DETERMINATION OF THE ELEMENTAL CONTENT OF THE SEED AND PHYSICOCHEMICAL PROPERTIES OF THE SEED OIL OF *ANNONA SQUAMOSA* L. (Aw-Za)

Ye Min Htet[1,2], Maung Maung Khin[3], Hnin Hnin Aye[4]

Abstract

This research work deals with the determination of elemental contents and physicochemical properties of *Annona squamosa* seeds were collected from Paukkhaung Township, Bago Region. The elemental contents of seeds sample was determined by Energy Dispersive X-ray Fluorescence (EDXRF) and Atomic Absorption Spectroscopy (AAS) methods. The EDXRF spectrum of the seeds sample indicated that the seeds contain 0.474 % K, 0.210 % P, 0.198 % Ca, 0.168 % S, 0.003 % Fe and Mn, 0.002 % Zn and Cu, 0.001 % Os and 98.938 % C-OH in relative abundance. According to EDXRF results, K is higher content than other elements such as P, Ca. The elements S, Fe, Mn, Zn, Cu and Os were observed as the trace elements. Quantitative elemental analysis of sample was done by using AAS methods. The sample contains calcium (9.107 ppm), copper (0.024 ppm), magnesium (8.025 ppm), zinc (0.780 ppm), manganese (0.543 ppm), chromium (0.001 ppm). Moreover, the toxic element such as cadmium (0.164 ppm) and lead (0.395 ppm) were observed. *A. squamosa* seeds were extracted with petroleum ether by two methods using Soxhlet and mechanical expeller. The yield percent of oil obtained by two methods were 25.62 % and 22.84 % respectively. The functional group of extracted oil sample was confirmed by Fourier Transform Infrared (FT IR) spectroscopic method. According to the spectral data which indicated that the extracted oil sample from *A. squamosa* seed may be triglyceride. Moisture content of *A. squamosa* seed was determined by oven drying method and it was found to be 4.65 %. In addition, the seed oil obtained by mechanical expression method was chosen for the determination of physicochemical properties. Physicochemical properties of *A. squamosa* seed oil (degummed oil) were determined by American Oil Chemistry Society (A.O.C.S) method such as acid value (1.89 mg KOH/g), iodine value (79.43 mg I_2/g), saponification value (186.53 %), peroxide value (7.74 mg/1000g), viscosity (42.68 mm^2/s), specific gravity (0.816) were observed.

Keywords: *Annona squamosa*, degummed oil, physiochemical properties

[1.] Assistant Lecturer, Department of Chemistry, Pyay University
[2.] PhD Student, Department of Chemistry, University of Yangon
[3.] Dr, Lecturer, Department of Chemistry, Taunggoke Degree College
[4.] Dr, Professor Department of Chemistry, Pyay University

Introduction

Annona squamosa L. is a small, well-branched tree or shrub from the family Annonaceae that bears edible fruits called sugar-apples. *A. squamosa*, an important fruit crop is widely distributed in Myanmar, West Indies and native of the tropical Americas. The sugar apple has been widely planted in home gardens of South Florida because of its high quality fruit and good adaptation to the area. Some fruit is found in local markets but commercial production is on a very limited scale. The common name of the *A. squamosa* is sugar apple and also been known as sweetsop (English). The color of the fruit is pale green to blue-green with a deep pink bluish in some verities. The flesh is light fragrant and sweet, creamy white to light yellow. The custard apple seeds which appears in brown to black color and are hard and shiny. Sweetsop seeds which appears in brown to black color and are hard and shiny. When the extraction of oil from sweetsop seeds, 1000 g of seeds contained 260 mL of oil. The percentage of oil content obtained is 26 %. Extracted seed oil was also used as a biodiesel and it can be supplied energy in farm, industries and transport. So, it should be used as renewable energy from the feed-stocks of raw materials and saving the maintenance of environmental pollution from non-renewable sources (Doddabasawa and Ravikumar 2014).

Scientific Classification of *Annona squamosa* L.

Kingdom	: Plantae
Order	: Magnoliales
Family	: Annonaceae
Genus	: *Annona*
Scientific name	: *Annona squamosa* L.
English name	: Sweetsop, Custard apple, Sugar apple
Myanmar name	: Aw-za

(Natural Resources Conservation Service (NRCS), 2008).

Chemical Constituents of *Annona squamosa* L. Seed

Major mineral present in *A. squamosa* seed is potassium followed by phosphate, calcium, sulphur, iron, manganese, zinc, copper and osmium. The seeds of *A. squamosa* obtain annotemoyin-1, annotemoyin-2, squamosin and cholesteryl glucopyranoside. The free fatty acid composition of *A. squamosa*

seed oil was shown in Table 1. The second largest constituent of *A. squamosa* seed is the oil which contain about 26 % of the seed (Siddalingappa and Omprakash. 2015).

Table 1: Free Fatty Acid Composition of *Annona squamosa* Seeds

Free Fatty Acid	Percentage
Lauric acid	0.08
Palmitic acid	17.79
Stearic acid	4.29
Oleic acid	39.72
Linoleic acid	29.13
Linolenic acid	1.37
Arachidonic acid	1.06
Behenic acid	2.01

(Siddalingappa and Omprakash, 2015)

Pharmacological and Other Uses of *Annona squamosa* L. Seed

The crush seeds are used in folk for their insecticidal activity, parasites activity, head lice and worm. The methanolic extract of *A. squamosa* seed are active against *Pseudomonus aeruginosa* and *Escherichia coli*. Antimicrobial constituents present in the seeds of *Annona squamosa* are annotemoyin-1, annotemoyin-2, squamosin and cholesteryl glucopyranoside (Win Min Oo, Myat Mon khine, 2017). The anthelmintic activity of the *A. squamosa* seed extract against the adult earthworm. *A. squamosa* seed contained oil and these oil can produce biodiesel (Bhattacharja, 2016).

Materials and Methods

Sample Collection and Preparation

The samples of *A. squamosa* seeds were collected from Paukkhaung Township, Bago Region.

Study on Physicochemical Properties of *Annona squamosa* L. Seeds

The relative abundance of elements (%) in *A. squamosa* seeds were determined by Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer (Shimadzu EDX-8000) at the West Yangon University. Trace Elemental Contents in *A. squamosa* seeds were determined by Atomic Absorption Spectroscopy (AAS), Analyst-800, (Perkin Elmer Co. Ltd.,) method at the URC. Moisture content was determined by oven drying method. The *A. squamosa* seeds oil was extracted with petroleum ether by two methods using Soxhlet and mechanical expeller. The extracted oil sample was confirmed by Fourier Transform Infrared (FT IR) (PerkinElmer C93927 Spectrum Two) spectroscopic method at University of Yangon. Physicochemical properties such as acid value, iodine value, saponification value, peroxide value, viscosity and specific gravity of *A. squamosa* oil were determined by the method given in A.O.C.S (1995).

Pretreatment of Extracted *Annona squamosa* L. Seed Oil

Degumming

Degumming is important in preparation of biodiesel. There are two main types of gum, hydratable phosphatides (HP), which is easy to remove by water and non-hydratable phosphatides (NHP), which is hard to remove from oil. Some NHP are removed with hydratable phosphatides for complete removal (Aye Aye Cho, 2015).

Degumming process involved two steps: (i) water degumming and (ii) acid degumming.

Water degumming

300 mL of Aw-za seed oil were warmed to70 °C and it was added 100 mL of hot deionized water. This mixture was warmed to 70 °C for 20 minutes. It was finally separated by separating funnel to get oil without water soluble gum (Aye Aye Cho, 2015).

Acid degumming

Water degummed oil (300 mL) was added with 5 mL of 10 % (v/v) phosphoric acid followed by heating to 85 °C for 1 h with stirring. It was then stored for 1 h with stirring. It was then stored for 30 min. After addition of 0.1 M NaOH, it was stored for 30 min again. Finally, this mixture was separated

by separating funnel to get (276 mL) of acid degummed oil (Aye Aye Cho, 2015).

Results and Discussion

Elemental Analysis of *Annona squamosa* L. by EDXRF Method

The elemental contents of seeds sample was determined by EDXRF. The seeds sample contains K, P, Ca, S, Fe, Mn, Zn, Cu, Os and C-OH. According to EDXRF spectrum, K is higher content than other element such as P and Ca. The relative abundance of elements of seeds sample are shown in Table 2 and Figure 1.

Table 2: Relative Abundance of Elements (%) in *Annona squamosa* Seeds

No	Element	Relative Abundance (%)
1	K	0.474
2	P	0.210
3	Ca	0.020
4	S	0.168
5	Fe	0.003
6	Mn	0.003
7	Zn	0.002
8	Cu	0.002
9	Os	0.001
10	C-OH	98.938

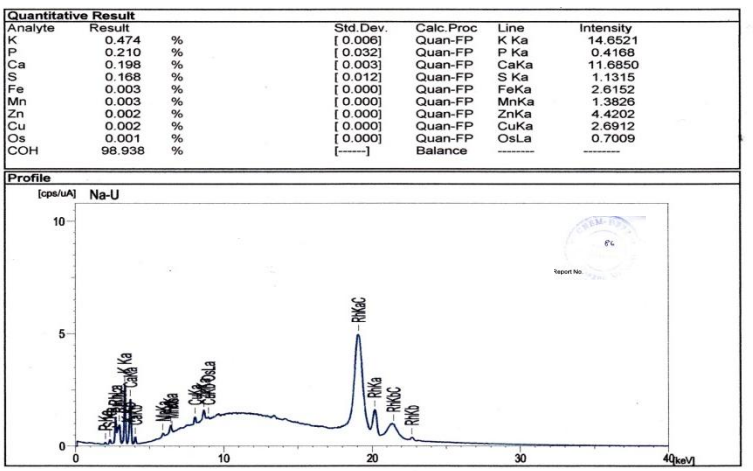

Figure 1: EDXRF spectrum of *Annona squamosa* L. seed

Trace Elements Contents in *Annona squamosa* Seed by Using AAS Method

Quantative elemental analysis of seeds sample was determined by using AAS method. The sample contain Ca, Cu, Mg, Zn, Mn and Cr. Moreover, the toxic elements such as Pb and Cd were observed. From the AAS measurements, the experimental data of elemental contents are shown in Table 3.

Table 3: Trace Elements Contents in *Annona squamosa* L. Seeds by AAS Methods

No.	Trace elements	Concentration (ppm)
1	Ca	9.107
2	Cu	0.024
3	Mg	8.025
4	Zn	0.780
5	Mn	0.543
6	Cr	0.001
7	Pb	0.395
8	Cd	0.164

FT IR Spectroscopic Study of the Prepared *Annona squamosa* Seed Oil

FT IR spectral data showed the characteristic peaks in the range of 4000 - 700 cm^{-1}. The FT IR spectrum of the prepared *A. squamosa* oil from *A. squamosa* seed is shown in Figure 2.

According to FT IR spectrum, the bands at 3006 cm^{-1} indicates the =C-H stretching vibration of sp^2 hydrocarbons. Asymmetric and symmetric -C-H stretching vibration of sp^3 hydrocarbons could be observed at 2922 and 2853 cm^{-1} respectively. The peaks at 1744 cm^{-1} indicates the -C=O stretching vibration of carbonyl group. The peak at 1456 cm^{-1} represents C=C stretching vibration of alkenic group. The bands at 1233 and 1163 cm^{-1} represents C-O-C stretching vibration of ester linkage. The peak at 721 cm^{-1} indicates the –C-H out of plane bending vibration of trans or E and cis or Z alkenic group. The FT IR assignments of the prepared *A. squamosa* oil from *A. squamosa* seed are shown in Table 4. From the study of FT IR spectral data, the prepared *A. squamosa* oil may be confirmed as a triglyceride.

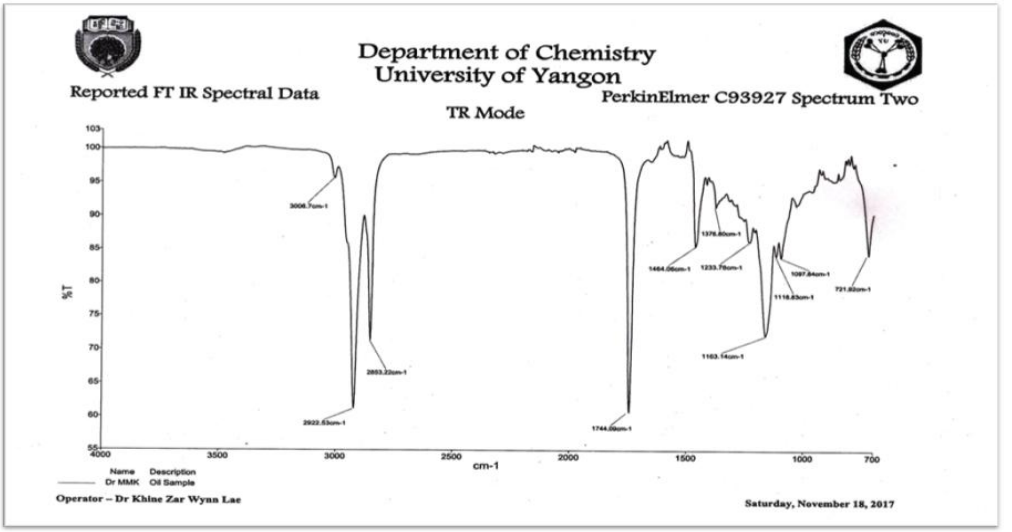

Figure 3: FT IR spectrum of *Annona squamosa* seed oil sample

Table 4: Characteristic Absorption Peak of FT IR Spectrum and their Assignments for *Annona squamosa* Seed Oil Sample

Literature value	υ_{max} (cm^{-1})	Assignment
3009.08	3006	=C-H stretching vibration of sp^2 hydrocarbon
2926.14 2854.77	2922, 2853	Asymmetric and symmetric –C-H stretching vibration of sp^3 hydrocarbons
1746.62	1744	C=O stretching vibration of carbonyl group
1463.07	1456	C-H in-plane bending vibration of sp^3 hydrocarbon
1164.09	1233, 1163	C-O-C stretching vibration
722.37	721	C-H out-of-plane bending vibration of trans or E and Cis or Z alkenic group

(Braz, 2011) and (Silverstein, 2003)

Composition of Moisture Content, Oil Content and Physicochemical Properties of *Annona squamosa* Oil

This research deals with the seed sample for the determination of moisture content and oil content. Moisture content of *A. squamosa* seed was determined by oven drying method. *A. squamosa* oil was extracted with petroleum ether by two methods using Soxhlet and mechanical expeller. The seed oil obtained by mechanical expression method was analyzed the physicochemical properties in which acid value (1.89 mg KOH/g), iodine value (79.43 mg I_2/g), saponification value (186.53), peroxide value (7.74 mg/ 1000g), viscosity (42.68 mm^2/s)and specific gravity (0.82) were observed. In accordance with the experiment, moisture content, oil content and physicochemical properties of oil sample are tabulated in Table 5.

Table 5: Moisture Content and Oil Content of *Annona squamosa* Seed

Sample types	No.	Parameter	Content
Seed	1	Moisture content (%)	4.65
	2	Oil content (%) (Solvent extraction)	25.62
	3	Oil content (%) (Mechanical expeller)	22.84

Table 6: Physicochemical Properties of *Annona squamosa* Oil

Sample types	**No.**	**Parameter**	**Content**
Degummed oil	4	Viscosity (mm^2/s)	42.68
	5	Acid value (mg KOH/g)	1.89
	6	Iodine value (mg I_2/g)	79.43
	7	Saponification value (%)	186.53
	8	Peroxide value (mg/1000g)	7.74
	9	Specific gravity	0.82

Table 7: Comparison between Physicochemical Properties of Some Edible oil and *Annona squamosa* Oil

Parameter	Degummed oil (Aw-za)	Peanut oil	Sesame oil	Sunflower oil
Viscosity (mm^2/s)	42.68	-	40.60	-
Acid value (mg KOH/g)	1.89	0.39	1.80	0.22
Iodine value (mg I_2/g)	79.43	102.02	111.60	94.35
Saponification value (%)	186.53	179.83	188.30	177.06
Peroxide value (mg/1000g)	7.74	9.99	-	9.99
Specific gravity	0.82	-	0.919	-

(Sibel, Anișoara-Arleziana, and Elisabeta, 2013)

Conclusion

In this present work, *A. squamosa* seeds samples were collected from Paukkhaung Township, Bago Region. The elemental contents of seeds sample were determined by EDXRF. The seeds sample contained K, P, Ca, S, Fe, Mn, Zn, Cu, Os and C-OH. According to EDXRF spectrum, K was higher content than other element such as P and Ca. Quantitative elemental analysis of sample was determined by using AAS method. The sample contained Ca, Cu, Mg, Zn, Mn and Cr. Moreover, the toxic elements such as Pb and Cd were observed. So that, *A. squamosa* seed is recommended unfit for human consumption.

The extracted oil sample was confirmed by FT IR spectroscopic method. In accordance with FT IR spectra, the extracted *A. squamosa* oil sample contains sp^2 hydrocarbon, sp^3 hydrocarbon, carbonyl group, alkenic group and ester linkage respectively. Therefore, the prepared *A. squamosa* oil from *A. squamosa* seed may be triglyceride.

In this research, the determination of moisture content and oil content were carried out from the seeds sample. Moisture content of *A. squamosa* seed was determined by oven drying method. *A. squamosa* oil was extracted with petroleum ether either by two methods using Soxhlet and mechanical expeller. According to the physicochemical properties of seed oil, viscosity,

saponification value, acid value, peroxide value and specific gravity are nearly agreed with the some edible oil, but iodine value is lower than some edible oil such as peanut oil, sesame oil and sunflower oil. Therefore, *A. squamosa* oil is not suitable for human consumption.

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References

A.O.C.S. (1995). *Official Method and Tentative Method of the American Oil Chemistry Society*. Section C, A.O.C.S. Official Method, pp. 31-45

Aye Aye Cho, (2015). "*Production of Biodiesel by Transesterification of Oil from the Groundnut Cake-meal*". PhD Dissertation, Department of Chemistry, University of Yangon, Myanmar

Bhattacharja, A. et al., (2016). "The Pharmacological Properties of *Annona squamosa* L.". *International Journal of Phamacy and Engineering*, Vol., **4**(2), pp. 692-699

J. Braz. (2011). "Chemical and spectroscopic characterization of a vegetable oil used as dielectric coolant in distribution transformers", *Journal of the Brazilian Chemical Society*, Vol. **22**, pp. 1-15

J.M. Nzikou, L. Matos, G. Bouanga-Kalou, and C.B. Ndangui. (2009). Chemical "Composition on the Seeds and Oil of Sesame (Sesamum indicum L.) Grown in Congo-Brazzaville", *Advance Journal of Food Science and Technology*, 1(1): pp. 6-11

Natural Resources Conservation Service (NRCS), (2008). "PLANTS Profile, *Annona squamosa* L."*The PLANTS Database*, United States Department of Agriculture

Robinson, T. (1983). "*The Organic Constituents of Higher Plant*". North Amberst, 5th Edition, Cordus Press, pp. 127-177

Silverstein, R.M., Webster, F.X. and Kiemle, D.J. (2003). Spectrometric Identification of Organic Compounds. New York: 7th Edition, John Wiley and Sons, Inc

Sibel GEACAI, Anişoara-Arleziana NEAGU, and Elisabeta BOTEZ. (2013). "A physico-chemical study for some edible oils properties" *Ovidius University Annals of Chemistry*, Vol **24** (2), pp. 121-126

Siddalingappa R. H., and Omprakash, D. H (2015). "Biodiesel Production Process Optimization from Sugar Apple Seed Oil (*Annona squamosa*) and its Characterization". *Journal of Renewable Energy*, Article ID 148587, **6**, pp. 1-6

Win Min Oo, Myat Mon khine, (2017). "Pharmacological Activities of *Annona squamosa*", *International Journal of Pharmacy and Chemistry*. Vol **3**(6): 86-93