Research Journal of Environmental and Earth Sciences 2(1): 13-18, 2010
ISSN: 2041-0492

Submitted Date: November 05, 2009 Accepted Date: November 23, 2009 Published Date: January 10, 2010

Proximate Composition and Physicochemical Properties on the Seeds and Oil of *Annona muricata* grown In Congo-Brazzaville

[1]A. Kimbonguila, [1,4]J.M. Nzikou, [1]L. Matos, [3]B. Loumouamou, [1]C.B. Ndangui,
[1]N.P.G. Pambou-Tobi, [2]A.A. Abena, [3]Th. Silou, [4]J. Scher and [4]S. Desobry
[1]ENSP-UMNG, Laboratory of Food Physicochemistry and Biotechnology, Pole of Excellence in Nutrition and Food, P.O. Box 69 Brazzaville, Congo
[2] Laboratory of Biochemistry and Pharmacology, Faculty of Science of Health, P.O. Box 69 University Marien Ngouabi, Brazzaville, Congo
[3]Equipe pluridisciplinaire de recherche en alimentation et nutrition, Centre IRD. P.O. Box 1286 Pointe-Noire, Congo
[4]ENSAIA-INPL, Laboratory of Engineering and Biomolecule, 2, Avenue de la forêt de Haye, 54505 Vandoeuvre-lès-Nancy, France

Abstract : Proximate composition and physicochemical analyses were carried out on the seed and extracted oil of *Annona muricata*. The results showed that the seed contained 7.7% moisture, 8.5% crude protein, 9.7% ash, 5.2% crude fiber, 40% fat and 34.1% carbohydrate. The seeds were found to be good sources of minerals. Potassium (357.14±1.84 mg/100 g) was the highest, followed in descending order by Calcium (149.1±2.10 mg/100 g), Phosphorus (136.0 ±1.52mg/100g), Sodium (17.35±2.47 mg/100 g), and Magnesium (12.57±1.42 mg/100 g).The physical properties of the oil extracts showed the state to be liquid at room temperature. The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 41.41%) and linoleic (up to 30.60%). *Annona muricata* oil can be classified in the oleic-linoleic acid group. The dominant saturated acid was palmitic (up to 20.33%). The oil extracts exhibited good physicochemical properties and could be useful as edible oils and for industrial applications.

Key words: *Annona muricata*, essential fatty acid, DSC, oil yield, physicochemical properties and proximate composition

INTRODUCTION

Annona muricata is a member of the family of Custard apple trees called Annonaceae and a species of the genus *Annona* known mostly for its edible fruits Anona. *Annona muricata* produces fruits that are usually called Soursop due to its slightly acidic taste when ripe. *A. 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.

Previous research on *Annona muricata* has focused on the bark of the tree and roots for pharmaceutical purposes (British Pharmacopeia, 1993). Little attention has not been paid to using the seeds for food purposes nor has any attempt been made to extract oil from the seeds. This study was, therefore, conducted to determine selected nutritional and physicochemical properties of the seeds and oil extracted from the seeds of *Annona muricata*.

MATERIALS AND METHODS

This study was led to the laboratory of engineering and biomolecule of the ENSAIA-INPL, Vandoeuvre-lès-Nancy (France) for the period of Jan. 5, 2009 to Feb. 27, 2009.

Materials: *Annona muricata*, grown in Mpissa, southern district of Congo,were bought from Total-Bacongo market, Brazzaville. Only seeds that were not damaged were chosen and stored under cool dry storage conditions until needed.

Methods: Proximate analysis of *Annona muricata* seed Moisture, crude protein (micro-Kjeldahl), crude fiber and oil (Soxhlet) contents were determined using the methods described by Pearson (1976), whereas the ash content was determined using the method of Pomeranz and Meloan (1994) and total carbohydrate was determined by difference. All determinations were done in triplicate.

Corresponding Author: J.M. Nzikou, ENSP-UMNG, Laboratory of Food Physicochemistry and Biotechnology, Pole of Excellence in Nutrition and Food, P.O. Box 69 Brazzaville, Congo

Oil extraction: Dried *Annona muricata* seeds were ground in a Moulinex Model SeB PREP'LINE 850 (Moulin cafe). For solvent extraction (soxlhet method), 50g of ground seeds were placed into a cellulose paper cone and extracted using light petroleum ether (b.p 40-60ºC) in a 5-l Soxhlet extractor for 8 h (Pena *et al*., 1992). The oil was then recovered by evaporating of the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60 ºC for 1 h and flushing with 99.9% nitrogen. For methanol/chloroform extraction of Bligh and Dyer (1959), 100g of the ground seeds were homogenised with a chloroform mixture methanol (1:1) and water. Two phases was obtained, aqueous layer (methanol-water) and organic layer (chloroform). Oil was recovered by evaporating of the solvent (chloroform) using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60ºC for 1 h and flushing with 99.9% nitrogen All experiments were done in triplicates and the mean and standard deviations were calculated.

Physical and chemical analysis of crude oil:

Thermal behaviour: The thermal property of the oil samples was investigated by differential scanning calorimetry using a Perkin-Elmer Diamond DSC (Norwalk,USA). The instrument was calibrated using indium and zinc. The purge gas used was 99.99% nitrogen with a flow rate of 100 ml/min and a pressure of 20 psi. Sample weights ranged from 5-7 mg and were subjected to the following temperature program: Frozen oil sample was heated at 50 ºC in an oven until completely melted. Oil sample was placed in an aluminium volatile pan and was cooled to -50ºC and held for 2 min, it was then heated from - 50 to 50ºC at the rate of 5ºC.min^{-1} (normal rate) (Che Man *et al*., 1995), and held -50ºC isothermally for 2 min and cooled from -50 to 50ºC at the rate of 5ºC per minute. The heating and cooling thermograms for the normal and the fast (hyper DSC) scan rates were recorded and the onset, peak, and offset temperatures were tabulated. These values provide information on the temperature at which the melting process starts, the temperature at which most of the TAG have melted, and the complete melting temperature of the oil, respectively.

Viscosity measurements: A rheometer as described by Nzikou *et al*. (2007) was used to measure the different oil viscosities. By this procedure, a concentric cylinder system is submerged in the oil and the force necessary to overcome the resistance of the viscosity to the rotation is measured. The viscosity value, in mPa.s, is automatically calculated on the basis of the speed and the geometry of the probe. Temperature (20ºC) was controlled with a water bath connected to the rheometer. The experiment was carried out by putting 3 ml of sample in a concentric cylinder system using 100 s^{-1} as shear rate.

Chemical analysis: Determinations for peroxide, iodine, and saponification values, unsaponifiable matter and free fatty acid contents were carried out using Pena *et al*. (1992) standard analytical methods. The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 µl of n-hexane 50 mg of oil followed by 50 µl of sodium methoxide using the method of Cocks *et al*. (1966). The mixtures were vortex for 5 s and allowed to settle for 5 min. The top layer (1 µl) was injected into a gas chromatograph (Model GC- 14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionisation detector and a polar capillary column (BPX70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 µm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240 °C and column temperature was 110 °C held for one minute and increased at the rate of 8 ºC.min^{-1} to 220 ºC and held for one minute. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample. The minerals were determined by atomicabsorption spectrophotometry. One gram samples, in triplicate, were dry ashed in a muffle furnace at 550ºC for 8 h until a white residue of constant weight was obtained. The minerals were extracted from ash by adding 20.0 ml of 2.5% HCl, heated in a steam bath to reduce the volume to about 7.0 ml, and this was transferred quantitatively to a 50 ml volumetric flask. It was diluted to volume (50 ml) with deionised water, stored in clean polyethylene bottles and mineral contents determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). These bottles and flasks were rinsed in dilute hydrochloric acid (0.10 M HCl) to arrest microbial action which may affect the concentrations of the anions and cations in the samples. The instrument was calibrated with standard solutions.

Statistical analysis: Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by Excel Version 8.0 software. Significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Proximate analysis of *Annona muricata* seed oil: Results obtained showed that the seeds contained 7.7% moisture, 40% crude oil, 8.5% crude proteins, 34.1% carbohydrate (by difference), 5.2% crude fiber and 9.7% ash (Table 1). The high percentage of oil makes this seed

a distinct potential for the oil industry. The oil output is higher than that obtained Onimawo (2002). Variation in oil yield may be due to the differences in variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used.

Minerals: The *Annona muricata* seeds contained significant amount of important minerals (Table 2). The Potassium concentration (357.14±1.4 mg/100 g dry mater) was the highest, followed in descending order by Calcium (149.1±2.10mg/100g dry mater), Phosphorus (136.0±1.52mg/100g dry mater), Sodium (17.35±42.45mg/100g dry mater), and Magnesium (12.57±1.42 mg/100g dry mater). Potassium is an essential nutrient and has an important role is the synthesis of amino acids and proteins (Malik and Srivastava, 1982). Calcium and Magnesium plays a significant role in photosynthesis, carbohydrate metabolism, nucleic acids and binding agents of cell walls (Russel, 1973). Calcium assists in teeth development (Brody, 1994). Magnesium is essential mineral for enzyme activity, like calcium and chloride; magnesium also plays a role in regulating the acid-alkaline balance in the body. Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance (Fallon and Enig, 2001).

Oil extraction: Characteristics of the oil were compared with *Annona muricata* varieties described by Onimawo (2002). The extracted oils were liquid at room temperature. The oil content of *Annona muricata* "Congo-Brazzaville" seeds for the two methods utilised and the level at which the differences are significant are shown in Table 3. The oil extraction with the Soxlhet method had the highest yield, due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix (Lumley and Colwell, 1991). The Blye and Dyer method, showed the low yield due to losses during the separation of the two phases, aqueous layer (methanol-water) and organic layer (chloroform). The results of the above authors agree with those of the present work.

Physical and chemical properties of oil:

Physical properties:

Differential Scanning Calorimetry (DSC): DSC is suitable to determine these physical properties. The results of thermal analysis of oils are presented in Table 4. The obtained peaks were asymmetries and may indicate the presence of three components in oil extracted from the two methods. The first peaks at low melting points appear at -23.77°C (H_f = +12.94 $J.g^{-1}$) for Bligh and Dyer method and -21.37°C (H_f = +11.55 $J.g^{-1}$) for Soxlhet method. These peaks correspond to triglycerides formed by poly unsaturated acids (PUFA). The second peaks appear to -7.19°C (H_f = +8.66 $J.g^{-1}$) for Blye and Dyer method and -5.09°C (H_f = +1.40 $J.g^{-1}$) for Soxlhet method, suggest the presence of mixed triglycerides groups with different melting points.

Table 1: Proximate analysis (g/100 g dry weight) of *Annona muricata* oil seed

Characteristic	Obtained values[a] (M ± S.D.)	Reported values[b] 1
Moisture content (%)	7.7±0.24	8.5
Crude protein[c](%)	8.5±0.52	2.4
Ether extract (%)	40±0.82	20.5
Crude fiber (%)	5.2±0.26	8.0
Ash content (%)	9.7±0.12	13.5
Total carbohydrate[d] (%)	34.1	55.1

[a] M±S.D. mean±standard deviation.
[b] (1) Onimawo (2002).
[c] Crude protein = N (%) x 6.25
[d] Non-fiber carbohydrate was estimated by difference of mean values i.e 100-(sum of percentages of moisture, ash, protein and lipid)

Table 2: Mineral elemental composition of *Annona muricata* seeds

Mineral Elements	Composition (mg/100g) of Seed
Calcium, Ca	149.1±2.10
Phosphorus, P	136.0±1.52
Magnesium, Mg	12.57±1.42
Potassium, K	357.14±1.84
Sodium, Na	17.35±2.47

Values are mean±S.D of triplicate determinations

Table 3: Physical and chemical properties of *Annona muricata* seed oil extracted using solvent process

Properties	Obtained values	
	Blye & Dyer	Soxlhet
Oil[a] (%)	$34.80±0.75^{B}$	$40.0±2.60^{A}$
PV	$0.74±0.32^{A}$	$0.89±0.75^{A}$
FFA (as % oleic acid)	$13.5±0.12^{A}$	$14.2±0.10^{A}$
IV (wijs)	$102.4±0.27^{A}$	$107.2±1.82^{A}$
Saponification value	$112±1.42^{A}$	$117±0.51^{A}$
Unsaponifiable matter Content (%)	$1.21±0.21^{A}$	$1.47±0.72^{B}$
Viscosity (mPa.s) at 20°C	$46.90±0.82^{B}$	$27.00±0.75^{B}$
Ea (KJ. mol^{-1})	14.36	21.46

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($P < 0.05$).
[a] Oil = weight of extracted oil x 100/weight of seed.
Abbreviations: PV: Peroxide Value, FFA: Free Fatty Acid, IV: Iodine Value.

Table 4: Melting behaviour of *Annona muricata* seed oil using different scan rates. Experimental conditions: temperature program set at -50 °C for 10 min, rising to 50°C at rate of 5°C.min^{-1}

	5 °C.min^{-1}	
Thermogram	Blye and	DyerSoxlhet
Peak 1 [°C]	-23.77	-21.37
ΔH_f [$J.g^{-1}$]	+12.94	+11.55
Peak 2 [°C]	-7.19	-5.09
ΔH_f [$J.g^{-1}$]	+8.66	+1.40

Viscosity: Viscosity is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, or resistance to pouring. Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. In optics to know the rheological proprieties of these oils, we studied the

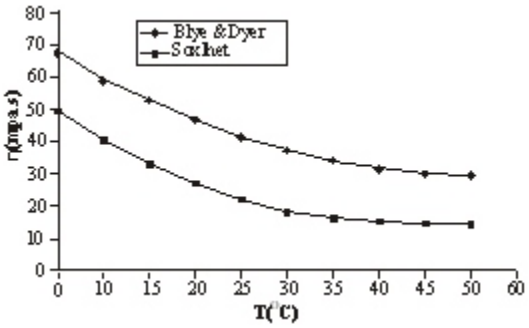

Fig.1: Effet of *Annona muricata.* temperature on seed oil

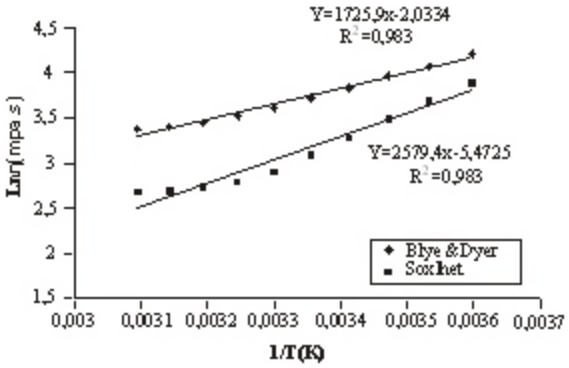

Fig. 2: Relationship between viscosity and temperature for *Annona muricata* seed oil extracted by Blye and Dyer and Soxlhet. Solid line Arrhenius model

influence of temperature on viscosity. Activation energies of the various classes of fatty acids contained in these oils were given Table 3. When the temperature increases, viscosity decreases exponentially (Fig. 1) some is the extraction method (Arslan *et al*., 2005; Nzikou *et al*., 2007). Viscosity varies between 49.40 and 14.60 mPa.s when temperature decreases of 50 to 5 ºC by Soxlhet method, by Blye and Dyer method, the viscosity of oil decreases of 67.70 to 29.60 mPa.s (Fig. 1 and Table 5). The viscosity of the oil obtained by Blye and Dyer method was highest, possibly because of the water that was absorbed by the gums (phospholipids) during extraction. This calculator calculates the effect of temperature on reaction rates using the Arrhenius equation.

$$\eta = A*\exp^{(-Ea/R*T)}$$

Where, η is the viscosity, A is constant, Ea is the activation energy (in KJ mol-1), R is the universal gas constant and T is the temperature (in degrees Kelvin). R has the value of 8.314 x 10-3 KJ mol^{-1} K-1. We should use this calculator to investigate the influence of temperature on viscosity. Linear regression analysis was applied to the logarithmic form of Arrhenius equation in order to determine the parameters of the relation (Fig. 2 and Table 6). lnη against 1/T, -Ea/RT is the slope from which Ea was evaluated. Activation energies of oils are given in Table 3. The highest value of activation energy is obtained by Soxlhet method (21.46 KJ.mol-1 and 14.36 $KJ.mol^{-1}$ by Blye and Dyer. The higher the activation energy, the more stable the fatty acid is.

Chemical properties: The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The high levels of %FFA, in two oils samples investigated (Table 3), indicate that these oils are not stable, because they worsen easily via oxidative rancidity.The unsaponifiable matter content of the Soxlhet method were significantly higher ($p<0.05$) than those of the Blye and dyer method (Table 3). There was no significant difference in the iodine and saponification values, in the two extraction methods ($p>0.05$). The

Table 5: Oil viscosity at various temperature in degree celsuis

	η (mPa.s)	
T (ºC)	Blye and Dyer	Soxlhet
5	67.70	49.40
10	58.90	40.40
15	52.90	32.95
20	46.90	27.00
25	41.30	22.15
30	37.40	18.30
35	34.20	16.40
40	31.70	15.50
45	30.10	14.80
50	29.60	14.60

Table 6: Energie plot derived from the Arrhenius equation

1/T (K^{-1}) Lnη (mPa.s)		
	Blye and Dyer	Soxlhet
0.00359712	4.21508618	3.89995042
0.00353357	4.07584109	3.69882978
0.00347222	3.96840334	3.49499126
0.00341297	3.84801768	3.29583687
0.0033557	3.7208625	3.0978375
0.00330033	3.6216707	2.90690106
0.00324675	3.53222564	2.79728133
0.00319489	3.45631668	2.74084002
0.00314465	3.40452517	2.69462718
0.00309598	3.38777436	2.68102153

Table 7: Relative percent composition of fatty acid in *Annona muricata*. seed oil

Fatty acid	Determined values	
	Blye and Dyer	Soxlhet
C14 :0	–	–
C16 :0	20.25 ± 1.34^{A}	20.41 ± 1.58^{A}
C16 :1	1.20 ± 0.45^{A}	1.44 ± 0.45^{A}
C18 :0	4.30 ± 0.18^{A}	4.13 ± 0.29^{B}
C18 : 1	41.53 ± 0.20^{B}	41.29 ± 0.53^{A}
C18 :2	30.34 ± 0.18^{A}	30.85 ± 0.34^{A}
C18 :3	2.38	1.88 ± 0.25^{A}
Saturated	24.55	24.54
Unsaturated	75.45	75.46

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($P < 0.05$).

Table 8: Comparison of the profile in fatty vegetable oil acids

Oils	C14 :0	C14 :1	C16 :0	C16 :1	C18 :0	C18 :1	C18 :2	C18 :3	C20 : 0
Palm	1.0	-	44.5	0.2	4.6	38.7	10.5	0.3	0.3
Safou	-	-	45.5	-	2.8	28	24.9	1.24	-
Maize	-	-	10.5	-	2.5	28	58.5	1.0	0.5
Groundnut	-	-	10.0	-	2.0	46.0	31.0	-	-
Cotton	0.9	-	23.0	-	2.2	17.7	55.8	-	-
Hazel nut	-	-	7.0	0.1	2.0	74.5	16.5	-	-
Soybean	-	-	11.0	-	4.0	22.0	54.3	7.5	-
Jatropha	-	-	15.6	1.0	5.8	40.1	37.6	-	-
A. muricata	-	-	20.33	1.32	4.22	41.41	30.60	2.13	-

slightly higher value of unsaponifiable matter in the Soxlhet method may be due to the ability of the solvent to extract other lipid associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic *et al*., 1978; Salunke *et al*., 1992).

Fatty acid composition: The major saturated fatty acids in *Annona muricata* seed oil were palmitic (20.33%) and stearic (4.22%) acids. The main unsaturated fatty acids are oleic (41.41%) and linoleic (30.60%) acids (Table 7). There was no significant difference ($p>0.05$) in the amounts of the major fatty acids in the two oil samples. The two oil samples of *Annona muricata* contained saturated and unsaturated acids (24.55% and 75.45%) respectively.

Annona muricata oil can be classified in the oleic-linoleic acid group. Linoleic acid which is one of the most important polyunsaturated fatty acids in human food because of its prevention of distinct heart vascular diseases (Boelhouwer, 1983). *Annona muricata* oil is predominantly made up of oleic and linoleic acids (41.41 and 30.60%) respectively. One notes 2.38% of linolenic acid C18:3 by the method Blye and Dyer and 1.88% (linolenic acid C18:3) by the method Soxlhet (Table 7). One also notes (Table 7) 1.20% of palmitolenic acid C16:1 by the method Blye and Dyer and 1.44% (palmitolenic acid C16:1) by the method Soxlhet.

Unsaturated total fatty acids are prevalent (75.45 %) (Table 7). This prevalence of the unsaturated fatty acids and the high values of the iodine index indicate that the oil of *Annona muricata* of Congo-Brazzaville is of *the unsaturated type.* The comparison of the composition in fatty acids of *Annona muricata* seed oil with that of vegetable oils (Table 8) indicates that this plant is rich in acids oleic (C18:1), linoleic (C18:2) and palmitic (C16:0); nevertheless, the presence of toxic subsistences, makes this oil unsuitable to consumption (Leatemia and Isman, 2004) and this oils is close to that of *Jatropha curcas* (Table 8).

CONCLUSION

This study showed that the *Annona muricata* seed is a good source rich in minerals and oil. *Annona muricata* seed oil is of unsaturated type and contains mainly the fatty acids oleic C18:1(41.41%) and linoleic C18:2 (30.60%). The oil can be classified in the oleic-linoleic acid group. High unsaponifiable matters content (1.34%) guarantees the use the oils in cosmetics industry. The oil extracts exhibited good physicochemical properties and could be useful for industrial applications.

REFERENCES

Arslan, E., M.E. Yener and A. Esin, 2005. Rheological characterization of tahin/pekmez (sesame paste/concentrated grape juice) blends. J. Food Eng., 69(2): 167-172.

Bastic, M., L. Bastic, J.A. Jabanovic and G. Spiteller, 1978. Hydrocarbons and other weakly unsaponifiables in some vegetable oils. J. Am. Oil Chem., Soc., 55: 886-892.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917

Boelhouwer, C., 1983. Trends in chemistry and technology of lipids. J. Am. Oil Chem. Soc., 60(2): 457-462.

British Pharmacopeia, 1993. Her Majesty Stationery Office, HMSO, London, 1: 478-498.

Brody, T., 1994. Nutritional Biochemistry, San Diego, CA: Academic Press. 2nd Edn., pp: 761-794.

Che Man, Y.B. and P.Z. Swe, 1995. Thermal analysis of failed-batch Palm oil by differential scanning calorimetry. J. Am. Oil Chem. Soc., 72(12): 1529-1532.

Cocks, L.V. and C. Van Rede, 1966. Laboratory handbook for oil and fats analysts. Academic Press. London, pp: 88.

Fallon, S. and M.G. Enig, 2001. Nourishing traditions. The cookbook that challenges policitally correct nutrition and the diet dictocrats. pp: 40-45.

Leatemia, J.A. and M.B. Isman, 2004. Toxicity and antifeedant activity of crude seed extracts of Annona squamosa (Annonaceae) against lepidopteran pests and natural enemies. Int. J. Tropi. Insec. Sci., 24: 150-158.

Lumley, I.D. and R.K. Colwell, 1991. Fats From Fatty Foods and Determination of Fat Content. In: Analysis of Fats and Fatty Foods. Rossell, J.B. and J.L.R. Pritchard, (Eds.), pp: 238-247.

Malik C.P. and A.K. Srivastava, 1982. Text book of plant physiology. New Delhi: Ludhiana Nzikou, J.M., M. Mvoula-Tsieri, L. Matos, E. Matouba, A.C Ngakegni, M. Linder and S. Desobry, 2007. *Solanum nigrum* L. seeds as an alternative source of edible lipids and nutriment in congo Brazzaville. J. Appl. Sci., 7: 1107-1115.

Onimawo, I.A., 2002. Proximate composition and selected physicochemical properties of the seed, pulp and oil of sour sop (*Annona muricata*). Plant Foods for Hum. Nutr., 57: 165-171.

Pearson, D., 1976. General Methods. In: The Chemical Analysis of Foods, pp: 6-26.

Pena, D.G., R. G.L. Anguiano and J.J.M Arredondo., 1992. Modification of the method 1 AOAC (CB-method) for the detection of aflatoxins, Bull. Environ. Contam. Toxicol., 49: 485-489.

Pomeranz, Y. and C. Meloan, 1994. Food Analysis: Theory and Practice, 3rd Edn.,Chapman and Hall, New York, pp: 778.

Russel, E.W., 1973. Soil Conditions and Plant Growth. Supergene Zone, M. Nedra, 19 (In Russian).

Salunke, D.K., J.K. Chavan, R.N. Adsule and S.S. Kadam, 1992. World Oil Seeds: Chemistry, Technology and Utilization. pp: 170-173.