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Food Chemistry 84 (2004) 207–212

Food Chemistry

www.elsevier.com/locate/foodchem

Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration

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Received 18 February 2003; accepted 1 May 2003

Abstract

The content, fractionation, solubility and functional properties of cowpea proteins were determined. The effects of pH and/or NaCl concentration on some of these functional properties were also investigated. The protein content of the seed was found to be 26.8%. Albumin is the major fraction of cowpea seed proteins. The minimal protein solubility was observed at pHs 4, 5 and 6, and the maximum was at pH 10. The emulsifying capacity, activity and emulsion stability, as well as foaming capacity and foam stability, were greatly affected by pH levels and salt concentrations. Lower values were observed at a slightly acidic pH and high salt concentration. The least gelation concentration of cowpea proteins was found to be 6% when the proteins were dissolved in 0.5 or 1.0 M NaCl. The total protein was highly viscous and dispersable with a water holding capacity of 2.20 ml H_2O/g protein, oil-holding capacity of 1.10 ml oil/g protein and bulk density of 0.82 g/ml, while dispensability was found to be 72.0%.

Keywords: Cowpea; Fractions; Total proteins; Solubility; Functional properties

1. Introduction

In developing countries, Plant proteins play significant roles in human nutrition where average protein intake is less than that required. Due to inadequate supplies of food proteins, there has been a constant search for unconventional legumes as new protein sources for use as both functional food ingredients and nutritional supplements (Onweluzo, Obanu, & Onuoha, 1994). Cowpea [*Vigna unguiculata* (L.) Walp] is indigenous to Africa and in Sudan it is usually interplanted with cereals as a mixed crop. High protein (18–35%) and carbohydrate (50–60%) contents, together with an amino acid pattern complementary to that of cereal grains, however, make cowpea a potentially important nutritional component in the human diet (Prinyawiwatkul, McWatters, Beuchat, & Phillips, 1996). Plant protein products, such as cowpea proteins, are gaining interest as ingredients in food systems throughout many parts of the world; the final success of utilizing plant proteins as additives depends greatly upon the favourable characteristics that they impart to foods. Therefore, the relationship of protein quality with processing parameters that affect the functional performance of protein products is worthy of extensive investigation (Jane, Rivas, & John, 1981). Solubility of a protein is one of the critical functional attributes required for its use as a food ingredient, because solubility greatly influences other properties, such as emulsification, gelation and foaming (Wang & Kinsella, 1976). Thus it determines the behaviour of a protein food product. For plant proteins to be useful and successful in food application they should ideally possess several desirable characteristics, referred to as functional properties, as well as providing essential amino acids (Wang & Kinsella, 1976). These properties are intrinsic physicochemical characteristics, which affect the behaviour of proteins in food system during processing, manufacturing, storage and preparation (Kinsella, 1979). Proteins have unique surface properties due to their large molecular size and their amphiphilic properties. However, the industrial applications of food proteins are limited, because proteins are generally unstable to heating, organic solvents and proteolytic attack (Sakamoto, Kumazawa, & Motoki, 1994). Therefore, if

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doi:10.1016/S0308-8146(03)00203-6

proteins could be converted into stable forms, their applications would be greatly broadened. Attempts have been made to modify plant proteins to improve their physical functionality, i.e. gelation, viscosity, emulsification and foaming (Sakamoto et al., 1994). Several molecular parameters, such as mass, conformation, flexibility, net charge and hydrophobicity, as well as interaction with other food components have already been shown to play an important part, in both their emulsifying and foaming properties (Nakai & Voutsinas, 1983). However, most chemical modifications are not applicable for the food industry. It has been reported that cowpea contains substantial quantities of good quality proteins with additional advantages of fibre, flavour and functional properties (Prinyawiwatkul, 1996). The objective of this study was to investigate the effect of pH and/or salt concentration on solubility and functional properties of cowpea proteins and to predict their compatibility prior to modification in different food systems.

2. Materials and methods

2.1. Materials

Cowpea [*Vigna unguiculata* (L.) Walp] seeds were obtained from the local market (elObeid, Sudan). The seeds were sun-dried, carefully cleaned and ground to a powder (0.4 mm screen). Groundnut and corn oils were obtained from Bittar Co. Ltd., Khartoum, Sudan. Unless otherwise stated, all reagents used in this study were of reagent grade.

2.2. Methods

2.2.1. Isolation of cowpea protein

Cowpea protein was isolated using the method of Prakash and Nandi (1978). The seeds were ground twice to pass a 0.4 mm screen. Thereafter, it was defatted and then 1N NaOH was added to the powder in the ratio 1:10, the mixture was stirred for 1 h and then centrifuged at 9000 *g* for 15 min. The pH of the supernatant was adjusted to 4.0 using 2N HCl and heated to 90 °C for 10 min. The heated liquor was cooled to room temperature and centrifuged at 9000 *g* for 15 min. The residue was dissolved in water and dialyzed against distilled water for 24 h at 4 °C and then freeze-dried.

2.2.2. Protein content

Nitrogen content of the defatted sample was determined by the micro-Kjeldahl technique, following the method of the AOAC (1980). Protein content of the sample was calculated by multiplying the nitrogen content by a factor of 6.25.

2.2.3. Protein fractionation

Protein fractions were extracted according to their solubilities in different solvents, as described by Landry and Moureaux (1970). Defatted cowpea flour (3.5 g) was extracted twice with 50 ml distilled water for 30 min at room temperature. The extract was centrifuged at 3000 *g* for 30 min and the supernatant was used for the determination of a water-soluble protein (albumin). The residue was then extracted successively in a similar manner with 1.0 M NaCl, 70% ethanol or 0.2% NaOH. The supernatant of each extract was collected separately and used to estimate the salt- (globulin), alcohol- (prolamin) or alkali- (glutelin) soluble fraction. The residue remaining after successive extractions represents the insoluble proteins.

2.2.4. Nitrogen solubility

Nitrogen solubilities of the proteins at 2% (w/v) in distilled water or 0.5 M NaCl were determined by the method of Beuchat, Cherry, and Quinn (1975) over a pH range from 1.0 to 11.0. The dispersions were stirred at different pHs at 24 °C for 45 min and then centrifuged at 5000 *g* for 15 min. Nitrogen contents of the supernatants were determined by the method of the AOAC (1980). Nitrogen solubility was expressed as percent of the nitrogen content of the sample.

2.2.5. Gelation capacity

Gelation capacity of cowpea proteins was determined according to the method of Coffmann and Garcia (1977) with a slight modification. Sample suspensions of 1–20 g/100 ml were prepared in 5 ml distilled water or 0.5 and 1.0 M NaCl. The test tubes containing these suspensions were then heated for 1 h in a boiling water bath, followed by rapid cooling under running cold tap water. The test tubes were then further cooled for 2 h at 4 °C. The least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall or slip.

2.2.6. Water- and oil-holding capacity

The method of Carcea Benecini (1986) was used with a slight modification. One gramme of protein samples was stirred in 10 ml of distilled water or corn oil and then centrifuged at 2200 *g* for 30 min. The volume of the supernatant was measured. The water-holding capacity is expressed as the number of g of water held by 1.0 g of protein sample. The oil-holding capacity is expressed as the number of g of oil held by 1.0 g of protein sample. Density of the oil was found to be 0.92 g/ml.

2.2.7. Apparent viscosity

Apparent viscosity of a 20% (w/v) of the proteins was determined at 25 °C using the method of Quinn and Beuchat (1975). Two samples at different temperature (25 and 70 °C) were tested. The apparent viscosity was

determined with a Brookfield (Model RVT) viscometer equipped with a No. 1 spindle. Apparent viscosity in centipoises (cps) was reported as the average of three readings.

2.2.8. Emulsion measurements

Emulsification capacity was determined according to the procedure of Beuchat, Cherry, and Quinn (1975) and is expressed as millilitres of oil emulsified per g of protein. Emulsion stability, at different pH, was determined according to the method of Pearce and Kinsella (1978).

2.2.9. Foam measurements

Foam capacity and stability at different pH levels or NaCl concentration were determined according to the Aruna and Prakash method (1993). One hundred millilitres of distilled water at different pH or NaCl, of different concentrations, were separately added to 2 g of defatted cowpea protein isolate and the mixture was homogenized at 300 rpm for 5 min in a Virtis homogenizer at 27 °C and transferred to a measuring cylinder. The volume of foam at 30 s was calculated, and the volume increase is expressed as percent foam capacity. The foam stability was determined by measuring the decrease in volume of foam as a function of time up to a period of 120 min.

2.2.10. Bulk density

The bulk density was determined according to Wang and Kinsella (1976), using samples of 10 g and a 25 ml graduated cylinder. Bulk density was calculated as g/ml.

2.2.11. Dispersibility

The dispersibility of cowpea protein isolate was measured according to the method of Karuna, Kulcarni, and Ingle (1991).

3. Results and discussion

3.1. Protein content, fractionation and solubility

The protein content of cowpea was found to be 26.8% with a moisture content of 8.9%. Fig. 1 shows cowpea protein (CP) fractions on the basis of solubility. CP were fractionated, on the basis of solubility, into albumin (71.4%), globulin (11.1%), prolamin (2.20%) and glutelin (11.0%). The results obtained indicated that about 95.7% of the total protein could be extracted by solvents and the remaining percentage accounted for the non-protein nitrogen and insoluble proteins. Bhattacharya, Bal, and Mukherjee (1994) obtained similar results from tamarind kernel by using different solvents (H_2O, NaCl, ethanol or NaOH). A confirmatory result, obtained by Hamada (1997), showed that defatted rice

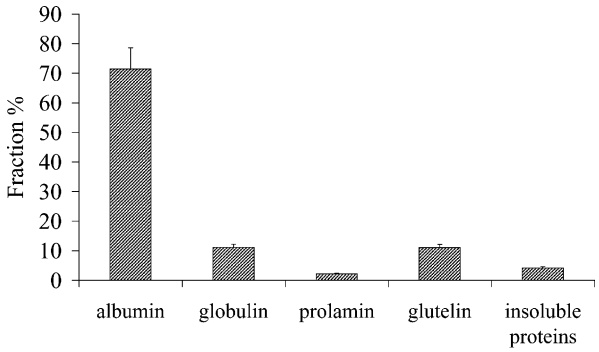

Fig. 1. Fraction percentages on the basis of solubility of cowpea proteins.

bran was fractionated according to solubility into different fractions, i.e. albumin, globulin, prolamin, and glutelin. Fig. 2 shows the variations in nitrogen solubility at different pH levels of cowpea protein isolate (CPI). The minimum nitrogen solubility of CPI was 5% at pHs 4 and 5 (Fig. 2). However, after addition of 0.5 M NaCl, the protein solubility at the same pH level was increased to 18% (data not shown). On either side of pHs 4 and 5, there was a sharp increase in the solubility of the total proteins. At pH 3, about 82.0% of the nitrogen was soluble; without addition of 0.5 M NaCl it was about 44%, while at pH 10 about 96.0% of CPI was soluble. Total protein isolate studied showed good solubility at alkaline and acidic pH and the presence of salts enhanced solubility only at the isoelectric pH, which is an important characteristic for food formulations (Idouraine, Yensen, & Weber, 1991). Prakash and Narasinga (1986) reported similar observations.

3.2. Functional properties of cowpea proteins isolate (CPI)

3.2.1. Water- and oil-holding capacity

CPI had a water-holding capacity of 2.20 ml H_2O/g protein (Table 1) that is similar to that reported by Prakash and Narasinga (1986) and within the range of the commercial values of protein concentrates (1.90–2.20), as reported by Lin and Zayas (1987). This is likely due to the fact that the CPI had great ability to swell because it contained proteins and crude fibre as

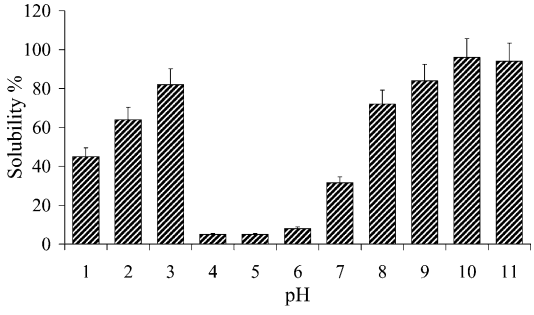

Fig. 2. Effect of pH levels on solubility of cowpea protein isolate.

Table 1
Some functional properties of cowpea proteins isolate

Functional properties	Value[a] (±SD)
Emulsifying activity (%)	50.00 (±1.21)
Dispersibility (%)	72.00 (±1.91)
Viscosity (cps) at 20 °C	3.90 (±0.43)
at 70 °C	8.00 (±1.20)
Water holding capacity (ml/g)	2.20 (±0.09)
Oil holding capacity (ml/g)	1.10 (±0.08)
Bulk density (g/ml)	0.82 (±0.04)

[a] Values are means of triplicate samples.

major components, which could be responsible for the increased water-holding capacity (Kinsella, 1979). The oil-holding capacity of CPI was 1.10 ml oil/g protein (Table 1). CPI showed a lower oil-holding capacity than soybean (Kinsella, 1979) chickpea flour (Marina, 1986). Kinsella (1979) explained the mechanism of fat absorption as a physical entrapment of oil and several authors have related the oil absorption capacity to the nonpolar side chains of the protein as well as to the different conformational features of the proteins. Our results suggested that CPI had both good water- and good oil-holding capacity.

3.2.2. Viscosity and dispersibility

Cowpea protein isolate (CPI) had a viscosity of 3.90 cps at room temperature. Heating CPI at 70 °C for 15 min resulted in an appreciable increase in viscosity to 8.00 cps. It has been reported that heating of aqueous proteins activated the protein solution to a progel state, which is characterized by a marked increase in apparent viscosity. Moreover, further increase in apparent viscosity was observed after cooling the progel (Rivero & Pauda, 1983). The present study indicated that, temperature greatly affected the apparent viscosity of the proteins, which is likely to affect the conformational characteristics of the proteins since viscosity is conformation dependent (Idouraine et al., 1991). The reconstitution property of CPI, in terms of dispersibility, was found to be 72.0% (Table 1). It was reported that higher dispersibility enhances the emulsifying and faoming properties of proteins, which was observed during making of bread, macaroni and cookies (Kinsella, 1979).

3.3. Gelation capacity

Gelation capacity of CPI is shown in Table 2. When CPI was only dissolved in water and tested for gelation capacity, it was found to be unable to form a gel, even after cooling. On the other hand, addition of 0.5 or 1.0 M NaCl to CPI at a concentration of 6% (w/v) had a profound effect on the gel-forming ability of the proteins, which was found to form a firmer gel. Accordingly, the least gelation concentration for CPI was 6% (w/v). Results obtained suggested that, gelation is not only a function of protein quantity but seems also to be related to the type of protein as well as to the non-protein components and protein solubility; a similar conclusion was reached by Sathe and Salunkhe (1981) in their study on the great northern bean.

Table 2
Gelation capacity of cowpea protein isolates in water or NaCl solution at different concentrations[a]

Isolate concentration (g/100 ml)	GC in H_2O	GC in NaCl (M)	
		0.5	1.0
1	−	−	−
2	−	−	−
4	−	+	−
6	−	+	+
8	−	+	+
10	−	+	+
12	−	+	+
14	−	+	+
16	−	+	+
18	−	+	+
20	−	+	+

[a] Values are means of triplicate samples. Symbols: −, no gel; +, strong gel.

3.3.1. Emulsifying properties

The effect of pH or NaCl concentration on emulsion capacity of CPI is shown in Fig. 3. It was found that CPI had a minimum capacity (40 ml oil/g protein) at pHs 4 and 5 (Fig. 3a) with an increase, on either side of pHs 4 and 5, and was observed to be 82 at pH 3 and 150 ml oil/g protein at pH 10. Emulsion capacity was

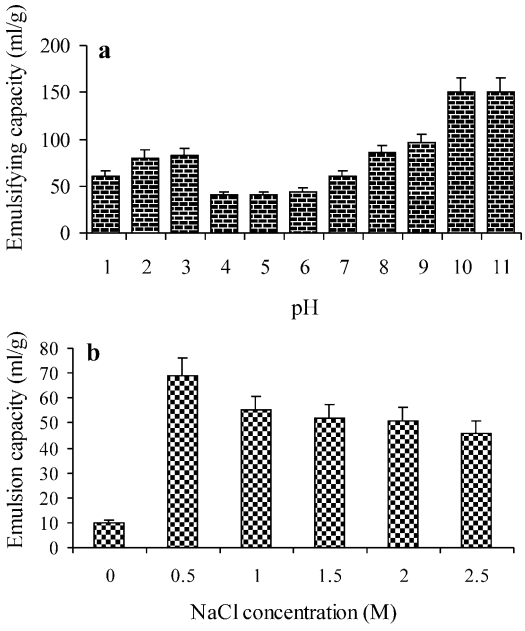

Fig. 3. Effect of (a) pH or (b) NaCl solution, at different concentrations, on the emulsion capacity of cowpea protein isolate.

pH-dependent and alkaline pH improved the emulsion capacity more than did acidic pH. Dependence of emulsion capacity on pH was expected, as it is known that emulsion capacity of a total protein depends on the hydrophilic-lipophilic balance, which is affected by pH (Sathe, Deshpande, & Salunkhe, 1982). Addition of NaCl at a concentration up to 0.5 M (Fig. 3b) increased the emulsification capacity of the proteins, due to the fact that addition of NaCl improved solubility of the proteins, even at the isoelectric pH and, accordingly, improved the emulsifying capacity. Beyond this salt concentration, the emulsification capacity gradually decreased due to the salting effect of NaCl. Chobert, Harb, Nicolas, Gaertner, and Puigserver (1987) have reported similar results. The effect of pH and time on emulsion stability (ES) of cowpea proteins (CPI) is shown in Fig. 4. It was found that CPI had a minimum stability at neutral pH that decreased greatly with time and reached 60% when the emulsion stood for 120 min. ES of the proteins gradually improved at acidic pH and was found to be 82% when the emulsion stood for 120 min, while alkaline pH showed a very stable emulsion even after standing for 120 min (100%). Differences observed might account for the variations of the hydrophilic–lipophilic balance of the proteins along the pH gradient. Similar observations on the pH dependence of ES have been reported (Sathe et al., 1982). Moreover, the relationship between ES and pH for CPI was similar to that between nitrogen solubility and pH. This was in agreement with the general correlation between ES and nitrogen solubility found in previous studies (Crenwelge, Dill, Tybor, & Landmann, 1974; Hung & Zayas, 1991). Hung and Zayas, (1991) suggested that various factors, including pH, droplet size, net charge, interfacial tension, viscosity and protein conformation, could affect the values of ES.

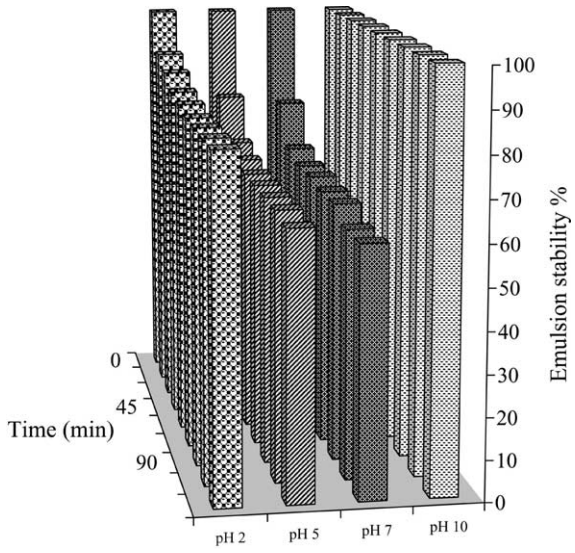

Fig. 4. Effect of time and pH on emulsion stability of cowpea protein isolate.

3.4. Foaming properties

Foam capacity of CPI is shown in Fig. 5. The foam capacity (FC) of CPI (Fig. 5a) was pH-dependent and the protein isolate was unable to foam at pH 5 (Fig. 5a). The lowest FC was attributed to the protein behaviour at its isoelectric point. Beyond pH 5, FC significantly increased, especially at pH 10 (92%). The higher FC at pH 10 was likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air–water interface to encapsulate air particles and to then enhance the foam formation (Aluko & Yada, 1995). The profile of FC against pH for the protein was more or less similar to that of its nitrogen solubility against pH. Addition of NaCl at a concentration up to 4.0 M (Fig. 5b) gradually improved FC of the protein and a higher increment was observed at this concentration. Lower concentration of NaCl was observed to cause a gradual decrease of FC. This may be attributed to the fact that addition of NaCl at a concentration up to 4.0 M enhances the protein solubility by weakening the hydrophobic interaction of the protein while low salt concentration had an adverse effect on FC. The effect of pH and time on foam stability (FS) of CPI is shown in Fig. 6. Regardless of the mixture pH, FS of the protein gradually decreased with time. At pHs 2 and 5 the protein stability

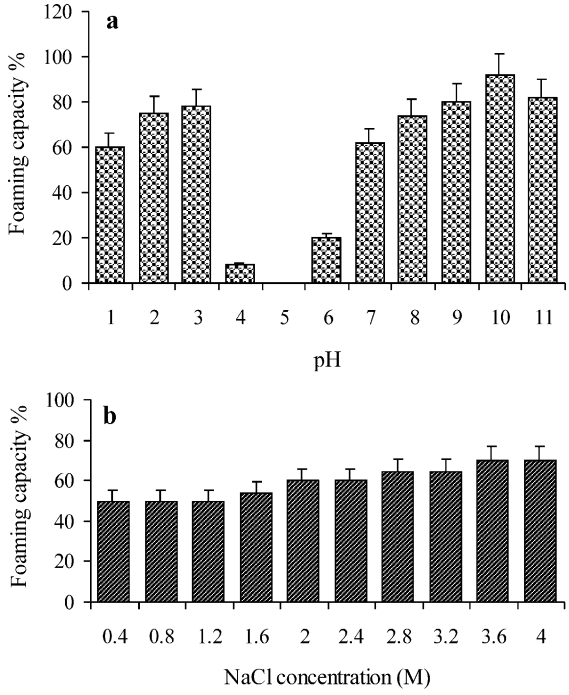

Fig. 5. Effect of (a) pH or (b) NaCl solution, at different concentrations, on foaming capacity of cowpea protein isolate.

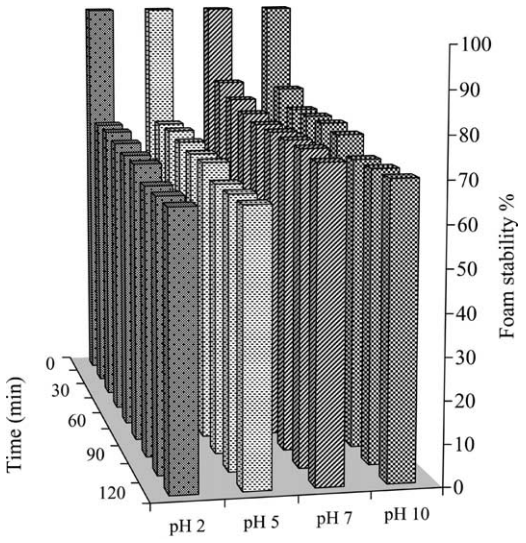

Fig. 6. Effect of time and pH on foam stability of cowpea protein isolate.

gradually decreased and reached 65% when the foam stood for 120 min, while at alkaline pH it reached 70%. An improvement in FS of the proteins at alkaline pH is likely to be due to increased solubility and surface activity of the soluble protein. Results revealed that the foaming properties of the proteins were pH-dependent.

In conclusion, a mixture of proteins isolated from cowpea was found to be highly soluble at alkaline pH. Therefore, emulsifying and foaming properties are higher than those of other proteins. Moreover, water-, fat-holding capacities, and other properties are good. Therefore, it can be used in food formulation systems.

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