight modification. Waxy rice powder was mixed with water to its 40% original concentration to make a dough, then mixed with porcine blood at the ratio of 3:5 (blood:dough); 0.15% calcium chloride was added to accelerate coagulation. The mixture was heated at 60, 75, 90, 105 or 120℃ for 30 min. The cooled sample was cut as a 1.5×4×10 mm cube, then fixed by 3% glutaraldehyde, followed by 1% osmium tetraoxide, dehydrated with a critical point dryer (Hitachi HCP-1, Japan) and stuck on an aluminum stub with ion coater (Eiko IB-2, Japan), then observed with SEM (Hitachi S-550, Scanning Electronic Microscope, Japan).

Electrophoresis

The extraction of glutelin from waxy rice was implemented by the method of Tseng (1987). An aliquot (8 ml) of a concentration of 4.5 mg/ml of each protein solution (glutelin, plasma, and haemoglobin) was poured into test tube. The samples were heated at 60, 75, 90, 105 or 120℃ for 30 min. After cooling, they were centrifuged (1,500×g, 25 min). The supernatant was collected for determination by the method of SDS-PAGE (Laemmli, 1970) with 4% stacking gel and 12.5% separating gel.

Statistical analysis

Each experiment was done at least three times, and each determination was duplicated. The experimental data were analyzed using SAS software (SAS, 1986).

RESULTS AND DISCUSSION

The composition of porcine blood cake

As shown in table 1, A, B and C represented different brands of blood cake, which were bought from a local supermarket; D is the control made by us. The differences in composition were due to the pretreatment, the ratios of raw materials, and processing conditions. When the blood for A, B and C was collected, 1.5-2.0% table salt was added as an anticoagulent; for D we used 0.9% NaCl and 0.5% sodium citrate which therefore showed lower ash and salt contents.

Table 1. Composition of porcine blood cake

Product* composition	Treatments			
	A	B	C	D
Moisture (%)	51.6	54.1	51.2	50.2
Crude protein (%)	5.4	4.5	5.6	5.1
Crude fat (%)	0.56	0.67	0.32	0.41
Ash (%)	0.7	1.1	1.6	0.6
Salt (%)	1.1	1.1	1.1	0.4

* Product A, B, and C were purchased from local supermarkets. Product D was made according to figure 1.

The fat content was related to the ratio of waxy rice to porcine blood, but the higher contents in commercial cakes A and B may have been because they were sprayed with a thin layer of food grade oil in order to increase attractiveness to customers and to prevent them sticking to their packaging.

Changes in water-holding capacity during heating

The water-holding capacity of diluted porcine blood, 60% blood and 40% water, is shown in figure 2. With the addition of 0.15% calcium chloride ($CaCl_2$), the treated blood before heating, gelled at ambient temperature. The water-holding capacities at room temperature, 60℃ and 75℃, were 8.6, 16.3 and 27.2%, respectively. When the temperature was increased to 75℃, the blood gels shrank and expelled water. When the heating temperature was higher than 105℃, water-holding capacity decreased. In non-$CaCl_2$ treatment (control), the diluted blood remained liquid-like when unheated at 60℃; at 75℃, it began to gel, with a water-holding value of 32.1%. The highest value (36.4%) appeared at 105℃, but decreased at 120℃ (33.7%). Without added $CaCl_2$ blood gelled at 75℃, and its gel nature was more stable than when $CaCl_2$ was added.

Degree of gelatinization

When water in the system is abundant and the starch is heated, its hydration raises dramatically. The starch granule swells due to absorbtion water and

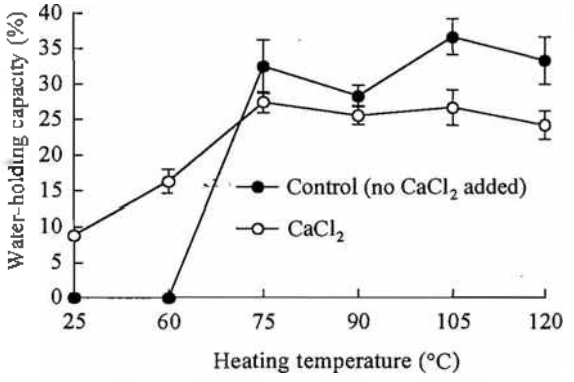

Figure 2. Water-holding capacity of porcine blood gel at various heating temperatures. * Addition of $CaCl_2$: 0.15%; Heating time: 30 min.

enlarges its volume many times. This is so-called gelatinization. When starch gelatinizes, its viscosity and solubility increase. And it can be easily hydrolyzed by amylase. The relative gelatinization of waxy rice, heated at different temperatures for 30 min. is shown in figure 3. The degree of gelatinization increased with increasing a temperature (25-90℃), and presented a significant linear result ($p<0.05$, $r=0.95$). The gelatinization reached 100% at temperatures above 90 ℃.

Moisture absorption of waxy rice

The whole grain of waxy rice was tested for its moisture absorption (figure 4) which increased with rising temperature, from 3.0% at 60℃ to 198% at 120℃. Generally speaking, the enthalpy (ΔH) and moisture absorption of rice grain is higher than that of rice powder (Norman and Marshall, 1989). We observed that there was a competitive relation for water between waxy rice and porcine blood in the process of manufacture of porcine blood cake. Before heating, the waxy rice grain was soaked in porcine blood. With rising temperature, changes in the water-holding capacity of porcine blood gel and gelatinization of heated waxy rice was in a state of dynamic distribution in the system. In a specific range of temperature, porcine blood gel had a reasonable water-holding capacity, and the moisture absorption of waxy rice greatly increased in the same range. Eventually porcine blood gel, at a higher temperature, expelled water which was immediately absorbed by waxy rice grain, and its gelatinization proceeded. If the porcine blood and waxy rice was steam-cooked this process took much more time than with an autoclave, which could greatly shorten the processing

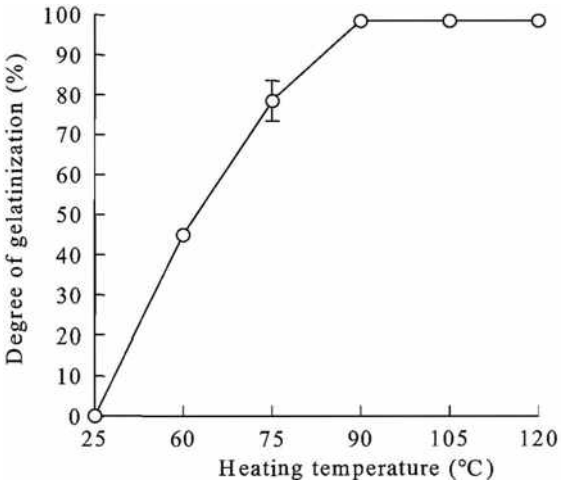

Figure 3. Effect of heating temperature on the degree of gelatinization of the waxy rice flour. * Heating time: 30 min

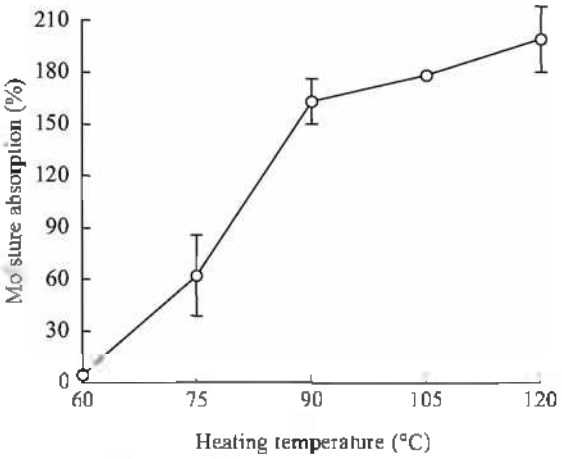

Figure 4. Moisture absorption of waxy rice grain at various heating temperatures. * Heating time: 30 min.

time. Figure 5 shows two kind of porcine blood cake with or without $CaCl_2$ addition. If $CaCl_2$ was not added, the cake was darker in colour and less gelatinized.

It could be that blood with $CaCl_2$ added might gel before heating, and so have a smoother appearance. When the heating temperature rose the water-holding capacity was less than when no $CaCl_2$ was added. At 90℃, blood gel with $CaCl_2$ released water which was transferred into waxy rice and the gel was driven into the gaps between the waxy rice granules.

Bownsey et al. (1989) studied the gel formed from a mixture of starch and egg white, and concluded the same result. The no added $CaCl_2$ porcine blood gelled by heating at 90℃ or above, and had a higher water-holding capacity, so affecting the degree of

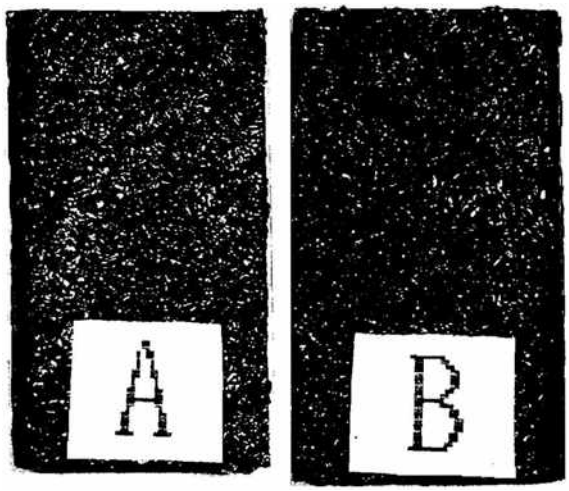

Figure 5. The appearance of porcine blood cake. A: no $CaCl_2$ added; B: $CaCl_2$ added

gelatinization of waxy rice in porcine blood cake.

Changes in iron content

The changes in iron content of porcine blood gel at heating to 60-120℃ are shown in table 2. There was no significant change between sample heated at 60℃ for 30 min and unheated sample ($p>0.05$). With increasing temperature (75-120℃), the non-haem iron rose significantly, especially in the samples heated 105 and 120℃ in which it was higher by 170% and 203%, respectively. Wang (1992) showed that non-haem iron was increasing with increasing of heating temperature, and Schricker and Miller (1983) found similarly when heating beef.

Thermal characteristics

The thermal characteristics of waxy rice and porcine blood are shown in table 3. The gelatinization temperature (To, Tp and Tc) of waxy flour was higher than that of the rice starch. It might be that the rice protein covering the surface of rice grains affected water penetration, in the same way as cell walls, consequently needing higher heating temperature to improve the penetration of water to initiate the gelatinization (Marshall et al., 1990). The waxy rice starch was more easily penetrated by water, due to the glutelin extracted from the waxy rice. Marshall (1992) pointed out that the gelatinization temperature and enthalpy of waxy rice grain were higher than waxy rice flour. Waxy rice flour, porcine whole blood and a mixture of both at the ratio of 5:3, as in porcine blood cake, were prepared for DSC analysis. The results are shown in figure 6. Porcine blood had higher heat absorption than waxy rice and the mixture. When waxy rice flour was mixed with porcine blood, its peak temperature of heat absorption was raised from 68.15℃ to 72.20℃. Evans and Haisman (1979) pointed out that the non-starch polymers formed a matrix, compete with starch for water absorption, and prevent water from transferring and penetrating, so raising the temperature of gelatinization. Tseng (1987) found the same result with bovine serum albumin and egg white added to waxy rice flour.

Table 2. Effect of iron content of porcine blood gel at various heating temperatures

Heating temperature (℃)	Iron content (μg/g wet sample)			Nohaem Fe increased (%)**
	Total	Nonhaem iron	Haem iron*	
Unheated	339±8	24.7±0.7[a]	316±14	-
60	325±7	24.5±0.2[a]	300± 3	0
75	328±2	31.3±0.5[b]	296± 3	21.7
90	329±3	35.4±0.3[c]	294± 3	43.2
105	325±7	66.8±1.1[d]	260± 7	170.5
120	330±7	74.9±2.0[e]	255± 6	203.7

* Haem iron content=total iron content-nonhaem iron content.

** Nonhaem Fe increase %=(nonhaem Fe in heated sample-nonhaem Fe in unheated sample)/nonhaem Fe in unheated sample.

[a,b,c,d,e] Data with different superscripts are significantly different ($p<0.05$).

Table 3. The differential scanning calorimetric characteristics of waxy rice flour, starch and porcine blood proteins (Mean±SE, n=3)

	Gelatinization and denaturation temperature (℃)		
	To	Tp	Tc
Waxy rice flour	59.5±0.2	69.1±0.3	84.3±0.9
Waxy rice starch	57.9±0.5	67.9±0.5	80.1±0.6
Porcine haemoglobin	77.1±0.6	83.7±0.0	93.3±0.0

To: onset temperature; Tp: peak temperature; Tc: conclusion temperature.

Scanning electron microscopy

In order to understand the changes of blood proteins and waxy rice flour in the heating process, they were mixed in the ratio of 3:5 and heated at various temperatures. After sampling, fixation, dehydration and ion coating, the samples were observed with SEM.

When $CaCl_2$ was added into the mixture of porcine whole blood and waxy rice flour, they formed a gel due to Ca^{+2} accelerating the formation of fibrin from fibrinogen (Ferry, 1945). The major network was contributed by plasma. However, haemoglobin was attached or arranged on/in the network as shown in figure 7. Wang (1992) showed plasma was heat-denatured and aggregated easier than haemoglobin; the latter was a mosaic on the plasma aggregating matrix observed by SEM, and the texture of blood curd. The

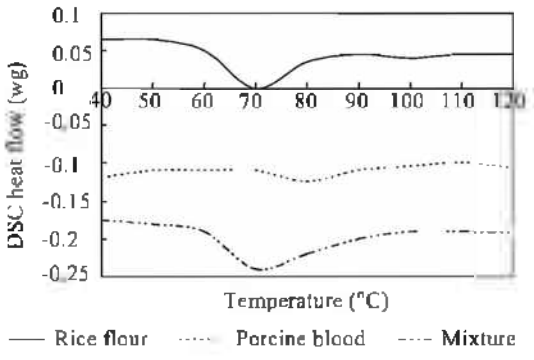

Figure 6. DSC curves of blood, waxy rice flour and their mixture. * The heating rate was 10℃/m.

granules of waxy rice flour gradually absorbed water and then swelled at increasing temperature (60-90℃). At the same time, plasma proteins were also denatured and aggregated to form a fine network as shown in figure 7B-D. When the heating temperature rose over 105℃, the waxy rice flour granules were burst, deformed, and mingled with plasma proteins. The shrunk haemoglobin, caused by heating, were mosaic on/in the network. Adhikari (1993) thought the arrangement and network of micro- or macro-structure of the constituents in food, caused in processing, were closely related to food texture and rheological property.

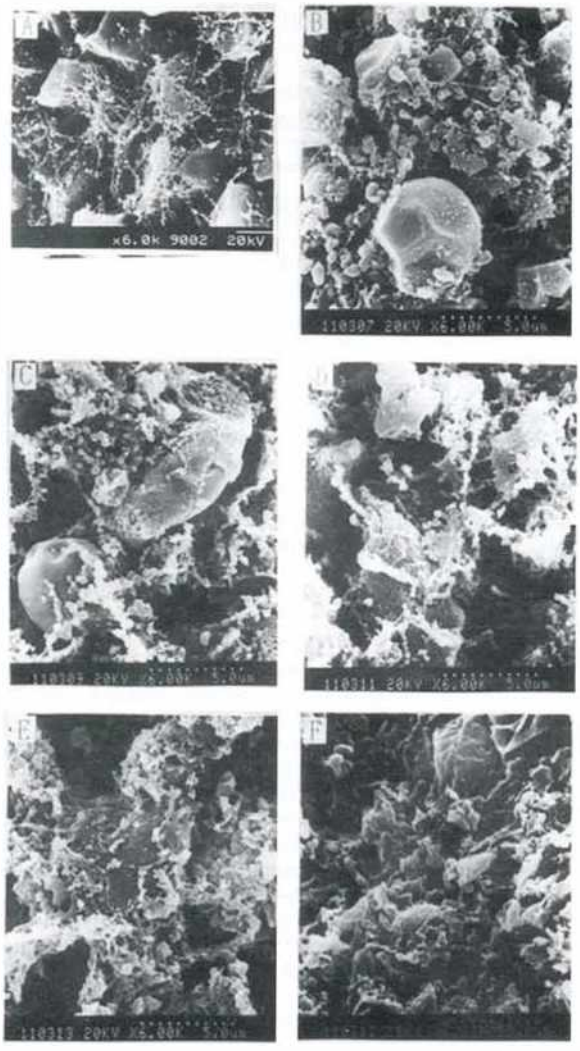

Figure 7. Photographs of SEM of mixture of porcine blood and waxy rice flour at various heating temperatures for 30 mins. (A) unheated; (B) 60℃; (C) 75℃; (D) 90℃; E 105℃; (F) 120℃.

Electrophoresis

Porcine plasma was mixed with equal volumes of haemoglobin and rice glutelin. After heating at various temperatures (60-120℃), the samples were centrifuged, and the supernatant was obtained for electrophoresis. The results are shown in figure 8 and 9. In figure 8, photograms of SDS-PAGE of supernatant of the mixture of porcine plasma and haemoglobin showed protein bands faded out with increasing heating temperature (75-120℃). They had nearly disappeared at 120℃, and only undefined protein bands with large molecular weights were left at the top of the stacking gel. As shown in figure 9, photograms of supernatant of the mixture of porcine plasma and waxy rice glutelin showed the protein bands were significantly faded at heating temperatures over 75℃. With heating temperature increasing from 90-120℃, protein bands almost disappeared. From these findings, rice glutelin and porcine plasma separately had reasonable heating stability, but each disappeared at lower heating temperature when mixed with other proteins. It could be speculated that there was an interaction among different protein molecules, based on the observation of very big molecules at the top of the stacking gel.

CONCLUSION

The mixing ratio of 5 parts waxy rice and 3 parts porcine blood steam-cooked at 120℃ for 30 min is suitable to make a satisfactory porcine blood cake.

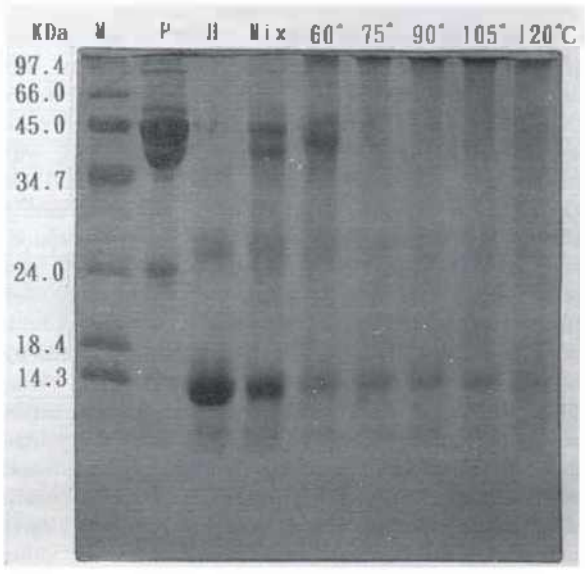

Figure 8. Photograms of SDS-PAGE of centrifuged supernatant of porcine plasma-haemoglobin at various heating temperatures. P: porcine plasma; H: porcine haemoglobin.

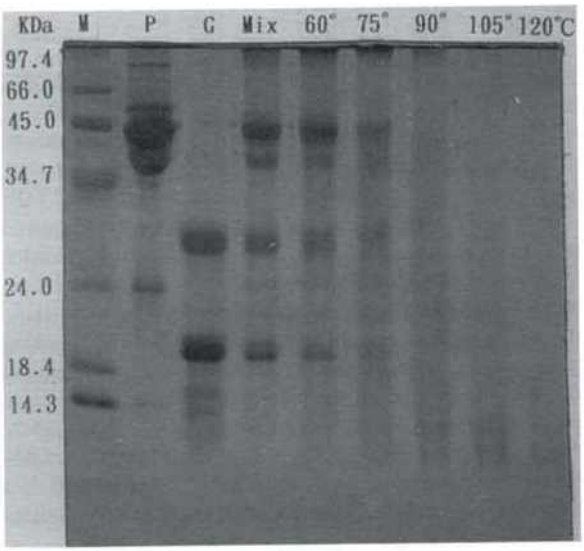

Figure 9. Photograms of SDS-PAGE of centrifuged supernatant of porcine plasma-glutelin at various heating temperatures. P: porcine plasma; G: glutelin.

The addition of 0.15-0.20% $CaCl_2$ aids gelling before heating. However, blood without added $CaCl_2$ gelled at 75℃, but its gel nature was more stable than when it was added. The highest value of water-holding capacity appeared at 105℃, but gradually decreased at 120℃. The gelatinization and moisture absorption of waxy rice were linearly increased during the heating process. So, there was a competitive relation for water between waxy rice and porcine blood in the process of manufacturing blood cake. Non-haem iron was increased with temperature (60-120℃). When waxy rice flour was mixed with porcine blood, its peak temperature of heat flow, determined with DSC, was raised from 68.15 to 72.2℃. This result could be explained by porcine blood forming a matrix during heating, competing with starch for water absorption, and raising the needed temperature for rice gelatinization. With SEM observation, the waxy rice flour granules were burst, deformed and mingled with plasma protein. The shrunk haemoglobin caused by heating was on/in the blood cake's network. With SDS-PAGE, we found that there was an interaction among porcine plasma, haemoglobin and waxy rice glutelin, based on the finding that rice glutelin and plasma had a reasonable heating stability, but each disappeared from the electrophoretic bands at lower temperature, when they were mixed with the other proteins.

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