Roselle Seeds: A New Protein Source

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Chemical analyses of the whole mature seeds of roselle (*Hibiscus sabdariffa* L.) revealed that the seeds contain protein (25.20%) and lipids (21.10%). Eighteen amino acids were detected and quantified for the first time. The most limiting amino acids were found to be tryptophan, valine, isoleucine, and threonine with chemical scores of 45.33, 52.54, 55.34, and 58.80, respectively, while the most abundant essential amino acids were found to be leucine, lysine, and phenylalanine. Oleic acid was the most predominant fatty acid followed by palmitic and stearic acids. Elemental analyses revealed that K, Na, Mg, and Ca were the major elements. Gossypol was found only as traces.

Roselle (*Hibiscus sabdariffa* L.) (Figures 1 and 2) is already a commerical crop. It is grown mainly for its pleasant red color calyxes, which are used mostly for making jam, jelly, and bottled drinks (Esselen and Sammy, 1973). On the other hand, the seed of roselle is reported to be edible (Wilson and Menzel, 1964), and the yield of seeds is reported to be 500–1000 kg/acre for an Indian cultivar (Mohiuddin and Zaidi, 1975). The review of literature revealed that, with the exception of a few controversial reports on seed oil fatty acid composition (Cornelius et al., 1970; Mohiuddin and Zaidi, 1975; Ahmad et al., 1979; Ahmed and Hudson, 1982), no data on the other seed components were reported. The present study reports on the amino acids, fatty acids, minerals, fiber, total carbohydrate, gossypol, and cyclopropenoids components of an Iraqi roselle cultivar known for its simple growth requirements and high calyxes and seed yield.

MATERIALS AND METHODS

Seed Sample. Mature sun-dried seeds were visually sorted for uniform size, color, and maturity.

Seed Flour. For seed flour preparation about 600 seeds were ground to a fine powder in an electric mill. The powder thus obtained was used for all subsequent analyses.

Moisture and Total Nitrogen. Moisture was determined by weight difference of a known amount of flour before and after drying at 105 °C for 18 h and expressed as percent moisture. Total nitrogen (N) was determined by the Kjeldahl method using 30% hydrogen peroxide in the digestion process as an oxidizing agent (Koch and McMeekin, 1924). Protein was calculated as $N \times 6.25$ and reported as percent protein. Amino acids, crude fiber, ash, gossypol, and cyclopropenoid compounds and minerals were determined as previously reported (Al-Wandawi, 1983).

Lipids and Fatty Acid Composition. Lipids were determined by extraction of roselle seed flour with redistilled hexane (1:20 w/v) in a Soxhlet extractor for 4 h. Fatty acid analysis was carried out by transesterification of about 25 mg of lipids with methanol–HCl reagent (procedure A). In procedure B, about 150 mg of lipids was saponified (in the dark and under a fine stream of high-purity nitrogen) for 5 min, and fatty acid esters were prepared as in procedure A. In procedure C, 25 mg of lipids was transesterified with 5 mL of BF_3–methanol complex reagent, the mixture was refluxed for 4 min, and the fatty acid esters were recovered by adding 5 mL of 2% aqueous NaCl solution and 10 mL of peroxide-free diethyl ether. In procedure D, the lipid samples were saponified (as in procedure B) and transesterified as in procedure C.

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Table I. Chemical Composition of Roselle Seed[a]

component	%[b] (dry wt basis)
protein ($N \times 6.25$)	25.20
lipids	21.10
crude fiber	16.30
starch[c]	2.25
ash	5.19
total carbohydrate[d]	26.64
moisture	5.57
gossypol: free	trace
bound	trace

[a] Whole mature sun-dried seeds. [b] Average of triplicate analyses. [c] Percentage of starch in defatted and sugar-freed seed flour. [d] Total carbohydrate is determined as 100 − (moisture + crude fiber + protein + lipids + ash).

Table II. Amino Acid Composition[a] of Whole Mature Seeds of Roselle

amino acid	g/16 g of nitrogen
Lys	5.56
His	1.87
Arg	10.75
Trp	0.68
Asp	10.16
Thr	2.94
Ser	4.37
Glu	23.45
Pro	3.29
Gly	5.08
Ala	4.09
Cys	2.50
Val	3.85
Met	1.35
Ile	3.21
Leu	6.31
Tyr	3.45
Phe	5.20

[a] Data are the average of duplicate analyses.

The fatty acid esters thus obtained by the above procedures (A–D) were analyzed on a Packard Model 419 gas chromatographic instrument and as previously reported (Al-Wandawi, 1983).

RESULTS

The average values of the major contents of the whole mature seeds of roselle are shown in Table I. The seeds contain protein (25.20%), lipids (21.10%), crude fiber (16.30%), starch (2.25%), ash (5.19%), total carbohydrate (26.64%), and moisture (5.57%). Gossypol, which is a toxic phenolic compound present in cotton seed, is found only as traces in roselle seed. Table II shows the average values of 18 amino acids detected in roselle seed for the first time. Glutamic acid, arginine, and aspartic acid were the major

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Figure 1. General view of roselle plants in the field.

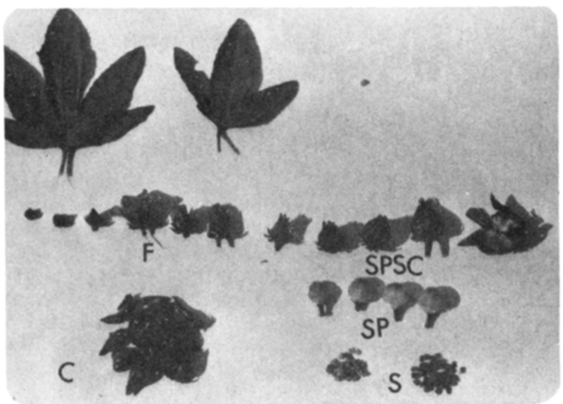

Figure 2. Close-up shows roselle flower (F), roselle calyxes (C), roselle seed pod surrounded by calyx (SPSC), roselle seed pod (SP), and roselle seeds (S).

Table III. Comparison of Chemical Score Values of Essential Amino Acids of Roselle Seeds with That of Okra Seeds

essential amino acids (EAA)[a]	roselle, Iraqi cultivar	okra	
		Emerald[b]	Ibtaira[b]
leucine	70.90	75.06	79.10
isoleucine	55.34	54.31	57.41
cysteine and methionine	67.54	71.93	76.34
valine	52.54	54.05	67.03
tryptophan	45.33	64.00	56.67
phenylalanine	92.86	76.43	70.36
lysine	83.00	117.91	133.13
histidine	89.05	84.76	87.62
threonine	58.80	60.00	70.00
tyrosine			

[a] Data for EAA in reference protein (whole egg) and the method used for calculation of EAA in roselle and okra seeds were reported by Osborne and Voogt (1978). [b] Al-Wandawi (1983).

amino acids and accounted for 23.45%, 10.96%, and 10.36% of the total recovered amino acids respectively. The essential amino acids leucine, lysine, and phenylalanine accounted for 6.43%, 5.67%, and 5.30% of the total recovered amino acids, respectively. Table III shows the chemical scores for the essential amino acids of the whole mature roselle seed. Table IV shows the fatty acid composition of seed oil of roselle. Oleic acid was found in the largest amount (73.14%), followed by palmetic acid (22.20%) and stearic acid (3.77%). The elemental composition is presented in Table V. The elements K, Na, Mg, and Ca were the major elements, while Fe, Zn, Sr, and Ni were present in abundant amounts in relation to quantities needed in the diet.

Table IV. Fatty Acid Composition[a,b] of Roselle (*H. sabdariffa* L.) Seed Oil

fatty acid	procedures[c]				mean	SD[d]
	A	B	C	D		
lauric	0.01	trace	0.02	0.01	0.01	0.005
myristic	0.22	0.40	0.36	0.30	0.32	0.08
palmitic	17.85	20.03	22.47	28.46	22.20	4.68
stearic	3.46	4.93	2.27	4.43	3.77	1.17
oleic	77.16	73.95	75.04	66.41	73.14	4.68
linoleic	0.05	0.23	0.64	0.06	0.25	0.28
arachidic	0.25	0.46	0.21	0.24	0.29	0.12

[a] Data are the average of duplicate analyses. [b] Values expressed as percent of total recovered fatty acids. [c] See the text for details of transesterification methods. [d] SD = standard deviation.

Table V. Mineral Compositions of *H. sabdariffa* Seed[a]

element	mg/100 g (dry wt basis)[b]
calcium	300
copper	<1
chromium	<1
iron	8.2
magnesium	580
manganese	3.5
nickel	1.0
potassium	1600
rubidium	<1
sodium	740
strontium	1.6
zinc	6.8

[a] Data are the average of three determinations on separate samples. [b] The seed flour was defatted and dried for 2 h at 105 °C before analysis.

DISCUSSION

Amino Acids. No data in literature on the amino acid composition of roselle seeds were available for comparative purposes. Therefore, since roselle is considered to be related to okra (Beattie, 1937) and because the importance of okra as a new protein source was reported (Karakoltsidis and Constantinides, 1975), the amino acid composition of roselle seed will be discussed in relation to that of okra seed. Thus the protein content ($N \times 6.25$) of roselle seed was found to be 25.20% in comparison to 20.58% reported for mature okra seed (Karakoltsidis and Constantinides, 1975). Amino acid composition and the chemical scores for the essential amino acids of the whole mature roselle seed are presented in Tables II and III, respectively. Data in Table III suggest that the most limiting amino acids (based on analytical evidence) are tryptophan (chemical score, 45.33), valine (52.54), isoleucine (55.34), and threonine (58.80). The chemical scores for phenylalanine, histidine, and lysine are 92.86, 89.05, and 83.00, respectively. The overall results suggest that roselle and okra seeds have almost the same limiting individual amino acids and that with the exception of lysine, which is higher in okra, and phenylalanine, which is higher in roselle, the chemical scores of other essential amino acids are very similar in both roselle and okra seeds.

Lipids and Fatty Acids. The pods of roselle contain oil-bearing seeds. The yield of oil from the seeds was reported to be 16–21% (Mohiuddin and Zaidi, 1975; Ahmad et al., 1979; Ahmed and Hudson, 1982). The results of the present study showed that the oil content of an Iraqi roselle cultivar was in the range of 20.5–21.7%. The results of the gas chromatographic analysis of the methyl esters of roselle fatty acids obtained (Table IV) revealed that oleic acid ($C_{18:1}$) by far was the most predominant fatty acid (66.41–77.16%) followed by palmitic acid ($C_{18:0}$;

17.85–28.46%) and stearic acid ($C_{18:0}$; 2.27–4.93%) of the total fatty acids. Lauric acid ($C_{12:0}$) and arachidic acid ($C_{20:0}$), which were present as minor fatty acids, have not been reported before. Linoleic acid ($C_{18:2}$), which was reported to represent 46.0% (Cornelius et al., 1970), 44.4% (Mohiuddin and Zaidi, 1975), 14.6% (Ahmad et al., 1979), and 30.1–37.5 (Ahmed and Hudson, 1982) of the total fatty acids, was found in this study to represent only about 1% of total recovered fatty acids. Cyclopropenoid fatty acids (CPFA), which are found in seed lipids of the order Malvales that comprises several important sources food for man and animals (Berry, 1980), were also reported to occur in roselle seed oil. Thus sterculic and malvalic acids were respectively 2.9% and 1.3% of total recovered fatty acids (Ahmad et al., 1979). However, recent investigation by Ahmed and Hudson (1982) on the fatty acid composition of seed oil from different seed collections (or cultivars) respresenting different growing areas reported that dihydrosterculic and malvalic acids were in the range of 1.0–1.6% and 0.4–2.0% of the total fatty acids, respectively. On the other hand, cotton seed, which represents one of the important sources of edible oil and feed for livestock, was reported to contain about 1% cyclopropenoid fatty acids (Berry, 1980); therefore, a comparison was made between roselle seed oil, okra seed oil, and cotton seed oil by using the Halphen test (Association of Official Analytical Chemistry, 1975. The data revealed that roselle seed oil contains cyclopropenoid materials half the amount present in okra seed and one-fifth that found in cotton seed. Gossypol, the phenolic compound found in cotton seed and known to cause undesirable physiological effects on nonruminants (Pons, 1977), was found in roselle seed only as traces.

Other Components. Crude fiber, ash, total carbohydrates, starch, and minerals were quantitated; but no data in the literature were available for the purpose of comparison. However, if the results are discussed in relation to okra, the results suggest that in roselle seed, crude fiber, total carbohydrates, and ash content were 16.30, 96.64, and 5.19%, respectively, in comparison to 27.3, 23.5, and 4.7% for okra seed. Both roselle and okra seeds were contained only traces of free and total gossypol, which is well below the tolerence level for this compound reported in the literature (Pons, 1977). Finally, animal feeding studies for the nutritional and safety of roselle seeds are in progress, and the preliminary results of this survey have been reported recently (Farjou et al., 1983).

Registry No. K, 7440-09-7; Na, 7440-23-5; Mg, 7439-95-4; Ca, 7440-70-2; Fe, 7439-89-6; Zn, 7440-66-6; Sr, 7440-24-6; Ni, 7440-02-0; Mn, 7439-96-5; starch, 9005-25-8; gossypol, 303-45-7.

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Antioxidative Effect of Nitrite in Cured Meat Products: Nitric Oxide–Iron Complexes of Low Molecular Weight

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The activity of iron–nitric oxide complexes of low molecular weight toward lipid peroxidation was studied in a linoleate–β-carotene model system. Cysteine, and especially cysteine in the presence of iron ions, caused a rapid increase in β-carotene destruction. Compared with cysteine, the complex of cysteine–Fe–NO had no prooxidant effect. Addition of the cysteine–Fe–NO complex to the model containing cysteine–Fe^{2+} or hemin inhibited β-carotene oxidation by 77% and 86%, respectively. The activity of hemin–NO on lipid oxidation was studied and compared under the same experimental conditions and concentrations as that of hemin. In the range of tested concentrations (1.8–9.0 μM), hemin acted as a prooxidant toward carotene oxidation and hemin–NO as an antioxidant. The antioxidative effect of hemin–NO was maintained even in the presence of lipoxygenase and myoglobin. The possible role of nitric oxide on the stabilization of lipid peroxidation in muscle foods is discussed.

Lipid peroxidation in raw muscle food is catalyzed by both a nonenzymatic and an enzymatic process. Heme proteins and non heme iron complexes have been implicated as prooxidants in meat lipid peroxidation (Tappel, 1962; Liu, 1970; Sato and Hegarty, 1971; Koizumi et al., 1976; Pearson et al., 1977; Igene et al., 1979). It was discovered more recently that muscle tissue of both warm- and cold-blooded animals contains a membrane-associated enzyme that catalyzed lipid peroxidation, the microsomal system required for the activation NADPH or NADH and iron complexed to ADP (McDonald et al., 1979). In cooked meat products non-heme iron of low molecular weight compounds are the main prooxidants (Sato and Hegarty, 1971; Igene et al., 1979).

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