

Full Length Research Paper

Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (var 1 and 2) of Congo Brazzaville

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Amaranthus hybridus is a vegetable which is eaten in Congo Brazzaville and in other countries. Two varieties of *A. hybridus* seeds (var 1 and 2) were selected for this study. Average oil content varies between 11 and 14%. *A. hybridus* seeds are also rich in proteins (17%) and minerals. Red oils obtained have a high saponification value (130-190) and the iodine value is between 100 and 113. The quantity of unsaponifiable matter (5 - 7%) in these oils is important. The fatty acids composition gives the following average profile: 18: 2n-6 > 18: 1 n-9 > 16: 0 > 22: 6n-3 > 18: 0. *A. hybridus* seeds oils also have long chain poly unsaturated fatty acids such as DHA (5.63-21.46%) and the results indicated that the *n-6/n-3* ratios were 1.48 to 5.63. The triacylglycerols analysis shows that oils extracted by Bligh and Dyer method contains 6 major TAGs in *A. hybridus var1*: LLnLn > OLL > POL > OLL > PLL > LLL and *Amaranthus hybridus var2*: LLnLn > OLL > PLL > POL > OLL > LLL. The *A. hybridus* seeds can be used as cattle food and baby complement food. These oils have nutritive and dietetic potentialities.

Key words: *Amaranthus hybridus* oil, Congo Brazzaville, TAG, chemical methods extraction.

INTRODUCTION

Amaranthus hybridus named “bari” is cultivated in several areas of the world including South America, Africa, India, China and the United States (He, 2002). In Congo, their leaves are eaten as spinach or green vegetables. In Nigeria, *Amaranthus* leaves combined with condiments are used to prepare soup (Oke, 1983; Oke, 1979). These leaves boiled and mixed with a groundnut sauce are

eaten as salad in Mozambique (Oliveira, 1975) or pureed into a sauce and served over (farinaceous) vegetables in West Africa (Martin, 1979).

Amaranthus hybridus seeds are small and lenticular in shape, with each seed averaging 1.0 -1.5 mm in diameter and 1,000 seeds weighing 0.6-1.2 g (Jain and Hauptli, 1980; Saunders and Becker, 1984). The seed oil contains three important fatty acids: 16: 0 (22.2%), 18: 1n-9 (29.1%) and 18: 2n-6 (44.6%) but leaves oil has a different composition: 18: 3n-3 (56.5-62%), 18: 2n-6 (15.5-24.7%) and 16: 0 (13.5-15.5%), (He, 2003; Guil-Guerrero, 2000).

Several authors have shown that *Amaranthus hybridus* oil contained squalene (He, 2002, 2003a, 2003b). The squalene concentration in this oil ranged from trace to 7.3% is much higher than in other oilseeds. Indeed, squalene a steroidal hormone precursor is used for cosmetics and it's employed as lubricant for precision instruments, such as computer disks (Budin, 1996; Sun,

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Abbreviations: DHA, docosa hexaenoic acid; DSC, differential scanning calorimetry; FAME, fatty acid methyl ester; IV, iodine value; L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; PUFA, poly unsaturated fatty acid; PV, peroxide value; S, stearic acid; SFA, saturated fatty acid; SV, saponification value; TAG, triacylglycerol; and UFA, unsaturated fatty acid.

1995). It was also reported that this compound has important beneficial effects on cancers (Rao, 1998) and reduce cholesterol level in the blood (Smith, 2000; Miettinen, 1994). *Amaranthus* seed has been suggested as an alternative to marine animals as a natural source of squalene (He, 2002).

For this study, two *Amaranthus hybridus* seeds cultivars were chosen; var1 (white) and var2 (black), and three chemical methods are used to extract the oil. The aim is to investigate the seeds and oils physicochemical characterization in order to deduce the nutritional and/or nutraceutical properties of these oils.

MATERIALS AND METHODS

Seed material

Two cultivars *Amaranthus hybridus* seeds were obtained from Brazzaville market.

Lipid extraction

Lipid extraction was carried out by three methods; Soxhlet (Horowitz, 1984), Bligh and Dyer (Bligh, 1959) and Folch (Omoti, 1987; Folch, 1957). After removing solvent, using a Rotavapor apparatus, the seed oil obtained was drained under a stream of nitrogen and then stored in a freezer (-20°C) for subsequent physicochemical analyses.

Analytical methods

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean \pm standard deviation ($\bar{E} \pm \text{S.D.}$).

Dry matter

This was determined according to the (AOAC, 1990).

Oil content

The weight of oil extracted from 40 g of seed powder was determined to calculate the lipid content. Result was expressed as the percentage of lipids in the dry matter of seed powder.

Protein content

Total protein was determined by the Kjeldahl method. Protein was calculated using the general factor (6.25) (AOAC, 1990). Data were expressed in percentage of dry weight.

Ash and mineral contents

To remove carbon, about 2 g (powdered) of each cultivar, in a porcelain container, was ignited and incinerated in a muffle furnace at about 550°C for 8 h. The total ash was expressed in percentage of dry weight. The mineral constituents (Ca, Na, K and Mg) present in the *A. hybridus* seeds of each cultivar were analysed separately, using an atomic absorption spectrophotometer (Hitachi Z6100,

Japan).

Lipid indices

Standard procedures of American Oil Chemist Society were used for indices values AOAC (AOAC, 1997), procedures were applied for acidic value (standard 969.17,1997), iodine value (standard 993.20, 1997), peroxyde value (standard 965.33, 1997), saponification value (standard 920.160,1997).

Fatty acid composition

The oils were converted into methyl esters using a KOH/MeOH method (Standard FIL, 182:1999). The extracted fatty acid methyl esters (FAME) were dissolved in hexane for GC analyses. GC analyses were performed on a PerichromTM 2000 system gas chromatograph (Saulx-les-Chartreux, France), equipped with a flame ionization detector hydrogen and a capillary column (BPX70 SGE Australia Pty Ltd, 25m \times 0.25 mm \times 0,5 μm film). The injector and detector temperature were set at 260°C . The column temperature was programmed from 70 to 180°C at $39.9^{\circ}\text{C}/\text{min}$, and is kept at this temperature during 8 min. It then underwent a second heating up to 220°C ($3^{\circ}\text{C}/\text{min}$). Nitrogen was the carrier gas (1.1 bars). The software Winilab (Périchrom, Saulx-les-Chartreux) was used to integrate the chromatograms, the peak areas of triplicate injections were measured. The identification of the peaks was achieved by retention times and by comparing them with authentic standards analysed under the same conditions.

Differential scanning calorimetry (DSC)

Thermal properties were determined with a Perkin- Elmer differential scanning calorimeter, DSC -7 equipped with a thermal analysis data station (Perkin- Elmer Corp., Norwalk, CT). Nitrogen was the purging gas flowed at 20 ml min^{-1} . The calorimeter was calibrated according to standard procedures set in manufacturer user book using indium and distilled water. The sample ($\sim 15 \text{ g}$ weight into an aluminium pan) was quickly cooled to -60°C with a speed of $15^{\circ}\text{C}/\text{min}^{-1}$, maintained at this temperature for 10 min, and heated to 80°C with a heating speed of $5^{\circ}\text{C}/\text{min}^{-1}$. Melting point and enthalpies ΔH (Jg^{-1}) were calculated for each peak by Pyris software (Perkin- Elmer Corp., Norwalk, CT). The DSC measurements were carried out in triplicate.

Viscosity determination

Viscosity was followed at 25°C or at other temperature with a Stress Tech Rheologica Rheometer (Rheologica Instruments AB, Lund, Sweden) conducted with a steel cone-plate (C40/4) under a shear stress increased from 7 to 20 Pa.

Triacylglycerols (TAG) analysis

Triacylglycerols were analysed by HPLC. HPLC Characteristics are: Shimadzu LC-BA pump - Rhéodyne Valve 7125 20 μL . Furnace with Peltier effect igloo-lash; evaporator detector with light diffusion DEDL Sedex 55 ($T = 40^{\circ}\text{C}$, $P = 3 \text{ bars}$); kromacil column C18 50 \times 4,6 mm 5 μm batch; DT 048 collar n³-2 CL 35. Analysis condition: Column temperature: 30°C ; eluent is MeCN/CH₂Cl₂ (67/33); 7,5 μL of sample injected in 5 ml of MeCN/CH₂Cl₂:25/25. The peaks were recorded and the under peaks areas measured.

Table 1. *Amaranthus hybridus* seed and its oils physico chemicals properties.

	<i>Amaranthus hybridus</i> var1			<i>Amaranthus hybridus</i> var2			Mean
	Sox	B&D	Folch	Sox	B&D	Folch	
Lipids (%)	8.77 ± 1.06	10.52 ± 0.31	13.95 ± 1.6	8.46 ± 0.2	10.36 ± 0.27	11.34 ± 0.75	10.57
AV	3.92±0.12	2.92±0.57	2.38±0.43	3.7±0.08	2.39±0.07	1.74±0.7	2.84
SV	147.9±5.1	176±3.8	185.6±2.9	150±1.4	153.1±1.95	134.85±0.98	157.91
IV	112.92±2.7	111.12±1.54	109±1.37	113.4±3.2	111.76±2.87	109.98±2.36	111.36
PV	4.3± 0.8	3.3 ± 0.15	2.8 ± 0.2	4.8 ± 0.7	3.8 ± 0.1	2.3 ± 0.5	3.55
Unsaponifiables	5.93±0.17	7.21±0.47	5.37±0.53	5.27±0.57	5.75±0.3	6.07±0.95	5.93
Viscosity at 25 °C	22.3±0.5	23.07±0.37	591±0.56	20.9±0.16	35.26±0.3	59.1±1	36.62
Fatty acids (mPa s)							
16:0	13.61	14.6	19.35	16.47	16.56	18.47	16.51
18:0	2.25	1.68	2.95	2.72	2.59	3.48	2.61
SFA	16.06	16.42	22.56	19.43	19.29	22.21	19.33
18:1n-9	28.25	29.27	30.64	27	27.89	26.44	28.25
22:1n-11	0.47	0.42	0.23	0.45	0.85	1.79	0.70
MUFA	28.79	29.76	31.04	27.51	28.8	28.23	29.02
18:2n-6	31.72	34.22	37.13	40.61	40.65	40.48	37.47
18:3n-3		0.47	0.69		0.68	0.74	0.65
20:5n-3				0.28			0.28
22:6n-3	21.46	17.78	6.58	9.92	9.2	5.63	11.76
PUFA	54.1	53.07	45.58	52.17	51.16	48.04	50.69
n-3	21.83	18.25	7.83	10.88	10.51	7.25	12.76
n-6	32.27	34.82	37.75	41.29	40.65	40.79	37.93
PUFA/SFA	3.33	3.23	2.04	2.7	2.63	2.17	2.68
n-6/n-3	1.48	1.91	4.82	3.8	3.87	5.63	3.59
Crude ash	4.86 ± 0.08			4.02 ± 0.38			4.44
K	48.59			18.52			33.5
Na	11.88			3.75			7.82
Mg	223.45			970			596.73
Ca	12.58			22.14			17.36
Crude proteins	17.60 ± 1.88			18.99 ± 1.47			18.29
Dry matter (%)	90.75 ± 0.96			89.39 ± 0.88			90.07

AV: acid value in % oleic acid
SV: Saponification value mg KOH g⁻¹
IV: Iodine value
PV: Peroxide value meq O₂ kg⁻¹
Viscosity at 25 °C in mPa.s
Minerals in mg/100 g

RESULTS AND DISCUSSION

Oil content

Substrate and oils physicochemical characterization are summarised in Table 1. Oil content ranges from 8.77 to 14% for *A. hybridus* var1 and for *A. hybridus* var2 it ranges from 8.5 to 11.54%, which are in agreement with

those obtained by Becker (11%) (Becker, 1981) and it are higher than those measured by He (2002, 2003a, 2003b). Soxhlet and Bligh and Dyer extractions give similar oil contents; 8 and 10%, respectively for the two varieties. Maximum oil content is obtained by Folch method (13.95%). *A. hybridus* seeds oil contents are very low compared to other tropical plants such as *Canarium schwenfurthii* which has an average lipid content 36.1% and *Balanites aegyptiaca* almonds, 48.3%. It is also lower

Table 2. Activation energy in KJmol^{-1} for fatty acids classes in *Amaranthus hybridus* seed oils.

	<i>Amaranthus hybridus</i> var1			<i>Amaranthus hybridus</i> var2			Mean
	Folch	B&D	Soxhlet	Folch	B&D	Soxhlet	
E_{a1}	17.75	3.78	2.96	17.75	2.52	-2.54	7.04
E_{a2}	0.94	1.88	0.83	0.94	1.03	0.88	1.08
E_{a3}	19.62	12.89	2.23	19.62	8.15	2.65	10.86

than that of some usual oilseeds such as cotton (16-28%) and corn (30-65%) (Kapseu, 1997).

Ash content and minerals

Ash content is 5% for *A. hybridus* var1 and 4% for *A. hybridus* var2 (Table 1). In a general way, these seeds are rich in minerals elements. Mg level is very high for the two cultivars with 970 mg/100 g for var2. Na is in small proportion between 4 and 12 mg/100 g. Calcium content is also considerable. Following ranking order for the elements is:

Mg>K>Ca>Na for *A. hybridus* var1

Mg>Ca>K>Na for *A. hybridus* var2

Proteins content and dry matter

The crude protein content of *A. hybridus* seed ranges from 17.6 to 19% of dry matter (Table 1). These values are similar to those obtained by Afolabi (1981), and higher than in most common seeds except soybeans. Dry matter content is about 91%.

Acid value

Acid value of oils varies between 1.74 and 3.92, which is in conformity with the specifications of edible oils (1 - 7% of oleic acid). It varies certainly according to the extraction method; with high acidity by Soxhlet method (4%) due to a beginning of oxidation and low for Folch (2%). This low acidity value characterizes a rather stable oil at the temperature (Codex Alimentarius, 1993, 1981).

Saponification value

A significant difference is noted on the saponification value. It varies between 147.9 and 185.6 for var1 and between 134.85 and 153.1 for var2 (Table 1). The mean average value (160) is lower than that of the *Dacryodes edulis* pulp oil (201) (Omoti, 1987), (230) (Kapseu, 1997) and of those of the usual oils such as soybean (189-195), peanut (187-196) and cotton (189-198) (Codex Alimentarius, 1993, 1981).

Iodine value

The iodine value for two cultivars varies between 109 and 113 (Table 1). These values are approximately the same as usual oils such as from soybean (120-143) and sunflower (110-143) (Codex Alimentarius, 1993, 1981), but higher than the other nonconventional oilseeds such as *Coula edulis* (90-95), *Dacryodes edulis* (60-80) and *Canarium schweinfurthii* (71.1 - 94.9), (Omoti, 1987; Kapseu, 1997; Kapseu, 1999; Abayeh et al., 1999).

Peroxide value

The peroxide value of those oils lies between 3 and 5 (Table 1). These values are below 10 which characterises the majority conventional oils (Codex Alimentarius, 1993, 1981).

Viscosity

At 25°C the viscosity of these oils varies between 20.9 and 59.1, indicating rather fluid oils. The oil obtained by Folch method is more viscous (59.1 mPa.s). With a view to evaluating activation energies of various fatty acids classes contained in those oils, the influence of temperature on viscosity was studied. When the temperature increases, viscosity decreases exponentially whatever the method of extraction (Isaac, 2004). The Arrhenius's equation was used to determine activation energy from the viscosity results:

$$\eta = A e^{-E_a/RT}$$

A is the frequency factor called also exponential pre factor energy

E_a = barrier to cross before the elementary flow can begin in kJ mol^{-1}

R = 8.31 $\text{Jmol}^{-1}\text{K}^{-1}$ (perfect gas constant)

T = absolute temperature (K)

In a plot of $\ln \eta$ against $1/T$, $-E_a/RT$ is the slope from which E_a was evaluated. Activation energies of oils are given in Table 2. Three values for each oil were generally observed. These oils contain three fatty acids classes. Activation energies E_{a1} (17.75) and E_{a3} (19.62) of Folch

Table 3. Linoleic and Linolenic acid content of usual oils and fats alimentaries (from CIQUAL databases, AFSSA Report, 2003).

Oil /Fat	Linoleic acid	Linolenic acid	18:2n-3 + 18:3n-3
Peanut oil	30.5	0	30.5
Rapeseed oil	21.2	9.6	30.8
Corn oil	55.9	0.9	56.8
Walnut oil	56.7	12.3	69
Grapeseed oil	67.3	0.3	67.6
Soybean oil	52.6	7.3	59.9
Sunflower oil	64.1	0.05	64.15
Olive oil	12.9	0.85	13.75
Mixed oil	47	1.2	48.2
A. hybridus var1	34.36	0.59	34.95
A. hybridus var2	40.58	0.71	41.29
Butter oil	1.16	0.46	1.62
Cream	0.52	0.12	0.64
African sheabutter	5.98	nd	5.98
Goose fat	12	nd	12
Palmkernel fat	2.7	nd	2.7
Cooking butter	12.4	1.24	13.64
Pig fat	8.1	nd	8.1

oil and Soxhlet oil have low activation energies. E_{a2} is low for all oils. The higher the activation energy the more the fatty acid is stable.

Unsaponifiable matter content

Unsaponifiable matter of *A. hybridus* seed oils extracted by the various methods varies between 5 and 7.21%. Unsaponifiable matter content in these oils is higher than in other oils such as shea butter (5.1%), avocado (2.8%), *Dacryodes edulis* (2.3%), red *egusi* seeds (1.6%), *Canarium schweinfurthii* Engl. (1.3%), sesame (1.2%), white melon (1.1%), corn (0.92%), cotton (0.52), palm (0.34%), peanut (0.33), palm kernel (0.22) and coco kernel (0.09) (Kapseu, 1997). Unsaponifiable matter content is high which adds value to these oils.

Fatty acids composition of oils and health use

Fatty acid compositions of *A. hybridus* seeds oil were determined. Results (Table 1) showed that the oil contained five majors' fatty acids: palmitic acid (14-20%), stearic acid (2-3.5%); oleic acid (26.5-31%), linoleic acid (32-41%) and docosahexaenoic acid (DHA) (7-21%). These results are slightly similar with those obtained by others authors: palmitic acid (22%), oleic acid (29.1%), linoleic acid (44.6%) (He, 2003). The fatty acids composition for the two cultivars have the following average profile: 18:2n-6 > 18:1n-9 > 16:0 > 22:6n-3 > 18:0.

Saturated fatty acids (SFA) content is the same (about 22%) in Folch method for the two varieties than in other extraction methods, 16% for var1 and 19% for var2. Unsaturated fatty acids (UFA) proportion is high (76-82%). The n-6/n-3 ratio varies between 1.5 and 5.63, which is in conformity with FAO/WHO recommendations (FAO/WHO, 1994). Linoleic acid content is high giving nutritional or dietetic properties to these oils. PUFA content are also high (46-54%) than that of other nonconventional oilseeds: avocado (15.5%), *Canarium schweinfurthii* (28.8%), *Dacyodes edulis* (25.2%) and of sheabutter (6,9%) (Chalon, 2001). According to Table 3, *A. hybridus* oils PUFA are more important than usual oils. PUFA/SFA ratio ranged from 2.04 to 3.33; with an average value of 2.68, we have unsaturated oils. The good value of PUFA/SFA ratio and iodine value make it possible to classify these as potentially oleic/linoleic oils having nutritional properties. Long chain poly unsaturated fatty acids such as docosa hexaenoic acid DHA (7-21%) are also present.

A. hybridus seed oils can be considered as source of n-3 polyunsaturated fatty acid (PUFA). The omega-3 polyunsaturate, docosahexaenoic acid (DHA), plays a number of biologically important roles, particularly in the nervous system, where it is found in very high concentrations in cell membranes. In infants, DHA is required for the growth and functional development of the brain, with a deficiency resulting in a variety of learning and cognitive disorders. During adulthood DHA maintains normal brain function and recent evidence suggests that reduced DHA intake in adults is linked with a number of neurological disorders including schizophrenia and

Table 4. Thermal analysis of *A. hybridus* seed oil.

	<i>Amaranthus hybridus</i> var1			<i>Amaranthus hybridus</i> var2		
	Soxhlet	Bligh & Dyer	Folch	Soxhlet	Bligh & Dyer	Folch
Peak 1 [°C]	-24.14	-25.52	-19.5	-32.2	-33.1	-17.3
ΔH [J/g]	42.22	0.66	6.82	5.04	48.25	14.04
Peak 2 [°C]	-22.12	-14.63	-13.32	-22.23	-23.18	-17.11
ΔH [J/g]	20.44	9.14	2.03	0.93	8.5	10.5
Peak 3 [°C]		-20.78	-21.1	-18.96	-13.85	
ΔH [J/g]		17.93	16.3	9.45	0.77	
Peak 4 [°C]					-17.24	
ΔH [J/g]					60.32	

Table 5. TAG content of *A. hybridus* seed oil extracted by Bligh and Dyer method.

<i>A. hybridus</i>	LLnLn	LLL	OLL	PLL	OOL	POL
var1	62.65	2.73	9.28	5.63	5.81	6.79
var1	27.05	6.03	16.44	16.06	7.09	12.91

depression. n-3 polyunsaturated fatty acids may protect against vascular diseases, however, their high accumulation in membranes may increase lipid peroxidation and subsequently induce deleterious effects in patients suffering from oxidative stress (Turner, 2003).

According to Table 3, walnut, grapeseed and sunflower would be the best oil of its natural high content of PUFA. In contrast, coconut oil and palm oil are the higher saturated oils. Saturated fatty acids contribute to increase cholesterol and predisposed to cardiovascular diseases. *A. hybridus* seed oils could be solution for cold use dietetic with essential polyunsaturated fatty acids guaranteed.

The importance of *A. hybridus* oil in the composition of breast milk was reported by Rocquelin et al. (1998). Indeed, Congolese women take in their food large amounts of fruits and vegetables such as *A. hybridus*. It was shown that the DHA percentage found in the maternal milk were very close to the amount of PUFA in *A. hybridus*. Fatty acid compositions of these oils are beneficial for breast fed infant development. n-6/n-3 ratio varies between 1.48 to 5.63, which is in conformity with WHO recommendation (1-10) (FAO/WHO, 1994). Thus, these oils have good dietetic properties.

Thermal analysis of oils

Thermal profiles parameters of *A. hybridus* oils are summarised in Table 4. Var1 oil presents two major melting point at about -25°C corresponding to unsaturated fatty acids and at -21.5°C corresponding to saturated fatty acids. Two other melting point appeared at -19.5°C and -14°C. In var2 Oil, thermal analysis reveals two important fusion peak in oil extract with Bligh and Dyer method at -33.1°C and at -17°C. Other melting point

appeared at -23°C, -17°C and -13.85°C. Tan (2002) indicates that triacylglycerols thermal parameters depend on the fatty acids distribution on glycerol. Generally, triacylglycerols with three saturated fatty acids have higher melting point than those of three unsaturated fatty acids.

Triacylglycerols (TAG) structure of oil

Triacylglycerols analysis shows that the *Amaranthus hybridus* seeds oil extracted by Bligh and Dyer method contains 6 major TAG (Table 5):

A. hybridus var1: LLnLn > OLL > POL > OLL > PLL > LLL
A. hybridus var2: LLnLn > OLL > PLL > POL > OLL > LLL

PPP and SSS TAG are not present in these oil. When long chains saturated fatty acids such as palmitic or stearic acid are esterified in position *sn*-2, TAG have hypercholesterolemic and arteriogenic properties (Kapseu, 1996; Ulbricht, 1991).

Conclusion

Protein content of *A. hybridus* seeds is high and it is rich in mineral elements particularly Mg. *A. hybridus* seeds can be suggested as an alternative to maintain and reinforce the immune system in humans and also used as cattle food. *Amaranthus hybridus* seeds oils are rich in PUFA. The presence particularly of DHA differentiates this oil from other nonconventional and usual oils. The total unsaturation was important. It is concluded that *A. hybridus* seeds oils has an important nutritional value. LLnLn have higher value to confer to these oil nutritional

and dietetic properties. Unsaponifiable matter content is high and shows good prospects for the exploitation of *A. hybridus* oil the cosmetic industry.

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