



ILJS-15-026

Evaluations of Seed Oil from Nigerian grown Sweet Orange for its Nutritional quality and Industrial Applications

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Abstract

Oil was extracted from the dried, powdered seeds of orange (*Citrus sinensis*) by cold extraction in n-hexane. 5 g of ground seed sample was used to determine the Oil content using Soxhlet extractor at 60 °C. The seed yielded 35.00 ± 0.54 % of the oil. The physicochemical properties of oil, such as specific gravity ($0.8695 @ ^\circ\text{C}$), viscosity ($1.3008 \pm 0.006 @ 26 ^\circ\text{C}$), refractive index ($1.453 @ 25 ^\circ\text{C}$), free fatty acid (2.31 ± 0.01 %), acid value (3.14 ± 0.05 mgKOH/g), saponification value (192.56 ± 0.39 mgKOH/g), iodine value (108.03 ± 0.16 g/100g), and peroxide value (9.38 ± 0.07 meq/kg) were determined. The oil was also subjected to gas chromatography – mass spectrophotometric (GC-MS) analysis which revealed that the oil was rich in Linoleic acid (C18: 2) 89.25 % and contain other fatty acids at different percentages as follows: Palmitic acid (C16:0) 1.57 %; Oleic acid (C18:1) 7.96 %; Linolenic acid (C18: 3) 1.21 %. There was a strong correlation between the parameters examined which was statistically significant at the 0.01 level (2-tailed) ($r = 0.994$, $N = 3$). This shows the physicochemical parameters examined are interdependent. The results obtained from the physicochemical characterization of the oils also show that the property of orange seed oil compares with the other seed oils and has high potentials for use both as domestic (edible as nutritional source) and industrial oil (Cosmetics production, pharmaceutical use as Omega 3 precursor).

Keywords: Orange Seed, Oil yield, Physicochemical, Fatty acid, Extraction

1. Introduction

Orange (*Citrus sinensis*) is a part of Citrus fruits that belong to the *Rutaceae* family (Reazai *et al.*, 2014). It is among the most popular fruits grown in Africa and has a very long history of production and use. However, in the recent past, industrial technologies began to develop in order to convert citrus fruits into commercial products [Hamilton & Raie, 1980]. Each year, millions of tons of citrus fruits are delivered to factories for processing and juice production. In general, peels, seeds, and pulps (about 50 % of the fruit) are dealt with as wastes, while potentially; they can be a source of valuable byproducts (Adaway *et al.*, 1999).

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With rapid development of science and technology, more areas of use of citrus oils have been found, for which more detailed information on chemical composition and properties are required. All plants contain oils mainly in their seeds. In higher plants, these stored oils are in the form of triglycerides and are accumulated in either the embryo or endosperm of the seeds tissue or both.

Seed oils are important sources of food nutrients, industrial raw materials and nutraceuticals. The characteristics of oils from different sources depend mainly on their compositions; no oil from a single source can be suitable for all purposes thus the study of their constituents is important. Many consumers are looking for variety in their diets. They are aware of the health benefits of fresh fruits and vegetables that are food sources rich in antioxidants (Aberoumand & Deokule, 2008). Oil yielding crop plants are actually very important for economic growth of the agricultural sector. The oil seeds containing unusual fatty acids are very important for the industry, as they can be used in pharmaceuticals, cosmetics, detergents, soaps, textiles and so on (Hosamani & Sattigeri, 2000).

The world's demand for vegetable oil is constantly increasing due to increase in the world population. The production of vegetable oils and fats, which is around 30 metric tons, is not enough to meet the needs of people, since fats and oil are required industrially for the manufacturing of soap and other industrial purposes (Charles & Gordon, 2005; Weiss, 2000). Some organic polar compounds that form part of the food stored in seed and nuts have different levels of food and medical value. These can be obtained from the seed oil when extracted (Akubugwo & Ugbogu, 2007; Ali *et al.*, 2008). Researchers (El Adawy *et al.*, 1999; Reazai *et al.*, 2014) have worked extensively on the extraction and characterization of seed oils of fruits grown elsewhere, but sparse data are available on the one grown in Africa especially in Nigeria and where such data are available (Nwobi *et al.*, 2008; Okoye *et al.*, 2011), the free fatty acid content differ both in type and quantity.

This research work is part of the efforts to confirm a statement in AOCS (1947) 'It is important to obtain the specific data for samples of oil from a particular area, because there exists a range of free fatty acid content of these oils due to geographical origin'. This research determined the quantity and quality of the free fatty acid present in the seed oil of sweet orange grown in Nigeria. The results are compared to other results of seed

oils from citrus grown elsewhere. This adds to ways of increasing fats and oil production. This research was achieved by the extraction of oil from orange seeds, analyzed the physiochemical properties of the seed oil and their free fatty acid content with a view to search for the quality and quantity of essential fatty acids present.

2. Materials and Methods

Sample Collection and preparation

The Orange fruit (*Citrus sinensis*) were sourced from the popular wholesale market in Ilorin, Kwara state, Nigeria, on a very large scale in bags. The fruits were carefully selected to ensure that they were fresh and pest free. The fruits were all washed thoroughly with water and liquid wash, then rinsed with distilled water. The clean oranges were peeled to remove the skin so that there would be access to the fresh skin of the fruits. All the fruits were squeezed to extract the juice alongside the seeds which were the required part. The seeds were separated from the juice, washed thoroughly to free the seeds from the bulbs, drained, sun dried within four days to obtain a constant weight and ground to powdered form. Analytical grade chemicals and solvent (BDH) were used in this study.

Extraction of Oil

The oil was then extracted from the seeds using double distilled n-hexane by adopting the method described by Association of Official Analytical Chemist (AOAC, 1998). 5 g of the pulverized seeds were packed in a muslin cloth and inserted into the Soxhlet extractor and n-hexane was used as the extraction solvent for a period of eight hours. At the end of the extraction period, the solvent was recovered with the aid of a two – stage vacuum pump, 180 ml oil capacity (model VE 215) free air displacement rotary evaporator at 54 – 59 °C temperature range. The residual oil was oven dried at 75 °C for one hour. The extract was transferred to a desiccator and then stored in an air -tight container until needed for further analysis.

Physicochemical Properties of the Oils

Percentage Yield: Percentage Oil Yield is calculated from the ratio of the mass in gram of the oil extracted to the mass in gram of ground sample before extraction as follow:.

$$\text{Percentage Yield of oil} = \frac{\text{weight of the extracted oil} \times 100}{\text{Weight of the ground sample}}$$

Specific gravity: A Density bottle, clean and dry of 25 ml capacity was weighed (W_0), Then the dry bottle was filled with the oil, stopper and then reweighed to give (W_1), the oil was substituted with water after washing and drying the bottle, then, weighed to give (W_2) (Abbas *et al.*, 2008).

The expression for specific gravity (Sp.gr) = $\frac{W_1 - W_0}{W_2 - W_0}$. Refractive index: Few drops of the

sample were transferred into the glass slide of the refractometer. Water at 30 °C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated thrice and the mean value noted and recorded as the refractive index (Akpan *et al.*, 2006).

Viscosity: A clean, dried viscometer with a flow time above 200 seconds for the fluid to be tested was used. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample, the viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force drawn up to the upper timing mark of the viscometer, after which the instrument turned to its normal vertical position, the viscometer was placed into a holder and inserted to a constant temperature bath set at 29 °C and allowed approximately 10 minutes for the sample to come to the bath temperature, the suction force was applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time was found by timing the flow of the sample as it was flowing freely from the upper timing mark to the lower timing mark, and was recorded.

Free fatty acid value: 0.25g of the oil sample was weighed and transferred into a conical flask, 50ml of hot ethanol was added and the mixture boiled together and gently stirred so as neutralize the fat, three drops of phenolphthalein indicator was added and the mixture titrated with a standard solution of NaOH of 0.04 M until a pink colour obtained (Deferne and Pate., 1998).

Calculation: Lipid FFA = $\frac{V \times F \times M}{10 \times W}$, Where V = titre value; F = equivalent weight of FFA expressed in Oleic acid (acid group molecular weight = 282); M = molarity of the NaOH

Acid value: The acid value was determined using the method described by Ronald and Ronald (1991). Equal volumes (25 ml) of diethyl ether and ethanol were mixed together and 1 ml of 1% phenolphthalein indicator solution was added and was then neutralized with 0.1 M potassium hydroxide solution. One gram of oil sample was dissolved in the neutralized solvent mixture and titrated with 0.1 M potassium hydroxide solution with constant shaking until a pink colour which persisted for 15 seconds is obtained.

This is given as: Acid Value = $\frac{V \times C \times M}{W}$, where V = the volume of KOH, C = the concentration, M = the molar mass and W = the weight of the sample.

Saponification Value: This was carried out using the method described by AOAC (1998). 2 g of the oil sample was added to a flask with 30 cm³ of ethanolic potassium hydroxide solution and was then attached to a reflux condenser and heated on a water bath for 1 hour with occasional shaking to ensure the sample was fully dissolved. After the sample had cooled, 1cm³ of phenolphthalein indicator was added and titrated with 0.5M hydrochloric acid until a pink endpoint was reached. A blank determination was done and saponification value was calculated using the equation:

Saponification Value = $\frac{(b - a) \times M \times 56.1}{\text{sample weight (g)}}$, Where a = sample titre value ; b = blank titre value ; M = molarity of the HCl ; 56.1 = molecular weight of KOH

Iodine value: The determination iodine value was carried out according to the IUPAC method (IUPAC, 1979). With the aid of a dropping pipette, about 0.5 g of the oil was accurately weighed into a glass stopper flat bottom flask and 10 ml carbon tetrachloride added to the oil to dissolve. Exactly 20 ml Wijs' solution was added and the stopper which had been moistened with potassium iodide solution inserted. The mixture was mixed and allowed to stand in a dark cupboard for 30 minutes. 15 ml of freshly prepared 10 % potassium iodide solution and 100 ml water was added and mixed. The mixture was titrated with 0.1 M standard sodium thiosulphate solution and using starch as an indicator just before the end point. A blank titration was also carried out. The iodine value is given as:

Iodine Value = $\frac{(b - a) \times 1.269}{\text{weight of sample (g)}}$, Where a = sample titre value ; b = blank titre value

Peroxide value: Determination of peroxide value was carried out using the method described by Pearson (1981) and Ranken (1988). The test which was carried out in subdued daylight involved weighing 1 g of the oil into a clean dry boiling tube and adding 1 g powdered potassium iodide and 10 ml of a solvent mixture consisting of 2 volumes of glacial acetic acid and one volume of chloroform. The boiling tube was placed in a boiling water bath so that the liquid boils within 30 seconds and allowed to boil for more than 30 seconds. The whole content was then poured into a titration flask containing 20 ml freshly prepared 5% potassium iodide solution and the tube washed twice with 25 ml portions of water with the washings added to the titration flask. It was then titrated with 0.002 M sodium thiosulphate solution using starch as indicator. A blank titration omitting the oil was also carried out. The peroxide value is calculated from the equation:

$$\text{Peroxide value} = \frac{2(a - b)}{\text{weight of sample used (g)}}, \text{ Where } a = \text{sample titre value; } b = \text{blank titre value}$$

All physicochemical properties determinations were carried out in triplicates.

Fatty acid methyl esters (FAMES) preparation

Oil (1 ml) was dissolved in 20 ml petroleum ether and 2 ml methanolic KOH (2 M). The mixture was shaken for 2 minutes and allowed to stand for about 30 minutes. The upper layer was removed and washed with water (Nickavar *et al.*, 2003; AOCS, 2009). It was then dried in the oven at 50 °C, until a constant weight was achieved. The resulting Fatty acid methyl esters (FAMES) were subjected to GC-MS analysis.

Statistical analyses

Data are presented as means \pm standard deviation (SD) (N=3). A Pearson correlation was run to determine the relationship between the parameters using IBM SPSS statistics 20.

3. Results and Discussion

Obtained results for various tests carried out on the sample are tabulated below,

Table 1: Physicochemical properties oil extracted from Nigerian grown *citrus sinensis*

Test	Result
Percentage yield (%)	35.00 ± 0.54
Refractive index (25°C)	1.453
Specific gravity (25°C)	0.8695
Viscosity (26 °C) (st)	1.3008 ± 0.006
Free fatty acid (%)	2.31 ± 0.01
Acid value (mgKOH/g)	3.14 ± 0.05
Saponification value (mgKOH/g)	192.56 ± 0.39
Iodine value (g/100g)	108.03 ± 0.16
Peroxide value (meq/kg)	9.38 ± 0.07

Table 1 reported the physicochemical characteristics of the sample seed oil. The seed gave a high yield of oil 35.00 ± 0.54%. The refractive index values obtained 1.453 is in close agreement with values reported for conventional oils from palm kernel (1.449 - 1.451) and lower than soybean (1.466 - 1.470). The high refractive index of this oil confirms the high number of carbon atoms in their fatty acids (Falade *et al.*, 2008). Eromosele and Pascal (2003) reported that refractive index increases as the double bond increases. This can also be used to predict increase in double bond in the oil seed. These also fall within the range of standard value of 1.4 - 1.473 for fish oils as reported by Abdulkadir *et al.*, (2010). The oils had specific gravity of 0.8695 which is lower than 0.918 specific gravity reported for groundnut and 0.939 reported for neem seed (Akpan *et al.*, 1999) and 0.9587 reported for castor seed oil (Akpan *et al.*, 2006). These are very close to 0.89 - 0.92 reported for edible oils (Odufoye, 1998). The viscosity of the orange seed oil was found to be 1.3008 ± 0.006 st at 26 °C and was far lower than 9.4248 st for crude castor oil and 6.4842 st for refined castor seed oil measured at 28°C (Akpan *et al.*, 2006).

There was a strong correlation between the parameters studied. This was statistically significant at the 0.01 level (2-tailed) ($r = 0.994$, $N = 3$). The free fatty acid value of 2.31 ± 0.01 falls below the maximum limit of 5% for free fatty acids in high grade palm oil in Nigeria [NIFOR, 1989]. The acid value is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. Acid values are used to measure

the extent to which the glyceride in the oil has been decomposed by lipase and other actions such as light and heat (Demian, 1990). The acid value of 3.14 ± 0.05 is slightly outside the recommended value for cooking oil, 0.00 to 3.00 mgKOH/g of oil, (Oderinde *et al.*, 2009) and lower than the value obtained for olive oil 17 mgKOH/g (Davine and Williams, 1961) and higher than 2.39 ± 0.065 , 1.2 ± 0.065 and 0.83 ± 0.01 reported for castor seed oil, *Jatropha curcas* L. seed oil and cotton seed oil respectively (Warra *et al.*, 2011).

The saponification value obtained for orange (192.56 ± 0.39) is less than 213 mg KOH/g reported for neem seed oil (Akpan *et al.*, 2006) and 253.2 mgKOH/g reported for coconut oil (Oshinowo, 1987), This shows that all the fatty acid present in the oil studied contain a lower chain length. Higher saponification value justifies the usage of fat or oil for soap production (Adeyemo, 2004). The iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. The iodine value is high and this reflected the presence of a high percentage of unsaturated fatty acids in the seed oil. The iodine value obtained is a little above 100 and so it could be classified as semi drying oil.

The iodine values 108.03 ± 0.16 g/100g compete favourably with iodine value of both white and red *Sesamum indicum* seed oils, 103 and 116 g/100g respectively (Mohammed & Hamza, 2008). Peroxide value obtained 9.38 ± 0.07 mEq/Kg is closer to 9.99 mEq/Kg obtained for peanut oil, sunflower seed oil and rape seed oil and far less than 22.49 mEq/Kg for corn oil (Anioara *et al.*, 2013) and 17 mEq/Kg obtained for unripe plantain peel oil. Peroxide value is an index of rancidity, thus low peroxide value indicates resistance of the oil to peroxidation during storage. Higher peroxide value corroborated the fact that the oil has less resistance to lipolytic hydrolysis and oxidative deterioration when compared with oil with lower peroxide value.

Table 2: Fatty Acid composition of lime seed oil

Fatty acid (%)	Orange
Palmitic acid (C16:0) 1.57	1.57
Oleic acid (C18:1) 7.96 %	7.96
Linoleic acid (C18: 2) 89.25 %	89.25
Linolenic acid (C18: 3) 1.21 %	1.21

The fatty acids of *orange seed* oil were linoleic (89.25 %), oleic (7.96%), palmitic (1.57%) and linolenic (1.21%), (Table 2). This is in contrary to the results reported by Nwobi *et al.* (2006) of acid value 82 % with oleic acid 56 %, linoleic acid, trace quantity, without linolenic acid for African sweet orange seed oil. Reazai *et al.* (2014) also reported average acid value of 0.51 mg KOH per g of oil and linoleic acid between 32.2 and 36.3 % while linolenic range between 3.2 and 4.1 for the sweet orange samples grown in Kerman Iran. In another study, the main fatty acids were oleic (12.8 – 70.1%), linoleic (10.5 – 58.8 %), and palmitic (5.1 – 28.3 %), however, content of other fatty acids were negligible (Matthaus and Ozcan, 2012).

This seed oil contained a high amount of linoleic acid (89.25%), which makes it prone to oxidation, but which may have favorable nutritional implications and beneficial physiological effects in the prevention of coronary heart disease and cancer (Oomah *et al.*, 2000) as linoleic acid is one of the three essential fatty acids. High ratio polyunsaturated / saturated fatty acid (57.62) is regarded favorably in the reduction of the serum cholesterol and atherosclerosis and the prevention of heart diseases (Rudel *et al.*, 1998; Ruggeri *et al.*, 1998). Linoleic acid is one of the most important polyunsaturated fatty acids in human food because of its prevention of distinct heart and vascular diseases. Apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, it also prevents high blood pressure. Linoleic derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds (Matos *et al.*, 2009). Finally, the percentage of oleic acid (7.96%) in the oil makes it desirable in terms of nutrition (Corbett, 2003).

4. Conclusion

This study has revealed that orange seed oil (*citrus sinensis*) is a rich source of some important nutrients that have very positive effect on human health and give a considerable yield of oil which revealed it to be a good source of the essential fatty acids. The high linoleic acid content makes the oil nutritionally and industrially valuable. The oil can protect against UV light, this justifies their use in the cosmetic industry. The use of orange seed oil for industrial applications could necessitate its exposure to high thermal treatments that could

lead to changes in quality characteristics of the oil. So, a study of thermo-oxidation effects on physic-chemical parameters of the seed oil needs to be undertaken.

Acknowledgements

The authors acknowledge the reviewers for their suggestions and comments in improving the quality of the manuscripts.

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