Original Research Article

EFFECT OF BOILING AND FERMENTATION ON PHYSICOCHEMICAL PROPERTIES, FATTY ACID AND MICRONUTRIENTS COMPOSITION OF *Hibiscus sabdariffa* (Roselle) SEEDS

ABSTRACT

Aim: To ascertain the effects of boiling and fermentation on the physicochemical properties, fatty acid, mineral and vitamin composition of *Hibiscus sabdariffa* (HS) seeds.

Study design: Completely randomized design

Place and Duration of Study: Rivers and Anambra states, Nigeria, between February and September, 2019.

Methodology: Two portions of 200 grams of HS seeds each were subjected to boiling and fermentation. The three samples were designated HSR, HSB and HSF for raw, boiled and fermented HS seeds respectively. Standard methods were used in determining the physicochemical properties and micronutrient composition, while fatty acid constituents were identified using a gas chromatography.

Results: The acid, free fatty acid, peroxidase values and specific gravity were significantly increased ($p<0.05$), while iodine value was significantly reduced ($p<0.05$) after boiling and fermentation. Saponification value showed a mixed trend, while refractive index was not significantly ($p>0.05$) altered. Lauric (5.51–33.79%), palmitic (27.23–30.87%) and myristic (12.69–35.00%) acids were the predominant saturated fatty acids in HSR, HSB and HSF samples respectively. Oleic, linoleic, alpha-linolenic and arachidonic acids were the unsaturated fatty acids present in the samples. Boiling increased oleic acid level, while fermentation caused a drastic reduction (>90%) in its amount. Linoleic acid level improved up to 43% after fermentation. Magnesium, iron and sodium amounts significantly ($p<0.05$) reduced after boiling and fermentation, while zinc, calcium and molybdenum levels were significantly ($p<0.05$) improved after boiling. Na/K ratios for all the samples were greater than 0.60, while Ca/Mg values ranged between 0.82 and 3.46, below the recommended value (1.0). Vitamins B1, B3, B12 and D were significantly reduced ($p<0.05$) after boiling and fermentation, while fermentation significantly increased ($p<0.05$) vitamins B2, A, E and K levels.

Conclusion: HS seeds were shown to possess good physicochemical properties that can enhance its utility in the industry. Boiling and fermentation maximized the usefulness of HS seeds as quality nutritional plant.

Keywords: [Hibiscus sabdariffa seeds, processing, physicochemical properties, fatty acid, minerals, mineral ratios, vitamins]

1. INTRODUCTION

Hibiscus sabdariffa (Roselle) Linn.is part of Malvaceae family believed to be from East Africa, Asia (India to Malaysia) or Tropical Africa. *Hibiscus sabdariffa* (HS) Linn seeds are cultivated in many countries such as Egypt, India, Mali, Malaysia, Nigeria, and Sudan have been found to contain high amount of protein, dietary fiber, vitamins, lipids, and minerals [1 – 5]. Seeds of HS have already been noted as prolific and were reported early in the century among African food grains, as being consumed in Northern Nigeria after grinding into a course meal. They are highly regarded as a nourishing food [6]. They are crushed and boiled in water to the consistency of a thin porridge and eaten as a sauce with staple foods among the Banyoro of Uganda. In the Sudan, HS seeds are used as a seasoning after fermentation, and in the South of Sudan the seeds are ground into flour [6].

Figure 1: *Hibiscus sabdariffa* seeds

In the northern regions of Cameroon, HS seeds are used to make “Mbuja” a condiment produced by Fermentation. Mbuja is also known as Bikalga (Burkina Faso), Dawadawa botso (Niger), Datou (Mali), Furundu (Sudan) [7].

According to Anioara-Arleziana *et al*. [8], physicochemical properties are imperative in determining the overall stability and quality of food materials. Some of the important physicochemical properties are acid value, specific gravity, iodine value, saponification value, peroxide value and refractive index. They are used to monitor the compositional quality of oils. Fatty acids are inherent in plant oils and the property of such oil is usually a function of the constituent fatty acids, which may either be a non-essential fatty acid (omega – 9) or the essential fatty acids (omega – 3 and 6) gotten from the diet [9]. Great proportions of unsaturated fatty acids are predominant in triglycerides from plant sources of oils, and the extent of unsaturation is related to the extent of oxidative deterioration. Therefore, determination of fatty acid composition of oils highlights the characteristics and stability of the oil.

Micronutrients are useful properties of food substances that enhance quality nutrition [10]. Minerals are very important in human nutrition for proper metabolic activities and enzymatic actions in the body. Magnesium is involved in regulating the acid-base balance in the body, utilization of iron and enzyme activity, while calcium and magnesium play major roles in carbohydrate metabolism, nucleic acids and binding agents of cell walls. Potassium is essential in synthesis of amino acids and proteins. Calcium helps in teeth development. Iron is very essential in formation of haemoglobin in red blood cells; hence it can help in stimulation of erythropoiesis. Vitamins can contribute to normal growth of body cells and skin, proper immune function, normal vision, cell development, gene expression and maintenance of epithelial cell functions [11].

Processing of seeds (such as boiling and fermentation) or other plant parts can either adversely affect or improve their nutrient composition. Also, bioavailability, usefulness and utilization of nutrients in food sources are seriously affected by the degree, nature or extent of processing they pass true. Boiling and fermentation have been shown to significantly alter the quantities of nutrients and anti-nutrients in seeds [12]. The impact of temperature on the stability, viscosity, peroxide value, iodine value to assess the quality and functionality of the oil have been studied by Farhoosh *et al*. [13] and Li *et al*. [14]. The objectives of this study include determination of physiochemical properties, and assay of fatty acids, minerals and vitamins compositions of raw and processed *Hibiscus sabdariffa* seeds.

2. MATERIAL AND METHODS

2.1 Sample Collection and preparation

Dried *Hibiscus sabdariffa* seeds were collected from Mangu Local Government Area, Plateau State, Nigeria. They were properly cleaned by removing all dirt and sorting out damaged seeds. The cleaned dried seeds were put in a container and stored properly for further use. A portion of the raw seeds was pulverized into a fine powder with an electric blender and stored in a lid-tight container for further analyses in the laboratory.

2.2 Processing of *Hibiscus sabdariffa* Seeds

2.2.1 Boiling

This was done according to the method modified from Mariod *et al.* [15]. *Hibiscus sabdariffa* (3 x 200 g) of raw seeds of HS was boiled in 500 mL distilled water for forty (40) minutes till they become softened when squeezed between the fingers. The cooked seeds were drained of water, dried, pulverized into fine powders and stored in a tight-lid container for further analyses.

2.2.2 Fermentation

This was carried out using a modified method of Parkouda *et al.* [7]. After boiling and draining off water from boiled seeds, the seeds were covered in a tight-lid container and allowed to ferment for 3 – 4 days. The fermented seeds were dried, ground into powder with an electric blender and stored in a tight-lid container in a refrigerator.

2.3 Determination of physicochemical parameters

Standard methods were used in determining the physicochemical properties. Acid, saponification, peroxide and iodine values were determined using the methods of A.O.A.C. [16]. Refractive indices were analyzed using Abbe refractometer at 25 ^{0}C according to Oderinde and Ajayi [17]. pH was measured electrometrically according to APHA [18] using an electric pH meter. Thiobarbituric acid value was determined as mg malondialdehyde per kg sample.

2.4 Determination of Fatty Acid Composition

The fatty acid constituents were identified on a Gas Chromatography (Agilent 6890N) equipped with Flame Ionization Detector and a 30 x 0.32m DB-225 silica capillary column (J and W Scientifics, USA). The split injector (1 mL) and detector were operated at a temperature of 230 °C and 25 °C respectively, while the oven temperature of 160 °C/2min was increased to 230 °C on a scale of 4 °C/min. Nitrogen was the carrier gas at a flow rate of 1.5 mL/min. The peaks were compared with standard methyl esters while the percentage area was recorded with standard Chemstation system.

2.5 Determination of Mineral Composition

Mineral composition was determined using Agilent FS240AA Atomic Absorption Spectrophotometer (AAS) according to the method of American Public Health Association [19].

2.6 Determination of Vitamins

Retinol and tocopherol (vitamins A and E) were determined calorimetrically using the method of Kirk and Sawyer [20]. Determination of thiamine, riboflavin, niacin and cobalamin (vitamins B1, B2, B3 and B12 respectively) and vitamin K were by spectrophotometric method while pyridoxine and ascorbic acid (vitamins B6 and C respectively) were determined by titrimetric method according to Kirk and Sawyer [20]. These methods are as described by AOAC [16].

2.7 Data Analysis

All data obtained in this study were subjected to statistical analyses using One-way Analysis of Variance (ANOVA) to test for differences between the raw and processed groups. All the values were reported as means ± standard deviation (SD) and the results were considered significant at P-values of less than 0.05 ($P<0.05$) i.e. at 95% confidence level.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties

The mean values of the physicochemical properties of the oils of raw (HSR), boiled (HSB) and fermented (HSF) *Hibiscus sabdariffa* seed oils are shown in Table 1. Generally, acid value can be related to the quality of the fatty acids in oil in terms of stability and shelf life. The acid values in this study were higher than the acid value for raw, sun-dried and roasted groundnut oil (2.35, 1.79 and 2.52 mgKOH/g respectively) as reported by Ayoola and Adeyeye [21]. It was however, lower than that found in *Duranta repens* seed oil (21.01 mgKOH/g) as reported by Agomuo *et al.* [10] and *plukeneti aconophora* (11.5mg KOH/g) as shown by Akintayo and Bayer [22]. The low acid values of the raw, boiled and fermented seeds of HS (2.51, 4.02 and 4.66 mgKOH/g respectively) strongly suggest that the oil may be very suitable for manufacture of soap, cooking, manufacture of margarine, mayonnaise, salad oils and cosmetics. The free fatty acids (FFA) for HSR sample is similar to that of raw groundnut oil (1.18%) [21]. The FFA values were all below the maximum limit of 5.0% reported for Nigerian palm oil [23]. An increase in the level of FFA in the samples may be as a result of hydrolysis of triglycerides which may occur by the action of lipase enzyme, an indicator of processing and storage conditions (i.e., high temperature and relative humidity, tissue damage) [24]. FFAs are sources of flavours and aromas. Samples with lower FFA values tend to be soluble in water and volatile with characteristic smell, while samples with higher FFA values are more prone to oxidation in their free form and their breakdown products (aldehydes, ketones, alcohols, and organic acids) provide characteristic flavors and aromas [24]. The low levels of percentage FFA in the three samples (1.26, 2.01 and 2.05% respectively) indicate that the oils from them may be useful edible oils that may be stored for a long time without spoilage via oxidative rancidity. The peroxide value of the samples were higher than that reported for water melon seed (3.24 mleq/kg) by Gladvin *et al.* [25] and different refined groundnut species (1.30 – 1.73 mleq/kg) by Nkafamiya *et al.* [26] but lower than those of *Opuntia dilleni* (15.60 mleq/kg) by Njoku *et al.* [27], crude groundnut oils (22.06 - 25.03 mleq/Kg) [26], *Duranta repens* leaf oil (20.00 mleq/kg) and *Duranta repens* seed oil (12.29 mleq/kg) by Agomuo *et al.* [10]. The peroxide values of the samples of *Hibiscus sabdariffa* seeds increased possibly as a result of boiling and fermentation. The peroxide value has been identified as the most common indicator for lipid oxidation [28] and [29] demonstrated that peroxide values greater than 10 mleq/kg is an indication that the oils are highly prone to auto-oxidation as a result of presence of trace elements or moisture. Such oils can be unstable and may easily go rancid. The peroxide values of all the oil samples were less than the standard peroxide value (10 mleq/kg) for vegetable oil deterioration and thus, suggest that they can be put on storage for an elongated time without becoming rancid or deteriorating. The lower level of the peroxide value of the raw sample suggests that it may have higher shelf life than the processed samples. Fresh oils have value less than 10 mleq/kg and value between 20 and 40 mleq/kg leads to rancid taste [30]. The low peroxide value indicated slow oxidation of these oils as suggested by Demian [31]. The iodine value for raw seeds of HS (72.07 gI_2/100g) was higher than 52.0 gI_2/100g for palm oil [32], *Opuntia dinelli* (63.33 gI_2/100 g) by Njoku *et al.* [27] and similar to the iodine value of *Durana repens* leaf oil (72.65 gI_2/100 g) by Akubugwo *et al.* [33], while those of boiled (46.47 gI_2/100g) and fermented (40.07 gI_2/100g) were lower. The values of the boiled and fermented samples were higher than that in *Cocos nucifira* (9.60 gI_2/100g), *Pentaclethra macrophylia* (20.50 gI_2/100g) and *Treculia africana* (27.50 gI_2/100g) as reported by Akubugwo *et al.* [33], whereas the iodine value of the raw sample was higher than them all. The iodine value of the boiled sample is similar to that of crude Kampala Michika oil (46.88 gI_2/100 g) [26] and *Durant repens* seed oil (44.84) [10], while that of the fermented sample was similar to that of *Citrullus vulgaris* with 38.1% [34] and Hausa melon seed with 38.50% [35]. The iodine values of all the samples were lower than that found in both crude and refined gargajiya oil (81.94 and 97.13 gI_2/100 g respectively) by Nkafamiya *et al.* [26] and water melon seed oil (112 gI_2/100 g) [25]. The reduction in iodine value after processing was similar to the trend found in raw and heat-processed groundnut oil where the iodine value reduced from 110.7 gI_2/100 g for raw groundnut to 100.7 gI_2/100 g for roasted groundnut oil as reported by Ayoola and Adeyeye [21]. With the classification of Duel [36] for oils and fats, (drying oils: IV 200-130, Semi drying: IV 130-100 and Non-drying: IV lower than 100), the samples all had iodine values less than 100 and therefore can be classified as non-drying oils in terms of industrial importance and also, as classified by Aremu *et al*. [37]. The iodine value is the generally accepted parameter used in showing the degree of unsaturation and number of carbon-carbon double bonds in fats or oils [38]. This value may be useful in determining the amount of double bonds present in the oil which in turn reflects the susceptibility of the oil to oxidation. The lower iodine values in the boiled and fermented samples in this study may imply few unsaturated bonds found in them and hence low susceptibility to oxidative rancidity [39]. The decrease in iodine value after processing (boiling and fermentation) may suggest lipid oxidation, which could be as a result of presence of metal ions and other factors, which enhances or promotes oxidation after the formation of hydroperoxide [40,41]. The SV of the raw sample of HS seed oil was similar to that of Winsor orange-coloured cashew nut seed oil (212.00) by Aremu and Akinwumi [42], *Jatropha curcas* seed oil (208.50) by Igwenyi [43] and yellow melon seed oil (210.00) by Egbebi [44]. The SV of the boiled sample of HS seeds was similar to that of melon seed oil (148.50) reported by [45] and Almond seed oil (151.55) as reported by Ogunsuyi and Daramola [46], while that of fermented sample was similar to coconut oil (248-265) [47] and *C. nucifera* (246.00) as reported by Amoo *et al*. [48]. The saponification value of oils is of interest when considering using the oil for industrial purposes [49]. Saponification value is applicable in tracking adulteration [50]. The larger the saponification values of oil, the better their soap-making abilities [51]. The saponification values greater than 200 mgKOH/g may indicate high proportion of unsaturated short chain fatty acids in the samples and may promote stability of the oil. This shows that they have a very high potential use in soap making and food industries. Denniston *et al*. [52] reported that high saponification value indicated the presence of greater ester bonds, suggesting that the fat molecules were intact. These properties make it useful in soap making industry. Furthermore, the high saponification values indicate oxidation and its decrease suggest the onset of oxidation. Rossel [53] reported similar observation. TBA values are used in assessing the level of oxidation of fats and oil (lipid oxidation) in terms of the amount of malondialdehyde (secondary product of oxidation of fats and oil) present in a sample. The presence of thiobarbituric acid in the samples suggests that some forms of oxidation had taken place as suggested by Lukaszewicz *et al*. [54]. These values may be useful in carrying out sensory tests aimed at ascertaining rancidity in food systems as suggested by these authors [55 – 57]. The raw and fermented samples had higher TBA values than the boiled sample in this study. The refractive index (RI) of the samples were 1.40, 1.42 1nd 1.40 for raw, boiled and fermented samples respectively. These values were less than the standard values for refined and virgin oils (1.4677–1.4707) according to CODEX-STAN [32]. However, they were higher than the RI of melon seed oil (1.35) as ascertained by Edidiong and Ubong [58], while the RI of the boiled sample was found to be same as that of cashew nut seed oil (1.420) by Aremu and Akinwumi [42]. The RI of an oil denotes the ratio of speed of light to its speed in the oil/fat itself, at a particular wavelength. The RI is important during quality control by indicating isomerization and hydrogenation which are necessary when ascertaining the purity of a substance [10]. The pH ranged from 4.67 – 6.17, with the fermented sample being the most acidic (4.67). The pH of the raw sample is similar to the pH of *Duranta repens* seed oil (6.16) as determined by [10]. The decrease in the pH may be attributed to the effect of microorganisms, which produces carbon dioxide during fermentation, thereby making the samples more acidic. This can be influenced by the duration of the fermentation process. The pH and acid values are used to assess the quantity of free fatty acids present in oils and can as well, determine their shelf life and stability [10]. The SG of the raw sample was similar to those of Koto/Pteryogota seed oil (0.930), *Pteryogota macrocarpa* (0.928) and Luffa gourd seed (0.930) as reported by Amoo and Agunbiade [59] and Oluba *et al.* [60] respectively. The boiled and fermented samples had SG values similar to Castor seed oil (0.959) and Cashew nut seed (0.964) as determined by Akpan *et al.* [61] and Aremu *et al*. [62]. The SG in the current study were higher than found in Melon seed oil (0.850) by Edidiong and Ubong [58], groundnut seed oil (0.914) by Musa *et al*., [63] and pumpkin seed oil (0.830) by Akubugwo *et al.* [33]. Whereas, they were found to be lower than SG of *Duranta repens* seed and leaf oils (1.64 and 1.02) [10] and Almond seed oil (1.71) by Akpambang e*t al*. [64]. The result showed that oils of the sample in the present study are less dense than water (1 g/cm^3) and therefore may find application in cream production, because it could make the oils flow and can easily be spread on the skin [45]. SG can be used alongside other figures in assessing the purity of oil [65].

Table 1: Physicochemical analysis of the oil of raw, boiled and fermented *Hibiscus sabdariffa* seeds

Parameters	HSR	HSB	HSF
Acid value (mgKOH/g)	2.51 ± 0.01^a	4.02 ± 0.04^b	4.66 ± 0.12^c
Free fatty acid (%)	1.26 ± 0.01^a	2.01 ± 0.21^b	2.05 ± 0.01^b
Iodine value (gI_2/100g)	72.07 ± 2.04^c	46.47 ± 4.01^b	40.07 ± 3.10^a
Peroxide value (mleq/kg)	4.40 ± 0.20^a	9.6 ± 0.50^c	8.25 ± 1.45^b
Saponification value (mgKOH/g)	210.10 ± 8.57^b	148.72 ± 7.11^a	256.68 ± 10.20^c
Thiobarbituric acid (mg.mal/kg)	3.58 ± 0.06^b	2.63 ± 0.30^a	3.58 ± 0.10^b
pH	6.17 ± 0.01^c	5.20 ± 0.03^b	4.67 ± 0.07^a
Refractive index	1.40 ± 0.01^a	1.42 ± 0.00^a	1.40 ± 0.02^a
Specific gravity	0.93 ± 0.02^a	0.99 ± 0.05^b	0.97 ± 0.01^b

Values are means of three determinations ± standard deviation (SD). At ($P < 0.05$), means with different superscripts in a row are significantly different from each other.

3.2 Fatty acid profile

The fatty acid profile of the samples (HSR, HSB and HSF) is presented in table 2. The results showed that Lauric (5.51 – 33.79%), palmitic (27.23 – 30.87%) and myristic (12.69 – 35.00%) acids were the predominant saturated fatty acids in HSR, HSB and HSF samples respectively. Oleic, linoleic, alpha linolenic and arachidonic acids were the unsaturated fatty acids present in the samples. Oleic acid was found in all the samples (HSR – 15.85%, HSB – 21.50% and HSF – 0.79%), linoleic in HSB (19.85%) and HSF (34.84%), alpha linoleic in HSF (2.18%) and arachidonic acid only in HSR (2.36%). Generally, the levels of lauric, palmitic and myristic acids in this study were higher than those reported by Rao [2] for mesta (*Hibiscus sabdariffa*) seeds and *Duranta repens* leaf oil [10]. Kostik *et al*. [66] reported higher amounts of lauric acids in coconut (48%) and palm kernel (41%) oils, and lower amounts of myristic acid in corn oil (0.6%), cottonseed (0.4%) and Safflower (0.5%). However, Ahmad *et al.* [67] reported similar amount of palmitic acid in HSB (30.87%) for *Hibiscus sabdariffa* seed oil; Agomuo *et al.* [10] in HSR and HSB in myristic acid for *Duranta repens* seed oil. Also, Al-Wandawi *et al*. [68] and Ahmed and Hudson [69] reported similar palmitic acid levels in Iraqi karkade cultivars (17.85–28.46) and crude karkade seed oil (20.5%) respectively. The stearic acid levels in HSR and HSB were lower than that of *Duranta repens* leaf (6.78%) and seed (8.05%) oils [10], Canola type 2 oil (6.9%) [66] and mature stems of *Opuntia dilleni* [27], but similar to soybean (4.00%), peanut (4.50%), and Canola type 1 (5.2%) oils [66] and crude Karkade seed oil (5.8%) by Abu-Tarboush [70]. The stearic acid level of HSF (19.23%) was much higher than all the oils mentioned above. HSR, HSB and HSF had stearic acid levels higher than mesta seed (2.4%), sunflower seed (2.0%), linseed (3.5%), cotton seed (2.0%), palm kernel (2.0%) and coconut (2.0%) oils reported by Kostik *et al*. [66]. The ratio of unsaturated to saturated fatty acids (SFAs) was found to be low, compared to another study by Soheir and Deba [71]. This may be as a result of geographical factors, growing conditions, degree of maturation etc. This implies that the samples had more SFAs and short chain FA may be used in chemical industries for soap and cosmetic production [72]. However, many studies have reported the harmful impacts small chain fatty acids on the human body by mainly lowering HDL cholesterol and increasing LDL cholesterol [73]. The oleic acid content in HSR and HSB were higher than that in coconut oil (8.8%) [66], egusi melon oil [74] and *Duranta repens* seed oil (11.47%) [10]; but lower than in crude Karkade seed oil [70], and groundnut oil (44.90%), cashew seed oil (34.47%) and pumpkin seed oil (36.10%) [74]. The oleic acid composition in this study is comparable to that of Safflower (16.6%) and Linseed (22.5%) [66] and rubber seed oil (23.74%) [74]. In comparison to the findings of Kostik *et al.* [66], the linoleic acid contents of the samples in this study were higher than those in coconut (0.5%), palm kernel (1.25%) and olive (7.0%) oils, much lower than those in corn (48.0%), soybean (49.5%), sunflower (59.5%) oils and similar to those in linseed (20.5%), peanut (20.0%) and canola variety 1 (18.8%) oil. Bello and Anjorin [74] also reported linoleic acid content in groundnut oil (32%) and cashew seed oil (34.47%) similar to HSF (34.84%) in this study. Also, Okra seeds contain 31.48% linoleic acids [75]. From the results of this study, the samples had lower amounts of unsaturated fatty acids. However, the unsaturated fatty acids were more concentrated in HSB (41.35%) and HSF (37.81%) samples, but they were all lower than the saturated fatty acids. Polyunsaturated fatty acids are essentially fatty acids needed for normal growth, physiological functioning and maintenance of the body. Linolenic acid is an omega – 3 polyunsaturated fatty acid (PUFA) involved in the regulation of biological functions and management of a many human diseases like hearth and inflammatory diseases [76]. However, further increase in PUFA may predispose the oil to oxidation [77]. The presence of oleic acid, linoleic, alpha linolenic and arachidonic acids suggests that the samples may find industrial applicability for pharmaceutics, soaps, shampoo and cosmetics productions. Unsaturated fatty acid improves lipid profile, whereas excess consumption of SFAs may cause obesity and elevated cholesterol levels [78]. Boiling and fermentation increased the levels of the SFAs – magaric, myristic and stearic acids, while the level of lauric acid reduced after boiling and fermentation. Varied effects of boiling and fermentation on the unsaturated fatty acids were observed in the study. While boiling increased the amount of oleic acid, fermentation caused a drastic reduction (> 90%) in its amount. However, the amount of linoleic acid improved by up to 43% after fermentation. These alterations may be as a result of the breakage of the fatty acid bonds or their complete degradation. Fermenting microorganisms may also contribute to the breakdown of fatty acids.

Table 2: Fatty acid profile of raw, boiled and fermented *Hibiscus sabdariffa* seed

Fatty acid	% Composition		
	HSR	HSB	HSF
SATURATED FATTY ACIDS			
C8 = Caprylic acid	0.91	ND	ND
C12 = Lauric acid	33.79	5.51	10.14
C14 = Myristic acid	13.36	12.69	35.00
C16 = Palmitic acid	27.23	30.87	ND
C17 = Magaric acid	1.47	4.49	ND
C18 = Stearic acid	4.17	5.10	19.23
C20 = Arachidic acid	1.14	ND	ND
UNSATURATED FATTY ACIDS			
C18:1 = Oleic acid	15.58	21.50	0.79
C18:2 = Linoleic acid	ND	19.85	34.84
C18:3 = Alpha linolenic acid	ND	ND	2.18
C20:4 = Arachidonic acid	2.36	ND	ND

ND = Not detected

3.3 Mineral Composition and Mineral ratios

The results of the mineral composition of HSR, HSB and HSF are presented in table 3. Magnesium, iron and sodium amounts (21.35, 24.08 and 17.24 mg/kg respectively) in HSR were significantly higher (p<0.05) than in HSB (5.95, 10.00 and 8.40 mg/kg respectively), which was also significantly higher than in HSF (3.74, 5.28 and 2.79 mg/kg respectively). Zinc and calcium contents of HSR (11.06 and 20.60 mg/kg respectively) were significantly higher (p<0.05) than in HSB and HSF, while lead and molybdenum contents were significantly higher in HSB (0.40 and 0.19 mg/kg) than in HSR and HSF. Lead (0.07 – 0.1 mg/kg), cobalt (0.09 mg/kg) and molybdenum (0.02 – 0.19 mg/kg) were found to be in least amounts. The variations in the levels of the macronutrients may be as a result of different geographical locations, methods of cultivation, soil types, processing methods etc, which they were subjected to. During boiling, there are tendencies of these nutrients to be leached into the boiling water, thereby causing their loss. The decrease in magnesium after boiling is in consonance with the observation of Tounkara *et al.* [79] which investigated the effect of boiling of the physicochemical properties of Roselle seeds in Mali. However, there was a disagreement in the results for other elements. There was a significant reduction (P<0.05) in Na/K level through the processing stages (HSR>HSB>HSF). Ca/K, Ca/Mg, Zn/Cu and Fe/Cu values in HSB were significantly elevated (P<0.05) after boiling when compared to HSR and HSF. All the Na/K values were greater than 0.60 (4.03, 2.29 and 1.09) for HSR, HSB and HSF respectively. This is the ratio that favours none enhancement of high blood pressure disease in man [80]. This denotes that the samples may not be suitable for managing high blood pressure. To bring this ratio low, consumption of foods rich in potassium is highly encouraged. The Ca/Mg values ranged between 0.82 and 3.46 whereas the recommended value is 1.0 [80]. Therefore, only HSB (3.46) and HSF (1.51) had the recommended Ca/Mg level. Both Ca and Mg would need adjustment for good health.

Table 3: Mineral Composition of raw, boiled and fermented *Hibiscus sabdariffa seeds*

Parameters	Concentration (mg/kg)		
	HSR	HSB	HSF
Magnesium	21.35 ± 0.68^{c}	5.95 ± 0.05^{b}	3.74 ± 0.58^{a}
Lead	0.12 ± 0.02^{a}	0.40 ± 0.10^{b}	0.07 ± 0.02^{a}
Manganese	2.45 ± 0.07^{b}	ND	0.08 ± 0.02^{a}
Copper	1.02 ± 0.06^{b}	0.22 ± 0.01^{a}	0.18 ± 0.01^{a}
Iron	17.24 ± 0.63^{c}	8.40 ± 0.10^{b}	2.79 ± 0.12^{a}
Zinc	8.25 ± 0.09^{b}	11.06± 0.59^{c}	0.90 ± 0.10^{a}
Cadmium	0.43 ± 0.03^{b}	0.47± 0.09^{b}	0.10 ± 0.00^{a}
Molybdenum	0.02 ± 0.02^{a}	0.19± 0.01^{b}	ND
Sodium	24.08 ± 1.21^{c}	10.00± 0.26^{b}	5.28 ± 0.19^{a}
Potassium	5.98 ± 0.21^{b}	4.37± 0.46^{a}	4.84 ± 0.07^{a}
Calcium	17.46 ± 0.13^{b}	20.60± 0.20^{c}	5.64 ± 0.09^{a}
Aluminum	0.96 ± 0.68^{b}	ND	0.01 ± 0.01^{a}

Values are means of three determinations ± standard deviation (SD). At (P < 0.05), means with different superscripts in a row are significantly different from each other. HSR = Raw *Hibiscus sabdariffa* seeds; HSB = Boiled *Hibiscus sabdariffa* seeds and HSF = Fermented *Hibiscus sabdariffa* seeds. ND = Not detected

Table 4: Mineral ratios of raw, boiled and fermented *Hibiscus sabdariffa* seeds

Mineral Ratios	HSR	HSB	HSF
Na/K	4.03[c]	2.30[b]	1.09[a]
Ca/K	2.92[b]	4.76[c]	1.17[a]
Ca/Mg	0.82[a]	3.46[c]	1.53[b]
Zn/Cu	8.14[b]	50.03[c]	5.01[a]
Fe/Cu	16.98[a]	38.08[b]	15.40[a]

Values are means of three determinations ± standard deviation (SD). At $P < 0.05$, means with different superscripts in a row are significantly different from each other.

3.4 Vitamin composition

The results of the vitamin composition of HSR, HSB and HSF are presented in figure 2. Vitamin B1 (thiamine) (23.45 mg/kg) and vitamin D (calciferol) (133.17 mg/kg) contents in HSR were significantly higher (p<0.05) than in HSB and HSF which did not differ significantly (p>0.05) from each other. Vitamins B2 (riboflavin), B6 (pyridoxine), E (tocopherol), A (retinol), C (ascorbic acid) and K contents in HSF (1.96 mg/kg, 102.50 mg/100g, 7.00 mg/kg, 42.53 mg/kg, 2.86 mg/kg and 6.52 mg/kg respectively) were significantly higher (p<0.05) than in HSR and HSB. Vitamin B12 (cobalamin) was significantly lower (p<0.05) in HSF than in HSB and HSF. Vitamins B2 (riboflavin), E (tocopherol), A (retinol), C (ascorbic acid) and K contents in HSR and HSB were not significantly different (p>0.05) from each other. Vitamin B6 content in HSR (53.74 mg/100g) was significantly lower than in HSB and HSF. The vitamin compositions of the seeds of HS show that they are good sources of vitamins and the presence of these vitamins can contribute to normal growth of body cells and skin, proper immune function, normal vision, cell development, gene expression and maintenance of epithelial cell functions [11]. Vitamin B6 was present in a reasonable amount and it helps in formation of red blood cells and maintenance of brain function. This vitamin also plays an important role in the proteins that are part of many chemical reactions in the body. Vitamin B12 is involved in formation of red blood cells and vitamin K aids in blood clotting [81]. Vitamin C is important for proper body function and its deficiency may interfere with the normal formation of intracellular substances which could lead to impaired growth and development in the body. It is also crucial in the maintenance and repair of tissues such as bones, skin and teeth. The antioxidant vitamins (A, C and E) were present in the raw, boiled and fermented seeds of HS and they neutralize free radicals that can accumulate in the body which in turn, leads to aging and some diseases. Therefore, the seeds of HS may possess ameliorative potentials if supplemented with other anti-oxidant rich plants against diseases linked with oxidative stress. The reduction in vitamin B1 (thiamine) and B3 (Niacin) contents after boiling is in agreement with the earlier reports of Fadahunsi [82] on Bambara groundnut flour, Prinyawiwatkul *et al.* [83] on cowpeas and Barampama and Simard [84] on beans. Further decrease in the amount of thiamine after fermentation is also in line with Fadahunsi [82] on Bambara groundnut flour, Philips *et al*. [85] on fermented cowpea and Wang and Hesseltine [86]; Murata *et al*. [87] on fermented soybeans; Van Veen *et al*. [88] and Keuth and Bispring [89] on fermented wheat. This decrease in the amount of vitamin B1 may be due to rapid utilization of vitamin B1 for optimum growth and other functions at a higher rate than its synthesis by the fermenting organisms [86]. For Vitamin B2 (Riboflavin), there was a reduction in its amount after boiling (though not significant). Similar report was given by Fadahunsi [82] for Bambara groundnut flour, Philips *et al*. [85] and Uzogara *et al*. [90] for boiled cowpea, whereas Deosthale [91] reported such in chicken peas and green peas. The significant ($p<0.05$) increase in vitamin B2 after fermentation is in tandem with Fadahunsi [82] on Bambara groundnut flour and Philips *et al*. [85] on fermented cowpea. Fermentation also significantly increased the amount of the antioxidant vitamins (A, C and E) and K. This increase in vitamins was also reported by Akinyele and Akinlosotu [92] on fermented cowpeas and Eka [39] on fermented locust bean. However, it disagrees with Barampama and Simard [84] which reported a decrease in the vitamin content of beans after fermentation. The variations in the levels of the vitamins may be as a result of different geographical locations, methods of cultivation, type of soil, processing methods e.t.c., which they were subjected to.

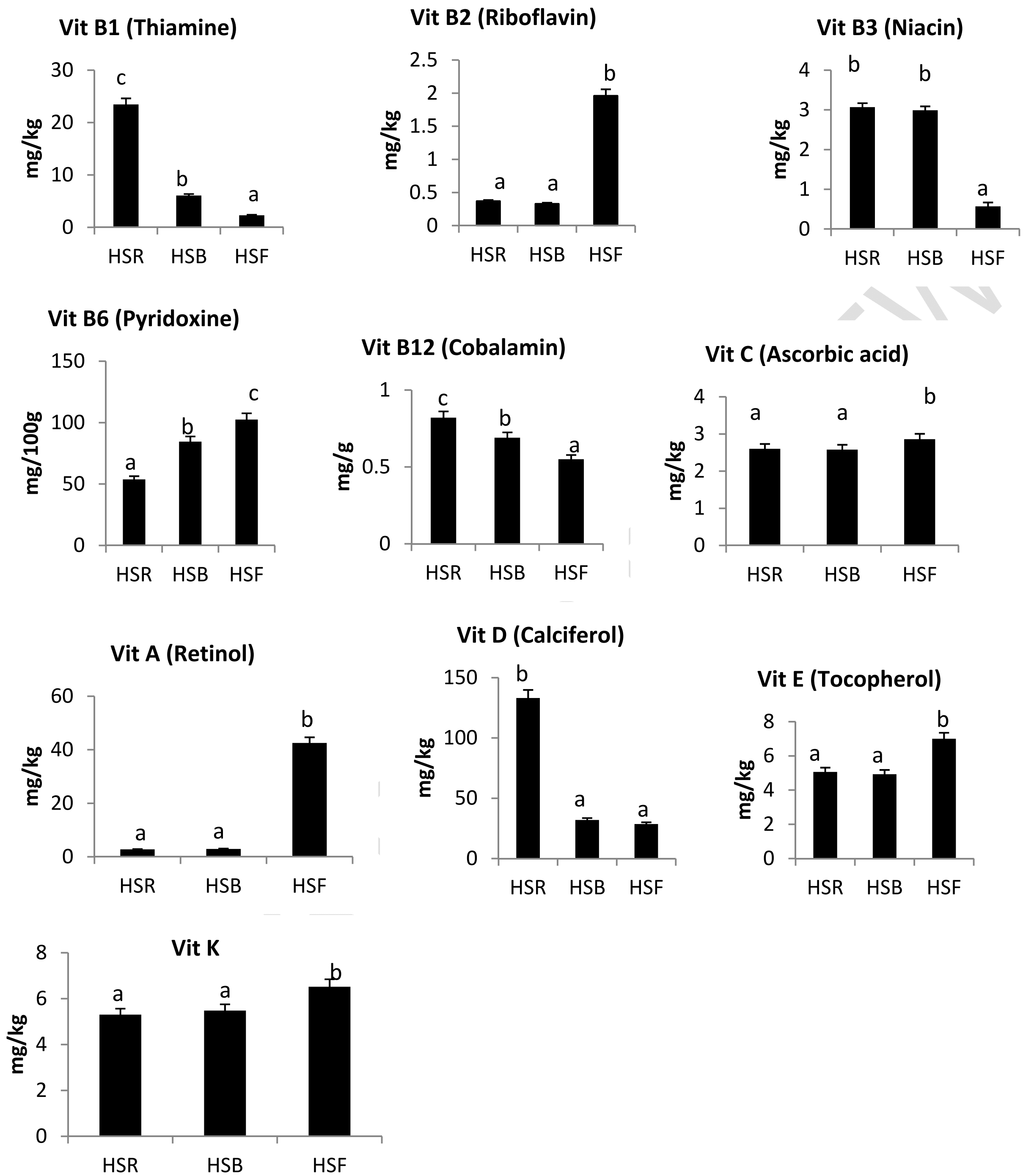

Figure 2: Vitamin composition of HSR, HSB and HSF. Values are means of three determinations ± standard deviation (SD). At $P < 0.05$, bars with different superscripts in a chart are significantly different from each other

4 CONCLUSION

The results of the physicochemical analysis showed that raw and processed *Hibiscus sabdariffa* seed oils are suitable for industrial applications in terms of shelf life, stability, density and resistance to auto-oxidation. The seeds are majorly composed of saturated fatty acids and also some polyunsaturated fatty acids. Minerals and vitamins were detected in reasonable amounts. Processing caused varied alterations in the micronutrient composition of HS seeds, most of which maximizes its usefulness as quality nutritional plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors.

CONSENT (WHERE EVER APPLICABLE)

None

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

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APPENDIX

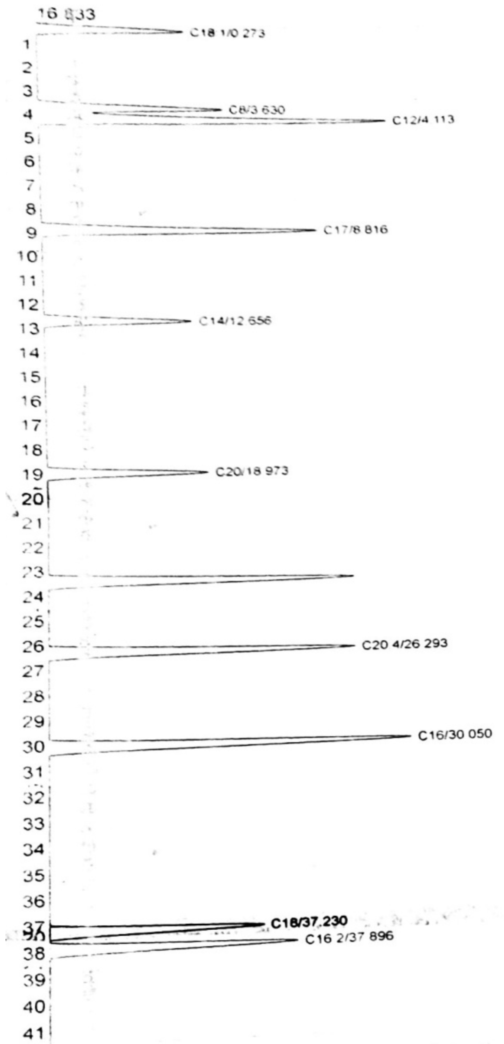
A Chromatogram of fatty acid profile of raw *Hibiscus sabdariffa* seed

Client: Charles
Client ID: DA134
Collected: 24/01/18
Description: FID
Column: RESTEK 15METER MXT-1
Carrier: HELIUM AT 5 PSI
Components: fame standard cpt
Data file: Charles fatty acid profile ()
Sample: fatty acid profile
Comments: TYPE YOUR COMMENTS HERE

HSR

Events

Time Event



Component	Retention	Area	Height	External	Units
C18 1	0 273	4636 9515	273 847	12 6256	ppm
C8	3 630	15478.1068	339 145	0 7386	ppm
C12	4 113	11295.9556	632 310	27 3788	ppm
C17	8 816	8838 2628	500.548	1 1903	ppm
C14	12 656	4803.1439	272 736	10 8266	ppm
C20	18 973	5160.5610	293 146	0 9214	ppm
C20 4	26 293	9544 9712	539 008	1 9148	ppm
C16	30 050	11160 0115	630 662	22.0624	ppm
C18	37 230	6529.9332	371 001	3 3792	ppm

B Chromatogram of fatty acid profile of boiled *Hibiscus sabdariffa* seed

Lab name: Springboard Lab
Client: Charles
Client ID: DA134
Collected: 25/09/18
Method: Syringe Injection
Description: FID
Column: RESTEK 15METER MXT-1
Carrier: HELIUM AT 5 PSI
ontrol filename: DEFAULT CON
Data file: Charles Fatty acid profile ()
Sample: fatty acid profile
Comments: TYPE YOUR COMMENTS HERE

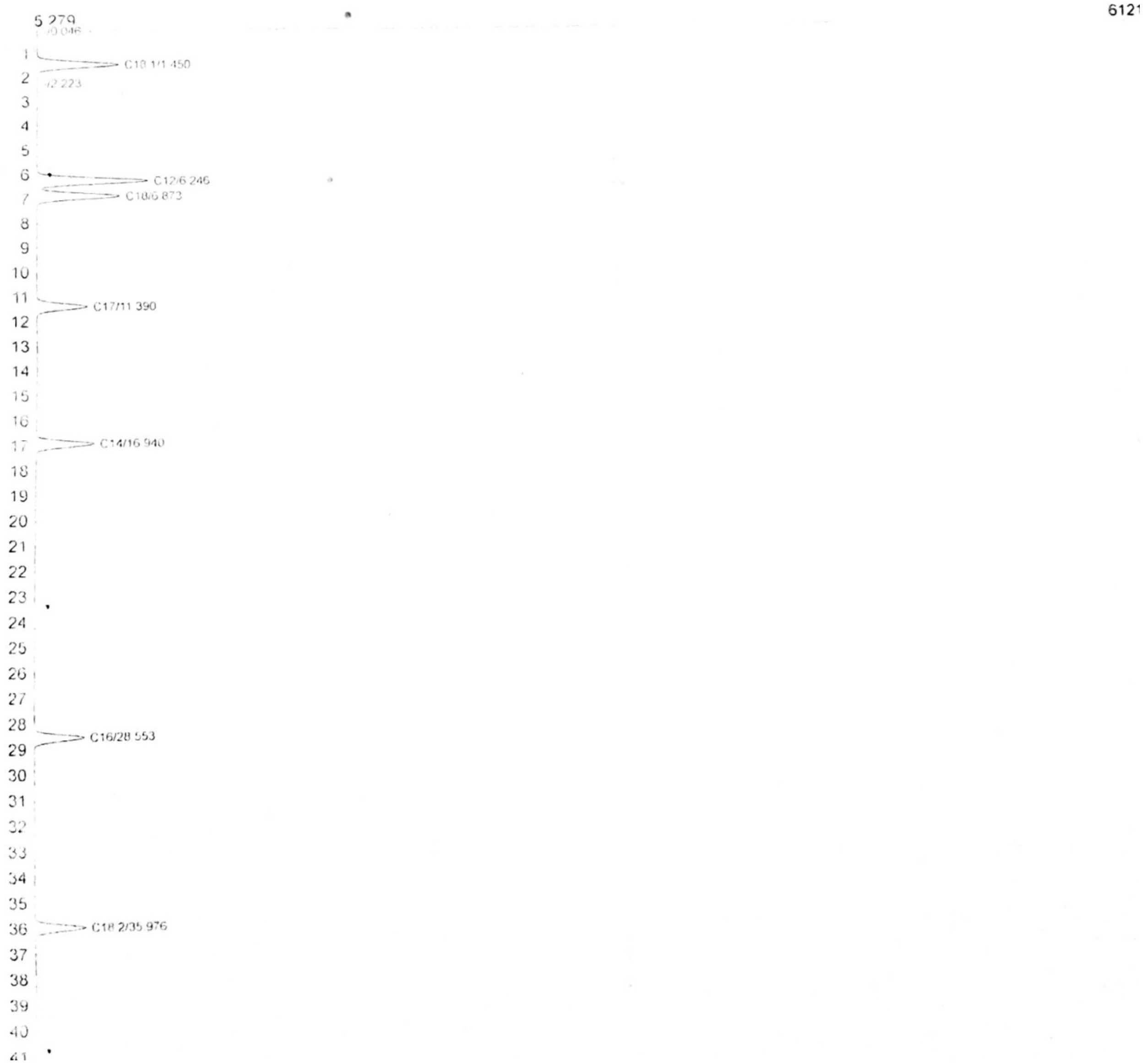
HSB

Temperature program

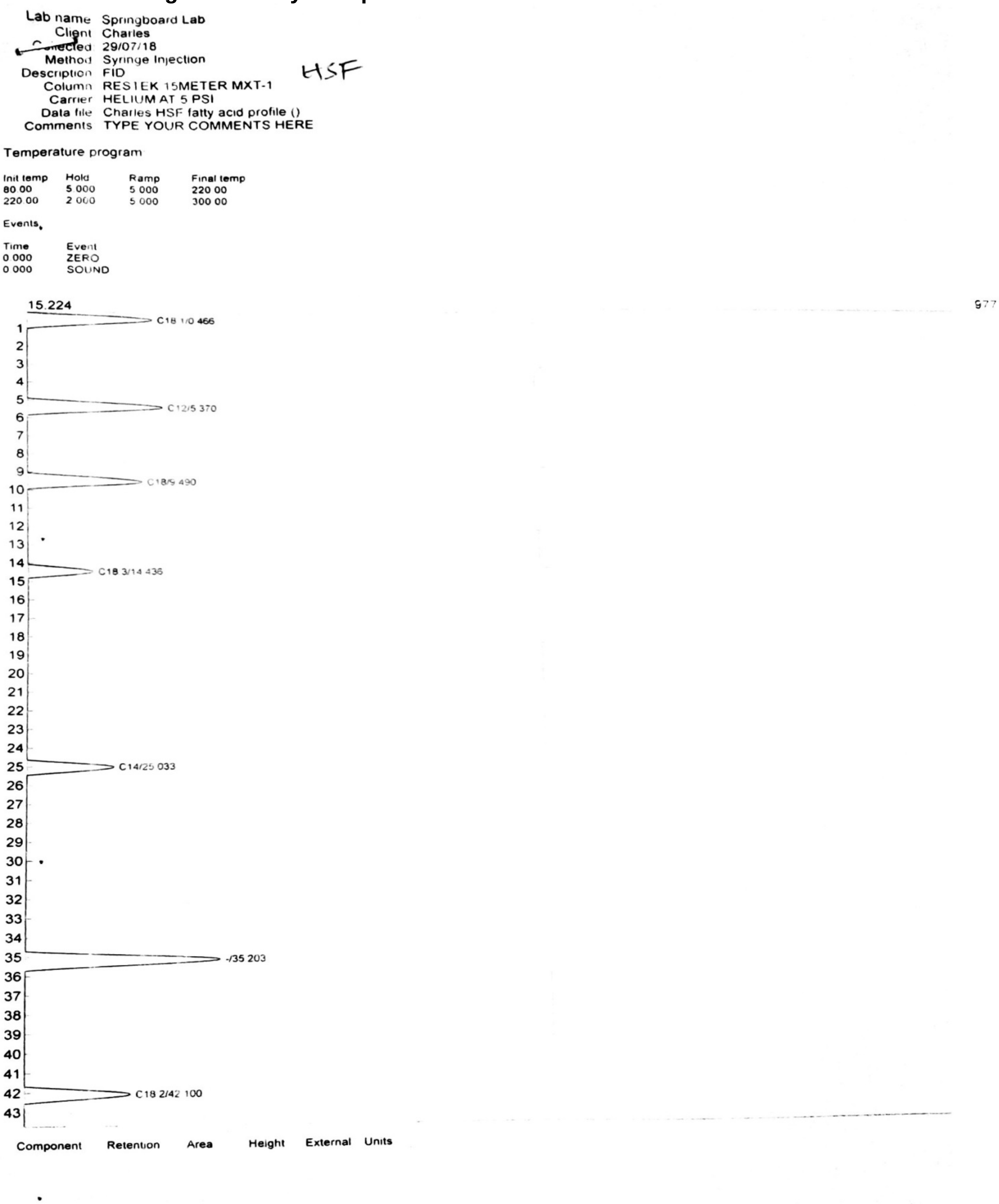
Init temp	Hold	Ramp	Final temp
70 00	10 000	10 000	220 00
220 00	5 000	5 000	280 00

Events

Time	Event
0 000	ZERO
0 000	SOUND



C **Chromatogram of fatty acid profile of fermented *Hibiscus sabdariffa* seed**



1

2