EFFECTS OF pH AND SODIUM CHLORIDE CONCENTRATION ON THE FUNCTIONAL PROPERTIES OF LOCUST BEAN PULP FLOUR

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P.I. Akubor* and E.O. Adedeji

Department of Food Science & Technilogy, Federal University Wukari, PMB 1020, Taraba State, Nigeria

*Corresponding author: akuborpeter@gmail.com

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Abstract: The effects of pH and NaCl concentration on the functional properties of African locust bean pulp flour were investigated. Flour was prepared from locust bean pulp. The pH of the flour was adjusted with HCl or NaOH solution from 2 - 12. The NaCl solution was added to the flour from 0.1 to 1.0M. The functional properties of the flour samples were evaluated. The locust bean pulp flour had minimum nitrogen solubility of 3% at pH 4.0. At pH 2.0, the nitrogen solubility was 7 % and increased to 10 % at pH 8 and thereafter, decreased. The locust bean pulp flour had maximum water absorption capacity of 321% at pH 2.0 and minimum of 292% at pH 12. The oil absorption capacity increased from 257% in water to 262% at pH 2.0; dropped to 250% at pH 4.0 and increased to maximum 270% at pH 12. The locust bean pulp flour had minimum foaming capacity of 7.5% at pH 4.0 and maximum of 22% at pH 8.0. The addition NaCl up to 0.2 M enhanced the nitrogen solubility, foaming capacity and foam stability of locust bean pulp flour but these parameters decreased at concentrations above 0.2M. The addition of NaCl up to 0.3 M improved the water absorption capacity and oil absorption capacity of locust bean pulp flour. The addition of up to 0.4M increased the emulsion activity and emulsion stability of locust bean pulp flour. Sodium chloride improved the emulsion activity in the pH range of 2-8. At any level of flour concentration, the flour gelled at 0.2M NaCl concentration. At 0.4 and 0.6 M NaCl concentrations, the least gelation concentrations were 8 and 16%, respectively. The locust bean pulp flour gelled at 16% and above at any NaCl concentration.

Keywords: Functional properties, locust bean pulp, sodium chloride, pH, interaction effect

Introduction

African locust bean (*Parkia biglobosa*) tree grows widely in many parts of the Sahel, particularly, the drier parts of West Africa (Akoma *et al*., 2001). In Nigeria, the tree grows in the wild throughout the savanna from Guinea through Sudan to Sahel (Addy *et al*., 1995). The tree produces 25-52 kg pods (Addy *et al*., 1995). In Northern Nigeria alone, about 200,000 tons of locust bean pods are produced yearly (Addy *et al*., 1995). Similarly, large quantities of the pods are produced in Oyo and Kwara states of Nigeria (Addy *et al*., 1995). A mature locust bean pod contains yellow dry and powdery pulp in which dark brown seeds are embedded. The pulp is rich in carbohydrates, minerals, vitamins and essential phytochemicals such as flavonoids, carotenoids, polyphenols, saponinsetc (FAO, 1988). The pulp is usually licked for its sweet taste but only to a small extent. The pulp is usually washed away when the seeds are processed into condiment called *dawadawa* or *iru*. This condiment serves as source of protein intake among the low income groups and rural populations of West Africa. The locust bean seed has been extensively studied (Omafuvbe *et al*., 2004). However, little has been done on the utilization of the pulp except for its use in making local dishes and drinks (Akoma *et al*., 2001). In West Africa, the pulp is prepared into flour and used in soups and stews or eaten with cereals as porridge (FAO, 1988).

Due to its high contents of phytochemicals, vitamins and minerals (FAO, 1988), locust bean pulp could be processed into flour and potentially used in similar ways to efforts reported on other flours. However, novels and food ingredients such as locust bean pulp flour must be functionally reliable to be acceptable for use. Functionality is any property of food or food ingredient other than the nutritional ones that affects its utilization (Pomeranz, 1991). Quality attributes of developed food products are generally affected by the functional properties of the flour (Akubor, 2016). Even though the methods used in the measurement of functional properties are open to various criticisms on the basis of rigor and trial error (Onimawo & Akubor, 2012), functional properties provide guide for use of flours in different food formulations. Functional properties are linked to carbohydrate, fat and protein constituents of foods (Ocheme *et al*., 2015). They are influenced by food particle size, protein shape, pH, salt concentration, temperature and storage (Onimawo & Akubor, 2012). Therefore, studies to characterize the effects of pH and sodium chloride concentration on the functional properties of locust bean pulp flour are needed. This would provide useful information for processors wishing to use the flour for specific food formulations. Therefore, the objectives of this study were to determine the effects of pH and sodium chloride concentration on some functional properties of locust bean pulp flour and to evaluate interactions between the factors.

Materials and methods

Preparation of locust bean pulp flour

Mature and ripe African locust bean (*Parkia biglobosa*) fruit pods were plucked from locust bean trees in a local farm in Ugwaka –Ollah Township, Kogi State, Nigeria. The locust bean pods (20 kg) were sorted, cleaned of extraneous materials and split open manually. The yellow pulps along with the attached seeds were removed from the hulls, sun dried at 30±2^{o}C for 48 h and pounded lightly in a mortar with a pestle. The pulp pieces were separated from the seeds, milled in a hammer mill and sieved through 60 mesh sieve (0.05 mm) (British standard). The pulp flour was packed in high density polyethylene bags and stored in a refrigerator (10±2^{o}C) until required.

Evaluation of functional properties

The Water and oil absorption capacities were determined as function of pH and NaCl concentration as described by Okaka & Potter (1977). The pH of the water or oil added to the flour was adjusted with 1M HCl or 1M NaOH. The emulsion activity and emulsion stability were determined using the methods of Onimawo & Akubor (2012). The pH (2-14) of the distilled water used in preparing the emulsion was adjusted with 1M HCl or 1M NaOH. To determine the effect of NaCl concentration on emulsion capacity and emulsion stability, flour samples were prepared in distilled water containing 0.2-1M NaCl concentrations. No adjustment of pH was made in NaCl samples. The foaming capacity and foam stability were measured as function of pH and NaCl concentration by the methods of Sathe *et al*. (1982). The volume of foam at 30 sec of whipping was expressed as foaming capacity (FC). The volume of foam was recorded one hour after whipping to determine foam stability (FS) as percent of the initial foam volume. The least gelation concentration was determined as function of pH and NaClconcentration as described by Kinsella (1981) with slight modification (Badifu & Akubor, 2001). The pH of the distilled water used in preparing the flour dispersions was adjusted with 1 M HCl or 1M NaOH.

Statistical analysis

Data were subjected to analysis of variance in completely randomized design using Statistical Package for Social Sciences (SPSS) software (version 15, 2007). Means where significantly different were separated by the least significant difference (LSD) test (Steel & Torrie, 1980). Significance was accepted at $p< 0.05$.

Results and Discussion

Effect of pH on functional properties

The effect of pH on nitrogen solubility of locust bean pulp flour (LBPF) is shown in Fig. 1. The locust bean pulp flour had minimum nitrogen solubility of 3% at pH 4.0, with values increasing at above or below this pH value. At pH 2.0, above 7% of the nitrogen was soluble while at pH 8.0, solubility increased to 10%. Beyond pH 8.0, there was significant ($p<0.05$) drop in nitrogen solubility. This solubility behavior was similar to that reported for soy flour (McWatters & Holmes, 1977). Below and above the isoelectric point, proteins have positive or negative charge which enhances solubility (Kinsella, 1981). At the isoelectric point, the charge is zero, the attractive forces predominate and the molecules tend to associate (Onimawo & Akubor, 2012). This perhaps, explains the relatively minimal solubility of the locust bean pulp flour at certain pH values. Foods with proteins which are soluble at pH 4-8 could be used in beverages such as vegetable milk (McWatters & Holmes, 1977). This result showed that the proteins of locust bean pulp flour were not very soluble. Protein solubility characteristics are influenced by origin, processing conditions, pH, ionic strength and presence of other ingredients (Kinsella, 1981). In this study, the functional attributes of locust bean pulp flour such as emulsion and foaming (Table 1) that could be adversely affected by low protein solubility were, indeed found to be low.

The effects of pH on some other functional properties of locust bean pulp flour are shown in Table 1. The locust bean pulp flour had maximum water absorption capacity of 321% at pH 2.0. Beyond pH 2.0, water absorption capacity decreased steadily. The water absorption capacity of locust bean pulp flour at pH 2.0 was higher than that in water (318%). At pH 2.0, the locust bean pulp flour protein may have been denatured (unfolding of the protein molecules) by the acidic medium. This might have increased accessibility of locust bean pulp flour to water. The oil absorption capacity increased from 257% in water to 262% at pH 2 and dropped to 250% at pH 4. Thereafter, the oil absorption capacity increased to a maximum of 270% at pH 12. The locust bean pulp flour had minimum oil absorption capacity at PH 4.0 which was the point of minimum nitrogen solubility of the flour (Fig. 1).

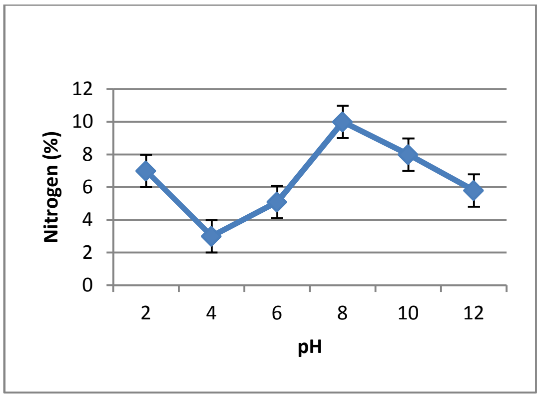

Fig. 1: Nitrogen solubility profile of locust bean pulp flour

The emulsion activity of locust bean pulp flour was 18% at pH 2.0 and 12% at pH 4.0 (point of minimum nitrogen solubility). With progressive increase in pH in the alkaline region, emulsion activity increased to maximum value of 24% at pH 12.0. At pH 4.0, the locust bean pulp flour protein was slightly soluble and would not contribute to the repulsive surface charge in oil droplets which are necessary for emulsion formation (Chavan *et al*., 2007). Altering the electrical charge of the protein molecules probably caused structural rearrangement which may have increased the number of potential binding reactive sites in locust bean pulp flour (Kinsella, 1981).The emulsion stability of locust bean pulp flour exhibited similar trend to emulsion activity except that maximum emulsion stability was at pH 4. The maximum emulsion stability at pH 4.0 could be due to the native conformation of locust bean pulp flour protein at pH 4.0 (point of minimum nitrogen solubility) (Pomeranz, 1991).

Generally, there was improved FC in the acid and alkaline regions. A minimum foaming capacity of 7.5% was obtained at pH 4.0 and maximum of 22% at pH 8.0. The foaming capacity at pH 4.0 may be due to decreased protein solubility. Similar observations were reported for lupin seed proteins (Sathe *et al*., 1982), African oil bean flour (Akubor&Chukwu, 1999) and African breadfruit kernel flour (Badifu & Akubor, 2001). The improved foaming capacity at pH 2.0 and pH 8.0 could be explained by the higher solubility of locust bean pulp flour protein at these regions (Fig. 1). Native protein is associated with higher FS than denatured proteins (Alobo, 2003). At pH 4.0, the locust bean pulp flour proteins would carry no charge and would be more stable than at other pH values where repulsion would decrease the stability (Kinsella, 1981). The foam stability and emulsion stability would therefore, be expected to be maximum at pH 4.0

Table 1: Effect of pH on some functional properties of Locust Bean Pulp Flour

pH	Properties					
	WAC (g/g)	OAC (g/g)	EA (%)	ES (%)	FC (%)	FS (%)
0	3.18^{a}	2.57^{a}	14.3^{f}	13^{g}	8.5^{f}	61.9^{b}
2	3.21^{a}	2.62^{a}	18^{d}	15^{f}	13^{d}	55^{b}
4	3.15^{a}	2.50^{a}	12^{g}	50^{a}	7.5^{g}	70^{a}
6	3.13^{a}	2.54^{a}	17^{a}	22^{e}	10^{e}	56^{d}
8	3.05^{a}	2.59^{a}	20^{c}	25^{b}	22^{a}	52^{f}
10	3.00^{a}	2.61^{a}	21^{b}	38^{c}	14^{c}	55^{e}
12	2.92^{a}	2.70^{a}	24^{a}	40^{b}	18^{b}	40^{g}

Values are means of 3 replications. Means within a column with the same superscript were not significantly different ($p > 0.05$). WAC = Water absorption capacity, OAC = Oil absorption capacity, EA = Emulsion activity, FC = Foaming Capacity, FS = Foam stability

Table 2: Effect of sodium chloride concentration on the functional properties of Locust Bean Pulp flour (LBPF)

NaCl Conc. (Mol^{-1})	Properties						
	NS (%)	WAC (g/g)	OAC (g/g)	EA (%)	ES (%)	FC (%)	FS (%)
0.0	10^{e}	3.18^{a}	2.57^{a}	14.3^{g}	13^{i}	8.3^{g}	61.9^{b}
0.1	12^{c}	3.25^{a}	2.70^{a}	16.4^{f}	18^{g}	10.0^{c}	52^{d}
0.2	15^{a}	3.40^{a}	2.85^{a}	19.1^{de}	25^{d}	14.0^{a}	71^{a}
0.3	13^{b}	3.80^{a}	2.94^{a}	22.0^{b}	38^{b}	11.0^{b}	54^{c}
0.4	11^{d}	3.40^{a}	2.75^{a}	25.0^{a}	40^{a}	8.5^{d}	50^{e}
0.5	9^{f}	3.10^{a}	2.60^{a}	21.0^{c}	27.5^{c}	7.5^{c}	41^{f}
0.6	8^{g}	3.00^{ab}	2.50^{a}	20.0^{d}	24.1^{k}	6.0^{f}	35.5^{g}
0.7	6^{h}	2.98^{b}	2.42^{a}	18.2^{e}	21.0^{e}	4.0^{g}	30.00^{h}
0.8	4^{i}	2.90^{b}	2.40^{a}	17.5^{e}	20.0^{f}	3.5^{g}	28.0^{i}
0.9	3^{j}	2.85^{b}	2.35^{a}	14.1^{b}	12.0^{h}	2.5^{h}	24.0^{j}
1.0	2^{k}	2.75^{b}	2.31^{a}	12.5^{h}	9.0^{j}	2.0^{h}	20.0^{k}

Values are means of 3 replications. Means within a column with the same superscript were not significantly different ($p > 0.05$). NS = Nitrogen solubility. Abbreviations as defined in Table 1

Effect of NaClon functional properties

The effects of NaCl concentrations on the functional properties of locust bean pulp flourare shown in Table 2. The addition of NaClup to 0.2M enhanced the nitrogen solubility of locust bean pulp flour but decreased the nitrogen solubility at NaCl concentrations above 0.2M. Low concentrations of NaClenhanced protein solubility (salting- in effect) while high NaCl concentrations decreased it (salting-out effect) (Oshodi & Asa, 1993). The addition of NaCl up to 0.3M improved the water absorption capacity and oil absorption capacity of locust bean pulp flour. At concentrations above 0.3M, there were concomitant decreases in water absorption and oil absorption capacity. Similar results were reported for pigeon pea flour (Oshodi & Asa, 1993). Chloride ions are bound to the protein which increased the net charge of the molecules (Adeunwa *et al*., 2012). This results in unfolding of the protein network (denaturation) which in turn leads to increased accessibility to water. The enhancement of water absorption capacity by low levels of NaCl would be added advantage in the use of locust bean pulp flour for the preparation of breads and cakes (Akubor, 2016). The addition of up to 0.4M NaCl increased the emulsion activity and emulsion stability of locust bean pulp flour. Concentrations above 0.4M decreased the emulsion activity and emulsion stability. The beneficial effects of low concentration NaCl on emulsions have been reported for African breadfruit kernel flour (Badifu & Akubor, 2001). The increases in emulsion activity and emulsion stability by low concentration of NaCl were probably due to salting-in effect of NaCl on proteins. Salting –in effect is a biochemical process whereby proteins (amino acids) are extracted from a product with high salt concentration due to denaturation of proteins by precipitating the proteins (Kinsella, 1981). At NaCl concentrations above 0.4M, the emulsion activity and emulsion decreased as there was likely to be salting- out of the proteins (Kinsella, 1981). In salting-out, the proteins are extracted with low salt concentration which does not denture the protein. The proteins are not precipitated but remained in solution (Onimawo & Akubor, 2012). The high emulsion activity and emulsion stability of LBPF at low NaCl concentration is desirable in nutrition. For instance, hypertension is linked to high level of NaCl in foods. Addition of NaCl up to 0.2M concentration increased the foaming capacity and foam stability of LBPF but greater concentrations of NaCl decreased them. Similar result was reported for orange seedflour (Apata & Akubor, 1999). The foaming capacity and foam stability of LBPF were higher at 0.2M than in water. The beneficial effects of low concentrations of NaCl on foaming capacity of soy flour and fish protein concentrate were reported (Kinsella, 1981). Low concentrations of NaCl enhanced the LBPF protein solubility whereas high concentrations decreased it (Table1). Since foaming capacity appears to be due to solubilized proteins (Chnma *et al*., 2012), the differing effects of salt concentrations may be explained on this basis.

Effect of flour and NaCl concentration on least gelation concentration

The effect of NaCl concentration on least gelation is shown in Table 3. At 0.2M NaCl concentration, for any level of flour used, the locust bean pulp flour gelled. However, at 0.4 and 0.6M NaCl concentrations, the least gelation concentrations were 6 and 14%, respectively. The locust bean pulp flour only gelled at higher flour concentrations at above 0.2M NaCl. The improved gelation capacity of locust bean pulp flour at 0.2M NaCl concentration indicated by a lower least gelation concentration of 2% (w/v) could be due to higher solubilization of LBPF protein by the salt solution whichcaused over lapping of the functional groups between adjacent protein molecules. This condition is necessary for network or gel formation (Badifu & Akubor, 2001). The least gelation concentration of locust bean pulp flour increased at higher NaClconcentrations. It has been suggested that hydrogen and ionic bonds are responsible for the stabilization of gel and that the addition of NaCl will decrease the viscosity of the gel if the NaClconcentration is high enough to neutralize the charges establishing the gel (Oshodi & Asa, 1993). Thus, NaCl at above 0.2 was able to neutralize the charges establishing gel formation in LBPF and decreased the viscosity which leads to higher least gelation concentration. This implied that in LBPF based food systems where high gelling and thickening is desirable, it is unnecessary to add high amount of salt. However, if high salt concentration is necessary, it is advisable to increase the locust bean pulp flour concentration.

Table 3: Effect of NaCl concentration on the least gelation concentration of locust bean pulp flour

Flour conc. (%,w/v)	Least gelation conc. (5,w/v) NaCl conc. (Mol^{-1})			
	0	0.2	0.4	0.6
2	-	+	-	-
4	-	+	-	-
6	-	+	+	-
8	-	+	+	-
10	-	+	+	-
12	+	+	+	-
14	+	+	+	+
16	+	+	+	+

+ = Gelled, – =Not gelled

Table 4: Effect of interaction of pH and NaCl concentration on the emulsion activity of Locust Bean Pulp flour

NaCl conc. (Mol^{-1})	pH						
	0	2	4	6	8	10	12
0.0	14.3^{d}	8^{d}	12^{b}	17^{a}	20^{a}	21^{a}	24^{a}
0.2	19.1^{b}	16^{b}	14^{a}	13^{b}	11^{b}	10^{b}	10^{b}
0.4	25.0^{a}	15^{b}	11^{c}	12^{c}	10^{bc}	10^{b}	9^{c}
0.6	20.0^{b}	12^{c}	10^{d}	12^{c}	9^{c}	8^{c}	7^{d}
0.8	17.5^{c}	19^{a}	9^{e}	8^{d}	7^{d}	6^{d}	4^{e}
1.0	12.5^{e}	9^{d}	8^{f}	6^{e}	5^{e}	6^{d}	4^{e}
$Lsd_{0.05}$	0.90	1.28	0.89	0.90	1.08	1.0	0.94

Values are means of 3 replicates. Means within a column with the same superscript were not significantly different ($p > 0.05$). Least significant difference (Lsd)

Interaction of pH and NaCl concentration on emulsion activity

Sodium chloride improved emulsion activity of locust bean pulp flour in the pH range of 2-8 (Table 4). Beyond this range, the emulsion activity decreased. The emulsion activity at pH values of 2, 10 and 12 decreased with NaClconcentrations.

Conclusion

Locust bean pulp flour has potential for use in various food products. However, the functional properties of locust bean pulp flour are affected by pH and sodium chloride concentration. Sodium chloride at high concentrations (>0.4M) decreased the water and oil absorption, emulsion and foaming properties of locust bean pulp flour. These properties may limit the use of locust bean pulp flour to low sodium chloride food systems. Further studies are in progress in our laboratory to determine the acceptability of food products supplemented with locust bean pulp flour.

Conflict of Interest

Researchers hereby declare that there is no conflict of interest whatsoever in this research.

References

Adegunwa MO, BakareHA & Akinola OF 2012. Processing effects on chemical functional and pasting properties of cowpea flour from different varieties. *Nig Food J.*, 30(1): 67-73.

Addy EOU, Salami CI, IgboeliLC & Emawa HS 1995. Effect of processing on nutrient composition and antinutritive substances of African locust bean (*Parkia filicoidea*) and baoba seed (*Adansonia digitata*). *Plant Foods Hum Nutr.,* 48(1): 13-17.

Akoma OO, Onuoha JA, Akoma AO & Zigis AA 2001. Physicochemical attributes of wine produced from the yellow pulp of *Parkia biglobosa* using traditional juice extraction techniques. *Nig Food J.,* 19: 76-79.

Akubor PI 2016. Chemical composition, functional and pasting properties of cashew pomace and wheat flours. *Intern J Agric and Veter Sci.,* 2(1): 28-37.

Akubor PI & Chukwu JK1999. Proximate composition and selected functional properties of fermented and unfermented oil bean (*Pentaclathramacrophylla*) seed flour. *Plant Foods Hum Nutr*., 54: 227-238

Alobo AP 2003. Proximate composition and selected functional properties of defatted papaya (*Carica p* Papaya) kernel flour. *Plant Foods Hum Nutr*., 58: 1-7.

Apata MI & Akubor P 199. Chemical composition and selected functional properties of sweet orange (C*itrus sennsis*) seed flour. *Plant Foods Hum Nutr*., 49: 213-219.

Badifu GIO & Akubor PI 2001. Influence of pH and sodium chloride on selected functional and physical properties of African breadfruit (*Treculia Africana* Decne) kernel flour. *Plant Foods Hum Nutr*., 56: 105-115.

Chavan UD, Mekenzie DB & Shachidi F 2007. Functional properties of protein isolates from black pea (*Lathyrus maritimu*). *Food Chem*., 20:177-187.

Chinma CC, Abu JO, James S & Iheanocho M 2012. Chemical, functional and pasting properties of defatted starches from cowpea and sorghum and application in stiff porridge preparation. *Nig Food J.*, 30(2): 80-88

FAO 1988. *Traditional Food Plants.* FAO, Food and Nutrition Paper No. 42, pp. 388-393.

Kinsella JE 1981. Functional properties of protein: Possible relationship between structure and function in foods. *Food Chem*., 7: 273-288.

Mcwatters KH & Holmes MR 1977. Influence of pH and salt concentration on nitrogen solubility and emulsion properties. *J. Food Sci*., 770-774.

Ocheme OB, Adedeji OE, LawalG & Zakari UM 2015. Effect ofgermination on functional properties and degree of starch gelatinization of sorgum flour. *J. Food Res*., 4(2): 159-165.

Okaka JC & Potter NN 1977. Functional properties of cowpea/wheat flour blend in bread making. *J. Food Sci*., 42: 828-833.

Omauvbe BO, Falade SO, Osuntogun AB, & Adewusi SRA 2004. Chemical and biochemical changes on African locust bean (*Parkia biglobosa*) and melon (*Citrillus vulgaris*) seeds during fermentation to condiments. *Pak. J. Nutr*., 3(3): 140-145.

Onimawo IA & Akubor PI 2012. *Food Chemistry* (Integrated Approach with Biochemical Background). 2ndedn. Joytal Printing Press, Agbowo, Ibadan, Nigeria, pp. 158-298.

Oshodi AA & Asa, MO 1993 . Effects of some salts on water absorption, gelation, foaming capacity/stability and protein solubility of pigeon pea (*Cajanus cajan*) flour. *Nig. J. Sci*., 27: 1-8.

Pomeranz Y 1991. Fuctional properties of food components.1stedn.Academic press Ltd, London, pp. 116-121.

Steel RGD & Torrie JH 1980. *Principles and Procedures of Statistics*. A Biometric Approach, 2nd edn. Auckland, New Zealland, MigrawHill, pp. 345-57.

Sathe SK, Desphande SS & Salunkle DK 1982. Functional properties of winged bean proteins. *J. Food Sci*., 47: 503-506.