

Effect of Soaking, Sprouting and Cooking on Chemical Composition, Bioavailability of Minerals and *in vitro* Protein Digestibility of Roselle (*Hibiscus sabdariffa* L.) Seed

Abu El Gasim A. Yagoub¹, Mohammed A. Mohammed¹ and Asma A. Abu Baker²

¹Faculty of Agriculture, University of Zalingei, P.O. Box 06, Sudan

²Faculty of Natural Resources and Environmental Sciences, University of Juba, Sudan

Abstract: Chemical composition, bioavailability of minerals and *in vitro* digestibility of proteins in karkade seed (*Hibiscus sabdariffa* L.) as affected by soaking, sprouting and cooking were studied. The different methods showed varied deviation of nutrients and antinutrients from the raw seeds. Sprouting and cooking significantly increased protein content and decreased starch and soluble carbohydrates levels. K, Na and all the trace elements studied were decreased by processing methods. Cooking was more effective in improving bioavailability of minerals studied than soaking or sprouting. Total polyphenols reduced more by cooking, while phytic acid did not change significantly by processing. *In vitro* protein digestibility was significantly reduced by all processing methods, with soaking gave the highest percentage of reduction and cooking the lowest percentage. The results also indicated that domestic processing methods changed total acidity and fat acidity as well as N solubility in water and 1 M NaCl. Amino acid profile of the karkade seed indicated that sulfur amino acids and threonine are the limiting amino acids. With respect to FAO pattern, considerable proportion of the essential amino acids were retained on processing, except for lysine on soaking and sprouting and sulfur acids on sprouting and cooking.

Key words: *Hibiscus sabdariffa* seed, domestic processing, chemical composition, *in vitro* digestibility of protein, amino acids profile, bioavailability of minerals

Introduction

Roselle (*Hibiscus sabdariffa* L.), locally known as karkade, is an annual herb that belongs to family malvaceae. The plant originated in tropical Africa (Mc Clean, 1973). In Sudan, the plant is mainly grown under rain fed conditions in the western part of the country. Two red cultivars are grown in the Sudan; Elfashir and Elrahad. The large thick calyces; not the seeds; are mostly used in the preparation of cold and hot beverages. Karkade; as an oilseed; attracting attention as a potential provider of good quality protein and fat for the future (El-Adawy and Khalil, 1994; Abu Tarboush *et al.*, 1997). Protein-calorie deficiency is now viewed as the major nutritional problem in most developing countries including Sudan. Due to the high price of animal proteins, much importance is now placed on plant foods as a source of proteins in all the developing countries. Karkade is regarded as both cash and food crop in some parts of the Sudan. The seeds which are unpalatable in their native state rendered consumable by the people in western Sudan. Whereas, some of the people roast the seeds and other boil them before consumption as food. Other communities of western Sudan ferment the seeds.

Processing methods, such as soaking, sprouting and cooking has been reported to improve the nutritional and functional properties of plant seeds (Jirapa *et al.*, 2001; Yagoub and Abdalla, 2007). Therefore, investigation of

the effects of different home processing methods on the nutritive value may increase utilization of karkade seeds in the food system and hence participate in finding solution for protein problems.

The aim of this study was to assess the efficiency of processing methods, such as soaking, sprouting, cooking on the changes in chemical composition of karkade seed and hence the effect on *in vitro* protein digestibility and bioavailability of minerals.

Materials and Methods

Karkade seeds (*Hibiscus sabdariffa* L.) purchased from the local market in Nyala (South Darfur, Sudan) were employed for this study.

Preparation of soaked, sprouted and cooked karkade seeds:

Karkade seeds were sorted out and cleaned. One batch of the raw seeds was milled (0.4 mm sieve). An other batch was cooked in boiling distilled water until softened on squeeze between fingers (~ 20 minutes). The cooked seeds were drained, dried at 70°C and milled to pass 0.4 mm sieve. The last batch was soaked in a solution of sodium azide (0.005 M) for 12 hours. Part of the soaked seeds, after draining water, was dried and milled as before. The other part was transferred to two wet kenaf sacks and left to germinate at room temperature (~ 27°C) for two different intervals (24 and 48 hours). By the end of the sprouting periods the seeds

were removed from the sacks, dried and milled as before. All prepared flours from karkade seed samples (raw and processed) were bottled and kept at 4°C until further analysis.

Chemical analysis

Proximate analysis: Lipids, ash, total carbohydrates and total nitrogen (micro-Kjeldahl) were determined according to AOAC (1990). Protein was calculated as $N \times 6.25$. Moisture content was determined by drying samples at 105°C overnight (AOAC, 1990) and then dry matter was calculated. Crude fiber content was determined by acid/alkali digestion method of Southgate (1976).

Starch and soluble carbohydrates: A starch and soluble carbohydrates content of samples were determined according to the method described by Yagoub *et al.* (2004). The 10% ethanol sample extract and the residue remaining were hydrolyzed with 1 M H_2SO_4 . The glucose content from the starch and soluble carbohydrates hydrolysates were quantified using the Dubois *et al.* (1956) method. The starch was expressed as:

$$\text{Starch \%} = \text{glucose \%} \times 0.9$$

pH: pH values karkade seed and furundu flours were measured directly in a homogenate prepared with 10% (w/v) flour in distilled water, using a glass electrode pH-meter (HANNA-pH 210).

Total titratable acidity: Total titratable acidity was estimated according to AOAC (1990).

Fat acidity: Fat acidity was determined according to the method described by Parades-Lopez and Harry (1989).

Total minerals: Minerals were determined in samples' extracts prepared by the dry-ashing method as described by Pearson (1981). The amounts of zinc, manganese, copper and ferrous were determined according to the analytical method of atomic absorption spectroscopy (Perkin-Elmer 1100 V). Phosphorus was determined by the ammonium molybdate/ammonium vanadate method of Chapman and Pratt (1968). Calcium and magnesium were determined by the titration method of Chapman and Pratt (1961). Sodium and potassium were determined according to AOAC (1990) using flame photometer (Corning EEL).

HCl-extractability of Minerals (Bioavailability): Minerals in the samples were extracted by the method described by (El Maki *et al.*, 2007). One gram of the sample was extracted using 10 mL of 0.03 N HCl with shaking at 37°C for 3 hours. Then, the extract was filtered and the

clear supernatant was dried at 100°C, incinerated at 550°C for 4 hours. Thereafter, the samples were cooled and 5 ml of HCl were added and heated gently on a sand bath for 10 minutes. After cooling samples were diluted to 100 ml. Individual elements were determined as before. Extractability of each element was calculated as a percentage of the total amount of the element.

Phytic acid: Phytic acid was determined by the method applied by Wheeler and Ferrel (1971). A standard curve of ferric nitrate was plotted. Phytate phosphorus was calculated from the standard curve assuming a 4:6 Fe to P molar ratio.

Total polyphenols: Total polyphenols present in raw and processed karkade seeds were determined using the Prussian Blue assay, as described by Price and Butler (1977). Tannic acid was used as a reference standard.

In vitro protein digestibility: *In vitro* protein digestibility of the samples was measured according to the method described by Monjula and John (1991), in which a pepsin digestion method was used in the determinations. The digestible protein was analyzed for nitrogen using the micro Kjeldahl procedure (AOAC, 1990) and expressed as a percent of the total N.

Amino acid analysis: Amino acid composition of samples was measured on hydrolysates using amino acid analyzer (Sykam-S7130) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stein (1963). Two hundred milligrams of sample were taken in hydrolysis tube. Then 5 mL 6 N HCl were added to sample into the tube, tightly closed and incubated at 110°C for 24 hours. After incubation period, the solution was filtered and 200 mL of the filtrate were evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one milliliter of 0.12 N, pH 2.2 citrate buffer, the same as the amino acid standards. Aliquot of 150 μ L of sample hydrolysate was injected in a cation separation column at 130°C. Ninhydrine solution and an eluent buffer (The buffer system contained solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 ml/min. The buffer/ninhydrine mixture was heated in the reactor at 130°C for 2 minutes to accelerate chemical reaction of amino acids with ninhydrine. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

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Table 1: Effect of Soaking, Sprouting and Cooking on Chemical Composition of Karkade Seed (percent*)

Sample	Protein	Oil	Crude fiber	Ash	NFE**	Starch	Soluble carbohydrates
Raw seed	32.283 ^c (0.012)	19.900 ^d (0.185)	22.297 ^c (0.306)	5.027 ^a (0.105)	21.103 ^a (0.101)	2.387 ^a (0.060)	10.173 ^a (0.061)
Soaked seed	32.490 ^a (0.017)	20.627 ^b (0.090)	22.623 ^c (0.205)	4.517 ^b (0.086)	21.500 ^b (0.557)	2.373 ^{ab} (0.031)	9.860 ^b (0.151)
Sprouted seed: 24 h	32.430 ^{ab} (0.078)	21.087 ^a (0.158)	23.307 ^b (0.180)	4.540 ^b (0.121)	20.500 ^c (0.794)	2.297 ^{bc} (0.032)	9.777 ^b (0.081)
48 h	32.343 ^{bc} (0.046)	20.800 ^b (0.100)	24.377 ^a (0.216)	4.537 ^b (0.064)	21.277 ^c (0.928)	2.253 ^c (0.025)	9.830 ^b (0.120)
Cooked seed	32.330 ^c (0.052)	20.367 ^c (0.145)	24.473 ^a (0.114)	4.427 ^b (0.064)	19.660 ^b (0.114)	2.303 ^{bc} (0.045)	9.700 ^b (0.100)

*: Means of three replicate samples. Values in parentheses are standard deviations. Means followed by the same letter are insignificantly different according to DMRT ($p \leq 0.05$). Calculations on free moisture basis. **: NFE; Nitrogen free extract

Table 2: Mineral composition of the raw and processed karkade seeds

Sample*	Na %	K %	Ca %	Mg %	P %	Zn mg/100g	Cu mg/100g	Mn mg/100g	Fe mg/100g
Karkade seed	0.129 ^a (0.029)	1.481 ^a (0.020)	0.064 ^{ab} (0.002)	0.121 ^a (0.007)	0.549 ^a (0.001)	10.283 ^a (0.071)	9.497 ^a (0.131)	20.164 ^a (0.150)	23.353 ^a (0.078)
Soaked seed	0.105 ^b (0.001)	1.289 ^b (0.010)	0.063 ^b (0.001)	0.115 (0.004)	0.552 ^a (0.003)	8.852 ^c (0.107)	8.056 ^b (0.068)	17.399 ^b (0.109)	21.500 ^b (0.557)
Sprouted seed: 24 h	0.106 ^b (0.001)	1.291 ^b (0.008)	0.065 ^{ab} (0.004)	0.120 ^a (0.006)	0.550 ^a (0.006)	8.018 ^b (0.028)	7.053 ^c (0.231)	15.210 ^d (0.066)	20.500 ^{bc} (0.794)
48 h	0.106 ^b (0.001)	1.291 ^b (0.008)	0.065 ^{ab} (0.004)	0.120 ^a (0.006)	0.552 ^a (0.007)	8.018 ^d (0.028)	7.053 ^c (0.204)	15.613 ^c (0.032)	21.277 ^b (0.928)
Cooked seed	0.105 ^b (0.002)	1.291 ^b (0.100)	0.074 ^a (0.009)	0.114 ^a (0.021)	0.552 ^a (0.004)	8.896 ^c (0.074)	6.933 ^c (0.208)	15.130 ^d (0.431)	9.660 ^c (0.114)

*: Means of triplicate samples. Values in parentheses are standard deviations. Means followed by the same letter are insignificantly different according to DMRT ($p \leq 0.05$). Calculations on free moisture basis

Statistical analysis: Means from triplicate determinations were analyzed using analysis of variance (ANOVA) to determine the significance differences (Snedecor and Cochran, 1987) followed by Duncan's Multiple Range Test ($p \leq 0.05$) when the F-test demonstrated significance (Duncan, 1955).

Results and Discussion

Chemical composition: Table 1, shows results of proximate composition as well as starch and soluble carbohydrates of karkade seed as affected by domestic processing methods. Soaking (12 hours), sprouting (24-48 hours) of presoaked seeds and cooking of seeds in water resulted in significant ($p \leq 0.05$) differences of nutrients from the raw seeds. Protein, oil, crude fiber and total carbohydrates are almost significantly ($p \leq 0.05$) increased, while ash, starch and soluble carbohydrates are decreased. The changes observed are due to leaching of soluble components in to soaking and cooking water and as a consequence of enzyme activities during sprouting (Obizoba and Ath, 1992; Saikia *et al.*, 1999; Yagoub and Abdalla, 2007).

Minerals composition: Results revealed that karkade seed has considerable amount of potassium. Potassium content of the raw seed was 1.481% (Table 2), which decreased significantly ($p \leq 0.05$) to

1.289 and 1.291% after soaking and cooking, respectively. This loss in potassium may be attributed to its leaching out into soaking and cooking water. Sprouting of the presoaked seeds did not affect the content of potassium. Sodium followed the same trend observed for potassium. Other major elements did not affect significantly by processing. On the other hand, Zinc, copper, manganese and iron content of karkade seed were 10.28, 9.50, 20.16 and 23.35 mg/100g. All trace elements studied were decreased significantly ($p \leq 0.05$) on soaking and further on sprouting, which might be ascribed to loss in soaking medium. Many workers reported reduction in major and trace elements in the soaked and cooked grains (Saikia *et al.*, 1999; Duhan *et al.*, 2000; El Maki *et al.*, 2007). As observed, cooking resulted in more significant loss in trace elements than soaking, which could be attributed to effect of heat on changing the insoluble chemical species of some trace elements into soluble ones; thus extracted more in the cooking water.

Bioavailability of minerals: The bioavailability of Na, K, Ca, Mg and P for the raw karkade seed were 97.17, 62.06, 16.93, 6.57 and 39.13%, respectively (Table 3). HCl-extractability of K was improved by cooking to 86.10%, but soaking and sprouting reduced it.

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Table 3: Effect of domestic processing on bioavailability (%) of some selected elements of karkade seed

Sample	Na	K	Ca	Mg	P	Zn	Cu	Mn	Fe
Karkade seed	97.20	62.96	16.93	6.57	39.13	30.12	8.79	21.70	7.31
Soaked seed	94.20	22.16	10.68	6.73	24.82	19.97	6.34	10.17	4.42
Sprouted seed:									
24 h	93.30	30.58	26.06	6.64	24.55	18.89	4.92	12.60	6.79
48 h	94.39	18.87	25.50	6.54	32.07	28.10	13.3	22.10	7.06
Cooked seed	94.20	86.10	21.22	6.68	39.67	25.88	3.30	42.12	10.07

*Means of duplicate samples

Table 4: Effect of soaking, sprouting and cooking on phytic acid (mg/100 g), total polyphenols (mg/100 g) and *in vitro* protein digestibility (%) of karkade seed

Sample	Phytic acid	Total polyphenols	<i>In vitro</i> protein digestibility
Karkade seed	888.333 ^a (3.512)	878.33 ^a (9.860)	51.465 ^a (0.654)
Soaked seed	889.333 ^a (4.618)	879.67 ^a (3.510)	29.337 ^d (0.586)
Sprouted seed: 24h	886.000 ^a (2.000)	884.33 ^a (2.080)	31.273 ^c (0.653)
48h	888.000 ^a (4.000)	880.67 ^a (1.829)	28.704 ^d (0.655)
Cooked seed	885.667 ^a (7.094)	854.00 ^b (6.000)	46.833 ^b (1.731)

*: Means of triplicate samples. Values in parentheses are standard deviations. Means followed by the same letter are insignificantly different according to DMRT ($p \leq 0.05$). Calculations on free moisture basis

Extractability of Ca was improved by almost all processing methods. None of the processing methods studied improved bioavailability of P or Mg. The changes observed in bioavailability of some major elements may be ascribed to the slight decrease in phytic acid (Table 4). Considering that P, Ca, Mg and K represent the elements of the molecular structure of phytic acid and phytin (Ryden and Selvendran, 1993). Results of the study showed that HCl-extractability of Mn, Cu, Zn and Fe were 21.7, 8.79, 30.12 and 7.31%, respectively (Table 3). Soaking profoundly decreased bioavailability of Mn to half that present in the raw seed and sprouting for 48 h of the soaked seeds increased it. Cooking has doubled extractability of Mn (42.12%) and that of Fe increased to 10.07%. Moreover, Cu improved by sprouting for 48 h reaching 13.30%, but Zn bioavailability did not improved by any of the processing methods studied.

Total polyphenols and phytic acid: Cooking decreased significantly ($p \leq 0.05$) polyphenol content inherent in the karkade seed but other processing methods studied did not (Table 3). Heat degradation, leaching out effects, change in chemical reactivity and formation of insoluble complexes might be the factors that resulted in the significant reduction of these antinutrients by cooking (Saikia *et al.*, 1999; Alonso *et al.*, 2000; Yagoub *et al.*, 2004). Moreover, the phytic acid content of karkade seed (888.33 mg/100g) is unaffected by soaking, sprouting and cooking (Table 3).

***In vitro* protein digestibility:** Table 3, gives *in vitro*

protein digestibility of karkade seed as 51.47%, which decreased significantly ($p \leq 0.05$) by 45% in the soaked seeds. As a result digestibility of proteins in the sprouted seeds was also decreased, bearing in mind that sprouting was done after soaking. This decrease may be ascribed to unfold of karkade seed proteins that may increase surface contact of imbedded hydrophobic amino acids with water molecules. Thus protein solubility decreased (Fig. 3) and consequently digestibility decreased.

On the other hand, cooking significantly ($p \leq 0.01$) decreased *in vitro* protein digestibility in karkade seed by 9%. Resistance to proteolytic degradation has been attributed to the presence of bound carbohydrates or polyphenols or to protein conformation (Venkatesh and Prakash, 1993; Genovese and Lajolo, 1996; Alonso *et al.*, 2000). Protein digestibility was found to decrease with formation of isopeptides and highly polymer protein fractions during heat treatments (Yagoub *et al.*, 2004).

Total titratable acidity, fat acidity and pH: Fig. 1 and 2, shows the total titratable acidity, fat acidity and pH of the raw and processed karkade seeds. Result revealed that the total acidity of the raw seed (pH 6.06) was 1070.70 mg/100g and fat acidity was 634.10 mg/100g. Mature oilseeds may have subjected to hydrolysis by the time they are harvested, giving rise to significant amounts of free fatty acids (Nawar, 1996).

Cooking of the karkade seed significantly ($p \leq 0.05$) decreased the total and fat acidity and as the result the pH increased to 6.27. Similar effects was observed during soaking. This decrease in acidity may be ascribed to leaching of the acidic constituents in to cooking and soaking water. Sprouting for 24 and 48 hours of the soaked karkade seeds slightly increased the total acidity and also increased fat acidity but with insignificant magnitude; suggesting activity of hydrolytic indigenous enzymes of the seedling. As a result the pH decreased in both sprouts to 5.93.

Protein solubility: Protein solubility, in water and 1 M NaCl, of the raw and processed karkade seed flours is presented in Fig. 3. Results show that the proteins extracted in water and 1 M NaCl solution of the raw seed (5.78 and 19.42 mg/ml, respectively) decreased significantly ($p \neq 0.05$) by soaking, sprouting for 24 and 48

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Table 5: Amino acid profile of karkade seed as affected by processing (g/100 g protein)

Amino acid	Karkade seed					FAO/WHO**
	Raw	Soaked	24 h sprouted	48 h sprouted	Cooked	
Glycine	6.02	6.28	6.10	6.21	5.64	
Alanine	5.41	5.16	5.08	5.13	4.86	
Valine*	5.83	5.61	5.47	3.86	5.63	5.0
Leucine*	7.99	7.92	7.73	7.80	7.68	7.0
Isoleucine*	4.24	4.18	4.05	4.20	4.09	4.0
Serine	4.05	4.39	4.37	4.35	4.39	
Threonine*	3.34	3.43	3.36	3.41	3.40	4.0
Methionine*	1.11	1.35	0.68	0.96	0.91	3.5
Cystine	1.75	2.05	2.11	ND	1.46	
Penylalanine*	5.35	5.43	5.15	5.19	5.15	6.0
Tyrosine	1.79	0.85	1.11	1.11	1.68	
Aspartic acid	11.42	12.22	12.01	12.08	11.32	
Glutamic acid	18.27	20.49	20.19	18.82	18.58	
Lysine*	4.84	3.11	3.52	3.68	4.81	5.5
Arginine	11.69	11.57	11.87	11.43	11.16	
Histidine	2.22	2.10	3.22	3.66	3.58	

*Essential amino acids; ND, Not determined; **: FAO/WHO reference protein pattern (FAO/WHO, 1975)

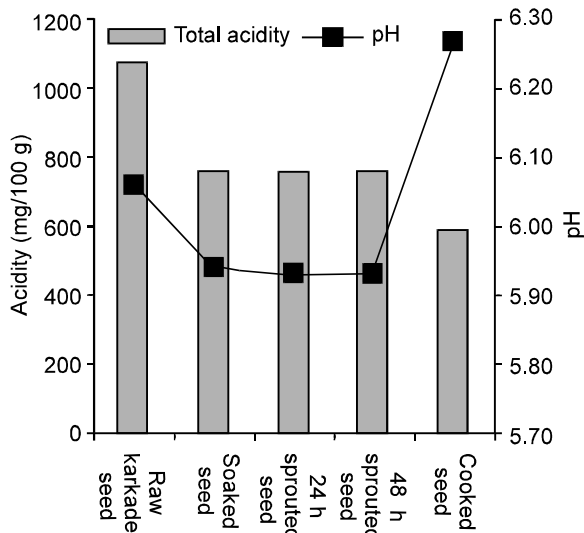


Fig. 1: Effect of soaking, sprouting and cooking on total acidity and pH of karkade seed

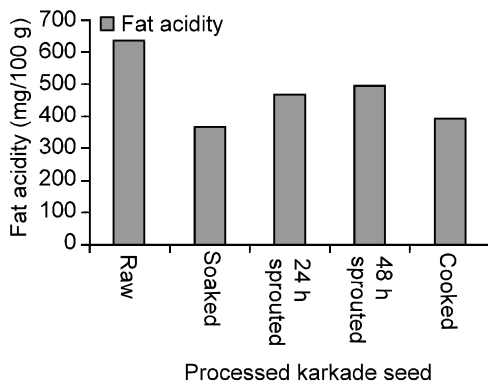


Fig. 2: Effect of soaking, sprouting and cooking on fat acidity of karkade seed

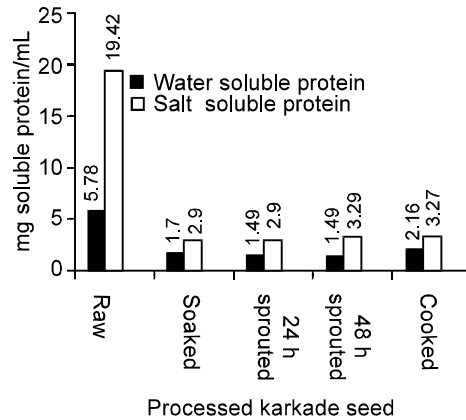


Fig. 3: Nitrogen solubility of the raw and processed karkade seed

hours and cooking. Denaturation of seed proteins and the increase in the amounts of the insoluble protein aggregates with carbohydrates and polyphenols on processing was found to reduce protein solubility (Genovese and Lajolo, 1996; Alonso *et al.*, 2000; Yagoub and Abdalla, 2007).

Amino acids composition: The results of the amino acids composition of the raw and processed karkade seeds are compared in Table 5. Glutamic acid, aspartic acid and arginine were the major amino acids in karkade seed and had values of 18.27, 11.42 and 11.69 g/100g protein, respectively. Relative to the FAO reference protein pattern (FAO/WHO, 1975), the limiting amino acids were found to be the sulfur amino acids (Methionine+cystine) and threonine. The lysine content of the karkade seed was 4.84 g/100 g protein, which is slightly lower than that of the FAO reference protein. Other essential amino acids are in consistent with those of the reference protein. The amino acids profile of the

karkade seed studied is comparable to those obtained by El Faki *et al.* (1991) and El-Adawy and Khalil (1994). It is clear that the sulfur-containing amino acids in karkade seed is not only maintained but increased in the soaked seed, which compares favorably with FAO reference protein in this respect. Contrast to that the lysine content of the raw seed was decreased on soaking. An increase in the concentrations of the acidic amino acids, aspartic and glutamic acids (accounting for 9.90 and 12.20%, respectively) was also apparent as a result of soaking. Sprouting of the soaked seeds for 24 hours further decreased sulfur amino acids contents; accounting for 17.94%. Other changes in the 48-hour sprout included a decline in valine and glutamic acid contents by 31.20 and 8.00%, respectively and an increase in ammonia by 58.03%. Simultaneously, with a rise in ammonia level of karkade seed on cooking a decrease in aliphatic amino acids, glycine, alanine and valine and sulfur amino acids was noticed. Moreover, an increase in histidine in the sprouted and the cooked seeds was observed. Other amino acids did not affect considerably by cooking or sprouting of the seed. Jirapa *et al.* (2001) stated that sprouting had little effect on amino acids composition of plant seed. Transamination and deamination reactions might be responsible for the slight changes in amino acid profile of the karkade seed on processing.

In conclusion the study indicate that *in vitro* protein digestibility and content and bioavailability of almost all minerals studied of the raw karkade seed are reduced profoundly by soaking the seeds for a period of 12 hours, which in turn extended that in the sprouted seeds. So that a careful programmed study to optimize the soaking period of karkade seeds is required.

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