

Research Article

Comparison of Stability between Kaso (*Tetracarpidium conophorum*) and Safou Pulp (*Dacryodes edulis*) Oils Encapsulated in Maltodextrins 6DE

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Abstract: Safou pulp and Kaso oils have a good nutritional value and olive oil's properties are well known. Totox value and the content of thiobarbituric reactive species were measured over a period of 2 months, at 50°C, to evaluate oxidation of free and maltodextrin-encapsulated oils. Freeze-dried particles were more efficient against oil oxidation than spray-dried particles. The comparison between Safou and Kaso using olive oil as reference demonstrates the best oxidative stability of this first one at 50°C. These oils stored over 3 years at 20°C showed the high amount of Peroxide value and modifications of the fatty acid fraction and composition.

Keywords: *Dacryodes edulis*, kaso, maltodextrin, oxidation, safou pulp oil, storage, *Tetracarpidium conophorum*, totox

INTRODUCTION

Safou (*Dacryodes edulis*) is a currently consumed fruit in Africa (Guinea gulf). Safou oil interest was demonstrated with nursing mother studying the growth of infants (Rocquelin *et al.*, 1998a, b). The diversification of edible applications has already been at work. Indeed safou pulp was successfully used as an ingredient in nutritious biscuits to replace partially margarine (Mbofung *et al.*, 2002).

The greatest problem for fruit preservation is its fragility. It softens at room temperature, between 28 to 80°C by the action of endogenous enzymes including cellulase, pectin esterase, polygalacturonase (Okolie and Obasi, 1992) and its shelf life is shortened. 30 to 80% of harvested fruits are lost each year by pulp softening. Oil extraction for safou at harvest time is a good way to partially reduce the fruit losses in Africa. Previous studies have shown that the common storage was not efficient in extending shelf-life (Emebiri and Nwufu, 1990).

African walnut or Kaso (*Tetracarpidium conophorum* Mull (Arg)) is an equatorial perennial climber shrub often found growing wild in moist forests. The climbers are provided in green capsules containing four shelled seeds which become greenish yellow when totally ripe (Akpuaka and Nwankwor, 2000). The ripe nuts are cooked and consumed as

snacks, along with boiled corn. Two classes of Isolectins (TCA I and TCA II) are extracted from African walnut (Animashaun *et al.*, 1994). The nut consumption presents some specificity as there is poisoning components like alkaloids and other toxic factors as oxalates, phytates, tannins... (Enujiugha, 2003). But the extracted oil presents a great nutritional interest as the fatty acid composition shows 70% of linolenic acid and over 40% of triglycerids were identified as trilinolein (Tchiegang *et al.*, 2001). Neither, the oil storage nor stability was studied.

Oxidation is a major cause of edible oil degradation, resulting in sensory changes known as oxidative rancidity, frequently responsible for oil rejection by consumers (Azeredo *et al.*, 2004).

The aim of the present study is to compare the stability of free and encapsulated Safou pulp and Kaso oils for two months at 50°C using olive oil as reference on one hand. On the other hand, a long time storage of oils at 20°C was investigated.

MATERIALS AND METHODS

Oil extraction: Fruits from Brazzaville (Congo) were crushed in one batch with a coffee grinder and the oil was extracted by the Bligh and Dyer (1959) method using methanol/chloroform (2/1, v/v). Commercial

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Extra virgin olive oil was obtained for industrial production.

Oil/maltodextrin emulsion: Oils emulsions in maltodextrin 6 DE solutions and drying were done as follows.

For each liter of emulsion, 50 mL of each oil were added to 100 g of 6DE maltodextrin solution (10% w/w in water). The mix was emulsified in a Stefan UMC 5 mixer under vacuum (Stephan GmbH, Germany). Finally, two liters of emulsion were done from each oil, 1 L was spray-dried and 1 L was freeze-dried.

Encapsulation methods:

Spray drying: The mixture was spray-dried in a Niro utility model (Niro Atomizer Lts, Columbia MD, USA). The dryer was operated at an air inlet of 170±5°C and outlet 95±5°C. The wet bulb temperature was at 43±2°C.

Freeze-drying: The mixture was frozen at -35°C and freeze-drying was operated during 2 days at 50 mtorr and -10°C on a Lyovac GT3 (Leybold Heraeus®; Orsay, France).

Fatty acid composition: The fatty acid content was measured before and after entrapment and during storage 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™ 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.

Separation and quantification of lipid classes: The Triacylglycerol (TAG), Diacyl Glycerol (DAG), Monoacylglycerol (MAG) and Free Fatty Acid (FFA) were separated by thin layer chromatography-flam ionisation detection Iatrosan TH10 apparatus model MK-IV (Iatron, Japan). Samples were dissolved in 0.3 mL hexane then applied in a band on silica gel 60 plates. The developing solvent was diethylether/hexane/formic acid (60:40:1). Separated bands were revealed with 1% 2.7-fluorescein in methanol solution and were visualized under an ultraviolet lamp (254 nm). Fractions corresponding to each lipid type were scraped

from the plates and methylated. To determine the mass-balance, lipid fractions were applied to a silica gel 60 column (Silica gel 100-200 mesh, ICN Biomedical, Eschege, Germany) (Linder *et al.*, 2005). Each measurement was in a triplicate.

Storage: After drying, powder samples of 10 g were stored in Petri dishes in darkness, at 44% HR in a saturated buffer of K₂CO₃ atmosphere 50°C. Triplicate samples were used for each measurement. Native oils were stored in 50 mL colored bottles in darkness, at 44% HR in a saturated buffer of K₂CO₃ atmosphere 20°C for 3 years.

Indices and oxidation: To assess the stability of encapsulated oils vs. time, 5 g of powder was dispersed in 100 mL hexane/sulphuric ether (1/1) and agitated for 30 min, at 500 rpm, on a G24 shaking table (New Brunswick Company Inc., New Brunswick, NJ). The supernatant was collected and vaporized. Collected oil served for indices measurements.

Standard procedures of the American Oil Chemist Society (1999) 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 (1999) standard Cd 8b-90. p-Aniside value was measured according to AOAC standard Cd 18-90.

Thiobarbituric acid reactive species measurement: A malondialdehyde (MDA, 5-10 mg) solution was freshly prepared by hydrolysing malonaldehyde bis (dimethyl acetal) (1, 1, 3, 3-tetramethoxypropan) (Sigma-Aldrich; Germany) in 0.1 M HCl (Strange *et al.*, 1997). The hydrolysis of malonaldehyde yields MDA at variable concentration, which serves for standard curves.

(2.5 mL) of Trichloroacetic Acid (TCA) 5% was added to 1 g of maltodextrin containing oil in a tube. The same quantities of TCA were used in standards, 1.5 mL aqueous Thiobarbituric Acid (TBA) (4, 6 Dihydroxypyrimidine-2-Thiol) (Sigma-Aldrich; Germany) (0.08%) was added in each tube both standards and assays. The tubes were heated in a 60°C water bath for 30 min. Absorbency was measured at 443 nm in a Spectrophotometer Ultraspec 4000 (Pharmacia, Biotech; UK). Each measurement was carried out several times.

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

Encapsulation process, fatty acid content and oil characteristics: Two encapsulation processes were used to entrap oils. No significant differences were observed between native and encapsulated oil before storage process for reference oil (olive) and safou pulp

Table 1: Comparison of fatty acid content in native olive, kaso and safou pulp oils and after spray and freeze-drying processes

	Control before entrapment		Assay after entrapment	
	Fatty acids	Entrapment	Spray-drying	Freeze-drying
Safou	C16:0	45.0±0.5	45.30±0.20	44.00±0.80
	C18:1	27.0±0.8	26.90±0.10	29.00±0.70
	C18:2	26.5±0.4	26.50±0.20	25.70±0.30
	C18:3	1.5±0.3	1.30±0.40	1.30±0.20
Olive	C16:0	10.8±0.6	10.60±0.20	10.80±0.80
	C16:1	0.9±0.3	0.90±0.10	0.90±0.20
	C18:0	2.9±0.5	2.90±0.10	2.90±0.40
	C18:1	71.3±0.5	71.20±0.20	71.30±0.30
	C18:2	6.5±0.9	5.90±0.80	6.30±0.30
Kaso	C18:3	0.7±0.4	0.60±0.00	0.70±0.20
	C14:0	2.0±0.8	2.21±0.83	1.72±0.81
	C16:0	2.5±0.4	3.05±0.60	2.10±0.10
	C18:1	10.7±0.7	12.68±0.32	10.30±0.80
	C18:2	13.0±0.5	15.78±0.65	13.84±2.83
	C18:3	71.0±0.8	65.22±4.16*	71.45±0.72
	C20:0	0.8±0.1	-	0.60±0.10

The value is the mean±S.E.M. (n = 3); *: p<0.05; **: p<0.01; ***: p<0.001

Table 2: Indices level of native oils of olive, kaso and safou pulp before and after spray-and freeze-drying process

Indices	Native oils before entrapment			Assays after entrapment					
				Spray-drying			Freeze-drying		
	Olive	Safou	Kaso	Olive	Safou	Kaso	Olive	Safou	Kaso
Peroxide value (meq/kg)	3.4±0.6	3.2±0.1	4.5±1.1	3.2±0.6	3.6±0.1	6.3±1.2**	3.4±0.3	3.3±0.10	5.1±1.2
Iodine value (mgKOH/g)	84.3±0.7	79.6±0.1	181±1.0	84.5±0.5	77.0±0.8*	173±2*	84.4±0.5	79.0±0.50	180±1.0
p-aniside value	3.1±0.1	7.7±0.4	11.0±1.6	3.5±0.6	8.9±0.7	15.1±1.5*	3.2±0.9	8.9±0.11	12.0±0.6

Spray drying helps decrease the iodine value of safou pulp oil and kaso oil; Peroxide value of kaso and safou pulp oils then increased; Olive oil was the less oxidized during entrapment process; The value is the mean±S.E.M. (n = 12); *: p<0.05; **: p<0.01; ***: p<0.001

oil (Table 1). The encapsulation process does not modify the ratio of saturated and unsaturated fatty acid C16 and C18 in the olive and safou pulp oils. However, kaso oil ratio was slightly modified. The C18:3 ratio decreased for 8.6±1.7% (p<0.01). According to the observation, the lost ratio of C18:3 was reported at first sight on C18:2 and C18:1 contents (Table 1).

In a previous study, we have shown that the best encapsulation rate was observed for the ratio 50:50 (safou oil: maltodextrin) and the encapsulation efficiency was up to 60% (Dzondo-Gadet *et al.*, 2005). The (50:50) ratio was applied for the continuation of the experiments comprising olive and kaso oils because if the amount of maltodextrin was below that of oil, the emulsion was unstable and coalescence led to a poor encapsulation rate (from 20 to 24.5% of loss for safou pulp oil).

Table 2 shows the changes of indices values after encapsulation process. Spray-drying heating seems to increase the level of fatty acids saturation. The Pv and Av of kaso oil increases respectively by 29.0±2.1% (p<0.001) and 36.0±3.3% (p<0.05), while its Iv slightly decreases by 8.0±1.1% (p<0.05). Those of safou were slightly modified and those of olive oil were unmodified. Nevertheless, freezing do not induce changes in any oil. It seems that the oxidation level was correlated with the C18:3 content. As described by Tchegang *et al.* (2001), the oils containing low level of C18:3 (olive and safou) were slightly oxidized and kaso

oil was strongly oxidized as it contains ~70% of C18:3. As previously shown, the freeze drying process seems to be better than spray drying to prevent oil oxidation.

Indices changes during storage: Totox value was used as an indication of overall oxidative stability and was correlated with the extent of oil deterioration (Poiana, 2012). For native products, the total oxidation (2Pv+1Av) was of 9.9 for olive, 14.1 for Safou and 20 for Kaso. Generally the Totox value was below 10 for the best storage. But for some like fish oils, Totox value could be higher than 26 (IFFO, 1981). During the first month, the oxidation was regular and then went up for kaso and safou or reached a plateau for olive. After 9 weeks at 50°C, the Totox value increased twice in olive and 4 times in safou pulp oil and 7 fold for kaso (Fig. 1). The Totox value seems to be close to Pv as the Av of olive was decreased in time. For Kaso and safou pulp oils, Av and Pv increases were linked. It's well known that Totox value was affected by light. It was shown that the level of Av in Camelia oil during 350 days was negligible when oil was stored at 8°C in a dark place. The involment of light lead to a 3 times increase in Pv value during a month of storage. Then combined effect of light and temperature helped increase the Totox value significantly up to 10 fold (Abramovic and Abram, 2005). When sunflower oil is heated, Totox value grow up to 100 during 2 h (Poiana, 2012).

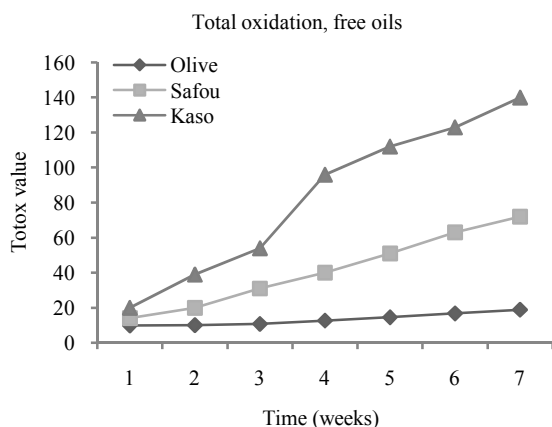


Fig. 1: Totox value (2Pv + 1 Av) of native oils of olive, kaso and safou pulp oil vs. time stored at 50°C. The results are the mean±S.E.M. (n = 12)

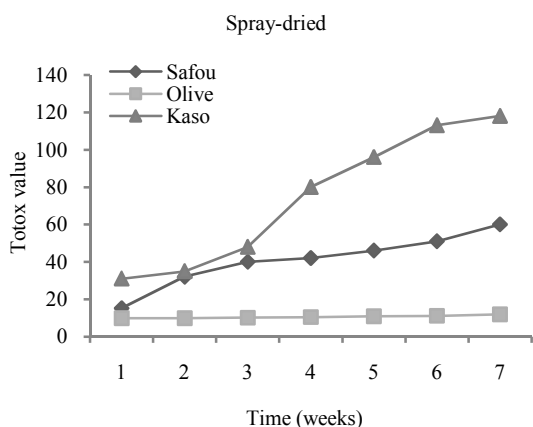


Fig. 2: Totox value (2Pv + 1 Av) of spray-dried oils of olive, kaso and safou pulp oil vs. time stored at 50°C. The results are the mean±S.E.M. (n = 12)

As is commonly known, Pv reveals primary byproducts and Av reveals secondary byproducts. It seems that the secondary byproducts were linked with the bond number of the fatty acids. The more unsaturated oils there are, the more oxidation we get in our experimentations. By comparison, oils stability could be presented in this order Olive<Safou<Kaso.

These changes would have a strong effect on the nutritional quality of the oil and would modify significantly the nutritional and sensory value of Safou pulp and Kaso oils. Lipid oxidation negatively affects the flavor, odor, color and nutritional value of foods during storage and may also limitate the utilization of oil in processed and fortified foods as well as nutritional supplements. The unpleasant flavor of oxidized oils has been attributed to oxidation products of unsaturated fatty acids (Ramadan and Mörsel, 2004). It is then essential to protect safou oil stored at 50°C against oxidation. This is because the storage at high temperature is not unusual in African countries where safou or kaso oils are produced.

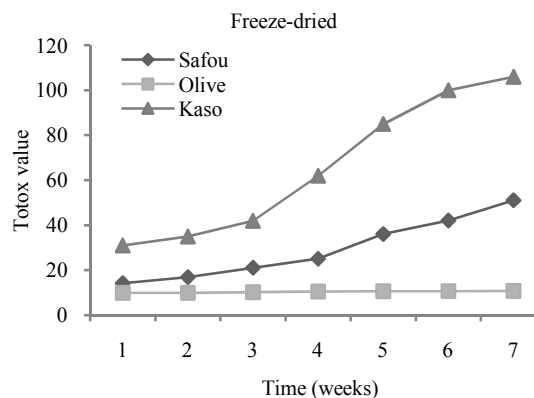


Fig. 3: Totox value (2Pv + 1 Av) of freeze-dried oils of olive, kaso and safou pulp oil vs. time stored at 50°C. The results are the mean±S.E.M. (n = 12)

When oils were encapsulated and stored at 50°C in the darkness, at RH 44%, the changes were like those described above in native oils. But the number was lower in entrapped oils. Indeed, kaso Totox value was 14.2% (p<0.05) (Fig. 2) lower in spray-dried and 21.4% (p<0.001) (Fig. 3) lower in freeze-dried particles. For safou, the decrease was of 15% (p<0.01) in spray-dried (Fig. 2) and 35% (p<0.01) for freeze-dried particles (Fig. 3). The values of olive oil were lower, the same at the end of experiments (Fig. 2 and 3). So olive oil was totally preserved by maltodextrins during our experiments. Freeze-dried particles protect oil from oxidation clearly better than spray-dried particles. This can be explained by the heating of the spray-dried solution, during which fat globules were destabilized and coalesced to produce bigger globules easily oxidable, due to the decrease of structural tortuosity in the atomized particle. As oxygen is hydrophobic, bigger globules reduce the presence of hydrophilic maltodextrin wall and favor oxidation (Desobry *et al.*, 1997). However, the protection of kaso oil even by freeze-drying was limited in time and by the combination of temperature and oxygen.

TBARS index: It's widely known that, degradation of fatty acids lead to byproducts (e.g., Dialdehydes, Thiobarbituric acid reactive species). These byproducts form complexes together or with other chemical species or continue their degradation to volatile compounds and even CO₂. The evolution of TBARS was close to the production and the disappearing of the byproducts in oil.

In previous works, at 50°C, the TBARS value of native safou pulp oil shows an evolution with a maximum after 5 weeks then reached a plateau indicating a steady state where oxidation rate was equal to the degradation rate of the byproducts, not the end of oxidation (Dzondo-Gadet *et al.*, 2005). In the present

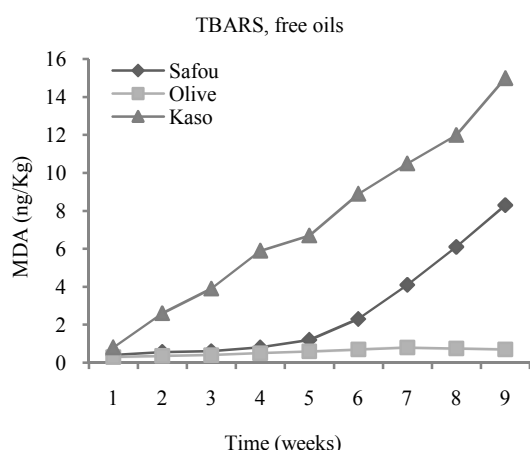


Fig. 4: The TBARS in natives oils stored at 50°C. MDA, malone dialdehyde. The results are the mean±S.E.M. (n = 8)

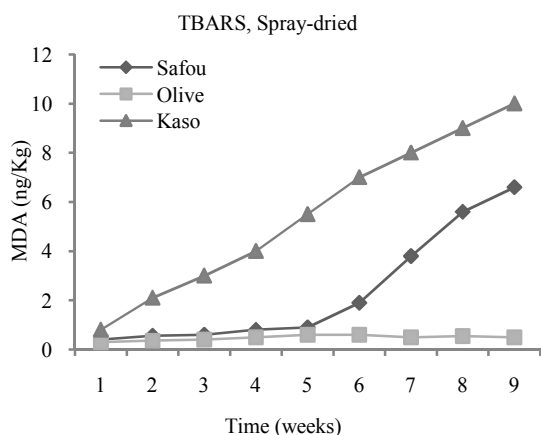


Fig. 5: The TBARS of spray-dried oils stored at 50°C during 2 months. MDA, malone dialdehyde. The results are the mean±S.E.M. (n = 8)

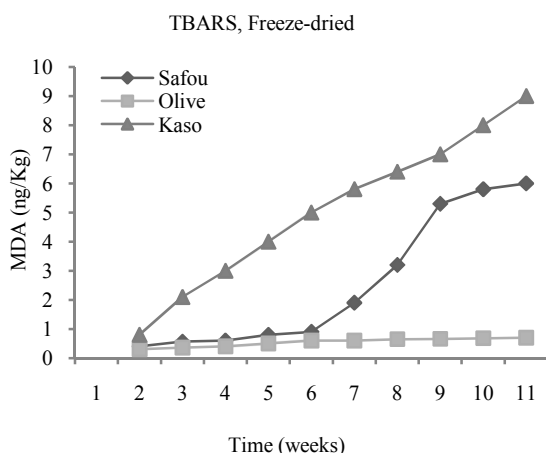


Fig. 6: The TBARS of freeze-dried oils stored at 50°C during 2 months. MDA, malone dialdehyde. The results are the mean±S.E.M. (n = 8)

Table 3: Very long time storage of natives oils at 20°C, 44% HR and darkness

	Fatty acids	Control	3 years after
Safou	C16:0	45.0±0.5	48.53±2.51
	C18:1	27.0±0.8	30.88±1.35
	C18:2	26.5±0.4	19.78±2.12
	C18:3	1.5±0.3	0.71±0.17
Olive	C16:0	10.8±0.6	19.57±1.15
	C16:1	0.9±0.3	0.47±0.09
	C18:0	2.9±0.5	2.45±0.21
	C18:1	71.3±0.5	71.86±7.40
Kaso	C18:2	6.5±0.9	4.95±0.51
	C18:3	0.7±0.4	0.60±0.13
	C14:0	2.0±0.8	5.31±0.80
	C16:0	2.5±0.4	12.60±0.60
	C18:1	10.7±0.8	27.88±0.80
	C18:2	13.0±0.8	8.78±0.80
	C18:3	71.0±0.8	45.33±4.60*
	C20:0	0.8±0.8	-

The results are the mean±S.E.M. (n = 3); *: p<0.05; **: p<0.01; ***: p<0.001

work (different lot of fruits) the TBARS shift after the 5th week disappears and the curve begins to grow up in agreement with our first hypothesis. The difference could be due also to the antioxidant content of each experimental oil. The maximum was of 7 weeks for olive oil with a lesser number. For kaso, the level was regular up to 15 ng/kg at the end of experiments (Fig. 4).

Entrapment leads to a limitation of oil oxidation. Olive and safou pulp oils present in spray and freeze-dried have the number varying from 25 to 50% (p<0.05) lower than native oils. The oxidation went step by step during the 2 months. For example, during the first month (freeze-dried), safou (MDA) <1 ng/kg, arounding the 6th week (MDA) <3 ng/kg, Then the amount became regular (Fig. 5) for spray-dried or reached a plateau for freeze-dried (Fig. 6). Kaso oil presents a TBARS level of 33% (p<0.001) lower in spray dried (Fig. 5) and 40% (p<0.001) lower in freeze-dried particles (Fig. 6). One more time, we have effective protection by encapsulation process at 50°C.

Oil behavior after very long storage:

Fatty acid composition: After 3 years in a dark place at 44% HR; 20°C, fatty acid composition was really changed following the C18:3 content. The least modified was olive which doubled its C16:0 ratio (from 10.8 to 19.57%) lost 2% of C18:2 and kept intact its C18:1 content. Safou pul oils won 3% of C16:0, lost 7% of C18:2 and lost also the half of C18:3 (Table 3). The most modified was kaso which lost 25% (p<0.05) of C18:3 (from 71 to 65.22%), doubled its C14:0 (from 2 to 5.3%) and C18:1 content (from 10.7 to 17.88%) and won 3 times its C16:0 content (from 2.5 to 12.6%) and for 7% the C18:1 content. The storage led to oil saturation. Our findings are in agreement with literature as the fatty acids ratio changes during storage were largely described for many kind of oil (Molteberg *et al.*, 1996).

Table 4: Iatrosacan analysis of fatty acids fractions after 3 years of storage

	Control				Assays			
	TAG	DAG	MAG	FFA	TAG	DAG	MAG	FFA
Olive	99.7±1.6		0.30±0.10		99.3±2.0	0.32±0.1	0.268±0.0	0.11±0.0
Safou	98.6±0.9	0.71±0.16	0.70±0.13	0.03±0.0	93.0±1.7**	3.41±0.9	2.380±0.8	1.20±0.4
Kaso	99.5±1.2	0.21±0.02	0.19±0.01	0.10±0.0	81.2±1.6**	3.60±0.7	5.100±0.9	10.10±1.5**

The results are the mean±S.E.M. (n = 3); *: p<0.05; **: p<0.01; ***: p<0.001

Table 5: Evolution of oil indices stored at 20°C HR 44%, in the darkness, measurements were done after 3 years

Indices	Assays after 3 years		
	Olive	Safou	Kaso
Peroxide value (meq/kg)	13.2±0.6	26.3±0.1	182.1±1.2
Iodine value (mg KOH/g)	84.5±0.5	77.0±0.8	165±3

The results are the mean±S.E.M. (n = 6)

Change in fatty acid fractions: The Iatrosacan analysis of fatty acid fraction demonstrates a strong loss of TAG fraction, in kaso. With 81.2% (p<0.01) of TAG and 10% (p<0.01) of FFA, kaso oil was unable to be used for edible applications. The oil storage led to the level of FFA (22). Safou pulp oil was also strongly affected. With 93% (p<0.05) of TAG and 1.2% (p<0.05) of FFA, safou pulp oil became very limited in edible applications. Olive oil that kept 99.3% (p<0.01) of FFA with 0.1% (p<0.01) of FFA could found edible applications yet in this view (Table 4).

Change in indices values: Peroxide value of oils was very affected. The amount of olive oil Pv was 4 times (p<0.001) and safou pulp oil Pv increase was by 9 fold (p<0.001) and kaso oil Pv increased by 36 fold (p<0.001). The iodine value (Iv) of olive oil was unmodified. The safou pulp Iv decreased by 2% (p<0.05) and for 16% (p<0.05) in kaso oil. These changes seems to limit these oils in edible applications (Table 5).

Long time storage demonstrates the strong oxidability of oils without antioxidants added. However the stability could be modified by additives. Indeed to prevent commercial oil oxidization, anti free radicals supplements were generally used as primary antioxidants TBHQ (Tert-Butylhydroquinone), or a single oxygen quencher β carotene, or a metal chelator citric acid, or an UV absorber (2-2'-hydroxy-5'-metilfenil) benzotriazol or Tinuvin P® (Nissiotis and Tasioula-Magari, 2002).

CONCLUSION

Olive and safou pulp oils, could be satisfactorily encapsulated in maltodextrins 6DE and the oxidative stability was possible at low temperatures (<50°C) for a month. Kaso oil has a very bad oxidative stability at 50°C. Preservation of the nutritional value of kaso and safou pulp oils could be ensured by freeze-drying or spray-drying encapsulation in a maltodextrin system.

Moreover, exportation of encapsulated safou oil could be an economic income for African countries due to the oil nutritional interest for equilibrated diets in European or North American countries. It could be used as food complement or additive for the growth of infants.

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