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Yield and Chemical Characterization of Congolese Mansa (*Solanum americanum* Miller) Oil Extracted from Plant by Solvents and Enzymes

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Abstract: New sources of edible oils extracted from new agricultural are continually researched. Mansa, *Solanum americanum* Miller, a typical culture widely produced in some african countries as Congo would be able to attaint this purposes. Mansa seeds contains up to 35% w/w of oil extracted by solvents, 2.7% in roots and 1.3% in leaves. The major fatty acid is linoleic acid (66.5%) followed by oleic acid (14.6%) and palmitic acid (12.6%). The minor fatty acids are DHA and EPA (0.17 and 0.23%, respectively). The omega 6/3 ratio (ω 6/3) is lower than 5. Enzymatic extractions by pectinase yield 18% w/w of oil. Besides this lower oil extraction compared to solvent extraction, the enzymatic extraction would be interesting as a way to maintain the minors fractions contents of great functional activities since these minors' fractions are generally susceptible of heat oxidation.

Key words: Lipids, edible oil, omega 3, mansa, *Solanum americanum* miller

INTRODUCTION

The production of edible oil is considerate one of the biggest markets in the food industries. Extensive research has been done in this field, not only about their caloric effects in the daily diets but also about their origin and their saturated and unsaturated fatty acids content. Food lipids are either consumed in the form of visible fats or as constituents of basic foods. The largest supply of vegetable oil comes from the seeds of soybean, cottonseed and peanut, canola, sunflower and the oil-bearing trees of palm, coconut and olive[1]. New sources of oil are of improving interest as a way to reduce their costs, optimize their chemical composition and spread out their production.

Congolese diet is rich in vegetable leaves. The currently consumed, are saka-saka (*Manihot esculentus*), ngayi-ngayi (*Hibiscus sabdarifa*), coco (*Gnetum africanum*) and mansa (*Solanum americanum* Miller).

Mansa (*Solanum americanum* Miller) or morelle is an annual branched herb up to 35-90 cm high, with bright green leaves, slightly toothed on margins. Flowers are small and white. Fruits are small green balls, turning to black and glowing when ripe. It belongs to the family of solanacea and is widely produced in some african countries, as Congo.

The common weed previously identified as *Solanum nigrum* is *Solanum americanum*. The most conspicuous differences between the two species concern influorescence and mature fruits. *Solanum americanum* has an umbellate or almost umbellate influorescence while *Solanum nigrum* present a short but distinct internodes between branchs. The fruit of *Solanum americanum* is shiny black when mature-hence the common name, glossy nightshade-while *Solanum nigrum* has dull black fruits. *S. americanum* is most common in the tropics and *S. nigrum* in cooler regions[2].

Mansa supposed to be a source of fat acids, but there are some concerns. It contains solanine, a glyco-alkaloid poisoning substance with the highest concentration in unripened berries[3-5]. The solanine content increases in leaves as the plant matures. When ripe, the berries are the least toxic part of the plant and have been eaten in some

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tropical countries without ill effects. This characteristic and their participation in mansa's oil might be evaluated in further works and researches.

The accumulation of body lipids in *Solanum nigrum* depends of the removal of embryo sac in the developing seeds[6]. Enzymes play an important role in this matter either by the lipids accumulation or as a technique for lipids extraction. Others application of mansa has been related. More recently, *Solanum americanum* cells were successfully used as a natural semiconductor sensitizer in photoelectrochemical application to convert visible light into electricity[7].

The aims of this study has been to evaluate the extraction of oil from mansa seeds, leaves and roots and to carry out the composition of mansa's oil in order to evaluate the edibility application of them in the future.

MATERIALS AND METHODS

Preparation of mansa seeds, leaves and roots for oil extraction: Dried fruits (ripe berries) were from Brazzaville (Congo) and leaves and root were extracted from mansa's plant growth experimentally in Nancy (north-eastern of France). Seeds were crushed in a coffee grinder, then separate in two batches which will serves for the same treatment. The first group was heated to inhibit the lipase endogenous activity enzymes. Leaves and roots were washed 3 times with distillated water and drained at room temperature, then were crushed and preserved for further analysis.

Solvent and enzymatic oil extraction procedures: The solvent system for oil extraction was generally chosen according to the composition of the working material. For the seeds with a low humidity and reduced as powder the system more adapted for extraction was the Sohxlet system. The mansa dried seeds were finely grinded (followed by heating or not) and the oil was extracted with soxhlet extractor for 5 h using petroleum ether as solvent. For the leaves and roots of mansa, the oil was extracted by the Bligh and Dyer[8] method using methanol/chloroform (2:1; v/v) as solvent system in the crushed raw material.

For the pectinase treatment and extraction, the Brazzaville dried seeds were heated at 105°C for 2 h to inactivated endogenous enzymes. Another part of the grinded seeds were used without heating. The resulting powder was diluted with distillated water (1/3 w/v). The pH of the slurry was corrected by adding HCl 1N and the enzymatic digestion has been conducted over night at 40°C in an incubator over stirring. The basic pH of the slurry (6.21) was adjusted to 5.5 and the pectinase (26.10^3 AU/g-SIGMA, USA) was used for 0.2% (v/v) as described[9]. The purpose has been to reduce viscosity of the slurry, hydrolyze the cell membrane and release easily the internal oil for further extraction.

The mixture was centrifuged at 10,000 × g under nitrogen in a j2-HS Beckman centrifuge (Beckman Instruments; Gagny, France). The oil was delicately obtained on the supernatant. The pellet was washed twice with distillated water to recovered remained oil and centrifuged again. The oil was collected as before.

Determination of fatty acid composition: Fatty Acid Methyl Esters (FAME) were obtained by transmethylation of total lipid aliquots (50 mg) with 1 mL of borontrifluoride in methanol (8% w/v) for 10 min in a shaking water bath heated at 90°C as described by Ackman[10] previously to submit the samples to gas chromatography analysis.

The analysis of fame was carried out by Gas Chromatography in a PerichromTM 2000 system (Saulx-les-Chartreux, France), equipped with a Flame Ionization Detector (FID) and a fused silica capillary (25 m × 0.25 mm, × 0.5 μm, BPX70 SGE Australia Pty. Ltd.). Column temperature was kept at 145°C for 20 min, then warmed up from 145 to 210°C flowing out at 5°C min^{-1} and hold at 210°C for 15 min. The ended injection port was maintained at 230°C and the detector at 260°C. The fatty acids were identified by comparison of their retention times with appropriate standards PUFA-1 Marine source (Supelco, N° 4-7033, Bellefonte, PA-USA), PUFA-2 Animal source (Supelco, N° 4-7015-U, Bellefonte, PA-USA). Each measure was in a triplicate.

Physical-chemical characterization of fatty acid fractions from Mansa oil: Standard procedures were used to characterize crude and heated fatty acids extracted by solvents and pectinases. The usual indices[11] determinate in oils were acid value (standard method 969.1), iodine value (standard 993.20), saponification value (standard 965.33) and peroxide value (standard 920.160).

Thermal properties and rheological properties of and fatty acid fractions from Mansa oil: Thermal analyses were performed with a Perkin-Elmer Differential Scanning Calorimeter, DSC-7, equipped with a thermal analysis data station (Perkin-Elmer Corp, Norwalk, CT, USA). Nitrogen was the purge gas and flowed at 20 mL min^{-1}. The calorimeter was calibrated according to standard procedures established in the manufacturer user book using indium and distilled water. Samples of 15 mg were weighed into aluminium pans and cooled and/or heated at 2.5°C min^{-1} from -60 to +60°C. The heat-of-fusion enthalpies ΔH (J g^{-1}) were calculated for each peak by

the Pyris software (Perkin-Elmer Corp, Norwalk, CT, USA). DSC measurements were carried out in triplicate.

The apparent viscosity of mansa seed oil was measured with a Stresstech viscosimeter (Rheological Instruments AB, Sweden), with a temperature increasing from 5 to 45°C, at 1°C min^{-1}. The applied stress was constant at 50 mPa.

Student's t-test was used for statistical validity of results and the coefficient of variation between each measurement do not exceeded 2%.

RESULTS AND DISCUSSION

Fatty acids composition: The Table 1 shows the composition of mansa fatty acids. The oil presents a very low saturated fraction (14.5-17.2%) compared to others oils produced in Africa and described by others works. Saturated fatty acids in safou (*Dacryodes edulis*) pulp oil is greater than 43% Dzondo-Gadet *et al.*[12], 32% in gombo (*Abelmoschus esculentus*) seed oil Camciue *et al.*[13], 46% in shea butter and 48% in palm oil Kapseu and Parmentier[14]. It contains essential fatty acids as 14.5% of oleic acid and 67.7% of linoleic acid, well above safou and gombo oils contents. The composition of mansa seed oil has similarities to sunflower oil which contains 67.4% of linoleic acid[15].

The unsaturation index of mansa seed oil (PUFA/SFA value) was in the range of 3.95 and 4.86 in agreement with WHO recommendations that suggest a relationship between ω 6/3 in the order of 5.0. Generally, it's admitted that African oils could present ratios up to 53/1 because of abiotic factors[16].

The minor fractions of fatty acids in the seed oil were the omega 3 as linolenic acid, EPA and DHA which were lower than <1%. The seed oil average could be given as linolenic acid [C18 (3) <1%] <palmitic acid (C16 (0); 10-12%) <oleic acid (C18 (1); 14.5%) < linoleic acid (C18 (2); 66-69%).

Fatty acids level and location: The level of mansa seed oil obtained by pectinase treatment (18%) is half of soxhlet (35%) Table 1. Generally the enzyme is function of vegetal wall composition and the extraction efficiency was near or equal of soxhlet extraction[12]. It seems that pectine is not a major component of cell wall of mansa seeds as the slurry is not very viscous like safou. The poor enzyme efficiency in oil yield is compensated by the gain of 3.6±0.5% ($p<0.05$) of linoleic acid (Table 1).

In the present case, the heating to prevent endogenous enzyme cross linking was unnecessary as it lows the minor fraction containing omega 3, principally linolenic acid for 95.8±0.9% ($p<0.05$) and 96.8±0.5% for EPA and DHA Table 1. The extraction process does affect significantly the composition of oil in contrast of safou and kolo oils[12].

Considering the organs, seeds gave 35% of oil while leaves up to 1.3% and roots to 2.7% Table 2 even oil extraction from leaves and roots was on raw materials and by Bligh and Dyer method. The oil in leaves and roots seems to be the same like seed oil according to those major components in saturated fraction and MUFA. The minors fatty as C17(0), C17(1) C20(0) and C20(1) appeared in leaves and roots. New fatty acids in leaves and root are current as described for gombo[17].

Table 1: Oil yields of Congolese Mansa seed oil extracted by solvents and pectinase. The results are the mean±SEM (n = 3)

	Extraction process			
	Soxhlet		Pectinase	
Fatty acids	Crude	Heated	Crude	Heated
Saturated Fatty Acids (SFA)				
C14: 0	0.15±0.01	0.30±0.01	0.23±0.02	0.10±0.01
C16: 0	12.60±0.81	12.95±0.93	10.05±0.79	12.15±0.94
C18: 0	4.40±0.06	4.39±0.82	4.20±0.44	4.30±0.41
∑ saturated (SFA)	17.15	17.64	14.48	16.55
C16: 1ω7	0.20±0.03	0.20±0.07	0.30±0.01	0.12±0.08
C18: 1ω9	14.60±1.01	18.30±1.16	14.51±1.51	10.78±1.66
C20: 1ω9	0.30±0.01	0.4±0.1	0.40±0.01	0.31±0.19
∑mono unsaturated (MUFA)	15.1	18.9	15.21	11.21
18: 2ω	66.5±3.01	26.07±2.11	68.91±1.56	62.06±3.17
18: 3ω3	0.91±0.18	0.04±0.01	0.95±0.05	0.04±0.01
20: 5ω3	0.17±0.01	0.08±0.01	0.23±0.01	0.07±0.01
22: 6ω3	0.17±0.02	0.07±0.00	0.22±0.01	0.07±0.00
∑poly unsaturated (PUFA)	67.75	62.26	70.31	62.24
PUFA/SFA rate	3.95	3.53	4.86	3.76

Table 2: Saturated and unsaturated fractions of mansa oil extracted from different parts of plant. The results are the mean±SEM (n = 3)

	Location		
Fatty acids	Seeds	Leaves	Roots
Oil yield (% w/w)	35	1.3	2.7
Saturated Fatty Acids (SFA)			
C14: 0	0.15±0.01	0.96±0.23	0.9±0.02
C16: 0	12.60±0.81	6.99±0.71	12.43±0.79
C17: 0		1.18±0.80	1.05±0.13
C18: 0	4.40±0.06	2.63±0.12	5.09±0.44
C20: 0		1.02±0.09	1.53±0.56
∑ saturated (SFA)	17.15	12.78	21
C16: 1ω7	0.20±0.03	0.20±0.07	0.59± 0.01
C17: 1ω8		1.2±0.1	1.1±0.1
C18: 1ω9	14.60±1.01	18.44±1.16	19.74±1.51
C20: 1ω9	0.30±0.01	1.40±0.15	0.40±0.01
C22: 1ω11		1.21±0.17	1.00±0.22
∑mono unsaturated (MUFA)	15.1	22.45	22.83
18: 2ω6	66.5±3.01	55.15±2.21	41.70±1.56
18: 3ω3	0.91±0.18	1.41±0.28	4.67±0.85
20: 5ω3	0.17±0.01	2.44±0.24	1.42±0.91
22: 6ω3	0.17±0.02	3.91±0.13	1.39±0.41
∑poly unsaturated (PUFA)	67.75	62.91	49.18
Others		1.86	6.99

In comparison to seed oil, the linolenic acid level in Mansa is higher in mature leaves up to 49% and for 5 times in roots. In the same order, the EPA (20:5 ω3) [leaves × 14, roots × 8) and DHA (22:6 ω3) (leaves × 23, roots × 8) contents burst was higher in leaves Table 2. The enrichment in omega 3 in leaves, pods and flowers is well known for gombo[17]. The oil extracted from leaves and roots could be very interesting because of their content in very long chain. Indeed these fatty acids are known for their health benefits by lowing coronary diseases and cholesterol.

Stability of Mansa seed oil: Analytical results showed that the iodine, acid and peroxide values ranged respectively at 106.37±0.44; 0.04±0.0 Table 3 and 0.06±0.01 meq/kg contribute to the stability of oil.

The enzyme treatment leads to protect oil from oxidation during extraction process. The amount of acid value was for 4 times in crude oil and for 3 times in heated oil between soxhlet and pectinase treatments Table 3. This tendency is confirmed by iodine and peroxide values. These results are in agreement with those previously described as oil oxidation during extraction process for Safou and Kolo oils[12].

The high saponification values suggest the possibility of industrial applications for food industries (table or cooking oils) or even in others industrial applications (i.e., skin cream or liquid soap) as described for safou[18].

Thermal behavior and viscosity: The Table 4 shows the thermogram of mansa seed oil. It revealed 3 melting points ranging from -11.68 to +6.1°C in crude oil extracted by pectinase treatment. When the oil was extracted by soxhlet, the thermogram revealed one more point at +32.01°C. The third point became of +23.69°C. It seems that the soxhlet extraction lead to oil oxidation resulting in broken fatty acids to give saturated fraction with very low carbone number. This hypothesis is confirmed by the heating pretreatment. Indeed it appeared a very high melting point up to 56.34°C.

The negative values confirm that the oil is provided in long chain fatty acids. The very low melting point at -16 or -22°C corresponds to the PUFA fraction of oil. The melting point at -0.3 or +0.5°C is correlated to the MUFA fraction. Generally high metling point corresponds to saturated fatty acids. They are present in crude oil extracted by soxhlet and after heating Table 4.

The three melting points of pectinase extracted oil traduce the low saturation of Mansa seed oil. The 4th point obtain after soxhlet extraction traduce the begining of deterioration. The 5th point confirm this hypothesis as it is present only when heated even the changes were not visible on fatty acid profile.

Table 3: Effect of extraction process on oil indices. The value are the mean±SEM (n = 6)

	Extraction process			
	Soxhlet		Pectinase	
Indices	Crude	Heated	Crude	Heated
Acid value (% oleic acid)	0.04±0.00	0.06±0.01	0.010±0.00	0.020±0.00
Saponification value	177.0±0.8	172.8±1.5	181.2±0.4	171.7±0.2
Iodine value	105.2±0.9	107.75±0.81	106.37±0.44	108.0±0.51
Peroxide value [meq O_2/kg]	0.06±0.01	0.08±0.02	0.03±0.01	0.19±0.02

Table 4: Thermal behavior of mansa seed oil. The value are the mean ±SEM (n = 3)

	Extraction process			
	Soxhlet		Pectinase	
Thermogram	Crude	Heated	Crude	Heated
Peak 1 [°C]	-16.46	-22.33	-11.68	-23.70
Onset	-26.58	-30.22	-18.42	-33.14
ΔH [J g^{-1}]	2.89	4.52	2.46	6.32
Peak 2 [°C]	-0.30	-8.82	+0.50	-10.02
Onset	-1.49	-17.88	-1.10	-17.85
ΔH [J g^{-1}]	1.11	1.70	1.20	1.23
Peak 3 [°C]	+23.69	+6.35	+6.10	+9.83
Onset	+23.05	-0.15	+2.60	+9.03
ΔH [J g^{-1}]	0.26	2.19	2.20	4.76
Peak 4 [°C]	+32.01	+21.36	-	+22.34
Onset	+27.83	+15.18	-	+17.22
ΔH [J g^{-1}]	2.99	3.70	-	4.07
Peak 5 [°C]	-	56.34	-	+52.32
Onset	-	55.781	-	+51.77
ΔH [J g^{-1}]	-	1.68	-	1.56

The thermogram is thightly correlated to the apparent viscosity value measured: 17.1±0.2 mPa/s for heated oil by soxhlet or enzyme treatment. The crude oil present an apparent viscosiy of 22.5±0.3 and oil 31.5±0.8 mPa/s for pectinase extracted oil at 20°C.

CONCLUSION

Although experimentally plants of mansa have been growth very well in France in climate's condition quite different from Africa, indicating the possibility of exploiting this culture elsewhere of Africa. Mansa seeds contains up to 35% w/w (linoleic acid as major fatty acid) of oil, extracted by Soxhlet, 2.7% in roots and 1.3% in leaves. Enzymatic extractions by pectinase yield 18% w/w of oil in seeds. Nevertheless the enzymatic extraction would be interesting to maintain the minors' fractions contents of great functional activities. These minors' fractions are generally susceptible of heat oxidation. The solanine toxicity on the other hand would be evaluated in

enzymatic extraction as heat has an antagonist effect over solanine. The results summarized here would indicate mansa as a possible source of edible oils, but fundamental and technical researches toward this objectives might be conducted in order to answer scientific questions and optimize several technical aspects in that way.

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