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The Effect of Fermentation on the Quality of Oil Extracted from the Seeds of *Annona squamosa*

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Abstract: A comparative study was carried out to evaluate the fatty acid profile and physicochemical qualities of oil extracted from the unfermented and fermented seeds of *Annona squamosa*. *A. squamosa* powdered, fermented and unfermented seeds were extracted using soxhlet extraction. The fatty acid composition was identified using GC-MS. The physiochemical properties were determined using standard analytical methods. Unfermented oil had a total saturated fatty acid (TSFA) of 37.41%, of which palmitic acid was the most predominant. Total monounsaturated fatty acid (TMUFA) content of the fermented oil sample was 58.48% while total polyunsaturated fatty acid (TPUFA) content was 2.76%. Total amount of unsaturated fatty acids (TUFA) found in the fermented oil was greater than TSFA. The main MUFA identified was oleic acid, which ranged from 49.12 to 55.01% for both oils. The physicochemical properties showed that fermented oil had higher free fatty acid, iodine value and peroxide value of 3.810%, 108.508 mgI_2/g and 0.830 mMol/kg respectively while unfermented oil had a higher saponification value of 180.112 mg KOH/g. The results indicated that the two oils from *A. squamosa* contained high amounts of PUFA and MUFA but the fermented oil showed higher TUFA than SFA, indicating suitability for consumption and industrial usage.

Keywords: *Annona squamosa*, Fermentation, Extraction, Fatty acids profile, physicochemical.

I.

Introduction

The desire for unconventional oils to supplement those already accessible around the world has increased [1]. This may be related to the requirement to close the gap between the supply and demand for commercially available oils and fats [2].

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Fruit seeds are good sources of edible oils and fat due to their nutritional content and caloric content, so there is a large market for them for both industrial and human consumption, this has prompted extensive research into new sources of vegetable oils [3-4].

A wide range of agricultural goods are produced in Nigeria because of the country's favorable climatic conditions and fertile soil. More than 70% of the total land space is suitable for cultivation [5]. Oil seeds are a significant agricultural product and primary source of edible oils. However, some types of plants, which are not planted for food, are being neglected and underutilized [5].

The fermentation process has been documented to improve the nutritive composition of seeds via enzymatic metabolism controlled by microorganisms [6]. There has also been a report of the improved yield of essential oil obtained using fermentation techniques [7]. Sugar apple, or *Annona squamosa* (*Annonaceae*), is widely cultivated and indigenous to the West Indies in Thailand's northeast and throughout India's tropics, primarily for its taste. Although the active ingredient has not been reported, the plant's seed is well known for eradicating head lice [8]. It is deciduous and has numerous lateral branches. It can grow to a maximum height of 6 m and thrives in humid environments. Its edible seeds account for 30% of the fruit's overall weight [9]. These seeds have an oil content ranging between 14 - 49%, with a high percentage of unsaturated fatty acids. According to previous reports, *A. squamosa* seed oil can be used for the production of alkyds, plasticizers, and soap. These seeds are toxic and bitter. Oil with low levels of polyunsaturated fatty acids and saturated but a high concentration of monounsaturated fatty acids. Stearic acid, linoleic acid, oleic and palmitic acid, are some of the principal fatty acids found in sugar apple seed oils [10]. Sugar apples are not well grown in Nigeria, and so the seeds are underutilized. It is known that fruits' rheological characteristics have a significant role in determining their overall quality [11-12]. The oil seeds have substantial quantities of total tocopherol and are a strong origin of unsaturation in fatty acids, particularly linoleic acids and oleic [11-12]. This study intends to observe how seeds of *A. squamosa* are affected by fermentation and to compare the fatty acid profile and the physicochemical parameters of the two oils to determine which will be more suitable for consumption or industrial purposes.

II. Materials and Methods

A. Collection of *Annona squamosa* Fruits

Ripe and fresh fruits of *Annona squamosa* were obtained from Ota-Efun, Osogbo, Osun State, Nigeria (07^{o} 32′ 30.2496″ N, 04^{o} 31′ 41.7036″ E).

B. Preparation of unfermented Sugar Apple seeds

A. squamosa seeds were extracted manually from the ripe fruits and placed into clean non-metallic containers. The pulp was removed from the seeds with the aid of distilled water. The seeds were then oven dried at 40^{o}C, powdered and kept as the unfermented seeds in preparation for extraction.

C. Preparation of fermented Sugar Apple seeds

A. squamosa seeds were extracted manually from the ripe fruits and placed into clean non-metallic containers. The seeds were traditionally fermented according to the method of [13], which involved covering the seeds with banana leaves and storing them in a dark cupboard for 5 days. The seeds were afterwards dried in an oven at 40^{o}C, powdered and kept as fermented seeds in preparation for extraction.

D. Extraction of Oil

Extraction was done with n-hexane via a soxhlet extractor for 6 hours. Ten grams of the powdered fermented and unfermented seeds of *A. squamosa* were extracted. The oil was concentrated with a rotating evaporator at 40°C.

E. Chemical Analysis on Samples

The [14] procedures were used to determine iodine value, saponification value, specific gravity, peroxide value, free fatty acid, acid value, viscosity, and refractive index.

F. Fatty Acid Composition (FAC)

A gas chromatograph (Perkin Elmer model 8700, USA), outfitted with an FID (flame ionization detector) and capillary column SP-2340 polar (60 m x 0.25 mm). At a flow rate of 3.5 mL/min, oxygen-free nitrogen was employed as the carrier gas. With a ramp rate of

4°C/min, the end temperature is 220°C at the following temperatures: 260°C for the injector, 270°C for the detector, 130°C for the beginning oven temperature. The injection volume for the sample was 2.0 L. By comparing the peak area's retention time to that of pure standards (Sigma Chemical Co, St. Louis, MO, USA), under identical conditions, fatty acid methyl esters were quantified and identified. The results in the lipid fraction were presented as a proportion of different fatty acids.

G. Statistical Analysis

The experiment's design was entirely random, and an analysis of variance (ANOVA) was performed on the data. Using the Duncan multiple range test, significant means were separated at the 5% level of significance. The differences between the triplicates were shown by the standard deviations.

III. Results and Discussion

The percentage yields of the fermented and unfermented seed oil of *A. squamosa* were 43.682% and 20.872% respectively. Table 1 showed the fatty acid composition of the oil produced from fermented and unfermented seeds of *A. squamosa*. The result obtained indicated the presence of ten and nine components in the fermented and unfermented oil respectively. Oleic acid had the highest composition in both fermented and unfermented oils, 55.01% and 49.12%, respectively, followed closely by palmitic acid, 30.68% in the fermented oil and 37.41% in the unfermented oil. Table 1 also showed three saturated fatty acids (STF) in the fermented oil (Tridecanoic acid, stearic acid and palmitic acid), while for the unfermented, two STF were observed (stearic acid and palmitic acid).

From Table 1, the unfermented had a total saturated fatty acids (TSFA) content of 37.41% while the fermented had the least content of TSFA at 31.86%. Palmitic acid, which makes up a percentage of the saturated fatty acids present, is the most predominant (37.19%). The value of monounsaturated fatty acids (MUFA) in the unfermented oil was 50.98%, whereas the fermented oil had a value of 58.48%, which was the highest of the two. The finding indicated that fermented oil contained the most notable polyunsaturated fatty acids (PUFA) (2.76%), while 2.31% was observed in the unfermented. Linolelaidic acid (1.63%) was the most notable PUFA in the fermented oil sample. Additionally, it was shown that the amount of total unsaturated fatty acids (TUFA) in the fermented oil (61.24%) was more than all the TSFA combined. The outcome showed that the TUFA was higher in the fermented oils having a value of 61.24% than that of the unfermented (53.29%).

The total monounsaturated fatty acids (TMUFA) observed in the unfermented seed oil was 50.98%, while oleic acid had the highest value of 49.12%. The commonest PUFA recorded in the unfermented sample is octadecanoic acid with 1.09%. TUFA observed in the unfermented seed oil was 53.29%. Saturated fatty acids have been documented to have a detrimental effect on man [15]. The study's findings suggested that unfermented oil had a higher value of TSFA than the fermented oils, making the fermented oil from the seed of sugar apple preferred oil to the unfermented based on the health impact of TSFA. Oleic acid was the most predominant MUFA found in both the fermented and unfermented oils. Studies have shown the benefit of oleic acid in cancer, autoimmune diseases, anti-inflammatory diseases, and wound healing [16]. Low-density lipoprotein (LDL) cholesterol levels rise when saturated fat is consumed. LDL cholesterol and the risk of cardiovascular disease (CVD) have a positive relationship [15]. This demonstrated that the oil obtained from unfermented seeds, if routinely consumed, builds up in the body and harms the human body. Compared to other saturated fatty acids, stearic acid was linked to decreased LDL cholesterol [15]. MUFA of the fermented and unfermented seed oil of sugar apple according to Table 1 was determined to be 58.48% and 50.98%, respectively.

Table 2 presents the results of the physicochemical properties of unfermented and fermented oils from the seed of *A. squamosa*. The oils were yellowish in colour with a slight increase in the specific gravity for the unfermented (0.930 gm/cm^3). For the fermented seed oil, there was a slight decrease in the refractive index (1.4665). [17] reported that the composition of fats and oils affected their refractive indices. The viscosity of the fermented oil (30.537 Pas/sec) was not significantly higher than that of the unfermented (29.448 Pas/sec). This demonstrated that the fermented oil sample's viscosity was thicker and more potent than the unfermented oil samples.

An essential indication of oil quality is acid value (AV). AV is defined as the quantity of KOH required to neutralize free fatty acid (FFA) [18]. The AV value of the fermented seed oil was 1.910 mg KOH/g, which was significantly above that of the unfermented seed oil (0.85 mg KOH/g). The FFA for fermented oil, with a value 3.810%, was higher than the unfermented oil, with a value of 1.68%. The usual term for the mass-fraction of total fat as a percentage is the free fatty acid content [19]. The amount of unsaturation in fatty acids can be frequently estimated using the iodine value [20-21]. The iodine value for the fermented oil was 108.508 mgl$_2$/g which was higher than unfermented oils which was 74.673 mgl$_2$/g. This indicated that the degree of unsaturation observed in the fermented oil was higher than that of the unfermented oil. This concurs with a higher MUFA and PUFA recorded in the fermented oil.

The primary oxidation of oils is measured by their peroxide value (PV) [22]. The peroxide value provides the first indication of oil and fat rancidity. The most vulnerable types of oils to autoxidation are those with high levels of unsaturation. [20] suggest that PV measurement is the most reliable way to evaluate oxidative rancidity. The PV value recorded in the fermented oil 0.830 of mMol/kg was higher the unfermented oil 0.810 mMol/kg. As a result, the oil made from unfermented seed had the least amount of autoxidation but with no significant difference.

The fermented oil recorded a higher saponification value of 180.112 mg KOH/g while the unfermented oil had a lower value of 130.02 mg KOH/g. Research has revealed that the more suitable an oil is for the production of soap, the greater its saponification value, making the fermented seed oil more suitable for soap production [23]. The unfermented oil had the least PUFA of 2.31%, while the fermented oil, with a value of 2.76%, had the highest amount of PUFA. Omega-6 fatty acids of which linoleic acid is one, have been proven via systematic and scientific study to lower cholesterol, which help to improve the condition of the heart [24]. The fermented oil sample was found to have the highest amount of linoleic acid with 0.81%, while the unfermented oil sample was found to have the lowest amount with 0.33%. In addition, the metabolism of linoleic acid might result in the formation of arachidonic acid (AA), which is crucial in the development of inflammatory mediators [25]. As PUFAs are known to possess a tendency to lessen tumor motility and invasive potential, in this study, a high value of PUFA obtained in the fermented and unfermented oils will make them suitable for reducing cancer [26].

TUFA values for both oil samples were higher than TSFA. Oleic acid and palmitic acid were found in considerable concentrations in the fermented oil from *A. squamosa* seeds. These findings support those made public by [27] who recorded a higher proportion of palmitic acid with respect to saturated fatty acid. There was a clear indication that the fermentation process increased the amount of unsaturated fatty acids and lowered the amount of saturated fatty acids in the fermented oil. Oils with an unsaturated fatty acid to saturated fatty acid ratio greater than 0.4 are found suitable for lowering cardiovascular diseases [28]. Thus, the ratio of 1.92 observed in the fermented oil and 1.42 obtained in the unfermented suggested that the oils were healthy and good for consumption but the fermented oil was better.

The measured refractive index and specific gravity measurements were consistent with those of a number of crude vegetable oils which are 1.47 and 0.92 gm/cm^3 respectively [29]. The refractive index of oil shows the possibility of rancidity developing in the oil. The higher the value of the refractive index (RI), the higher the chance of spoilage due to oxidation. A value of 1.467 was observed as the RI in the fermented oil while that of the unfermented oil was 1.4655, but there was no significant difference at P<005, indicating that the fermented oil has a higher chance of going rancid than the unfermented. This was similar to the work of [30]. They recorded a 1.4668 specific gravity value. The specific gravity of oil is impacted by the oil's composition. An increase in the aromatic compound is an indication of an increase in SG. The SG for both fermented and unfermented seed oil of *A. squamosa* with values of 0.929 and 0.930 gm/cm^3 respectively was significantly not different. [31] reported the specific gravity of *A. squamosa* to be 0.79. The lower density of the fermented and unfermented oils than that of water indicates they will float on water. In another way, when the saturated compound in an oil increased, SG also increases. This is in agreement with findings where the unfermented oil with a lower specific gravity (0.929) had a higher value of total saturated compounds [32].

The fermented seed oil had a higher viscosity of 30.537 Pas/sec than the unfermented seed oil, with a value of 29.448 Pas/sec. The difference in viscosity should be a result of different fatty acid composition [33]. This demonstrated that the fermented oil sample had a higher viscosity than the unfermented oil sample. The acid value and FFA were used as indicators for the edibility of oil. For use as food, oil should have an acid value of less than 4.0 mg KOH/g. The values obtained in the study were lower than the recommended, indicating their edibility. Low acid value denotes that the oil is more resistant to hydrolytic rancidity [31]. The AV in the fermented oil was 1.910 mg KOH/g, while the unfermented oil had a lower AV of 0.85 mg KOH/g. The value of AV indicated that the fermented oil had a higher chance of going rancid than the unfermented oil. [31] reported the acid value of sugar apple seed oil to be 0.83 mg KOH/g, which was similar to what was reported in the work. The oil tends to oxidize more quickly the higher the unsaturation or iodine value [20-21, 34]. Iodine value ranged from 74.673 to 108.508 mgl_2/g with fermented seed oil having the highest iodine value. Consequently, oil's oxidative rancidity increased with fermented oil. The higher iodine value in the fermented oil is indicative of the level of unsaturation in the oil. This is comparable to that provided by [31] who reported an iodine value of 107.18 mgl_2/g and also in correlation with the work of [30], who recorded an iodine value for sugar apple seed oil of 90.55 mgl_2/g. Oils with an iodine value below 100 are known as non-drying oils, while those that are above 100 mgl_2/g but below 130 mgl_2/g are semi-drying oils, and above 130 mgl_2/g are termed drying oils. This suggested that the oil from fermented sugar apple seed (*A. squamosa*) was semi-drying oil and the unfermented oil was non-drying. These oils are great raw sources for the supply of vegetable oil-based creams. The fermented oil can be grouped as liquid oil and might be useful in soap production [35].

The maximum FFA value (3.81%) was found in fermented seed oil, whereas the lowest FFA (1.68%) was found in unfermented seed oil. Both samples' FFA values differ greatly from one another. According to [36], the values obtained, which is less than 7%, makes it be useful to humans, The higher the quality of the oil, the lower a sample's PV. The variation in peroxide value (0.810 to 0.830 mMol/kg) with unfermented oil records the least value, but the values are not significantly different. The higher value of PV in the fermented seed oil indicate that it is more prone to rancidity. The low peroxide value in the unfermented oil indicated oil sample stability and a value ranging from 20 to 40 mEq/Kg leads to rancidity [37]. The Codex Alimentarius Commission has set the acceptable limit for nuts and seed oils at 10 mEq/Kg [38]. However, the values obtained in this study were not above the permissible limit of 10 mEq/Kg, which suggests that the oil will not easily go rancid. It is also the formation of free fatty acids and glycerol, which are each soluble in aqueous solution [23]. The high saponification values reported in this study suggested an occurrence of molecular weight and long-chain fatty acids in both oil [39]. Fermented seeds oil had the highest saponification value of 180.112 mg KOH/g while the unfermented seed oil had the lowest value for saponification (130.02 mg KOH/g), making the unfermented seed oil more suitable for soap production [23]. [30] reported the saponification value of *A. squamosa* to be 189.21 mg KOH/g.

Table 1: Fatty acid profile of oil from fermented and unfermented seeds of *A. squamosa*

		Fermented	Unfermented
Fatty acids	Fatty acid number	Composition (%)	Composition (%)
Tridecanoic acid	13.0	0.230 ± 0.001^{a}	ND
Vinyl caprylate	18.2	0.710 ± 0.011^{a}	0.890 ± 0.021^{b}
Palmitic acid	16.0	30.680 ± 0.021^{a}	37.190 ± 0.021^{b}
Linolelaidic acid	18.2	1.630 ± 0.001^{a}	ND
0ctadecanoic acid	8.1	2.500 ± 0.001^{a}	ND
Stearic acid	18.0	0.950 ± 0.031^{b}	0.220 ± 0.001^{a}
Linoleic acid	18.1	0.810 ± 0.011^{b}	0.330 ± 0.001^{a}
Oleic acid	18.1	55.010 ± 0.021^{ab}	49.120 ± 0.001^{a}
Eicocenoic acid	20.1	0.160 ± 0.001^{a}	ND
Vinyl caprylate	18.2	ND	0.890 ± 0.021^{a}
Octadecadienoic acid	18.2	ND	1.090 ± 0.031^{a}
Octadecenoic	8:1	ND	1.340 ± 0.012^{a}
Heptanoic acid	7:1	ND	0.290 ± 0.031^{a}
13-Tetradece- 11- yn- 1- ol	14.2	0.42± 0.001^{a}	ND
TSFA		31.86	37.410
MUFA		58.48	50.980
PUFA		2.76	2.310
TUFA		61.24	53.29

Total unsaturated fatty acids (TUFA), Total saturated fatty acids (TSFA), Monounsaturated fatty acids (MUFA) and Polyunsaturated fatty acids (PUFA).

Table 2: Physicochemical properties of *A. squamosal* oil from unfermented and fermented seeds

PARAMETER	Fermented	Unfermented
Refractive index (@30^{O}C)	1.467 ± 0.001^{a}	1.4665 ± 0.012^{a}
Specific gravity (gm/cm^3)	0.929 ± 0.011^{a}	0.930 ± 0.012^{a}
Viscosity (Pas/sec)	30.537 ± 0.021^{a}	29.448 ± 0.001^{a}
Acid value (mg KOH/g)	1.910 ± 0.011^{b}	0.85 ± 0.001^{a}
Free fatty acid (%)	3.810 ± 0.013^{c}	1.68 ± 0.002^{a}
Iodine value (mgI_2/g)	108.508 ± 0.031^{b}	74.673 ± 0.011^{a}
Peroxide value (mMol/kg)	0.830 ± 0.018^{a}	0.810 ± 0.011^{a}
Saponification value (mg KOH/g)	180.112 ± 0.014^{b}	130.02 ± 0.021^{a}

Free fatty acid (FFA)

IV. Conclusion

The effect of the fermentation process was evident in the oil extracted from the seed as well as the increase in the physicochemical parameters determined. The findings of this experiment showed that the TUFA values were greater than those of SFA, indicating that the oil produced from the fermented oil of *A. squamosa* is edible and nutritious for human use. The unfermented oil's lower PV and FFA values are a sign of less auto-oxidation, which aids in promoting heart health, as shown by the FFA value. Additionally, the two oils showed significant levels of PUFA, which have the ability to block the formation of cancer cells, while the concentration of oleic and palmitic acids was strikingly high, demonstrating the oil's quality in terms of its health benefits.

This study has shown that the seed oil of *A. squamosa* is a good source of unsaturated fatty acids, which are very good for the body. Studies have also revealed that the seed contains some toxics such as cyanide, cyanogenic glycoside, and amygladin, the removal of these toxins from the seed is necessary to enjoy the health benefits of the high level of unsaturated fatty acids present. In other way, because it has a good source of monounsaturated fatty acids, the oil can be used as biodiesel. The fermented oil has a better monounsaturated fatty acid, which makes it a better candidate for biodiesel than the unfermented oil. The removal of cyanide present in the seed that could have found its way into the oil will make the fermented oil a very good source of drugs and food because it has a larger proportion of unsaturated fatty acids.

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