

Research Article

Chemical Composition of Fermented Karkade (*Hibiscus sabdariffa* L.) Calyces and Leaves

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Abstract

Roselle (*Hibiscus sabdariffa* L.) an important base fiber crop is a member of the Malvaceae family. This study dealt with calyces and leaves of karkade (*Hibiscus sabdariffa* L.) to compare the effect of fermentation on its chemical composition. The calyces of karkade were obtained from El-Nasr Hibiscus Factory, Khartoum North, and the leaves for the same cultivar were collected from the experimental field of Khartoum University farm, Faculty of Agriculture. Calyces and leaves of karkade were fermented traditionally for 3, 5 and 7 days and compared with unfermented (control) sample. The moisture contents of calyces and leaves had dropped by 10% and 25% respectively, protein content increased in calyces from 8.53% to 10.57% and decreased from 8.75% to 7.07% in the leaves. The fermentation time increased the fiber content by 37% in calyces and slightly affected that of leaves. There was no significant difference in the oil content of calyces, with increasing of 36% in the content of leaves. The ash content increased significantly ($p < 0.05$) from 8.57% to 10.67% while that of leaves was not significantly affected ($p > 0.05$). Carbohydrate content decreased by 16% in the calyces, but it was increased 6% in the leaves. Mineral contents of karkade calyces and leaves were found to be generally dropped throughout the fermentation time. Fermentation increased the protein, fiber and ash contents of karkade calyces and decreased the moisture and carbohydrate contents with no effect on oil content. Among the fermentation treatment periods tested 5 days' fermentation, gave the best results and accordingly it is recommended to be used.

Keywords: Calyces; Chemical Composition; Fermentation; Karkade; Treatment

Introduction

The plant of karkade (*Hibiscus sabdariffa* L.) belongs to the family Malvaceae and the species *Sabdariffa* L. It is from a Turkish origin and is an annual plant that grows in the tropical and semi-tropical areas. The growth of this plant is concentrated in western Sudan and specially in the great kordofan state in the traditional rainfall zone. Kordofan produces 95% of karkade production in Sudan, karkade is grown as an essential and principle monetary crop in eastern kordofan that contains the cities of Um-Ruaba and Al-Rahad, this area is known to produce and export the highest quality types of karkade in the world The crop is also planted around Al Obeid and eastern kordofan and the sandy areas of southern kordofan. karkade is also planted around Al-Fashir in northern Darfur and around Nyala and Eldeain and Boram in southern Darfur. Furundu, a meat substitute, is traditionally prepared by cooking the karkade seed and then fermenting it for 9

days. Food fermentation can be classified in a number of ways (Dirar ,1993):by categories (1) alcoholic beverages fermented by yeast; (2) vinegars fermented with acetobacter; (3) milks fermented with lactobacilli; (4) pickles fermented with lactobacilli; (5) fish or meat fermented with lactobacilli; and, (6) plant proteins fermented with molds with or without lactobacilli and yeast. The principal production of Karkade in the Sudan is eastern kordufan sands in an area encompassing EL Rahad and Ummruaba. Karkade is grown on smaller scale around EL Obeid, in western kordufan near EL Fashir and Nyala. Sudan became the world major supplier of roselle dried calyces in 1960, In 1968 about 1,500 metric tons were exported [1]. Roselle can be used in many ways. In western Sudan, karkade is grown successfully as rain crop, not for its seeds but for its calyces which are widely used to prepare invigorating refreshing drinks. In addition, the water extraction of the calyces is added to color and alter flavor of sorghum or millet flour drinks (nasha). In the regions where it is grown, the leaves, young shoots and calyces are eaten raw as salad or cooked and the seeds are roasted. The tender leaves are as well essentially consumed as a pot-herb and the seeds can be conveniently converted to accept-

able meaty-tasting fermented food [2].

Material and Methods

Materials

Hibiscus sabdariffa L. (El-Rahad cultivar) calyx's samples were obtained from El-Nasr Hibiscus Factory Khartoum north and the leaves samples for the same cultivar were collected from the experimental field, of Khartoum University Farm, Khartoum North, Sudan.

Methods

Sample Preparation

Calyces and leaves of karkade were carefully cleaned, freed from foreign material, and stored. The calyces and leaves were ground to fine particle to pass 0.4 mm sieve, and then divided into four portions, one was kept raw (control, unfermented) and the other portions were taken to subsequent step to produce fermented substrate.

Natural Fermentation

Three ground portions were mixed with distilled water (1:6w/v). The mixture was incubated at 37°C in incubator for 3, 5, and 7 days, and the fermented mixtures were dried at 70°C and ground to pass 0.4 mm sieve.

Samples Preparation for Chemical Analysis

The raw and fermented samples were prepared as described by the Official Methods of Analysis [3]. Approximately, 100g of each sample was pulverized, using a super mill until a standard fine powder was obtained. The ground samples were kept each into an air33 tight container and stored at room temperature (28 – 32°C). Moisture Content (M.C), Crude Protein (C.P), Crude Fiber (C.F), Crude Oil (C.O), total ash, total carbohydrate, Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), Iron (Fe), Phosphorous (P), pH, total titratable acidity, ascorbic acid, total polyphenols, tannin and phytic acid inhibitors were systematically estimated.

Proximate Analysis

Moisture Content

Moisture content of garlic bulbs samples were determined according to the [4]. Two grams' garlic bulb were weighed using balance in a clean dry and pre-weighed crucible and placed in an oven at 105°C. The crucible was transferred to a desiccator allowed to cool and then weighed. Additional placements in the oven were carried out until a constant weight was obtained. Moisture content was calculated using the following formula:

$$MC \% = \frac{(w_3 - w_1) \times 100}{W_2 - w_1}$$

Where:

MC = Moisture Content

W1 = Weight of empty crucible.

W2 = weight of crucible with sample

W3 = weight of crucible with sample after drying.

Crude Protein

The crude protein of garlic samples was determined using the micro-kjeldahl method according to [4] as follows:

0.2 gram of the sample were weighed and placed in small digestion flask (250ml). Two catalyst tablets were added (anhydrous sodium sulphate + copper sulphate) then, 3.5ml of approximately 98% H₂SO₄ were added. The contents of the flask were then heated on electrical heater for 2 hours till the color changed to blue-green. The tubes were then removed and allowed to cool. The digested sample was transferred to the distillation unit and 15 ml of 40% NaOH were added. The ammonia was received in 100ml conical flask containing 10 ml of 2% boric acid plus 3-4 drops of methyl red indicator. The distillation was continued until the volume reached 50 ml. The content of the flask was titrated against 0.02N HCl. The titration reading was recorded. The crude protein was calculated using the following equation:

$$CP \% = \frac{(T - B) \times N \times 14 \times 100 \times 6.25}{WS \times 1000}$$

Where:

CP = Crude protein

T = Titration reading

B = Blank titration.

N = HCl normality

WS = Weight of sample

1000 = to convert to mg

6.25= Protein factor for garlic

Fat Content

Fat content of garlic samples were determined according to the method [4], using soxhlet apparatus. An empty clean and dry extraction round bottomed flask was weighed. About 2 grams of sample were weighed and placed in a clean extraction thimble and covered with cotton wool. The thimble was placed in the extractor. Extraction was carried out for 8 hours with petroleum ether. The heat was regulated to obtain at least 15 siphoning per hour. The residual ether was dried by evaporation. The flask placed in an oven at 105°C till it dried completely and then cooled in a desiccator and weighed. The fat content was calculated using the following equation:

$$FC = \frac{(W2 - W1) \times 100}{W3}$$

Where:

FC = Fat content

W1 = weight of the empty extraction flask

W2 = weight of extraction flask with fat

W3 = weight of sample.

Crude Fiber

Crude fiber of garlic samples was determined according to [4]. Two grams of defatted sample were treated successively with boiling solution of H_2SO_4 of 0.26 N and KOH of 0.2N. The residue was then separated by filtration, washed and transferred into a crucible then placed into an oven adjusted to 105°C for 18 - 24 hours. The crucible with sample was weighed and ashed in a muffle furnace at 500°C and weighed. The crude fiber was calculated using the following equation:

$$CF \% = \frac{W1 - W2 \times 100}{W3}$$

CF = Crude fiber

W1 = weight of crucible with the sample before ashing.

W2 = weight of crucible with sample after ashing

W3 = weight of sample

Ash Content

Ash content of garlic samples were determined according to method [4]. Two grams of sample were placed in a clean dry pre-weighed crucible, and then the crucible with its content ignited in a muffle furnace at 550°C for 3 hours or more until light grey ash was obtained. The crucible was removed from the furnace to a desiccator to cool and then weighed. Ash content was calculated using the following equation:

$$AC = \frac{W2 - W1 \times 100}{W3}$$

Where:

AC = Ash content

W1= weight of empty crucible

W2= weight of crucible with ash

W3= weight of sample

Determination of Minerals Content

Minerals of each sample were extracted according to Pearson's method [5]. Two gms of sample were placed in a muffle fur-

nace at 550°C for 4 hr, samples were cooled and 10 ml of 5N HCl were added, then boiled gently for 10 min using sand bath, diluted to volume (100 ml) with distilled water and taken for minerals determination.

Potassium and Sodium Contents

Potassium and sodium contents of each extracted sample were determined according to the Standard Official Methods of Analysis [3] using corning 400, flame photometer. One ml of the extract was diluted in a 50 ml conical flask with distilled water. The standard solutions of the KCL and NaCL were prepared by dissolving 2.54, 3.33g of KCL and NaCL respectively, each in 1000 ml distilled water. Ten mls of this solution were taken and diluted with 1000 ml distilled water to give a 10 ppm concentration. The flame photometer was adjusted to zero degree using distilled water as a blank and to 100 degree using standard solution. Calculations of the alkaline metals were effected by.

$$K \text{ or } Na (\%) = \frac{[F.R \times D.F \times 100]}{103 \times S \times 10}$$

Where:

F.R = flame photometer reading.

D.F = dilution factor.

S = sample weight.

Calcium and magnesium contents

Calcium and magnesium determinations were carried out for each sample extract according to [6]. Calcium was determined by taking 2 ml of the extracted sample and placed in a 50 ml conical flask. Ten mls of distilled water were then added to the contents in the flask. Three to four drops of 4N NaOH were added with small amount of meroxide indicator (0.5g of ammonium purpurat was mixed with 100g of powdered K_2SO_4) giving a pink color. The contents of the flask were titrated with 0.01N EDTA (ethylene diamine tetra-acetic acid) until a violet color, indicating the end point was obtained. Calcium and magnesium were determined together by taking 2 ml of the extract in 50 ml conical flask. Twenty mls of distilled water, 10 drops of buffer (6.75g ammonium chloride in 57 ml conc. Ammonia diluted to 100 ml with distilled water) and 3-4 drops of Eriochrome Black T (E.B.T) indicator (0.1g eriochrome + 0.9g hydroxylamine hydrochloride were dissolved in 20 ml of about 95% ethanol) were added to the extract giving purple color. The mixture was titrated with 0.01N EDTA until a blue color indicating the end point was reached. The magnesium content was estimated by subtracting the Ca content from (Ca + Mg) content. Calculation;

$$Ca \text{ or } Mg (\%) = \frac{[T.R \times N(EDTA) \times D.F \times M.wt \times 100]}{106 \times S \times 2 \times \text{valency}}$$

Where:

T.R = titration reading.

N(EDTA) = normality of EDTA.

D.F = dilution factor.

M. wt = molecular weight of the elements estimated.

S = sample weight.

Iron Content

Iron content of each extract was determined according to [7]. Ten mls of the extract were taken and put into a 50 ml conical flask. One ml of each of 30% H_2SO_4 and of 7% $K_2S_2O_8$ (potassium persulphate) were carefully added. Then 1.5 ml of 4% KCNS was transferred to the contents of the conical flask which was then allowed to stand for 20 minutes until a red color developed. The color density was measured using colorimeter (Lab System Analyzer – 9 filters, J. Mitra and Bros Pvt. Ltd.) at 540 nm.

Phosphorous Content

Phosphorus (P) was determined using the molybdo-vanadate method [8]. Two ml of the mineral extract were pipetted into a 50 ml volumetric flask. Ten ml of ammonium molybdate-ammonium vanadate reagent (22.5 g of $(NH_4)_6NO_7O_{24} \cdot 4 H_2O$ in 400 ml distilled water + 1.25 g ammonium vandate in 300 ml boiling water + 250 ml conc. HNO_3), then diluted to 1 liter] were added. The contents of the flask were mixed and diluted to volume. The intensity of the color was read after 30 min at 470 nm using Spectrophotometer (Model 6305 – England). A standard curve was plotted to calculate the phosphorus content.

$$P (\%) = R \times D. F \times 100 \\ 106 \times S$$

Where:

R = Curve reading

D.F= Dilution factor

S = Sample weight

Statistical Analysis

The data collected were subjected to analysis of variance and whenever appropriate the mean separation procedures of LSD was employed [9]. The SAS program [10] was used to perform the GLM analysis.

Results and Discussions

Proximate Composition

The effects of fermentation time (days) on proximate composition of karkade calyces and leaves are shown in Tables 1,2. Expectedly, the moisture content of the unfermented karkade calyces

were lower than that of the unfermented karkade leaves ($p < 0.05$). The value of the moisture content of karkade calyces compares favorably with that observed by [11,12]. The moisture contents of karkade calyces decreases as fermentation time increase (Table 1), likewise the content of moisture in karkade leaves decreases with fermentation time increase (Table 2). The drop in moisture content of karkade calyces throughout the fermentation periods is a proximately 10% compared to 25% a drop in that of karkade leaves. The protein contents of the unfermented karkade calyces and leaves were almost similar (Tables 1,2). These results are within the range of results obtained by [13] and higher than the value obtained [11]. The protein contents of karkade calyces increased with the increase in fermentation time (Table 1). Throughout the fermentation periods investigated the calyces fermented for 7days had the highest protein content, however, the increase of protein content in karkade calyces fermented traditionally may be due to increase in the mixture/variety of microbial species involved which might have secreted some extracellular enzymes [14,15]. The results of this study agrees with the earlier reports by [16] who reported that, fermentation increase protein content of the plant.

Similar results were observed by [12]. To the contrary fermentation lead to a substantial decrease (19.2%) in the protein content of karkade leaves. Furthermore, the protein contents of fermented leaves became constant from the 3rd day of fermentation till the end of fermentation period (7th day). The reduction of protein content might be attributed to the protein incorporated by the microorganisms. Initially (day 0) there was no considerable difference between unfermented karkade calyces and leaves in the fiber content (Tables 1,2), In this study the fiber content of karkade calyces was found to be close to the results obtained [11] and [17]. The fiber content of karkade calyces increased progressively ($p < 0.05$) with increase in fermentation periods (Table 1). By the end of fermentation period the change in the fiber content of karkade calyces mounted to 60%. On the other hand, the fiber contents of karkade leaves was not affected ($p > 0.05$) by fermentation period (Table 2), such results are in agreement with those reported by [17]. The oil content of the unfermented karkade calyces is nearly similar to that of karkade leaves (Tables 1,2). The value of the oil content in karkade calyces was found to be similar to the values ranges given by [13]. The oil content of karkade calyces was not affected ($p > 0.05$) by fermentation periods. These results are in agreement with the results observed by [12]. On the other hand, the oil content of karkade leaves increases progressively ($p < 0.05$) with increase in the fermentation time, resulting in a total change of 56% by the end of fermentation time (7 days). Obviously, the ash content of the unfermented karkade calyces was lower than that of unfermented karkade leaves (Tables 1,2). The ash content of karkade calyces fermented for 3 days was lowered compared to the calyces fermented for 5 days and 7 days. Conversely, there was no considerable differences ($p < 0.05$) in the ash content of karkade leaves throughout fermentation periods.

Clearly, it can be shown that the carbohydrate content of the unfermented karkade calyces were higher by about 14% than that of unfermented karkade leaves (Table 1,2). These results are in agreement with the data reported by [13]. There was a significant decrease ($p<0.05$) in carbohydrate content of karkade calyces among fermentation periods, especially from unfermented and sample fermented for 3days. This therefore supports the fact that the protein increase could be as a result of hydrolyses of starch to glucose and there was used by the same organisms as a carbon source to synthesize microbial biomass rich in protein. On the other hand, there was a considerable increase in carbohydrate content of karkade leaves especially the leaves fermented for 3days, while the content of carbohydrate from 3days and till the end of fermentation times (7days) showed considerable differences. The results of this study favorably compared to the results reported by [11].

FT	moisture (%)	protein (%)	Fiber (%)	Oil (%)	Ash (%)	CHO (%)
0	9.47 ^a ±0.21	8.53 ^c ±0.22	11.40 ^c ±0.53	0.83 ^a ±0.02	8.57 ^b ±0.15	61.21 ^a ±0.51
3	9.23 ^a ±0.06	9.84 ^b ±0.22	15.99 ^b ±0.56	0.83 ^a ±0.02	8.90 ^b ±0.70	55.20 ^b ±1.40
5	9.07 ^a ±0.57	10.04 ^a ±0.04	16.29 ^b ±0.61	0.82 ^a ±0.02	10.37 ^a ±0.32	53.42 ^b ±0.89
7	8.57 ^b ±0.61	10.57 ^a ±0.06	18.14 ^a ±0.53	0.82 ^a ±0.02	10.67 ^a ±0.12	51.23 ^c ±0.42

* FT = fermentation time (days).

* Means in the same column bearing different superscript letters were significantly different ($p<0.05$).

* n = 3

Table 1: Effect of fermentation time (days) on the proximate composition of karkade calyces.

FT	Moisture (%)	Protein (%)	Fiber (%)	Oil (%)	Ash (%)	CHO (%)
0	12.33 ^a ±0.32	8.75 ^a ±0.00	10.97 ^a ±0.33	0.75 ^d ±0.05	13.50 ^a ±0.00	53.67 ^b ±0.50
3	12.20 ^a ±0.61	7.07 ^b ±0.06	10.18 ^a ±0.20	0.82 ^c ±0.03	13.60 ^a ±0.10	56.13 ^a ±0.68
5	10.83 ^b ±0.72	7.07 ^b ±0.06	10.80 ^a ±0.53	0.98 ^b ±0.03	13.73 ^a ±0.06	56.92 ^a ±1.12
7	9.77 ^c ±0.15	7.07 ^b ±0.06	10.80 ^a ±0.34	1.17 ^a ±0.29	13.87 ^a ±0.75	57.33 ^a ±0.12

* FT = fermentation time (days).

* Means in the same column bearing different superscript letters were significantly different (p<0.05).

* n = 3

Table 2: Effect of fermentation time (days) on the proximate composition of karkade leaves.

Mineral Composition

The effects of fermentation time (days) on the mineral contents of karkade calyces and leaves are shown in tables 3,4. From tables 3,4, it can be seen that the sodium content of the unfermented karkade calyces was found to be similar to that of unfermented karkade leaves. Clearly it can be shown that the sodium contents in both karkade calyces and leaves were not affected (p>0.05) by fermentation at 3 days and then affected significantly (p<0.05) at 5 and 7 days by the same values. There was a significant difference (p<0.05) in the potassium contents between unfermented karkade calyces and leaves (Tables 3,4). The content of potassium in karkade calyces was found to agree with the results obtained by [18], while the potassium content of karkade leaves was higher than the data reported by [13], this variation may be due to the genetic factors. On the other hand, it can be obviously observed that the potassium contents of karkade calyces significantly decrease (p<0.05) among fermentation periods except between 3 and 5 days. Conversely, the potassium contents of karkade leaves significantly decreased at 3 days of fermentation, whilst the values of potassium became constant from 3rd day and till the end of fermentation time (7 days). Clearly, the calcium content of the

unfermented karkade leaves were higher than that of unfermented karkade leaves (Tables 3,4).

The value of calcium content in karkade leaves was found to be similar to the values ranges given by [1] and [13] There was a significant decrease (p<0.05) in the calcium contents of karkade calyces throughout the fermentation periods. On the other hand, the calcium contents of karkade leaves (Table 4) was not affected by 3 days of fermentation, and then significantly decreases till the last day of fermentation (7 days). Expectedly, the magnesium content of the unfermented karkade leaves were higher than that of unfermented karkade calyces (p<0.05). there were no differences in the magnesium content of karkade calyces fermented for 3 days while the values decrease significantly through fermentation time till the end (Table 3). these results are lower than those reported by [13,19]. In contrast, the contents of magnesium in karkade leaves were not affected (p>0.05) throughout the fermentation periods. Obviously, the iron content of the unfermented karkade leaves was higher than that of unfermented karkade calyces. There were no considerable differences in the iron content of karkade calyces among fermentation periods, these values are in agreement with the range values observed by [13] and higher than those reported by [1,18].

FT	Na (%)	K (%)	Ca (%)	Mg (%)	Fe (%)	P (%)
0	0.07 ^a ±0.00	0.43 ^a ±0.06	0.45 ^a ±0.05	0.006 ^a ±0.00	0.15 ^a ±0.01	0.27 ^a ±0.05
3	0.07 ^a ±0.00	0.33 ^b ±0.06	0.30 ^b ±0.05	0.006 ^a ±0.00	0.14 ^a ±0.00	0.26 ^a ±0.05
5	0.06 ^b ±0.00	0.30 ^b ±0.10	0.23 ^c ±0.06	0.005 ^b ±0.00	0.13 ^a ±0.01	0.20 ^b ±0.02
7	0.06 ^b ±0.01	0.23 ^c ±0.06	0.13 ^d ±0.06	0.004 ^c ±0.00	0.11 ^a ±0.01	0.19 ^b ±0.03

* FT = fermentation time (days).

* Means in the same column bearing different superscript letters were significantly different (p<0.05).

* n = 3

Table 3: Effect of fermentation time (days) on the minerals content of karkadeCalyces.

FT	Na (%)	K (%)	Ca (%)	Mg (%)	Fe (%)	P (%)
0	0.07 ^a ±0.00	0.53 ^a ±0.06	1.15 ^a ±0.04	0.15 ^a ±0.01	1.63 ^a ±0.03	0.32 ^a ±0.04
3	0.07 ^a ±0.00	0.40 ^b ±0.10	1.13 ^a ±0.02	0.15 ^a ±0.01	1.55 ^b ±0.05	0.29 ^a ±0.01
5	0.06 ^b ±0.01	0.40 ^b ±0.00	1.07 ^b ±0.06	0.13 ^a ±0.01	1.55 ^b ±0.03	0.26 ^b ±0.01
7	0.06 ^b ±0.00	0.40 ^b ±0.10	0.98 ^c ±0.02	0.10 ^a ±0.01	1.50 ^b ±0.02	0.24 ^b ±0.01

* FT = fermentation time (days)

* Means in the same column bearing different superscript letters were significantly different (p<0.05).

* n = 3

Table 4: Effect of fermentation time (days) on the minerals content of karkade leaves.

On the other hand, the contents of iron in karkade leaves were affected significantly (p<0.05) by fermentation times especially at 7 days which had the lowest content of iron (Table 4). As shown in Tables 3,4 there were no considerable differences (p<0.05) in phosphorous content between the unfermented karkade calyces and leaves. Also it can be observed that there was no significant difference in the phosphorous contents in karkade calyces and leaves at 3rd day and then significantly (p<0.05) decreased at 5 and 7 days when compared to unfermented sample. However, the contents of phosphorous in karkade calyces are in fair agreement with the results observed by [1] and [13], also the data in agree with [18]. While the contents of phosphorous in karkade leaves are in agreement with the results reported by [1] and higher compared to those obtained by [13]. Generally, the minerals content of karkade calyces and leaves decrease throughout fermentation periods, this result may be assertion to the microorganisms which might have use some of the minerals for their metabolic activities [20]. On the other hand, and from the nutritional point of view, karkade calyces and leaves are seen to be a rich source of calcium and potassium. They are also a fairly good source of iron.

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