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Irvingia Fat Ageing: Study of Chemical Characteristics Related to MIR Spectroscopy

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Abstract

The *Irvingia gabonensis* kernels, which have been extensively studied for their numerous virtues, including the ability to act against the accumulation of fats in the body [1], contain an oil. The aging of this oil under two different conditions of conservation was the subject of our work. One of the results was an increase in the content of long-chain carbonaceous fatty acids during aging for 11 months of storage at low temperature (6˚C) and at 30˚C. This behavior does not find a concordant explanation by the comparative analysis of the chemical indices. Hence, there is the need to use the Medium Infra-Red spectroscopy (MIR) which allowed to clarify the information of the saponification index, to justify the weakness of the formation of peroxides in the case of the conservation at 30˚C and to confirm the information given by the peroxide index. It also allowed to understand the formation of the long carbon chains by the "cis-trans" isomerization and the homolytic cuts which intervene within the matrix of the fat by the analysis of the number of $-CH_2$ and $-CH_3$ groups in the two conditions of conservation. This study reveals that the rapid solidification of *Irvingia gabonensis* oil at room temperature [2] is an advantage for its preservation at room temperature but a great weakness when the fat is stored at low temperature.

Keywords

Ageing, *Irvingia gabonensis*, MIR, *Cis-Trans*, Isomerism

1. Introduction

Irvingia fat is obtained from the fruits of a tree indigenous to the humid forest zone of the Gulf of Guinea [3]. The kernels of the fruit, called "péké" in northern Congo Brazzaville (Cuvette Ouest and Sangha Departments), produce a highly valued food additive. Ground and crushed, they are used to thicken soups and stews. An edible oil is extracted from the seeds and is used in cooking; it is solid at room temperature and can replace cocoa butter for use in soap making, but above all it has the same characteristics as she a butter. Unlike most Irvingia, whose fruits have a bitter pulp, those of the *gabonensis* are juicy, sweet and are eaten raw [4]. This fat is kept by rural populations for long periods in the open air without showing signs of deterioration.

The wild mangoes cotyledons, also known as Irvingia gabonensis, are very rich in oil, over 60% [5]. This oil, solid at room temperature, is a whitish yellow food fat. The lipid content varies according to the trees, from 37.5 to 75 g/100g; the approximate composition of fatty acids is as follows: lauric acid 20% - 59%, myristic acid 33% - 70%, palmitic acid 2%, stearic acid 1% and oleic acid 1% - 11% [6].

In this work, we followed the aging of their *Irvingia gabonensis* fat by comparing the chemical characteristics correlated to MIR spectroscopy. Two conditions were selected: a follow-up at 30˚C of a follow-up at 30˚C average temperature in a cupboard to simulate rural conservation and 6˚C average temperature in a cold room.

Also, we have linked the information obtained from the chemical characteristics through the analysis of the indices and the fatty acid composition to the spectral data in order to find a link between the chemical alteration of the fat, which is very rarely observed in rural areas, and the storage conditions, and then to find ways to understand the reason for the effectiveness of open-air storage encountered in rural areas.

Against all expectations, our results tend to validate the open-air preservation found in rural areas rather than the low-temperature preservation recommended for fats, especially non-conventional oilseeds. However, during ageing, Irvingia fat shows a behavior that is in agreement with its composition in FA, nearly 10% of unsaturated fatty acids.

In the course of our work, we have shown that conservation at low temperatures, however highly recommended, is not good for Irvingia gabonninsis fat, and which we have shown is an exception case for fat. It is an innovation in the preservation of oils and fats.

2. Material and Methods

2.1. The Fruits

Two samples of *Irvingia gabonensis* fats, R01 and R03, were extracted from the "all comers" almonds from in Mbomo Sub-Prefecture (Cuvette Ouest Department). These almonds were purchased dry, and their drying over a wood fire

was not the subject of our study. They were then crushed using an electric crusher equipped with a rotor calibrated at 2500 revolutions per minute. The crushed material (powder) obtained is then placed in a cartridge and the whole introduced into an extraction device (soxhlet). The extraction is carried out 12 months earlier for the sample R01 and 3 months for the sample R03 that is to say a gap of time of activation of ageing of 9 months between the two samples.

2.2. Oil Extraction Process

2.2.1. Solvent

The extraction solvent is hexane. The extraction is done for 3 hours in a soxhlet [7].

2.2.2. Oil

The oil obtained, of light yellow color, becomes solid at room temperature [8]. It is placed in two transparent bottles not sheltered from light in order to respect the storage conditions encountered in rural areas.

2.3. Fatty Acid Composition

Fatty acid esters are obtained after direct methylation: 2 drops of oil in 1 mL of hexane in the presence of 0.4 mL of 1N sodium hydroxide (in methanol) heated for one minute. Then 0.4 mL of 1N hydrochloric acid in methanol is added followed by 1 mL of hexane [9].

The chemical composition is determined by GC/FID using an AGILENT 5890 apparatus equipped with a Supelco FAMES column of 100 m length with an internal diameter of 0.25 mm and a thickness of 0.25 μm. This apparatus, flows dihydrogen (H_2) as a carrier gas at 0.7 mL·min^{-1}. It is equipped with an oven whose temperature rises to 140˚C then the rise continues during 5 minutes at a rate of 4˚C·min^{-1} until 240˚C. The temperature of the injector is at 280˚C according to a split of 1/30 and injecting a volume of 1 μL, that of the detector is at 300˚C at a rate of 40 mL·min^{-1} for the dihydrogen (H_2) and 450 mL·min^{-1} for the air finally a Make-up of 45 mL·min^{-1} for the nitrogen (N_2).

2.4. Physico-Chemical Analyses

The main physico-chemical indices were determined with reference to international and French standards [10]. We have thus realized the acid index IA (NF EN ISO 660), the saponification index IS (NF EN ISO 3657), the peroxide index IP (NF T 60-220).

These main indices [11] have been correlated to spectroscopic methods. Medium Infra-Red (MIR) spectra were recorded with a NICOLET 760 Magna IRTF spectrophotometer.

2.5. Ageing

After analysis, the fat samples were divided into two fractions of equal quantities and stored for 11 months under different conditions in order to judge the degree of alteration.

2.6. MIR

Medium Infra-red Spectroscopy MIR: a drop of the sample is placed between two KBr pellets; then the spectrum is recorded with a resolution of 8 and 32 acquisitions. The spectra of the main fatty acids (FA) were compared with those obtained for each sample. The analysis of the MIR spectra was correlated with the saponification index on the one hand and the degree of oxidation on the other hand.

3. Results and Discussion

3.1. Analysis of the Chemical Composition

The chemical composition confirms that the fat of *Irvingia gabonensis* is a fatty substance rich in saturated fatty acids [12]. We note from **Table 1** that the storage time seems to have little influence on the FA composition of Irvingia samples.

We obtain a quasi-stability of the fatty acid composition during this storage aging study. This result is rather surprising because the conditions of conservation not sheltered from the light should favour modifications within this fat, in particular in the conditions of conservation at 30˚C. However, this behavior is in agreement with the great poverty of this fat in unsaturated fatty acids [13] which are most often fragile to light. It seems as if Irvingia fat stored at 6˚C improves in quality, and after 11 months of storage, sample R01, having reached a maturity of 23 months, shows a C18:1 content that has decreased by more than 50% under storage conditions at 30˚C. This result suggests that Irvingia fat cannot be preserved longer at 30˚C.

The conditions of conservation at 6˚C are in view of these results better adapted for the Irvingia fat, however, it occurs a phenomenon which seems quite strange, that of an increase in the contents of unsaturated fatty acids as if it

Table 1. Chemical composition in fatty acids of each sample after 11 months of storage.

Samples	R03			R01		
Conditions	Activation	6˚C	30˚C	Activation	6˚C	30˚C
Age	3 months	14 months	14 months	12 months	23 months	23 months
C10:0	1.21	1.05	1.16	1.05	0.89	1.05
C12:0	38.18	35.81	37.63	36.58	34.13	36.56
C14:0	52.32	52.36	52.04	53.62	54.18	53.23
C16:0	5.30	6.10	5.39	5.33	6.44	5.42
C18:0	0.76	0.84	0.74	0.75	0.87	0.79
C18:1	1.53	2.56	2.07	1.52	2.28	0.79
C18:2	0.41	0.68	0.48	0.47	0.83	0.63
C18:3	-	0.15	0.12	-	0.14	0.11
Total	99.71%	99.55%	99.63%	99.32%	99.76%	98.58%

occurred in the fat of elimination reactions. Indeed, the C18:1 and C18:2 are clearly increased at 6˚C, respectively by 67% and 66% for the sample R03. The same behavior is noticed for the other sample under the same conditions of conservation. We can thus suspect that there is elongation of the carbon chains of the FA [14] during the ageing of the Irvingia fat and these whatever the conditions of conservation.

3.2. Chemical Indices Analysis

The analysis of the chemical composition opens up the possibility of the elongation of the carbon chains during the ageing by storage of Irvingia fat. The need for analysis of chemical indices [15] is necessary to verify this trend. The stability of Irvingia samples is confirmed by the small difference in the peroxide value.

This observation is in agreement with the GA composition of this fat, which is very low in unsaturated fatty acids, given that peroxides are formed preferentially on double bonds.

After 11 months of conservation we note that the peroxide index shows a behavior related to the conservation conditions. Indeed, the conservation at 6˚C (low temperature) translates a decrease of the quantity of peroxides present in the initial fat, that is to say, more than 50% of decrease (**Table 2**). This observation shows that fresh Irvingia fat is better preserved at low temperature. However, the peroxide content of R01 increases whatever the storage conditions, but this increase is more accentuated at 6˚C. However, peroxides are formed preferentially on the double bonds, and these are quite low in number in the fat (10% of IFA) but also, should be little active given that the temperature is low [16].

Moreover, this increase in the peroxide content of R01 can be explained by an embrittlement during the storage of the structure which causes the solid aspect of the Irvingia fat, thus making the few double bonds available for the formation of peroxide. Being in adequate temperature conditions, the peroxides thus formed evolve very little towards the degradation compounds which explains the exponential increase noted. This interpretation suggests that the Irvingia fat loses its consistency during storage at 6˚C. This is a rather strange behavior that could explain the fact that the fat is enriched in long carbon chain fatty acids. At 30˚C, there is probably little steric stress around the double bonds, making them

Table 2. Chemical indices of each sample after 11 months of conservation.

Samples	R03			R01		
Conditions	Activation	6˚C	30˚C	Activation	6˚C	30˚C
Age	3 months	14 months	14 months	12 months	23 months	23 months
I_A	3.40 ± 0.15	2.05	1.98	9.18 ± 0.26	10.69	7.87
I_p	0.39 ± 0.01	0.17	2.86	0.43 ± 0.01	3.95	2.24
I_s	239.51 ± 2.0	244.26	253.10	223.51 ± 3.0	240.94	207.61
I_e	236.11 ± 1.85	242.21	251.12	214.33 ± 1.74	230.25	199.74

available for peroxide formation. However, these evolve very quickly towards the degradation products, which explains the lower increase observed [17].

We note that during storage at 6˚C, the indices displayed increase for both samples, which proves that there has been an effective modification of the Irvingia fat matrix.

At 6˚C, R01 shows the greatest increase, 17 units, which shows that the average length of the carbon chains of FA decreases [18]. On the other hand, we note that the same sample at 30˚C shows a loss of 16 units on its saponification index, which means that the carbon chains are lengthening [19]. We note that the saponification index does not give more precision on the real behavior of the fat matrix.

The chemical reactions that tend to release the FA from the triglycerides take place in Irvingia fat and are responsible for the increase in its acidity [20]. R03 shows an identical behavior in both storage conditions, a decrease of the acid number. This behavior suggests that after three months of storage, the maximum acidity of this sample of Irvingia fat is reached, so that by placing it at low temperature, under slow kinetic conditions, the FA already released are used in the rearrangement reactions within the fat matrix, which explains the decrease in this index. The phenomenon is even more accelerated under 30˚C conditions, resulting in a larger decrease in the acid number for this sample. Thus the decrease in acidity is correlated to the increase in the saponification index, the released FA that cause the increase in acidity undergo homolytic cleavage hence, the decrease in acidity but also the increase in the saponification index on the one hand. The released FA are integrated thanks to the isomerism "cis-trans" in the formation of long carbon chains on the other hand.

In addition, the profiles of the behavior of the sample R01 in the two conditions of conservation are opposed. Indeed, at 6˚C the acidity of the fat increases, while it decreases at 30˚C. This is evidence that low temperature conditions favor the hydrolysis reactions of AFT, thus releasing the FA [21]. This interpretation is not sufficient, as there is an inhibition of the reactions in the fat which tends to use the released FA for other combinations, hence their accumulation in the fat at 6˚C. Indeed, this result is in agreement with the analysis of the peroxide value which increases in the fat stored at low temperature.

3.3. Spectroscopic Analysis: MIR

The analysis of the FA composition revealed a rather peculiar behavior; the increase of the unsaturated fatty acids content. The various indices determined could not allow us to justify it, it is thus important to proceed to a spectral analysis. Indeed, the increase in unsaturated fatty acid content should result in an increase in the degree of unsaturation [22] within the Irvingia fat matrix. This phenomenon can be verified by analysis of the spectra obtained in the MIR. The first piece of information from the MIR is that the carbon chains are longer in the low temperature conditions. The vibrational band near 727 cm^{-1} (**Figure 1(b)**) is in favor of R01 and the intensity of this band is inversely proportional to

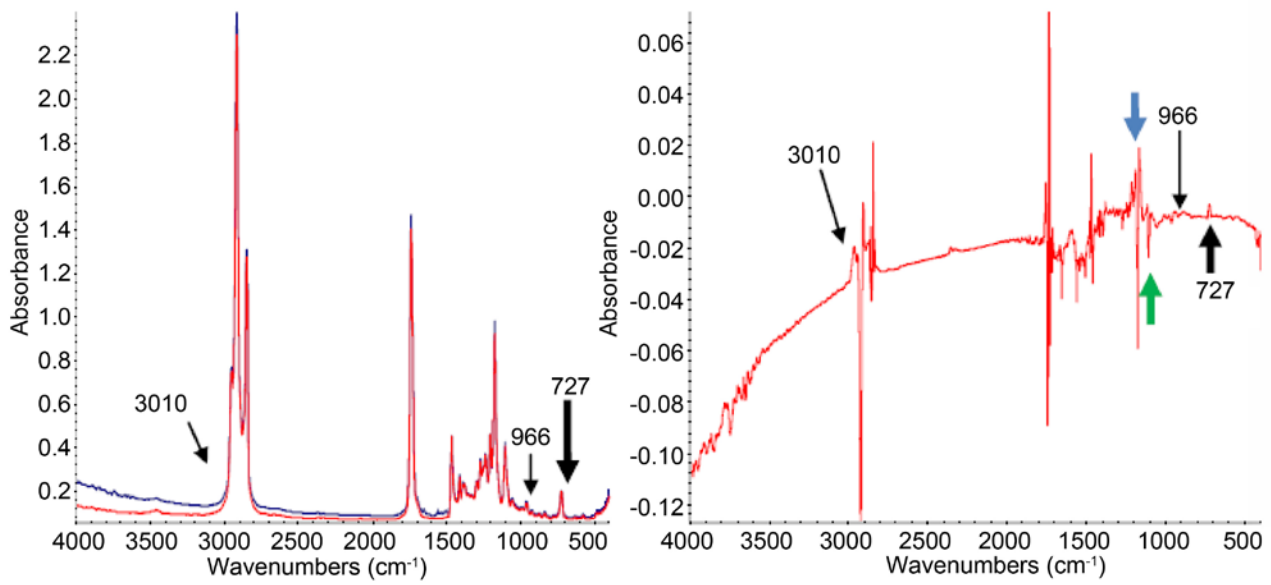

Figure 1. R03 sample MIR spectra (correction of spectra on the left and subtraction of R03 at 30˚C (red) and R03 at 6˚C (blue) on the right).

the length of the carbon chain [23].

This result confirms the analysis of the saponification index, indeed the ratios of increase of this index are 1.02 at 6˚C and 1.06 for that at 30˚C. It implies that the carbon chains shorten more in the conditions of 30˚C than at low temperature. This favors a strengthening of the solidification of the *Irvingia gabonensis* fat. The second piece of information is that the Irvingia fat has the same content of "cis" FA in both conditions of conservation. This does not mean that this content has not changed but, if there was a variation it would have occurred in the same way in both conditions. Indeed, the band near 3010 cm^{-1} (**Figure 2**, left) remains almost linear. On the other hand, the band near 966 cm^{-1} which is attributable to the vibrations of the "*trans*" double bonds [24] is in favor of conservation at 30˚C (**Figure 2**, right). This implies that under the 30˚C storage conditions, "cis-trans" isomerization is preponderant. This result is in agreement with the high number of peroxides that formed under these conditions. Indeed, at low temperature, the "*trans*" FA formed evolve very quickly towards degradation compounds, this result is corroborated by the increase of the saponification index of 17 units translating shorter carbon chains.

Also, the transition from "*cis*" to "*trans*" FA is carried out with the same kinetics under both storage conditions. The difference in alteration is in the kinetics of the evolution of "*trans*" FA towards degradation products. This difference is more important at 6˚C, hence a poor preservation of the fat.

The bands attributable to the vibrations of the esters are also in favor of conservation at 30˚C, we note moreover, a band near 1180 cm^{-1} (blue arrow) attributable to the vibrations of the C-O group of the carboxylic acids which is also important under these conditions. There is still a strong presence of carboxylic acids in the fat stored at 30˚C [25]. Indeed, if the C-O vibration band of carboxylic

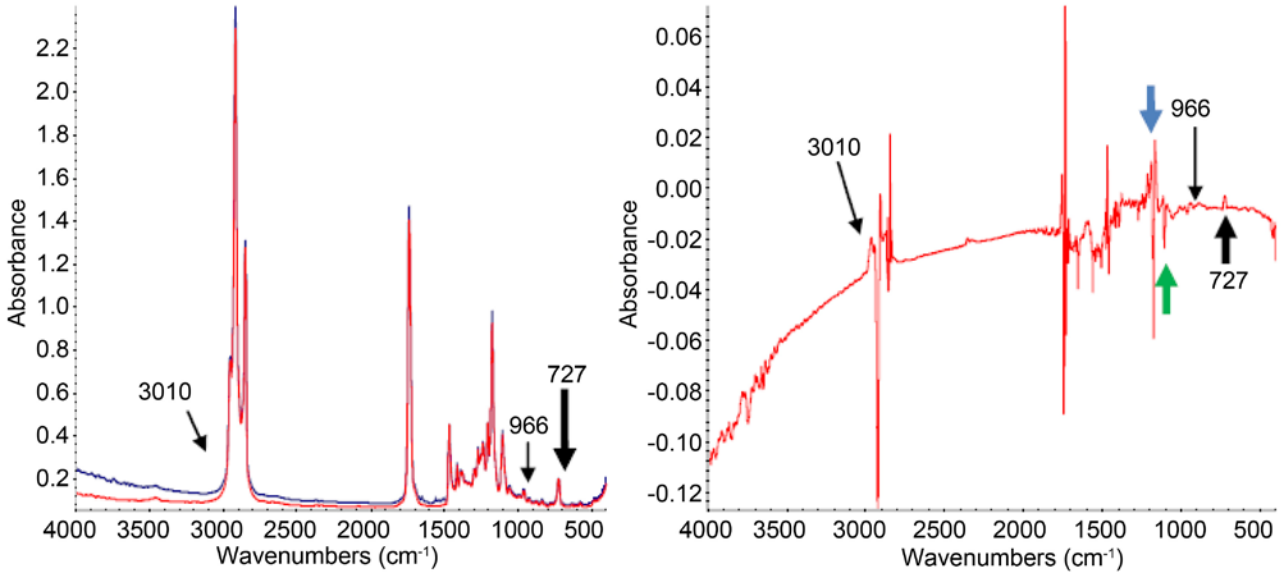

Figure 2. MIR spectra of sample R03 (correction of spectra on the left and subtraction of R03 (red) and R03 (blue) on the right).

acids is more intense in the conditions at 30˚C, this shows that fatty acids in these conditions are more released than at low temperature. However, their incorporation in chain extension reactions is faster. Therefore, the value of I_A is lower at 30˚C [25].

However, the band attributable to vibrations of the C-O group of the esters (green arrow) which appears near 1150 cm^{-1} is in favor of the conditions at 6˚C. This proves that the esters formed by oxidation of carboxylic acids accumulate in the Irvingia fat. It is as if the oxidation products are not formed in the same proportions according to the storage conditions. Thus, there are many more esters formed at low temperatures because the kinetics of the reactions are slower and many fewer esters at 30˚C because the accumulation of "trans" FA favors the addition reactions at the origin of carbon chain elongation of fatty acids [26].

Figure 3 allows us to confirm that much more esters are formed under the conditions of conservation at low temperature, indeed, the vibration band near 1150 cm^{-1} (red arrow) is in favor of the sample at 6˚C, as well as the vibration band near 1750 cm^{-1} (green arrow) attributable to the C=O vibration of esters [26]. These results prove that Irvingia fat oxidizes more under low temperature conditions unlike most oilseeds which oxidize less at low temperature. One approach to explain this is to consider that during its solidification Irvingia fat traps oxygen molecules from the air which are therefore at the origin of this slow oxidation.

The bands between 2986 and 2850 cm^{-1} attributable to the vibrations of the $-CH_3$ and $-CH_2$ groups are in favor of the R03 sample, but the intensity of these bands is proportional to the length of the carbon chains, which means that the FA of the R03 sample are relatively longer, this result invalidates the saponification index analysis. Indeed, when we consider only the saponification index values obtained 11 months after the beginning of the aging process, we notice that

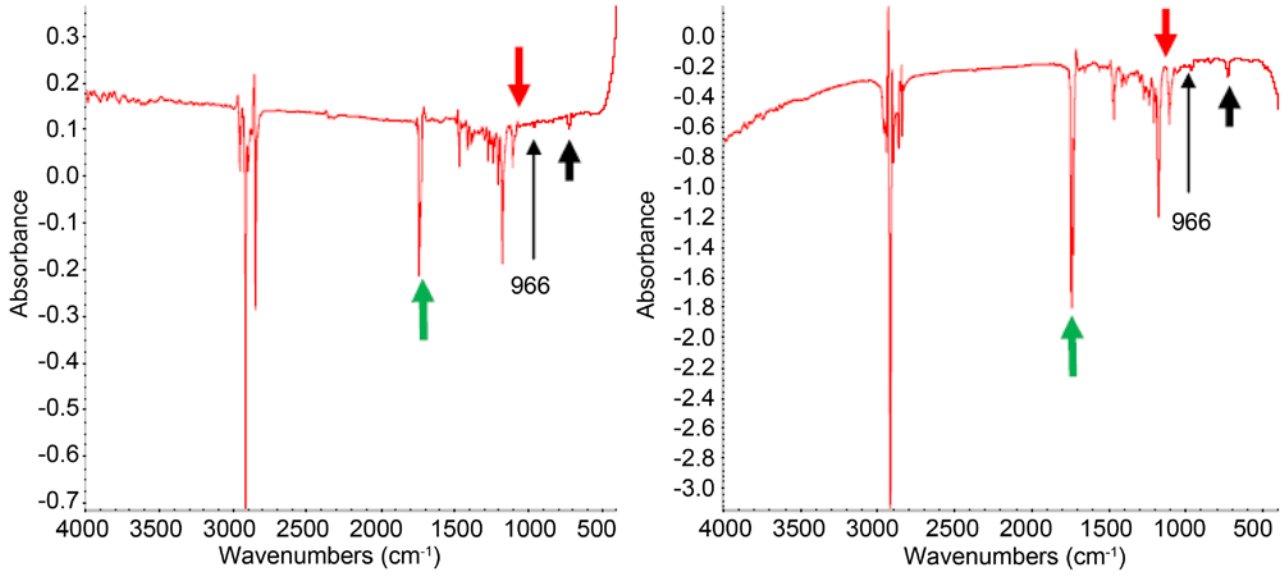

Figure 3. MIR spectra of sample R03 (correction of spectra on the left and subtraction of R03 (red) and R03 (blue) on the right).

the R01 sample shows the lowest value. This result means that this sample should have the longest carbon chains, which is confirmed by the intensity of the band near 720 cm^{-1} (black arrow) [25] which is in favor of R03. The carbon chains of sample R01 are longer than those of sample R03. We note that the number of $-CH_2$ and $-CH_3$ groups is greater in sample R03 than in sample R01 but that the latter is not formed of long carbon chains. It is very likely that other structures are formed than the lengthening of the carbon chains in which these groups are incorporated during aging. Indeed, the number of $-CH_2$ and $-CH_3$ groups cannot be changed during storage because the storage conditions are such that no material is lost [27].

The band near 966 cm^{-1} is in favor of the R03 sample whatever the storage conditions. This result proves that "*trans*" FA are more present in fresh irvingia fat than in aged fat, so that all the "*cis*" FA in irvingia fat are transformed into "*trans*", and then the "*trans*" formed disappear during storage. This explains their low presence in the sample R01

However, this result does not explain the observed increase of IFA contents.

This suggests that as the compact structure of the irvingia fat loses consistency during storage, the condensed "cis" IFA unwind and then convert to "*trans*". Thus the conditions of oxidation and rearrangement within the matrix of the fat becoming favorable, this one begins its degradation which is fast and less at 30˚C whereas it is slow and very pushed at low temperature.

4. Conclusions

The work presented had the ambition to shed light on the reason for conservation in the open air and not sheltered from the light by the rural people in farming areas. Irvingia fat is preserved in these localities at room temperature in the form of medium-sized pellets, certainly in order to increase as much as possible

the various encumbrances in order to lengthen the time of its conservation. Only the external surface of the pellet is grated while ensuring the conservation of the round shape of the whole.

Indeed, the work explains the decrease of the acid number determined in the conditions of conservation at 30˚C by an incorporation of the released FA in the formation of a more complex structure during the storage after conversion "*cis-trans*". We are thus in an environment with a large number of $-CH_2$ and $-CH_3$ groups, which explains the decrease in the saponification index, reflecting the lengthening of carbon chains.

By combining all the parameters studied, we can think that during the ageing of the Irvingia fat, a series of reactions follow one another without however foreseeing with exactitude the order of this succession. Thus, a release of fatty acids is carried out but also, homolytic cuts at the level of these fatty acids and thus the formation of secondary degradation compounds which are very often stable because they have a short carbon chain and compounds with longer carbon chains which participate in the formation of the network crystalline and are obtained through isomerization "cis-trans". However, the rapid solidification at room temperature of the fat traps substances promoting oxidation or autooxidation. These substances remain active but the kinetics of their activity is strongly linked to the storage conditions. Thus at 6˚C the interior of the fat not being solidified in a homogeneous way is the seat of chemical reactions with slow kinetics and important consequences; contrary to 30˚C the interior of the solidified fat being more compact, the reactions which take place there have a fast kinetics with less consequences.

From this combination of chemical and physical methods, it appears that the irvingia fat is much more stable under the conditions of 30˚C than at low temperature. This surprising result proves the accuracy of the realism of the rural populations consuming this commodity.

In the course of our work, there were questions of grasping the aging of *Irvingia gabonensis* fat during storage. It would therefore be interesting to redo this study by approaching aging under conditions of conservation in the open air or subjected to UV to simulate the action of daylight.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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