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Study of the Maturation of *Dacryodes Edulis* Oil Placed in Two Different Storage Conditions

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Abstract The maturation of oil extracted from the safou oil *Dacryodes Edulis* preserved at low temperature (6°C) and at 30°C is monitored during a 11 month storage, comparing physical chemistry. Thus, analysis of saponification, the information provided by the peroxide and behavior of acid value and ester indices have yielded an overall behavior of the oil in the safou storage thereof being correlated to the variation of the levels of AG.This study reveals that as the safou fruit, the oil contained in the pulp degraded itself quickly during the maturation, especially in storage conditions at moderate temperature.

Keywords: *maturation, Dacryodes edulis, moderate temperature*

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1. Introduction

Safou oil is extracted from the dry pulp/seeds of the fruit named "safoutier". The "safoutier" is a dioecous tree belonging to the *Burseracées* family [1].

The pulp is the only part eatable and contains 59% of water. Hundred grams of dry matter contains: 32-44 g of oil using a press for the extraction (more than 60% for extraction using solvents), 14-26 g of proteins, 32-38 g of carbon hydindex, fibres and others materials and 4-10 g of ashes. The oil content in the pulp is high. The oleic acid (45-60%), the palmitic acid (30-35%), the linoleic acid (15-20%) and the stearic acid (2%) formed around 95% of the content of the pulp. Without movement, the oil separates in two phases: an inferior one semi solid and a liquid layer at the top.For both layers, the fatty acid composition is the similar. [2].

The non-saponificable part is around 2% and mainly contains sterols (20%, mainly sitosterol), triterpenic alcohols (34%) and low quantity of tocopherols [3].

Contrarily to others oleaginous plant, the oil extracted from the seed (content for 100g of dry matter: 10-15g) is the same as the oil extracted from the pulp [4].

The safou fruit is very difficult to conserve and huge amounts of it are wasted after harvests [5].Several authors think that we can solve this by drying the pulp to use it daily or by extracting the oil [1].The drying step consists in evaporating excess of water of a product or food [6].

Along this study, we monitored the maturation of two different oil of safou (different origins) under two different conditions. Storage conditions are different (30°C in a cupboard in Brazzaville, Congo and 6°C in a cold room in Clermont-Ferrand, France).

To do this, we coupled some physico-chemical characteristics to the behavior of fatty acid content, and we found that the oil from the pulp safou is readily biodegradable and conservation does not exceed 8 months under Brazza but can be extended a little beyond 12 months at moderate temperature.

2. Material and Methods

2.1. Fruits

One the one hand, fruits were bought on a market at Dolisie (Niari department) and on the other hand on a market ("total market") at Brazzaville. Fruits were stoned, dried using the sun and crushed thanks to an electric grinder (rotor calibrated at 2500rpm). The powder obtained was inserted in a specific shell and introduced in a soxlhet apparatus.

2.2. Oil Extraction Process

2.2.1. Solvent

The solvent used is the hexan and the extraction last 3 hours in a soxhlet apparatus.

2.2.2. Oil

The light green oil extracted was semi-solid at room temperature. Samples named "young", *A09* and *A10*, came from the Niari department. These samples were extracted 3 month before the study and stored in non transparent flasks and in a dark environment at 30°C.

Samples named "adults" *A04* and *A05*, are mine-run bought on the market place "total market" in Brazzaville and their origin is not defined, maybe from the Pool. The extraction took place 10 months before the study and was stored in the conditions as *A09* and *A10*.

Finally, sampled named "old", *A02* and *A03*, «mine-run" bought on the total market were extracted 28 months before the starting of analysis. These samples, stored at 30°C in a dark environment [7], are used as reference for the maturation study.

However, two categories of pulp were considered for the oil extraction:

- Dry pulp with "endocarp" which gave samples *A02*, *A04* and *A1*,
- Dry pulp without "endocarp" which gave samples *A03*, *A05* and *A09*. For these last ones, drying kinetic was faster.

2.3. Fatty Acid Constitution

Esters from fatty acid were obtained after a direct methylation: 2 drops of oil mixed with 1mL of hexan and 0,4mL of NaOH 1N (in methanol) and heated for 1 minute. Then, 0,4mL of chlorhydric acid 1N in methanol is added followed by 1mL of hexan [8].

The chemical constitution is determined by Gas Chromatography coupled with a FID detector on an AGILENT 5890 equipment working with a Supelco FAMES column (length 100m, intern diameter 0,25 mm, thickness 0,25 µm). This equipment use hydrogen (H_2) to push the mixture through the column at 0,7 $mL.min^{-1}$. It has a heater where temperature increase until 140°C and then the temperature increases again for 5 minutes with a rate of $4°C.min^{-1}$ until reaching the temperature of 240°C. The injector temperature is 280°C with a 1/30 split (for a 1µL injection) and the detector temperature is 300°C where flow index are 40 $mL.min^{-1}$ for the hydrogen (H_2), 450 $mL.min^{-1}$ for the air and a "Make up" of 45 $mL.min^{-1}$ for the nitrogen (N_2).

2.4. Physical and Chemical Analysis

Major physical and chemical index were determined to refer to international and French standards [9]. The acid rate was done I_A (NF EN ISO 660), the saponification rate I_S (NF EN ISO 3657) and the peroxide rate I_P (NF T 60-220).

2.5. Maturation

Oil samples were split in two parts at the end of the analysis and stored 11 months in different conditions to be able to prove the degree of degradation.

Samples stored in a cold room and in a dark environment are named «CF» in reference to their storage place (Clermont-Ferrand). Samples stored at Brazzaville in a cupboard, in a dark environment too and at 30°C are named "BZV".

2.6. Part Unable for the Saponification

The part of the oil that cannot be treated by saponification is constituted by tocopherols (vitamin E): antioxidant agent [10]. It is determined using the NF T 60 - 205 - 1 norm.

3. Results and Discussion

3.1. Physical and Chemical Index Analysis

The different index [9] were measured for each sample, before storage and after an 11 months storage in two different conditions.

Table 1 sum up all physical and chemical indexes for the safou oil at the beginning of the study. We notice that oldest samples (28 months) and the eldest ones (3 months) have the lowest acid index compared to the samples "adults" (10 months). It seems that fatty acid liberated in the fresh oil disappear along the storage. This behaviour is the proof that many events occurs during the safou oil maturation [11].

However, the peroxide rate which is an important value to characterize the fatty acid state seems to be modified (analyzing samples "young" and "adults"). Samples named "old" have a high peroxide rate. This behaviour displays that the safou oil is not in favour of the formation of peroxides during the storage [12].

Moreover, the safou oil density is low for the samples "adults" and the samples "young" and "old" have higher densities, with the highest for the sample "old". Saponification and refraction index are not very modified. During the maturation (storage) we shall have behaviour close to these samples:

- An increase for the content of C16:0 and a decrease for the content of C18:0.
- A decrease for the content of C18:1 and an increase for the content of C18:2.

3.1.1. Physical Index Study

Over-all, we notice that the density of the safou oil stored at Brazzaville is higher than the density of the homologue one at low temperature. This behaviour is not dependent of the sample age and confirmed by the samples "old" that have the highest densities. Thus, during the storage, the density increases and in particular along the storage at 30°C.

The samples "adults" had the lowest densities values at the beginning (Table 1) increase too independently along the storage. Indeed, the sample *A05* (initial value *0,8953*) has a value of *0,9217* eleven months later at 30°C (and *0,9142* at 6°C). This is the same phenomenon for the sample *A04* (*0,8982*) which display the value *0,9124* at 30°C and *0,9097* at 6°C.

Physical index have the same profile: refraction and density increase. These results let us think that carbon chain are cut or lengthen responsible to the increase of density and have an effect on the reflective power [13].

Table 1. Sum Up Of Physical And Chemical Index Determined At The Beginning Of The Study Considering Storage Conditions. Calculations Were Done 3 Times

Sample	A 11	A 09	A 05	A 04	A03	A02
Time	3 months	3 months	10 months	10 months	28 months	28 months
I_A	2,72±0,15	4,59±0,26	23,89±0,15	39,44±0,15	9,81±0,15	8,84±0,15
I_P	7,32±0,04	5,67±0,15	4,39±0,15	7,07±0,10	26,67±0,67	26,0±0,47
I_S	211,86±3	202,05±6	196,99±4	205,20±5	210,52±4	218,34±3
I_E	209,14±1,99	197,47±1,85	135,37±2,79	173,10±1,81	200,92±1,32	209,50±1,29
I_R	1,4691±0,0003	1,4649±0,0002	1,4629±0,0005	1,4595±0,0004	1,4683±0,0002	1,4695±0,0002
Density	0,9107±0,0002	0,9119±0,0003	0,8982±0,0002	0,8953±0,0004	0,9156±0,0002	0,9278±0,0003
% AG						
C16 :0	49,70	59,15	46,14	44,86	40,96	40,81
C18 :0	2,93	3,35	3,52	3,76	10,97	11,55
C18 :1	26,39	28,05	33,65	27,84	18,81	19,15
C18 :2	18,54	6,64	14,29	21,30	26,77	26,00
C18 :3	0,74	0,45	0,45	0,84	0,81	0,79
Total	98,73	97,64	98,05	98,60	98,32	98,30

Table 2. Physical Indexes For Safou Oil Samples11Months Later. (CF = Sample Stored aA 6°C; BZV = Sample Stored At 30°C)

Sample	A11 CF	A11 BZV	A09 CF	A 09 BZV	A05 CF	A05BZV	A04 CF	*A04 BZV*	*A03BZV*	*A02BZV*
Time	14 months	14 months	14 months	14 months	21 months	21 months	21 months	21 months	39 months	39 months
I_R	1,4723	1,4622	1,4723	1,4712	1,4651	1,4723	1,4785	1,4671	1,4743	1,4633
Density	0,9147	0,9170	0,9152	0,9236	0,9142	0,9217	0,9097	0,9124	0,9381	0,9227

Table 3. Repartition Of The Saponification Rate Samples11 Months Later. (CF = Sample Stored At 6°C; BZV = Sample Stored At 30°C)

Sample	*A11 CF*	*A11 BZV*	*A09 CF*	*A 09BZV*	*A05 CF*	*A05BZV*	*A04 CF*	*A04 BZV*	*A03BZV*	*A02BZV*
Time	14 months	14 months	14 months	14 months	21 months	21 months	21 months	21 months	39 months	39 months
I_S	179,64	209,79	190,33	207,87	178,39	195,76	178,35	192,37	203,57	213,01
I_E	176,13	204,91	183,89	200,74	140,55	169,01	154,48	152,49	191,59	201,49
I_A	3,51	4,88	6,64	7,12	23,91	26,75	37,80	39,75	11,98	11,52
I_P	141,92	89,15	176,98	111,06	70,73	36,08	53,01	26,99	48,20	88,06

We know that the variation of a physical value is a criteria linked to a fatty acid degradation. It is displayed that this degradation is more important at Brazzaville at 30°C (Table 2).

3.1.2. Chemical Index Study

- **Saponification rate**

The behaviour we expect to have is reached for this rate, higher values obtained at 30°C (Table 3). We notice that globally the saponification rate decreased (Table 4). This result confirm conclusion concerning physical index analysis.

Table 4. Saponification Rate Behaviour Under Brazzaville Conditions Samples11 Months Later. (CF = Sample Stored At 6°C; BZV = Sample Stored At 30°C)

sample	Is before	Is after	rate
A 11 BZV	211,86	209,79	0,99
A 11 CF	-	179,64	0,85
A 09 BZV	202,05	207,87	1,03
A 09 CF	-	190,33	0,94
A 04 BZV	205,20	192,37	0,94
A 04 CF	-	178,35	0,87
A 05 BZV	196,99	195,76	0,99
A 05 CF	-	178,39	0,91

Indeed, the saponification rate is linked to the fatty acid weight. The density increased. The higher the saponification rate, the lower the mean molecular weight, conveyed by shorter carbon chain [14].

The density study at low temperature displays that there is no increase, which means that the saponification rate must decrease. This fact is efficiently observed (Table 4).

These results allow us to assert that carbon chain lengthen during the maturation of the safou oil, independently of storage conditions. However, results obtained from the samples A09 and A05 could be explained by the fact that the oil is extracted from a pulp without “endocarp”.

Both samples “old” *A02 (0,96)* and *A03 (0,97)* show that the maturation of the oil implies a decline in the saponification rate and so lengthen carbon chain of fatty acid.

The fact that homologue CF displays low saponification rate (Table 4) after 11 months storage could explain that the molecular transformation does not occurs similarly in both storage conditions.

- **Esters index**

As for the saponification index, we can estimate the oil oxidation using the ester index. The samples stored at Brazzaville displays a higher oxidation (Table 3) and values from the samples “adults” are the lowest. This is the proof that fresh oil degrades quickly which is observed by an increase in the oxidation index with a maximum reached before decreasing between 12 and 24 months storage and independently of storage conditions. So, the oxidation is not a continuous phenomenon and we can see that index for samples (old” are higher than index for samples “adults”.

- **Acid index**

The free acid quantity increases in the oil (Table 3). However this liberation is stronger at 30°C and slackens

after 12 months at low temperature (6°C). The acid index did not increase after 10 months but the saponification index decreased. This is the proof that fatty acids formed the first 10 months are used to lengthen carbon chain or other chemical reaction (more at 30°C rather than 6°C).

Studying samples "old", *A02 (1,30)* and *A03 (1,25)* we can say that after two years storage, the acidity lightly increase and so a new AG release in the oil. It acts as if the TAG hydrolysis start again or some TAG of the oil hydrolyzes only after 2 years. It seems that each TAG has a reactional specificity for the hydrolysis for the release of fatty acid.

- **Peroxide index**

Globally the peroxide index is higher at low temperature (Table 3) meaning that peroxides formation is favoured at low temperature. Indeed, peroxides are unstable compounds and their stability is linked to the temperature. At 30°C, the temperature is sufficient to turn peroxides into primary and secondary compounds [15].

These results show that storage at low temperature accelerates the oil degradation. Indeed, the peroxide index is an indicator to characterize a fatty acid degradation [10]. The higher this index, the higher the degradation of the fatty acid. However, this could be a false interpretation since the more deteriorated fatty acid could contain the fewer quantity of peroxide.

The samples «young» have high index. The appearance of peroxides depends on the age of the safou oil (Table 4). Consequently, the safou oil is deteriorated quickly. The analysis of homologue CF samples displays a stronger tendency (Table 5). Independently to the storage conditions, the safou oil deteriorates and the peroxides accumulation has a fast kinetic. A low temperature is in favour of the peroxide accumulation. The analysis of samples "adults" displays a low quantity of peroxides. This behaviour could be explained by a slower accumulation of peroxides after 10 months due to fewer sites able to create peroxides (refer to ester index).

Table 5. Rate Peroxide Rate After Storage At 6°C/Peroxide Rate Before Storage At 30°C

sample	Ip before	Ip after	Rate
A 11 BZV	7,32	89,15	12,18
A 11 CF	-	141,92	19,39
A 09 BZV	5,67	111,06	19,59
A 09 CF	-	176,98	31,22
A 04 BZV	7,07	36,08	5,10
A 04 CF	-	70,73	10,00
A 05 BZV	4,39	26,999	6,14
A 05 CF	-	53,01	12,07
A 02 BZV	26,00	88,06	3,39
A 03 BZV	26,67	48,20	1,81

3.2. Non Saponificable Part

[3-16] show that the non saponificable part of the safou oil i slow, around 1,2%. Results in Table 6 are close to this value and we can think that along the maturation, reaction occurred with the tendency to increase the non saponificable part of the oil. Compounds in this part of the oil are very important for the storage and especially antioxidant which protect the oil from different attacks [17].

The non saponificable part increases only if saponificable compounds are turned into non saponificable compounds due to chemical reaction or structure changes.

Table 6 show that the homologue samples CF have high values and especially for the samples "young" *A11 (1,82%)* and *A09 (1,43%)*. This is in accordance with the peroxides index. The samples "young" display the higher values (for peroxides index) and it assumed that the peroxides formation in the oil is responsible for the non saponificable part of the oil. This is confirmed by the behaviour of the samples "old" which have a low peroxide index. We have a link between the non saponificable part of the oil and the peroxide index.

Table 6. Repartition Of The Content Of Non-saponifiable For Samples In The Two Different Conditions Samples11 Months Later. (CF = Sample Stored At 6°C; BZV = Sample Stored At 30°C)

Sample	*A11 CF*	*A11 BZV*	*A09 CF*	*A 09BZV*	*A05 CF*	*A05BZV*	*A04 CF*	*A04 BZV*	*A03BZV*	*A02BZV*
Time	14 months	14 months	14 months	14 months	21 months	21 months	21 months	21 months	39 months	39 months
Non-sapo	1,82	1,61	1,43	1,29	1,34	1,81	1,31	1,83	1,29	1,39

Table 7. Sum Up Of AG Index Along The Storage In The Working Conditions Samples11 Months Later. (CF = Sample Stored At 6°C; BZV = Sample Stored At 30°C)

Sample	*A11 CF*	*A11 BZV*	*A09 CF*	*A 09BZV*	*A05 CF*	*A05BZV*	*A04 CF*	*A04 BZV*	*A03BZV*	*A02BZV*
Time	14 months	14 months	14 months	14 months	21 months	21 months	21 months	21 months	39 months	39 months
%AG										
C14:0	0,08	0,13	0,08	0,08	0,02	0,03	0,09	0,1	0,07	0,12
C16:0	46,22	42,7	44,62	44,07	45,92	46,93	45,44	45,90	42,94	45,9
C16 :1	0,17	0,17	0,16	0,17	0,22	0,23	0,24	0,24	0,29	0,26
C17:0	0,17	0,14	0,17	0,17	0,17	0,18	0,14	0,14	0,15	0,17
C18:0	3,04	2,18	3,13	3,04	2,43	2,36	1,88	1,84	3,72	2,13
C18 :1	34,59	30,08	35,3	35,46	28,11	23,46	30,09	29,71	30,25	31,84
C18 :2	14,08	22,08	14,93	15,39	20,81	20,52	19,84	19,81	20,66	17,24
C18 :3	0,56	0,51	0,54	0,50	1,11	1,01	0,75	0,65	0,48	0,38
C20:0	0,18	0,64	0,21	0,17	0,18	0,17	0,12	0,11	0,16	0,13
Total	99,09	98,63	99,14	99,05	98,97	94,89	98,58	98,50	98,72	98,17

The study of the samples “adults” show that at 6°C, the non saponificable part are lower than others in Brazzaville conditions. This confirms former results concerning the peroxide index. Old safou oil produce less and less peroxides because of a lack of sites able to produce them. However, the non saponificable part is more important for homologue samples stored at 30°C and this show that compounds (different of peroxides and non saponificable too) are cumulated in the oil along the maturation. To conclude, it is possible to say that during the maturation, peroxides formed at the beginning are turned into non saponificable compounds.

- **Fatty acid analysis**

The analysis in Table 7 confirms that even if the oil has a high part of non-saturated fatty acid, it has a high content of C16:0. Indeed, storage conditions do not affect the dominance of this fatty acid in the safou oil whatever sample age [18].

Globally, the storage of the safou oil does not affect the composition (fatty acid), results in accordance with [12]. However, the part of the C18:3 increased and especially for the samples “adults” after 11 months storage. This is in discordance with results expected. Indeed, the C18:3 part is a fatty acid non saturated in three parts and its stability is generally low and this value decrease for the samples “old”. It seems that along the storage, the fatty acid part increase for 24 months and decreases faster whatever storage conditions.

4. Conclusion

Fresh oil extracted from the safou pulp suffers a fast degradation, intensified along the maturation. This forms peroxides, high amount of fatty acid are liberated in the oil and carbon chain are lengthen. After 10 months storage, carbon chain cut occurs in the oil which confirm that saponification index of the samples “old” are higher than others.

To conclude, we can underline that the safou oil is fragile and degrades fatty acid dates quickly whatever storage conditions. The storage time cannot be more than 8 months at 30°C and 12 months at low temperature. Consequently, this oil must be stored in particular conditions due to its low content of antioxidants [1]. For a better storage, it is possible to insert additional compounds such as antioxidants after the 4th month of maturation at 30°C and after the 8th month at low temperature.

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