Characteristics of Roselle Seeds as a New Source of Protein and Lipid

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Chemical composition and nutritional studies of roselle seeds (*Hibiscus sabdariffa*) were performed. The results of amino acid analysis indicated roselle seed to be a potential high-protein source. Lysine content was found to be similar to that of the FAO reference protein. The most limiting amino acids in roselle seed were found to be valine, isoleucine, and tryptophan. Also, the results of lipid analysis indicated roselle seeds to be a good source of lipid. Fatty acid analysis revealed that linoleic, oleic, palmitic, and stearic acid were the major fatty acid constituents. The most predominant inorganic elements in roselle seed were found to be K, Na, Mg, and Ca. Roselle seeds exhibited high digestibility by a trypsin–pancreatin system. Analysis of tannins and phytic acid in roselle seed revealed low levels. The results of Osborne classification indicated that the major protein fraction consisted of globulins. Also, roselle protein gave a sharp spectrum with a maximum absorbance at 272 nm. The present study showed that roselle seed proteins were highly soluble at acid and alkaline pH values, with a minimum solubility at about pH 4. Roselle seed meals had excellent foaming capacity, foam stability, and water absorption capacity.

Keywords: *Roselle seed; in-vitro digestibility; antinutritional factors; functional properties*

INTRODUCTION

Plant proteins contribute 50–75% of the total dietary protein needs. Cereal grains, oilseeds, and pulses are the three groups of plants that supply most of the protein in the world (Dimler, 1971). Due to the shortages of protein and lipid sources as a food supply, considerable research is indicated to investigate and explore new sources.

Roselle *(Hibiscus sabdariffa)* is one of the botanical species cultivated for its pleasant red color calyxes which are used for making common drinks called karkade (Al-Wandawi et al., 1984). It was reported by Earle et al. (1960) and Watt and Breyer (1962) that roselle seed has a high content of oil and protein. The total protein content of roselle seed was found to be 25.20% (Al-Wandawi et al., 1984) in comparison to 20.58% reported for mature okra seed (Karakoltsdis and Constantinides, 1975), which is considered to be a potential rich protein with a high lysine level (Al-Wandawi, 1983). The chemical scores for the essential amino acids of Iraqui whole mature roselle seed were reported by Al-Wandawi et al. (1984). Fatty acid composition of roselle seed oil was reported by several groups of workers (Subbaram et al., 1964; Cornelius et al., 1970; Mohiuddin and Zaidi, 1975; Ahmad et al., 1979; Ahmed and Hudson, 1982). Fatty acid composition was determined by gas–liquid chromatography of the methyl esters. No data in the literature on the physical properties of roselle seed protein were available for comparative purposes.

The objectives of the present study were to evaluate the chemical composition of Egyptian roselle seed cultivars and to study the functional properties and nutritive quality of roselle seed meals.

MATERIALS AND METHODS

Roselle Seed Flour. Roselle seeds *(H. sabdariffa)* were obtained from Tahrier goveronrate, Egypt. The seeds of three different cultivars, a light red, an early dark red, and a late dark red, were used in the present study. The seeds were ground, using an electric mill, to a fine powder. A Soxhlet apparatus was used for deffating the meals, which were used for subsequent analysis.

Chemical Analysis. Moisture, ash, total carbohydrates, and total nitrogen (micro-Kjeldahl) were determined according to AOAC (1980) methods. Protein was calculated as N × 6.25. Non-protein nitrogen was measured as nitrogen soluble in a 12% TCA filtrate of samples (Patel et al., 1990). Fat content was determined according to the Soxhlet method (Egan et al., 1981).

Mineral contents were determined by digesting the samples with concentrated nitric acid and perchloric acid (1:1 v/v). Na, K, and Ca were determined using a flame photometer (Corning 410), while Mg, Mn, Zn, Fe, and Cu were determined using an atomic absorption spectrophotometer (Perkin-Elmer Instrument Model 2380).

Amino acids were determined using amino acid analyzer (Aminochrome II OE 914, Hungary). Tryptophan content was determined according to the spectrophotometric method (Bencze and Schmid, 1957).

Fatty acids were determined by gas–liquid chromatography (Model GC 4 CM, Shimadzu) of the methyl esters.

The ultraviolet (UV) absorption spectrum of approximately 0.1% (w/v) protein in 1 M sodium chloride was recorded at room temperature in a Bausch and Lomb Spectronic 2000 over the range 220–300 nm.

The Osborne classification of protein was done according to the method of Abd El-Aal et al. (1986) using distilled water, 1 M NaCl, 70% ethanol, and 0.2 M NaOH solutions for albumins, globulins, prolamins, and glutelins, respectively. Non-protein nitrogen was determined in a separate sample and subtracted from the albumin value. The remaining residue, after the successive extractions, was quantitatively transferred into a Kjeldahl flask and digested with concentrated H_2SO_4 to determine the residual protein.

Nutritional Analysis. *Antinutritional Factors*. Phytic acid was determined according to the method of Wheeler and Ferrel (1971), while tannic acid was determined colorimetrically as described in AOAC (1980). Hemagglutinin activity was measured using 4% trypsinated rabbit red blood cells as described by Liener and Hill (1953).

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Table 1. Chemical Composition of the Whole Roselle Seed Cultivars

component	% (dry weight basis) for cultivars, mean[a] ± standard deviation		
	light red	early dark red	late dark red
total protein	31.02[a] ± 1.32	30.11[a] ± 0.78	30.94[a] ± 0.94
non-protein nitrogen	2.50[a] ± 0.11	2.60[a] ± 0.09	2.06[b] ± 0.03
total ash	6.89[a] ± 0.23	5.80[b] ± 0.12	6.52[a] ± 0.32
total lipids	21.60[c] ± 0.57	22.53[b] ± 0.64	23.26[a] ± 0.78
crude fiber[b]	4.12[a] ± 0.14	3.44[b] ± 0.08	1.23[c] ± 0.06
total carbohydrates	36.37[a] ± 1.86	38.12[a] ± 1.12	38.05[a] ± 1.05
glucose	3.82[b] ± 0.12	3.50[b] ± 0.08	4.74[a] ± 0.09
sucrose	17.90[a] ± 0.86	19.75[a] ± 1.16	14.99[b] ± 1.11
starch	14.65[b] ± 0.94	14.86[b] ± 1.06	18.32[a] ± 0.74
moisture	9.25[b] ± 0.98	11.66[a] ± 1.08	11.45[a] ± 086

[a] Means followed by a different letter within a row are significantly different according to an lsd test at 0.05 level. [b] Crude fiber is determined as 100 − (total protein + total ash + total lipids + total carbohydrates).

In-vitro protein digestibility of a double digestive enzyme system (trypsin−pancreatin) was determined according to the procedure of Salgo et al. (1985).

The biological values of roselle seed flours were determined on the basis of the amino acid composition. The procedure of Hidvégi and Békés (1985) was used to determine the chemical score (CS), Mitchel essential amino acid index (MEAAI), FAO/WHO index, Mørup and Olesen's index (MOI), Gaussian index (GI), and transformed Gaussian index (TGI).

Functional Properties. Nitrogen solubility as a function of pH was determined according to the method of Shen (1975). Water absorption was measured according to the procedure of Sosulski (1962). Values are expressed as grams of water absorbed by 100 g of sample. Oil absorption was determined as described by Sosulski et al. (1976) using refined corn oil, and the data are expressed as millilters of oil per 100 g of sample. Emulsification capacity was measured according to the method of Beuchat et al. (1975). The method described by Lawhon et al. (1972) was used to determine the foaming capacity and stability.

Statistical Analysis. Data were analyzed using analysis of variance, and least significant differences (lsd) were followed to make the multiple comparisons (Steel and Torrie, 1980). Significant differences were determined at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Chemical analysis of the whole roselle seed cultivars is presented in Table 1. The seeds contained 30.11−31.02% protein, 2.06−2.60% non-protein nitrogen, 5.80−6.89% total ash, 21.60−23.26% total lipids, 1.23−4.12% crude fiber, 36.37−38.12% total carbohydrates, and 9.25−11.66% moisture. The cultivars were similar in their total protein and total carbohydrate contents. Significant differences ($P \leq 0.05$) among seed cultivars were observed in total lipids and crude fiber content. The present study indicated that roselle seed could be used as a protein and lipid source. Al-Wandawi et al. (1984) found that roselle seed contained 25.20% protein. Also, the yield of oil from the seeds was reported to be 16−21% (Mohiuddin and Zaidi, 1975; Ahmed and Hudson, 1982).

The results of the amino acid contents of roselle seed cultivars are presented in Table 2. The results reveal that all amino acids were either similar or only slightly different for all cultivars. There were no significant ($P \geq 0.05$) differences among the roselle seed cultivars in amino acid contents. Glutamic acid, aspartic acid, and arginine were the major amino acids in all cultivars and had value ranges of 21.30-21.78%, 10.50−10.91%, and 10.13−10.58% of the total amino acids, respectively. The total concentration of essential amino acids in the FAO reference protein (Renner and Abd El-Salam, 1991) was

Table 2. Amino Acid Profile of Roselle Seed Cultivars (Grams per 16 g of N)

amino acid	light red	early dark red	late dark red
aspartic acid	10.91	10.50	10.52
threonine[a]	4.86	4.74	4.39
serine	4.40	4.65	4.70
glutamic acid	21.30	21.78	21.38
proline	4.14	4.13	4.21
glycine	4.27	4.27	4.32
alanine	4.69	4.55	4.72
cystine[a]	2.64	2.91	2.88
valine[a]	3.26	3.32	3.32
methionine[a]	1.13	1.20	1.15
isoleucine[a]	3.24	3.03	3.64
leucine[a]	7.32	7.29	7.28
tyrosine[a]	3.46	3.39	3.54
phenylalanine[a]	5.09	5.25	5.15
lysine[a]	5.37	5.46	5.56
histidine	2.97	2.74	2.77
tryptophan[a]	0.37	0.29	0.34
arginine	10.58	10.50	10.13
classified distribution of amino acids			
essential amino acids	36.74	36.88	37.25
nonessential amino acids	63.26	63.12	62.75
hydrophobic (nonpolar) neutral amino acids	29.24	29.06	29.81
hydrophilic (polar) neutral amino acids	19.63	19.96	19.83
basic amino acids	18.92	18.70	18.46
acidic amino acids	32.21	32.28	31.9
aromatic amino acids	8.55	8.64	8.69
sulfuric amino acids	3.77	4.11	4.03

[a] Essential amino acids according to FAO/WHO (1975).

Table 3. Fatty Acids Composition of Roselle Seed Oil (Percent of Total Fatty Acid)

fatty acid	variety		
	light red	early dark red	late dark red
lauric	0.903	0.801	1.003
myristic	0.309	0.431	0.501
palmitic	24.206	20.613	25.050
stearic	5.687	4.599	5.982
oleic	26.326	31.596	28.057
linoleic	42.124	41.311	38.873
linolenic	0.445	0.649	0.534

36 g/100 g of protein, while it was 36.74, 36.88, and 37.25 g/100 g of protein for the light red, early dark red, and late dark red cultivars, respectively. Relative to the FAO reference pattern, the limiting amino acids were found to be valine, isoleucine, and tryptophan, while total sulfur-containing amino acids were not limiting. In the present study, the lysine content of roselle seed cultivars was found to be similar to that of the FAO reference protein, so that it could be used as a supplement food mixture for poor lysine sources.

Fatty acid compositions of roselle seed oil cultivars are shown in Table 3. Linoleic acid, oleic acid, palmitic acid, and stearic acid were the major fatty acid constituents in the Egyptian cultivars. Fatty acid compositions in the three cultivars correlate closely with each other. The cultivars were found to contain 38.8−42.1% linoleic, 26.3−31.6% oleic, 20.6−25.1% palmitic, 4.6−6.0% stearic, 0.8−1.0% lauric, 0.4−0.6% linolenic, and 0.3−0.5% myristic acid. These results indicate that the ratio of saturated to unsaturated fatty acids is 1:2, which differs from that (1:3) found by Mohiuddin and Zaidi (1975). The results of the present study were in good agreement with data reported by Cornelius et al. (1970) and Subbaram et al. (1964). On the other hand, variations were noticed between the Egyptian seed oil (different cultivars) and the Sudanese and Indian seed oils previously reported by Ahmad et al. (1979) and Ahmed and Hudson (1982). The deviation is that the

Table 4. Mineral Composition of Roselle Seed Cultivars (Milligrams per 100 g of Defatted Seed Flours)

element	light red	early dark red	late dark red
sodium	620	590	680
calcium	390	393	362
potassium	1300	1390	1350
magnesium	460	480	420
manganese	4.10	3.90	4.00
copper	tr[a]	tr	tr
iron	9.10	9.00	8.80
zinc	5.60	5.50	5.90

[a] tr, trace.

Table 5. Antinutritional Factors and In-Vitro Protein Digestibility of Roselle Seed Cultivars[a]

sample	% tannins	hemagglutinin (HU/g of sample)	% phytic acid	% in-vitro digestiblity
light red	1.37 ± 0.11[b]	nil	1.10 ± 0.09	79.00 ± 2.84
early dark red	1.13 ± 0.08	nil	0.92 ± 0.12	80.10 ± 1.68
late dark red	1.15 ± 0.06	nil	1.18 ± 0.16	81.30 ± 2.26

[a] Average of three determinations. [b] ± standard deviation.

present study indicates a higher percentage of linoleic acid ($C_{18:2}$) and a lower percentage of oleic acid ($C_{18:1}$). Since linoleic acid formation is favored in cooler growing areas (Hilditch and Williams, 1964) and the biosynthesis of oleic acid is related to that of linoleic acid (Morris, 1970), differences between the Egyptian seed oil and both Sudanese and Indian cultivars might be attributed to both factors.

Elemental compositions of the roselle seed cultivars are shown in Table 4. The elements K, Na, Mg, and Ca were the major inorganic constituents, while Mn, Fe, and Zn were present in abundant amounts. Note that potassium was the major inorganic cation. The early dark red cultivar contained higher levels of K, Mg, and Ca than the light red and the late dark red cultivars, while Na was higher in the late dark red cultivar. Trace amounts of copper were found in roselle seed cultivars. The values reported for the elements Ca, Mg, Mn, Cu, and Fe were in good agreement with those reported by Al-Wandawi et al. (1984), while the values for Na and K were slightly different. Since some flours in the baking industry are very deficient in some element, in particular, calcium, the fortification of flours with roselle seed flour might improve their dietary properties.

The results of antinutritional factors and in-vitro protein digestibility of roselle seed cultivars are shown in Table 5. There are no distinct differences between cultivars in the content of tannins, hemagglutinin activity, phytic acid, and digestibility. Analysis of tannins in roselle seed revealed low levels (1.13–1.37%) compared with faba beans (1.57%), which has been reported by Ziena et al. (1991). Also, the present study indicates the absence of hemagglutinin activity in roselle seeds. Such activity has been found in some legumes, such as beans (Vander Poel et al., 1990; Egbe and Akinyele, 1990). Phytic acid analysis revealed the levels to be 0.92–1.18%, which is considerably lower than that reported for beans (Khalil and El-Adawy, 1994) and higher than that reported for faba beans (Ziena et al., 1991). The values of in-vitro protein digestibility by the trypsin–pancreatin system for roselle meals were close to that reported by Rahma (1983) for cottonseed flour, which had 80.19% digestibility by the pepsin–pancreatin system. No literature data were found regarding antinutritional factors and in-vitro digestibility of roselle seeds for comparison purposes.

The biological values of roselle seed protein cultivars

Table 6. Biological Values of Roselle Seed Protein

method of evaluation	light red	early dark red	late dark red
chemical score (CS), %	24.66	19.33	22.66
Mitchel essential amino acid index (MEAAI), %	61.08	59.05	61.10
FAO/WHO index, %	22.22	17.78	20.00
Mørup and Olesen's index (MOI)	105.78	107.30	109.70
Gaussian index (GI)	16.95	18.59	46.07
transformed Gaussian index (TGI)	65.14	66.35	79.56
first limiting amino acid	tryptophan	tryptophan	tryptophan
second limiting amino acid	methionine	methionine	methionine

Table 7. Osborne Classification of Roselle Seed Proteins (Percent)[a]

protein fraction	light red	early dark red	late dark red
albumins	24.02 ± 1.20[b]	22.6 ± 1.12	25.01 ± 1.86
globulins	43.79 ± 2.61	45.13 ± 2.45	41.90 ± 2.00
prolamins	8.21 ± 0.61	8.9 ± 0.82	7.20 ± 0.63
glutelins	14.50 ± 1.13	13.57 ± 0.98	18.00 ± 1.23
non-protein nitrogen	8.06 ± 0.11	8.65 ± 0.09	6.64 ± 0.03
residual protein	1.42 ± 0.06	1.15 ± 0.08	1.25 ± 0.07

[a] Average of three determinations. [b] ± standard deviation.

are shown in Table 6. These values depend, in large measure, on the relative proportions of the essential amino acids (Padhye and Salunkhe, 1979). The biological values of the late dark red cultivar were higher than those of the light red and early dark red cultivars for all measurements except the FAO/WHO index and the chemical score. The first and second limiting amino acids in roselle cultivar seed proteins, based on chemical scores, were tryptophan and methionine, respectively. It was reported, by Al-Wandawi et al. (1984), that the most limiting amino acids of the Iraqui roselle seed cultivar, based on the chemical score, were tryptophan (chemical score, 45.33), valine (52.54), isoleucine (55.34), and threonine (58.80).

The classification of roselle seed proteins according to the method of Osborne (1924) is given in Table 7. Globulins represent the major protein fraction (41.90–45.13%), followed by albumin (22.6–25.01%) and then by glutelin (13.57–18.00%). Prolamin was found to be the lowest fraction (7.20–8.90%). These data indicate that roselle seed proteins have characteristic high molecular weight molecules. No publications were found regarding the Osborne classification of roselle seed protein.

The UV spectra of roselle seed proteins in 1 M sodium chloride solution are given in Figure 1. Roselle seed proteins gave a sharp spectrum with a maximum absorbance at a range of 271–272 nm and a minimum at a range of 244–246 nm. The ratios of the absorbance at the maximum to that at the minimum was 1.42, 1.26, and 1.27 for light red, early dark red, and late dark red, respectively. It has been reported by Layne (1957) that proteins which did not conjugate with nucleic acid and other UV-absorbing impurities gave a ratio of 1.5. This criterion indicates that roselle flour proteins were contaminated with nucleic acid and other UV-absorbing impurities. The percentage of nucleic acid was calculated from the conversion tables, according to the procedure of Chaykin (1966). It was found that the light red cultivar contained 0.71% nucleic acid, while the early and late dark red cultivars contained 1.45% and 1.40% nucleic acid, respectively.

The functional properties of defatted roselle seed cultivars are given in Table 8. The meals absorb more water than oil; the water absorption capacity was about twice the oil absorption capacity. This may give an

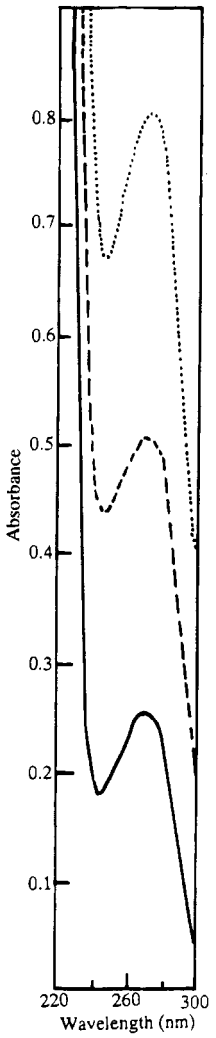

Figure 1. Ultraviolet (UV) absorption spectrum of roselle seed protein cultivars: (—) light red; (- - -) early dark red; (-·-) late dark red.

Table 8. Functional Properties of Roselle Seed Cultivars[a]

functional property	light red	early dark red	late dark red
water absorption, g of H_2O/100 g of sample	254 ± 4.60[b]	254 ± 4.90	220 ± 4.29
fat absorption, mL of oil/100 g of sample	159 ± 3.26	158 ± 3.12	125 ± 3.71
emulsification capacity, mL of oil/g of sample	46 ± 1.60	47 ± 1.80	53 ± 1.60
foam capacity, % volume increase	57 ± 2.30	56 ± 2.10	54 ± 1.93
foam stability, mL at			
30 min	54 ± 2.20	53 ± 2.14	51 ± 2.60
60 min	48 ± 2.16	49 ± 2.60	41.5 ± 2.51
90 min	43 ± 2.01	46 ± 1.98	30 ± 2.40
120 min	39 ± 2.15	43 ± 1.90	25 ± 1.95

[a] Average of three determinations. [b] ± standard deviation.

advantage to roselle seed meals in some foods, especially comminuted meat and baked doughs, which require meals with good water absorption capacity. In general, fat and water absorption capacities of the light red and early dark red samples were higher than those of the late dark red sample. These differences may or may not be attributed to different structural configurations of the proteins. There appear to be no published studies concerning the functional properties of roselle seed meals. Therefore, since cottonseed belongs to the same family and is considered to have modest functional properties (Rahma, 1983), the present data will be

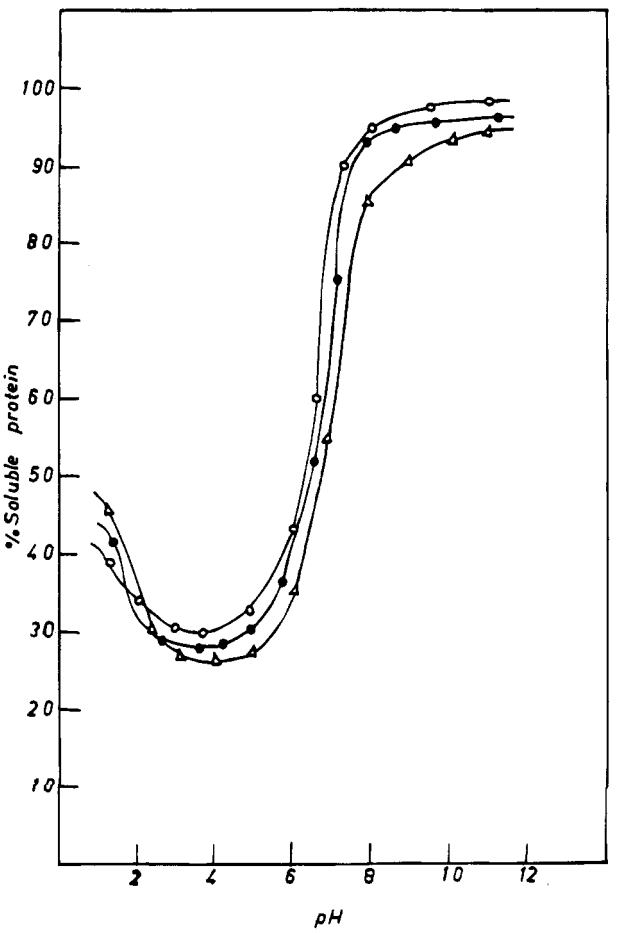

Figure 2. Protein solubility of roselle seed cultivars at different pH values: (●) light red; (○) early dark red; (△) late dark red.

discussed in relation to those of cottonseed. Rahma (1983) reported that the water and fat absorption capacities of cottonseed meal were 280 g of H_2O/100 g of flour and 220 mL of oil/g of flour, respectively. The present study indicates that the emulsification capacity of the roselle meals is lower than that of cottonseed meal (62.84 mL of oil/g of meal) as reported by Rahma (1983). Kinsella (1976) reported that emulsification capacity depends upon the amount of soluble protein in the solutions. The foam capacities (percent volume increase) of roselle meals were 57%, 56%, and 54% for light red, early dark red, and late dark red, respectively, compared with 49% for cottonseed meal as reported by Rahma (1983). Approximately the same trend was observed for foaming stability. In general, foam stability at room temperature tends to be decreased as the time increased (see Table 8). These decreases may be due to collapsing and bursting of the formed air bubbles (Kinsella, 1976).

The solubility of roselle protein was markedly influenced by pH (Figure 2). The solubility profile reveals its isoelectric point for future precipitation studies. A U-shaped pattern for roselle meals was observed, suggesting the existence of only one isoelectric point typical of other vegetable proteins such as soybean and groundnut. The results indicated that there were slight differences among the roselle cultivar proteins in protein solubility. Minimum solubility of all roselle cultivar proteins (<30%) occurred at pH 4, which might be the region of the isoelectric point, and solubility increased at more acid and alkaline pH values. At pH 1.5, approximately 37–43% of roselle protein nitrogen was

soluble, whereas 90–95% was soluble at pH 9. Theoretically, partial hydrolysis could account for some of the enhanced solubility at acid and alkaline pH values (De Witt, 1989; Kinsella, 1984).

It is worthwhile to indicate that there are no data in the literature of roselle seed protein solubility for comparative results.

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