



Research Article

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Physicochemical properties of Roselle (*Hibiscus sabdariffa* L.) seeds oil (Elrahad-1) in North Kordofan, Sudan

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Abstract

The aim of this study was to clarify the physicochemical property evaluation of Roselle seed oil. Two samples (red and white calyces) of Roselle seeds (Elrahad-1) were obtained from El-obied Agricultural Research Station. The Roselle seed oil was extracted by soxhlet method. The standard procedure of the Association of Official Analytical Chemists (AOAC) was used to determine the physicochemical properties (oil%, specific gravity, refractive index, viscosity, saponification value, acid value, iodine value, peroxide value and fatty acids). The yield of the extracted oil for both red and white was found to be 21.1%. The oil had a refractive index (1.467, 1.466), saponification value (189.7, 189.1), iodine value (119, 119), peroxide value (4.6, 4.7), acid value (3.57, 3.55), viscosity (22.5, 22.5), specific gravity (0.90, 0.90). The main unsaturated fatty acids in the oil are Oleic (47.0555%, 47.8868%), Linoleic (30.5836%, 30.7931%) and Elaideic acid (14.359%, 15.1603%) and the saturated acids are Palmitic acid (3.9494%, 3.9198%) and Myristic acid (1.9609%, 1.9845%). These values were arranged for red and white calyces respectively. Comparing the study results with the standards and guideline of edible oils set by the FAO/WHO and the Sudanese standard and the metrology organization (SSMO) the study recommended that the Roselle seed oil can be an economic source of healthy edible fat and for other food industry applications and suggest further study on the effect of storage time on the physicochemical characteristic of the oil.

Keywords: Roselle seed, Elrahad-1, Edible oil.

Introduction

Roselle (*Hibiscus sabdariffa* L.) belongs to the family Malvaceae, locally called “Karkade” is an important annul crop grown successfully in tropical and sub-tropical climates.¹ There are more than 300 species of hibiscus around the world, one of them is Roselle (*Hibiscus sabdariffa* Linn.), which is a member of the plant family Malvaceae.² There are two main types of Roselle are recognized *Hibiscus sabdariffa* var altissima and *Hibiscus sabdariffa* var sabdariffa. Altissima is nearly branchless and can grow up to 3-5 m in height, its flowers are yellow, and calyces are red or green with high fiber but not used for food. The other distinct type of *Hibiscus sabdariffa*, grows in a bush with many branches. The flowers are axillaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column. The calyx enlarges at maturity. The more economically important is var. altissima, which is cultivated for its jute-like fiber in India, East Indies, Nigeria and South America, whereas var. sabdariffa is another distinct type of Roselle and is also widely exploited for its calyces and fiber.³ Roselle plants are suitable for tropical climates with well-distributed rain- fall of 1500-2000 mm/year, from sea-level to about 600 m in attitude. The plant tolerates a warmer and more humid climate with night time temperature not below 21 °C, and is most susceptible to damage from frost and fog. In addition, it requires 13 hours of sunlight during the first months of growth to prevent premature flowering.⁴ Roselle is a very versatile plant similar to the coconut tree.⁵ Roselle can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico.^{3, 6, 7}

Materials and Methods

Plant materials

Two samples of Roselle seeds (ELrahad-1) were obtained from El-Obeid Agricultural Research Station in May 2013 (Red calyces and White calyces). The Roselle seeds were ground into powder using a Grinder (Model EM- 11, Sharp, Japan).

Preparation of extract

50 g of (*Hibiscus sabdariffa* L.) seed powder were placed in a cellulose paper cone soxhlet and extracted with Hexane for 8 hours.⁸ The oil was recovered by evaporating off the solvent using rotary evaporator (Model N-1Eyela, Tokyo Rikakikal Co. Ltd. Japan). The crude extract oil obtained was 17 ml.

Viscosity measurements

The experiment was carried out by placing 3 ml of sample oil in a concentric cylinder system using $100s^{-1}$ as shear ratio.

Specific Gravity

The dry pycnometer was filled with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. After the insertion of the stopper the pycnometer was immersed in a water bath at 30 °C for 30 minutes. After the bottle was removed from the bath it was cleaned and dried thoroughly. The cap of side arm was removed and the specific gravity bottle was weighed in 30 °C.

$$\text{Specific gravity at } 30\text{ }^{\circ}\text{C} = \frac{A-B}{C-B}$$

Where:

A= weight in gm of specific gravity bottle with oil at 30 °C.

B= weight in gm of specific gravity bottle at 30 °C.

C= weight in gm of specific gravity bottle with water at 30 °C.

Refractive Index

After the prisms were cleaned and dried, few drops of the sample were placed on the prism; the prism was closed and allowed to stand for 2 minutes. The instrument was adjusted, lighted and the refractive index was determined.

Saponification Value

A sample of oil was mixed thoroughly; 2.0 g was taken from the sample and placed in 250 ml conical flask. 25 ml of alcoholic potassium hydroxide solution was added to the sample in the conical flask. Blank determination, along with the above sample was conducted. The sample flask was connected with blank flask and kept in the water bath. They were gently but steadily boiled until saponification was completed, which was indicated by the absence of any oily matter, and appearance of the solution. Clarity may be achieved within an hour of boiling. After the flask and condenser have cooled some waste was washed down

inside the condenser with about 10 ml of hot ethyl alcohol, neutral to phenolphthalein, the excess of potassium hydroxide was titrated with 0.05N hydrochloric acid and 1.0ml phenolphthalein indicator was used.

$$\text{Saponification Value} = 56.1 (B-S) N/W$$

Where:

B= volume in ml of standard hydrochloric acid required for the blank.

S= volume in ml of standard hydrochloric acid required for the sample.

N= normality of the standard hydrochloric acid.

W= weight in gm of the oil taken for test.

Acid value

3 g of the cooled oil sample was weighed in a 250 ml conical flask, 100 ml of freshly neutralized hot ethyl alcohol, 1.0 ml of phenolphthalein indicator solution were added, the mixture was boiled for 5 minutes, titrated while hot against standard alkali solution and shake vigorously during the titration,

$$\text{Acid Value} = (56.1)V.N/W$$

Where:

V= volume in ml of standard potassium hydroxide.

N= normality of the potassium hydroxide solution.

W =weight in gm of the sample.

Iodine Value

2 ml of oil was weighed into 250 ml conical flask with glass stopper to which 10 ml of chloroform was added, the content was mixed well, the weight of the sample exceeded 25-35 % of w/w solution over the actually needed, 25 ml of w/w solution was pipette, the glass stopper was replaced after wetting with potassium iodide solution, swirled for proper mixing and the flasks were kept in dark for 90 minutes, blank was carried simultaneously, after standing 15 ml of potassium iodide solution was added followed by 10 ml of recently boiled and cooled water was rinsed in the stopper, liberated iodine was titrated with standardized sodium thiosulphate solution with starch as indicator until the blue colour was formed. Blank determination was carried out in the same manner as tested sample. Slight variations in temperature appreciably affect titer of iodine solution as chloroform has high coefficient of expansion. It was thus necessary that blanks and samples determinations were made at the same time.

$$\text{Iodine Value} = 0.01269(B-S) N/W$$

Where:

B= volume in ml of standard sodium thiosulphate solution required for the blank.

S= volume in ml of standard sodium thiosulphate solution required for the sample.

N= normality of the standard sodium thiosulphate solution.

W= weight in gm of the sample.

Peroxide Value (PV)

5 g of the oil was placed in 250 ml conical flask and fitted with ground –glass stopper. 30ml of a mixture of chloroform and glacial acetic acid (2:3v/v) was placed in the conical flask, shake exactly to 1.0 min to dissolve the oil, 0.5 ml of saturated potassium iodide solution and 30 of water were added . The flask content was titrated with 0.01 N sodium thiosulphate, the titrant was added slowly with continuous vigorous shaking until the yellow colour was unchanged and a blank test under the same condition was carried.

$$PV = 10 (n_1 - n_2)/m$$

Where:

n_1 = volume in ml of standard sodium thiosulphate solution required for the sample.

n_2 = volume in ml of standard sodium thiosulphate solution required for the blank.

m = weight of sample

Fatty Acid Composition in Oil by Gas Chromatography

4 g of clear sample oil was transferred to a 100 ml round-bottomed flask; 40 ml of methanol, 0.5 ml of methanolic potassium hydroxide solution and a boiling chip were added, the mixture was fitted under reflux condenser and stirred until boiled. The solution should become clear (5-10 minutes). Cooled under running water and the content was transferred to 125 ml separating funnel, 20 ml of heptane and 40 ml of water were added, shaken and allowed to separate. The esters passed to the upper heptane layer. The aqueous layer was extracted again with 20 ml heptane. The two extracts were combined and washed with several 20 ml portions of water, separated, dried the ester solution over anhydrous sodium sulfate, filtered through cotton

wool into a 50 ml conical flask and evaporated the solution to approximately 20 ml on a water bath while a stream of nitrogen was passed. This solution was injected directly into the column of gas chromatography.

Results and Discussion

The oil percentage (21.1%) shown in the table (1) agreed with the value (20%) reported by AL-Wandawi *et al.*⁹ and (21.71%) reported by Salama and Ibrahim¹⁰. This high value for proximate analysis make the Roselle seeds a rich source of nutrients. Variations in oil yields may be due to the differences in the extraction methods used and location of the plant. Mean while the relatively high fat content shown in the table (2) indicated that these seeds could become an excellent economic source for edible oil production.

The saponification value is high as shown in table (1) and this suggests the use of the oil in the production of liquid soap, shampoos and lather shaving creams. The Iodine value shown in the table (1) is the measure of the degree of unsaturation of the oil. The refractive index shown in table (1) reflects the degree of unsaturation. Free fatty acid and peroxide values are a valuable measure of the oil quality. The low peroxide value shown in table 1 indicates that the oil is stable. The low free fatty acids content as shown in table 2 is indicative of the low enzymatic hydrolysis; this could be advantageous as oil high free fatty acid develops off flavor during storage. The major saturated fatty acids in Roselle seed oil were Myristic (1.9609%, 1.9845%) and palmitic (3.9494%, 3.9198%) acids and the main unsaturated fatty acids are oleic (47.0555%, 47.8868%), linoleic (30.5836%, 30.7931%) and elaidic (14.3159%, 15.1603%) for red and white calyces as shown in table 2. The standards and guideline for sunflower oil as set by the Codex Alimentarius Commission of the FAO/WHO and SSMO were shown in table 3.¹¹⁻¹³ Fig.1 and fig. 2 show the chromatogram of the components in Roselle seed oil for red and white calyces respectively as analyzed by GC.

Table 1: Physicochemical properties of Roselle seed oil

Properties	Red calyces Roselle	White calyces Roselle
Oil%	21.1%	21.1%
Specific gravity	0.90	0.90
Refractive index	1.447	1.446
Viscosity	22.5	22.5
Saponification value	189.7	189.1
Acid value	3.577	3.5533
Iodine value	119	119
Peroxide value	4.6	4.7

Table 2: Fatty acids percentage in Roselle seed oil

Fatty acid	Determined value %	
	Red calyces	White calyces
Myristic acid	1.9609	1.9845
Palmitic acid	3.9494	3.9198
Linoleic acid	30.5836	30.7931
Oleic acid	47.0555	47.8868
Elaidic acid	14.3159	15.1603
Stearic acid	0.2518	0.2744
Arachidic acid	0.2583	0.4404
C cis-8,11,14-Eicosatric acid	0.7062	0.6735
Cis-11-Eicosenoic acid	0.9185	0.8672

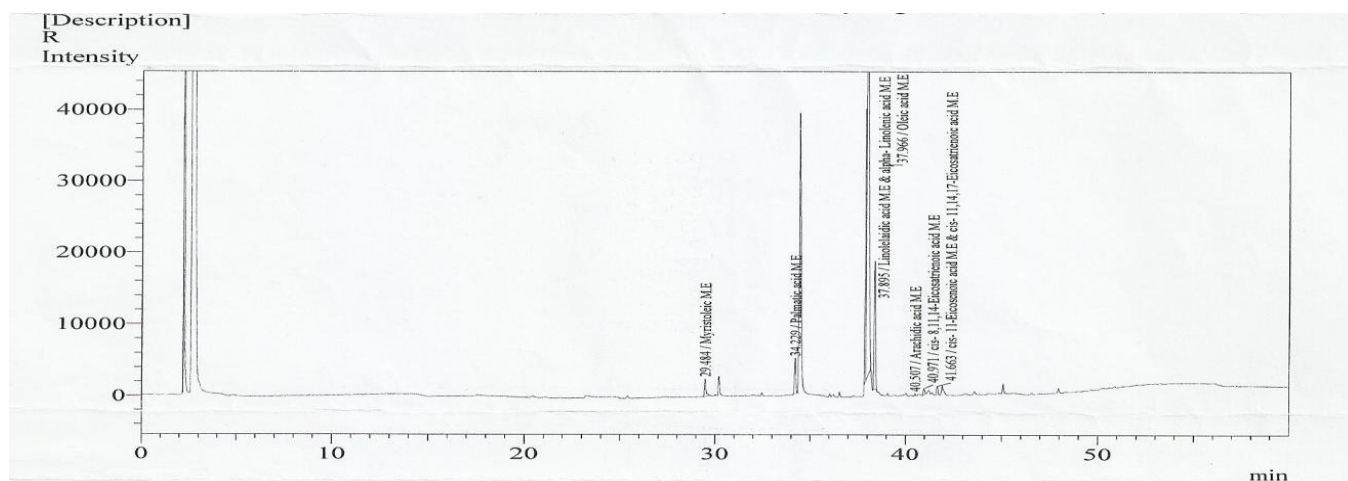


Figure 1: Fatty acids in Roselle seed oil (red calyces) by GC

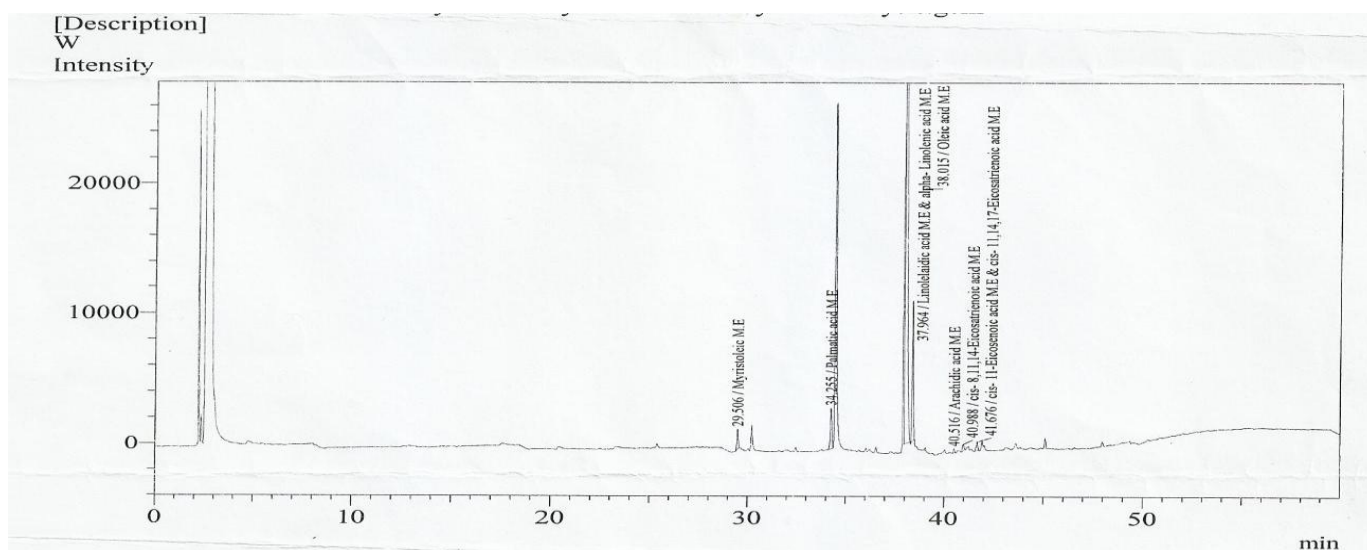


Figure 2: Fatty acids in roselle seed oil (white calyces) by GC

Table 3: Standard set for edible sunflower oil

Standard and guideline	Refractive Index(at 40 °C)	Viscosity (at 35 °C)	Acid value (mgKOH/gm.oil)	Peroxide value (meq/kg)	Iodine value (wijs method)***
FAO/WHO (1993)	1.467-1.469	Not limited	≤0.6* 4.0** ≤	≤10* 15** ≤	110-143
SSMO (2003)	1.461-1.469	Not limited	≤0.6* 4.0** ≤	≤10* 15** ≤	110-143

*: Upper limit set for refined oil. **: Upper limit set for virgin oil. ***: As indicated by AOAC official methods of analysis 1984 chapter 28.023

Conclusion

The Roselle seed is a byproduct of the Roselle processing industry and this unwanted byproduct was shown in this study to be a rich source of nutrients and it can be used for the preparation of food products as the low free fatty acids content is indicative of low enzymatic hydrolysis. The Roselle seeds oil is of unsaturated type and contains mainly fatty acids oleic C18:1(47.0555, 47.8868) and linoleic C18:2(30.5836, 30.7931). The physicochemical properties refractive index, acid value, iodine value and peroxide value were found to be in the range set by FAO/WHO and SSMO as shown in table 3 and this may the Roselle seed oil is edible oil.

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