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Study on Furundu, a Traditional Sudanese Fermented Roselle (*Hibiscus sabdariffa* L.) Seed: Effect on in Vitro Protein Digestibility, Chemical Composition, and Functional Properties of the Total Proteins

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Furundu, a meat substitute, is traditionally prepared by cooking the karkade (*Hibiscus sabdariffa* L.) seed and then fermenting it for 9 days. Physicochemical and functional properties of raw and cooked seed and of furundu ferments were analyzed. Furundu preparation resulted in significant changes in karkade seed major nutrients. Total polyphenols and phytic acid were also reduced. The increase in total acidity and fat acidity coupled with a decrease in pH indicates microbial hydrolysis of the major nutrients; proteins, carbohydrates, and fats. In vitro digestibility of the seed proteins reached the maximum value (82.7%) at the sixth day of fermentation, but thereafter it significantly decreased. The effect of furundu preparation on N solubility profiles and functional properties, such as emulsification and foaming properties and other related parameters, is investigated in water and in 1 M NaCl extracts from defatted flour samples. The results show that cooking followed by fermentation affects proteins solubility in water and 1 M NaCl. The foaming capacity (FC) from the flour of raw seed decreased as a result of cooking. Fermentation for 9 days significantly increased the FC of the cooked seed, restoring the inherent value. Foam from fermented samples collapsed more rapidly during a period of 120 min as compared to the foam from raw and cooked karkade seeds; stability in 1 M NaCl was lower as compared to those in water. In water, the emulsion stability (ES) from the fermented samples was significantly higher than that of the raw seed flour. Addition of 1 M NaCl significantly decreased the ES of the fermented samples.

KEYWORDS: ***Hibiscus sabdariffa*; fermentation; furundu; in vitro protein digestibility; physicochemical and functional properties**

INTRODUCTION

Proteins are structural components that play a major role in the texture of foods through their functional behavior (*1*). The acceptability of proteins as ingredients depends largely on their functional properties rather than their nutritional values. Desirable physicochemical properties attributed to proteins in product formulation systems, which contain other food components such as carbohydrates, depend in part upon the molecular size and structure of the proteins (*2*).

With an increasing world population, the food supply, particularly proteins, is presently precarious. More food proteins are needed from both conventional and nonconventional sources. In recognition of the worldwide need for more dietary protein, especially for low-income groups in developing countries, there have been efforts to develop low-cost protein foods of plant origin (*3*).

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Roselle (*Hibiscus sabdarrifa* L.), locally known as "karkade", is grown as a rain-fed crop for its calyces. The seed, a byproduct, was reported to be a promising new source of protein (*4*). In western Sudan the seed is subjected to a solid-state fermentation (SSF) process to produce a meat-substitute food known as furundu. The SSF has been reported to improve the nutritional and sensory value of a wide variety of legumes and oilseeds (*3*).

Although the karkade seed is reported to possess fairly good functional properties (*4*, *5*), its unpalatability may prevent its incorporation into food systems. Manipulation of the karkade seeds by the local peoples through a natural fermentation process may modify, in addition to the nutritional value, the functional characteristics of their proteins. It would be fruitful to investigate such changes in functional properties, which may help to find use in foods. This will enhance the value of karkade seed as a food ingredient.

The present study was carried out with the aim of assessing changes in the physicochemical and functional properties of karkade seed due to furundu fermentation.

MATERIALS AND METHODS

Karkade Seed. Karkade seeds were purchased from the local market. They were cleaned and freed from foreign materials. Some of these seeds were milled to pass through a 60-mesh sieve to obtain karkade seed flour and stored in tightly sealed containers at 4 °C until analysis. The remaining part of the seeds was subjected to natural furundu fermentation process.

Furundu Preparation. Furundu was prepared following the method adopted by the local peoples of western Sudan with some modifications. Karkade seeds were cooked in distilled water at 100 °C for 25 min (i.e., the time required to soften the seeds). After draining away the cooking water, some of the cooked seeds were dried in an oven set at 60 °C for 24 h and then milled to pass through a 60-mesh sieve. The obtained flour was kept in tightly sealed containers and stored at 4 °C. The rest of the cooked seeds were cooled. After cooling, appropriate plastic containers (locally, earthenware pots "burma" are used) were packed by pressing the cooked seeds into them, covered tightly, and fermented for 9 days at room temperature (~33 °C). Sample portions were withdrawn at intervals of 3 days, dried at 60 °C for 24 h, milled to pass a 60-mesh screen, and kept at 4 °C for further analysis. Samples from three different fermentation runs were obtained.

Preparation of the Defatted Flour. Full-fat flours of both the raw and cooked karkade seeds and furundu samples (3, 6, and 9 days ferments) were extracted by rotary shaking with hexane at a 1:10 solute-to-solvent ratio for 17 h at room temperature (~33 °C). The defatted meals were air-dried, overnight, at 60 °C, then powdered and passed through a 60-mesh sieve, and kept at 4 °C until analysis.

Proximate Analysis. Lipids, ash, total carbohydrates, and total nitrogen (micro-Kjeldahl) were determined according to AOAC methods (*6*). Protein was calculated as N × 6.25. Moisture content was determined by drying a sample at 105 °C overnight (*6*), and then dry matter was calculated. Crude fiber content was determined according to the acid/alkali digestion method of Southgate (*7*).

Nonprotein Nitrogen. Nonprotein nitrogen (NPN) was determined according to Kjeldahl as described by Paredez-Lopez and Harry (*8*). It was measured as nitrogen soluble in 12% trichloroacetic acid (TCA).

pH. pH values of karkade seed and furundu flour 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 an AOAC method (*6*). It was expressed as milligrams of KOH per 100 g of material.

Fat Acidity. Fat acidity was assessed according to the method described by Paredez-Lopez and Harry (*8*). Fat acidity was expressed as milligrams of KOH per 100 g of sample.

Soluble Carbohydrates. Soluble carbohydrates of the samples were determined according to the method described by Paredez-Lopez and Harry (*8*). They were quantified using the phenol−sulfuric acid method of Dubois et al. (*9*) with glucose as a standard.

Starch. The starch content of samples was determined according to the method of Faithful (*10*), with slight modification. One hundred milligrams of defatted flour, in a beaker, was extracted with 10 mL of ethanol (10% v/v) by continuous shaking for 30 min to remove soluble carbohydrates. The mixture was centrifuged at 3000 rpm for 5 min at room temperature (~33 °C), and the supernatant was decanted. The residue was washed thoroughly with a 1 M H_2SO_4 solution and then centrifuged. Fifteen milliliters of 1 M H_2SO_4 was added to the clean residue, and the mixture was covered and heated in a boiling water bath for 45 min. After cooling, the contents were washed into a 100 mL volumetric flask, and the volume was made up to mark. After settlement, a 10 mL aliquot was taken and made up to 100 mL in a volumetric flask. The glucose of the hydrolysate was quantified using the Dubois et al. (*9*) method. The starch was expressed as

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

Total Polyphenols. Phenolic compounds present in karkade seed and furundu samples were determined using the Prussian Blue assay, as described by Price and Butler (*11*). Tannic acid was used as a reference standard.

Phytic Acid. Phytates of the samples were determined according to the method of Wheeler and Ferrel (*12*) as described by Lajolo et al. (*13*).

Phytate was extracted from samples with a 3% trichloroacetic acid (TCA) solution containing 10% (w/v) sodium sulfate and precipitated using ferric chloride (0.2% Fe^{3+}). The iron recovered by boiling with NaOH and then with HNO_3 was quantified by reading the intensity of the colored complex formed, after the addition of potassium thiocyanate, in a Jenway 6305 spectrophotometer at 480 nm. The iron content was calculated from the ferric nitrate standard curve, and the data were extrapolated to phytic acid. A ratio of iron to phosphorus of 4:6 was assumed.

In Vitro Protein Digestibility. The in vitro protein digestibility of the samples was measured according to the method developed by Saunders et al. (*14*), in which a double-digestive pepsin−pancreatin system was used in the determinations. The digestible protein was analyzed for nitrogen using the semimicro-Kjeldahl procedure (*6*) and expressed as a percent of the total N.

Osborne Classification of Proteins. The proteins from the defatted flours of karkade seed and furundu samples were fractionated according to the technique of Osborne as described by Abd Elal et al. (*15*) using distilled water, 1 M NaCl, 70% ethanol, and 0.2% NaOH solutions for albumins, globulins, prolamins, and glutelins, respectively. The nitrogen content of each fraction was determined using the micro-Kjeldahl procedure (*6*). The residue left after extraction was also analyzed for nitrogen content. Each fraction was expressed as a percent of the total nitrogen.

Nitrogen Solubility. Nitrogen solubility, both in water and in 1 M NaCl, of karkade seed (raw and cooked) and furundu samples was determined following the method described by Prakash (*16*). The water soluble nitrogen in the defatted flour was extracted by rotary shaking with distilled water at a 1:10 solute-to-solvent ratio for 1 h, at room temperature (~33 °C). The slurry was centrifuged at 3000 rpm for 30 min at room temperature. The nitrogen value of the supernatant obtained was determined according to the micro-Kjeldahl procedure (*6*) and expressed as milligrams of protein per milliliter of solution.

Nitrogen Solubility Profile. Nitrogen solubility profiles of karkade seed and furundu total proteins were determined by extraction in water and a 1.0 M NaCl solution over a pH range of 1−12 according to the method described by Quinn and Beuchat (*2*). A 2% (w/v) defatted flour suspension was shaken for 10 min before the desired pH was maintained by the addition of 2 N HCl or 2 N NaOH over a 60 min period of constant shaking at room temperature. The suspension was centrifuged at 3000 rpm for 20 min at room temperature. The soluble nitrogen in the supernatant was determined according to the Kjeldahl procedure (*6*). The percentage of soluble nitrogen was calculated and plotted against corresponding recorded pH values. The pH of minimum extractability was determined on the basis of the midpoint of a

Table 1. Proximate Composition of the Whole Karkade Seed and Furundu Preparation Products (Grams per 100 g)[a]

sample[b]	total protein	crude oil	crude fiber	ash	total carbohydrates[c]	DM[d]
KS	28.59b (0.01)	22.15a (0.05)	12.29a (0.18)	5.13a (0.12)	27.79a (0.05)	95.95
KSc	28.90a (0.28)	22.56b (0.06)	12.27a (0.13)	4.85b (0.09)	27.39b (0.42)	95.67
9DF	28.66b (0.03)	24.60c (0.06)	14.48b (0.09)	5.52c (0.05)	22.77c (0.42)	96.05

[a] Means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.01$). [b] KS, raw karkade seeds; KSc, cooked, dried karkade seeds; DF, days of fermentation. [c] Calculated by difference. [d] Dry matter composition.

regression line if the region was flat and on the basis of the experimental pH minimum if the region was sharp (*16*).

Functional Properties Measurements. *Water Absorption Capacity (WAC).* The WAC of samples was determined according to the method of Lin et al. (*17*) with a modification described by Wang and Kinsella (*18*). A 10% defatted flour suspension was stirred in a 50 mL centrifuge tube using a glass rod for 2 min at room temperature. After 30 min of shaking, the tube was centrifuged for 25 min at 3700 rpm at room temperature. The freed water was carefully decanted in a graduated measuring cylinder and the volume recorded. The WAC was corrected for the loss of soluble components and expressed as milliliters of water retained by 1 g of defatted flour.

Fat Absorption Capacity (FAC). The FAC of samples was measured according to the method of Lin et al. (*17*). The FAC was expressed as milliliters of oil retained per 1 g of defatted flour.

Bulk Density. The bulk density of the defatted flours of samples was determined according to the method of Wang and Kinsella (*18*) and expressed as grams per milliliter.

Foaming Capacity and Foam Stability. Foaming capacity and foam stability of karkade seed and furundu samples were determined according to the method described by Venktesh and Prakash (*19*). A 3% defatted flour suspension was stirred in a kitchen blender for 6 min, and the volume of foam at 30 s was calculated; the increase in volume is expressed as a percent foam capacity. The foam stability was determined by measuring the decrease in volume of foam as a function of time for up to a period of 30 min.

Emulsification Capacity (EC). The EC was determined according to the method of Beuchat et al. (*20*). The EC was expressed as milliliters of oil emulsified by a gram of defatted flour.

Emulsifying Activity (EA) and Emulsion Stability (ES). EA was determined according to the method described by Venktesh and Prakash (*19*). Thirty milliliters of distilled water and 10 mL of refined peanut oil were added to 1.5 g of defatted flour, and the mixture was stirred. The contents were homogenized in a Virtis homogenizer at 2000*g* for 1 min. An aliquot of 0.10 mL was withdrawn immediately and at regular intervals of time from the bottom of the container and diluted to 10 mL with 0.1% sodium dodecyl sulfate; the absorbance was measured at 500 nm in a Jenway 3536 spectrophotometer. To measure the absorbance, the emulsion was diluted so as to read within 1.00 absorbance in a spectrophotometer where the reading is multiplied by a dilution factor, and the resulting absorbances are plotted on the *Y* axis. A graph of absorbance against time was plotted. The time for the initial absorbance (emulsifying activity) to decrease by half was recorded as emulsion stability.

Statistical Analysis. Data representing a mean value of triplicate samples were subjected to analysis of variance, and means were separated according to Duncan's multiple-range test (*21*).

RESULTS AND DISCUSSION

Proximate Composition. The effect of furundu preparation on the proximate composition of karkade seed is shown in **Table 1**. Cooking followed by fermentation resulted in deviation of nutrients from the raw seed. The differences found in protein fat contents found after cooking are probably due to leaching of soluble components (minerals, carbohydrates, and proteins).

Table 2. Proximate Composition of the Raw, Cooked, and Fermented Karkade Seed Defatted Flours (Grams per 100 g, Dry Basis)[a]

sample[b]	total protein	residual fat	crude fiber	total carbohydrates	ash
KS	38.73 (0.01)	4.98 (0.12)	15.35 (0.11)	34.31 (0.27)	6.63 (0.06)
KSc	39.50 (0.36)	4.65 (0.12)	15.37 (0.16)	33.80 (0.44)	6.62 (0.10)
3DF	39.38 (0.15)	4.76 (0.12)	17.73 (0.07)	31.51 (0.08)	6.63 (0.00)
6DF	40.04 (0.05)	4.79 (0.14)	18.10 (0.15)	30.28 (0.14)	6.80 (0.12)
9DF	40.12 (0.05)	4.78 (0.12)	18.23 (0.11)	30.28 (0.20)	6.60 (0.12)

[a] Means of three replicates. Values in parentheses are standard deviations. [b] KS, raw karkade seeds; KSc, cooked, dried karkade seeds; DF, days of fermentation.

The slight increase in dry matter (0.4%) on fermentation of karkade seeds suggests addition of microbial cells.

Table 2 shows that nutrient composition from the defatted flours of the raw and cooked seed and of furundu ferments was higher than that of the full-fat flours.

Nitrogenous Constituents. Table 3 shows that total protein (29.79%), true protein (28.44%), nonprotein nitrogen (1.35%), and water soluble protein (6.81%) of raw karkade seed were changed during furundu preparation to varied extents. The changes in nitrogenous constituents has been reported by workers to be a result of leaching out effects during cooking (*26*) and as a consequence of proteolytic activity of enzymes during fermentation (*8*, *27*).

Nitrogen-Free Constituents. Cooking significantly decreased ($P \leq 0.01$) total carbohydrates of karkade seed, and fermentation further reduced their level to 23.71% (**Table 3**). The decrease in soluble carbohydrates (**Table 3**) is a result of the leaching out effect during cooking. The significant ($P \leq 0.01$) loss observed in both soluble carbohydrates and starch contents after 9 days of fermentation is likely due to their utilization by microorganisms as a source of energy.

Total Acidity, Fat Acidity, and pH. Table 4 shows that the pH of the raw karkade seed (6.12) was significantly increased by cooking (pH 6.28), accompanied with a decrease in total acidity and fat acidity. This suggests leaching of acidic components into cooking water. Fermentation for 9 days (9DF) significantly ($P \leq 0.01$) decreased the pH to 5.64, concurrently with an increase in total acidity and fat acidity. The increase in fat acidity of the cooked seed after fermentation indicates the hydrolysis of triglycerides by microbial lipases.

Antinutritional Factors. Changes in total polyphenols and phytic acid of karkade seed due to furundu fermentation are shown in **Table 5**.

Total Polyphenols. Cooking resulted in a significant ($P \leq 0.01$) reduction of these antinutrients to 0.95%. Fermentation further decreased their level. Leaching of these molecules as well as change in their chemical reactivity or the formation of the insoluble complex with proteins could explain the reduction of polyphenols during furundu preparation.

Phytic Acid. Cooking karkade seeds was found to significantly ($P \leq 0.05$) decrease the level of phytate by 2.30%. Evidence indicates the low susceptibility of phytates to cooking (*8*). Fermentation for 9 days significantly ($P \leq 0.05$) decreased the phytate of the cooked seed (0.85%) by nearly 13%. Microbial production of phytase could be responsible for the loss of phytates (*28*).

In Vitro Protein Digestibility (IVPD). Changes in IVPD during furundu preparation are shown in **Table 5**. Results revealed that cooking decreased significantly ($P \leq 0.01$) the IVPD of the karkade seed to 69.84%.

Table 3. Effect of Furundu Preparation on Nitrogen-Containing and Nitrogen-Free Compounds of the Whole Karkade Seed (Grams per 100 g, Dry Basis)[a]

sample[b]	total protein	nonprotein nitrogen	true protein	water soluble protein	total carbohydrates	soluble carbohydrates	starch
KS	29.79b (0.01)	1.35b (0.01)	28.44c (0.00)	6.81a (0.01)	28.96a (0.05)	11.07a (0.40)	18.31a (0.05)
KSc	30.21a (0.28)	0.86d (0.01)	29.35a (0.29)	5.41c (0.02)	28.63b (0.42)	10.78b (0.43)	18.29a (0.04)
3DF	29.58d (0.11)	1.18c (0.02)	28.40b (0.19)	5.34c (0.02)	25.81c (0.15)	7.47cd (0.31)	18.02b (0.12)
6DF	29.65c (0.08)	1.65a (0.02)	28.00d (0.16)	6.04b (0.02)	24.31d (0.39)	7.40d (0.36)	17.53c (0.04)
9DF	29.84b (0.03)	1.35b (0.02)	28.49c (0.13)	4.64d (0.01)	23.71e (0.50)	5.58e (0.47)	17.55c (0.01)

[a] Means of three replicate samples. Values in parentheses are standard deviations. Means not sharing a common letter within a column are significantly different according to DMRT at $P \leq 0.01$. [b] KS, raw karkade seeds; KSc, cooked, dried karkade seed; DF, days of fermentation.

Table 4. Total Acidity, Fat Acidity, and pH of Karkade Seed and Furundu Preparation Products[a]

	mg of KOH/100 g, dry basis			degree of contribution in TA (%)		
sample[b]	TA	FA	AC_d[c]	FA	AC_d	pH
KS	429.80d (39.20)	102.60d (7.50)	327.20	23.90	76.10	6.12b (0.00)
KSc	297.90e (39.60)	74.20e (2.90)	223.70	24.90	75.10	6.28a (0.00)
3DF	487.90c (52.00)	129.2c (1.60)	358.70	26.50	73.50	5.88c (0.01)
6DF	549.50b (19.40)	275.00b (14.50)	274.50	50.05	49.95	5.46e (0.00)
9DF	606.70a (67.40)	386.50a (32.20)	220.20	63.70	36.30	5.64d (0.01)

[a] Values are means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.01$). [b] KS, raw karkade seeds; KSc, cooked, dried karkade seed; DF, days of fermentation. [c] AC_d, acidity due to components other than fats.

Table 5. Effect of Furundu Preparation on Total Polyphenols (TPP), Phytic Acid (PA), and in Vitro Protein Digestibility (IVPD) of Whole Karkade Seed[a]

sample[b]	TPP* (%)	PA** (%)	IVPD* (%)
KS	0.98a (0.01)	0.87a (0.02)	77.75b (0.18)
KSc	0.95b (0.01)	0.85b (0.01)	69.84d (0.44)
3DF	0.93c (0.02)	0.76c (0.01)	72.55c (0.35)
6DF	0.93c (0.00)	0.74d (0.001)	82.73a (0.18)
9DF	0.93c (0.01)	0.74d (0.00)	68.92d (0.52)

[a] Values are means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT (*, significant at 1%; **, significant at 5% level). Calculations on dry basis. [b] KS, raw karkade seed; KSc, cooked, dried karkade seed; DF, days of fermentation.

Table 6. Nitrogen Solubility in Water and 1 M NaCl of Raw, Cooked, and Fermented Karkade Seed Defatted Flours (Milligrams of Protein per Milliliter of Solution)[a]

sample[b]	nitrogen soluble in water	nitrogen soluble in 1 M NaCl
KS	8.86a (0.08)	21.00b (0.14)
KSc	7.08c (0.11)	10.05c (0.24)
3DF	7.12c (0.14)	10.32a (0.14)
6DF	8.16b (0.07)	11.55d (0.14)
9DF	6.23d (0.04)	12.25bc (0.21)

[a] Values are means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.01$). [b] KS, raw karkade seed; KSc, cooked, dried karkade seed; DF, days of fermentation.

Results show that the level of IVPD of the cooked seed was significantly ($P \leq 0.01$) increased in the sixth day fermented sample (82.94%). Fermentation has been reported to dissociate and degrade proteins (*25*) and favor the accessibility of proteases, resulting in increased digestibility (*29*). Protein digestibility was decreased significantly ($P \leq 0.01$) in the ninth day ferment compared to that of the raw seed. This could be a result of the increase in the glutelin fraction (**Table 8**). Fermentation may denature these fractions, increasing hydrophobicity, and may favor aggregation. Protein digestibility has been reported to decrease with increased highly polymeric glutelin fractions (*30*).

Nitrogen Solubility. Nitrogen solubility, in water and in 1 M NaCl, of the defatted flours of the karkade seeds (raw and cooked) and furundu ferments of 3 days (3DF), 6 days (6DF), and 9 days (9DF), is given in **Table 6**. The data show that the proteins extracted in water and in 1 M NaCl of the raw seed (8.86 and 21.00 mg/mL, respectively) were significantly ($P \leq 0.01$) reduced by cooking. Heat treatments were found to facilitate formation of insoluble protein complexes with polyphenols and carbohydrates and also enhanced denaturation of proteins, resulting in decreased solubility (*19*). This agrees with the notable increase in prolamin and glutelin fractions (**Table 7**).

The N solubility in water and in 1 M NaCl of the cooked seed (7.08 and 10.50 mg/mL, respectively) was significantly ($P \leq 0.01$) increased by fermentation. Nitrogen solubility in water reached the maximum value at the sixth day, but maximum salt solubility was not seen until the ninth day. The reduction in the N solubility in water could probably be due to the decrease in the level of nonprotein nitrogen (**Table 3**).

Percent Nitrogen Extracted in Distilled Water at Various pH Values. Percent soluble nitrogen extracted in distilled water over a range of pH from 1 to 12 from the defatted flours of karkade seed (raw and cooked) and furundu samples (3, 6, and 9 day ferments) is presented in **Figure 1a**.

Total proteins of the raw karkade seed defatted flour showed a U-shaped solubility curve, suggesting the existence of only one isoelectric point (PI) typical of other studies on similar oilseed proteins (*4*, *5*). However, minimum solubility of the karkade seed proteins (of average value of 7.1% w/w of the total N) occurred at pH 3.5, which might be the pH of the PI. Moreover, N solubility increased to both sides of the isoelectric region (pH 3−4.5), apparently at more acid and alkaline pH,

Table 7. Protein Fractions of Raw, Cooked, and Fermented Karkade Seeds (Percent)[a]

sample[b]	albumin	globulin	prolamin	glutelin	insoluble protein	total protein recovered	nonprotein nitrogen
KS	54.80a (0.41)	14.11a (0.10)	0.81c (0.05)	8.86b (0.26)	20.25c (0.35)	98.83	8.43b (0.10)
KSc	32.76b (0.23)	8.76e (0.21)	1.06b (0.05)	31.42a (0.55)	25.67b (0.21)	99.67	5.35d (0.03)
3DF	32.58b (0.32)	9.04d (0.14)	2.44a (0.07)	32.20a (0.16)	25.47b (0.08)	101.73	7.38c (0.11)
6DF	29.40c (0.35)	10.76c (0.08)	2.41a (0.06)	31.65a (0.33)	26.13a (0.14)	100.35	10.35a (0.03)
9DF	27.34d (0.19)	12.73b (0.20)	2.44a (0.06)	32.12a (0.28)	26.39a (0.24)	101.02	8.42b (0.14)

[a] Values are means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.01$). [b] KS, raw karkade seed; KSc, cooked, dried karkade seed; DF, days of fermentation.

Table 8. Changes in Bulk Density (BD), Water Absorption Capacity (WAC), and Fat Absorption Capacity (FAC) of Karkade Seed Defatted Flour due to Furundu Preparation[a]

sample[b]	WAC[c] (mL/g)	FAC (mL/g)	BD (g/mL)
KS	3.11b (0.04)	3.54b (0.03)	0.790a (0.04)
KSc	2.53d (0.02)	3.52c (0.02)	0.61b (0.02)
3DF	3.11b (0.01)	3.52c (0.03)	0.55d (0.01)
6DF	3.16a (0.03)	3.55a (0.02)	0.55e (0.01)
9DF	3.02a (0.02)	3.54b (0.05)	0.56c (0.02)

[a] Values are means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.01$). [b] KS, raw karkade seed; KSc, cooked karkade seed; DF, days of fermentation. [c] Water absorbed was corrected for the soluble components in the defatted flours.

with maximum solubility observed at pH 10. The water extract of the cooked karkade seed flour has an N solubility profile similar to that of the raw sample. However, N solubility levels were slightly reduced over a wide range of pH from 1 to 11, with a pronounced reduction found at pH 8−10. The PI of the raw seed (pH 3.5) was slightly displaced in the cooked sample (pH 3.75).

Differences in N solubility profiles of raw and heat treated samples could arise due to (1) denaturation of the protein, (2) surface charge variation as a result of protein−protein interaction at elevated temperature, and (3) masking of charged amino groups due to either complexation with reducing sugars or free phenolic acids present in the microenvironment of the protein (*23*).

The amounts of karkade seed proteins extracted at pH 1−6 did not show improvement by fermentation process. Cooking followed by fermentation resulted in a significant reduction in the amounts of karkade seed proteins extracted at the alkaline region. Carbonaro et al. (*22*) reported that neutralization of the positive charge of the basic amino acid residues, by exposure of more hydrophobic groups, imparts stabilization to the insoluble form of protein; thus, a loss of solublization occurred.

Percent Nitrogen Extracted in 1 M Sodium Chloride Solution at Various pH Values. Karkade seed proteins extracted in 1 M NaCl solution, at various pH values (**Figure 1b**), do not show a solubility minimum like that obtained in water (**Figure 1a**). Instead, the range of the isoelectric region is broadened (from pH 1.50 to 4.00). Addition of 1 M NaCl significantly increased the extractability of the karkade seed proteins from an average of 18.70% in water to 36.70% at pH 1.00−6.50, but significantly decreased the extractability (from 63.10 to 55.30%) at pH 7.00−12.00. At pH values higher than the PI the proteins have negative net charges. Sodium chloride can bring about charge differences on the protein, leading to changes in solubility. The exposed surface charge of the protein, which is charge protected at lower pH, and the alkali peptization at higher alkaline pH are predominantly responsible for the differences in solubility (*23*).

Salt solubility of the cooked karkade seed protein significantly decreased at the acid and alkaline regions of the profile (**Figure 1b**), compared to that in water (**Figure 1a**). Cooking heat may denature the salt soluble proteins and thus reduce their solubility (**Table 6**).

Extractability of the cooked seed proteins in 1 M NaCl at pH 1.5−4.0 was unaffected by fermentation, but the extractability at pH 4.5−12 was significantly decreased. Fermentation may denature the proteins of the cooked karkade seed, increasing hydrophobicity, and may favor aggregation and thus solubility loss.

Protein Fractions. Osborne classification of karkade seed proteins revealed variation in protein fraction during furundu fermentation (**Table 7**). Karkade seed albumin represents the major protein fraction (54.80%), followed by globulin (14.11%) and then by glutelin (8.86%). Prolamin was found to be the lowest fraction (0.81). Elbashir (*24*) reported 64.00% albumin, 11.30% globulin, 3.30% glutelin, and 1.00% prolamin for a Sudanese variety of karkade seed. Compared to the raw karlade seed, significant reductions in the levels of albumin and globulin fractions were observed after cooking and then fermentation. As a result, a relative increase in prolamin, glutelin, and insoluble protein fractions occurs in the cooked and fermented samples. The variations in protein fractions observed are the consequences of changes in the molecular mass of the different proteins. The dissociation, denaturation, or hydrolysis of the proteins (*25*) may modify their solubility, and they are extracted in other conditions.

Water Absorption Capacity. The WAC of the total proteins from the defatted flours of karkade seed (raw and cooked) and furundu samples is presented in **Table 8**. The WAC of the defatted flour from karkade seed (KS) was 3.11 mL of H_2O/g. Cooking prior to fermentation significantly ($P \leq 0.01$) decreased the WAC of the KS to 2.52 mL of H_2O/g of flour. The WAC of the cooked seed (2.52 mL/g) was increased significantly in the fermented samples (3.11 mL/g). Changes in the surface characteristics of protein molecules and starch granules during the preparation of furundu may be reflected in the differences observed in WAC.

Fat Absorption Capacity. Table 8 shows that karkade seed (KS) had an FAC of 3.54 mL of oil/g of flour. Cooking was significantly ($P \leq 0.01$) reduced the FAC of the KS to 3.52 mL of oil/g of flour. Fermentation of the cooked seed did not show improvement in the FAC, compared to that of the raw seed.

Bulk Density (BD). The BD of the defatted flours from karkade seed (raw and cooked) and furundu ferments (3DF, 6DF, and 9DF) is shown in **Table 8**. Cooking significantly ($P \leq 0.01$) decreased the BD to a value of 0.61 g/mL, accounting for a 22.40% loss of the original value (0.79 g/mL). Fermenta-

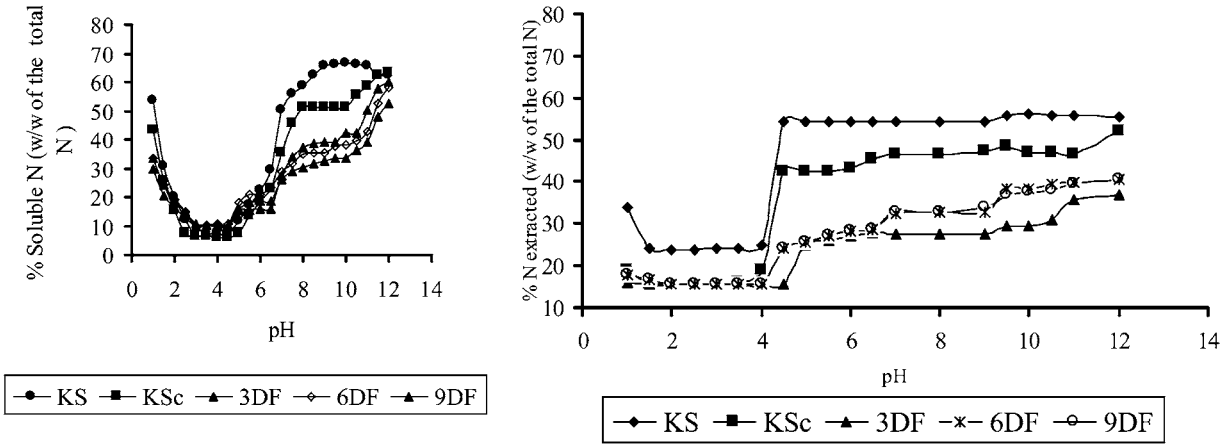

Figure 1. (**a**) Percent nitrogen extracted as a function of pH of raw, cooked, and fermented karkade seed total proteins from the defatted flours; (**b**) percent nitrogen extracted in 1 M NaCl solution as a function of pH of raw, cooked, and fermented karkade seed defatted flours. Values are means of three replicates. KS, raw karkade seed; KSc, cooked, dried karkarde seed; DF, days of fermentation.

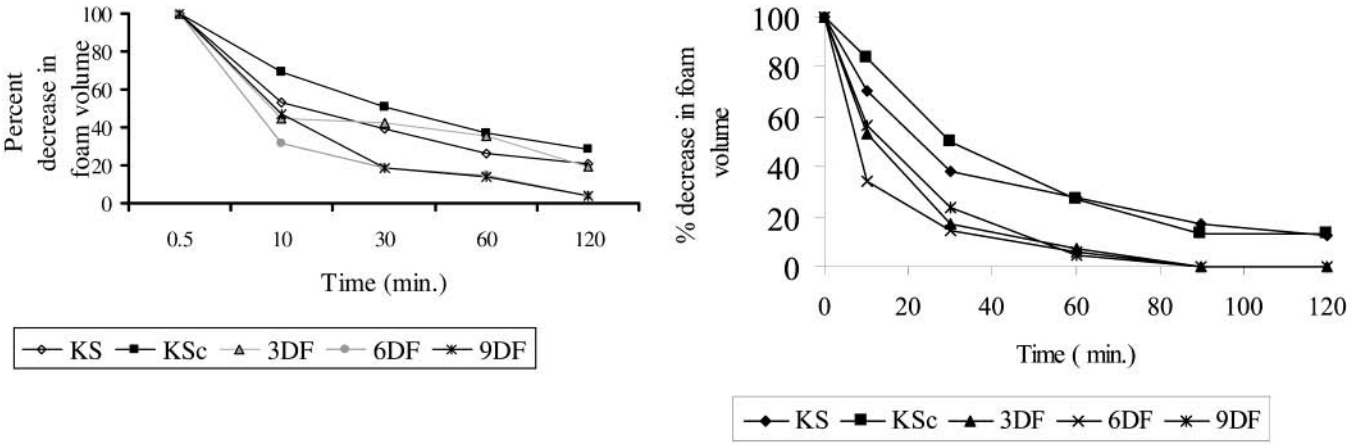

Figure 2. (**a**) Percent decrease in foam volume in water as a function of time of the total proteins from the defatted flours of karkade seed and furundu samples; (**b**) decrease in foam volume in 1 M NaCl solution as a function of time of the total proteins from the defatted flours of raw, cooked, and fermented karkade seed. Values are means of triplicate samples. KS, raw karkade seed; KSc, cooked, dried karkarde seed; DF, days of fermentation.

tion for 9 days significantly ($P \leq 0.01$) decreased the BD of the cooked seed flour to a value 0.56 g/mL. BD depends on combined effects of interrelated factors such as intensity of attractive interparticles forces, particle size, and number of contact points (*5*).

Foaming Properties. Changes in foaming capacity (expressed as a percent increase in foam volume) and foam stability (expressed as a percent decrease in foam volume) of the total proteins from the defatted flour of karkade seed due to furundu preparation are presented in **Table 9**.

Foaming Capacity (FC). Cooking significantly ($P \leq 0.01$) decreased the FC of the raw karkade seed (KS) flour from 46.50 to 29.00% (**Table 9**).

Fermentation of the cooked karkade seed for 6 days resulted in a significant ($P \geq 0.01$) decrease in FC (20.60%). The 9 day fermentation of the cooked seed significantly ($P \leq 0.01$) increased the FC to 53.80%, completely restoring the initial foamability of the karkade seed defatted flour. Fermentation has been reported to dissociate proteins (*25*). Dissociated proteins have more capacity to foam (*19*). In addition, constituents other than proteins may aid in the formation of whipped foam (*17*). Addition of 1 M NaCl decreases the FC of all of the flours observed in water.

Foam Stability (FS). FS, measured as a percent decrease in foam volume for up to a period of 30 min, of the total proteins from the defatted flours of the karkade seed (KS), cooked seeds (KSc), and furundu ferments of 3, 6, and 9 days (3DF, 6DF, and 9DF) is shown in **Table 9**.

Results showed that FS of the total proteins from the defatted flour of the KS whipped in water has a value of 41.90%. A

Table 9. Functional Properties of Raw, Cooked, and Fermented Karkade Seed Defatted Flours in H_2O and 1 M NaCl[a]

sample[b]		FC[c] (%)	FS[c] (%)	EC[c] (mL/g)	EA,[c] absorbance at 500 nm	ES[c] (s)
KS	H_2O	46.50a (2.12)	41.90b (1.20)	58.10b (0.80)	23.40d (0.14)	1515f (21)
	NaCl	31.33b (1.48)	38.30c (0.45)	57.08b (0.40)	11.50e (0.14)	2450a (70)
KSc	H_2O	29.00b (1.41)	50.00a (0.70)	59.00b (0.50)	33.00b (0.28)	1525f (35)
	NaCl	20.13c (0.88)	50.00a (0.56)	56.70b (0.74)	24.00c (0.35)	1590d (42)
3DF	H_2O	24.00c (1.41)	38.60c (0.62)	64.20a (0.62)	33.70b (0.21)	1835c (42)
	NaCl	21.40c (1.78)	10.25f (0.40)	62.82a (0.42)	25.70c (0.42)	1609d (33)
6DF	H_2O	20.60c (0.96)	25.00d (0.88)	65.40a (0.80)	33.80b (0.14)	1832c (49)
	NaCl	23.53c (0.90)	14.29e (0.40)	65.86a (0.68)	24.70c (0.44)	519e (15)
9DF	H_2O	53.80a (1.77)	12.70ef (1.28)	63.50a (0.85)	41.20a (0.43)	1880b (28)
	NaCl	31.10b (1.22)	23.91d (0.36)	61.96a (0.40)	24.40c (0.22)	526e (19)

[a] Values are means of three replicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.01$). [b] KS, raw karkade seed; KSc, cooked, dried karkade seed; DF, days of fermentation. [c] FC, foaming capacity; FS, foam stability; EC, emulsification capacity; EA, emulsification activity; ES, emulsion stability.

significantly ($P \leq 0.01$) higher value of 50.00% was found for the cooked karkade seed flour. Fermentation, however, significantly ($P \leq 0.01$) decreased the FS of the KSc sample, the extent depending on lengthening the incubation time. In 1 M NaCl the FS significantly decreased in the 3DF and 6DF samples (to 10.25 and 14.29%, respectively) but significantly increased in the 9 DF sample (to 23.91%) compared to their stability in water.

The decrease in foam volume as a function of time of the karkade seed (raw and cooked) and furundu total proteins from the defatted flours, in water and in 1 M NaCl, is traced via **Figure 2**. The KS defatted flour has stable foam in water. Cooking significantly increased the stability of the foam in the raw karkade seed. Fermentation significantly decreased the stability of foam in KSc, and extending the fermentation time showed poor stability of foams with respect to time. Fermentation for 6 days severely impairs the stability of foams in 1 M NaCl solution (**Figure 2b**). Foaming properties were found to be affected by the solubility of proteins and salting-in and -out of proteins (*31*).

Emulsifying Properties. The emulsification capacity (EC), emulsifying activity (EA), and emulsion stability (ES) of the total proteins from the defatted flours of the raw, cooked, and fermented karkade seeds are presented in **Table 9**.

Emulsification Capacity. Results showed that 1 g of defatted karkade seed flour had an ability to emulsify 58.1 mL of oil (**Table 9**). Cooking of the karkade seed did not affect the EC. Fermentation of the cooked karkade seed for 3, 6, and 9 days significantly ($P \leq 0.01$) increased the EC to 64.20, 65.50, and 63.50 mL of oil/g, respectively. Addition of 1 M NaCl did not affect the EC of the raw, cooked, and fermented karkade seed. Proteolytic activity of enzymes has been reported to improve the emulsification capacity of proteins (*32*).

Emulsifying Activity and Emulsion Stability. EA (expressed as an absorbance at 500 nm) and ES (expressed as time for the initial absorbance to decrease by half) of the total proteins from the defatted flours for the karkade seed (KS), cooked seed (KSc), and furundu ferments (3DF, 6DF, and 9DF) are shown in **Table 9**.

Cooking significantly ($P \leq 0.01$) increased the EA of the karkade seed from 23.40 to 33.00. Fermentation for 3 and 6 days did not affect the EA in the KSc sample, but a 9 day period significantly ($P \leq 0.01$) increased it to 41.20. One molar sodium chloride solution significantly ($P \leq 0.01$) decreased the EA in all samples.

The ES of the raw karkade seed (1515 s) was unaffected by cooking conditions. The ES of the KSc sample significantly increased in the fermented samples, reaching the maximum value at the ninth day (1880 s). Addition of 1 M NaCl into water significantly increased the EA in the raw and cooked karkade seed samples, but it significantly decreased in the fermented samples. Moreover, a graphic presentation of emulsion stability of samples in water and 1 M NaCl is shown in **Figure 3**. In water, both KS and KSc are similar, indicating a gradual fall. This stabilizes in the presence of 1 M NaCl, and the fall is reduced with significantly increased ES values for KS (2450 s) and KSc (1590 s). In water the fermented samples showed a significant increase in stability of the KSc, to higher values of 1835, 1832, and 1880 s for 3DF, 6DF, and 9DF, respectively. The ES in the presence of 1 M NaCl significantly decreased in all of the fermented samples, displaying a gradual fall.

Cooking and fermentation may denature the proteins and may increase the surface hydrophobicity. The nature of the hydrophobic groups that are exposed to the surface may largely

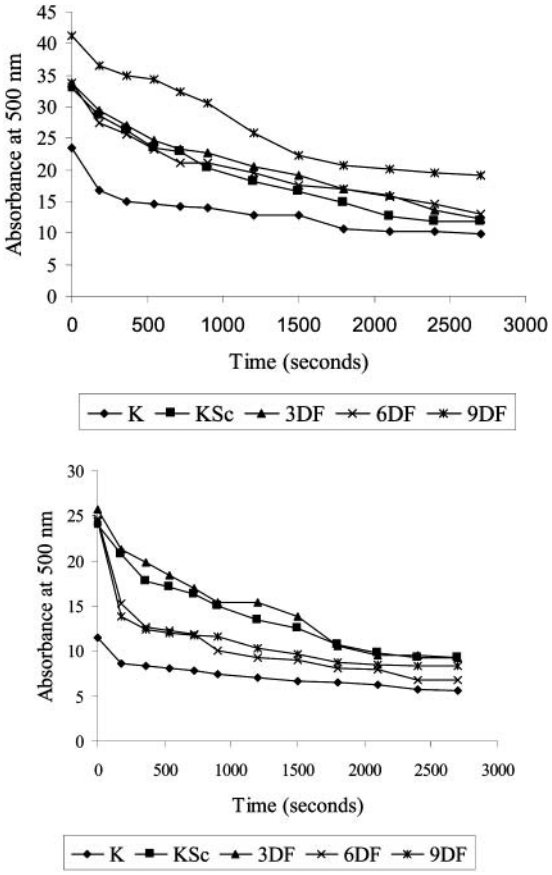

Figure 3. (**a**) Emulsification stability as a function of time of raw, cooked, and fermented karkade seed total proteins from the defatted flours in water; (**b**) emulsification stability as a function of time of raw, cooked, and fermented karkade seed total proteins from the defatted flours in 1 M NaCl. KS, raw karkade seed; KSc, cooked, dried karkarde seed; DF, days of fermentation.

determine the emulsification properties of the proteins and may effect a balance between net charge on the protein and hydrophobicity (*19*, *31*). Damodaran (*32*) has found a positive correlation between emulsifying activity and surface hydrophobicity of proteins.

Caution should be exercised in the interpretation of the results because the karkade seed contains considerable amounts of carbohydrates, such as starch and other materials in the fractions.

The data obtained from the present study indicate that modification of the karkade seed proteins by traditional fermentation for 6 days significantly increased the in vitro protein digestibility Also, it imparts superiority to emulsification properties, whereas 3 days of fermentation is satisfactory to attain this improvement. Nevertheless, whether the furundu product described in this study has the potential for practical application as a protein source remains to be demonstrated.

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