

Food Chemistry 67 (1999) 445-452

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeonpea (Cajanus cajan) and cowpea (Vigna unguiculata) protein isolates. II. Functional properties

Mwanjala A. Mwasaru*, Kharidah Muhammad, Jamilah Bakar, Yaakob B. Che Man

Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Received 25 July 1996; received in revised form and accepted 17 May 1999

Abstract

The functional properties of pigeonpea and cowpea protein isolates were determined as a function of extraction technique and pH conditions of the extraction medium. The isolates extracted using the micellization technique (MP) showed significantly $(P<0.05)$ higher solubility than those extracted using the isoelectric point precipitation technique (IP) and, for the latter, solubility was negatively correlated with the extraction pH. The MP isolates exhibited significantly higher water absorption than the isoelectric isolates extracted at pH 8.5 but lower than the isolates extracted at pH 11.5 and 12.5. Cowpea MP exhibited higher oil absorption than the IP but pigeonpea MP was lower in this property than the IP extracted at pH 8.5 and higher than those extracted at pH 9.5-11.5. The MP isolates exhibited better emulsifying properties than the corresponding IP isolates and this property was drastically impaired at extraction pH 12.5. Pigeonpea MP exhibited lower foam expansion than the IP isolates except for the isolate extracted at pH 12.5, but was higher in foam stability. Cowpea MP showed higher foam expansion than the IP isolates which decreased with increasing extraction pH for the latter, but foam stability was only slightly affected. The MP isolates exhibited better gelation properties than the IP isolates extracted at pH above 9.5 and the least gelation concentration increased with increasing extraction pH. The solubility and exposed hydrophobicity best predicted the whipping properties, emulsion stability, and least gelation concentration of the isolates. \odot 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Tropical legumes, such as pigeonpea and cowpea, are crops that are well adapted to the semi-arid zones of Kenya because of their drought tolerance. A review of available literature reveals that more effort has been invested in the nutritional and chemical evaluation of these legumes than the studies of those physicochemical and biochemical properties that bear upon their technological transformation and postharvest stability (Hulse, 1991). It has been demonstrated in Part I of this work that extraction technique and conditions had significant effects on the physicochemical properties of pigeonpea and cowpea protein isolates such as chemical composition, colour, thermal properties and hydrophobicity. Previous studies have indicated significant differences in functional properties between micelle and

The present study was, therefore, aimed at determining the effects of extraction techniques and conditions on the functional properties of protein isolates extracted from commercial pigeonpea and cowpea samples from Kenya, and to establish models for predicting some functional properties of the isolates using some basic physicochemical characteristics.

isoelectric protein isolates extracted from faba bean, chickpea and fenugreek (Abdel-Aal, Shehata, Mahdy & Youssef, 1986), safflower (Paredes-Lopez & Ordorica-Falomir, 1986b) and chickpea (Paredes-Lopez, Ordorica-Falomir & Olivares-Vasquez, 1991). As pointed out by Mitchell and Ledward (1986), many past developments of fabricated foods have been as a result of inspired creativity and trial and error manipulation of ingredients with little understanding of the underlying science. There is, therefore, need for the food technologist to understand the behaviour of individual ingredients in the formulated foods.

^{*} Corresponding author.

2. Materials and methods

Protein isolates from pigeonpea and cowpea extracted by the isoelectric and micellization techniques as described in Part I of this paper were used. The isoelectric isolates were designated the code IP followed by the pH of extraction and the micelle isolates were designated as MP. Suproplus 651 soy protein isolate (Protein Technologies International, Zwaanhofweig, Belgium) was used for comparison whenever applicable.

2.1. Protein pH -solubility

The pH-solubility was determined according to AACC method $46-23$ (1983) with some modifications. Samples (ca 1.0 g) were accurately weighed into 50 rnl centrifuge tubes and dispersed in 20 ml of water adjusted to pH between 2 and 12. The dispersions were mechanically shaken for 1 h, centrifuged at 8000 g for 15 min and the supernatants collected. The residue was resuspended and centrifuged twice in 10 ml water. The combined supernatants were analyzed for nitrogen by the Kjeldahl method (AACC 46-12, 1983) and reported as nitrogen solubility index (NSI), defined as $\%$ soluble nitrogen/total nitrogen.

2.2. Functional properties

Water and oil absorption capacities were determined by the centrifugation method of Lin, Humbert and Sosulski (1974). Emulsifying properties were determined according to the method of Yasumatsu, Sawada, Moritaka, Toda and Ishii (1972) with modifications (Wang $\&$ Kinsella, 1976). Whipping properties were determined according to the method described by Kabirullah and Willis (1982). Gelation properties were determined according to the method described by Coffmann and Garcia (1977).

2.3. Statistical analysis

Analysis of variance, Duncans multiple range test, and backward stepwise multiple regression were done using the Statistical Analysis System package (SAS, 1987).

3. Results and discussion

3.1. pH-Solubility

The nitrogen pH -solubility profiles of pigeonpea (Fig. 1) and cowpea isolates (Fig. 2) showed three general regions: one of minimum solubility (pH 4 -8) essentially the isoelectric pH range and two of solubility maxima at pH 2 and 12. The isolates presented similar solubility

Fig. 1. Nitrogen solubility profiles of pigeonpea micelle (MP) and isoelectric (IP) protein isolates.

Fig. 2. Nitrogen solubility profiles of cowpea micelle (MP) and isoelectric (IP) protein isolates.

profiles, qualitatively, but significant quantitative differences were observed among the isolates. Micelle isolates exhibited significantly ($P < 0.05$) higher solubility than the corresponding isoelectric isolates over all pH values away from pH 4. The highest solubilities were observed at pH 12 for pigeonpea MP (79.22%) and at pH 2 for cowpea MP (75.65%) and the lowest at pH 4 for the IP 12.5 isolates. Previous studies had shown that MP was superior to IP in terms of solubility for saf flower (Paredes-Lopez & Ordorica-Falomir, 1986a), and chickpea (Paredes-Lopez et al., 1991). The pH-solubility curves, especially those of the isoelectric isolates, were characterized by a broad range of low solubility over the pH 4 -8 range with solubilities of less than 10% , and the solubilities of IP $10.5-12.5$ were relatively low $(<50\%)$, even at pH 2 and 12. An inverse relationship was apparent between protein solubility and the extraction pH of the IP isolates. A narrower pH range of insolubility has been reported as desirable for protein extractability (Kilara, Humbert & Sosulski (1972). The low solubility of the isoelectric isolates observed in the present study paralleled the increase in the degree of protein denaturation with increasing extraction pH previously demonstrated by the differential scanning calorimetry studies reported in part I of this paper. Profiles with low solubility over a broad range of pH are indicative of severe protein denaturation and insolubilization (Hermansson, 1979; Kinsella, 1979; Lillford, 1983; Nakai, 1983) which have been shown to markedly affect the functional properties of proteins.

3.2. Water absorption capacity

The water absorption capacities of pigeonpea and cowpea isolates are shown in Table 1. IP 8.5 of both legumes exhibited the lowest water absorption capacity and this property increased with increasing extraction pH for the IP isolates. Pigeonpea MP exhibited significantly $(P < 0.05)$ higher water absorption capacity than IP 8.5, was similar to IP 9.5 and 10.5, but was lower than IP 11.5 and 12.5. For cowpea, MP exhibited higher water absorption than IP 8.5 but was lower than the rest of the IP isolates. The water absorption capacities of pigeonpea and cowpea isolates in the current study compared unfavourably to those of a commercial soy isolate (Suproplus 65 1) which gave a value of 4.04 ml H_2O/g . However, they compared favourably to isolates from great northern bean (2.73 g/g) (Sathe & Salunkhe, 1981), safflower $(1.80-2.82 \text{ ml/g})$ (Paredes-Lopez & Ordorica-Falomir, 1986b), faba bean, chickpea and fenugreek (1.84, 1.88, and 2.61 ml/g, respectively) (Abdel-Aal et al., 1986) and rapeseed (1.33 g/g) (Mansour, Peredi & Dworschak (1992). The low water absorption capacities of the pigeonpea and cowpea isolates in the present study were probably due to the ovendrying method used which yielded isolates with a horny

Table 1 Water absorption capacities (ml H_2O/g protein) of pigeonpea and cowpea protein isolates^a

Isolateb	Pigeonpea	Cowpea
MP	$1.24 \pm 0.01e$	$1.24 \pm 0.01e$
IP 8.5	0.84 ± 0.00 f	0.85 ± 0.01 f
IP 9.5	1.25 ± 0.01 de	$1.28 \pm 0.00d$
IP 10.5	1.26 ± 0.02 de	$1.68 \pm 0.02c$
IP 11.5	$2.10 \pm 0.01a$	$1.68 \pm 0.03c$
IP 12.5	$2.13 \pm 0.04a$	$1.73 \pm 0.04b$

^a Means followed by the same letter are not significantly different $(P < 0.05)$. Values given are means of duplicate determinations.

 b MP=micelle protein; IP=isoelectric protein.

gelatinized texture which may have hindered their hydration. It had been previously observed that increasing the pH of extraction resulted in an increase in the degree of protein denaturation. The consequent conformational changes probably resulted in the exposure of previously hidden hydrophilic amino acid side chains and may account for the observed increase in the water absorption capacity with increasing extraction pH. Similar observations have been previously reported for chickpea isolates (Paredes-Lopez et al., 1991) and pea isolate (Sumner, Nielson & Youngs, 1981). Differences in the contents of non-proteinaceous material (NFE) of the isoelectric isolates, may also have contributed to the observed differences in water absorption among the isolates as previously observed for isolates from adzuki bean (Tjahjadi, Lin & Breene, 1988), sun flower (Kilara et al., 1972) and great northern bean (Sathe & Salunkhe, 1981). Solubility of protein has also been shown to exhibit an inverse relationship with water absorption (Quinn & Paton, 1979), a factor which may account for the lower water absorption capacity of the more soluble IP 8.5 isolates in the present study.

3.3. Oil absorption capacity

As shown in Table 2, pigeonpea isolates generally exhibited significantly ($P < 0.05$) higher oil absorption capacities than the corresponding cowpea isolates. For pigeonpea, MP exhibited significantly ($P < 0.05$) higher oil absorption capacity than IP 9.5, 10.5 and 11.5, was similar to IP 12.5, and was lower than IP 8.5. For cowpea, MP exhibited significantly higher oil absorption than all IP isolates, indicating that the effect of extraction conditions on this property was also influenced by the botanical source of the protein. Paredes-Lopez et al. (1991) have reported that chickpea micelle isolate exhibited higher oil absorption capacity than the isoelectric isolate. The oil absorption capacities of pigeonpea and cowpea isolates in the present study were lower than that of a commercial soy isolate (3.29 ml oil/g) . They were, however, comparable to the 1.7 and 2.0 ml

	-14 -	anie	
--	-------	------	--

Oil absorption capacities (ml oil/g protein) of pigeonpea and cowpea protein isolates^a

^a Means followed by the same letter are not significantly different $(P<0.05)$. Values given are means of duplicate determinations.

 b MP = micelle protein; IP = isoelectric protein.

oil/g protein for chickpea MP and IP isolates, respectively (Paredes-Lopez et al.), $1.59-2.58$ ml/g for adzuki bean isolates (Tjahjadi et al., 1988) and $2.00-2.22$ ml/g for cowpea IP isolates (Sefa-Dedeh & Stanley, 1988). Oil absorption has been attributed to physical entrapment of oil within the protein isolates (Kinsella, 1976) and non-covalent bonds such as hydrophobic, electrostatic and hydrogen bonding are forces involved in lipid-protein interaction.

3.4. Emulsifying properties

The emulsifying properties of pigeonpea and cowpea isolates are presented in Figs. 3 and 4, respectively. The MP of both legumes showed significantly $(P < 0.05)$ higher emulsifying activity and foam stability than the IP. Pigeonpea IP exhibited a gradual increase in emulsifying activity as the extraction pH was increased from 8.5 to 11.5, followed by a steep decline at pH 12.5. The

Fig. 3. Emulsifying properties of soy protein (SP), pigeonpea micelle protein (MP) and isoelectric protein (IP) isolates.

Fig. 4. Emulsifying properties of soy protein (SP), cowpea micelle protein (MP) and isoelectric protein (IP) isolates.

emulsion stability of cowpea IP exhibited an increase as the extraction pH was increased from 8.5 to 9.5 which stabilized between pH 10. 5 and 11.5, followed by a marked decrease at extraction pH 12.5. Cowpea IP isolates exhibited a significant decrease in emulsifying activity as the extraction pH was increased from 8.5 to 12.5 with a drastic decline at pH 12.5. A trend similar to that of emulsifying activity was observed for emulsion stability. All pigeonpea and cowpea isolates were similar to, or better in emulsifying activity and emulsion stability than a commercial soy isolate except for pigeonpea IP 12.5 and cowpea IP 11.5 and 12.5. Previous studies have shown that chickpea IP isolate exhibited superior emulsifying activity to the MP isolate but the latter exhibited better emulsion stability (Paredes-Lopez et al., 1991). MP of faba bean, chickpea and fenugreek performed better than the IP isolates in terms of emulsifying properties (Abdel-Aal et al., 1986) and safflower MP showed better emulsion stability, but no significant differences were apparent in emulsifying activity (Paredes-Lopez & Ordorica-Falomir, 1986b). The results obtained in the current study and previous studies tend to indicate that the responses of the emulsification functionality to extraction technique and conditions are dependent on the botanical source of the proteins. Differences in the emulsifying activity of protein may be related to their solubility and conformational stability (Abdel-Aal et al.; Tjahjadi et al., 1988). Paredes-Lopez et al. observed that the sample with the lowest solubility exhibited the lowest emulsifying activity and the highest emulsion stability, an observation partly consistent with the results obtained in the present study since IP 12.5 which exhibited the lowest pH-solubility showed the lowest emulsifying activity but was also the lowest in emulsion stability. Multiple regression analysis, however, showed no significant $(P<0.05)$ correlation between solubility and the emulsifying activity of the isolates, probably because of the extremely low solubility of the protein isolates used in the present study. This latter observation was therefore at variance with that of Li-Chan, Nakai and Wood (1984), who had reported that, for meat proteins, solubility parameters were more influential in predicting the emulsion properties of samples with low solubility $(<50\%$). Hydrophobicity of proteins has also been reported to influence their emulsifying properties (Aluko & Yada, 1993; Kato & Nakai, 1980; Kinsella, 1979; Li-Chan et al.; Nakai, 1983; Townsend & Nakai, 1983). The roles of exposed hydrophobicity (Se) and solubility at pH 8 (So), in determining the emulsifying properties of pigeonpea and cowpea protein isolates, were investigated using backward stepwise multiple regression (Table 3). The following regression models were obtained for predicting their emulsifying properties.

$$
ES = 26.48477 + 0.000597 SeSo
$$
 (1)

Table 3

Backward stepwise multiple regression models for predicting emulsion stability (ES), foam expansion (FE) and foam stability (FS) of pigeonpea and cowpea protein isolates using exposed hydrophobicity (Se) and solubility (So) parameters

Dependent variable	Independent variable	Regression coefficient	F -value	F -probability
ES (n = 12, F-probability = 0.046, R^2 = 0.342	SeSo Constant	0.000597 26.48477	5.189	0.0459 0.0027
ES (n = 12, F-probability = 0.0129, $R^2 = 0.477$)	ln(SeSo) Constant	11.87115 -74.18519	9.114	0.0129 0.0757
FE (n = 12, F-probability = 0.044, R^2 = 0.346)	ln(SeSo) Constant	4.60477 -16.72255	5.288	0.0443 0.4011
FE (n = 12, F-probability = 0.030, R^2 = 0.0653)	Se So SeSo Constant	0.01371 4.52415 -0.00178 -4.25908	5.021	0.0231 0.0124 0.0218 0.6657
FS (n = 12, F-probability = 0.006, R^2 = 0.541)	So Constant	0.69339 66.49304	11.764	0.0064 0.0001
FS (n = 12, F-probability = 0.003, $R^2 = 0.607$)	SeSo Constant	0.000263 66.70417	15.416	0.0028 0.0001
FS (n = 12, F-probability = 0.021, R^2 = 0.576)	Se So Constant	0.01452 0.60318 63.90394	6.111	0.4087 0.0275 0.0001
FS (n = 12, F-probability = 0.0052, R^2 = 0.559)	(lnSe)So Constant	0.08941 66.46039	12.671	0.0052 0.0001

$ES = -74.18520 + 11.87115 \ln(SeSo)$ (2)

where $ES =$ emulsion stability. The regression models indicated that SeSo accounted for 34.2% and ln(SeSo) 47.7%, of the observed variability in emulsion stability and both parameters were positively related to emulsion stability. By virtue of the R^2 value, model (2) is a better predictor of the emulsion stability of the isolates. Aluko and Yada; on the other hand, reported significant correlations between aliphatic hydrophobicity and emulsifying activity index, and between aliphatic hydrophobicity, aromatic hydrophobicity and protein solubility, and emulsion stability of cowpea isolates. The cowpea isolate used by these workers was highly soluble $(>90\%)$ and this probably accounts for the discrepancy between their results and those obtained in the current study.

3.5. Whipping properties

The foam expansion and stability, used as indices of the whipping properties of pigeonpea and cowpea isolates are presented in Figs. 5 and 6, respectively. Pigeonpea MP exhibited significantly $(P<0.05)$ lower foam expansion than the IP isolates except for IP 12.5. The foam stabilities were characterized by small but significant differences, with MP showing the highest stability. Cowpea MP showed significantly $(P < 0.05)$ higher foam expansion than the IP isolates. The foam stability of cowpea MP was significantly higher than that of IP 9.5, 10.5 and 11.5. Foam expansion of cowpea and pigeonpea IP isolates decreased with increasing extraction pH but foam stability was only slightly

affected. It was observed that a commercial soy isolate was superior in foam expansion to all pigeonpea isolates and cowpea IP 9.5-12.5. Soy isolate exhibited better foam stability than pigeonpea isolates extracted at pH $10.5-12.5$ and cowpea IP $9.5-11.5$. Previous studies have indicated that safflower MP gave better foaming capacity than the IP (Paredes-Lopez & Ordorica-Falomir, 1986b) whereas chickpea IP was better in foam expansion and stability than the MP (Paredes-Lopez et al., 1991). It has also been reported that the solubility of soy proteins is closely correlated with foam expansion but that foam stability is related to the degree of denaturation (Kinsella, 1979). The results obtained in the present study are consistent with the above relative to foam expansion but are at variance with those pertain-

Fig. 5. Whipping properties of soy protein (SP), pigeonpea micelle protein (MP) and isoelectric protein (IP) isolates.

Fig. 6. Whipping properties of soy protein (SP), cowpea micelle protein (MP) and isoelectric protein (IP) isolates.

ing to foam stability, which appeared relatively indifferent to the degree of denaturation, as determined by differential scanning calorimetry. Horiuchi, Fukushima, Sugimoto and Hattori (1978) reported a good correlation between surface hydrophobicity and foam stability. It has also been reported that optimum foaming of protein seems to be associated with dispersibility and hydrophobicity values above 20% and 700, respectively (Townsend & Nakai, 1983). The latter workers also concluded that, regardless of the degree of dispersibility, proteins with low hydrophobicity exhibited poor foaming capacity. This conclusion was in agreement with the results obtained in the present study in that the isolates with the lowest exposed hydrophobicity values (IP 12.5) also exhibited the lowest foam expansion. The roles of protein solubility and exposed hydrophobicity on the whipping properties of pigeonpea and cowpea isolates were investigated using backward stepwise multiple regression (Table 3). The following regression models were obtained for predicting the whipping properties

$$
FE = 4.60477 \ln(SeSo) - 16.72255 \tag{3}
$$

 $FE = 0.01371$ Se $+ 4.452415$ So $- 0.00178$ SeSo

$$
-4.25908 \t(4)
$$

$$
FS = 66.49304 + 0.69339 \text{ So}
$$
 (5)

 $10 \text{ FS} = 66.70417 + 0.000263 \text{ SeSo}$ (6)

 $\text{FS} = 63.90394 + 0.01452 \text{ Se} + 0.60318 \text{ So}$ (7)

$$
FS = 66.460039 + 0.08941 \text{ (lnSe)So}
$$
 (8)

where $FE = f$ oam expansion, $FS = f$ oam stability, $Se =$ exposed hydrophobicity, and $So = nitrogen$ solubility index at pH 8.

Model 4 was deemed the best predictor of foam expansion of pigeonpea and cowpea protein isolates. This was because the independent variables So and Se, and their interaction (SeSo) contributed the highest proportion (65.3%) of the variability in foam expansion. By applying the same logic, model Eq. 6 $(R²=0.607)$ was considered the best predictor of foam stability of these isolates. These models indicated that the foam expansion of the isolates was strongly influenced by solubility and the interaction between exposed hydrophobicity and solubility. On the other hand, foam stability was correlated to the interaction of these two parameters. The above observations are consistent with those previously made by Aluko and Yada (1993) for cowpea isolate.

3.6. Gelation properties

The least gelation concentrations (LGC) of pigeonpea and cowpea isolates are given in Table 4. No significant $(P<0.05)$ differences were observed between the MP and IP 8.5 and 9.5, for both pigeonpea and cowpea protein isolates. For the IP isolates, the LGC increased at extraction pH above 9.5. The commercial soy isolate exhibited LGC of 12%, which was higher than all the isolates in the present study. The increase in LGC of IP isolates extracted at $pH > 9.5$ suggested that the gelling ability of the isolates decreased with increasing degree of denaturation, which had earlier been shown to be positively correlated with extraction pH (see Part I). Voutsinas, Nakai and Harwalkar (1983) reported that protein gelation was significantly affected by exposed hydrophobicity and the square of sulfhydryls of proteins. The roles of hydrophobicity and solubility in predicting the gelation properties of pigeonpea and cowpea were investigated using backward stepwise multiple regression (Table 5). Sulfhydryls were excluded from the analysis since it had been shown earlier that pigeonpea and cowpea isolates were similar and low in sulfur amino acid contents. The following regression models were obtained for predicting the gelation properties of pigeonpea and cowpea isolates.

Table 4

Least gelation concentration (%) of pigeonpea and cowpea protein isolates^a

Isolate ^b	Pigeonpea	Cowpea
MP	6.00a	6.00a
IP 8.5	6.00a	6.00a
IP 9.5	6.00a	6.00a
IP 10.5	8.00b	10.00c
IP 11.5	10.00c	10.00c
IP 12.5	10.00c	10.00c

^a Means followed by the same letter are not significantly different $(P<0.05)$. Values given are means of triplicate determinations.

 b MP = micelle protein; IP = isoelectric protein.

Table 5

$$
LGC = 12.0803 - 0.00195Se
$$
 (9)

 $LGC = 9.45291 - 0.22942S$ o (10)

 $LGC = 41.76839 - 4.44728\text{InSe}$ (11)

 $LGC = 24.82135 - 1.810841n(SeSo)$ (12)

LGC = $12.11995 - 0.001496$ Se $- 0.13650$ So (13)

 $LGC = 13.71915 - 0.00234$ Se $- 0.40423$ So

$$
+ 0.000122SeSo \tag{14}
$$

where LGC = least gelation concentration, Se =exposed hydrophobicity, $So = nitrogen$ solubility index at pH 8.

The regression models indicated that LGC was negatively correlated with exposed hydrophobicity and that this variable accounted for 61.9% [model Eq. (9)] of the observed variability in LGC; solubility was also negatively correlated with LGC and accounted for 45.7% of the variability [model Eq. (10)]. The regression model incorporating ln(SeSo) [model Eq. (12)], which accounted for 78.6% of the variability in LGC, was selected as the best predictor of the gelation properties of pigeonpea and cowpea protein isolates.

Acknowledgements

Financial support from Japan International Cooperation Agency (JICA) and Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya is gratefully acknowledged.

References

- Abdel-Aal, E. S. M., Shehata, A. A., Mahdy, E. A. R., & Youssef, M. M. (1986). Extractability and functional properties of some legume proteins isolated by three methods. Journal of the Science of Food and Agriculture, 37, 553-559.
- Aluko, R. E., & Yada, R. Y. (1993). Relationship of hydrophobicity and solubility with some functional properties of cowpea (Vigna unguiculata) protein isolate. Journal of the Science of Food and Agriculture, 62, 331-335.
- AACC (American Association of Cereal Chemists) (1983). Approved methods of AACC. St. Paul, MN: The Association.
- Coffmann, C. W., & Garcia, W. W. (1977). Functional properties and amino acid composition of a protein isolate from mung bean flour. Journal of Food Technology, 12, 473-484.
- Hermansson, A. M. (1979). Methods of studying functional characteristics of vegetable proteins. Journal of the American Oil Chemist's Society, 56, 272-279.
- Horitichi, T., Fukushima, D., Sugimoto, H., & Hattori, T. (1978). Studies on enzyme-modified proteins as foaming agents: Effects of structure on foam stability. Food Chemistry, 3, 35-42.
- Hulse, J. H. (1991). Nature, composition and utilization of grain legumes. In ICRISAT. Uses of tropical grain legumes: Proceedings of a consultants meeting, 27-30 March 1989. ICRISAT, Hyderabad, India. Patancherti, A.P., India: International Crops Research Institute for the Semi Arid Tropics Center.
- Kabirullah, M., & Willis, R. B. H. (1982). Functional properties of acetylated and succinylated sunflower protein isolate. Journal of Food Technology, 17, 235-249.
- Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence method and its correlation with surface properties of proteins. Biochimica et Biophysica Acta, 624, 13-20.
- Kilara, A., Humbert, E. S., & Sosulski, F. W. (1972). Nitrogen extractability and moisture absorption characteristics of sunflower seed products. Journal of Food Science, 37, 771-773.
- Kinsella, J. E. (1976). Functional properties of proteins in foods. Critical Reviews of Food Science and Nutrition, 7, 219-280.
- Kinsella, J. E. (1979). Functional properties of soy proteins. Journal of American Oil Chemists' Society, 56, 242-257.
- Li-Chan, E., Nakai, S., & Wood, D. F. (1984). Hydrophobocity and solubility of meat proteins and their relationship to emulsifying properties. Journal of Food Science, 49, 345-350.
- Lillford, P. J. (1983). Extraction processes and their effect on protein functionality. Plantarum Qualitas Plant Food for Human Nutrition, 32, 401±409.
- Lin, M. Y. J., Humbert, E. S., & Sosulski, F. W. (1974). Certain functional properties of sunflower meal products. Journal of Food Science, 39, 368-370.
- Mansour, E. H., Peredi, J., & Dworschak, E. (1992). Preparation and functional properties of rapeseed protein products. Acta Alimentarius, 21, 293–305.
- Mitchell, J. R., & Ledward, D. A. (1986). Preface In: J. R. Mitchell & D. A. Mitchell, Functional properties of food macromolecules. London and New York: Elsevier Applied Science Publishers.
- Nakai, S. (1983). Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. Journal of Agricultural and Food Chemistry, 31, 676-683.
- Paredes-Lopez, O., & Ordorica-Falomir, C. (1986a). Production of safflower protein isolates: composition, yield and protein quality. Journal of the Science of Food and Agriculture, 37, 1097-1103.
- Paredes-Lopez, O., & Ordorica-Falomir, C. (1986b). Functional properties of safflower protein isolates: water absorption, whipping and emulsifying characteristics. Journal of the Science of Food and Agriculture, 37, 1104-1108.
- Paredes-Lopez, O., Ordorica-Falomir, C., & Olivares-Vasquez, M. R. (1991). Chickpea protein isolates: physicochernical, functional and nutritional characteristics. Journal of Food Science, 56, 726-729.
- Quinn, J. R., & Paton, D. (1979). A practical measurement of water hydration capacity of protein materials. Cereal Chemistry, 51, 38-39.

SAS (1987). SAS user's guide: statistics. Cary, NC: SAS Institute.

- Sathe, S. K., & Salunkhe, D. X. (1981). Functional properties of the great northern bean (Phaseolus vulgaris L.) proteins: emulsion, viscosity, and gelation properties. Journal of Food Science, 46, 71-74.
- Sefa-Dedeh, S., & Yiadom-Farkye, N. A. (1988). Some functional characteristics of cowpea (Vigna unguiculata), bambara beans (Voandzeia subterranea) and their products. Canadian Institute of Food Science and Technology Journal, 21, 266-270.
- Sumner, A. K., Nielsen, M. A., & Youngs, C. G. (1981). Production and evaluation of pea protein isolate. Journal of Food Science, 46, 364±367.
- Tjahjadi, C., Lin, S., & Breene, W. M. (1988). Isolation and characterization of adzuki bean (Vigna angularis cv Takara) proteins. Journal of Food Science, 53, 1438-1443.
- Townsend, A., & Nakai, S. (1983). Relationships between hydrophobicity and foaming characteristics of food proteins. Journal of Food Science, 48, 588-594.
- Wang, L. C., & Kinsella, J. E. (1976). Functional properties of novel proteins: alfalfa leaf protein. Journal of Food Science, 47, 286-292.
- Voutsinas, L. P., Nakai, S., & Harwalkar, V. R. (1983). Relationships between protein hydrophobicity and thermal functional properties of food proteins. Canadian Institute of Food Science and Technology Journal, 16, 185-190.
- Yasumatsu, K., Sawada, K., Moritaka, S., Toda, J., & Ishii, K. (1972). Whipping and emulsifying properties of soybean products. Agricultural and Biological Chemistry, 36, 719-727.