Evaluation of the Fatty Acid Profile, Physicochemical, Proximate and Phytochemical Properties of Soursop Seed Oil

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Abstract

This study involves ***evaluation of fatty acid profile, physicochemical, proximate and phytochemical properties of soursop seed oil.*** The oil was extracted using soxhlet extraction methods with n-hexane. The physicochemical, proximate and phytochemical properties of the soursop seed oil were determined using standard methods, while GC-MS was used to determine the fatty acid profile of the oil. The percentage oil yield was 37.42%. The physicochemical properties of the soursop seed were: specific gravity (0.92), refractive index (1.465), viscosity at 100 ^oC (3.456 *pa s*), flash point (200.53 ^oC), pour point (-15 ^oC), kinematic viscosity @ 40 ^oC (8.01 mm^2/s), saponification value (118.9 mg KOH/g oil), acid value (5.61 mg KOH/g oil), iodine value (109.24 gI_2/100g oil), peroxide value (2.09 meq O_2/kg oil), and pH (5.86). The fatty acid profile of the oil indicated 0.01%, 18.60%, 1.79%, 4.59%, 40.98%, 32.11%, 1.23%, 0.49%), 0.07%, and 0.13% for myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic (C18:3n3), arachidic acid (C20:0), behenic acid (C22:0), and Lignoceric (C24:0) respectively. The proximate compositions of the oil were: moisture content (5.94%), crude fibre (1.19%), crude protein (8.10%), fat (33.08%), ash content (9.60%), and total carbohydrate (42.09%), while the result of the phytochemical analysis of the oil indicated the following: alkaloids (-), flavonoids (+), tannins (-), saponnins (+), steroids (+), terpenoids (++), and cardiac glycoside (+).The oil is of oleic category and could be used for biodiesel production, as well as a source of energy and protein to the body and some bioactive compounds needed by pharmaceutical companies. It is recommended for domestic and industrial applications.

Keywords: Soursop oil, fatty acid, physicochemical, and proximate

Introduction

The demand for vegetable oil for consumption, or for production of soap, detergent, paint, biodiesel, biolubricant etc by industries increases with increasing population. Also, the depletion of the world's crude oil reserve, increasing crude oil prices, and issues related to conservation have brought about renewed interest in the use of bio-based materials such as oil from plants and animals. Generally, fats and oils are major sources of energy to human, generating greater amount of calories than carbohydrates in food. Aside from generating energy, they are known to be sources of some vitamins such as A, D, E, K, and essential fatty acids which the human body cannot synthesize. Fats and oil are derived from both animals and plants. The plant sources (vegetable oil) are more acceptable for human consumption than the animal sources due to the health issues, such as coronary heart diseases, associated with the animal sources of oil. Nutritionally, vegetable oil provides calories and vitamins in human diet in an easily digested form and at a lower cost. Vegetable oils also contain additional health beneficial phytochemicals such as saponin, flavonoids, tanins, steroids, terpenoids, phlobatanin, anthraquinone, etc. (Siyanbola *et al.*, 2013).Majority of the phytochemicals have been known to bear valuable therapeutic activities such as insecticidals, antibacterial, antifungal, anticonstipative, spasmolytic (Siyanbola *et al.*, 2013).

In plants, fatty matter is concentrated only in some parts such as seeds, fruits and tubers, stone fruits, sprouts, representing a reserve substance that the plant uses during its development as a source of energy. Although the oilseeds field is very wide, plants that can be used as raw materials in vegetable oil industries are few because many of them have low oil content - being unprofitable, others with higher oil content present difficulties in oil extraction because of the special structure of the plant (Ionescu *et al*., 2013). Despite the broad range of sources for vegetable oils, the world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively. In Nigeria, palm oil, groundnut oil, and coconut oil are the major oils consumed. These conventional sources of vegetable oil have little impact in meeting the increasing demand of vegetable oil for both human and industrial use. It has become imperative to improve the bio-based oil data base, exploring most especially the underutilized oilseeds such as pawpaw, gmelina, soursop, calabash seed etc, for seed oil availability for the various purposes (Popoola *et al*., 2016).

Annona muricata is a member of the family of Custard apple trees called *Annonaceae* and a specie of the genus *Annona* known mostly for its edible fruits. *Annona muricata* produces fruits that are usually called Soursop due to its slightly acidic taste when ripe. Annona muricata trees grew natively in the Caribbean and Central America but are now widely cultivated and in some areas, escaping and living on their own in tropical climates throughout the world. It is commonly known as ‘chop-chop’ in Edo state Nigeria, which is a corrupted form of the English name sour sop. It is, however, found in the rain forest and derived savannah zone in Nigeria either domesticated or growing wild. The fully mature fruit is green or light greenish yellow. The ripe, mature fruit is soft to the touch; ripeness is better detected by touch than by color. The outside of the fruit is thorny while the pulp is white and juicy with brownish seeds (Onimawo, 2002; Kimbonguila *et al*., 2010).

The extraction and use of vegetable oils has for centuries played an important role in the manufacture of a large number of industrial products and food items. Currently, two main processes for the extraction of oil from seeds are of industrial importance: the hydraulic process and further purification and the chemical process using organic solvents. Other methods are screw presses, extrusion methods and solvent extraction method (Syed *et al*., 2011). High yield of oil from vegetable seeds is very important for the oil to be sustainable industrially and for commercial purposes. A number of parameters affect the oil yield in solvent extraction method. Such important parameters are extraction temperature, extraction time, solvent to solid ratio, particle size and to lesser extent, agitation speed which is not usually considered in soxhlet extraction method (Syed *et al*., 2011). Vegetable oil properties such as physicochemical properties, nutritional, vitamin, and phytochemical contents, as well as antinutrients, and antioxidant factors determine the suitability of the oil for human consumption, production of paint, soap, detergent, biodiesel, biolubricants, etc. Thus, this study evaluated the physicochemical properties, proximate, and phytochemical contents of soursop seed oil.

Experimental

Ripe and healthy Soursop fruits were purchased from Eke Awka, Anambra State, Nigeria. The soursop fruits were cut into pieces and the seeds were manually separated from the fruit, collected and washed to remove particles of the pulp and then dried at room temperature for 4 hours. The black hard epicarp of the seeds was cracked manually and the fleshy part was removed and gathered together. The fleshy part of the seed was sun dried for 7 days until constant weight was obtained. The cleaned seeds were milled to powder by grinding with a grinder (Bellini Energi Nutrient Blender/Grinder PRO – BL6880/2018). Hundred grams (100g) of the ground sample was weighed into cellophane bags and preserved for oil extraction for further analysis.

Oil Extraction

Oil extraction was done using soxhlet apparatus with n-haxane as extracting solvent at 60°C for 75 minutes with solvent/solid ratio of 6:1 (w:w% of solvent and sample). The percentage yield of the soursop seed oil (SSO) was calculated using Equation 1.

$$\%\ \text{Yield} = \frac{\text{Weight of Oil (g)}}{\text{Weight of Sample (g)}} \times \frac{100}{1} \qquad (1)$$

Determination of the Fatty Acid Profile of the Oil

Gas Chromatography mass spectrometer (GC-MS) was used to determine the fatty acid contents of the oil samples according to the methods employed by Uzoh and Onukwuli (2014). Oil analysis was carried out with Thermo Finnigan Trace GC/Trace DSQ/A1300, (E.1Quadropole) equipped with a SGE-BPX5 MS fused silica capillary column (film thickness 0.25µm) for GC-MS detection, using an electron ionization system with ionization energy of 700eV. Helium at a flow rate of 10mL/min was used as a carrier gas. The temperature of injector and transfer line were respectively set at 220^{o}C and 290^{o}C, while the temperature of the oven was programmed to rise at 3^{o}C/min from 50^{o}C to 150^{o}C, then held isothermal for 10min, and finally increased to 250^{o}C at 10^{o}C/min. Diluted samples (i/100, v/v, in methylene chloride) of 1.00µL was manually injected in the slitless mode. Individual components were identified based on the comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching their mass spectral of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data.

Determination of the Physicochemical Properties of the Oil

The extracted soursop seed oil (SSO) was characterized for its physicochemical properties such as density, specific gravity, pH, viscosity, flash point, kinematic viscosity, refractive index, saponification value, iodine value, peroxide value, acid value, and free fatty acid using American Society for Testing Material, ASTM 6751(1973) and ASTM D4067 (1986), as well as AOAC (2000).

Proximate Analysis

Moisture, crude fat, and crude fibre were determined in accordance with the official methods of the Association of Official Analytical Chemists (AOAC, 1999), while nitrogen was determined by the micro-kjeldahl methods (Pearson,1976) and percentage of nitrogen was converted to crude protein by multiplying by 6.25.

Qualitative Phytochemical Screening

The sample was soaked in 120 ml of ethanol at room temperature for two days. The extract was filtered using a Whatman filter paper No. 42 (125mm), and then through cotton wool. The extract was evaporated into dryness using a hot water bath for 72 hours, and then used for phytochemical analysis. Preliminary phytochemicaI tests were done for alkaloids, steroids and saponins according to Sofowora (1993), while terpenes, flavonoids and tannins were carried out according to Trease and Evans (1989).

Results and Discussion

The percentage oil yield from the soursop seeds (37.42%) was appreciably high. This implies that the soursop seeds which are normally regarded as waste may be considered economical for commercial production of oil in Nigeria. Therefore, the seed oil could be put for domestic and industrial applications, thereby converting waste to wealth.

The fatty acid composition of soursop seed oil (SSO) presented in Table 1 and Table 2 indicated that the oil comprises 23.89% of saturated acids and 76.11 % unsaturated acids. The study revealed that palmitic acid was the dominant saturated fatty acid in the SSO, while oleic acid (monounsaturated) was the dominant unsaturated fatty acid in the studied oil, which implies that the oil belongs to oleic category. This result is comparable to total unsaturated fatty acid (TUFA) of 76.66 and 73.50% reported by Pinto *et al*. (2018) for soursopseed oil. It is also comparable to TUFA of 74.13-79.3% and 78.08% reported by Samaram *et al.* (2013) and Senrayan and Venkatachalam (2018) for pawpaw seed oil with oleic acid as the dominant fatty acid in both studies. Vegetable oil with relatively higher percentage of unsaturated fatty acid has better cold flow behaviour (Verma *et al*., 2016) and high oxidation stability, making them good candidates for synthesis of alkyl esters (biodiesel) and biolubricant.

Table 1: Fatty acid profile of Soursop seed oil

Common name	IUPAC name	Formula	Composition (%)
Myristic acid	Tetradecanoic acid	C14:0	0.01
Palmitic acid	Hexadecanoic acid	C16:0	18.60
Palmitoleic acid	Hexadecenoic acid	C16:1	1.79
Stearic acid	Octedecanoic acid	C18:0	4.59
Oleic acid	Octadecenoic acid	C18:1	40.98
Linoleic acid	9,12-Octadecadienoic acid	C18:2	32.11
Linolenic	α-Linolenic	C18:3n3	1.23
Arachidic acid	Eicosanoic acid	C20:0	0.49
Behenic acid	Docosanoic acid	C22:0	0.07
Lignoceric	tetracosanoic acid	C24:0	0.13

Furthermore, the result for the fatty acid profile indicated that the oil has high molecular weight due to high concentration of unsaturated fatty acids. Also, the presence of omega-3 make this oil useful in supplying the necessary compound needed as a starting point for making hormones that regulate blood clotting, contraction and relaxation of artery walls, and inflammation. It inhibits the synthesis of prostaglandin resulting in reduced inflammation and prevention of certain chronic diseases (National Center for Biotechnology Information, 2022).

Table 2: Saturated fatty acid (SFA) and unsaturated fatty acid (UFA) concentration for Soursop seed oil

Saturated Fatty Acid (SFA)	Concentrations (%)	Unsaturated Fatty Acid (USFA)	Concentrations (%)
Myristic	0.01	Palmitoleic	1.79
Palmitic	18.60	Oleic	40.98
Stearic	4.59	Linoleic	32.11
Arachidonic	0.49	Linolenic acid	1.23
Behenic	0.07		
Lignoceric	0.13		
Total SFA	**23.89**	**Total UFA**	**75.24**

The physicochemical properties of the soursop seed oil is presented in Table 3. From the table, it can be seen that the pH of the oil (5.86) is slightly acidic. Acid value indicates whether the oil is in good non-degradable state or not. The maximum acceptable level for acid value is 4 mg KOH/g oil, and any value below this value simply means that the oil is acceptable for consumption according to AOAC (1990). The soursop seed oil has acid value of 2.22 mg KOH/g, which is within the standard range. Thus, the oil is in good non-degraded state and can be consumed by human. The refractive index of the oil (1.465) is within the standard range of 1.4 - 1.6. According to AOAC (1990), Oil fraction with saponification value of ≥ 180 mg KOH/g oil possesses low molecular weight fatty acid. So, the soursop seed oil which has saponification value of 1118.90 mg KOH/g can be said to have high molecular weight fatty acid which implies that they may be useful in soap making. The peroxide value of the studied seed oil (2.09 meq O_2/kg) is within the standard range of 2-10meq O_2/g as reported by AOAC, (1990), indicating that the oils may not be susceptible to oxidative degradation**.** Iodine value is the measure of the properties of unsaturated organic compound, according to Pearson (1981). It indicates the reactivity of double bond.

Table 3: Physicochemical properties of soursop seed oil

Parameters	Values
Specific gravity at 60°C	0.92
Refractive index at 30°C	1.465
Viscosity at 100 °C (*pa s*)	3.456
Flash point (°C)	200.53
Pour Point (°C)	-15
Kinematic viscosity @ 40 °C (mm^2/s)	8.01
Saponification value (mg KOH/g oil)	118.90

Acid Value (mg KOH/g oil)	5.61
Iodine value (gI_2/100g oil)	109.24
Peroxide value (meq O_2/kg oil)	2.09
pH at 30 ^oC	5.86

AOAC (1990) reported that oil with low iodine value which fall within the standard range of (80 – 100 gI_2/100g oil) have low degree of unsaturation and they are classified as the non-drying oil. Whereas oils that have high iodine value have high degree of unsaturation and they are classified as drying oil according to (Atasie *et al.*, 2009). Therefore, the studied seed oil with iodine value of 109.24gI_2/100g oil has high degree of unsaturation, so it is non-drying oil.

The proximate composition of soursop seed oil is presented in Table 4. It can be seen from the table that carbohydrate was the predominant component followed by fat (ether extracts), ash, protein and then crude fibre (the least). Carbohydrate which is the most important constituents of soursop seed oil makes the oil a rich source of energy for the human system (Khan et al., 2008). Thus the oil can supply the high energy needed for metabolic processes when consumed.

Table 4: Proximate composition of soursop seed oil

Nutrients	Values (%)
Moisture	5.94
Protein	8.10
Fat (Ether extracts)	33.08
Ash	9.60
Fibre	1.19
Carbohydrate	42.09
Total	**100.00**

The low moisture content (5.94%) implies that the soursop seed oil can be stored for long time without spoilage. It is known that the lower the moisture contents of a food, the higher the nutrient as well as the shelf life (Moore, 2020). Thus, the oil is expected to contain appreciable amount of nutrients. Dietary fibre is known to influence digestion and absorption processes in the small intestine. Since the crude fibre value of the soursop oil is not high, it's consuption would not aid digestion and absorption processes. The seed oil contain appreciable amount of protein, therefore can serve as source of protein to the body for cell growth and maintenance of body and cell. The high level of lipids or fat (ether extracts) content implies that the soursop seed is not safe for the heart and not good for high blood pressure patients because it contains a high level of fatty acids and cholesterol.

Phytochemicals possess pharmacological potentials which include antioxidant properties, antimicrobial efficiency and effective modulation of detoxifying enzymes and hormonal activities, as well as stimulation of the immune system (Narasinga, 2003).The results of the phytochemical screening of the oil sample indicated the presence of flavonoids, saponins, cardiac glycosides, steroids, and terpenoids (highest concentration), while alkaloids and tannins, were absent in the sample. These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al*., 2006). Stereoidal compounds are of importance due to their relationship with some hormones such as sex hormones (Okwu, 2001). Steroids, glycosides, and terpenoids have been reported to exert inhibiting activity against most bacteria (Camacho-Corona *et al*., 2008; Al-Bayati and Suleiman, 2008). Also, the presence of terpenoid shows that there are isoprene units as monomeric constituents of the seed oil and could be useful in the development of techniques for sustainable pest control and abiotic stress protection (Tholl, 2015).

Table 5: Qualitative phytochemical contents of soursop seed oil

Phytochemicals	Values
Alkaloids	-
Flavonoids	+
Tannin	-
Saponins	+
Steroid	+
Terpenoids	++
Cardiac glycoside	+

Keys: - (absent), + (slightly present), ++ (moderately present), +++ (highly present)

Conclusion

The study showed that the soursop seed regarded as waste has high oil yield with interesting properties. It may be considered economical for commercial production of oil in Nigeria. The oil is dominated by unsaturated fatty acid of oleic type suitable for biodiesel production. The seed oil contained interesting nutrients and phytochemicals which could be used for industrial production of food supplements, cosmetics, improved drugs and other valuable products.

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