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Effect of salts on some of the functional properties of bovine plasma protein concentrate

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The effects of salts, NaCl, Na_2SO_4, KCl and CH_3CO_2Na on some of the functional properties of bovine plasma protein concentrate (BPPC) were investigated. Results showed that the least gelation concentration of 6.0% observed in distilled deionized water was improved to between 2% and 4% in the presence of the salts used. The foaming capacity of about 36.0% in water considerably increased to between 52.0% to 158.0% depending on the type and concentration of the salts. The water absorption capacity decreased at low salt concentrations compared with values in distilled water and increased with increase in salt concentration while the emulsion capacity decreased with an increase in salt concentration. The variation of protein solubility with pH was found to depend on salt type and concentration. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Despite the growing shortage of proteins of animal origin suitable for human consumption, vast quantities of blood are wasted annually due to apathy in the food industry (Akers, 1973). With the rise in world population, particularly in the developing countries, it is logical to find some means of making the maximum use of by-products of the meat industry. By-products such as blood, are known to be a source of nutritional and functional proteins. However, bovine blood has been used only in limited quantities for direct human consumption because of its intense colour and characteristic taste (Lee *et al.*, 1987). Methods for the separation of blood into plasma and red cell fractions by centrifugation have been developed commercially (Nazlink & Lawne, 1983). More recently, preparation of colourless globin from bovine haemoglobin was reported (Lee *et al.*, 1990). There is now growing interest in utilizing blood from slaughtered animals owing to its high content of quality plasma proteins and nutritionally available iron (Lee *et al.*, 1987).

The presence of salt may increase the total water content of the protein system at specific water activity values, although it may reduce the preferential binding of water to the protein (Sathe & Salunkhe, 1981). These effects are markedly dependent on the nature of the anion and cation components (Sathe & Salunkhe, 1981; Altschul & Wilcks, 1985). The effect of salt is significant because, in many foods, salt concentrations are approximately 0.2–0.3 M (Altschul & Wilcks, 1985).

The present report is on the effect of NaCl, Na_2SO_4, CH_3CO_2Na and KCl on some of the physicochemical properties of bovine plasma protein concentrate.

MATERIALS AND METHODS

The bovine plasma protein concentrate (BPPC) was prepared as described by Oshodi and Hall (1993). The salts used were NaCl, Na_2SO_4, KCl and CH_3CO_2Na, all British Drug Houses products. The required concentrations of the various salt solutions used were prepared by weighing 0.5, 1, 2, 5, 10, 15 and 20 g of the salts which were dissolved in 99.5, 99, 98, 95, 90, 85 and 80 g of distilled, deionized water, respectively.

The variations of protein solubility with pH for BPPC in the various salt concentrations were determined using the method described by Sathe and Salunkhe (1981) and a supernatant was obtained whose protein content was determined by the Biuret method (Wiechselboven, 1946).

The least gelation concentration, water absorption and foaming properties of the BPPC were determined using the methods of Sathe *et al.* (1982), replacing water with appropriate salt solutions.

The emulsion capacity and stability were determined by the method described by Sathe and Salunkhe (1981). The results are means of at least triplicate determinations.

Table 1. Least gelation concentrations of BPPC flour in various salt solutions

Concentration of salt (%) in water used	Least gelation concentrations (%)			
	NaCl	Na_2SO_4	KCl	CH_3CO_2Na
0.0	6	6	6	6
0.5	4	3	3	4
1.0	4	2	2	4
2.0	3	2	2	4
5.0	3	2	2	2
10.0	2	2	2	2
15.0	2	2	2	2
20.0	2	2	2	2

RESULTS AND DISCUSSION

Table 1 shows the variation of least gelation concentration with percentage of salts. Table 1 indicates that the least gelation concentration of the bovine plasma protein concentrate (BPPC) is 6% in distilled deionized water. The addition of salts resulted in a decrease in least gelation concentration which depended on the concentration and type of salts under consideration and values obtained varied between 2% and 4%. It is observed that the addition of salts at the low concentration of 0.5% improved the gel-forming property of BPPC and that this effect is more pronounced with the use of Na_2SO_4 and KCl. These results will enhance the uses of BPPC in various food applications such as in comminuted sausage products and in new product development where gelation may be needed to provide increased gel strength.

The results for water absorption capacity of BPPC in the different salt solutions are presented in Table 2. The water absorption capacity in distilled, deionized water is found to be 94% which is lower than the values reported for some soybean (130% and 227%) and sunflower (107% and 137%) protein concentrates (Lin *et al.*, 1974), and the protein concentrate of *Abenopus breviflorus* benth (201%) seed flour (Oshodi, 1992), but higher than the value reported for fluted pumpkin (85%) protein concentrate (Oshodi & Fagbemi, 1992). The low water absorptivity of BPPC may make it less susceptible to heat denaturation (Kinsella *et al.*, 1985). Table 2 further shows a progressive decrease in water absorptivity with increase in salt concentrate generally up to 5% salt, after which the water absorptivity starts to increase. The degree of decrease or increase in water absorption capacity varies with the type of salt. This may be due to the fact that the effects of salts vary with the cation and anion species involved (Kinsella *et al.*, 1985). The observed trend at low salt concentration may be due to masking of charges which may reduce electrostatic interaction and hydration but increase hydrophobic interaction. At high salt concentrations, electrostatic interactions are apparently of little importance with regard to the amount of water bound to protein because competition between the ions and proteins for water becomes predominant (Shen, 1981; Altschul & Wilcks, 1985; Kinsella *et al.*, 1985). In addition, at low concentrations of salts (ions), macro-

Table 2. Water absorption capacity of BPPC flour in various salt solutions

Concentration of salt (%) in water used	Water absorption capacities (%)			
	NaCl	Na_2SO_4	KCl	CH_3CO_2Na
0.00	94.5 ± 1.5	94.5 ± 1.5	94.5 ± 0.7	94.5 ± 1.5
0.50	40.5 ± 0.5	80.0 ± 2.0	80.0 ± 0.5	40.6 ± 0.4
1.00	32.5 ± 0.5	70.0 ± 1.0	80.0 ± 0.8	30.5 ± 0.5
2.00	30.5 ± 1.5	60.0 ± 0.0	40.5 ± 0.5	20.0 ± 1.0
5.00	20.5 ± 1.5	60.5 ± 0.6	20.5 ± 0.5	20.0 ± 1.5
10.00	20.0 ± 1.0	340.0 ± 2.5	30.0 ± 0.3	80.5 ± 0.5
15.00	80.0 ± 1.0	386.0 ± 2.0	60.0 ± 1.5	100.0 ± 1.5
20.00	300.5 ± 1.5	388.0 ± 1.5	100.0 ± 1.5	200.0 ± 2.0

Table 3. Foaming capacity of BPPC flour in various salt solutions

Concentration of salt (%) in water used	Foaming capacity (%)			
	NaCl	Na_2SO_4	KCl	CH_3CO_2Na
0.0	31.5 ± 0.5	31.5 ± 0.5	31.5 ± 0.5	31.5 ± 0.5
0.5	52.5 ± 0.4	70.0 ± 1.2	88.0 ± 1.0	70.6 ± 1.2
1.0	71.8 ± 0.7	87.5 ± 1.5	92.0 ± 2.1	66.8 ± 0.5
2.0	68.0 ± 0.9	84.0 ± 1.1	87.8 ± 1.1	70.5 ± 1.5
5.0	97.0 ± 1.0	108.6 ± 0.8	95.6 ± 0.7	78.0 ± 2.5
10.0	108.0 ± 1.4	116.8 ± 1.7	110.0 ± 1.8	77.5 ± 1.0
15.0	117.0 ± 0.6	126.0 ± 0.5	121.5 ± 0.7	84.0 ± 0.6
20.0	111.0 ± 1.5	66.6 ± 1.3	114.0 ± 1.2	70.0 ± 1.1

molecules preferentially bind the ions; that is, more ions are associated with the macromolecule than is the case in bulk solution. At high concentrations of salt, the macromolecule preferentially binds water; that is, the proportion of water to salt in the vicinity of macromolecules is greater than the ratio in bulk-phase solvent (Kinsella *et al.*, 1985). Salts bound to protein may depend on the ions, and the ability of the ions to enhance hydration. When protein attracts such ions (electrostatic effect), the ions may carry hydration water into the vicinity of the protein. The high water absorptivity in the presence of a Na_2SO_4 concentration higher than 5% may, therefore, be due to the high hydration potential of the Na_2SO_4 (Kinsella *et al.*, 1985). The lower water absorptivity at low salt concentrations may be an advantage in drying and storage stability of BPPC while the higher water absorptivity at high salt concentrations may be an advantage in the production of meat analogues where the capacity of the matrix to imbibe and hold moisture and to stimulate the juiciness and texture of the product is critical. However, care has to be taken as the high water binding capacity caused by addition of salts may cause the protein to imbibe a disproportionate amount of water and dehydrate other components in the food system or vice versa. For example, in bread making, the water-binding capacity of an added protein extender must be compensated to ensure proper hydration of flour proteins (Kinsella *et al.*, 1985; Kinsella, 1976).

The effect of salts on the foaming capacity is presented in Table 3 and indicates that foaming capacity depends on type of salt under consideration. For NaCl, KCl and Na_2SO_4, there is an increase in the foaming capacity with increase in concentration of salt from

Table 4. Foaming stability of BPPC flour in various salt solutions (after 2 h)

Concentration of salt (%) in water used	Foaming stability (%)			
	NaCl	Na_2SO_4	KCl	CH_3CO_2Na
0.0	6.0±0.2	6.0±0.2	6.0±0.2	6.0±0.2
0.5	24.0±0.6	33.5±0.7	42.0±0.3	30.0±1.2
1.0	41.8±0.4	38.0±0.7	27.5±1.0	28.0±0.0
2.0	38.8±1.1	35.0±0.7	27.5±1.0	28.0±0.0
5.0	40.0±0.5	18.0±0.6	36.0±0.3	37.5±0.5
10.0	43.5±2.2	53.5±1.4	45.5±0.8	34.5±0.3
15.0	47.3±0.9	77.0±0.8	50.0±0.5	43.0±0.7
20.0	59.5±1.4	37.0±0.5	46.0±0.7	37.5±0.5

Table 5. Emulsion capacity of BPPC flour in various salt solutions

Concentration of salt (%) in water used	Emulsion capacity (%)			
	NaCl	Na_2SO_4	KCl	CH_3CO_2Na
0.0	27.3±0.8	27.3±0.4	27.3±0.5	27.3±6.3
0.5	23.0±0.5	26.5±0.3	22.5±0.6	26.4±0.7
1.0	21.5±0.3	26.9±0.8	22.9±0.1	24.6±0.5
2.0	21.3±1.3	26.2±0.3	23.2±0.2	24.1±0.9
5.0	20.9±0.9	25.6±0.2	24.8±1.1	23.8±0.3
10.0	20.3±0.4	25.0±0.5	21.6±0.7	23.6±0.1
15.0	20.9±0.3	24.9±0.7	20.1±0.6	22.4±0.6
20.0	22.0±0.7	24.7±0.6	18.6±0.4	19.5±0.5

Table 6. Emulsion stability of BPPC flour in various salt solutions (volume of water separated at room temperature(25±2.5°C) after 24 h)

Concentration of salt (%) in water used	Emulsion stability (%)			
	NaCl	Na_2SO_4	KCl	CH_3CO_2Na
0.0	0.0	0.0	0.0	0.0
0.5	16.0±0.5	13.6±0.7	21.9±0.5	15.0±0.4
1.0	18.5±0.4	17.7±1.0	20.4±0.5	18.5±0.2
2.0	16.9±0.7	18.8±0.6	21.8±0.7	18.6±0.9
5.0	24.6±0.9	18.9±0.4	20.6±0.6	19.7±0.5
10.0	25.4±0.2	19.5±0.5	21.5±0.2	19.8±0.3
15.0	21.6±0.4	22.3±0.8	26.2±0.7	20.4±0.6
20.0	23.8±1.2	33.6±0.8	24.3±0.6	22.6±0.5

0.5 to 15.0% and then a drop at 20% salt concentration. This may be due to the fact that salts usually reduce surface viscosity and rigidity of protein films but increase spreading rate, thereby weakening interpeptide attractions and increasing foam volume for certain proteins (Altschul & Wilcks, 1985). Salts at appropriate concentrations aid foaming, presumably by aiding diffusion and spreading at the interface, but high levels of salts will depress foaming (Altschul & Wilcks, 1985), for example, depression at 20% salt concentration. On the whole, all the salts used increase the foaming capacity of BPPC but the lowest effect is observed with CH_3CO_2Na and this may not be unconnected to the hydrolysis of CH_3CO_2Na in water. The improved foaming capacity in the presence of salts may consequently improve the functionality of BPPC in its uses for the production of cakes (Johnson *et al.*, 1979; Lee *et al.*, 1993) and whipped toppings where foaming is an important property (Kinsella, 1979). The results for foaming stabilities after 2 h are shown in Table 4 which indicates that all salts used at various concentrations had significantly improved the foaming stability.

The values obtained for emulsion capacity and stability are presented in Tables 5 and 6, respectively, and they indicate that BPPC has good emulsion capacity and stability in the absence of salts. Emulsion capacity and stability depend on concentration and the types of salt under consideration. Table 6 shows further that, after 24 h, no water was separated from emulsion produced in the absence of salts, whereas there was water separation in all samples containing salts, indicating a decrease in emulsion stability in the presence of salts. The degree of water separation varies from salt to salt. In the presence of NaCl and Na_2SO_4, the volume of water separated increases with increase in salt concentration, while in the presence of KCl and Ch_3CO_2Na, the volume of water separated was almost constant up to 10% of these salts. Three separate mechanisms that appear to be involved in the formation of a stable emulsion may be (i) reduction of interfacial tension, (ii) formation of a rigid interfacial film and (iii) electrical charge (McWatters & Cherry, 1981). The surfactancy of proteins is related to their ability to lower the interfacial tension between water and oil in the emulsion. The surface activity is a function of the ease with which protein can migrate to, adsorb at, unfold and rearrange at an interface and presumably salts reduce the surface activity of BPPC and thereby increase the interfacial tension which leads to a decrease in emulsion capacity. Salts may also reduce charge repulsion between the proteins and enhance hydrophilic association at the interface (Kinsella, 1979). The decrease in emulsion stability as noticed in Table 6, may be due to increased contact leading to coalescence which thereby reduces stability (Parker, 1987).

The variations of protein solubility with pH in the presence and absence of salts are shown in Figs 1–4. The balance of hydrophobic and electrostatic interactions can be manipulated, not only by pH and ionic strength, but also by the nature of the salts used. Neutral salts are known to exert striking effects on the solubility, the association–dissociation equilibrium, the enzyme

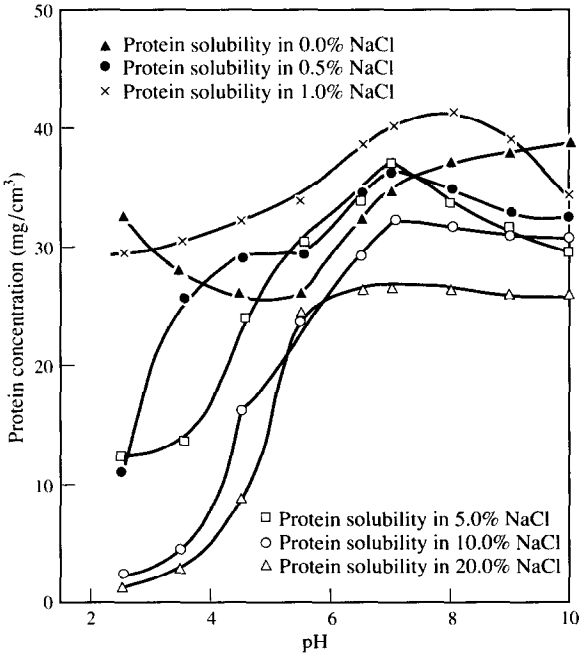

Fig. 1. Variation of protein solubility of BPPC with pH in various concentrations of NaCl solutions. Protein solubility in ▲, 0% NaCl; ●, 0.5% NaCl; ×, 1% NaCl; □, 5% NaCl; ○, 10% NaCl; △, 20% NaCl.

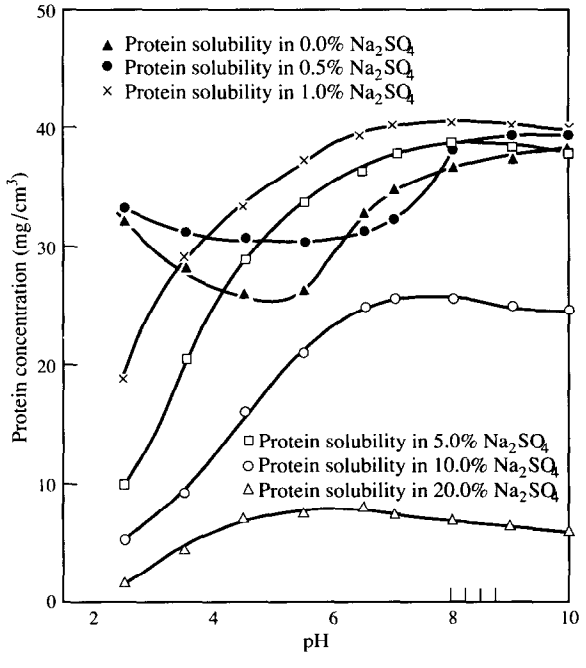

Fig. 2. Variation of protein solubility of BPPC with pH in various concentrations of Na_2SO_4 solutions. Protein solubility in ▲, 0% Na_2SO_4; ●, 0.5% Na_2SO_4; ×, 1% Na_2SO_4; □, 5% Na_2SO_4; ○, 10% Na_2SO_4; △, 20% Na_2SO_4.

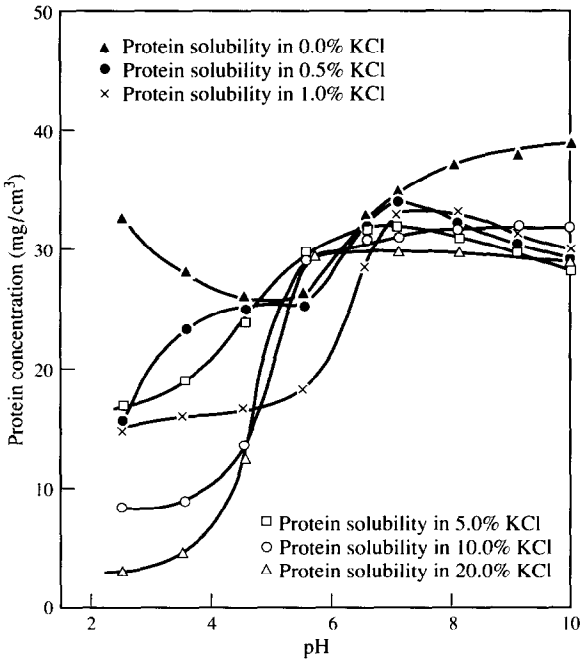

Fig. 3. Variation of protein solubility of BPPC with pH in various concentrations of KCl solutions. Protein solubility in ▲, 0% KCl; ●, 0.5% KCl; ×, 1% KCl; □, 5% KCl; ○, 10% KCl; △, 20% KCl.

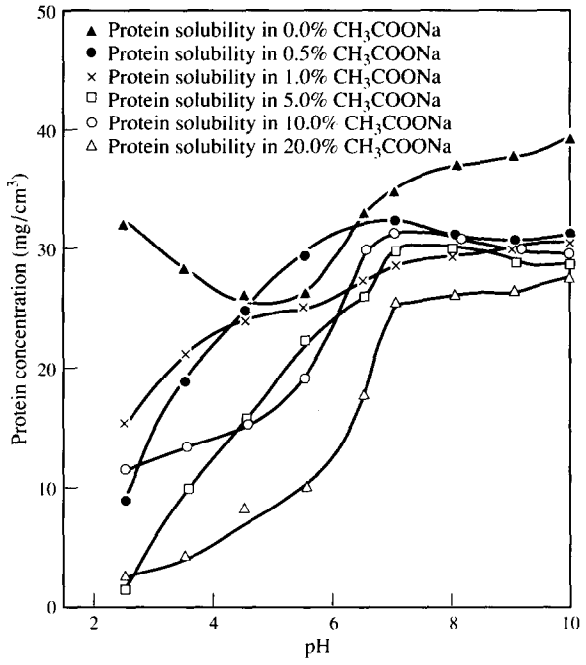

Fig. 4. Variation of protein solubility of BPPC with pH in various concentrations of CH_3CO_2Na solutions. protein solubility in ▲, 0% CH_3CO_2Na; ●, 0.5% CH_3CO_2Na; ×, 1% CH_3CO_2Na; □, 5% CH_3CO_2Na; ○, 10% CH_3CO_2Na; △, 20% CH_3CO_2Na.

activity, the stability of native and fibrillar structures and the rates of conformation change of proteins, polypeptides and nucleic acids (Von Hippel & Scheleich, 1969). Salts that are effective in increasing protein solubility (salting in) are also effective in destabilizing native globular and fibrous structures and in increasing the rate of denaturation of native structures. Conversely, salts that decrease solubility (salting out) have the reverse effect on the stability and the rate of denaturation of native proteins (Shen, 1981). Anions have greater effects on water structure than cations. The degree to which the water structure is affected depends on the size and charge density of the anions. Figures 1–4 indicate that the variations in protein solubility of BPPC depend on pH and concentration of salts. In the absence of salts, the solubility curve shows a minimum at about pH 5. In the presence of NaCl (see Fig. 1) it is observed that there is no pH of minimum solubility, but instead there are pH values of maximum solubility which depend on salt concentrations ranging from 0.5 to 10% NaCl. For 20% NaCl, the solubility increases with pH up to pH 7 and becomes constant. It is also observed that the solubilities of the protein in BPPC in 10% and 20% NaCl are lower at all pH values studied than in the absence of the salt. Figure 2 shows the effect of Na_2SO_4 on the solubility of protein in BPPC. The solubility increases up to pH 8 and becomes constant for salt concentrations between 0.5 and 20%. For concentrations of 10 and 20% Na_2SO_4, the solubility is significantly lower at all pH values studied than in the absence of this salt. Figure 3 indicates that, in the presence of KCl, the solubility of protein in BPPC is less than in its absence for pH values from 2 to 5 and 6.5 and 10. Between pH 5 and 6.5, the solubilities are comparable in the presence or absence of KCl. Instead of a pH of minimum solubility which is observed in absence of KCl at about pH 5, a pH of maximum solubility is indicated at about pH 7 for 0.5 to 5%. For 10 and 20% KCl, the solubility increases up to pH 6.5 and becomes virtually constant up to pH 10. Figure 4 shows the effect of CH_2CO_2Na on the solubility of protein in BPPC and indicates that for virtually all pH values the protein is less soluble in this salt solution than in its absence. It is also observed that, in the presence of CH_3CO_2Na, protein solubility increases up to pH 7 and then stays virtually constant between pH 7 and 10. It is observed generally that for all four salts used in these studies, the proteins in BPPC are less soluble in the acid region of pH than in the absence of these salts, whereas in the basic region the solubilities are more comparable to those obtained in absence of the salts. The solubility of protein depends on hydration and the degree of hydrophobicity of the protein molecules (Sathe & Salunkhe, 1981). Denaturation processes may cause reduction in the hydration of the protein, exposing more hydrophobic groups and thereby reducing the solubility of the protein in the lower pH regions. Perutz (1978) observed that the ionization of non-polar groups (that lie in the interior of the molecule) by alkali leads these groups to attract hydration shells which are misfits in the native

structure thus shifting the equilibrium towards the unfolded form. In addition, Perutz (1978) emphasized that the electrostatic interactions (ionization of interior non-polar groups) are more important in hydration of proteins than the surface charge. This may contribute to the improved protein solubility obtained in the alkaline pH region.

The above results show that the gelation, water absorption, foaming capacity/stability, emulsion capacity/stability and protein solubility of BPPC are affected by salts and that these effects depend on the types of salt and their concentrations. Therefore, salts may be selectively used to improve or inhibit these functional properties of bovine plasma protein concentrate.

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