Functional properties of selected legumes flour as influenced by pH

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The effect of different pH levels on the protein solubility, emulsifying and foaming properties of phaseolus, pigeon pea, cowpea and hyacinth bean flours was studied. The results obtained showed that the protein solubility and emulsifying and foaming properties at different pH levels of the selected legumes flour were varied. The minimum values of protein solubility, emulsifying capacity, emulsion stability and foaming capacity were found at pH 4.0. On either sides of this pH such properties were significantly increased. The solubility of the legumes flours was observed to be high at pH 8. The emulsifying activity (EA) improved above and below pH 4.0 and was higher in alkaline than acidic pH with a maximum value at pH 8.0. The highest emulsion stability (ES) of the flour was found at pH 6.0. At alkaline pH (8.0-12.0) the ES of the legumes flour was significantly decreased. The maximum foaming capacity (FC) for phaseolus (84%), pigeon pea (70%), cowpea (94%), and hyacinth bean (90%) flours was found at pH 12.0. The foam stability (FS) of the selected legumes flour at a given pH value was significantly ($p \leq 0.05$) decreased with time. The FS at pH 8.0 was low compared to pH 10 and 12.0. Generally, high FS was observed at acidic pH compared to alkaline pH region.

Introduction

In Africa and the rest of the developing world, where malnutrition due to inadequate protein is a prevalent problem, there is an urgent need to explore the utilization of plant proteins in the formulation of new food product or in

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conventional food (Khalid *et al*., 2003). This is predicated on the fact that animal protein such as meat, milk and eggs are expensive and relatively difficult to acquire (Chel-Guerrero *et al*., 2002). The partial replacement of animal foods with legumes has been shown to improve nutritional status due to lower cholesterol level in plant foods (Guillon and Champ, 1996). In addition, plant food diets increase the level of fibre intake which reduces the risk of bowel diseases, including cancer of the colon and also reduction in incidence of osteoporosis (Sirtori and Lovati, 2001). In the developed countries, plant proteins are now either regarded as versatile functional ingredients or as biologically active components more than as essential nutrients (Marcello and Guius, 1997). This evolution towards health and functionality is mainly driven by the demands of consumers and health professionals.

Legume proteins are characterized by a high content in polar amino acids with opposite charge (aspartic and glutamic acid on one side and lysine and arginine on the other side) (Duke, 1982). Therefore, it is reasonable to suppose that these amino acids are involved in the association-dissociation phenomena of protein sub-units that are at the basis of the solubility properties of legume oligomeric proteins. The high content of such amino acids i.e. lysine/arginine and aspartic/glutamic acids in the bean proteins would represent the condition for this mechanism of interaction to occur effectively (Marletta *et al*., 1992). Such a mechanism might be common among legume proteins, as all of these are characterized by a very high amount of both acid and basic amino acids (Duke, 1982). Mosses (1990) stated that cereal grains and legumes or oilseeds remain by far the predominant source of protein used for human food and for animal feed.

Functionality of food protein has been defined as those physical and chemical properties which affect the behaviour of proteins in food systems during processing, storage, preparation and consumption (Fennema, 1996). Functional properties constitute the major criteria for the adoption and acceptability of proteins in food systems. Many factors influence the functional properties of proteins, including moisture, temperature, pH, enzymes concentrations, reaction time, chemical additives, mechanical processing, ionic strength and amount, sequence, rate and time of additives had been studied (Johnson, 1970). The modern food processing industry is becoming increasingly dependent on the manufacture of fabricated foods rather than the preservation of commodities grown or reared on the farm. Protein functionality is dependent on hydrophobic, electrostatic, and steric parameters of the proteins, which are essential for defining the protein structure (Nakai, 1983). Therefore, this study was aimed to investigate the influence of different levels of pH on the functional properties of selected legumes flour.

Materials and methods

Phaseolus (*Phaseolus vulgaris*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata* (L.), and hyacinth bean (*Lablab purpureus*) were obtained from the local market, Khartoum, Sudan. Refined corn oil was brought from Bitar Company, Khartoum, Sudan. Unless otherwise stated all chemicals used in this study were of reagent grade.

Preparation of raw defatted legumes flour

The legumes seeds were first cleaned, freed from foreign matter and milled in a laboratory miller to pass through a 0.4 mm screen. To extract oil from legumes flour, cold extraction method was used according to the method of AOAC (1984). The defatted dried flour was ground to pass a 0.4 mm screen and stored at 0^oC for further analysis.

Measurement of solubility at various pH values

Protein solubility of the legumes flour at different pH levels was determined by the method of Aoki *et al*. (1981). The turbidity of the mixture was measured at 500 nm.

Measurement of emulsifying properties

The emulsifying properties of the samples were determined by the method of Pearce and Kinsella (1978). To prepare emulsion, 1.0 ml of corn oil and 3.0 ml of flour solution (2%) in 0.1M phosphate buffer (pH 7) were shaken together and homogenized in Ultra Turrax (Hansen Co., West Germany) at $12000 \times g$ for 10 min at 20^oC, then the mixture was poured into centrifuge tubes and centrifuged at $2000 \times g$ for 5 minutes and then poured into 50 ml measuring cylinders and stayed a few minutes until the emulsified layer was stable. The emulsifying activity (EA) as a function of pH was determined according to the formula:

$$EA\% = \frac{\text{Height of emulsified layer}}{\text{Height of total concentration in the cylinder}} \times 100$$

For the emulsion stability, about 50 μL of the homogenized mixture was taken from the bottom of the container of different times and pH and then diluted with 5 ml of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted mixture was then determined at 500 nm. The emulsion stability at different pH was expressed as the half-time of the initial turbidity of the emulsion (Kato et al., 1991). Values obtained are means of triplicate samples.

Foaming properties

The foaming capacity (FC) of the samples was determined by following the procedure described by Lawhon et al. (1972). Two grams of the sample were blended with 100 ml buffer at different pH levels (2, 4, 6, 8, 10 and 12) in a Moulinex blender at high speed for 2 minutes. The mixture was poured into a 250 ml measuring cylinder and the foam volume was recorded after 30 sec.

$$FC\% = \frac{\text{Volume after whipping-Volume before whipping}}{\text{Volume before whipping}} \times 100$$

The foam stability (FS) was determined as function of pH (2, 4, 6, 8, 10, 12) according to Ahmed and Schmidt (1979). The FS was recorded at 15 min interval after pouring the material in a cylinder.

$$FS\% = \frac{\text{Foam volume after 15 min}}{\text{Initial foam volume}} \times 100$$

Statistical analysis

Each determination was carried out on three separate samples and analysed in triplicate and figures were then averaged. Data was assessed by the analysis of variance (ANOVA) (Snedecor and Cochran, 1987). Duncan's multiple rang test was used to separate means. Significance was accepted at ($P \leq 0.05$) (Duncan, 1955).

Results and discussion

Effect of pH on flour solubility of selected legumes

The solubility of a protein is the thermodynamic manifestation of the equilibrium between protein–protein and protein solvent interactions (Damodaran, 1996). Solubility of a protein is one of the critical functional attributes required for use as food ingredient, because it greatly influences other

properties such as emulsification, gelation and foaming (Kinsella, 1976). Thus, it is an important property governing the functional behavior of proteins and their potential application to food processing. The results of the present study showed variation in the flour nitrogen solubility at different pH levels of selected legumes (Figure 1).
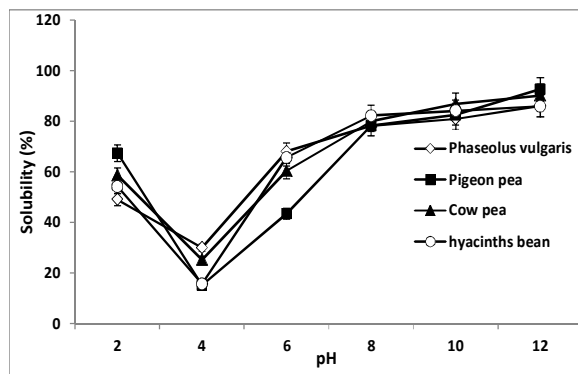

Fig. 1. Effect of pH on protein solubility of selected legumes flour

The minimum solubility was found to be 30.06, 15.22, 25.32, and 15.75 for phaseolus, pigeon pea, cowpea and hyacinth bean flours, respectively, at pH 4.0 which indicating that the isoelectric point of the flour protein is 4.0. On either sides of this pH the solubility was increased up to maximum values at the extreme levels of pH. Generally, the dependency of the solubility on pH has been attributed to the change in the net charges carried by the flour protein as the pH changes (Fagbemi *et al.*, 2006). The high net charge acquired at both acid and alkaline pH's caused arise in solubility due to unfolding of the flour protein with the degree of unfolding being greater at alkaline than the acidic pH (Damodaran, 1996). Minimum solubility at pH 4.0 and the increment of it on both sides of this pH were reported by many researchers (Hsu *et al.*, 1982; Narayana and Rao, 1991; Carbonaro *et al.*, 1993; Ali *et al.*, 2010). The low protein extractability at pH values of 4.0-6.0 were essentially attributed to the intermolecular attraction of protein molecules in the isoelectric zone (Molina *et al.*, 1994). However, part of this low dispersibility could be attributed to the formation of protein phytic acid complexes as reported for navy beans at similar pH values (Powrie, 1961). The occurrence of minimum solubility near the isoelectric pH is due primarily to the lack of electrostatic repulsion, which promotes aggregation and precipitation via hydrophobic interaction (Fennema, 1996). Legumes proteins contained high amount of polar amino acids with opposite charge (aspartic and glutamic acid on one side and lysine and arginine on the other side) (Duke, 1982). Therefore, it is reasonable to suppose that the

amino acids are involved in the association and disassociation phenomena of the protein subunits that are the basis of the solubility properties of legume oligomeric proteins. The result obtained for cowpea agreed with that reported by Padmashree *et al.* (1987) who stated that raw cowpea flour gave U shaped curve in pH range 2.0-10.0 with minimum nitrogen solubility (26%) at pH 4.0 and then increased considerably at acid (58%) and alkaline (96%) region. Also similar to those reported by Okaka and Potter (1979) for raw cowpea flour. The results obtained for phaseolus solubility at pH 4.0 agreed with those reported by Carbonaro *et al.* (1993). Selected legumes flour showed good solubility in both acidic and alkaline pH regions which can be considered as an important characteristic for food formulations. Since protein solubility largely affect other functionalities like emulsification, foaming and gelation (Kinsella, 1976), the high solubility of the flours indicated that they could have promising food applications.

Effect of pH on the emulsifying activity (EA) and emulsion stability (ES) of legumes flour

The emulsifying properties are usually attributed to the flexibility of solutes and exposure of hydrophobic domains. The formation and stability of emulsion is very important in food systems such as finely comminuted meats, soaps, cakes and salad dressings. The EA reflects the ability of the sample to rapidly adsorb at the water-oil inter phase during the formation of emulsion, thereby preventing flocculation and coalescence (Subago, 2006). The effect of pH on the EA and ES of selected legumes flour is shown in Figure 2.
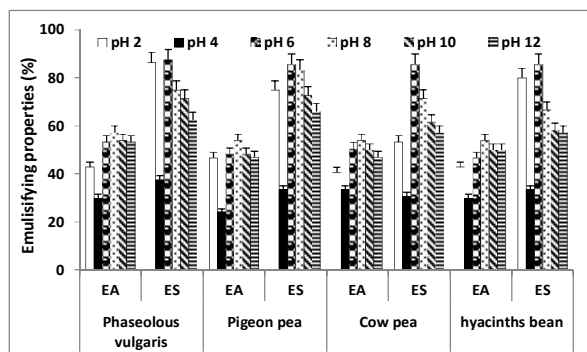
Fig. 2. Effect of pH on the emulsifying activity (EA) and emulsion stability (ES) of legumes flour

The studied samples showed minimum values of EA as 30.0, 24.23, 33.33 and 30.0% for phaseolus, pigeon pea, cowpea and hyacinth bean flours,

respectively at pH 4.0. This might be due to increased protein-protein interaction, which lowering the surface hydrophobicity and decrease the net charge and solubility of proteins. The EA improved above and below pH 4.0 and was higher at alkaline than acidic pH with maximum values at pH 8.0 due to high solubility of such legumes flours at same pH regions. The results obtained in this study agreed with those of Yim and Lee (2000) who found that the EA of soybean protein isolate was lower at pH between 4 and 5 and higher above pH 5.0. Also Khalid *et al.* (2003) found that sesame protein isolate had a minimum EA at pH 5.0 with increasing in EA at both sides of this pH. Monterio and Parkash (1994) observed higher EA at alkaline pH than acidic pH on soybean, sesame and peanut protein. Mahamuod (2004) reported that the EA was high at acidic pH compared to the alkaline one. The EA at pH 12.0 was lower than at pH values of 6, 8 and 10. This variation possibly due to varietal variation and methods applied to estimate EA. Emulsions are highly unstable systems but proteins play some important roles during emulsification especially in emulsion stability. The ES is important since success of an emulsifier depends on its ability to maintain the emulsion in subsequent processing steps such as cooking and canning (Akintayo *et al.*, 1998; Williams, 1999; Tsaliki *et al.*, 2004). The results obtained for ES of the selected legumes flour showed minimum values of ES at pH 4.0 (Figure 2). The highest ES of the flour was found at pH 6.0 with values of 87.5, 85.71, 85.71 and 85.71 followed by pH 2.0 with values of 86.33, 75.0, 53.33 and 80.0 for phaseolus, pigeon pea, cowpea and hyacinth bean flours, respectively. At alkaline pH (8.0-12.0) the ES of all legumes flour was significantly decreased. These results agreed with those of Khalid *et al.* (2003) who found that the ES of sesame protein isolate was higher at acidic pH (75.0 %) than at alkaline pH (62.0%) while it was minimum (37.8%) at pH 4.9. Paul and Polmer (1972) stated that low ES at pH 4.0 is possibly due to colloidal particles which carrying an electrical charge that promotes the stability of the colloid itself as well as in emulsions formed by causing particles of similar charge to repel each other by preventing the precipitation. Emmanuel *et al.* (1998) found that pigeon pea form unstable emulsions at pH 4.0. These variations might be due to differences in protein subunit, molecular weight distribution and amino acid composition.

Effect of pH on foaming capacity (FC) and foam stability (FS) of legumes flour

The foaming capacity (FC) of a protein refers to the amount of interfacial area that can be created by the protein while foam stability (FS) refers to the ability of protein to stabilize against gravitational and mechanical stresses (Fennema, 1996). Foam formation and foam stability are a function of the type

of protein, pH, processing methods, viscosity and surface tension. The selected legumes flour showed minimum FC at pH 4.0 with values of 25%, 20%, 26 % and 24% for phaseolus, pigeon pea, cowpea and hyacinth bean flours, respectively (Figure 3).
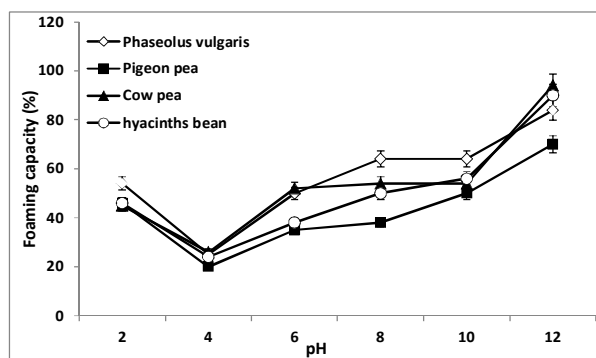

Fig. 3. Effect of pH on foaming capacity (FC) of selected legumes flour

Whereas, the maximum FC values for phaseolus (84%), pigeon pea (70%), cowpea (94%), and hyacinth bean (90%) flours were obtained at pH 12.0. These findings agreed with those of Pawar and Ingle (1988) who reported that the minimum FC of moth bean flour was 31% at pH 4.5 and the maximum FC at both alkaline and acidic pH values. The increase in FC of certain protein isolate might be due to increased solubility, rapid unfolding at the air-water interface, limited intermolecular cohesion and flexibility of protein surfactant molecules (Kinesella *et al*., 1985). The high FC at pH 12.0 is likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increase the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air-water interface to encapsulate air particles and to enhance the foam formation (Aluko and Yada, 1995). The FC was increased at alkaline pH region and the behavior of the FC versus pH values was completely agreed with the behavior of protein solubility versus pH values particularly at pH 12.0, which showed maximum protein solubility and foaming capacity. These observations confirmed the fact that foaming property was also depending on the protein solubility. Foam stability (FS) describes the ability of the proteins to form strong cohesive film around air vacuole that resists air diffusion from the vacuole. The effects of pH on the FS of selected legumes flour are shown in Figure 4a-d. The FS of selected legumes flour at a given pH value significantly ($p \leq 0.05$) decreased with time. After standing for 150 min at room temperature the FS was decreased at pH 2.0 from 100 to 40.74, 36, 35.46, and 42.55 for hyacinths bean (Figure 4a), phaseolus (Figure 4b), cowpea (Figure 4c) and pigeon pea (Figure 4d), respectively. Whereas, at pH 12.0 it

1298

decreased to 28.75, 27.75, 18.0 and 17.76 for phaseolus, pigeon pea, cowpea and hyacinths bean, respectively. The FS at pH 8.0 was low compared to that obtained at pH 10 and 12.0. Generally, high FS was observed at acidic pH compared to alkaline pHs. Similar results were reported by Mahamoud (2004). Aluko and Yada (1995) reported that better stability of the foam in the acidic pH range might be attributed to the formation of stable molecular layer in the acidic pH range, which contributes to the foam stability and elasticity. Also Idris *et al*. (2003) stated that the foam stability was greatly affected by the pH of the medium. The foam stability of winged bean flour was measured at several pH values. At pH values of 2.5, 6.6 and 7.8 the foam stability decreased markedly within 10 min and then decreased gradually. However, at pH 4.5 where the initial foam capacity was low it decreased steadily (Mohamoud, 2004). Wang and Kinsella (1976) found that the FS varied with pH being minimum in the isoelectric range (pH 3.0-4.0) and being maximum in narrow pH regions above the isoelectric range where protein is slightly negatively charged and showing a rapid decrease at alkaline pH values (pH 6.0). The latter effect may explain by charge repulsion between proteins with resultant lack of adhesion and also by some solubilization of alfalfa leaf protein in this range (Lu and Kinsella, 1972) thereby reducing the quantity of aggregated protein necessary to stabilize the foam (Wang and Kinsella 1976). Subago (2006) stated that the foaming stability of *lablab purpreus* was low.
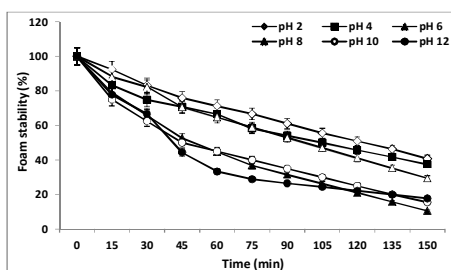

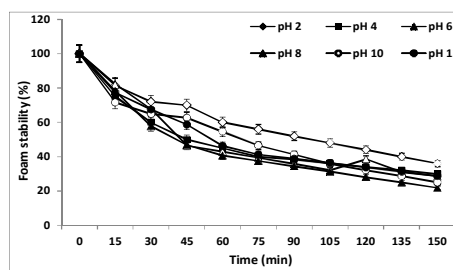Fig. 4a. Effect of pH on foam stability of hyacinths bean flour

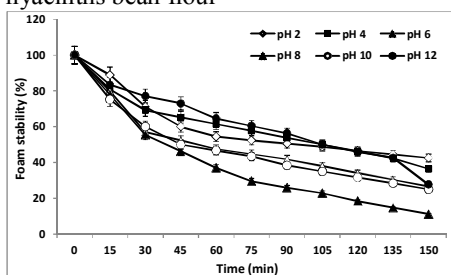Fig. 4b. Effect of pH on foaming stability of Phaseolus four

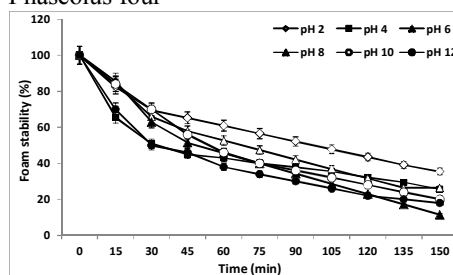**Fig. 4c.** Effect of pH on foam stability of cowpea flour

Fig. 4d. Effect of pH on foam stability of pigeon pea flour

Conclusion

The fairly good functional properties of the selected legume flours in wide pH range and particularly in alkaline pH region make such legumes potential ingredients for application in foods system, therefore, the partial replacement of animal foods with such legumes could improve the nutritional status of people in both developed and developing countries due to lower cholesterol level in plant foods. This evolution towards health and functionality is mainly derived by the demands of consumers and health professionals.

References

Ahmed, E.A. and Chmidt, R.H. (1979). Functional properties of peanut and soybean proteins as influenced by processing method. Peanut Sci. 6: 1-6.

Akintayo, E.T., Esuoso, K.O. and Oshadi, A.A. (1998). Emulsifying properties of some legume proteins. Int. J. Food Sci. Technol., 33: 239-246.

Ali, N. A., Ahmed, S. H., Mohamed, E. A., Mohamed Ahmed I. A. and Babiker, E. E. (2010) Changes in functional properties by transglutaminase cross linking as a function of pH of legumes protein isolate. Innovative Romanian Food Biotechnol., 7: 12-20

Aluko, R.E. and Yada, R.Y. (1995). Structure-function relationships of cowpea (*Vigna unguiculata*) globulin isolate, influence of pH and NaCl on physicochemical and functional properties. Food Chem., 53: 259-265.

AOAC (1984). Official methods of analysis. 14th ed. Association of Official Agricultural Chemists, Washington, D.C.

Aoki, H., Taneyama, O., Orimo, N. and Kitagawa, I. (1981). Effect of lipophilization of soy protein on its emulsion stabilizing properties. J. Food Sci., 46: 1192-1195.

Carbonaro, M., Vecchini, P. and Carnovale, E. (1993). Protein solubility of raw and cooked beans (*Phaseolus vulgaris*). Role of the basic residues. J. Agric. Food Chem., (41) 8: 1169-1175.

Chel-Guerrero, L. Perezflores, V., Bentacur-Ancona, D. and Davilla-Ortiz, G. (2002). Functional properties of flours and protein isolates from *Phaseolus lunatus* and *Canavalia ensiformis* seeds. J. Agric. Food Chem., 50: 584-591.

Damodaran, S. (1996). Amino acids peptides and proteins. In: Owen, R., Fennema (ed) food chemistry, 3rd edn. Marcel Dekker, Inc., 270 Madison Avenue. New York, 10016, USA pp. 327-425.

Duke, J.A. (1982). In handbook of legumes of world economic importance, Duke, J.A. Ed.; Plenum Press: New York, pp. 334-339.

Duncan, D.B. (1955). Multiple range and F-test. Biometrics, 11: 1-42.

Emmanuel, T.A., Kayode, O.E. and Aladesanmi, A.O. (1998). Emulsifying properties of some legume proteins. J. Food Sci. Technol., 33: 239-246.

Fagbemi, T.N. Oshodi, N.A. and Ipinmoroti, K.O. (2006). Effect of processing on the functional properties of full fat and defatted fluted pumpkin (*Telfairia occidentalis*) seed flours. J. Food Technol., 4: 70-79.

Fennema, R.O. (1996). Food chemistry, (3rd edition). Marcel Dekker, Inc. New York, Basel. Hongkong. pp. 365-39.

Guillon, F. and Champ, M. (1996). Grain Legunes and transit in humans. Grain Legumes AEP 11, 18-23.

Hsu, D.L., Leang, H.K., Morad, M.M., Finney, P.L. and Leung, C.T. (1982). Effect of germination on electrophoretic, functional and bread baking properties of yellow pea, lentil and faba bean protein isolate. Cereal Chem., 58: 344-350.

Idris, W.H., Babiker, E.E. and El Tinay, A.H. (2003). Fractionation, solubility and functional properties of wheat bran proteins as influenced by pH and/or salt concentration. Nahrung Food 47: 425-429.

Johnson, D.W. (1970). Oilseed proteins properties and application. Food Production and Devlopment 3, 78-80.

Kato, A., Shimokawa, K. and Kobayasi, K. (1991). Improvement of the functional properties of insoluble gluten by pronase digestion followed by dextran conjugation. J. Agric. Food Chem., 35: 200-205.

Khalid, E.K., Babiker, E.E. and El Tinay, A.H. (2003). Solubility and functional properties of sesame seed properties as influence by pH and/or salt concentration. Food Chem., 82: 361-366.

Kinesella, J.E., Damodaran, S. and Geman, B. (1985). Physico-chemical and functional properties of oilseed proteins with emphasis on soy proteins. In: A.M. Altschul and H.L. Wilcke (eds) New Protein Foods, New York, Academic Press, Inc. pp. 107-179.

Kinsella, J.E. (1976). Functional properties of protein in foods: A survey. Crit. Rev. Food Sci. Nutr., 7: 219-280.

Lawhon, J.T., Cater, C.M. and Mattil, K.F. (1972). A comparative study of the whipping potential of an extract from several oilseed flours. Cereal Science Today 17: 240-244.

Lu, P.S. and Kinsella, J.E. (1972). Extractability and properties of proteins from alfalfa leaf meal. J. Food Sci., 37:94-99.

Marcello, D. and Guius, C. (1997). Legume sees: protein content and nutritional value. Field Crops Res., 53: 31-45.

Marletta, L., Carbonaro, M. and Carnovale, E. (1992). *In vitro* protein and sulphur amino acids availability as measure of bean protein quality. J. Sci. Food Agric., 59: 497-504.

Mohamoud, N.M.O. (2004). Effect of Autoclaving on solubility and functional properties of chickpea (*Cicer areitinum* L.) flourm. M.Sc. Thesis, University of Khartoum, Sudan.

Molina, M.R., Argueta, C.E. and Bressani, R.J. (1974). Extraction of nitrogenous constituents from the jackbean (*Canavalia ensiformis*). J. Agric. Food Chem., 22: 309-312.

Monteiro, P.V. and Prakash, V. (1994). Functional properties of homogenous protein fractions from peanut (*Arachis hypogaea* L.). Journal of Agricultural and Food Chemistry, 42: 274-278.

Mosses, J. (1990). Nitrogen to protein conversion factor for ten cereal and six legumes or oilseeds. J. Agric. Food Chem., 38: 18-24

Nakai, S. (1983). Structure function relationships of food proteins with emphases on the importance of protein hydrophobicity. J. Agric. Food Chem., 31: 676-683.

Narayana, K. and Rao, M.S. (1991). Effect of acetylation and succinylation on the physicochemical properties of winged bean (*Psophocarpus tetragonolobus*) proteints. J. Agric. Food Chem., 39: 259-261.

Okaka, J.C. and Potter, N.N. (1979). Physicochemical and functional properties of cowpea powders processed to reduce beany flavour. J. Food Sci., 44: 1235-1240.

Padmashree, T.S., Vijayalak-Shmi, L. and Puttaraj, S. (1987). Effect of traditional processing on the functional properties of cowpea (*Vigna catjang*) flour. J. Food Sci. Technol. 24: 221-224.

Paul, P.C. and Palmer, H.H. (1972). Colloidal systems and emulsions. In: food theory and application. John Wiley and Sons, Inc. New York.

Pawar, V.D. and Ingle, U.M. (1988). Effect of germination on the functional properties of moth bean (*Phaseolus aconitifolius*) flours. J. Food Sci. Technol., 25, 1: 7-10.

Pearce, K.N. and Kinsella, J.E. (1978). Emulsifying properties of proteins: Evaluation of a hurbidimetric technique. J. Agric. Food Chem., 26: 716-723.

Sirtori, C.R. and Lovati, M.R. (2001). Soy proteins and cardio-vascular disease. Corr. Atheroscler Rep. 3: 47-53.

Snedecor, G.W. and Cochran, W.G. (1987). Statistical methods (17th ed). Ames, IA: The Iowa State University Press, pp. 221-222.

Subago, A. (2006). Characterization of hyacinth bean (*Lablab purpureus* (L.) sweet) seeds from Indonesia and their protein. Food Chem., 95 (1): 65-70.

Tsaliki, E., Pegiadou, S. and Doxastakis, G. (2004). Evaluation of the emulsifying properties of cottonseed protein isolate. Food Hydrocolloids, 18: 631-637.

Wang, J. and Kinsella, J.E. (1976). Functional properties of novel proteins. Alfalfa leaf protein. J. Food Sci., 41: 286-292.

Williams, P.A. (1999). Emulsions and foams: Interfaces, interactions and stability. Cambridge Press. Royal Soc. Chem. pp. 360-396.

Yim, M. and Lee, J. (2000). Functional properties of fractionated soy protein isolates by proteases from meju. J. Food Sci. Biotchnol., (9) 4: 253-257.