NOTE

Some Nutritional and Functional Properties of Karkade (*Hibiscus sabdariffa*) Seed Products

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Hibiscus sabdariffa (Roselle or Karkade) is a popular plant (belonging to the family Malvceae) in some Middle East countries. It is used mainly for its fiber and the calyces of the flower, which are used in cold and warm beverages. Karkade seeds contain considerable amounts of proteins (25–25.2%) and oil (20.3–21%) (Al-Wandawi et al 1984, Abu-Tarboush 1995). Nutritional properties of oil and protein of the whole seeds have been studied by Al-Wandawi et al (1984). Some antinutritional factors in the defatted flour and protein isolate of karkade were studied by Abu-Tarboush and Ahmed (1996). However, the nutritional properties of karkade seed products, as well as their functional properties, have not yet been studied. Therefore, the objectives of this study were to investigate the nutritional and functional properties of some karkade seed products: karkade defatted flour (KDF), protein concentrate (KPC), and protein isolate (KPI).

MATERIALS AND METHODS

Karkade Seed Preparation

Karkade seeds of the variety Al-Rahad were obtained from western Sudan. After cleaning, seeds were milled using an electric grinder to pass through a 0.30-mesh screen. Oil was extracted with *n*-hexane according to the method of El-Tinay et al (1988a). Defatted flours (KDF) were stored in a glass jar at 5°C until used.

Karkade Protein Concentrates and Isolates

Karkade protein concentrates and isolates (KPC and KPI) were obtained from defatted karkade flour as reported by El-Tinay et al (1988b).

Proximate Analysis and Amino Acids

Proximate composition of karkade seed product was determined according to standard methods (AOAC 1995). Total carbohydrates were calculated by difference. Amino acid analysis was performed using reverse phase-high pressure liquid chromatography (LC-10 AD, Shimadzu Corp., Kyoto, Japan) following hydrolysis of the sample with 6*N* HCl at 110°C for 24 hr (AOAC, 1995). Tryptophan was determined spectrophotometrically according to the method of Devaries et al (1980) after KOH hydrolysis of the sample (AOAC 1995).

Protein Analysis

The multienzyme method of AOAC (1995) was used to determine in vitro protein digestibility (IVPD). Computed protein efficiency ratio (C-PER) was obtained by using data from IVPD and essential amino acid composition of the protein according to standard methods (AOAC 1995). Animal Nutrition Research Council (ANRC) casein was included for comparison.

Physiochemical Characteristics and Fatty Acids

Specific gravity (25°/25°C), refractive index (27°C), peroxide, iodine, and saponification values were determined according to standard methods (AOAC 1995). Fatty acid composition was determined by gas-liquid chromatography (HP–5840A) equipped with a flame ionization detector. Fatty acid methylesters were prepared according to standard methods (AOAC 1995).

Functional Properties

The functional properties of karkade seed products (KDF, KPC, and KPI) were determined. Water and oil absorption capacities were determined according to the method of Lin et al (1974) with minor modification (Pardes-López and Ordorica-Falomir 1986). Bulk density was determined by the method of Wang and Kinsella (1976). Emulsifying activity was measured according to the procedure of Wang and Kinsella (1976). Protein solubility profile was determined according to the method described by Bryant et al (1988) with minor modifications.

Statistical Analysis

Data were analyzed using analysis of variance (Steel and Torrie 1980) and SAS programs (SAS 1986).

RESULTS AND DISCUSSION

Proximate Analysis

Karkade whole seed flour (KWSF) contained high amounts of protein (26.48%), crude oil (20.13%), carbohydrate (43.21%), and ash (4.83%). Such results were in agreement with the findings of Al-Wandawi et al (1984). The karkade protein isolate (KPI) showed a higher protein content (88.15%) than the other karkade products (defatted flour 50.63% and protein concentrate 62.24%). However, the protein content of KPI was less than that of *Moringa peregrina* protein isolate (97.8%) and soybean protein isolate (94.0%) reported by Al-Kahtani and Abou-Arab (1993).

Amino Acids

Amino acid compositions of KDF, KPC, and KPI are shown in Table I. The amino acid profiles of KPC and KPI were similar to that of KDF. Therefore, extraction of karkade proteins appeared to have no adverse affect on the amino acid profiles of KPC and KPI. Arginine, aspartic acid, and glutamic acid were found in high amounts in karkade seed products. The amino acid compositions of karkade seed products in this study were in agreement with the findings of Al-Wandawi (1984) for the karkade whole seed. Karkade is considered to be related to okra and results from this study on amino acid composition of karkade seed products were in agreement with the finding of Al-Wandawi et al (1983) for okra seeds.

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TABLE I
Amino Acid Composition of Karkade Seed Products (g/100 g of protein)

	Karkade Products[a]			Reference Protein[b]		
	KDF	KPC	KPI	Infants	Preschoolers	Adults
Essential Amino Acids						
Lysine	5.12 ± 0.198	5.34 ± 0.212	5.10 ± 0.407	6.6	5.8	1.6
Threonine	2.67 ± 0.592	2.79 ± 0.028	2.41 ± 0.282	4.3	3.4	0.9
Valine	4.57 ± 0.143	4.63 ± 0.099	4.55 ± 0.018	5.5	3.5	1.3
Methionine (M)	1.44 ± 0.039	1.28 ± 0.183	1.48 ± 0.014			
Cystine (C)						
M + C				4.2	2.5	1.7
Isoleucine	2.96 ± 0.306	3.09 ± 0.077	3.01 ± 0.168	4.6	2.8	1.3
Leucine	5.58 ± 0.568	5.75 ± 0.106	5.92 ± 0.499	9.3	6.6	1.9
Phenylalanine (P)	5.96 ± 0.138	5.50 ± 0.085	5.99 ± 0.293			
Tyrosine (T)	2.76 ± 0.252	3.25 ± 0.233	2.72 ± 0.259			
P + T	8.72	8.75	8.71	7.2	6.3	1.9
Histidine	1.91 ± 0.098	2.03 ± 0.233	1.80 ± 0.180	2.6	1.9	1.6
Tryptophan	0.76 ± 0.038	0.75 ± 0.208	0.76 ± 0.108			
Nonessential Amino Acids						
Arginine	10.65 ± 0.209	11.40 ± 0.367	9.58 ± 0.261			
Aspartic acid	10.87 ± 0.514	10.20 ± 0.021	10.28 ± 0.294			
Glutamic acid	23.31 ± 0.607	23.99 ± 0.049	24.00 ± 0.585			
Proline	3.91 ± 0.121	3.99 ± 0.169	4.30 ± 0.200			
Glycine	5.10 ± 0.172	4.79 ± 0.085	5.09 ± 0.211			
Alanine	5.24 ± 0.257	4.42 ± 0.127	5.56 ± 0.057			
Serine	4.88 ± 0.212	4.76 ± 0.049	4.70 ± 0.211			

[a] KDF = karkade defatted flour , KPC = karkade protein concentrate , KPI = karkade protein isolate.
[b] FAO/WHO/UNU 1985.

TABLE II
Physiochemical Characteristics of Crude Karkade Seed Oil

	Mean ± Standard Deviation
Refractive index	1.47 ± 0.000
Specific gravity	0.92 ± 0.002
Saponification value	194.85 ± 0.7919
Iodine value	81.45 ± 0.3818
Peroxide value	11.86 ± 2.2839

When compared to to reference proteins (FAO/WHO/UNU 1985), only phenyalnine+tyrosine of the KPI met the required levels for infants; phenyalnine+tyrosine, valine, isoleucine, and histidine are the only amino acids that met the requirements for preschool children. The data also indicated that karkade proteins contained adequate amounts of most essential amino acids except methionine for adults, and methionine appeared to be the limiting amino acid.

Protein Analysis

IVPD and C-PER of KDF, KPC, KPI, and casein were 82.14 and 1.70, 85.12 and 2.0, 87.09 and 2.06, and 88.95 and 2.50, respectively. Al-Kahtani (1995) reported IVPD of 76.32, 87.27, 88.45%, for soybean products, and 69.72, 80.13, and 82.48% for *M. peregrina* products, respectively. High IVPD indicates a high percentage of readily digested protein. The C-PER for karkade products were lower than that of casein and this could be due to the low content of some essential amino acids. Bryant et al (1988) reported C-PER of 2.17 and 2.14 for okra and soybean protein isolates, respectively.

Physiochemical Characteristics and Fatty Acids

The physiochemical characteristics of karkade crude oil are presented in Table II. The oil was high in saponification number but relatively low in iodine value. Values for refractive index and specific gravity are within the range values reported by Formo et al (1979) for most crude vegetable oils. Iodine value indicates the degree of unsaturation while saponification value reflects the average molecular weight. The peroxide value (an indicator of fat oxidation) was slightly high for crude oil, and this might be due to the conditions of handling and shipping of the seeds from their home country to Saudi Arabia.

TABLE III
Fatty Acid Composition of Crude Karkade Seed Oil

	% Mean ± Standard Deviation
Myristic (C14:0)	0.10 ± 0.000
Palmitic (C16:0)	19.98 ± 0.305
Stearic (C18:0)	5.80 ± 0.489
Arachidic (C20:0)	0.83 ± 0.131
Behenic (C22:0)	0.29 ± 0.068
Lignoceric (C24:0)	0.51 ± 0.170
Palmitoleic (C16:1)	0.26 ± 0.071
Oleic (C18:1)	36.77 ± 2.261
Linoleic (C18:2)	33.41 ± 2.521
Eicosatrienoic (C20:3)	0.34 ± 0.000
Erueic acid (C22:2)	1.71 ± 0.141

Table III shows the fatty acid composition of crude karkade seed oil. Palmitic acid was the major saturated fatty acid, while oleic acid followed by linoleic acid were the major unsaturated fatty acid. Al-Wandawi et al (1984) found that Iraqi karkade cultivars contained high amounts of oleic acid (66.41–77.16%) followed by palmitic acid (17.85–28.46%), and stearic acid (2.27–4.93%), but linoleic acid represented only ≈1% of total recovered fatty acids. Ahmed and Hudson (1982) reported that crude karkade seed oil (Al-Rahad variety) contained linoleic (37.4%), oleic (34.6%), and palmitic (20.5%) acids in higher amounts than other fatty acids. Karkade is considered to be related to okra. Okra seeds contain 33.53, 29.29, and 31.48% palmitic, oleic, and linoleic acids, respectively (Karakoltsidis and Constantinides 1975). The fatty acid profile of karkade can indicate its importance for human consumption as well as its suitability for vegetable oil processing.

Functional Properties

Results of several functional tests on karkade products are shown in Table IV. The product of KPI was the highest among other products in terms of water and oil absorption capacities. Water and oil capacities of some seeds were reported by several researchers (Wang and Kinsella 1976, Okezie and Bello 1988, Paredes-López et al 1991, Al-Kahtani and Abou-Arab 1993). The degree of water retention is considered to be useful as an indication of performance in several food formulations, especially those involving dough handling (Circle and Smith 1972). Water absorp-

TABLE IV
Functional Properties of Karkade Seed Products[a–c]

	KDF	KPC	KPI
Water absorption capacity (mL/g of protein)	2.43 ± 0.1155A	2.13 ± 0.153B	2.47 ± 0.058A
Oil absorption capacity (mL/g)	2.06 ± 0.1150B	1.93 ± 0.058B	2.77 ± 0.153A
Bulk density (g/mL)	0.37 ± 0.0121C	0.64 ± 0.009A	0.54 ± 0.008B
Emulsifying activity (%)	51.97 ± 0.9500C	54.83 ± 0.289B	56.83 ± 0.666A

[a] Means of three determinations ± standard deviation; adjusted to pH 7.0.
[b] Means followed by the same letter within a row are not significantly different ($P \le 0.05$).
[c] KDF = karkade defatted flour , KPC = karkade protein concentrate, KPI = karkade protein isolate.

tion capacity of isolates may be affected by conformation and environmental factors. Conformational changes in the protein molecules may expose previously enclosed amino acid side chains, thereby making them available to interact with water. Oil absorption of food products is an important functional property because it improves mouthfeel and flavor retention (Kinsella 1976). The bulk density of KPC was denser than that of KDF and KPI (Table IV). Al-Kahtani and Abou-Arab (1993) reported bulk density for soybean protein isolate (0.678) and *M. peregrina* protein isolate (0.677). The bulk density of cotton seed defatted flour was 0.29 g/mL (Rahma and Narasinga Rao 1983), whereas the bulk density of winged bean defatted flour and soybean defatted flour were 0.45 and 0.46 g/mL, respectively (Dench 1982). Bulk density depends on combined effects of interrelated factors (intensity of attractive interparticle forces, particle size, number of contact points) (Peleg and Bagley 1983). It also depends on type of solvents used to extract the protein products (Wang and Kinsella 1976) and on method of drying (Bryant et al 1988).

Emulsifying activities of karkade products are shown in Table IV. The emulsifying activity of KPI was significantly higher ($P \le 0.05$) than that of KDF and KPC. The emulsion activity of KPI was similar to that of Chinese rapeseed protein isolate (Xu and Diosady 1994), whereas it was higher than that of soybean protein isolate and lower than that of chickpea protein isolate (Paredes-López et al 1991). Proteins constitute an important group of emulsifiers because they reduce interfacial tension, form rigid interfacial films, and possess charged groups (McWatters and Cherry 1981).

The solubility profiles of KDF, KPC, and KPI were comparable to that of plant proteins reported by several researchers (Wolf 1970, Lawhon and Cater 1971, Wang and Kinsella 1976, Narayana and Narasinga Rao 1982, King et al 1985, Ahmed and Rammanathan 1988, Al-Kahtani and Abou-Arab 1993). Minimum protein solubility for all products occurred between pH 4 and 4.5 (the approximate isoelectric point for the proteins). On the other hand, maximum protein solubility was observed in the alkaline and acid ranges. KPI was more soluble between pH 2 and pH < 4 than KDF and KPC. However, KDF and KPC were more soluble between pH 6 and pH10. Protein solubility at various pH values may serve as a useful indicator of how well protein isolates will perform when they are applied to food systems and also the extent of protein denaturation due to heat or chemical treatment. The good solubility of Karkade protein isolates might contribute beneficial functional properties.

In conclusion, Karkade seeds might be potential sources for protein and oil, which would add some economic value to the existing uses of the plant and expand its cultivation.

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