

## Extraction and characteristics of seed oil from Papaya (*Carica papaya*) in Congo-Brazzaville

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**Abstract:** Papaya seeds were collected and dried. This study was carried out on papaya seed to clarify their proximate composition and the characteristics of the extracted oil including unsaponifiable matter and fatty acid composition. The seed is a rich source of protein (26.78%) and crude fiber (21.4%). Palmitic acid was the main saturated fatty acid (15.22%), while linoleic acid was the major unsaturated fatty acid (76.38%) in all lipid classes. The physical properties of the oil extracts showed the state to be liquid at room temperature. *Carica papaya* seeds have ash content of 3.2% (with the presence of following minerals: K, Na, Ca, P and Mg). However, Ca and P occur in appreciable quantities (1821±2.12 mg/100 g dry matter and 1156±1.8 mg/100 g dry matter, respectively).

**Key words:** *Carica papaya* seed, essential fatty acid, minerals, nutritive values, unsaponifiable matter

### INTRODUCTION

*Carica papaya* (Family Caricaceae) originated in Central America. It contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion (Brocklehurst and Salih, 1985). *Carica papaya* plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural (botanical) pesticides (McLanghlin *et al.*, 1992). The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 1991). *Carica papaya* L. leaf tea or extract has a reputation as a tumour-destroying agent (Walter, 2008). The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender, (Wilson, 1974). The seed is used for intestinal worms

when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence, (Elizabeth, 1994). Fresh, green pawpaw leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier (Atta, 1999). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion (Elizabeth, 1994). The green unripe pawpaw has a therapeutic value due to its antiseptic quality. It cleans the intestines from bacteria, more so that (only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B12). The tea, prepared with the green papaya leaf, promotes digestion and aids in treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2005).

Papaya is important for its fruit and it is only recently that it has been cultivated for this purpose. This new trend is an indication of increased consumption due to better awareness of the importance of the fruit in diet of the third world countries, such as Congo-Brazzaville. In Congo, like most other countries, the seeds of papaya fruits (about 15% of the wet weight) are discarded. Evidently, this is because of bad experiences when they are consumed by humans or animals. However, in order to make more

efficient use of papaya, it is worth in investigating the use of the seeds.

As part of a study on the utilization of papaya seeds, the characteristics of the seeds are now reported with a view to evaluating nutrients.

## 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 Apr. 1, 2010 to Jun. 30, 2010.

**Materials:** Mature *Carica papaya* fruits were collected from various locations in Diata and Baongo districts of Brazzaville. The fruits were cut into two longitudinal halves and seeds removed by hand. The testae of the seeds were removed by squeezing the seeds between two fingers. The seeds were dried in an oven at 60°C. The dried seeds were stored at -10°C until required for analysis.

**Methods:** Proximate analysis of *Carica papaya* seed Moisture, crude protein (micro-Kjeldahl), crude fiber, oil (Soxhlet) contents and refractive index of the oil (at room temperature) 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. The sample calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (2.44, 8.37 and 3.57, respectively) used in vegetable analysis (Asibey-Berko and Tayie, 1999). All determinations were done in triplicate.

**Oil extraction:** For solvent extraction (soxhlet method), 50g of papaya seed flour were placed into a cellulose paper cone and extracted using light petroleum ether (b.p. 40-60°C) in a 5-l Soxhlet extractor for 8 h (Pena *et al.*, 1992). The oil was then recovered by evaporating off 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°C for 1 h and flushing with 99.9% nitrogen. For methanol/chloroform extraction (Bligh and Dyer, 1959), 100 g of the papaya seeds flour 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 off 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 60°C 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°C in an oven until completely melted. Oil sample was placed in an aluminium volatile pan and was cooled to -50°C and held for 2 min, it was then heated from - 50 to 50°C at the rate of 5°C/min (normal rate) (Che Man and Swe, 1995), and held -50°C isothermally for 2 min and cooled from -50 to 50°C at the rate of 5°C per min. 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.* (2009) 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 (20°C) 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<sup>-1</sup> 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 °C/min to 220°C 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

Table 1 Proximate analysis (g/100 g dry weight) of papaya (*Carica papaya*) seed

Characteristic	Obtained values <sup>a</sup> (M±S.D.)	Reported values <sup>b</sup>
		1
Moisture content (%)	6.8±0.1	6.20
Crude protein <sup>c</sup> (%)	26.78 ±0.42	27.8
Ether extract (%)	31.13±0.24	28.3
Crude fiber	21.4±0.17	22.6
Ash content (%)	3.2±0.12	3.50
Total carbohydrate <sup>d</sup> (%)	10.69	11.67
Calorific value (Kcal/100g)	364.06	nd

<sup>a</sup>: M ± S.D. mean ± standard deviation; <sup>b</sup>: (1) Marfo *et al.* (1986); <sup>c</sup>: Crude protein = N (%) × 6.25; <sup>d</sup>: Total carbohydrate was estimated by difference of mean values i.e 100-(sum of percentages of moisture, ash, fiber, protein and lipid); nd: not determined

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 atomic absorption spectrophotometry. One gram samples, in triplicate, were dry ashed in a muffle furnace at 550°C 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 papaya seed:** Results obtained showed that the seeds contained 6.8% moisture, 31.13% crude oil, 26.78% crude protein, 10.69% carbohydrate (by difference), 21.4% crude fibre, 3.2% ash and 364.06 Kcal calorific value (Table 1). The seed is a rich source of proteins (26.78%), lipids (31.13%) and crude fiber (21.4%). These high values for proximate analysis make the papaya seeds a rich source of nutrients. Also, the high percentage of oil makes this seed a distinct potential for the oil industry. According to Marfo *et al.* (1986) 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:** It is of interest to note that the most prevalent mineral element in *Carica papaya* seeds is Calcium which is as high as 1821±2.12 mg/100 dry mater (Table 2), followed in descending order by Phosphorus (1156±1.8

Table 2: Mineral elemental Composition of papaya (*Carica papaya*) seed

Mineral Elements	Composition (mg/100 g) of Seed
Calcium, Ca	1821±2.12
Magnesium, Mg	28.7±1.20
Potassium, K	32.89±1.7
Sodium, Na	12.59±1.5
Phosphorous, P	1156±1.8

Values are mean ± S.D of triplicate determinations

mg/100 g dry mater), Potassium (32.89±1.7 mg/100 g dry mater), Magnesium (28.7±1.20 mg/100 g dry mater) and Sodium (12.59±1.5 mg/100 g dry mater). Potassium is an essential nutrient and has an important role in 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 *Carica papaya* varieties described by Marfo *et al.* (1986). The extracted oils were liquid at room temperature. The oil content of *Carica papaya* 'Congo-Brazzaville' seeds and the level at which the differences are significant are shown in Table 3. The oil extraction with the Soxhlet 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 Bligh 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 study.

### 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 3: Physical and chemical properties of papaya (*Carica papaya*) seed

Properties	Obtained values		Reported values <sup>a</sup>
	Bligh & Dyer	Soxhlet	Solvent extract
Oil <sup>b</sup> (%)	30.25±0.18 <sup>B</sup>	32±1.1 <sup>A</sup>	28.3
PV	0.03±0.17 <sup>A</sup>	0.05±0.24 <sup>A</sup>	nd
FFA (as % oleic acid)	0.98±0.12 <sup>A</sup>	1.2±0.2 <sup>A</sup>	0.94
IV (wijs)	73.32±0.35 <sup>A</sup>	72.78±0.22 <sup>A</sup>	74.8
Saponification value	195.82±0.42 <sup>A</sup>	198.5±0.21 <sup>A</sup>	197
Unsaponifiable matter Content (%)	0.78±0.31 <sup>A</sup>	0.81±0.27 <sup>B</sup>	0.72
Refractive index (at 25°C)	1.4692	1.4680	1.4678
Viscosity (mPa.s) at 20°C	38.40	27.30	nd
Ea (KJ/mol)	14.28	17.25	nd

nd, not determined; Means for the determined values in the same row followed by the same superscript letter are not significantly different (p<0.05); <sup>a</sup>: Marfo *et al.* (1986); <sup>b</sup>: Oil = weight of extracted oil x 100/weight of seed; PV: Peroxide Value; FFA: Free Fatty Acid; IV: Iodine Value

Table 4: Melting behaviour of papaya (*Carica papaya*) seed oil using different scan rates. Experimental conditions: temperature program set at -50°C for 2 min, rising to 50°C at rate of 5°C/min

Thermogram	5°C/min	
	Bligh and Dyer	Soxhlet
Peak 1 [°C]	-20.73	-21.53
ΔH <sub>f</sub> [J/g]	+2.19	+1.81
Peak 2 [°C]	-8.34	-9.77
ΔH <sub>f</sub> [J/g]	+62.26	+41.53

Table 4. The obtained peaks were asymmetries and may indicate the presence of two components in oil extracted from the two methods. The first peaks at low melting points appear at -20.73°C (ΔH<sub>f</sub> = +2.19 J/g) for Bligh and Dyer method and -21.53°C (ΔH<sub>f</sub> = +1.81 J/g) for Soxhlet method. These first peaks (at -20.73 and -21.53°C, Bligh & Dyer and Soxhlet methods, respectively), correspond to triglycerides formed by poly unsaturated acids (PUFA) and the last peaks appear to -8.34°C (ΔH<sub>f</sub> = +62.26 J/g) for Bligh and Dyer method and -9.77°C (ΔH<sub>f</sub> = +41.53 J/g) for Soxhlet method, suggest the presence of mixed triglycerides groups with different melting points.

**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 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.*, 2009). Viscosity varies between 58.40 and 22.10 mPa.s when temperature decreases of 50 to 5°C by Soxhlet method. By Bligh and Dyer method, the viscosity of oil decreases of 67.70 to 29.60 mPa.s (Table 5). The viscosity of the oil obtained by Bligh and Dyer method was highest, possibly because of the water that was absorbed by the

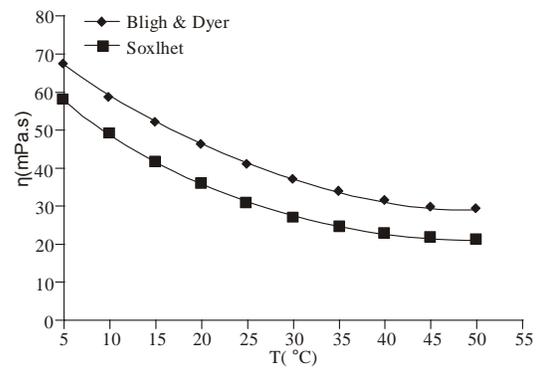


Fig. 1: Effet of papaya (*Carica papaya*) temperature on seed oil

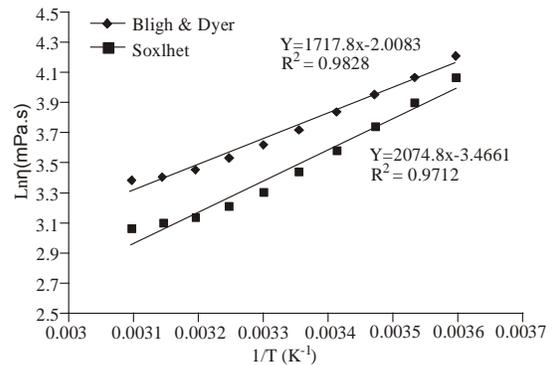


Fig. 2: Relationship between viscosity and temperature of papaya (*Carica papaya*) seed oil extracted by Bligh Dyer and Soxhlet

gums (phospholipids) during extraction. This calculator calculates the effect of temperature on reaction rates using the Arrhenius equation.

$$\eta = A * \exp(-E_a / R * T)$$

where, η is the viscosity, A is constant, Ea is the activation energy (in KJ/mol), R is the universal gas constant and T is the temperature (in degrees Kelvin). R

has the value of  $8.314 \times 10^{-3}$  KJ/molK. 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, Table 6).  $\ln \eta$  against  $1/T$ ,  $-E_a/RT$  is the slope from which  $E_a$  was evaluated. Activation energies of oils are given in Table 3. The highest value of activation energy is obtained by Soxhlet method (17.25 KJ/mol) and 14.28 KJ/mol by Bligh and Dyer method. 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 free fatty acid and the unsaponifiable matter content of the Soxhlet method were significantly higher ( $p < 0.05$ ) than those of the Bligh 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 low free fatty acids content is indicative of low enzymatic hydrolysis. This could be an advantage as oil high free fatty acids develop off flavour during storage (Bailey, 1954). The refractive index reflects the degree of unsaturation and chain length. Values obtained here (1.4692 for Bligh & Dyer and 14680 for Soxhlet methods) are expected of on oils with low iodine value and the presence of Oleic acid fatty in the proportion observed (Table 3). The percentage saponifiable is 197 for the two extractions methods (Bligh & Dyer and Soxhlet), while the non-saponifiables are 0.80. this is an indication that the steroidal and related components are low in the oil (Marfo *et al.*, 1986). This is a good oil property sought after for both nutritional and industrial purposes.

The slightly higher value of unsaponifiable matter in the Soxhlet 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 *Carica papaya* seed oil were palmitic (15.22%) and stearic (4.39%) acids and the main unsaturated fatty acids are oleic (76.38%) and linoleic (4.02%) (Table 7). There was no significant difference ( $p > 0.05$ ) in the amounts of the major fatty acids in the two oil samples. In the two oil samples of *Carica papaya* contained saturated and unsaturated acids (19.61 and 80.39%) respectively. The proportion of unsaturated fatty acids was greater than the saturated fatty acids. *Carica papaya* seed oil is predominantly made up of palmitic and oleic acids (15.22 and 76.38%), respectively. The results obtained are in

Table 5: Oil viscosity at various temperature in degree celsius

T (°C)	$\eta$ (mPa.s)	
	Bligh and Dyer	Soxhlet
5	67.70	58.40
10	58.90	49.40
15	52.90	41.95
20	46.90	36.00
25	41.30	31.15
30	37.40	27.30
35	34.20	24.80
40	31.70	23.50
45	30.10	22.80
50	29.60	22.10

Table 6: Energie plot derived from the Arrhenius equation

1/T (K <sup>-1</sup> )	Ln $\eta$ (mPa.s)	
	Bligh and Dyer	Soxhlet
0.00359712	4.21508618	4.06731589
0.00353357	4.07584109	3.89995042
0.00347222	3.95699637	3.73647843
0.00341297	3.84160054	3.58351894
0.00335570	3.72086250	3.43881425
0.00330033	3.62167070	3.30688670
0.00324675	3.53222564	3.21084365
0.00319489	3.45631668	3.13983262
0.0314465	3.40452517	3.09557761
0.00309598	3.38777436	3.06339092

Table 7: Relative percent composition of fatty acid in papaya (*Carica papaya*) seed oil

Fatty acid	Determined values	
	Bligh and Dyer	Soxhlet
Palmitic acid (C16:0)	15.39±0.24 <sup>A</sup>	15.05±0.18 <sup>A</sup>
Stearic acid (C18:0)	4.27±0.18 <sup>A</sup>	4.51±0.32 <sup>B</sup>
Oleic acid (C18:1)	76.11±0.10 <sup>B</sup>	76.64±0.23 <sup>A</sup>
Linoleic acid (C18:2)	4.23±0.29 <sup>A</sup>	3.81±0.42 <sup>A</sup>
Saturated acid	19.66	19.56
Unsaturated acid	80.34	80.44

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

agreement with previous study (Subramaniam and Achaya, 1957) on the indian and Mexican varieties. The major fatty acid (C18:1), may be of nutritional advantage (Friedman and Reid, 1973).

## CONCLUSION

*Carica papaya* seed is a rich source of nutrients. Palmitic and oleic acids were the principal fatty acids and the proportion of unsaturated fatty acids was greater than the saturated fatty acids. High fiber value, *Carica papaya* could be a rich source of dietary fiber which can have beneficial effects.

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