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Research Article

Thermo-oxidation and Long Time Storage of Nkamba Nut (*Ricinodendron africanum* var Nkamba) Oil Stored at Room Temperature

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Abstract: The Nkamba nut (*Ricinodendron africanum* var Nkamba), named *Kingoma-ngoma*, a member of Euphorbiaceae yield 67% of oil when extracted with soxlhet using hexane as solvent. The crude oil is rich in C18:1 (19.48%); C18:2 (39.44%) and C18:3 (31.63%). The behavior of oil is investigated when heated at 100°C and/or stored 4 years at room temperature. The oxidation was appreciated by chemical indices or photospectrometrically ($K_{232/270}$). The Totox value grew up from 5.33 in freshly extracted oils to 87.46 for aged oils. The level of hydroperoxides show a great amount of absorbance (0.857) for the primary byproducts in fresh oil heated. In the aged oils, there was a great level of ended byproducts up to 17.78. The fatty acid profile is modified with an amount of elaidic acid (0.0064 to 12.4%) and the strong decrease of the linolenic acid rate (31.63 to 1.07%). The thermogramm shows a peak of -27.1°C which is deplace to -15.1°C by progressive saturation of double bonds. Despite of natural antioxidants content, Nkamba nut oil is hardly oxidized in 4 years and then became unusual to human consumption.

Keywords: Nkamba nut oil, room temperature, storage, thermo oxidation, totox

INTRODUCTION

The *Ricinodendron africanum var* Nkamba; named *kingoma-ngoma* in the south west of DR Congo, belongs to the family of the Euphorbiaceae. The fruits and the seeds or nuts are edible in the south east of Congo Brazzaville; not in the harvest site in the left side of river Congo.

The oils extracted from Nkamba nuts are rich in PUFA. They contained 19.48% of C18:1; 31.63% of C18:3 and 39.44% of C18:2 (Dzondo-Gadet *et al*., 2014b). Lipid oxidation of oil contained PUFA-rich foods is a serious problem that leads to loss of shelf-life, consumer acceptability, functionality, nutritional value and safety. It affects the quality and nutritional value of foods (Dacaranhe and Terao, 2001). In human foods, the presence of fatty acid oxidized products, like the aldehydes have been implicated in aging, mutagenesis and carcinogenesis (Kampa *et al*., 2007). These aldehydes like Malondialdehyde (MDA) or 4-Hydroxy-2-Nonenal (4-HNE) are very toxic by their ability to crosslink to proteins and bind covalently to nucleic acids (Nair *et al*., 1986).

Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Arabshahi *et al*., 2007).

Many methods have been made to prevent the oxidative deterioration of lipids by using natural antioxidants. The main antioxidative mechanisms necessary to protect food systems from oxidation are radical scavenging, metal chelation and oxygen scavenging.

Some natural components such as vitamin E, carotenoids, flavonoids, anthocyanins and phenolic compounds are known to decrease oxidation processes (Ahn *et al*., 2008).

About the protective processes, the Modified Atmosphere Packaging (MAP) is a technique, which is widely used to extend the shelf-life and to improve the quality of perishable foods including meat, meat products, fish, fruits and fresh-vegetables. The storage of deep water pink shrimp (*Parapenaeus longirostris*) in modified atmosphere (40% CO_2/30% O_2/30% N_2 and 45% CO_2/5% O_2/50% N_2) was successfully studied. Generally, MAP atmospheres preserved the quality up to 9 days (Gonçalves *et al*., 2003).

The oils in industry were protected by adding TBHQ (Tri-Butylhydroquinone), or Tinuvin P® (2- (2'-

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hydroxy-5'-metylphenil) benzotriazol) according to Azeredo *et al*. (2004).

No paper is available on the stability and the storage of Nkamba nut oil. The aim of the present paper is to study the oxidation and the storage of oil in wild conditions. It well known that crude oil could contain natural antioxidants leading to reduce the level of oxidation.

MATERIALS AND METHODS

Seeds and oil extraction: Seeds harvested from Nkamba (south west of DR Congo; 19E5S) were crushed in a coffee grinder (Moulinex model SeB PREP'LINE 850). Thirty gram of ground seed were placed into a cellulose paper cone and extracted using n-Hexane (60°C) in a 250 mL soxlhet extractor for 6 h. The solvent was removed using rotary evaporator model Heidolph, laborota 4000 (Tuttlingen, Germany) at 50°C. Residual solvent was removed by drying in flushing with 99.9% nitrogen.

Lots and treatments:

Lot 1: Freshly extracted oil
Lot 2: Fresh oil heated 1 h at 100°C
Lot 3: Oil extracted in 2009 without treatment stored at room temperature
Lot 4: Oil extracted and heated 1 h at 100°C in 2009 and stored at room temperature

Color determination: Colors of nut and defatted nut were determined on a micro flash 200 d (data color International) which converts colors in numbers CIELAB (CIE, 1986) and giving to them the values ranged behind letters (L*, a*, b*).

The color of the oil was determined in triplicate, using the Lovibond method. Color was measured using the Lovibond (Lovibond PFX195, VWR International France). Each sample was taken in a cube and placed in the space provided in the tint meter. A sample of 5 mL was analyzed at 45°C (Bhattacharya *et al*., 2008) and the Gardner was automatically read on the apparatus.

Fatty acid composition: The fatty acid content was measured before and after heating at 100°C and during storage (3 years) to evaluate the damages during processes. Fatty Acids Methyl Esters (FAME) were obtained by transmethylation of total lipid aliquots (50 mg) with 1 mL of borontrifluoride in methanol (8% wt/vol) for 10 min in a shaking water bath heated at 90°C as described by Ackman (1998). The analysis of FAME was carried out in a Perichrom^{TM} 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.). Nitrogen was used as the gas carrier. For best separation, the column temperature was kept at 145°C for 20 min; then programmed at 5°C/min from 145 to 210°C and finally held at 210°C for 15 min. The injection port was maintained at 230°C and the detector at 260°C. The fatty acids were identified by analogy of their retention times with appropriate standards. Each measurement was in a triplicate.

Indices and oxidation: Standard procedures of the AOCS (American Oil Chemist Society) (1997) were used for indices. Iodine value (Iv) was measured according to AOAC standard Cd 1d-92 and Peroxide value (Pv) was measured according to AOAC standard Cd 8b-90. p-Aniside value was measured according to AOAC standard Cd 18-90.

The value of absorptivity at 232 and 270 nm (K232 and K270) was determined by spectrophotometry according to recommendations in AOCS Official Methods.

Thermal properties of Nkamba nut 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. 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 aluminum pans and cooled and/or heated at 2.5°C/min from -60 to +60°C. The heat-of-fusion enthalpies ΔH (J/g) were calculated for each peak by the Pyris software (Perkin-Elmer Corp, Norwalk, CT, USA). DSC measurements were carried out in triplicate.

Viscosity measurement: The dynamic viscosity of Nkamba nut oil was measured with a Malvern Kinexus Pro. Samples (3 mL) were paced in a CP2/50 SC0029SS plateau; with a temperature increasing from 5 to 45°C, at 1°C/min. The applied stress was constant at 50 Pa (Souhail-Besbes *et al*., 2007).

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

RESULTS AND DISCUSSION

The change of color during storage: Color is an important indication of product composition, purity and degree of deterioration (Kabri *et al*., 2011). The freshly extracted oil are yellow shiny. When heated at 100°C, oil became more and more clear. During the time of experiments, the value of L* grew up from 31.22 to 91.73 (Table 1). The oil at this point of view became white or transparent. The value of a* and b* do not vary a lot. The value of a* show that the oil went from green

Table 1: Color assessment

	L*	a*	b*	ΔE
Lot 1	31.22	0.27	11.97	15.7969174
Lot 2	61.78	0.28	12.01	16.0376869
Lot 3	73.19	2.14	17.12	16.5124832
Lot 4	91.73	-2.35	18.85	17.0029086

Table 2: Indices values

	Lot 1	Lot 2	Lot 3	Lot 4
Av	2.97±0.31	9.50±1.97*	23.73±1.01**	42.09±1.31***
Pv	2.01±0.19	15.64±1.56**	18.81±1.39**	19.77±1.56*
PA v	1.31±0.09	1.78±0.32	25.34±2.11*	27.92±2.97*
Totox v	5.33	33.06**	62.96***	87.46***

IA mg NaOH/g; IP méq O_2/kg; Totox v = 2Pv + PAv; *: p<0.05; **: p<0.001; ***: p<0.0001

Table 3: Hydroxyperoxides level in oil

	DO à λ = 232 nm	DO à λ = 270 nm
Lot 1	0.057	0.007
Lot 2	0.857**	0.063*
Lot 3	1.620***	3.945***
Lot 4	0.900***	17.780***

*: p<0.05; **: p<0.001; ***: p<0.0001

to red (0.27 to -2.35) leading to the alteration. The value of b* was the more stable from 11.97 to 18.85.

The color change during heating were carrying out by Lee *et al*. (2005) working on rose salmon. The value of a* varies from 0.0292 to 0.566 and those of hue hand (b*) varies from 1.245 to 1.299 leading to clarify oil.

Indices value: The peroxide value do not change a lot along a time of storage (Table 2). It's well known that the peroxide value describe one hyperbole as the gong of Gauss. So we can have the same number (15.64±1.56 or 18.81±1.39) with a different signification. Really, the oxidation is due to expression of the totox value. Indeed, the fresh oil level was a basic number of 5.33. When heated the amount of totox value was for 7 times. After 4 years the non-treated oil was presented a value of 62.96 leading to an amount of 12 fold. The treated oil stored at room temperature for 4 years was presented a value of 87.46 leading to an amount of 18 times. These results confirm that the measurement of oxidation must be the results of many methods to be sure absolutely.

The fish oil heated for 55 to 70°C show a level of PV from 26.78 to 41.79 meq/g. The temperature they use was under what we use, but the PV was increased for 1.5 times (Mohanarangan, 2012).

The total oxidation value is a quality parameter related to the presence of different compounds such as hydroperoxides, aldehydes, ketones, etc., which are mainly generated by PUFA degradation under pro-oxidant conditions, especially high temperatures, oxygen, metal compounds and light. The Totox value is therefore intrinsically related to the PUFA amount in the oil and to the extraction procedure. The maximum of Totox is fixed at 25; then we are high numbers in the aged oils (Rubio-Rodríguez *et al*., 2012). The Totox value obtained lead to accumulation of hydroxiperoxides in aged oils.

Evolution of hydroxyperoxides: The rate of hydroxyperoxides (lot 1) were very low from proximal products (λ = 232 nm) to distal products (λ = 270 nm). This result is under of Dandjouma *et al*. (2008) on oils of *Ricinodendron heulodotii* of the same family who obtained 2.78. When heated (lot 2) the early oxidized products in oil (0.857) were higher than the later products (Table 3). These results confirm that the oil was freshly oxidized. When stored 4 years, the ended products were more increased (3.945) than the proximal products (1.62) confirming that the oil was aged. The lot 4 confirm that the oxidation of oil was maximum (17.78) at this ended step. At this moment the proximal products could be necessary as trace state. But we were in native oils with a high protective potential as polyphenol, phytosterols as α tocopherol was present and lows slowly the effects of oxidation agents.

Vegetables and seed oils including soybean, safflower and corn, sunflower seeds, nuts, whole grains and wheat germ are the main sources of tocopherols, whereas animal products are generally poor sources of this vitamin. Absorption of vitamin E is dependent upon digestion and absorption of fat (Meydani, 1995). α-Tocopherol has the highest biological activity. It is the most effective chain-breaking lipid-soluble antioxidant. The ability of α-tocopherol to have an antioxidant, neutral, or pro-oxidant effect in foods depends on temperature, lipid composition, physical state (bulk phase or emulsion) and its concentration (Arab-Tehrany *et al*., 2012).

The level of hydroxyperoxides is so higher, but in refined oil, it would be so high. The natural content of oil (vitamin, polyphenols…) lead to limit its alteration. But after many years, the power of antioxidants became fragile.

Fatty acids evolution: When we compared the lot N°1 to the lot N°2, it appeared that there was a slight deterioration meaning by the low saturation traduced by a little amount of oleic acid from 22.08 to23.51% and of linoleic acid from 36.82 to 38.58% (Table 4). The deterioration is beginning and the natural antioxidants lows oxidization as described (Ahn *et al*., 2008).

At the end of the experiments (Lot N°3 and Lot N°4), the more dangerous was the high rate of trans fatty acids. Indeed, the level of elaïdic acid varies from 0.0064% in fresh oil to 8.1-12.4% in old oils. In the same time, the level of linolenic acid decrease strongly from 30.62 to 1.07%. In the same time working on fish oils, Mohanarangan (2012) shows the slight increase of saturated fatty acid from 6.11 to 6.28% with increased temperature (from 55 to 70°C). In the same work, the increase of oleic acid was shown for 1% and the loss of PUFA was up to 2%.

Recently we have investigated the storage of Kaso (*Tetracarpidium conophorum*) oil for 3 years. The

Table 4: Fatty acid change during experiments

Fatty acids	Lot N°1	Lot N°2	Lot N°3	Lot N°4
C14:0	0.0787	0.9056	15.77±1.54**	12.23±1.11**
C16:0	7.7006±0.41	7.0900±0.53	15.23±2.53**	16.70±1.94**
C16.1	0.0346	0.0197	10.00±1.56*	8.00±2.42*
C18:0	2.8142±0.13	3.5500±0.19	9.70±0.45*	8.80±1.23*
C18:1n9t	0.0064	0.0256	8.10±0.55**	12.40±1.73**
C18:1n9c	22.0823±0.22	23.5100±1.18*	11.30±0.11*	8.20±1.67*
C18:2n6c	36.8160±1.29	38.5800±1.44*	20.17±0.27*	22.17±0.27*
C18:3n6	0.2166	0.0179		
C18:3n3	31.6300±2.78	27.3800±1.71***	5.23±0.50***	1.07±0.89***
C18:3n4	0.4342	0.1517		
C21:0	0.4680	0.5445		
C20:2n6	0.0056	0.0430		
C22:0	0.0294			
C22:1n9c	0.0056			
C20:3n3	0.0375			
C22:2n6	0.0060			
C24	0.0075			
Others				10.43
∑AGS	11.1300	12.1200	50.70	37.73
∑AGPI	88.7000	87.9000	49.30	23.26
ω6/ω3	1.2500	1.4100	3.87	20.20

*: p<0.05; **: p<0.001; ***: p<0.0001

Table 5: Thermal behavior changes

	Lot N°1	Lot N°2	Lot N°3	Lot N°4
Peak (°C)	-27.100	-25.20	-18.40	-15.10
ΔH (J/g)	+1.094	+1.12	+29.38	+31.23

T° set at -30°C for 10 min, rising to +30°C at 5°C/min

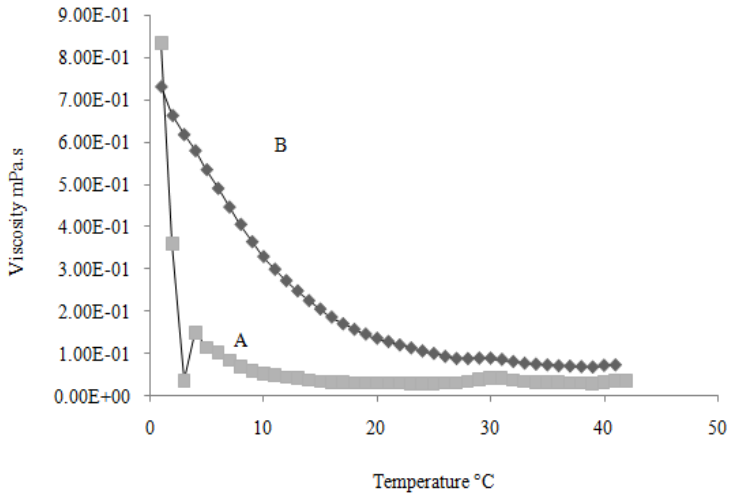

Fig. 1: Evolution of viscosity from freshly (A) extracted oil to 3 years aged oil (B)

levels of palmitic acid went from 2.5 to 12.6%; those of oleic acid were 10.7 to 27.88% and the decrease of linolenic acid was for 71 to 45.33% (Dzondo-Gadet *et al*., 2014a).

Naturally the fatty acid present as trace were the more fragile and desappeared from heating to long time storage. It appeared also that there were a little fraction of fatty acid up to 10.43% not recognized by the methods we used (Table 4). The fatty acid ratio ω6/ω3 was for 20.2 meaning that health benefits were lost and the oxidized oil became unpropre to consume.

Thermal behavior: The oil shows one melting point at -27.1°C which went at the right according to the oxidation (Table 5). It's well known that the melting points were the results of many fractions of oil as SFA, MUFA and PUFA. So when the oil is rich in double bonds, the peak is very low. But the progressive saturation of oxidized bonds lead to carry out the saturated fraction and then the number grew up neighboring zero. Indeed, it's well known that the lipid oxidation lead to decale the curve at the right (Gill *et al*., 2010).

Evolution of viscosity: The dynamic viscosity of freshly extracted oils was for 6 mPa.s at 20°C (Fig. 1A) and the oil are fluid with the increasing temperature. The aged oil (Lot 3) became slightly more viscous at 12 mPa.s (Fig. 1B) and heating do not lows totally the viscosity. Between 0 and 10°C, the aged oil (Fig. 1B) presents high resistance then the shift of viscosity is lower in oxidized oil.

CONCLUSION

The Nkamba nut oil is rich in PUFA. The heating or the long term storage (4 years) lead to strong deterioration of oil. The Totox value grew up from 5.33 in freshly extracted oils to 87.46 for aged oils. The level of hydroperoxides show a great amount of absorbance (0.857) for the primary byproducts in fresh oil heated. In the aged oils, there was a great level of ended byproducts up to 17.78. The strong oxidation (lot 4) lead to trans fatty acid accumulation as an amount of elaidic acid (0.0064 to 12.4%) and to the strong decrease of the linolenic acid rate (31.63 to 1.07%). The thermogramm shows a peak of -27.1°C which is deplace to -15.1°C by progressive saturation of double bonds. Despite of natural antioxidants content, Nkamba nut oil is hardly oxidized in 4 years and then became unusual to human consume.

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