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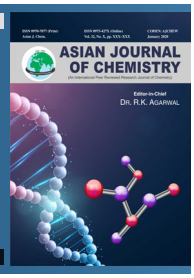

Citrus sinensis Seed as a Potential Source of Quality Edible and Industrial Oil

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Seeds of sweet oranges (*Citrus sinensis*) were analyzed for oil and fatty acid composition. The crude oil was extracted by the use of soxhlet extraction. The results showed that oil content is as high as 71 %. The physico-chemical assessment gave the following results: refractive index (1.457), peroxide value (4.12 meq/kg) and iodine value (34.06 Wijs). The ATR-IR spectrum of oil showed prominent bands at 3008.6, 2921.73, 2852.7, 1742.86, 1709.23, 1460, 1167.66, 1117.39 and 1051.16 cm^{-1} that identified fatty acid composition. The percentage compositions of various classes of fatty acids present in the oil were polyunsaturated fatty acids (PUFAs, 39.82 %), monounsaturated fatty acids (MUFAs, 57.08 %) and saturated fatty acids (SFAs, 3.09 %). The oil has linoleic acid (18:2, n-6) as the only PUFA and the second highest fatty acid composition (25.63 %). Palmitoleic acid is the highest MUFA (56.30 %) as well as the highest fatty acid composition while capric acid (C10:0) is the lowest fatty acid (0.02 %). These results and in addition to the low compositions of erucic acid, behenic acid and lignoceric acid indicated that the seed oil of sweet orange is possible suitable for both human consumption and industrial purposes.

Keywords: Polyunsaturated fatty acids, Monounsaturated fatty acids, Rancidity, Essential fatty acids.

INTRODUCTION

Lipids are chemical substances obtained from living things that are soluble in non-polar solvents. One important component of lipid are glycerides (fats and oils) which are saponifiable lipids because they contain ester linkages that can be hydrolyzed in basic solution to give an alcohol and the salt of a carboxylic acid. Another component of lipids is a long-chain aliphatic monocarboxylic acid called fatty acid. Lipids are a major component of the human diet needed for the structure and functioning of membranes that separate living cells from the surrounding environment as well as needed for energy production. All over the world, consumers are increasingly turning to natural health products to maintain or improve their health. The nutritional lifestyle of an individual is positively related to one's health conditions. The attention is now been drawn to the potential health benefits derived from the consumption of fatty acids so as to guide the consumers about the role of fatty acids play in nutrition, health and disease, in order to make informed choices about health care. This is because there is a relationship between blood cholesterol and intake of different fatty acids. Intake of fatty acids has an impact on human health [1]. Oil crops, therefore, all over the world, are becoming popular and important to agriculturists and their associated industries. Fatty acids are widespread and of different compositions in natural oils and fats. The composition of these fatty acids controls the functional and nutritional values of different vegetable oils, varying considerably depending on the plant species.

Hence most of the diets may not contain sufficient essential fatty acids (EFAs) not to talk about so many other factors that are likely to interfere with fatty acid metabolism in the body. Food scientists, therefore, are always searching for potential sources of oils and fats that may contain these essential fatty acids in substantial amounts in diets. Many vegetable oils such as coconut, peanut, pumpkin, sesame, walnut, olive and almond have been found useful and analyzed for their fatty acid compositions. However, sweet orange (*Citrus sinensis*) one of the commonest and recognized world's major fruit crops, grown all over the world, has not been known well for its importance

in terms of its seed oil and fatty acid composition. Orange fruit is one of the most popular fruits for consumers throughout the world due to the nutritional and medicinal values. They are produced in many countries all around the world with a tropical or subtropical climate. Brazil, USA, Japan, China, Mexico, India, and some countries of the Mediterranean region are the major citrus producers [2]. Orange is noted for the extraction of its juice rich in vitamin C. The peels containing abundant fragrant substances are extensively applied for processing into essential oils, which are used commercially for flavouring foods, beverages, perfumes and cosmetics [3]. The remaining parts of the orange fruit after extraction of juice and sometimes the extraction of the essential oil are discarded as waste. This work, therefore, seeks to evaluate the oil content and fatty acid composition of *Citrus sinensis* seed oil grown in Ghana for its possible suitability for human consumption and industrial purposes.

EXPERIMENTAL

Mature fruit samples (10 kg) of *Citrus sinensis* were purchased from the local markets in Cape Coast, Ghana. The fruits were cut into small pieces with a sharp knife and seeds were collected manually. The seeds were washed with tap water and dried at 40 ºC in an oven for 24 h. All reagents and solvents were of analytical grade and obtained from the Department of Chemistry, University of Cape Coast, Cape Coast, Ghana. Pure standards of fatty acid methyl esters (FAMEs) were obtained from Kwame Nkrumah University of Science & Technology where the GC-FID analysis was carried out.

Extraction of oil: The dried citrus seeds were de-husked/deshelled and crushed using a commercial blender. A total of 920.96 g of the well-crushed seeds was divided into four parts and separately fed in turns into a Soxhlet extractor fitted with a 1 L round-bottomed flask and a condenser. Each extraction was executed on a heating mantle set up for 6 h with 0.50 L of petroleum ether (40-60 %). The solvent was distilled off under vacuum using a rotary evaporator (Buchi B-169 vacuum-system, Switzerland) to obtain a total yield of 71 % oil.

Analysis of extracted oils

Physico-chemical parameters of oils: Determination of relative density, refractive index, iodine value, peroxide value, acidity and saponification value of the extracted oil were performed using standard methods [4,5].

Preparation of fatty acid methyl ester for GC-FID analysis: Sulphuric acid in methanol (10 %, 1 mL) was added to 0.92 g of oil in 15 mL centrifuge tube and shaken for some time. The solution was purged with nitrogen gas for 3 min. The tube was allowed to cool to room temperature and extracted with hexane (3×5 mL). The hexane layer was transferred into another centrifuge tube with pipette and allowed to evaporate leaving fatty acids methyl esters (FAME). Sample (0.4 µL) was injected into the GC machine with a syringe for analysis.

GC-FID analysis: Analytical GC was performed on GC-2010Plus series (Shimadzu, Japan) equipped with a flame ionization detector (FID) and an SP-2560 capillary fused silica column (100 m × 0.25 mm ID, film thickness: 0.25 µm). The column temperature was programmed as follows: Initially, 140 ºC, rate of rise 4 ºC/min up to 240 ºC and then isotherm for 15 min. The carrier gas was nitrogen at a flow rate of 1 mL /min^{-1}; the injector temperature was 260 ºC. Sample was injected and mixed C8-C24, 100 mg/mL FAME was used as the interior label for quantification.

FAMEs identification and quantification: The identification of unknown FAMEs was based on the comparison of their relative retention times with those of authentic standards of FAMEs. The fatty acid composition was reported as a relative percentage of the total peak area.

ATR-IR spectral analysis of oil: A portion of oil was fetched and used to fry peeled yam cut into pieces three times in a pan at constant temperature. ATR-IR spectra of oil samples before used and after frying each time were recorded to study any significant change in composition that could be used to monitor the oxidation process in the oil.

Statistical analysis: All analysis was carried out in triplicate and data reported as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA), using Minitab 2000 Version 13.2 statistical software (Minitab Inc., USA).

RESULTS AND DISCUSSION

ATR-IR spectral analysis: In ATR-IR spectrum (Fig. 1A), a band at 3008.6 cm^{-1} is assigned to C-H *str*. vibration of *cis*-double bond (C=C-H) and 2921.73-2852.7 cm^{-1} shows C-H asymmetric and symmetric stretching vibrations of the CH_2/CH_3 aliphatic bond. The bands 1742.86 and 1709.23 cm^{-1} showed the stretching vibrations of carbonyl ester (C=O) of triglycerides and bond stretching of free fatty acids, respectively [6]. The band at 1460 cm^{-1} represents the C-H bending vibrations of CH_2 and CH_3. Also, CH_2 bending vibrations were observed at 1377.80 and 1241.46 cm^{-1}. The absorption bands due to C-O ester bonds were observed at 1167.66, 1117.39 and 1051.16 cm^{-1}. Another prominent band that occurred at 721.56 cm^{-1} describes the CH_2 rocking vibrations. These absorptions were indicative of the presence of carboxylic acids which identified the composition of fatty acids in the oil.

Figs. 1A, B, C and D represent ATR-IR spectra of the oil sample at room temperature, frying first, second and third times. The spectra did not show any appreciable difference in the bands at room temperature and when used to fry first, second and third times. Hence, oil could be described to resist lipid oxidation and hydrolytic decomposition to avoid deterioration in quality. However, the band trend was much more similar at second and third frying with a disappearance of 1709.23 and 1708.68 cm^{-1} that were prominent in the spectra A and B, respectively. This could be explained in terms of the presence of a saturated aldehyde functional group or any other secondary oxidation resultant product that might cause the absorbance at 1708 cm^{-1} to overlap with the vibration at 1742 cm^{-1} of ester carbonyl group of triglycerides. Also, there was no significant absorption band between 968-966 cm^{-1}, which arises from (E)-HC=CH vibrational mode. This confirms the absence of (E)-HC=CH functional groups, believed to be associated with heart disease [1], in all the unsaturated fatty acid chains. However, absorption at 967 cm^{-1} arises after continuous heating signified a probable occurrence of isomerization in the unsaturated fatty acid chains as shown in Fig. 2.

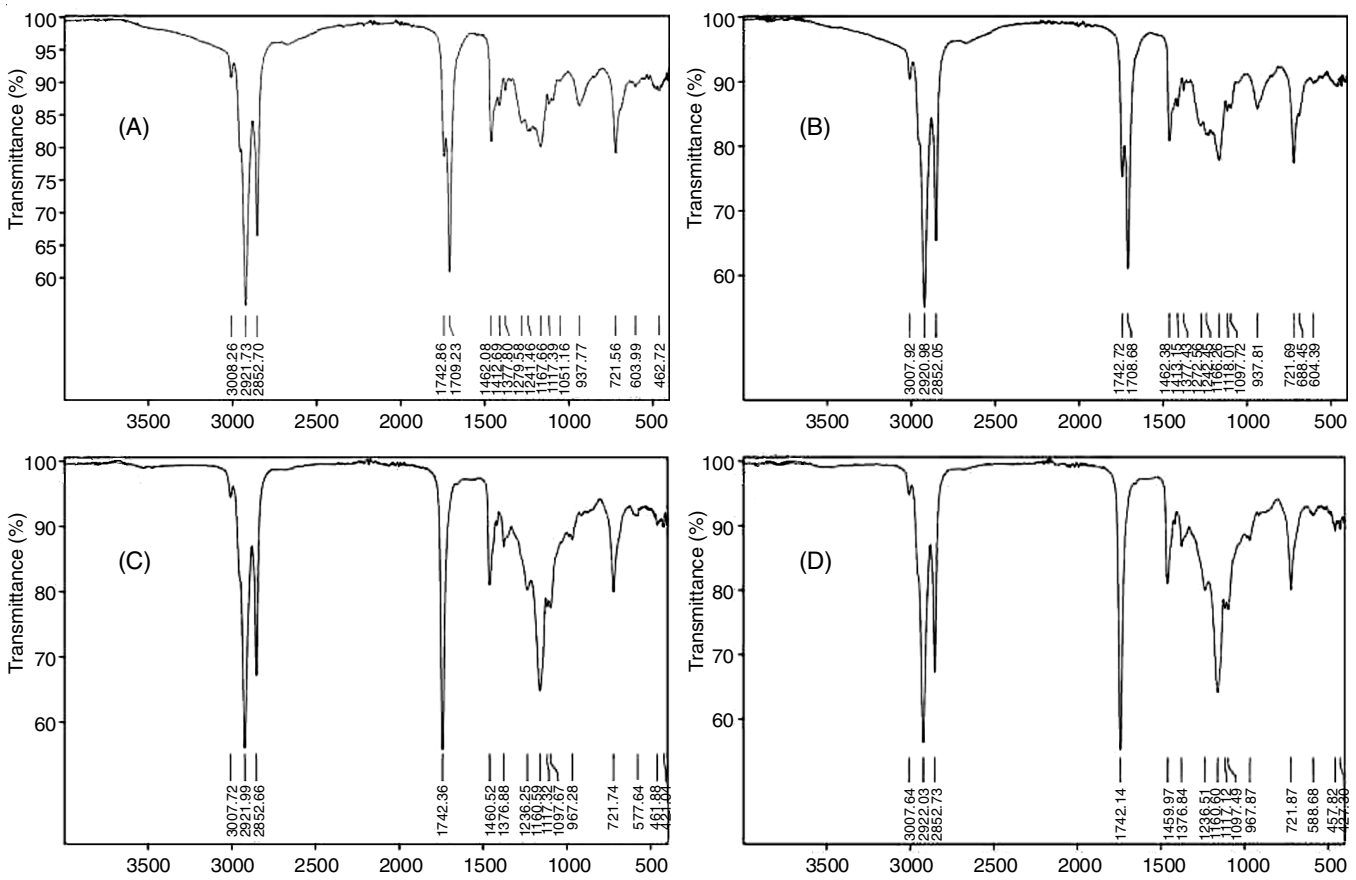

Fig. 1. FT-IR spectrum of oil (A) at room temperature (35 °C), (B) first, second (C) and third (D) frying

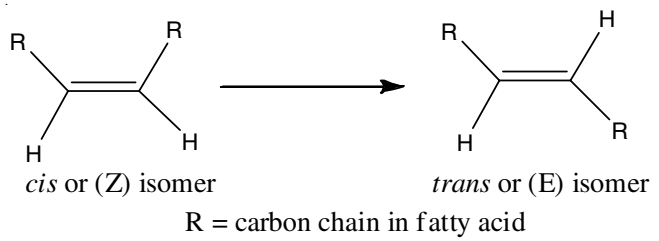

Fig. 2. Isomerization in unsaturated fatty acid chain

The petroleum-ether extracted oil content of *Citrus sinensis* seed oil recorded in this analysis was 71 %. The quantity of oil yield from *Citrus sinensis* seed grown in Ghana is comparatively overwhelmingly higher than those analysed from other countries such as Pakistan [2] and Nigeria [7-9] and this is an indication of very economical source of useful oil *hitherto*, considered as waste.

Physico-chemical properties: Some physico-chemical properties of the oil extracted from *C. sinensis* are shown in Table-1. The amount of oil obtained indicates that sweet orange seeds are good oil source, especially when compared to soybean seed which has approximately 20 % of lipid content. The seed moisture was 5.80 ± 0.1 below 10 %, an ideal value for the extraction of vegetable oils. Moisture content of (13.5 %) [10] and (3.83 %) [11] were obtained in *C. sinensis* seeds and *C. sinensis* embryo, respectively. High moisture content of oil fruit enhances enzyme activity. Vegetable oils are therefore susceptible to deterioration as a result of moisture and enzymes activities. The relatively low moisture content recorded in this study signifies a long shelf life for *C. sinensis* seed oil.

TABLE-1
PHYSICO-CHEMICAL PROPERTIES OF OIL AT ROOM TEMPERATURE (28 °C)

Parameter	Recorded value
Refractive index	1.457
Per cent yield	71.0 %
State at 28 °C	Liquid
Colour of oil	Light yellow
pH at 28 °C	5.05
Relative density (g/mL)	0.881 ± 0.001
Moisture content	5.80 ± 0.1
Saponification value (mg)	165.9 ± 0.01
Iodine value (g)	34.06 ± 0.03
Peroxide value (meq/kg)	1.77 ± 0.42

The refractive index (1.457) determined in the present analysis, agreed well with what was reported for cotton seed oil (1.458-1.466). This result is however slightly lower than those recorded for other seed oils such as mustard seed (1.461-1.469), groundnut (1.460-1.465), almond kernel (1.462-1.465), kapok seed (1.460-1.466) oils, including low-, and high-erucic acid rape seed (1.465-1.469), soybean (1.467-1.470), sunflower (1.467-1.469), safflower and grape seed (1.473-1.477) oils [7]. The peroxide value for the freshly prepared oil is 4.2 (meq/kg). Even though it is not possible to use the peroxide value alone to judge the quality of edible oils, because hydroperoxides decompose during storage, thus the peroxide value is a suitable parameter for measuring the deterioration and quality of oil over time. In general, oil producers target to produce oils with peroxide values as low as possible, without the formation of secondary reaction products. A higher peroxide value at the

beginning of storage period affects negatively the storage stability of oil. Hence, producers aim for a peroxide value for refined oils below 1.0, better 0.5 meq/kg oil and higher, up to 3 meq/kg for virgin oils [12]. The result obtained in this study was 1.77 ± 0.42 far less than 10 meq/kg. This indicates high-quality oil probably good for consumption. It may also not easily be prone to rancidity since rancidity often begins to occur when peroxide value is between 20-40 meq/kg [13,14].

The iodine value (IV) measures the relative degree of unsaturation in oil components. The greater the iodine value, the more unsaturation and the higher the susceptibility to oxidative rancidity. The iodine value of 34.06 ± 0.03 wijs obtained is low corresponding to a few amounts of double bonds present in the oil. This can be linked to the absence of many different kinds of long-chain polyunsaturated fatty acids and has considerable amounts of low molecular weight fatty acids compared to other oils such as peanut (IV 82-107), corn (IV 103-128), cottonseed (IV 99-113) or linseed (IV 155-205) oils. However, it is considerably less saturated than coconut oil (IV 7.7-10.5) [15]. Iodine value can also be used to predict the drying properties of oils. Low values indicate a non-drying nature of oils. The iodine value obtained is below 100 wijs classified the oil as non-drying and not suited for oil paints but likely to be good for soap making just like coconut oil. The iodine value obtained also gives an indication that oil cannot easily be prone to rancidity.

Saponification value (SV) measures the average molecular mass or chain length of fatty acid in any given oil sample [16]. It is known that saponification value increases with decreasing molecular weight of the oil. Higher the SV of oil, therefore, predominantly contain a high proportion of shorter carbon chain lengths of fatty acids with more glyceride molecules per gram. Oils with SV above 200 were reported to contain shorter carbon chain fatty acids (low molecular weight fatty acid). The SV of 165.9 mgKOH/g obtained in this study is not only below the recommended range (195-205 mg/ KOH/g) of oil for palm oil [17] and (188-198 mgKOH/g) for edible oils [14] but also lower than 200. This means that oil contains fewer of the lower molecular weight fatty acids or fewer number of ester bonds than oil palm since high saponification value indicates more glyceride molecules in oil [16]. Similarly, oils with better soap making ability are linked to high saponification value [16]. Hence, soap making ability of oil under study is likely to be poor as compared to palm oil.

Fatty acid composition: Characterization of 12 fatty acids composition in % of total methyl ester of fatty acids (FAMEs) of sweet orange seed oil were obtained by using gas chromatography. Fig. 3 shows all the fatty acids identified in the oil. Among the 12 fatty acids characterized (Table-2), 8 were saturated fatty acids (SFA: capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0); 3 were monounsaturated (MUFA: palmitoleic acid (C16:1), oleic acid (18:1) and erucic acid (C22:1) and 1 polyunsaturated fatty acid (PUFA: linoleic acid (18:2). All the saturated fatty acids constituted 3.09 % with MUFA and PUFA constituting 57.08 and 39.82 %, respectively. This shows that the oil predominated with unsaturated fatty acids

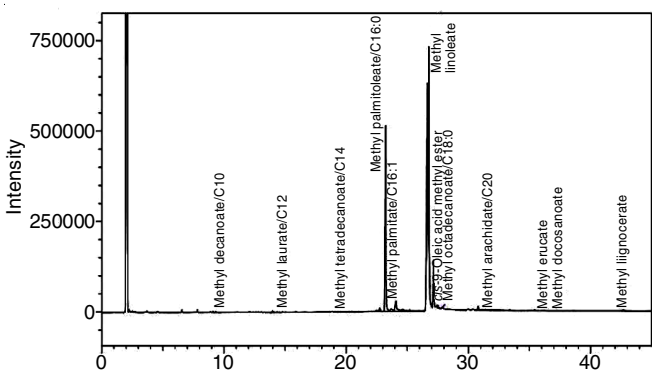

Fig. 3. GC-FID spectrum of fatty acids present in the *Citrus sinensis* seed oil

TABLE-2
PERCENTAGE OF FATTY ACID COMPOSITION OF ORANGE SEED OIL COMPARED WITH THOSE OBTAINED FROM PALM, PALM KERNEL AND COCONUT OIL

Fatty acid	Orange seed oil	Palm oil	Palm Kernel oil [Ref. 27]	Coconut oil [Ref. 19]
Caproic acid (6:0)	–	–	–	–
Caprylic acid (8:0)	–	–	–	–
Capric acid (10:0)	0.02	–	3.5	5.5
Lauric acid (12:0)	0.13	0.2	47.8	47.7
Myristic acid (14:0)	0.09	1.1	16.3	19.9
Palmitic acid (16:0)	2.06	44	8.5	–
Palmitoleic (16:1) acid	56.30	–	–	–
Stearic acid (18:0)	0.05	4.5	2.4	2.7
Oleic acid (18:1, n-9)	0.57	39.2	15.4	6.2
Linoleic acid (18:2, n-6)	39.82	10.1	2.4	1.6
Linolenic acid (18:3	–	0.4	–	–
Arachidic acid (20:0)	0.05	0.1	0.1	–
Behenic acid (22:0)	0.02	–	–	–
Erucic acid (22:1, n-9)	0.21	–	–	–
Lignoceric acid (24:0)	0.67	–	–	–
PUFAs	39.82	10.5	2.4	1.6
MUFAs	57.08	39.2	15.4	6.2
SFAs	3.09	49.9	82.1	92.1
PUFAs/ SFAs	12.88	0.21	0.03	0.02
MUFA/SFA	18.47	0.78	0.19	0.07
PUFA/MUFA	0.70	0.26	0.16	0.26

(96.9%). Similar study conducted on orange seed oil fatty acid profile, identified 11 fatty acids with palmitic acids (21.72-37.18 %), oleic (24.58-29.31 %) and linoleic acid (21.32-31.86 %) detected in larger amounts for four different varieties [18]. Palmitic acid (31.37 %), oleic (22.89 %) and linoleic (30.53 %) were also found as the major fatty acid composition of seed oil of *Citrus sinensis* [10]. This study identified palmitoleic acid, linoleic acid and palmitic acid as the major fatty acid components.

Saturated fatty acids such as lauric acid, myristic acid and palmitic acid (12:0, 14:0 and 16:0, respectively) are higher risk components of diets. However, stearic acid (C18:0) another saturated fatty acid is not associated with increased cholesterol levels because it is efficiently converted to unsaturated oleic acid (18:1) [1]. The presence of stearic acid and the low percentage composition (3.09 %) of these saturated fatty acids compared with the percentage unsaturated fatty acid composition in the orange seed oil could, to some extent, describe this oil as good. Unsaturated fatty acids are beneficial to the body. They are

either monounsaturated (MUFA) (*e.g*. omega-9 fatty acid) or polyunsaturated (PUFA) (*e.g*. omega-3 and omega-6 fatty acids). The inclusion of these long-chain polyunsaturated fatty acids in diets lowers the risk of heart diseases and strokes. These polyunsaturated fatty acids are principal components of plant lipids. The omega-6 fatty acid linoleic acid and the omega-3 fatty acid α-linolenic acid are absolutely essential fatty acids (EFAs) because they cannot be synthesized by mammals. They are therefore essential dietary components that have to be obtained from a diet, particularly by the consumption of fish and fish oils [19]. The results showed the presence of one essential fatty acid, Linoleic acid 18:2(n-6) whose percentage composition is 39.82% the second-highest in all. This value is far higher than those obtained for some common edible oils on the Ghanaian market such as palm oil, palm kernel oil and coconut oil (Table-2). However, a study on citrus seed oil from Pakistan, linoleic acid (36.26 %) and α-linolenic acid (3.44 %) were identified as the two essential fatty acids [2]. Other components include stearic acid (0.05 %), palmitic acid (2.06 %), oleic acid (0.57 %) and arachidic acid (0.05%). This clearly showed that the fatty acid composition of commodities varies from one geographic area to another.

The nutritional properties of oils and fats are dependent on their fatty acids composition, particularly the amount of oleic acid, linoleic acid, linolenic acid and erucic acid. High oleic acid oils have been shown to have equivalent heat stability to saturated fats and are, therefore, suitable substitute for them in commercial food-service applications entailing for long-life stability [20]. In addition, oils with high oleic acid content are noted for lowering cholesterol levels. Such oils are recommended for nutritional and industrial applications. Hence, a practice is either to reduce levels of polyunsaturated fatty acids or substitute them with monounsaturated fatty acids (C18:1) so as to enhance oil quality. Also, the nutritional quality of oils can be undertaken for improvement by increasing the dietary essential linoleic acid (C18:2) contents and decreasing the linolenic acid (C18:3) contents [20]. High level of linoleic acids in oil reduces the blood cholesterol level and plays an important role in preventing atherosclerosis. This study recorded a high percentage composition of linoleic acid, moderate content level of oleic acid and no traces of linolenic acid. The presence of the above two fatty acids and the absence of linolenic acid, an essential fatty acid, whose presence in the oil is likely to cause rancidity and off-flavor present the oil as possible good edible oil.

Erucic acid is a 22-carbon fatty acid that is harmful to human health. Studies on male adult rats have shown that erucic acid promotes myocardial lesions (fatty infiltration, that is, accumulation and retention of fat in cardiac tissues) [21]. Oils without this fatty acid are nutritionally rated in the highest level. However, high erucic acid oil is useful for industrial applications and a valuable raw material for manufacture of industrial products such as plasticizers, detergents, surfactants, polyesters, *etc*. [20]. Food and Drug Administration, in United States and European commission have precautionary established that the level of erucic acid in edible oils must not exceed respectively 2 % and 5 % [22,23]. This study shows some level of erucic acid (0.21 %) which is less than the recommended level. The oil, therefore, aside from its possible use as edible oil can be good for industrial applications.

Two other important fatty acids identified are palmitoleic acid and behenic acid. Palmitoleic acid (C16:1), an omega-7 monounsaturated fatty acid, is found in plants and marine sources [24,25]. Behenic acid is essentially used in skincare to provide soothing relief for dry and sensitive skin [26]. The presence of these two fatty acids gives a comparative advantage to oil than palm kernel oil and coconut oil. Thus, sweet orange oil is worthy of every necessary attention and consideration for its economical uses.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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