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Compared Ageing of Oil from Curcubitea Pepo in Two Different Storage Conditions

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Abstract: The aim of this study was to investigate the oil pumkin seeds of Curcubitea pepo from Congo-Brazzaville. The ageing of oil extracted from the seeds of the pumpkin *Curcubitea pepo* stored at two temperatures (6 and 30ºC) was monitored during storage for 11 months by comparison of physical and chemical characteristics correlated with spectroscopic data. Medium infrared spectroscopy confirmed saponification index data. Ultraviolet absorption confirmed peroxide index data. Antioxidant behaviour was monitored by fluorescence and the effect of ageing on the two major fatty acid families was analysed by a study of chemical composition correlated with differential scanning calorimetry measurements. This study showed an overall lengthening of the fatty acid carbon chains and allowed preferential storage conditions to be specified for this oil.

Key words: Ageing, chemical composition, *Curcubitea pepo*, DSC, MIR spectroscopy

INTRODUCTION

"Pumpkin" has become the generic name for all the oil-bearing members of the Cucurbitaceae family consumed in Congo-Brazzaville. These oil-rich pumpkins have found many uses as food (Silou *et al*., 2008). Consumed either directly or as edible ingredients, pumpkins are valued foods widely used by the populations of southern Congo and neighbouring countries (Silou *et al*., 2008). Their high oil content makes pumpkins the second most important source of oil used by rural populations in southern Congo after groundnuts.

Several studies underline the useful properties of pumpkin oil, including protection against prostate disorders (Goetz, 2009; Fruhwirth and Hermetter, 2007). The benefits of this oil give it an ever-growing place in the diet of the Congolese and of Africans generally (Mvoula-Tsieri *et al*., 2008). Although the nutritional qualities of pumpkin oil are proven (Silou *et al*., 2008) the production and storage of this oil in Congo-Brazzaville have not yet been well researched.

In this study we monitored the evolution of pumpkin oil of different origins in the course of storage under different conditions. We linked the physical and chemical data obtained from analysis of indices and Fatty Acid (FA) composition to spectral data in order to seek a chemical explanation for the effects observed and so characterise the influence of storage conditions on the ageing of pumpkin seed oil.

FA degradation is not viewed in the same light by chemists and nutritionists: monitoring the effects of such degradation is usually done by analysing only how the physical and chemical characteristics change, by comparing chemical and physical indices between two different states under set conditions (Fekarurhobo *et al*., 2009). However, the variations in the values of particular indices are interpreted differently by chemists and nutritionists. One way to link the two approaches is to measure variations irrespective of their perceived import and correlate them with spectroscopic data (Belton *et al*., 1988).

The peroxide index is a clear, unequivocal example: of two otherwise identical lipids, the one with the higher peroxide index is the more degraded (AOAC, 1999). We know that peroxides are very sensitive to light and heat and that their disappearance from the lipid is accelerated by a simple rise in temperature. The analysis of the

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variation in the peroxide index allows meaningful conclusions to be drawn only if the two lipids are studied in the same temperature conditions or if temperature changes are controlled. Conversely, this variation can lead to unwarranted conclusions if these conditions were very different. The lipid that presents the higher peroxide index may even prove to be the less degraded of the two, because its safer conditions of storage prevent the rapid disappearance of peroxides (Chan *et al*., 1976).

To avoid such situations, we coupled certain physical and chemical characteristics to spectral analysis. Our storage study was conducted in very different storage conditions (average temperature 30ºC in Brazzaville and 6ºC in a cold-room in Clermont-Ferrand). In this case a simple comparison of indices, though useful, could be misleading.

MATERIALS AND METHODS

Pumpkin seeds: De-hulled pumpkin seeds were bought at the market in Dolisie (Niari). The seeds originated from two regions, Lekoumou and Bouenza, in the south of Congo-Brazzaville. They were ground in an electric mill fitted with a rotor set at 2500 rpm. The resulting powder was then placed in a cartridge and loaded into a Soxhlet extractor.

Oil extraction process: The extraction solvent was hexane. The extraction was carried out for 3 h in a Soxhlet apparatus (Grondin *et al*., 2002). The pale yellow oil obtained was liquid at room temperature. The "young" samples W01 and W02 came from Lekoumou and Bouenza, respectively. The samples were extracted in 2008, 3 months before the study and then stored in opaque jars in the dark at 30ºC.

The "adult" sample W03 came from Lekoumou and was extracted in 2007, 12 months before the study and stored in the same conditions as W01 and W02. An "old" sample W04 from Bouenza was extracted in 2005, 28 months before the beginning of analysis. This sample, stored at 30ºC in the dark (Bacon and Douville, 2006), was used as a control in the ageing study.

All extractions of oils were done in Congo-Brazzaville and analysis was performed in France (Clermont Ferrand) from 2008 to 2010.

Fatty acid composition: FA esters were obtained by direct methylation: 2 drops of oil in 1 mL of hexane in the presence of 0.4 mL of 1 N sodium hydroxide (in methanol) was heated for 1 min; 0.4 mL of 1N hydrochloric acid in methanol was then added, followed by 1 mL of hexane (AOAC, 1999).

Chemical composition was determined by GC/FID with an AGILENT 5890 instrument equipped with a Supelco FAMES column 100 m long of inside diameter 0.25 mm and thickness 0.25 μm. Hydrogen was used as carrier gas at a flow rate of 0.7 mL/min. Oven temperature was programmed as follows: rise to 140ºC, then ramp for 5 min at 4ºC/min to 240ºC. The injector temperature was 280ºC, split ratio was 1/30, and injection volume was 1 μL. The detector temperature was 300ºC at 40 mL/min for hydrogen and 450 mL/min for air; nitrogen was the make-up gas at 45 mL/min.

Physical and chemical analysis: The principal physical and chemical indices were determined with reference to international and French standards (AFNOR, 2000). The acid index I_A (NF EN ISO 660), the saponification index I_S (NF EN ISO 3657), and the peroxide index I_P (NF T 60-220) were determined.

These principal indices were correlated with spectroscopic data. Medium Infrared (MIR) spectra were recorded with an IRTF NICOLET 760 Magna IR spectrometer. Ultraviolet (UV) absorption spectra were recorded with a Shimadzu UV-2101PC spectrometer fitted with an integrating sphere to collect the whole of the transmitted light. Differential Scanning Calorimetry (DSC) measurements were made with a DSC 822^e enthalpic differential analyser (Mettler Toledo). Fluorescence emission and excitation spectra were recorded using a Perkin-Elmer LS-55 spectrofluorimeter.

Ageing: After analysis the oil samples were divided into two equal fractions and stored for 11 months in different conditions to determine their degrees of degradation.

- Samples kept in a cold-room in the dark in Clermont-Ferrand and denominated "CF".
- Samples stored in a cupboard in the dark and at an average temperature of 30ºC in Brazzaville and denominated "BZV".

Spectroscopic methods:
MIR: one drop of sample was placed between two KBr plates and the spectrum recorded at a resolution of 8 and 32 acquisitions. The spectra of the main FAs were compared with those obtained for each sample. The MIR spectra were correlated with the saponification index and the degree of oxidation.

DSC: The sample (encapsulated) and the reference (empty capsule) were placed on platforms in the thermoelectric ceramic disk that transfers heat from the oven to the cell and the capsules. The oven temperature was raised or lowered linearly and the differential heat flow between the sample and the reference was measured using multizone thermocouples fixed under the platforms.

Fluorescence spectroscopy: the sample was diluted in hexane so that the UV absorbance was less than or equal

Table 1: Physical and chemical indices determined at the start of the study and 11 months later for different storage conditions*

Sample	W 01	W 01 CF	W 01 BZV	W 02	W 02 CF	W 02 BZV	W 03	W 03 CF	W 03 BZV	W 04	W 04 BZV
Age (months)	3	14	14	3	14	14	12	23	23	28	39
I_A	7.14	7.50	4.44	7.99	8.43	4.34	1.59	1.53	2.53	13.09	1.68
I_P	4.64	51.11	33.87	2.47	71.08	43.87	3.50	16.69	16.04	18.50	95.36
*I*s	201.00	201.00	196.46	199.76	199.76	196.89	193.92	193.92	195.06	218.64	208.37

*: Calculations were repeated up to three times (CF = sample stored at 6ºC. BZV = sample stored at 30ºC)

to 1.2. We used a CARY3 spectrophotometer (Varian). The fluorescence spectra were recorded with excitation and emission slits with passing band 5 nm and scan speed 120 nm/min for a 500-emission spectrum. The data were processed using FLwinlab software. The fluorescence emission spectra were studied for excitation wavelengths of 270, 300 and 340 nm. Excitation spectra were recorded for analysis wavelengths of 350 and 400 nm.

UV and visible spectroscopy: the sample was diluted so that the absorbance in the range 220-320 nm was between 0.8 and 0.2. Standard NF ISO 3656 is designed to show the presence of degradation compounds, both primary (linoleic hydroperoxide) and secondary (e.g., trienes and aldehydes) (AFNOR, 2000) in a lipid mixture.

Non-saponifiable fraction: The non-saponifiable oil fraction comprises, in particular, the tocopherols (the antioxidant vitamin E) (Léger, 1992). It was assayed using the standard NF T 60-205-1.

RESULTS AND DISCUSSION

Evolution of acid, peroxide and saponification indices: We measured different physical and chemical indices (AFNOR, 2000) for each of the samples before storage and after 11 months storage at 6 or 30ºC to evaluate optimal storage conditions (Table 1).

The high values of acid, peroxide and saponification indices observed for the "old" sample W04 show its degradation relative to the other samples and justify using it as an ageing control. The two "young" samples W01 and W02 displayed similar patterns, showing that their origin was not a factor of variability in ageing behaviour (Table 1).

The acid index is a marker of lipid degradation, as the free FA content in lipids increases in time (CAC, 2001). When stored at an average temperature of 30ºC, the quantity of free FAs in the oil fell, in particular in samples W01 (BZV) and W02 (BZV), reflecting an appreciable decrease in free FAs in these oils. This behaviour was also seen in the "old" sample W04. However, the "adult" sample W03 displayed the opposite profile.

The quantity of free FAs could be expected to increase in the Brazzaville conditions, the higher temperature favouring the release of FAs from triacid glycerides (TAGs). This occurred in the "adult" sample W03. These results suggest that the pumpkin seed oil stored at an average temperature of 30ºC kept well.

Measurement of peroxide indices is directly linked to the degradation of the oil considered (CAC, 2001). If two identical lipids are compared, the one with the lower peroxide index is the less degraded.

The values of the peroxide indices of all the samples increased considerably during storage. For the "young" samples W01 and W02, this index was higher in the CF storage conditions (6ºC) than in the BZV storage conditions (30ºC). These results confirm the conclusions suggested by the acid index analysis, i.e. that pumpkin seed oil is better stored at 30ºC than at low temperature. However, the "old" sample W04, displayed opposite behaviour, and the "adult" sample W03 presented a near-stable peroxide index. This difference may be due to a modification of the chemical composition of these "aged" samples relative to W01 and W02.

The saponification index is a variable that reflects the quality of a lipid. It is linked to the molar mass of the compound: the higher the saponification index, the lower the average molar mass, i.e., the shorter the average carbon chain lengths.

The overall fall in the saponification index observed corresponds to a lengthening of the carbon chains. This finding explains why the quantity of free FAs observed in the samples stored at average temperature 30ºC was lower than at 6ºC. The TAGs release FAs, but these are rapidly used in molecular rearrangement reactions to yield products with long carbon chains.

This result is consistent with the relatively low value of the peroxide index observed for the samples stored at 30ºC. The attack sites liable to produce chain lengthening are most likely located on peroxides (Bourgeois *et al*., 2001). In this case *cis-trans* isomerisation will occur (Ahlers *et al*., 1953). The presence of trans isomers in the oil is thus clear evidence of its degradation, since all natural unsaturated FAs are *cis* isomers (Fig. 1) (CAC, 2001).

Evolution of the chemical composition of the samples: The time variation in FA contents observed by GC analysis of the different samples was too weak to reveal any significant pattern of behaviour relative to storage conditions (Table 2).

However, this analysis shows the time course of levels of derivatives with "long chains" (C20:0) and of "unsaturated compounds". Two hypotheses can be advanced: (i) the long-chain compounds, in particular C20:0 are formed and their amounts increase, and (ii) levels of unsaturated compounds decrease.

Table 2: Chemical composition obtained by GC for different storage conditions

Sample	W 01 CF	W 01 BZV	W 01	W 02 CF	W 02 BZV	W 02	W 04 CF	W 04 BZV	W 04	W 05 BZV	W 05
Age(months)	3	14	14	3	14	14	12	23	23	28	39
% FA	-			-			-			-	
C16:0	15.76	15.21	14.47	16.23	14.3	14.99	11.29	10.52	10.53	19.92	19.19
C17:0	-	0.08	0.08	-	0.08	0.08	-	0.07	0.07	-	0.14
C18:0	12.92	12.62	12.26	13.19	12.57	12.57	12.59	11.85	11.91	19.99	19.71
C18:1	12.71	14.73	12.54	14.90	14.72	14.72	14.11	13.67	13.71	19.11	19.52
C18:2	56.83	56.15	59.38	54.83	56.4	56.4	60.32	62.26	62.22	38.14	36.37
C18:3	0.30	0.22	0.15	0.45	0.12	0.12	0.39	0.18	0.09	0.64	0.12
C20:0	-	0.32	0.30	-	0.32	0.32	0.29	0.47	0.39	0.63	0.70

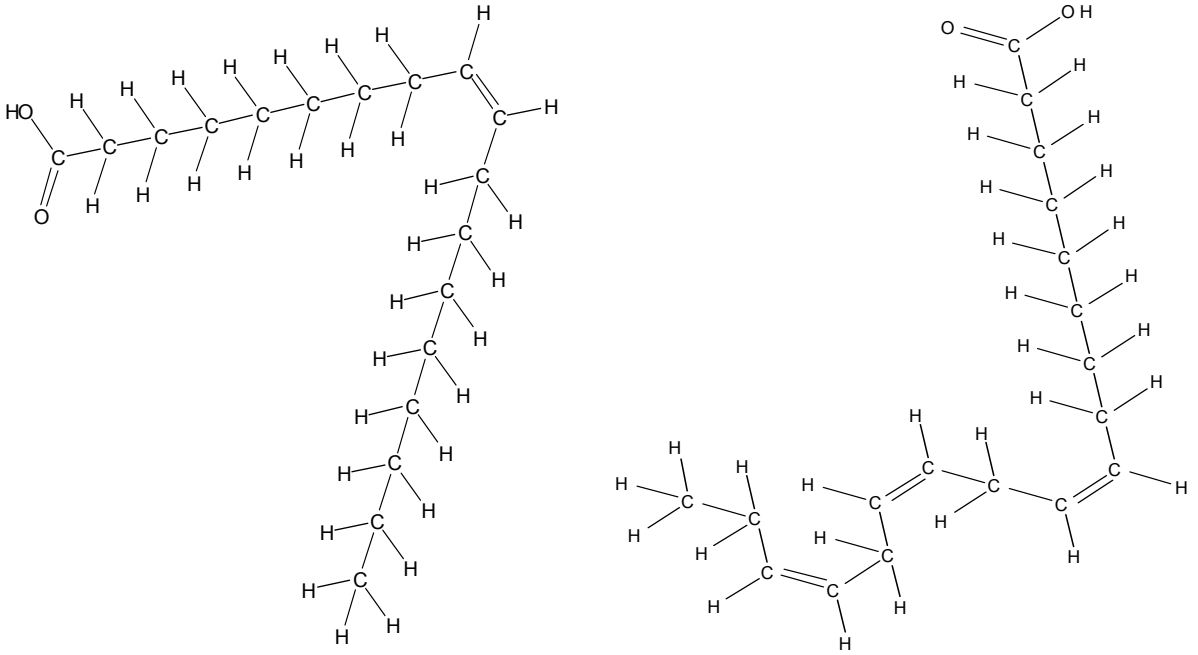

Fig. 1: Naturally-occurring molecules of oleic acid (left) and linolenic acid (right). They are always of *cis* configuration

Our first hypothesis is not well supported: C20:0 appeared, a long-chain FA was observed, but at practically the same level in both storage conditions studied. This reflects chain lengthening in both conditions. However, this observation is not corroborated by the saponification index.

Our second hypothesis is supported; the unsaturated FA levels, in particular that of C18:3 decreased at average temperature 30ºC. This result suggests that low temperature storage in the dark (Fekarurhobo *et al*., 2009) is best for pumpkin seed oil.

However, the analysis of chemical composition alone does not enable us to specify the best general conditions of storage for pumpkin seed oil. In the "young" samples the levels of C16:0 decreased while those of C18:2 increased, although the opposite might have been expected. Also, we found that C18:2 were more stable, irrespective of the storage conditions, but its level increased during storage from W01 to W03. However, it was rapidly degraded beyond 25 months storage as shown by W04 (Table 2).

From the FA levels in W04 after 39 months storage we can postulate an order of degradation of unsaturated FAs in pumpkin seed oil: first C18:3, then C18:2. However, C18:1, though unsaturated, remained intact after 23 months and then strongly increased, together with C18:0.

Behaviour of the non-saponifiable fraction of pumpkin seed oil: Table 3 shows that the level of non-saponifiables increased in all samples stored at 30ºC. This expected finding confirms that the oil was markedly degraded in these conditions (Bacon and Douville, 2006).

By contrast, the levels of non-saponifiables fell for the samples stored at low temperature except for the adult sample W03 in which this level increased, as in the samples stored at 30ºC in about the same ratio. Hence the pumpkin seed oil was more severely degraded when stored at 30ºC, and this degradation was more marked in "young" samples (Fig. 2).

Thus saponifiable compounds initially present in the oil evidently undergo changes and become

Table 3: Yield of non-saponifiable 6ºC determined at the start of the study and 11 months later for different storage conditions

Echant	W01	W 01 CF	W 01 BZV	W 02	W 02 CF	W 02 BZV	W 03	W 03 CF	W 03 BZV	W 04	W 04 BZV
Age(months)	3	14	14	3	14	14	12	23	23	28	39
Non-sap.	1.3496	0.9803	1.9091	1.1292	0.9616	1.8571	0.9202	1.4822	1.5247	1.6185	1.9784

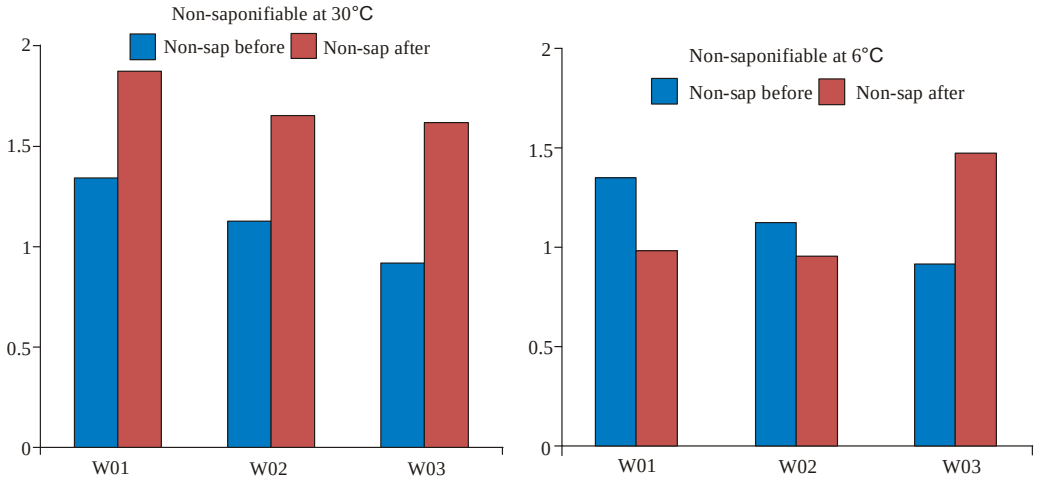

Fig. 2: Comparison of the time course of the non-saponifiable 6ºC fraction of samples in the two storage conditions to appraise variations between levels at the beginning of the study (*non-sap before*) and 11 months later (*non-sapo after*).

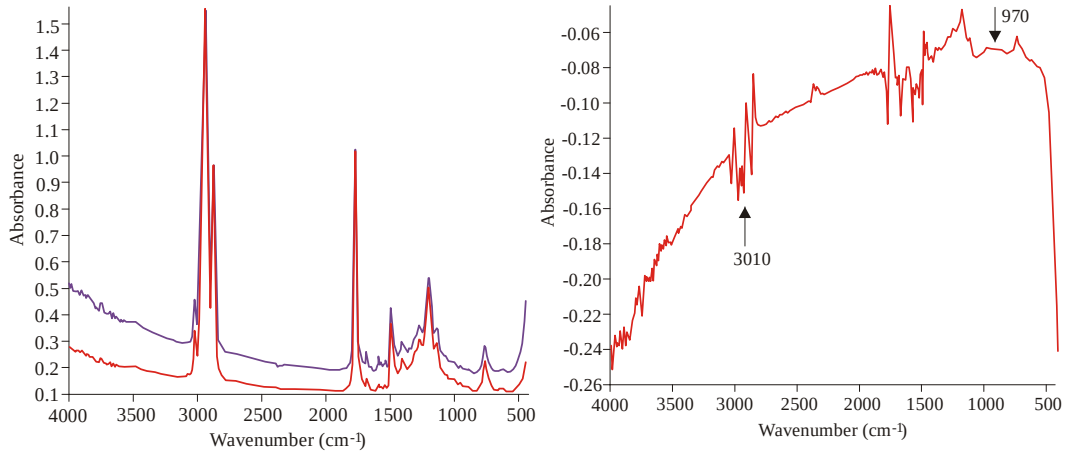

Fig. 3: Illustration of differences in MIR band intensity of sample W01 on the same scale (left: red W01 (BZV) (30ºC) and violet W01 (CF) (6ºC); right: subtraction of spectra of sample W01 stored at 30 and 6ºC.

non-saponifiable, resulting in a greater non-saponifiable fraction.

It seems that so long as peroxides are formed and go on to yield other compounds (Chan *et al*., 1976), the levels of non-saponifiable compounds increases continuously. When peroxide formation slows down, so does that of non-saponifiable compounds. Once the oil has reached a certain degree of degradation its non-saponifiable content no longer increases, irrespective of storage temperature. The compounds liable to switch from saponifiable to non-saponifiable have mostly done so and the non-saponifiable fraction therefore becomes stable. These results show that the degradation of peroxides leads to non-saponifiable compounds.

Methods of spectral analysis: The use of simple spectral analysis methods that require smaller amounts of material provide further data from which conclusions may be drawn. Conclusions resulting from the analysis of indices and of chemical composition show noteworthy differences.

Medium infrared spectroscopy: The spectra of the "young" sample W01 (Fig. 3) showed the changes that had occurred in the oil during storage. The oil underwent greater oxidation when stored at 30ºC, the absorbance due to C=O and C–O groups near 1753 and 1150 per cm was more intense in the oil stored at 30ºC. The low intensity of these bands in the oil stored at 6ºC confirms that in

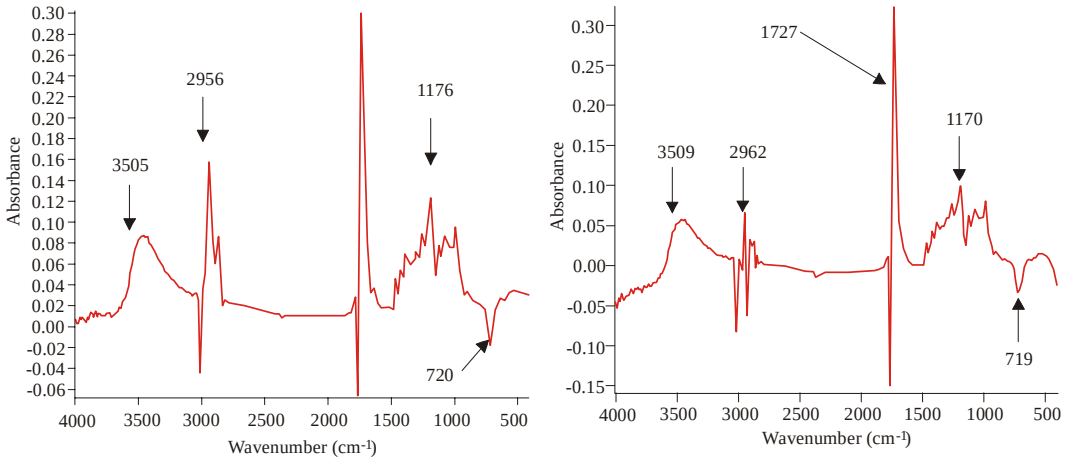

Fig. 4: Subtraction of two spectra to show the evolution of the different samples during storage: "old" sample W04-W03 (left) and W04-W01 (right) in storage conditions at 30ºC

these storage conditions the carbon chains were shorter (Ahmed and Helal, 1977), consistent with the conclusions drawn from the analysis of the saponification index.

We found that the sample W01 (CF) stored at 6ºC showed a more intense band near 3010 per cm attributable to the stretching vibration of the *cis* =C–H group, while the sample W01 (BZV) presented a shoulder near 970 per cm attributable to the *trans* =C–H group (Ahlers *et al*.,1953). This finding is consistent with the alkene radical addition reaction mechanism, and confirms the lengthening of the carbon chain, as evidenced by the analysis of the saponification index. The weak variation in the saponification index is thus corroborated by the weakness of the bands (right spectrum).

The spectra in Fig. 4 show that the $-CH_2-$ vibration band increased during storage, in particular from the "young" sample W01 stored at 30ºC to the "old" sample W04 (band at 2956 per cm on right-hand spectrum).

An evolution in the degree of oxidation (band at 3505 per cm for –OH and C–O of esters at 1176 per cm) was observed and an evolution in the intensity of absorption bands in regions 2954, 2924 and 2852 per cm was also observed. These correspond respectively to the asymmetrical stretching vibration of the $-CH_3$ group (2954), the asymmetrical stretching vibration of the $-CH_2-$ group (2924) and the symmetrical stretching vibration of the $-CH_2-$ group (719; 720) (Bertrand and Dufour, 2000). These bands were more intense in the right-hand spectrum than in the left-hand spectrum. This result confirms that the young samples were degraded faster than the adult samples, and is evidence that the carbon chains lengthen during storage; the intensity of the band at 720 per cm was inversely proportional to the length of the carbon chains (Safar, 1995).

At 1745 per cm, we found that the stretching vibration of the carbonyl groups intensified during storage. This effect is linked to the degree of oxidation of the oil and the relative length of the carbon chains (Belton *et al*., 1988). This result confirms that the variations in the saponification index, though small, reflected marked changes in the oil.

In the low frequency region, a band linked to the vibration of the ester group C–O was observed at around 1150 per cm. The intensity of this absorption band increased during storage; it is linked to the degree of oxidation and the relative length of the carbon chains (Bertrand, 2000).

The ratio of the saponification indices of samples BZV, W03 and W01 was 0.99, in favour of the "young" sample, confirming higher ester content. However, this ratio was 0.96 for the CF samples, showing that the sample W01 (CF) was less rich in esters than W01 (BZV), and so was less degraded.

The band near 3010 per cm increased in intensity (Fig. 4 right), while the band at 970 per cm barely changed. It seems that during ageing the FAs that are naturally of *cis* configuration isomerise to *trans*, and these *trans* compounds are then degraded and disappear. This explains the low level of peroxides in the oil stored at 30ºC. This result is consistent with the mechanism of alkene radical addition causing *cis* / *trans* isomerisation, and explains the rapid disappearance of peroxides in the conditions of storage at 30ºC (Chan *et al*., 1976).

The analysis of these MIR spectra provides an explanation for why the peroxide index and the acid index were low at 30ºC. As the reactions are radical-based, the released FAs were used up very rapidly in molecular rearrangements preferentially affecting the peroxides of *trans* configuration formed (Bourgeois *et al*., 2001). This result is evidence that any assessment of the ageing of oils based solely on variations in indices is insufficient and needs additional spectral analysis.

Table 4: Absorbance of bands examined for the control sample W04; "adult" W03 and "young" W01 to check for the presence of primary and secondary degradation products in the two storage conditions

	Conditions at 6ºC		Conditions at 30ºC	
Sample	Abs at232±10 nm	Abs at268±10 nm	Abs at232±10 nm	Abs at268±10 nm
W04	-	-	0.7	0.18
W03	0.28 and 0.2	0.07, 0.06 and 0.04	1 and 0.65	0.24, 0.28 and 0.2
W01	None	0.31 and 0.26	None	0.87 and 0.8

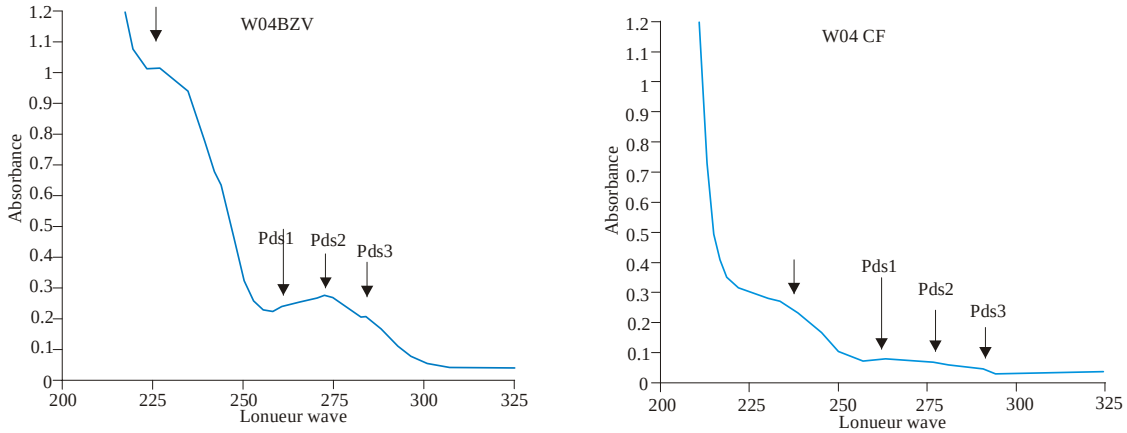

Fig. 5: Absorption bands for sample W04 stored at 6ºC (Pds: secondary degradation products)

UV-visible spectroscopy: We know that the products of lipid auto-oxidation show characteristic UV spectra (AFNOR, 2000). When a lipid is degraded, primary products (e.g., linoleic hydroperoxide) and secondary products (e.g., diketones, aldehydes and trienes) are formed. The primary products absorb at 232 nm and the secondary products at 268-280 nm.

Linoleic hydroperoxides and conjugated dienes can result from the decomposition of linoleic acid (Chan *et al*., 1976). They present a characteristic absorption band near 232 nm (arrow on far left). Secondary products of auto-oxidation, in particular ethylene diketones, present a characteristic band near 268 nm.

We used UV spectroscopy to check for the presence of these compounds in our samples during storage (Fig. 5). Analysis of peroxide indices revealed an increase in peroxides in the "young" samples, especially in those stored at 30ºC. By contrast, the opposite was found for the "old" samples irrespective of the storage conditions.

The "young" sample displayed no bands corresponding to primary degradation products (Fig. 5). The secondary compounds were thus detected first. Also, in the conditions at 30ºC the products formed were better specified (stronger intensities). However, the adult samples showed both primary and secondary types of degradation products (Table 4). This result shows that the formation of the secondary products from the primary products was very fast. This step may be inhibited or slowed by the accumulation of secondary compounds. This would explain the presence of primary products in the sample W04, with high absorption intensity. The fact that W04 displays only a blunt peak near 268 nm shows that during ageing the secondary degradation compounds evolve towards complex compounds in a broadened band. This result is consistent with the increase in the levels of non-saponifiable components

Comparative analysis of peroxide indices and UV spectroscopy leads us to the conclusion that samples of freshly extracted pumpkin seed oil favour the formation of peroxides irrespective of the storage conditions. The presence of peroxides is taken as an indication of the state of degradation of pumpkin seed oil (Murkovic and Pfannhanser, 2000), but is not sufficient, as peroxides are present in different storage conditions. Secondary degradation compounds are the first to accumulate in pumpkin seed oil, and the presence of compounds of primary degradation is thus sufficient evidence of advanced degradation of pumpkin seed oil. During ageing of pumpkin seed oil the secondary degradation products evolve towards products with chemically homogeneous (equivalent) structures. We conclude that storage conditions at low temperature are clearly best for pumpkin seed oil.

Fluorescence spectroscopy: The behaviour of antioxidant compounds, in particular vitamin E and polyphenols during storage was monitored by fluorescence (Quiles *et al*., 2002). These compounds are fluorescent, and when excited at a set wavelength produce an emission spectrum (Fruhwirth *et al.*, 2003).

Analysis of the non-saponifiable fraction showed that it increased during storage in all our samples, and especially at 30ºC. This result, though interesting, gives

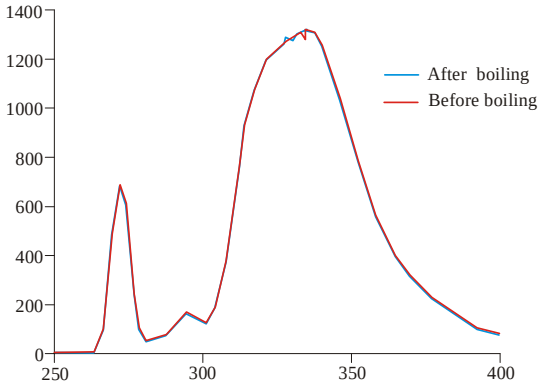

Fig. 6: Behaviour of standard α-tocopherol before and after boiling, at excitation wavelength 270 n

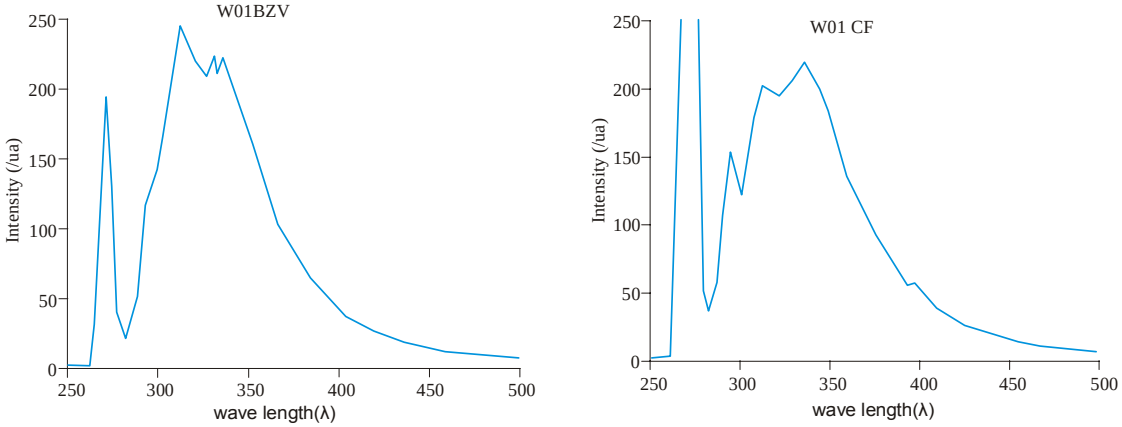

Fig. 7: Emission spectra of the sample W01 at λ_{exc} = 270 nm (the peaks at 300 nm can be ignored

us no information on the behaviour of vitamin E and the important polyphenol compounds in the non-saponifiable fraction that protect the oil against oxidation (Sedghi *et al*., 2008).

The fluorescence excitation spectra of standard vitamin E (α-tocopherol, Merck) at the same concentrations as in the oil were obtained by fluorescence spectroscopy (Fig. 6) at an emission wavelength of 330 nm (Cheikhousman *et al*., 2005).

A first measurement was made after dilution of α-tocopherol in HPLC-grade hexane, at its concentration in the oil. This concentration was obtained from the UV spectra of the oil. A second measurement was made after heating in the conditions in which the oil was extracted.

These results show that heating did not change the emission wavelength of the vitamin E. This is important as we can study its behaviour in oil in the knowledge that the hot extraction process has no effect on its fluorescence emission zone. Thus we can confidently assert that emissions near 330 nm can be attributed to vitamin E (Sikorska and Khmelinski, 2008).

We recorded the fluorescence spectra of each sample to monitor the behaviour of vitamin E and polyphenols.

We see that the sample stored at low temperature had the opposite profile to that stored at 30ºC (Fig. 7); emission in one case and absorption in the other. The absorption thus corresponded to a vitamin E that had lost its anti-oxidant power, and the emission was evidence that the vitamin E still exercised that power. This result was confirmed by the spectrum of standard vitamin E (Fig. 6) and by that of the "old" sample W04, which presented a profile close to that of W01 stored at 30ºC

Vitamin E absorbs on the left and emits on the right, evidence that the two samples are not subject to the same aggression and do not resist in the same way in the two storage conditions (Fig. 8). However, the "adult" sample presented the opposite profile to the "young" sample. During storage the vitamin E in the pumpkin seed oil seemed to have recovered its antioxidant power, especially in the storage conditions at 30ºC (Sikorska and Khmelinski, 2008).

This result enables us to estimate the best time to add antioxidants to pumpkin seed oil during storage to preserve it. Even when stored at low temperature, vitamin E loses its efficacy after 2 years. This is in line with the less severe degradation of pumpkin seed oil stored at 6ºC before 2 years (Fig. 9).

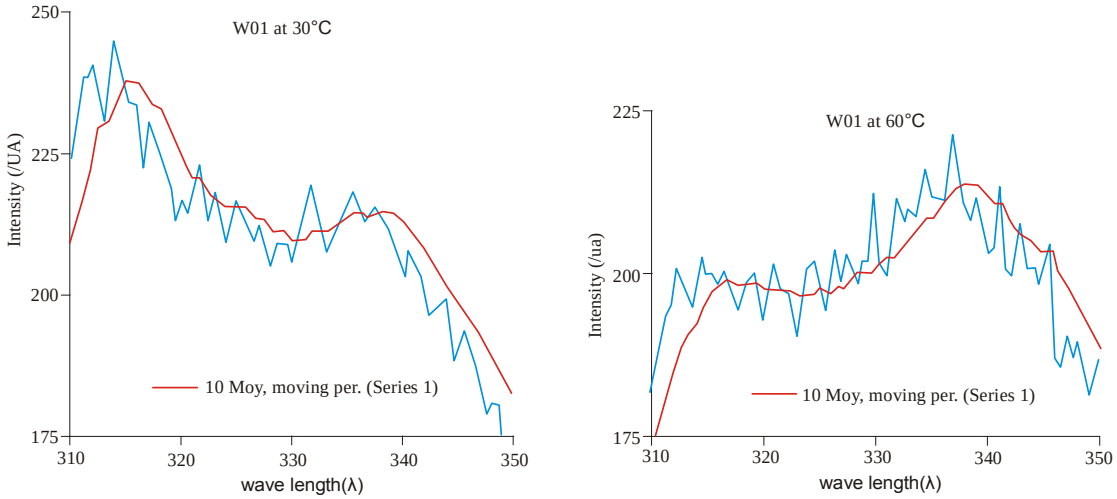

Fig. 8: Illustration of the overall behaviour of vitamin E in the case of sample W01

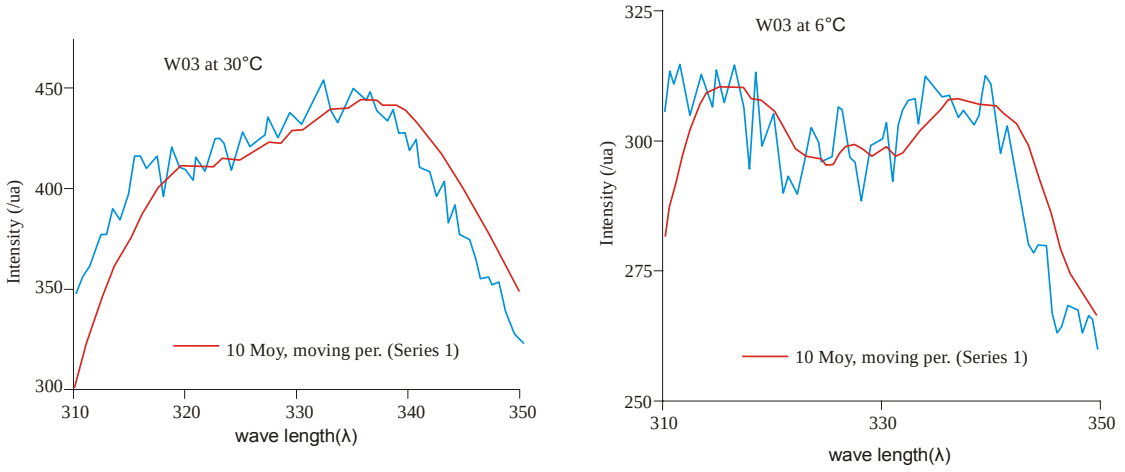

Fig. 9: Behaviour of E after 23 months in the two storage conditions: emission on the left, absorption on the right

This observation is consistent with the maximum emission intensities obtained with excitation at 270 nm. The greatest intensity was obtained for the sample W03 at 30ºC and the lowest for the "old" sample in the same conditions (Fig. 10). This indicates that the action of vitamin E and polyphenols was most intense between 12 and 23 months, the time when primary degradation products were accumulating. We note that these products undergo rapid secondary conversion and that this process is slowed by the build-up of these secondary products.

The emission spectrum recorded at excitation wavelength 350 nm confirms this observation. The polyphenols of pumpkin seed oil disappear very rapidly in oil stored at 30ºC, so that vitamin E is not regenerated in these conditions. However, at low temperature the disappearance of the polyphenols is slower, favouring the regeneration of vitamin E (Quiles *et al*., 2002; Fruhwirth *et al*., 2003).

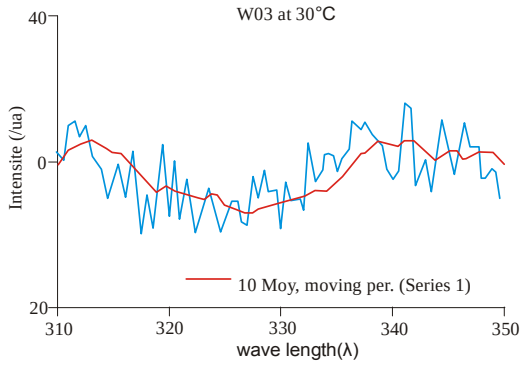

Fig. 10: Emission spectrum at excitation wavelength 270 nm showing the behaviour of vitamin E in the sample W04 stored at 30ºC. This sample was degraded, so low emission intensity was observed

Table 5: DSC peaks during heating of samples at 30ºC and 6ºC (Heating from −70ºC in 10 minutes to 30ºC at 5ºC per minute).

			Peak no.		
Sample			1	2	3
W04	BZV	*T* (°C)		- 30.1	3.9
		Δ*H* (J/g)		- 12.84	- 12.19
W03	BZV	*T* (°C)	- 44.3	- 32.8	0.3
		Δ*H* (J/g)	- 2.15	- 4.72	- 4.99
	CF	*T* (°C)	- 47.7	- 32.9	- 0.6
		Δ*H* (J/g)	- 5.78	- 0.99	- 0.54
W02	BZV	*T* (°C)	- 46.3	- 28.3	0.3
		Δ*H* (J/g)	- 1.34	- 18.15	- 16.94
	CF	*T* (°C)	- 47.5	- 27.6	1.5
		Δ*H* (J/g)	- 1.39	- 20.22	- 19.21

This result is consistent with the free-radical mechanism producing radicals that are resonance-stabilised by the benzene ring of the phenol compounds, and which then react by oxidation or self-condensation.

Differential scanning calorimetry: Differential scanning calorimetry (DSC) is one of the most often used analytical methods in the field of oils and fats. The overall behaviour of the main families of compounds that make up pumpkin seed oil at different storage temperatures was monitored by DSC (Tan and Che-Man, 2002a). Analysis of the chemical composition of pumpkin seed oil revealed noteworthy variations in FA levels.

Expressed per gram of plant matter, integration revealed three peaks during heating of the "young" sample W02 (BZV):

- One at -46.29°C corresponding to linolenic acid C18:3.
- One corresponding to a mixture of two closely similar compounds, linoleic acid C18:2 and oleic acid C18:1, with dominance of the first. This peak appeared at -28.28°C with a noteworthy difference between the two compounds, especially in quantity.
- One at -0.52ºC, presenting a slight shoulder. This was also due to a mixture of compounds with closely similar chemical structures: stearic acid C16:0 and palmitic acid C18:0. Palmitic was present in much larger quantities than stearic acid.

Similar results were also observed for W02 (CF): its first peak appeared at -47.47ºC instead of -46.29ºC, the second at -27.56°C instead of -28.28ºC and the third at +1.52ºC instead of +0.34ºC.

The increase in melting point indicated that the number of cross-links in the oil increased, i.e. that the number of links between molecules of the "oil" matrix increased, resulting in an increasingly crystalline structure. Thus the energy required to heat the pumpkin seed oil was greater (Tan and Che-Man, 2002b).

We kept to an analysis based on the melting points of the components. The crystallisation temperatures were very broad and so were harder to analyse, unlike the well-defined melting points (Table 5).

We can at least assert that the different values of enthalpy obtained are firm evidence that various complex reactions were taking place in the oil matrix. These reactions range from chain cleavage to chain lengthening, in an order that depends on the FA, and which results in a weak variation in the saponification index.

During ageing in storage the two major populations in pumpkin seed oil became homogenised: the different bands that characterised each constituent of the population merged into a single band, so that the number of peaks observed diminished (UserCom, 1/2000).

Comparing the samples W03 and W04 stored at 30ºC, we found that the activities of certain FAs decreased during ageing. After 2 years of storage the melting point curve of pumpkin seed oil showed only two peaks: one corresponding to C18:2 and the other to C18:0. Both peaks were broad, indicating that other compounds with closely similar chemical structures were also formed (UserCom, 1/2000). This result is consistent with the chemical composition: we showed that the level of C18:2 increased during storage and that this was due to stabilisation by the mesomer effect of the radical forming this FA, especially during the first two years of storage.

All the peaks visible at 23 months became practically invisible at 39 months, suggesting that most of the oil was degraded after 2 years in the Brazzaville conditions. This result is consistent with the florescence spectroscopy analysis of the vitamin E and polyphenols. It also confirms the increase in the non-saponifiable fraction. This is an encouraging finding for our rural areas where the local populations store oils in conditions far removed from those of refrigeration.

Examination of the cooling phase gave identical results. By contrast, examination of the heating phase confirmed that many intermediate compounds appeared during ageing of the pumpkin seed oil, and that the oil profile was markedly modified during the period when these intermediates were forming. On the other hand it is quite possible that this profile reverts to normal after the intermediates have disappeared. This step may well be the most important one in the degradation of the pumpkin seed oil during storage. It is marked by the disappearance of the unsaturated FAs and an increase in the non-saponifiable part of the oil, followed by an increase in the

levels of saturated FAs and above all the formation of long-chain saturated FAs, in particular C24:0, and also an increase in the levels of C20:0. These observations are supported by the fact that the melting points increase: the peak corresponding to C18:2 rose from −32.8ºC (W03) to −30.1ºC (W04) and the other from +0.3ºC (W03) to 3.9ºC (W04). This is evidence that the number of cross-links increases in the oil, causing its density and its refractive index to increase during storage.

The chemical composition indicated different levels of FAs, but in fact the oil was totally degraded after three years storage in the Brazzaville conditions, judging by the melting points, which are a criterion of purity. Clearly, a study based solely on the chemical composition gives results that are interesting but incomplete.

As the number of cross-links in the oil increases, the crystallisation temperature also goes up: the oil crystallises more easily, the energy of crystallisation greatly decreases, and we obtain a more organised crystal lattice. This is observed macroscopically as the oil becomes an amorphous, highly viscous paste smelling of varnish.

CONCLUSION

The ageing of pumpkin seed oil studied in this study shows us that mere analysis of physical and chemical indices, though important, is not sufficient to specify the state of degradation of plant oil, especially when storage conditions vary. Thus ageing is an unavoidable process that is more rapid in freshly extracted pumpkin seed oil and slower in less fresh oil. We also see that the origin may have no influence on the ageing of the oil, and that complex substances are formed. Also, the action of antioxidants in this oil is limited in time and could usefully be prolonged by additives, certainly before 22 months of storage at low temperature and much sooner in storage conditions at 30ºC.

The storage conditions at 30ºC were found to favour the degradation of pumpkin seed oil. At this temperature the FA carbon chains lengthen after *cis-trans* isomerisation. The presence of long-chain FAs and FAs with *trans* configuration severely impairs dietary quality (CAC, 2001).

Thus pumpkin seed oil is best stored at low temperature. At 30ºC it should not be stored longer than 2 years. Spectral analysis proved most useful, supporting or challenging the results obtained on the basis of physical and chemical indices or chemical composition, while giving insight into the processes at work in the oil matrix.

This study will be continued with a focus on the action of factors linked to storage conditions at 30ºC such as light (UV) and temperature, with the aim of promoting a large-scale production industry for the general good and in particular for the benefit of rural populations in Africa.

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