Decolorization of Porcine Red Blood Cell Globin With Ion Exchanger Method and Modification of Its Protein Functionalities

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ABSTRACT : Extended use of porcine blood in food ingredients depends on the decolorization of red blood cell concentrates and the modification of its functional properties. The purpose of this study is to compare the relative effect of cation ion exchanger for decolorization of porcine red blood globin. The globin extract is freeze-dried for determination of various functional properties, such as solubility, emulsion capability and foaming ability. Since the isoelectric point of blood globin is located at pH 6.8, which is the neutral pH ranges (6-8), so its functionalities are inferior around these pHs. This weakness has been the main reason, which limit the extended use of blood globin in food industry. Acetylation and succinylation of blood globin can be an alternative way to improve its functionalities. These results may provide new information to understand the decolorization mode by cation ion exchanger for the blood globin. With chemical, the functionalities of blood globin could be obviously improved. The above findings could enable food industry to extend the use of blood globin as a food ingredient. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1770-1774)*

Key Words : Porcine RBC, Globin, Decolorization, Ion Exchanger, Functionalities, Modification

INTRODUCTION

Recent years, the collection apparatus has reached to a large scale applying to industrial use. The collected blood is mainly to produce plasma powder, which may be widely used in meat products such as sausages (Wismer-Pedersen, 1988). Beside the plasma, the red blood cell concentrates (RBCC) is obtained too. RBCC contains mostly of hemoglobin, which is added in foods even with a little amount, will turn blackish brown after it is heated. If RBCC can be extensively utilized, the undesirable color should be disguised with decolorization or process technology (Wismer-Pedersen, 1988). Several methods have, therefore, been developed to remove the heme group from hemoglobin of RBCC. These include the acid-acetone method (Tybor et al., 1973), hydrogen peroxide decolorization (Wismer-Pedersen, 1987) and decolorization with carboxymethylcellulose (Sato et al., 1981), with sodium carboxymethylcellulose (NaCMC, Autio et al., 1984) and with sodium alginate (Hayakawa et al., 1986). With these methods, the functional properties of separated globin varied significantly. The solubility, emulsifying activity and foaming capacity were inferior in neutral pH 6-8 ranges (Yang, 1997). From the result of screening ion exchangers for decolorization of globin, we found the strong acid cation ion exchange may be potential tool for this purpose. No report is related to the decolorization method with ion exchange, so this study is attempted to examine the effect of strong acid type of cation exchanger Amberlite IR-120, and to improve the defects of functional properties of globin in normal pH ranges with chemical modification by acetylation and succinylation.

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MATERIALS AND METHODS

Separation of blood

Porcine blood added with 0.5% sodium citrate as a anticoagulant was obtained from a slaughterhouse in Chiayi, Taiwan and centrifuged (Hitachi Himac SCR 20B, Tokyo, Japan) at 4000×g for 20 min to separate plasma and corpuscles.

Decolorization of red blood cells with ion exchanger

Red blood cells were diluted to four times of the original volume with distilled water. After lyses, the RBC was centrifuged again to remove stroma, and then 4 ml of diluted RBC was mixed with 1 ml of each one of five ion exchangers (Amberlite IRA-400, -410, IR-120, Imac A-27, Diaion WA-30). After shaking for 30 min, the supernatant was filtered off and examined by a spectrophotometer at wavelength 650-500 nm. With this pre-test, the Amberlite IR-120 was chosen owing to its best decolorization effects. One percent of diluted RBC was driven with a peristalsis pump into two series-connected glass columns (short column 3.5 D×6 L/cm acted as prefilter, the other long column 3.5 D×16 L/cm), which were packed with Amberlite IR-120, and the separated fractions were collected each 10 min in test tubes for determination with spectrophotometer at wavelength 580-380 nm. The decolorizated RBC with ion exchanger method was freeze dried (Eyela FD-1,

Tokyo, Japan).

Chemical modification of blood globin

The acetylation of blood globin was done by the method of Nakamura et al. (1984), and the succinylation of blood globin was done according to Miyaguchi et al. (1989).

Colour and colour difference

A spectrophotometric glass cell (5 cm dia., 1 cm high) was filled with 5 ml fractions of ion exchanged globin solution, then put into a colour difference meter (Nippon Denshoku 300A, Tokyo, Japan) for measuring L-, a-, and b-values. Using a C 2° light source, a standard white calibration plate was determined for X=78.79, Y=80.16, Z=89.93.

Solubility

The solubility of the globin proteins was determined by the method of Lowhon and Cater (1971) with modification. One-percent stock solutions of the proteins were prepared with distilled water and aliquots (100 ml) adjusted to a pH value ranging from pH 2-9 with 1 N HCl and 1 N NaOH. After stirring for 5 min, the pH of the protein solutions was readjusted if necessary and the solutions were stirred again for another 5 min, then centrifuged at 6,000×g for 20 min. The protein concentration of supernatant was measured at 660 nm wavelengths by the method of Lowry et al. (1951) using 0.1-5 mg/ml bovine serum albumin (BSA) as a standard. The regression equation for the standard curve over this range of protein concentration was Y=-1.0152+2.2339X (r^2=0.9639), where Y=protein concentration (mg/ml), and X=absorbance.

Emulsifying activity index, EAI

The turbid metric method of Pearce and Kinsella (1978) and Saito et al. (1987) was used to obtain the emulsifying activity index (EAI). Four milliliters of soy salad oil (Taiwan Sugar Co., Kao-Hsung, Taiwan) was added to one aliquot (16 ml) of each 1% pH-adjusted protein solution (see "Solubility"), and homogenized (knife-chopped) with a homogenizer (NISSEI AM-10 Homogenizer, Tokyo, Japan) at 10,000 rpm for 3 min. The emulsion was immediately diluted to 100-fold with 0.1% Triton X-100 solution (Merck. Art/ 11869, Darmstadt. Germany) and then measured the turbidity (Mizutani and Nakamura, 1988). The absorbance of the diluted emulsion was measured at 500 nm and used as the index for emulsifying activity (EAI), where EAI=2×2.303 Abs/φC,
where φ= the oil volume fraction (4 ml) which is constant, and C=the weight of protein per unit volume of aqueous phase before the emulsion is formed (ca. 0.16 g).

Foaming capacity and foam stability

The foaming properties of each globin protein were measured by the method of Lowhon and Cater (1971) with slight modification. Samples (0.3 g) of each globin protein were dissolved in 100 ml distilled water and the pH adjusted to the range 2 and 9. Each solution was divided equally into two 100-mL graduated cylinders, which were then covered with aluminum foil and shaken vertically by hand, with 2 up-and-down shakes per second for 1 min (shaking distance 30 cm). After shaking, the cylinders were allowed to stand at room temperature and the volume of foam was measured after 30 s, 10 min, and 2 h. The mean foam volume at 30 s is referred to as "foaming capacity" and the mean foam volume at 10 min or 2 h is referred to as "foam stability".

Statistical analysis

Each experiment was done at least three times, and each determination was duplicated. The experimental data were statistically analyzed using the General Linear Models procedure of SAS software (SAS Institute Inc., Cary, NC, USA, 1986) and significant differences among means were determined using Duncan's multiple range test. Statements of significance were based on $p<0.05$ unless others indicated.

RESULTS AND DISCUSSION

Absorbance of globin fractions

The absorbances of globin fractions, treated with several kinds of ion exchangers, are shown in table 1. Amberlite IR-120 was observed to have the best decolorization effect among 5 kinds of ion exchangers. At wavelength 500-600 nm, the absorbance of Amberlite IR-120 showed the lowest absorbance values. The absorbance of globin fractions, treated with Amberlite IR-120, is shown in figure 1. At wavelength 580-380 ranges, the absorbance of the first 3 fractions was significantly decreased. At 580 and 540 nm, the absorbance of the fourth and following fractions were lower than that of untreated control,

Table 1. Absorbance of 1% porcine red blood cell concentrate treated by various ion exchangers

Ion exchange	Absorbance at different wavelength (nm)				
	650	600	580	540	500
IRA-400	1.042	0.472	-	-	2.058
IRA-410	0.971	0.398	-	-	1.974
IR-120	0.913	0.087	0.128	0.102	0.133
A-27	1.044	0.525	-	-	2.489
WA-30	1.196	0.603	1.911	2.542	2.181

"-" over ranges of maximum absorbance 2.000.

however, they were not significantly different. This might be affected by the saturation of ion exchanger.

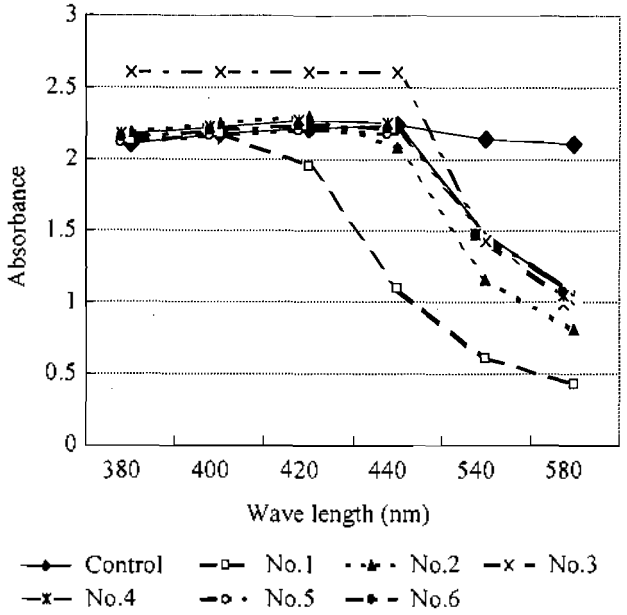
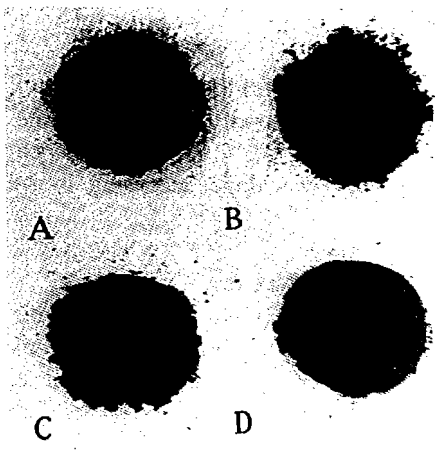


Figure 1. Absorbance of various fractions from ion-exchanged porcine RBCC

The photograph of decolorizated porcine red blood globin by ion exchanger and undecolorizated freeze-dried porcine red blood globin is shown in figure 2. The order of decolorization effect was as following: succinated>acetylated>ion exchanged>control.



A. decolorizated and freeze-dried PRBCC
B. Ion-exchanged PRBCC
C. Acetylated PRBCC
D. Succinylated PRBCC

Figure 2. Decolorizated and un-decolorizated freeze-dried porcine red blood cell concentrates (PRBCC)

Solubility

The solubility of 1% blood globin solutions measured by the method of Lowhon and Carter (1971) is shown in figure 3. Among treatments, the solubility at pH range (2-9) of ion exchange-treated globin isolates was generally inferior to that of control. However, succinylated globin isolate was more soluble at pHs of 6-7 and was less soluble at pHs of 2-5. The solubility of globin isolates was greatly affected by different pH levels. The effect of pH on solubility was also reported by Tybor et al. (1975), Saito et al. (1987), and Miyaguchi et al. (1989).

Emulsifying activity index

Figure 4 shows the emulsifying activity index (EAI) of porcine blood globin isolates. The effect of

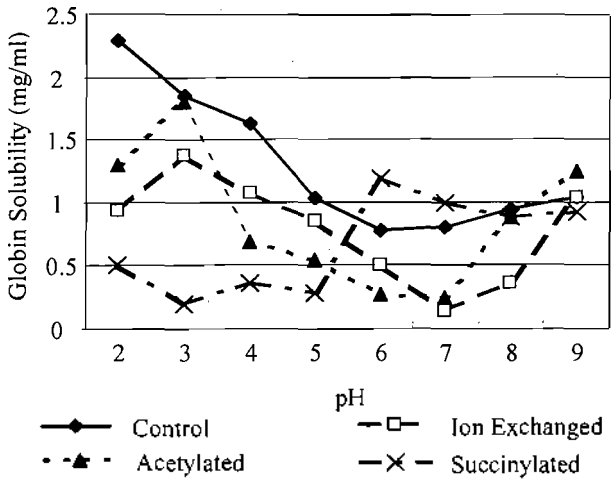


Figure 3. Solubility of 1% porcine RBCC treated by 4 different methods

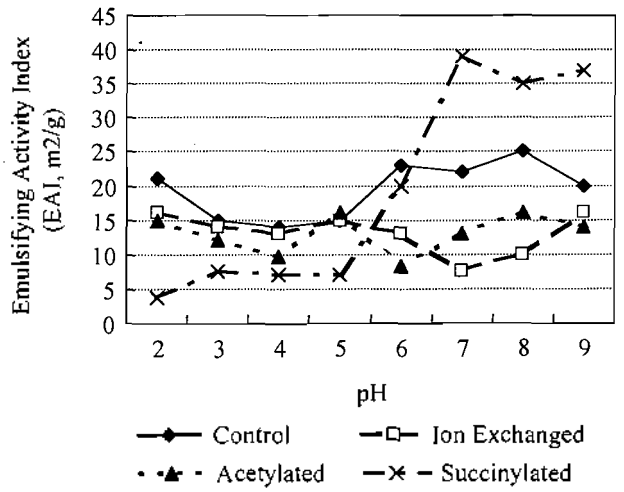


Figure 4. Emulsifying activity index of 1% porcine RBCC obtained by 4 treatments

pH on EAI was determined over the pH range 2 to 9. The EAI of all of globin isolates were greatly affected by pH. At pH 7-9, the EAI of succinylated globin isolates was the highest, and that of the ion exchanged globin isolates was the lowest at pH 7.

Foaming capacity

Figure 5 shows the foaming capacity (FC) of the blood protein isolates. The FC is significantly different among treatments and pHs ($p<0.01$). At pH of 4-9, the foaming volumes of control are greater than those of ion exchanged and chemical modified globin isolates, except for succinylated samples at pH 7-9, which foaming volumes were higher than those of other samples, but the foam from control nearly disappeared at pH 2-3.

Yang and Lin (1998) compared with other decolorization method, such as Na-CMC (sodium carboxymethyl cellulose, Autio et al., 1984; Yang and Lin, 1998), acid-acetone method (Tybor et al., 1973, 1975), H_2O_2 treatment (Wismer-Pedersen, 1987) and Alcalase treatment (Houlier, 1986). They found the color of RBC globin isolates from Na-CMC and Alcalase method were not effectively decolorizated, and acid acetone and H_2O_2 treated method have practical problems associated with harmful residues.

From the above results, the strong acid type cation ion exchange resin may be considered into the alternative methods of decolorization the blood globin. But batch treatment and the saturation degree of cation ion exchanger limited the potential of decolorization of blood globin isolates. The resin was needed to be refreshed to retrieve its ion exchange capability, which might be attributed to chelate the heme part of blood globin. Since the isoelectric point of blood globin is at pH 6.8, so its functional properties (such as solubility, emulsifying activity index and foaming capacity) are inferior around this neutral pH range between 6 and 8 (Yang, 1997). This weakness is the main barrier to limit extensive use of blood globin in foods. From this study, the succinylation of ion exchanged globin isolate shows the improved solubility, emulsifying activity index and foaming capacity of proteins at neutral pHs. Whereas, the acetylation of ion exchanged globin did not exert the effect.

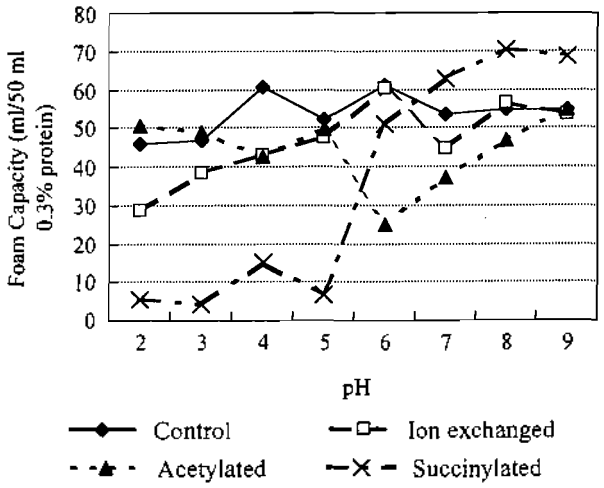


Figure 5. Foaming capacity of 1% porcine RBCC obtained from 4 treatments

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