Functional Properties of Porcine Blood Globin Treated with Various Discoloration Methods

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ABSTRACT

The functional properties of freeze-dried porcine blood globin isolates, which were obtained after discoloration with sodium carboxymethyl cellulose (Na-CMC), acid-acetone (A-A), hydrogen peroxide (H2O2) or Alcalase were compared. Porcine red blood cells (RBC) without discoloration were freeze-dried as controls. The solubility, emulsifying activity index, foaming capacity and gel strength of globin isolates were determined. The results showed that, the solubility of globin isolates were affected by different discoloration treatments and pH ranges (pH 2-9). The foaming capacity was significantly different among treatments and pH levels ($P < 0.01$). As for gel properties, the 10% solution of control RBC powder showed the greatest gel strength and breaking intension, followed by A-A-treated, Na-CMC-treated and H2O2-treated globin isolates. The Alcalase-treated globin isolates solution could not form gels.

INTRODUCTION

The major part of blood protein is hemoglobin in the erythrocytes, which even in trace amounts imparts a dark brownish color to foods. Large-scale use of hemoglobin in foods consequently would require discoloration or technological manipulation to cover the heme pigment in processing (Clark *et* al.,1987). The heme pigment can be removed from the globin by extraction with acidified acetone (Tybor *et* al.,1973; Tybor *et* al.,1975), or by absorption on certain agents such as carboxymethyl cellulose(Sato *et* al.,1981), sodium carboxymethyl cellulose and sodium alginate (Yang and Lin, 1996). Alternatively, the globin may be digested by proteolytic enzymes and separated from the heme by ultrafiltration or centrifugation (Hald-Christensen, 1978; Houlier, 1986). Another approach is oxidative destruction with hydrogen peroxide to oxidize hemoglobin to bile pigments (Wismer-Pedersen, 1987). If the above mentioned methods to obtain blood globin isolate are to be used in foods, however, their functional properties must be surveyed extensively. Thus, this study concentrates on different methods of discoloring blood globin isolates and compares the functional properties of the resulting solutions.

MATERIALS & METHODS

Preparation of globin protein isolates

1. sodium carboxymethyl cellulose method

The separation of blood globin from RBC was conducted by the method of Autio *et* al. (1984) and Yang and Lin (1996). The globin solution was freeze-dried into flakes and ground with a hammer mill (Cullatti DCFH 48, Canada) through a 2mm sieve plate.

2. Acid-acetone method

Blood globin was prepared from the RBC using hydrochloric acid and acetone (pH 4) procedure described by Tybor *et* al. (1973; 1975).

3. Oxided method with hydrogen peroxide

As described by Wismer-Pedersen (1987).

4. Alcalase hydrolysis method

The hydrolyzation of the RBC was conducted by the method of Houlier (1986).

Color and color difference A spectrophotometric glass cell (5 cm dia. 1 cm high) was filled with globin and plasma powder, then put into a color difference meter (Nippon Denshoku 300A, Japan) for measuring L-, a-, and b- values.

Solubility (Lawhon and Cater, 1971).

Emulsifying activity index, EAI (Pearce and Kinsella, 1978, Saito *et al.*, 1987)

Foaming capacity (Lawhon and Cater, 1971).

Gel strength The globin "elation method of Miyaguchi *et al.* (1992) was followed. Five grams of globin protein or plasma was dispersed in 50 ml of distilled water, adjusted to pH 7.0, then decanted into a 100 ml beaker and heated for 10 min in boiling water bath. After heating, the beaker was placed in a cool water bath (15°C) and allowed to cool for 30 min. The beaker with protein "elation was then placed on the loading table of a Rheometer (Fudoh Rheometer NRM-2010J-CW, Japan), with 2K sensor (0-200 g) and # 3 adapter (~ 10mm). the gel strength (g) and breaking intension (g/cm2) was recorded with a plotter (Fudoh plotter FR-801, Japan).

RESULTS & DISCUSSION

Color and color difference

The L-, a-, lo-value of freeze-dried blood globins obtained from various treatments are given in Table 1. Among the treatments, the L-, a-, lo-values all show significant different ($P<0.01$). Alcalase-treated globin turns brown as same as the untreated RBC. However, the H2O2- and acid-acetone-treated globin has a larger L-value than untreated RBC.

Especially, the acid-acetone-treated globin has a soil-like yellow. But the L-value of Na-CMC-treated globin is lower than that of untreated RBC, so its lightness also less. The a-value are greater in Alcalase- and Na-CMC-treated globin, which also displays more redness than untreated RBC. The acid-acetone-treated globin isolates have higher bvalues than others, followed by H2O2- and Alcalase-treated globin isolates, while the Na-CMC-treated and untreated RBC are not significantly different.

Solubility

The solubility of 1% blood globin solutions measured by the method of Lowry *et al.* (1951) is shown in Fig.1. Among the treatments, the solubility of Alcalase-treated globin isolates is superior to that of other globins. the solubility of Na-CMC-, H2O2-treated globin and RBC tend to the same at different pHs. However, the changes of acidacetone-treated isolates are most dramatically affected with pHs. At a pH of 2, it has the highest solubility, then declines as increases, untill at pHs of 8-9 it goes up again. This effect of pH on solubility was also reported by Tybor *et al.* (1975) and Saito *et al.* (1987). Except for the acid-acetone-treated globin isolates, which are less soluble at pHs of 6-8, the other treated globin solutionsa are less affected by changing pH.

Emulsifying activity index (EAI)

Figure 2 shows the EAI of porcine blood globin isolates. The effects of pH on EAI was determined over the range of pHs from 2-9. The EAI of all of the blood globin isolates are greatly affected by pH. At pH 6-8, the EAI of Na-CMC-, Alcalase-, and acidacetone- treated globin isolates remain at lower levels, and the H2O2- treated globin isolates were the lowest at pH 7.

Foaming capacity

Figure 3 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 2-3, the foaming volumes are greater than those at pHs of 4-9 (Alcalase-treated samples excluded).

Gel strength

The gel strength and breaking intension of all blood proteins shows in Table 2. The untreated RBC forms the strongst gel and exhibits the highest gel strength and breaking intension. followed by Na-CMC-treated and H2O2-treated globin isolates, but these bothdo not form a solid gel. The Alcalase- treated globin does not exhibit gelling with a suspension state. The data on gel strength and breaking intension are the same, so either one of them can be chosen to compare gelation.

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Table 1. The comparison of L-,a-,b- value of freeze-dried porcine blood globin isolate powder obtained from various treatments (n=4)

Item	Na-CMC treated	Acid-Acetone treated	H2O2 treated	Alcalase treated	RBC untreated
L-value	20.41±0.06d	41.66± 0.04a	33.16 ± 0.01b	24.81 ± 0.05c	24.89 ± 0.08c
a-value	10.73± 0.08b	5.34 ± 0.05e	9.34 ± 0.24c	13.29 ± 0.13a	8.70 ± 0.10d
b-value	4.99± 0.03c	12.11± 0.02a	7.23 ± 0 06b	7.19 ± 0.05b	4.93 ± 0 05c

a,b,c,d: Means at the same row with different superscripts are significant different (P <0.05).

Table 2. The gel strength and breaking intension of various porcine globin curds

Treatment	Gel strength (g)	Breaking intension (g/cm2)
Na-CMC treated	18 ± 4c	22± 5c
Acid-Aceton treated	29 ± 2b	36± 2b
H202 treated	20± 4c	25± 5c
Alcalase treated	---- *	---- *
RBC untreated	71+8a	90± 10a

* It was remained as suspension.

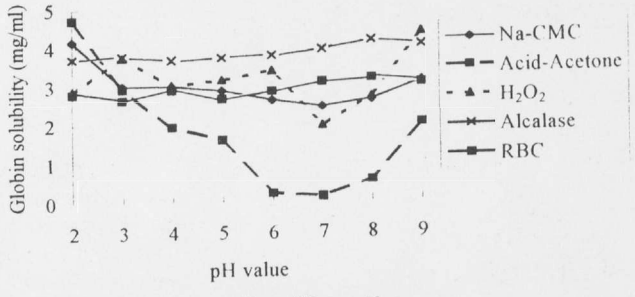

Fig. 1. The solubility of 1% globin at different pHs

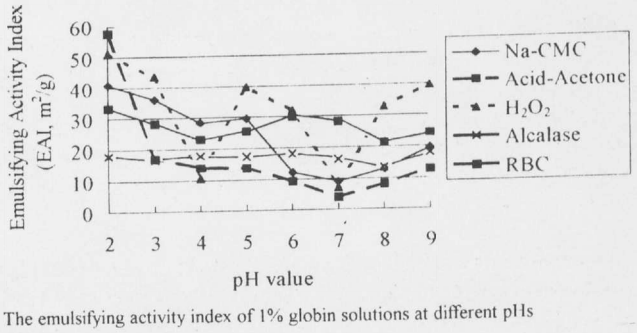

Fig. 2. The emulsifying activity index of 1% globin solutions at different pHs

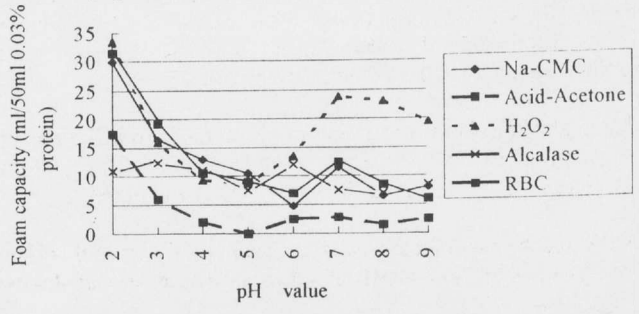

Fig. 3. The foaming capacity of 0.3% globin solutions at different pHs

NOTES