Effect of cation exchange on discolouration of globin in porcine red blood cells

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ABSTRACT: The purpose of this study is to investigate the relative effect of cation exchange on the discolouration of globin in porcine red blood cells. The globin extract was freeze-dried for determination of various functional properties, such as solubility, emulsion capability and foaming ability. Chemical modification of the globin extract by either acetylation or succinylation provided improvement in discolouration and the functional properties of globin extracted from porcine red blood cells. Cation exchange of porcine globin extract with Amberlite IR-120 resin followed by succination improved discolouration and functional properties at pH 6-8.

Key Words: Porcine Red Blood cells, Globin, Discolouration

INTRODUCTION
Animal blood is collected at abattoirs to provide plasma powder and red blood cell concentrate (RBCC). Although plasma powder is used widely as an additive in meat products such as sausages (Wismer-Pedersen, 1988), inclusion of red blood cell concentrate (RBCC) in meat products is limited by the undesirable dark colour. A number of discolouration technologies have been developed to remove the haem group from haemoglobin of RBCC. These techniques include discoloration with acid-acetone (Tybor et al., 1973), hydrogen peroxide (Wismer-Pedersen, 1987), carboxymethylcellulose (Sato et al., 1981), sodium carboxymethylcellulose (NaCMC, Autio et al., 1984) and sodium alginate (Hayakawa et al., 1986). However the functional properties of separated globin has been found to vary significantly with each of these discoloration technologies. For instance, Yang (1997) found that each of these techniques reduced the solubility, emulsifying activity and foaming capacity of globin within normal pH ranges (6-8). This report describes a new technology to discolor porcine RBCC using cation exchange to maintain the functional properties of globin within normal pH ranges by acetylation and succination.

MATERIALS AND METHODS
Porcine blood containing 0.5% sodium citrate as an anticoagulant was obtained from an abattoir in Chiai, Taiwan and centrifuged (Hitachi Himac SCR 20B, Tokyo, Japan) at 4000 × gravity for 20 minutes to separate plasma and red blood cells.

Red blood cells (RBC) were diluted to four times the original volume with distilled water and the lysed cells were centrifuged again to remove the cell stroma. Four ml of the supernatant was mixed with 1 ml of each of five cation exchange resins (Amberlite IRA-400, IRA-410, and IR-120; Imac A-27 and Diaion WA-30). After shaking for 30 min, the supernatant was filtered and examined by a spectrophotometer set at wavelength 650-500 nm. The best discolouration was achieved with the Amberlite IR-120 resin. Hence a one percent solution of the diluted RBC was pumped through two series-connected, cation exchange, glass columns (a short column, 3.5 D x 6 L /cm used as a pre-filter; and a long column, 3.5 D x 16 L/cm). Both columns were packed with Amberlite IR-120. The separated fractions were examined for optical density with a spectrophotometer set at wavelength 580-380 nm and freeze-dried (Eyela FD-1, Tokyo, Japan).

Chemical modification of the blood globin included acetylation using the method described by Nakamura et al. (1984), and succination according to Miyaguchi et al. (1989).

The functional properties of the separated globin were determined as the solubility, emulsifying activity and foaming index. Solubility of the globin proteins was determined by the method of Lowhon and Cater (1971). The turbidimetric method of Pearce and Kinsella (1978) and Saito et al. (1987) were used to obtain the emulsifying activity index (EAI) of the blood globin isolates. The foaming properties of each globin protein were measured using a modification of the method described by Lowhon and Cater (1971). Each of these functional tests involved dissolving a sample (0.3g) of each globin protein in 100 mL distilled water and adjusting the pH over the range 2 to 9.

Each functional test was conducted at least three times, in duplicate to compare freeze-dried red blood cell concentrate before cation exchange (control), after cation exchange with Amberlite IR-120 followed by either acetylation or succination. The experimental data were statistically analysed using the General Linear Models procedure of SAS software (SAS Institute Inc., Cary, NC, USA, 1986).

RESULTS AND DISCUSSION
Absorbance of globin fractions
The mean absorbance values of globin fractions in each of the five cation exchange resins is presented in Table 1. Amberlite IR-120 had the least absorbance...
value at a wavelength of 500-600 nm. A photograph representation of the freeze-dried red blood cell concentrate before cation exchange (control), after cation exchange with Amberlite IR-120, followed by either acetylation or succination is presented in Figure 1. The order of discoloration was as follows: succinated > acetylated > cation exchange > control.

Table 1. Mean absorbance\(^1\) (x10\(^{-3}\)) of 1% porcine red blood cell concentrate in different cation exchange resins.

<table>
<thead>
<tr>
<th>Resin(^2)</th>
<th>Wave length (nm)</th>
<th>650</th>
<th>600</th>
<th>580</th>
<th>540</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRA-400</td>
<td></td>
<td>1042</td>
<td>472</td>
<td>-</td>
<td>-</td>
<td>2058</td>
</tr>
<tr>
<td>IRA-410</td>
<td></td>
<td>971</td>
<td>398</td>
<td>-</td>
<td>-</td>
<td>1974</td>
</tr>
<tr>
<td>IR-120</td>
<td></td>
<td>913</td>
<td>87</td>
<td>128</td>
<td>102</td>
<td>133</td>
</tr>
<tr>
<td>A-27</td>
<td></td>
<td>1044</td>
<td>525</td>
<td>-</td>
<td>-</td>
<td>2489</td>
</tr>
<tr>
<td>WA-30</td>
<td></td>
<td>1196</td>
<td>603</td>
<td>191</td>
<td>2542</td>
<td>2181</td>
</tr>
</tbody>
</table>

\(^1\) Mean absorbance (x10\(^{-3}\)) above 2500.
\(^2\) Amberlite IRA-400, IRA-410 and IR-120; Imac A-27 and Diaion WA-30.

Fig. 1. Freeze-dried porcine red blood cell concentrate (RBCC)

A. RBCC prior to cation exchange.
B. RBCC following cation exchange with Amberlite IR-120.
C. RBCC following B and acetylation.
D. RBCC following B and succination.

Solubility
The solubility of cation exchange-treated globin isolates was generally inferior to that of the untreated control. The solubility of globin isolates varied with different pH levels as reported by Tybor et al. (1975), Saito et al. (1987), and Miyaguchi et al. (1989). For example, the succinicated globin isolate was more soluble at pH 6-7 compared to the control, but was less soluble at a pH range of 2-5.

Emulsifying activity index
The emulsifying activity index (EAI) of porcine blood globin isolates is presented in Figure 2. The EAI of all of globin isolates were greatly affected by pH. At pH 7-9, EAI values were higher for the succinated globin isolates. Conversely, the globin isolates treated with cation exchange only had the lowest EAI values at pH 7.

Fig. 2. Emulsifying activity index of 1% porcine red blood cell concentrates before (control) and after cation exchange with either acetylation or succination.

Foaming capacity
The foaming capacity (FC) of the blood protein isolates is presented in Figure 3. There was a significant interaction of treatment at different pH (P<0.01). At pH 4-9, the foaming volumes of the control isolates were greater than the cation exchanged and chemical modified globin isolates. The exception was the succinated samples which had increased FC values compared to other treatments at pH 7-9. However the FC of the succinated samples almost disappeared at pH 2-3.

Fig 3. Foam activity of 1% porcine porcine red blood cell concentrates before (control) and after cation exchange with either acetylation or succination.

In summary, cation exchange with Amberlite IR-120 resin provides a method of discoloring porcine blood globin. Discolouration of porcine red blood cells with cation exchange alone appeared to be limited by batch treatment and the saturation degree of the resin.
It was important to use fresh resin to limit chelation between the haem component of blood globin and the resin. Previous studies (Yang, 1997) have shown that the functional properties of porcine RBCC (such as solubility, emulsifying activity index and foaming capacity) are inferior around neutral pH levels (6-8) because the isoelectric point of blood globin is located at pH 6.8. This characteristic is the main limiting factor for the extensive use of blood globin in foods. The current study has demonstrated the benefit of succination following cation exchange of porcine RBCC to improve solubility, emulsifying activity index and foaming capacity at neutral pH levels. However, acetylation following cation exchange of porcine RBCC did not show similar benefits.

REFERENCES
