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Effects of NaCl, ionic strength and pH on the foaming and gelation of pigeon pea (*Cajanus cajan*) protein concentrates

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Abstract

Studies were carried out on the effects of pH, ionic strength, sample concentration and added salt (NaCl) concentration on two hydrodynamic properties — gelation capacity and foaming properties of pigeon pea protein concentrates. Results indicate that the protein concentrate has a good gelation capacity with a least gelation concentration of 6% (w/v). Using the least concentration endpoint (LCE) as an index of gelating ability, the gelation capacity of the flour improved in the presence of moderate NaCl concentration and low ionic strength. Acidic and neutral pH also enhanced gelation while alkaline pH decreased it. The foaming capacity of the flour could be improved in a medium of low ionic strength and by increasing the concentration of protein sample as well as by a moderate concentration (0.25% w/v) of NaCl. Better foaming capacity was observed in the acidic region than in the alkaline region. Foams were also observed to be more stable as concentration of NaCl, concentration of protein concentrate and ionic strength increased.

Keywords: Foaming; Gelation; Pigeon pea protein concentrates

1. Introduction

Pigeon pea (*Cajanus cajan*) also known as Congo pea, Red gram or Non-eye pea is perhaps one of the most widely grown agricultural legumes in tropical and subtropical countries (Summerfield & Robberts, 1985). It is much less popular in Nigeria due to lack of knowledge of its nutritional and industrial utilisation potentials. It has a great potential as a protein supplement to cereal-based diets and, because of this, the Protein Advisory Group (PAG) of the United Nations recommended, in 1972, that urgent attention be paid to research into the production and nutritional evaluation of pigeon pea and seven other grain legumes (PAG, 1972). Consequently the chemical composition (Aletor & Aladetimi, 1989; Elias, Cristales, Bressani, & Miranda, 1976; Mtanga & Sugiyama, 1974; Oshodi, Olaofe, & Hall, 1993; Singh, Sharman, Heodhar, & Sharman, 1975) and mineral content (Deosthale & Rao, 1981; Singh, Jain, Jambunathan, & Faris, 1984) of pigeon pea have been studied.

In order to successfully introduce a new supplementation into any food item, it is necessary to find out whether the supplementations possess appropriate functional properties for food applications and consumer acceptability. Functional properties of pigeon pea flour have been investigated (Oshodi & Ekperigin, 1989). However, the flavour and functional properties of food systems have been found to be impaired when legumes are used in large quantities as food supplements (Rooney, Gustafson, Clark, & Cater, 1972).

To overcome this inadequacy, proteins of the legumes rather than the legume flour have become increasingly attractive as protein supplements as they have superior functional properties, low flavour profile, relative freedom from toxic factors and indigestible carbohydrates (Berk, 1970).

The functional properties of protein preparations depend, not only on factors connected with the preparations themselves, such as kind of protein, type of preparation and the way by which it has been produced, but are also influenced by environmental factors (Hermmansson & Akesson, 1975).

While the functional properties of pigeon pea flour have been investigated (Oshodi & Ekperigin, 1989), there has been no reported work on the functional properties of the pigeon pea protein concentrates and the influence of environmental factors such as pH,

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temperature, ionic strength sample concentration and presence of other components like salt on functional properties. It is, therefore, the aim of this research to study some functional properties (foaming properties and gelation capacity of pigeon pea protein concentrates and the effects of some variable factors such as pH, sodium chloride concentration, ionic strength and sample concentration on them.

2. Materials and methods

2.1. Flour preparation

Pigeon pea seeds were purchased from Erekesan Market in Ado-Ekiti. The seeds were screened to eliminate the bad ones. Boiling water was added to the samples and left until the water cooled to room temperature (25°C). The seeds were then manually dehulled. The dehulled peas were dried in an air-oven at 30°C and dry-milled into the flour. The flour was stored in polythene bags and kept in a refrigerator at about 4°C.

2.2. Preparation of pigeon pea protein concentrate (PPPC)

The method employed for the preparation of PPPC is outlined in Fig. 1. The whole seed flour was subjected to alkaline extraction at pH 8.5 using dilute alkali (0.03 M NaOH) at room temperature, clarification of the extract by centrifugation and acid precipitation of the protein at pH 4.5 using 2 M HCl. Protein concentration of the whole seed flour and protein concentrate determined by

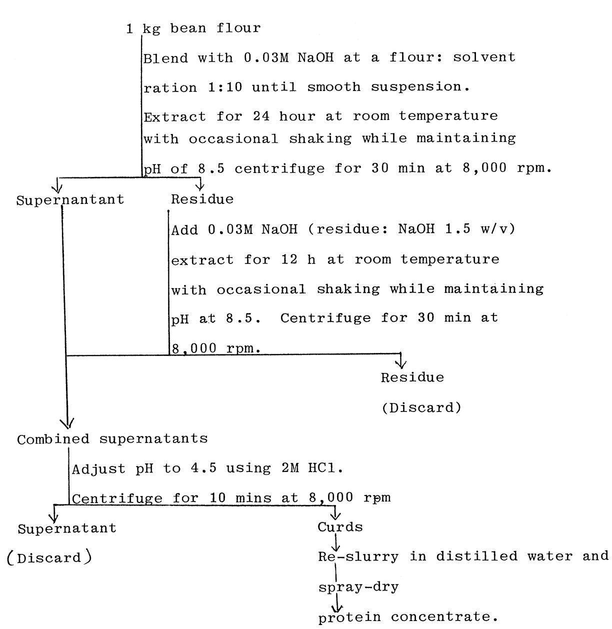

Fig. 1. Schematic diagram for the preparation of pigeon pea protein concetrate.

Kjeldahl method (AOAC, 1985) were found to be 21.5 and 72%, respectively.

2.3. Foaming properties

The foaming capacity for each sample suspension was determined by the method of Coffman and Garcia (1977) with slight modifications. Weighed amounts of flour were dispersed in 100 ml distilled water.

The resulting solution was vigorously whipped for 3 min in a Moulinex Blender. Volumes were recorded before and after whipping and the percentage volume increase calculated according to the following equation:

$$\%\ \text{volume increase} = \frac{(\text{vol. after} - \text{vol. before}) \times 100}{\text{vol. before}}.$$

Weights were also determined before and after whipping and the specific volume calculated according to Baldwin and Sinthavalai (1974).

Effects of sample concentration on foaming were evaluated by whipping 2, 4, 6, 8 and 10% (w/v) slurries as described above. To study effects of salt (NaCl) on foaming, 2% (w/v) slurries were employed. Salt concentrations of 0, 0.25, 0.5 and 1% were investigated.

Effects of pH were evaluated on 2% (w/v) slurries by adjusting pH to the desired value using either 0.5 M HCl or 0.5 M NaOH prior to whipping.

Effects of ionic strength were evaluated on 2% (w/v) slurries. Two grams of flour were dispersed in 100 ml of 0.25 and 0.75 M KCl solutions, respectively, and pH adjusted to 7 using either 0.25 M HCl or 0.25 M NaOH to produce media with ionic strengths of 0.5 and 1, respectively.

All experiments on foaming were carried out in duplicate.

2.4. Gelation

The method of Coffman and Garcia (1977) with slight modifications was employed for determining the gelation capacity for each solution. Sample suspensions 2–20% (w/v), were prepared in distilled water. Ten millilitres of each suspension was put in a test tube and heated for 1 h in a boiling water bath followed by rapid cooling in a bath of cold water. The test tubes were further cooled at 4°C for 2 h. The least gelation concentration was determined as that concentration when sample from the inverted test tube did not fall down or slip.

Effects of pH on gelation were investigated by adjusting pH values of each set of 2–20% (w/v) sample suspensions to a desired value using either 0.5 M NaOH or 0.5 M HCl.

Effects of ionic strength on gelation were investigated by preparing sample suspensions using 0.25 M and

0.75 M KCl solutions, respectively, as the dispersing medium. pH values were then adjusted to 7 using 0.25 M HCl or 0.25 M NaOH to finally produce ionic media with strengths of 0.5 and 1, respectively.

Effects of salt (NaCl) on gelation were investigated in the presence of 0.25, 0.5 and 1% (w/v) NaCl solutions.

All experiments on gelation were carried out in duplicate.

3. Results and discussion

3.1. Foaming properties

Table 1 presents results showing the effects of salt (NaCl) on the foaming capacity and stability of PPPC. Addition of NaCl improved foaming capacity, increasing it from 80% at 0% NaCl to 110% at 0.25% NaCl. The improvement could be due to:

(a) higher protein solubility of vegetable proteins in salt solutions (Hang, Steinkraus, & Hackler, 1970; Rahda, Pant, & Tulsiani, 1969); and
(b) the ability of NaCl to aid diffusion and spreading at the interface.

These effects are, however, concentration-dependent, as high concentrations of NaCl solutions above (0.25%, w/v) were found to depress foaming. The beneficial effect of low concentrations of NaCl on emulsions (Nath & Narasinga Rao, 1981; Ramanatham, Ran, & Urs, 1978; Wang & Kinsella, 1976) and foams (Hammansson, Sivik, & Skholderrand, 1971; Watts, 1937) has been reported. Low concentrations of salt enhance protein solubility whereas high concentrations decrease it (Narayana & Narasinga Rao, 1982). Since foam capacity appears to be due to solubilised protein, the differing effects of salt concentrations may be explained on this basis. As salt concentrations increased, foams formed were denser as indicated by decreased specific volume.

Table 2 shows that PPPC had greater foaming capacity at the low ionic strength of 0.5 compared to that at 1. The results suggest participation of ionic bonds in the foaming phenomena of PPPC. At high ionic strength, foaming properties become depressed because ions may reduce the coulombic forces between polypeptide chains in the protein molecules (Altshal & Wilcke, 1985).

Foaming capacity was dependent on sample concentration (Table 3) showing 152% increase in volume at 10% (w/v) concentration compared to 80% at 2% w/v. The results are in agreement with the observations of earlier workers on foaming properties of Great Northern bean proteins (Sathe & Salunkhe, 1981) and lupin seed proteins and protein concentrates (Sathe, Desphande, & Salunkhe, 1982). The specific volumes reveal that foams formed at higher protein concentrations were less dense compared to those formed at lower protein concentration. The foaming capacity of 80% observed in this work was high compared to the 32% reported for lupin seed proteins (Sathe et al.), 32% also reported for Great Northern bean flour (Sathe & Salunkhe, 1981) and 68% reported for pigeon pea flour (Oshodi & Ekperigin, 1989). This may be due to differences in proteins and the concentrations employed. Grahams and Phillips (1976) linked good foamability

Table 1
Effect of salt (NaCl) on the foaming capacity and stability of pigeon pea protein concentrates [a]

Salt concentration (%, w/v)	Specific volume (ml/g)	Volume whipping (ml)	% Increase	Vol. (ml) at room temp. (24°C) after time (h)				
				0.5	1	2	6	24
0.00	1.26	180 ± 2.0	80	110 ± 2.0	102 ± 1.5	102 ± 4.0	100 ± 0.0	100 ± 0.0
0.25	1.20	210 ± 2.0	110	180 ± 1.5	150 ± 2.0	120 ± 3.0	105 ± 0.0	100 ± 0.0
0.50	1.12	160 ± 1.5	60	150 ± 2.5	140 ± 2.0	132 ± 3.0	120 ± 1.0	100 ± 0.0
1.00	1.10	140 ± 2.0	40	140 ± 2.0	138 ± 2.5	130 ± 2.0	120 ± 1.0	100 ± 0.0

[a] Results are means of duplicate determinations.

Table 2
Effect of ionic strength on the foaming capacity and stability of pigeon pea protein concentrates [a]

Ionic strength (μ)	Specific volume (ml/g)	Volume after whipping (ml)	% Increase	Vol. (ml) at room temp. (24°C) after time (h)				
				0.5	1	2	6	24
0 (water)	1.26	180 ± 2.0	80	110 ± 1.0	102 ± 1.5	102 ± 1.0	100 ± 0.0	100 ± 0.0
0.5	1.14	200 ± 2.0	100	180 ± 2.0	165 ± 2.0	142 ± 1.5	116 ± 1.0	100 ± 0.0
1.0	1.13	150 ± 1.0	50	148 ± 2.0	140 ± 1.0	136 ± 1.5	120 ± 1.0	100 ± 0.0

[a] Results are means of duplicate determinations.

Table 3
Effect of sample concentration on the foaming capacity and stability of pigeon pea protein concentrates [a]

Sample concentration (%, w/v)	Specific volume (ml/g)	Volume after whipping (ml)	% Increase	Vol. (ml) at room temp. (24°C) after time (h)					
				0.5	1	2	6	24	36
2	1.17	180 ± 2	80	110 ± 1	102 ± 2	102 ± 1	100 ± 0	100 ± 0	100 ± 0
4	1.24	210 ± 3	110	150 ± 2	130 ± 1	105 ± 1	100 ± 0	100 ± 0	100 ± 0
6	1.25	226 ± 2	126	200 ± 2	150 ± 1	110 ± 2	100 ± 0	100 ± 0	100 ± 0
8	1.26	240 ± 2	140	240 ± 3	200 ± 2	145 ± 1	115 ± 2	100 ± 0	100 ± 0
10	1.37	252 ± 3	152	250 ± 3	242 ± 2	235 ± 2	180 ± 2	120 ± 4	100 ± 0

[a] Results are means of duplicate determinations.

with flexible protein molecules that can reduce surface tension, while highly ordered globular proteins, which are relatively difficult to surface-denature give low foamability. Hence, one may suggest that pigeon pea proteins may be high in flexible proteins.

Foaming capacity of PPPC was pH-dependent (Table 4). Generally there was improved foaming capacity in the acid and alkaline regions. While foaming capacity was highest at pH 2, it was least in the isoelectric region (between pH 4 and 6). The results closely agree with those observed for lupin seed proteins (Sathe et al., 1982), soy isolate (Eldridge, Hall, & Wolff, 1963), soy and sunflower proteins (Lin, Humbert, & Sosulki, 1974). The improved foaming capacities at the acid and alkaline regions could be explained by the higher solubilities of the pigeon pea proteins at these regions which had been earlier observed (Oshodi & Ekperigin, 1989). The specific volumes of the foams were nearly constant in the acid region through to the alkaline region, which means that densities of the foams were not all that pH-dependent.

Results in Table 4 also show that foam stability was better in the acid region than in the alkaline region, though foam stability in both regions was better than in the isoelectric region. The foams produced were also observed to be more stable as:

1. the concentration of added salt (NaCl) increased (Table 1);
2. the ionic strength of the medium increased (Table 2); and
3. the concentration of protein sample increased (Table 3).

3.2. Gelation

The simple gelation tests described in the Materials and Methods section were carried out to verify the gel forming ability of PPPC and factors affecting it. The least concentration endpoint (LCE) which is defined as the lowest protein concentration at which gel remained in the inverted tube was used as an index of gelation capacity. The lower the LCE, the better the gelating ability of the protein ingredient.

The LCE of 6% (w/v) for PPPC observed in this study (Table 5) is lower than that observed for the Great Northern bean proteins (10%, w/v) (Sathe & Salunkhe, 1981), lupin seed proteins (14%, w/v) (Sathe et al., 1982), mung bean protein isolate (10%, w/v) (Coffman & Garcia, 1977) and suggests that PPPC would be a better gelating agent than these other legume preparations. The swelling ability of the PPPC with LCE of 6% (w/v) is superior to that of the pigeon pea flour with LCE of 12% (w/v), as reported by Oshodi and Ekperigin (1989).

The improved gelating ability of PPPC in the presence of 0.25% NaCl (Table 5) indicated by a lower LCE of 2% (w/v), could be due to higher protein solubilisation of pigeon pea proteins by the salt solution thereby creating an effective overlapping of the functional groups between adjacent protein molecules, a condition necessary for a network or gel formation

Table 4
Effect of pH on the foaming capacity and stability of pigeon pea protein concentrates [a]

pH	Specific volume (ml/g)	Volume after whipping (ml)	% Increase	Vol. (m1) at room temp. (24°C) after time (h)					
				0.5	1	2	6	24	36
2	1.25	240 ± 3	140	238 ± 3	235 ± 4	210 ± 2	140 ± 2	105 ± 0	100 ± 0
4	1.22	190 ± 2	90	185 ± 2	170 ± 2	150 ± 1	108 ± 1	100 ± 0	100 ± 0
6	1.23	180 ± 2	80	170 ± 1	150 ± 2	115 ± 1	100 ± 0	100 ± 0	100 ± 0
8	1.25	210 ± 3	110	200 ± 2	160 ± 2	115 ± 1	100 ± 0	100 ± 0	100 ± 0
10	1.24	228 ± 2	128	220 ± 2	200 ± 2	165 ± 3	120 ± 1	100 ± 0	100 ± 0

[a] Results are means of duplicate determinations.

Table 5
Effects of sample and salt (NaCl) concentration on the gelation of pigeon pea protein concentrates

Sample concentration (%, w/v)	0% NaCl		0.25% NaCl		0.50% NaCl		1% NaCl	
	Gelation [a]	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance
2	–	Viscous	+	Gel (LCE)	–	Liquid	–	Liquid
4	–	Curdy	+	Gel	–	Liquid	–	Liquid
6	+	Gel (LCE) [b]	+	Gel	–	Viscous	–	Liquid
8	+	Gel	+	Gel	–	Viscous	–	Viscous
10	+	Firm gel	+	Firm gel	–	Curdy	–	Curdy
12	+	Firm gel	+	Firm gel	+	Gel (LCE)	–	Curdy
14	+	Very firm gel	+	Very firm gel	+	Gel (LCE)	+	Gel (LCE)
16	+	Very firm gel	+	Very firm gel	+	Firm gel	+	Gel
18	+	Solid gel	+	Solid gel	+	Firm gel	+	Gel
20	+	Solid gel	+	Solid gel	+	Very firm gel	+	Firm gel

[a] (–) No gelation, (+) gelation.

(Catsimpoolas & Meyer, 1970). The gelating ability of PPPC decreased at higher concentrations of salt. It has been suggested (Catsimpoolas & Meyer, 1971) that hydrogen and ionic bonds are responsible for the stabilisation of gel, and that addition of NaCI will decrease the viscosity of gel if the concentration of NaCl is high enough to neutralise the charges stabilising the gel. It therefore seemed reasonable to deduce that NaCl, at concentrations of 0.5% (w/v) and above, is able to neutralise the charges stabilising gel formation in PPPC, thereby decreasing viscosity and leading to a higher LCE. The LCE increased to 12% (w/v) and 14% (w/v) in the presence of 0.5% (w/v) and 1% (w/v) NaCl, respectively. In the same manner, increased ionic strength was found to decrease the gelating ability (Table 6) as there would be neutralisation of charges stabilising the gel in a high ionic medium. The LCE was observed to be 4% (w/v) and 8% (w/v) in ionic media of

Table 6
Effect of ionic strength (μ) on gelation of pigeon pea protein concentrate pH 7

Flour concetration	μ = 0.5		μ = 1.0	
(%)	Gelation	Appearance	Gelation	Appearance
2	–	Viscous	–	Viscous
4	+	Gel (LCE)	–	Viscous
6	+	Gel	–	Viscous
8	+	Gel	+	Gel (LCE)
10	+	Firm gel	+	Gel
12	+	Firm gel	+	Firm gel
14	+	Firm gel	+	Solid gel
16	+	Solid gel	+	Solid gel
18	+	Very solid gel	+	Very solid gel
20	+	Very solid gel	+	Very solid gel

Table 7
Effect of pH on gelation of pigeon pea protein concentrates

Flour concentration (%)	pH 2		pH 4		pH 6		pH 8	
	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance
2	–	Viscous	–	Liquid	–	Viscous	–	Liquid
4	+	Gel (LCE)	–	Liquid	–	Viscous	–	Liquid
6	+	Gel	–	Viscous	+	Gel (LCE)	–	Liquid
8	+	Firm gel	–	Curdy	+	Gel	–	Viscous
10	+	Firm gel	+	Gel (LCE)	+	Firm gel	–	Viscous
12	+	Solid gel	+	Gel	+	Firm gel	+	Gel (LCE)
14	+	Solid gel	+	Firm gel	+	Very firm gel	+	Gel
16	+	Very solid gel	+	Firm gel	+	Very firm gel	+	Firm gel
18	+	Very solid gel	+	Very firm gel	+	Solid gel	+	Firm gel
20	+	Very solid gel	+	Very firm gel	+	Solid gel	+	Very firm gel

0.5 and 1 strengths, respectively. These results agree well with those of Catsimpoolas and Meyer (1970) for soybean globulins.

Table 7, showing the effect of pH on gelation, indicates that pH 2 and 6 enhanced the gelating ability of the PPPC while alkaline pH decreased the gelating ability.

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