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Fururndu, a Meat Substitute from Fermented Roselle (*Hibiscus sabdariffa* L.) Seed: Investigation on Amino Acids Composition, Protein Fractions, Minerals Content and HCl-Extractability and Microbial Growth

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Abstract: Indigenous furundu is prepared by cooking the karkade (*Hibiscus sabdariffa* L.) seed and then fermenting it for 9 days. Physicochemical properties and HCl-extractability of minerals of the karkade seeds and of furundu ferments were analyzed. Microbial growth during fermentation was also screened. Furundu fermentation resulted in increase in total protein of the karkade seed, suggesting changes in dry matter composition. Significant decrease in karkade seed protein fractions, albumin plus globulin and prolamin and a significant increase in G1, G2 and G3 on fermentation was found. Also a decrease in total acidity and pH was observed. The amino acids profile of the karkade seed was dominated by glutamic acid, aspartic acid and arginine. The sulfur amino acids (Methionine and cystine) and threonine were the limiting amino acids in karkade seed and they remained limiting in furundu. Slight mobilization in amounts of some amino acids, simultaneously with increase in the level of ammonia during preparation of furundu was noticed. The contents of K, Na, Cu, Mn and Fe of the karkade seed were decreased significantly in furundu. The HCl-extractability of K, Mn and Fe in karkade seed was improved in furundu product. Fermentation of the cooked seed increased growth of bacteria, yeasts and moulds. The endospore-forming *Bacillus* species is predominately the active organism in the substrate medium.

Key words: *Hibiscus sabdariffa* L., fermentation, furundu, amino acids, protein fractions, HCl-extractability of minerals, microflora

Introduction

The world attitude is now oriented to develop low cost protein foods of plant origin, especially for the developing countries. The development of the indigenous plant-based fermented foods in the Sudan is based on the fact that the major and most serious food shortages common in rural Sudan are those related to cereals, the staple food and the major ingredients of the stew i.e. meat and milk. In Sudan the villagers have developed milk and meat substitutes primarily from plant material, which they manipulate by fermentation to give flavors and textures more or less simulating those of proteolytic meat and sour milk (Dirar, 1993). Therefore, one of the benefits of traditional fermentation that it provides methods of converting vegetable proteins to meat-like flavors and textures (Reddy *et al*., 1986; Dirar, 1994; Steinkraus, 1996).

The traditional Sudanese fermented food 'furundu', strictly defined as a meat substitute prepared from the seeds of karkade (*Hibiscus sabdariffa* L.), falls in the family of strong smelling West African fermented foods (Dirar, 1993). Furundu is produced by solid-state fermentation (SSF) process. Paredes-Lopez and Harry (1989) reported that SSF improved nutritional and sensorial value of plant seed substrates. Dirar (1993) appreciably reviewed on meat substitutes of plant origin in the Sudan; he stated that furundu has the flavor closest to that of fermented meat. The bilateral role of furundu as a source of protein and flavor is paralleled in some local fermented foods, such as kawal (Dirar *et al*., 1985) and sigda (El Faki *et al*., 1991) as well as in other ones in the world, such as the West African strong smelling flavor foods dawadawa and ugba (Odunfa, 1986; Steinkraus, 1996) and Tempeh (fermented soybean) of the oriental world (Desphande *et al*., 2000). However, the strong odors seemed to be the rule in food fermentation in which *Bacillus subtilis* dominates, this kind of bacteria is known to have a powerful proteolytic enzymes (Mackie *et al*., 1971). In this context, *Bacillus* species was suggested to be the active organism during fermentation of furundu (El Faki *et al*., 1991). In general, a mixed culture originating from the native microflora of the raw materials is in action in most of the indigenous food fermentations. However, starter culture fermentation is of first preference in the industrial scale because quality of finished products can be maintained (Sahlin, 1999). In line with this concept, investigations on kinds of organisms dominate furundu fermentation is claimed for future control of the fermentation process.

In a previous study some physicochemical and functional properties of the total proteins of furundu were assessed (Yagoub *et al*., 2004). Accordingly, the present study was designed to evaluate changes in protein quality through its solubility fractionation and amino acids composition and also to assess minerals extractability of the karkade seed in relation to fermentation process.

Materials and Methods

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

Processing of furundu: Karkade seeds were sorted out and cleaned. One batch of the raw seeds was kept in a freezer for microbiological tests and the other was milled (0.4 mm sieve), kept in tight containers and stored at 4°C until chemical analysis. Furundu was prepared in the laboratory following the method adopted by the local people of Darfur as described by Yagoub *et al.* (2004). Karkade seeds were cooked by boiling in distilled water. After cooking the water was drained away. Two parts of the cooked seeds were prepared and stored as before until microbiological and chemical tests. The last part of the cooked seeds was packed in plastic containers by pressing, covered tightly and fermented for 3, 6 and 9 days intervals at room temperature (~ 30°C). Portions of the ferments were reserved for microbiological tests, while the rest was prepared for chemical analysis as before. Samples from three different fermentation runs were prepared.

Total protein and moisture content: Total nitrogen (micro-Kjeldahl) was determined according to AOAC (1990). Protein was calculated as N% X 6.25. Moisture content was determined by drying samples at 105°C overnight (AOAC, 1990) and then dry matter was calculated.

Total minerals: Minerals were determined in extracts prepared by digesting the ash with 5 N HCl (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 vandate 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: 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 and 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 (De Boland *et al.*, 1975).

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

pH: pH values of 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).

Amino acids analysis: Amino acids composition of samples was measured on hydrolysates using amino acids 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 (amino acid standards H, Pierce. Inc., Bockford). Aliquot of 150 µL of sample hydrolysate was injected in a cation separation column at 130°C.
Ninhydrin 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/ninhydrin mixture was heated in the reactor at 130°C for 2 minutes to accelerate chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acids composition was calculated from the areas of standards obtained from the integrator and expressed as percentages.

Landry and Moureaux classification of proteins: The proteins from the defatted flour of karkade seed and furundu samples were fractionated following the technique of Landry and Moureaux (1981). Six protein fractions were extracted stepwise by a series of solvents, to obtain fractions of albumin, globulin, prolamin, prolamin-like protein (G_1), glutelin-like protein (G_2), true glutelin (G_3) and insoluble protein.
Then the nitrogen content of each fraction and the residue was determined using the micro-Kjeldahl procedure (AOAC, 1990).

Table 1: Amino acids composition (g/100 g protein) and protein content (%) of karkade seed and furundu product

Amino acid	Karkade seed		Furundu (9DF)**	FAO/WHO***
	Raw	Cooked		
Glycine	6.02	5.64	5.83	
Alanine	5.41	4.86	5.00	
Valine*	5.83	5.63	5.66	5.00
Leucine*	7.99	7.68	7.73	7.00
Isoleucine*	4.24	4.09	4.13	4.00
Serine	4.05	4.39	4.26	
Threonine*	3.34	3.40	3.42	4.00
Methionine*	1.11	0.91	0.93	3.50 (Methionine+cystine)
Cystine	1.75	1.46	1.48	
Penylalanine*	5.35	5.15	5.19	6.00 (Penylalanine+Tyrosine)
Tyrosine	1.79	1.68	1.80	
Aspartic acid	11.42	11.32	11.23	
Glutamic acid	18.27	18.58	18.52	
Lysine*	4.84	4.81	4.95	5.50
Arginine	11.69	11.16	11.31	
Histidine	2.22	3.58	3.99	
Ammonia	4.56	5.64	6.03	
Total protein#	32.28b (0.03)	32.33b (0.05)	33.03a (0.10)	

*Essential amino acids. **DF, Days of fermentation. ***FAO reference protein pattern (FAO/WHO, 1975). # Means of triplicate samples. Values in parentheses are standard deviations. Means followed by different letters within a row are significantly different according to DMRT ($p \leq 0.05$). Calculations based on free moisture.

Calculations:

$$\text{Protein fraction \%} = \frac{\text{\% soluble protein}}{\text{\% total protein}} \times 100$$

$$\text{\% soluble protein} = \frac{T \times TV \times N \times 14 \times 100}{a \times b \times 1000} \times 6.25$$

Where: T, Titer volume in milliliters; TV, Total volume of extract in milliliters; N, Normality of HCl; 14, Nitrogen equivalent weigh; 6.25, protein conversion factor; a, Number of milliliters digested; b, Grams of sample extracted.

Microbiological tests: Samples (10 g) of karkade seed at each stage of fermentation process were placed in 90 mL sterile 0.1% peptone water and shaken to prepare 10^{-1} dilution. Then a decimal dilution series was prepared in 0.1% peptone solution. Aliquots (0.1 mL) were used to inoculate on to the surface of agar media a spread plate technique. Additionally, aliquots (1.0 mL) were used in an agar pour plate procedure for total viable count of bacteria, staphylococcus and yeast and moulds. The agar media employed were: plate count agar incubated both aerobically and anaerobically; Mannitol salt agar- selective for staphylococci; potato dextrose agar containing 50 µg mL^{-1} chloramphenicol-selective for yeasts and moulds. Media after inoculation were incubated at 37°C (potato dextrose agar was also incubated at 28°C) and examined after 48 hours. Colonies on the agar plate were counted (cfu/g) and a proportional subsampling procedure was used to select colonies of bacteria for identification. Gram staining, spore staining, presence of active enzymes (Catalase and oxidase), differentiation of oxidation and fermentation of carbohydrates, examination for motility and growth in air tests were employed to identify the genera of bacteria (Harrigan, 1998).

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

Results and Discussion

Protein content and amino acids composition: Table 1, shows the protein content and the amino acids composition of the raw and cooked karkade seeds and furundu. The protein content of the karkade seed was 32.28%. Fermentation of the cooked karkade seed significantly ($p \leq 0.05$) increased the level of the protein to 33.03%. Leaching out effects and enzyme activity of microorganisms during furundu process may result in changes in dry matter constituents of the karkade seed that reflected in this increment. Glutamic acid, arginine and aspartic acids were the major amino acids in karkade seed and had values of 18.27, 11.69 and 11.42 g/100g protein, respectively. Relative to the FAO reference protein pattern (FAO/WHO, 1975), the limiting amino acids were 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 acid is comparable to the reference protein. Also, the amino acids profile of karkade seed is comparable

Table 2: Protein fractions (%)* of karkade seed and furundu product

	Protein fraction**						
Sample***	I + II alb. + glob.	III Prolamin	IV G1	V G2	VI G3	Insoluble protein	Protein recovered
Karkade seed	64.55^{a}(1.07)	3.63^{b}(0.00)	ND	0.93^{d}(0.08)	16.93^{e}(0.14)	14.23^{e}(0.11)	100.27
Cooked seed							
0DF	44.30^{b}(0.27)	2.12^{d}(0.10)	ND	2.98^{c}(0.23)	32.76^{c}(0.40)	16.20^{d}(0.31)	98.36
3DF	40.38^{c}(0.37)	4.01^{a}(0.16)	0.47^{a}(0.09)	3.48^{b}(0.13)	29.94^{d}(0.23)	19.53^{c}(0.08)	97.81
6DF	35.38^{d}(0.34)	3.21^{c}(0.49)	0.43^{c}(0.10)	4.78^{a}(0.16)	38.30^{a}(0.73)	20.59^{b}(0.13)	102.69
9DF	35.44^{d}(0.21)	2.16^{d}(0.00)	0.46^{b}(0.09)	4.68^{a}(0.16)	34.33^{b}(0.82)	21.29^{a}(0.13)	98.36

*Means of triplicate samples. Values in parentheses are standard deviations. Means followed by different letters within a column are significantly different according to DMRT ($P \leq 0.05$). Calculation on free moisture basis. **alb.: Albumin; glob.: globulin; G_1: Prolamin-like protein; G_2: Glutelin-like protein; G_3: True glutelin.; ND: Not detectable. *** DF: Days of fermentation.

Table 3: Content (%)* and HCl-extractability (%) ** of major elements of karkade seed and furundu product

	Major elements									
	Na		K		Ca		Mg		P	
Sample***	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Karkade seed	0.129^{a}(0.029)	97.17	1.481^{a}(0.020)	62.96	0.064^{a}(0.002)	16.93	0.121^{a}(0.007)	6.57	0.549^{a}(0.001)	39.13
Cookedseed										
0DF	0.105^{b}(0.002)	94.20	1.291^{b}(0.100)	86.10	0.074^{a}(0.009)	21.22	0.114^{a}(0.021)	6.68	0.552^{ab}(0.004)	39.67
3DF	0.107^{b}(0.004)	92.43	1.302^{b}(0.010)	27.53	0.068^{bc}(0.009)	19.15	0.119^{a}(0.006)	6.40	0.539^{c}(0.016)	28.01
6DF	0.106^{b}(0.002)	94.39	1.288^{b}(0.010)	72.39	0.072^{ab}(0.010)	19.31	0.116^{a}(0.006)	6.56	0.556^{a}(0.003)	40.65
9DF	0.108^{a}(0.003)	93.10	1.291^{b}(0.010)	69.40	0.072^{ab}(0.008)	19.22	0.122^{a}(0.004)	6.23	0.559^{a}(0.003)	35.42

*Means of triplicate samples. Values in parentheses are standard deviations. Means followed by the same letter within a column are insignificantly different according t o DMRT ($p \leq 0.05$). Calculations based on free moisture. **Means of duplicate samples. *** DF, Days of fermentation.

Table 4: Content (mg/100 g)* and HCl-extractability (%)** of minor elements of karkade seed and furundu product

	Minor element							
	Zn		Cu		Mn		Fe	
Sample***	Total	Available	Total	Available	Total	Available	Total	Available
Karkade seed	10.283^{b}(0.071)	30.12	9.497^{a}(0.131)	8.79	20.164^{a}(0.150)	21.70	23.353^{a}(0.078)	7.31
Cooked seed								
0DF	8.896^{d}(0.074)	26.41	6.933^{d}(0.208)	6.60	15.130^{e}(0.431)	42.93	19.660^{c}(0.114)	10.07
3DF	9.145^{c}(0.044)	21.27	6.963^{d}(0.210)	6.96	16.400^{d}(0.118)	38.53	21.207^{b}(0.538)	8.64
6DF	8.563^{e}(0.031)	26.40	7.643c(0.311)	7.15	16.763^{c}(0.064)	38.75	19.380^{d}(0.012)	9.87
9DF	10.407^{a}(0.032)	23.01	7.850^{b}(0.350)	7.26	17.130^{b}(0.288)	35.49	19.353^{d}(0.100)	8.33

*Means of triplicate samples. Values in parentheses are standard deviations. Means followed by the same letter within a column are insignificantly different according to DMRT ($p \leq 0.05$). Calculations based on free moisture .**Means of duplicate samples. ***DF, Days of fermentation.

to those obtained by El Faki *et al.* (1991) and El-Adawy and Khalil (1994). The ammonia content of the raw seed was 4.56 g/100g protein. Cooking of the karkade seed was increased histidine and ammonia but decreased glycine, alanine, valine and sulfur amino acids. Fermentation of the cooked karkade seed slightly increased histidine from 3.58 to 3.99 mg/100g protein with a concomitant increase in ammonia from 5.64 to 6.03 g/100g protein. Other amino acids almost remained unchanged. Transamination and/or deamination reactions might be responsible for the changes in amino acids profile of the karkade seed on processing.

Protein fractions: Landary and Moreaux classification of karkade seed and fururndu proteins is shown in Table 2. Fraction I + II (albumin plus globulin) and fraction III (prolamin) decreased significantly ($p \leq 0.05$) on cooking and further on fermentation. The G_1, G_2, G_3 and the insoluble protein fractions increased significantly ($p \leq 0.05$) in fururndu. The dissociation, denaturation or hydrolysis of the proteins may modify their solubility and hence extracted in other forms (Yagoub *et al.*, 2004).

Minerals composition and HCl-extractability: Results revealed that potassium content of the raw karkade seed was 1.481% (Table 3), which decreased significantly ($p \leq 0.05$) to 1.291% after cooking. The loss of potassium on cooking may be attributed to its leaching out into the discarded water. Sodium, Zinc, Copper, Manganese and iron followed the same trend observed for potassium. Losses into cooking water were reported (Saikia *et al.*, 1999; Duhan *et al.*, 2002). Other major elements did not affect by cooking. Moreover, fermentation of the cooked seed almost had no effect on contents of all major and minor elements studied. On the other hand, HCl-

Table 5: Phytic acid (mg/100g), total acidity (mg/100g) and pH of the karkade seed and furundu product

Sample*	Phytic acid	Total acidity	pH
Karkade seed	888.33^{a} (12.52)	1070.70^{a} (18.07)	6.06^{c} (0.05)
Cooked seed			
0DF	885.67^{a} (7.09)	588.93^{e} (9.00)	6.27^{b} (0.01)
3DF	885.33^{a} (5.68)	670.07^{d} (11.85)	6.60^{a} (0.02)
6DF	886.00^{a} (10.64)	712.43^{c} (9.78)	5.82^{d} (0.01)
9DF	885.67^{a} (6.31)	739.37^{b} (13.85)	5.81^{d} (0.01)

*Means of triplicate samples. Values in parentheses are standard deviations. Means followed by the same letter within a column are insignificantly different according to DMRT ($p \leq 0.05$). Calculations based on free moisture. DF; Days of fermentation.

Table 6: Total microbial viable count (cfu/g)* of karkade seed and furundu product

Sample**	Bacteria	*Staphylococcus* spp.	Yeasts and moulds
karkade seed	4.0 x 10^{4}	3.6 x 10^{3}	No growth
Cooked seed			
0DF	6.0 x 10^{2}	No growth	No growth
3DF	7.2 x 10^{4}	No growth	7.0 x 10^{4}
6DF	3.0 x 10^{5}	4.4 x 103	3.2 x 10^{5}
9DF	6.4 x 10^{6}	No growth	4.8 x 10^{6}

*Means of two isolates. **DF, Days of fermentation.

extractability of K and Ca in karkade seed (62.96%, 19.93%, respectively) was improved in furundu (69.40%, 19.22%, respectively). Results also show that the HCl-extractability of Mn and Fe in karkade seed were 21.70% and 7.31%, respectively, which both increased in furundu product to 35.49 and 8.33%, respectively. While, HCl-extractability of other trace elements did not improve.

The slight insignificant decrease in phytic acid observed in furundu (Table 5) may result in changes in HCl-extractability of Ca, K, Mn and Fe. Phytic acid was reported as having strong negative effect on the calculated availability of Ca, Fe and Zn (Mamro *et al.*, 2001).

Phytic acid: Table 5, illustrates that the phytic acid content of the karkade seed (888.33 mg/100g) is insignificantly ($p \geq 0.05$) reduced, in furundu (885.67 mg/100g). Cooking step during furundu preparation is the main cause for this reduction. El Maki *et al.* (2007) attributed the decrease in phytic acid during cooking to formation of insoluble complex between Phytates and other components and accordingly, the amount of free Phytate was reduced. Several studies indicate the involvement of some side chains of proteins in the formation of protein-phytic acid complexes (Reddy *et al.*, 1982).

Total titratable acidity and pH: Cooking of the karkade seed significantly ($p \leq 0.05$) decreased the total acidity and increased the pH from 6.06 to 6.27. Fermentation of the cooked seed for 9 days increased significantly ($p \leq 0.05$) the acidity with a decrease in the pH to 5.82 (Table 5). The decrease in acidity on cooking may be ascribed to leaching of the acidic constituents in to cooking water and the increase on fermentation may be due to action of the microbial enzymes on substrate proteins, carbohydrates and lipids. Yagoub *et al.* (2004) previously reported an increase in acidity during furundu fermentation.

Microbial succession during fermentation of furundu: Results revealed that the raw karkade seed contained total number of 4.0 x10^{4} cfu/g viable bacteria. Cooking of the karkade seed decreased the number to 6.0 x10^{2} cfu/g. Fermentation of the cooked seeds for 3, 6 and 9 days gradually increased the number to 7.2 x 10^{4}, 3.0 x 10^{5} and 6.4 x 10^{6} cfu/g, respectively; exceeding the viable bacterial count of the raw karkade seed (Table 6). The most dominating genus that has been isolated from the cooked karkade seed was the endospore-forming *Bacillus* species. This may suggests ability of *Bacillus* species isolated to withstand heat of cooking. *Bacillus*, however, continued domination within the first 3 days of fermentation and further on to the last 9th day of fermentation (Table 7).

Non-spore forming *Staphylococcus* species (Table 6), yeasts and moulds were absent in the cooked seeds; indicating susceptibility of these organisms to heat. *Staphylococcus* species *was* also almost absent during the fermentation period of 9 days. On the other hand, yeasts and moulds were steadily increased as increasing fermentation time from 7.0 x 10^{4} at the 3rd day to 3.2 x 10^{5} and 4.8 x 10^{6} cfu/g at the 6th and the 9th day, respectively. An increase in acidity and/or reduction in potential oxygen during fermentation process may provide suitable state for growth of yeasts and moulds. Since oxygen utilizing organisms were detected in the karkade seed substrate (Table 7).

In general, the dominance of *Bacillus* species almost during the whole course of furundu processing may be attributed to that, the cooking step adopted by the local people of Western Sudan may effectively remove much of the heat sensitive microflora and may favors the survival of endospore-forming *Bacillus* species. The elimination of yeasts may give the chance for amylase producing bacteria, such as *Bacillus* species, to utilize nutrients. Heat was found to change the nature of the proteins (De Man, 1990) and the carbohydrates (Davidson *et al.*, 1979). Thus may alter the course of fermentation by rendering proteins and carbohydrates more available to attack by microorganisms. Many indigenous fermentations of oilseeds in West Africa, such as agile and ogiri-sara, were reported to treat the seeds prior to fermentation in such a way similar to that

Table 7: Typing of bacteria during furundu preparation

Sample*	Isolate No.	Shape	Gram staining	Endospore staining	Growth in air	Catalase test	Oxidase test	Glucose (acid)	O/F test**	Motility	Genus
Karkade seed	1	Sphere	+	-	+	+	-	+	O	-	*Micrococcus*
	2	Rod	+	+	+	+	+	+	F	+	*Bacillus*
Cooked seed											
0DF	1	Rod	+	+	+	+	+	+	F	+	*Bacillus*
	2	Rod	+	+	+	+	+	+	+	+	*Bacillus*
3DF	1	Rod	+	+	+	+	+	+	F	+	*Bacillus*
	2	Rod	+	+	+	+	+	+	F	+	*Bacillus*
6DF	1	Rod	+	+	+	+	+	+	F	+	*Bacillus*
	2	Rod	+	+	+	+	+	+	F	+	*Bacillus*
9DF	1	Rod	+	+	+	+	+	+	F	+	*Bacillus*
	2	Rod	+	+	+	+	+	+	F	+	*Bacillus*

*DF: Days of fermentation. **O/F: Oxidation/fermentation.

for karkade seed during processing of furundu (Odunfa, 1985; Campbell-platt, 1987).

In conclusion, results of this study indicate that furundu raised from the cooked karkade seed have considerable amounts of essential amino acids and minerals. Limiting amino acids found in the raw seed remained limiting in furundu. The HCl-extractability of K, Ca, Mn and Fe were improved by furundu preparation. Also the microbial growth during fermentation of the cooked karkade seed is dominated by *Bacillus* species and yeasts and moulds. More subtyping of the active organisms is important.

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