Scientific Research Publishing

American Journal of Analytical Chemistry, 2025, 16(2), 15-22
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Physical Determination and Extraction of *Annona muricata* Seed Oil

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How to cite this paper: Don-Lawson, C., Okah, R. and Chituru, A.R. (2025) Physical Determination and Extraction of *Annona muricata* Seed Oil. *American Journal of Analytical Chemistry*, **16**, 15-22.
https://doi.org/10.4236/ajac.2025.162002

Received: January 8, 2025
Accepted: February 17, 2025
Published: February 20, 2025

Abstract

The physical examination of the fruit of soursop fruit (*Annona muricata*) selected from different parent trees was investigated. Three-stage modified Soxhlet method was used which includes a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the seed to be extracted, and a siphon mechanism, which periodically empties the condensed solvent from the thimble back into the percolator. The extraction of oil from the seed and the percentage yield was examined. The oil samples were characterized for physico-chemical properties. The maximum values of physical parameters found were fruit weight 3.7 ± 7.09, fruit length 12.2 ± 28.3 cm, with 15.2 ± 20.81 cm and 0.12 ± 18.91 g for pulp weight. The percentage oil yield of 48.5% was obtained due to the environmental factors such as the soil type, planting season and optimal temperature of the region of seed cultivation. The result of chemical properties showed maximum acid value 0.46 mg KOH, FFA of 0.33 mg, saponification of 189.4 mg KOH mg and peroxide value of 4.33 mg/g. The oil physical properties as discovered have a melting point of 32˚C, smoke point of 198˚C and flash point of 280˚C. The results obtained in this study further reveal the potential of oil from seed of soursop as a substitute for conventional vegetable oil due to its high flash point which is an indication of its low flammability and can be used as a good source of food, industrially can be used as an anti-microbial agent and for pest control.

Keywords

Soursop, Extraction, *Annona muricata,* Melting Point

1. Introduction

1.1. Background of Study

Annona muricata (also called sour sop, graviola, mullatha) is an indigenous fruit

tree in the humid low lands and plateau regions of west, central African and gulf of guinea countries. In south east Nigeria, the trees are grown around homesteads and flowering and fruiting take place between May and October [1]. The flowers are unisexual, subtended three lobed and conspicuous with caduceus low bract. According to Verheiji [2], the cotyledons are very much thickened and deeply folded or conduplicated appearing palmately lobed [3]. In aroma, the pulp is pine-apple-like but it's musky with a unique acidic flavour according to Sofowara, 2008. The fruits are ellipsoidal and their size varies approximately from 4 - 9 cm long and from 2 - 5 cm wide [4]. They could be an important source of pulp oil, seed oil and even whole fruit oil [5]. The pulverized seeds are effective pesticide against headlice, the extracted juice of ripe fruits is said to be diuretic, it is medically very effective in relieving liver ailments and leprosy according to Bouquet [6]. Research has shown that *Annona muricata* contains riboflavin which helps with ear aches, migraine and heart ache relief according to Diafouka [7]. The seed oil should take their place in the food industry, the pharmaceutical and cosmetics industry (soap, perfume, fat raw materials are needed). The cake remaining after the production of seed oil may be useful in human food industry (bakery baby foods).

As the fruit becomes more popular and is increasingly commercialized, such information is indispensable for proper valorization of the fruit. A major setback in the commercial utilization of sour sop fruit is the lack of adequate and consistent data. Most of the published data collected on the physical and chemical properties of the fruit are at variant from each other. Efforts made so far to optimize the economic and to a lesser extent the nutritional value of the fruit have emphasized its oil content (quality and extraction methods) and have largely ignored low other components, especially the proteins could also be utilized to supplement the nutritional needs of the consumer. The antioxidant property was attributed to the presence of flavonoid in the plant, the essential oil of the plant resin also exhibited high antioxidant activity, in a DPPH test system, the IC_{50} value of *A. muricata* oil was reported to be 2.29 mg/L while oxidation of linoleic acid was effectively inhibited by the plant (70%) in the beta carotene linoleic acid test according to Obame *et al.* [8]. However, the antioxidant capacity was ascribed to the mono sesquiterpenes present in the plant essential oil. The essential oil has also shown more potent antibacterial effects against bacteria such as staphylococcus, bacillus careens, *Escherichia coli*, salmonella enteric and proteins mirabilis than antifungal effect against candida albicans and this effect was found to be due to the presence of high content of terpene and pinene according to Abame *et al.* [9]. Like many other Nigerian plants, the leaves showed better activity than stem and root which lends credence to the wider application of leaves in Nigerian traditional medicine than the other plants parts according to Ajibesin *et al.* [10]. Anonna muricata oil was reported to decrease the HDL cholesterol level in serum of rats according to Leudeu *et al.* [11].

The generic name *Annona muricata* is derived from the Greek word, referring to resin droplets on the bark surface of its members. *Annona muricata* emphasiz-

ing the important of the nutritious fruit in the plants cultivation [12], the plant belongs to the family annonaceae, whose members are characterized by an ovary of two to five cells, prominent as in dusts in the dark, wood and intrastiminal disk [13]. The genus *Annona muricata* consists of about forty species [2]. However, eighty species encompass subspecies, varieties, forms and cultivars. More of action studies have recently been determined that these acetogenins are super inhibitors to enzymes processes that are only found in the membranes of cancerous tumor cells [14].

It is an indigenous fruit tree in the gulf of guinea and central African countries [15], the plant is widely cultivated, extending its area of distribution to Sierra lone, Uganda, Angola, Zimbabwe and Nigeria [12] it rarely grows wild. Thus, the exact natural area of distribution is obscure [2]. Annona muricata is a dioeciously more or hear shape, sometimes irregular, lopsided or curved due to improper carper development or insect injury. The inner surfaced is creamed coloured and granular and separates early from the mass of snow-white, fibrous, juicy argument

1.2. Aim of the Research

The aim of this research is to investigate the physical property and characterization of *Annona Muricata* seed oil.

2. Materials and Methods

The sour sop (*Annona muricata*) fruits were obtained from three different markets in Port Harcourt (Mile 1, 2, 3) which are located in Rivers State, Nigeria. The markets are the largest points of sales that accommodate a larger population of the residents. They are also known as the largest market places for the different fruits cultivation and sales.

2.1. Sample Collection and Preparation

Thirty fruits (30) fruits were collected randomly from ten (10) sellers m the market. Cleaned and stored in a basket made from rather and kept in a dry place until analysis.

2.2. Analysis Site

The analysis was carried out in Rivers Vegetable oil Company Limited laboratory situated in plot 80 Trans Amadi Industrial layout Port Harcourt Rivers State.

2.3. Physical Characteristics

The physical characteristics which were studied in triplicates include: fruit length width and seed thickness which were measured with veneer caliper. Also, fruit weight, pulp weight, seed.

Weights were determined with a weighing balance (RADWAG. AS220/C/2). The bulk density of seed as well as percentage shell and fiber. Only dehauled seeds were used for this research work.

2.4. Mass of the Entire Fruit and Seeds

Using the electronic weighing balance (RADWAG. AS220/C/2), the entire fruit was weighed to obtain the total mass, also withdrawn seeds were dried and measured by water displacement in a measuring cylinder.

2.5. Sample Preparation

After withdrawal of the seed collected, fruits were immediately washed under tap water and then drained on tissue paper before homogenizing in a Panasonic blender. The seeds were then dried in an air oven (memmert) at 105˚C ± 2˚C for five hours after which it was again blended into powder

2.5.1. Determination of % Oil Content (AOCS METHOD AL 3 - 7.5)

This method determines the substance extracted by petroleum ether under condition test

Procedure

1) A portion of the sample prepared was used as described in AOCS official method.

2) Approximately 40 g of the seed was weighed to the nearest 0.1 g into a large beaker with equal weight of diatomaceous earth well mixed with a large spatula.

3) Sample was thoroughly mixed by inverting the container several times. the rubber stopper will dislodge any of the ground samples which may cake on any of the side of the container.

2.5.2. Determination of Free Fatty Acid

This method determines the free fatty acid existing in the samples.

PROCEDURE

1) Sample was well mixed and entirely liquid before weighing. However, I ensured that the sample was not heated more than 10˚C over the melting point.

2) Specific amount of hot neutralized alcohol 2 ml of indicator was added.

3) It was titrated with standard sodium hydroxide, shaking vigorously until appearance of the first permanent pink colour of the same intensity as that of the neutralized alcohol before the addition of the sample. The colour must persist for 30 seconds.

4) 4 g of the ground mixture was weighted accurately to the nearest 0.001 g into a filter paper folded in such a fashion as to prevent escape of the meal.

5) A but tube was and extracted with petroleum ether as directed in AOCS official method Aa 4-38. The procedure was repeated for four hours without interruption before regrinding with mortar and pistle.

6) The flask was cooled and disconnected and the solvent evaporated in a water bath until no petroleum ether remains. A gentle stream of nitrogen was used to facilitate removal of solvent. It was cooled in room temperature carefully to remove any moisture or dirt from outside of flask and weighed. repeat heating until constant weight is obtained.

2.5.3. Procedure for Fats and Oil

1) 5.00 g Sample was weighed into a 250 ml Erlemmeyer flask with glass stopper and 30 ml of the 3.2 acetic acid chloroform solution to dissolve the sample. 0.5 ml of saturated KI solution was added using a suitable volumetric pipette.

2) The solution was allowed to stand with occasional shaking for exactly 60 seconds and then immediately 30 ml was added.

3) 0.1 N sodium thiosulphate was titrated. It was continued until the yellow iodine color has almost disappeared. About 0.5 ml of starch indicator solution was added.

4) The titration was continued with constant agitation especially near the end point until the just disappears. A Blank determination of the reagents was conducted daily.

5) The blank titration must not exceed 0.1 ml of the 0.1 N sodium thiosulphate solution.

2.5.4. Determination of Saponification Value

The saponification value is the amount of alkali necessary to saponify a definite quantity of sample.

PROCEDURE

1) Sample was filtered through dry filtered through dry filter paper to remove any impurities and moisture.

2) Sample was weighed of such size that the back titration is 45% - 55% of the blank. This usually requires a sample 4 - 5 g. Add 50 ml of alcoholic KOH with a pipette to drain for a definite period of time.

3) The air condense was connected and boiled gently but steadily until the samples are completely saponified This usually requires about I hour for normal samples to make certain the vapour ring in the condenser does not rise to the top of the condenser or loss may occur.

4) On cooling the flask and the condenser, the condenser was disconnected, about 1 ml of phenolphthalein indicator was added and titrated with 0.5NHCl until the pink colour disappears.

3. Results

The physical examination of the fruit of sour sop (*Annona muricata*) selected from different parent trees at different locations of vey high population density with very high industrial and local activities was investigated, a three stage modified soxhlet method was used which includes a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the seed to be extracted, and a siphon mechanism, which periodically empties the condensed solvent from the thimble back into the percolator for the extraction of oil from the seed and percentage yield was examined. The oil sample was characterized for physico chemical properties. The results obtained were fruit weight 3.7 ± 7 09 mg, fruit length 12.2 ± 28.3 cm, width 15.2 ± 20.81 cm and 0.12 ± 18.91 g for seed weight (see Table 1). The percentage oil yield of 48.5%. The result

of chemical *properties showed a maximum of acid* value 0.46 *mgKOH/g*, *%FFA*0.26, saponification of 189.4 mgKOH/g, peroxide value of 4.33 mg/g (see Table 2). The oil physical properties discovered melting point of 32˚C, smoke point of 185˚C and flash point of 278˚C. The results obtained in this study further reveal the potentials of oil from seed of sour sop fruit as a substitute for conventional vegetable oil and industrial application conventional vegetable oil and industrial applications, since most of the values fells within the range of value for the reference standard.

Table 1. Physical properties of sour sop fruit seed.

Parameter	Test result
Fruit Wt (mg)	3.7
seed wt	16.19
Fruit width (cm)	2.55
Fruit length (cm)	6.65
Fruit density (g/cm^3)	0.47
% seed moisture	49.52

Table 2. Physical characteristics of seed oil.

Parameter	Test result
% moisture	0.25
Specific gravity	0.906
Melt point (˚C)	32
% free fatty acid	0.33
Smoke point	198
Flash point	280
Freezing point	25.0

The physical examination of the fruit of sour sop were purchased from the mile 3 and mile 2 markets and crushed after removing the seed and dried in an open oven at 20C Sour sop seed was used for the extraction of oil to examine percentage yield. The physic chemical properties of the oil characterized showed no significant indifference detected in other oils from edible plant sources. In Table 3 the high percentage yield of 48.5% of the seed oil content of sour sop is as a result of the environmental cultivation factors such as humidity which are relatively low in the regions where the samples were obtained. The rate of moisture content also attributed to the increased oil content and the high fatty acid content as shown in Table 4. The high flash point of 280˚C is an indication that the oil is not highly flammable as compared to groundnut oil and soya bean oil, this also makes it a better source of lipids as food according to Abame *et al.* [9]. Medically, sour sop seed oil is highly anti cancerous because of its ability to destroy cancerous cells especially liver and breast cancer according to Leudeu [11] helps in sight improvement because it contains vitamins A C and E. It is anti inflammatory, anti diabetic

and also destroy intestinal parasites according to Missang *et al.* [16].

Table 3. Chemical characteristics of sour sop seed oil.

Parameter	Test result
Oil content	48.5
Acid value (mg KOH/g)	0.46
Saponification value (KOH/G)	189.4
Peroxide value (meg/Kg)	4.33

Table 4. Characteristic of soap from sour sop seed oil.

Parameter	Test result
% moisture	12.3
% free caustic alkaline as N_2O	0.05
Sodium chloride (as NaCl)	0.45
% total fatty matter	78.6
% matter insoluble in ethanol (MIIE)	0.53
% matter insoluble in water (M11W)	0.02
Foam value (Ross Mill) ml	257

4. Conclusions and Recommendation

Scientific information and knowledge on less familiar or underutilized crops such as sour sop fruit encouraged the utilization of nutritional and industrial potential. This study has shown the potential of seed of sour sop for the production of vegetable oil. The result of physico-chemical properties further confirmed the quality of the extracted oil. This study shows that sour sop (*A. muricata*) can be used as an alternative resource base for fats and oils. The physico-chemical characteristics and fatty acids composition of this oil suggest that they have some individual potential preference towards any of the fruits varieties, which should be based on the intended function of such a fruit in the overall process.

It showed that high quantities of these constituents are present in the sour sop seeds and could be incorporated in food products. It could as well be regarded as physiological mature stage of the fruits that should present the optimum values of the properties. Also, it helps the harvesters to reduce the collection of fruits that dropped naturally which results in contamination, pest and disease attacks, over-rope and under-ripe fruits. This shows that sour sop fruits which are a nutritious food could be harvested in this period for industrial and domestic uses.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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