

Research Article

Accelerated Ageing Effects on Curcubitea Pepo Seed Oil

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Abstract: Accelerated ageing of curcubitea pepo seed oil was followed by simulation of UV (light) and ambient oxygen actions held separately then simultaneously in order to know the effects of these parameters on this oil resistance to deterioration. Nine withdrawals had undergone analyses by titrimetry supported by spectroscopic analyses notably MIR (Medium Infrared), DSC (Differential Scanning Calorimetry) and also a gas chromatography for the composition in FA. This study has showed that pumpkin seed oil displays a weak resistance to UV which results among other in decrease of unsaponifiable compounds. Its oxidization takes place very quickly in the presence of ambient oxygen. However the accumulated action of the two factors weakens to the highest degree pumpkin seed oil and these results in polymerization. It has also enabled us to show the link between the formation of reticulations in the oil matrix and variations at the level of the molecular structure of pumpkin seed oil.

Keywords: Accelerated ageing, curcubitea pepo, DSC, MIR, reticulation

INTRODUCTION

The big family of Curcubitea and notably the pepo species has been the subject of several studies. Goetz (2009) proved for example that pumpkin seedoil delays the inflammations of prostate; it was also proved that this oil has a positive effect on the regulation of blood flow, thus its use for the bodily massages (Sedghi *et al.*, 2008). Fruhwirth and Hermetter (2007) highlighted the importance of the biologic activities bound to this oil. Grondin *et al.* (2002) prepared datasheets for some tropical Curcubitea oils. Mvoula-Tsieri *et al.* (2008) compared the FA and the TAG of some Curcubiteas of the Congo-Brazzaville and Nakavoua *et al.* (2011) followed the ageing of this Curcubitea oil in two regions of the Congo-Brazzaville during their storage in two very different conditions.

All these studies have contributed to know and make better known the Curcubitea oil, however, in comparison with other edible vegetable oils; the oil studied in this study represents less than 1% of the world consumption in oil and vegetable fat. Olive oil for example, although widely-known represents only 3% of the human consumption. It is far exceeded by

soya oil (27%), palm oil (20%), colza seed oil (15%) and sunflower oil (12%). in the same way, the oils being the subject of this study are not represented in international trade, given the fact that they are only used in the regions of their production. (*FAO Corporate document repository*).

If the content of 58-60% in mono-unsaturated oleic acid triggered a real "rush toward colza", then strong reasons suggest that Curcubitea oil has a bright future provided its popularization proves to be successful. Indeed its composition in oleic acid is very close to 20% on average. In the United States, different studies show that the mono-unsaturated fatty acids, notably the oleic acid, exercise an extremely favorable influence on blood cholesterol.

In Northern Europe, colza is used for frying. On the other hand, for the meantime in France, since this oil contains more than 2% of linolenic acid, it is only allowed for dressing. This suggests that Curcubitea oils could serve as dressing oil in France. This regulation is highly based on the strong thermal instability of unsaturated oils. The speed of lipidic peroxydation increases indeed with the number of unsaturations of the fatty acids, those of the omega 3 families being the

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most fragile. The interactions and competitions exist otherwise between unsaturated fatty acids (the literature suggests that a high content of linoleic acid has a protective effect on oleic acid). During thermal treatments in the presence of the air, oil undergoes a deterioration mainly of oxydative type, the formation of oxygenated compounds being important. One can mention other anecdotal reactions occurring in these conditions: the "trans" isomerization and the formation of non-oxidized polymers or of cyclic compounds.

The process of auto-oxidization, for example, stakes radical chain reaction that entails the oxidization of the unsaturated fatty acids of oil. This oxidization drives to a reduction of the nutritional value of oil by deterioration of the essential fatty acids (linolic acid). Besides, it leads to the formation of various products of decomposition that confers it an undesirable odor and taste, or even presumably certain toxicity (Chimi *et al.*, 1990).

Some studies have been made to evaluate the deterioration of greases and oils during storage, after heating or frying. Most of them show that the important factors are the temperature, the surface/volume and aliment/oil ratio, the nature of the fatty acids and the content in oil antioxidants (Quiles *et al.*, 2002).

Oil ageing being an irreversible process that takes place most often in the presence of two main factors namely light (UV) and oxygen in the air, during this study, we simulated and accelerated pumpkin oil ageing with two factors taken separately and then simultaneously. The analyses achieved by titrimetry were confronted to the one obtained thanks to two spectroscopic methods namely MIR and DSC.

The spectra of our oils were recorded to determine the change of the properties of these oils during storage from the spectra collected in MIR (degree of insaturation, chains length and esterification degree). The relation between the intensity of the peaks in the MIR spectra of these oils and the impact of the factor considered was also studied in order to conclude on the behaviors of this oil.

These results came in complement to the study of already achieved ageing during storage and allowed us to open a big door toward the thorough knowledge of this oil notably its food qualities (importance of cis isomery in relation to the Trans).

MATERIALS AND METHODS

Pumpkin seeds: Seeds were bought at a local market in Dolisie (Niari) with already removed hulls. The seeds originated from Bouenza region in the south of the Congo-Brazzaville. They were ground using an electric grinder fitted with a rotor set at 2500 rpm. The powder obtained was then placed in a cartridge and the whole was loaded into a Soxhlet extractor.

Oil extraction process:

Solvent: Hexane was the extraction solvent and the extraction was carried out for 3 h in a soxhlet (Grondin *et al.*, 2002).

Oil: The obtained oil, of pale yellow color, was liquid at ambient temperature. It was stocked for months before the study in a non-transparent small bottle safe from light at 30°C. This sample was named W02.

Fatty acids composition: Esters of fatty acids were obtained after direct methylation: 2 drops of oil in 1 ml of hexane in the presence of 0.4 mL of sodium carbonate at 1N (in methanol) heated for one minute. Then 0.4 mL of hydrochloric acid at 1N in methanol was added followed by 1 mL of hexane (AOAC, 1995).

The chemical composition was determined by GC/FID with the help of an AGILENT 5890 instrument equipped with a Supelco FAMEScolumn 100 m long having an inside diameter 0.25 mm and thickness 0.25 µms. This instrument produced dihydrogen (H₂) that was used as carrier gas at a flow rate of 0.7 ml/min. Oven temperature was programmed as follows: rise until 140°C, then ramp for 5 min at the rate of 4°C/min to 240°C. The injector temperature was at 280°C according to a split ratio of 1/30 and injection volume was 1 µL. The detector temperature was 300°C at the rate of 40 ml/min for dihydrogen (H₂) and 450 mL/min for air and finally diazote (N₂) was the Make up gas at 45 ml/min.

Physico-chemical analyses: The principal physico-chemical indices were determined with reference to international and French standards (fat Body. FRENCH INDUSTRIAL STANDARDS AUTHORITY. 2000). we thus carried out the acid index I_A (NF IN ISO 660), the saponification index I_S (NF IN ISO 3657) and peroxide index I_P (NF T 60-220).

These principal indices were correlated with spectroscopic methods. Medium Infrared (MIR) was recorded with an IRTF NICOLET 760 Magna IR spectrophotometer. The analysis of the Ultra-Violet (UV) absorption of the samples were recorded with a Shimadzu UV-2101PC spectrometer fitted with an integrating sphere permitting to collect the whole of the transmitted intensity. DSC measurements were made with a DSC 822e enthalpic differential analyser (Mettler Toledo). And finally fluorescence emission and excitation spectra were recorded using a Perkin-Elmer LS-55 spectrofluorimeter.

Accelerated ageing: Accelerated ageing was carried out inside a glass reactor. It was made of two jointed glass tubes with only one ground outlet. Water

circulated in the big coulingtube while the diluted sample in solvent (2/8; v/v) was placed in the small inside tube, this last was equipped with a gas duct that enables to carry out splash mixings.

Ambiant oxygen action could thus be observed with an air splashing at 200 20 mL/min, the one of the UV was observed by the introduction of the reactor in a surrounding wall equipped with a mercury steam lamp with an estimated capacity 2 times the African sun in the zenith. The enclosure temperature was set at 35°C and was maintained thanks to an automatic cooler (fan) placed over the equipment. To face any rise of temperature, the room was maintained at 18°C with an airconditioner.

A magnetic stirrer was used to homogenize the mixture. Some withdrawals were made at specific times in order to monitor the oil behaviour during the 24 h that lasted the experience.

9 withdrawals were obtained at the following time intervals (in minutes): 0; 30; 60; 120; 240; 360; 480; 1440 and 1440.'

The withdrawal 1440' differed from the 1440 because solvent was removed in the rotavapor, as this one also evaporated the volatile products.

Spectroscopic methods: Medium Infra Red (MIR) Spectroscopy: A drop of the sample was placed between two KBr plates, then the spectrum was recorded at a resolution of 8 and 32 acquisitions. The spectra of main Fatty Acids (FA) were compared to those obtained for each sample. The MIR analysis of the spectra was correlated on the one hand with the saponification index and with the degree of oxidization on the other hand (Safar, 1995).

DSC: The sample (encapsulated) and the reference (empty capsule) were placed on platforms in the thermoelectric ceramicdisk that serves to transfer heat from the oven toward 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 zone thermocouples fixed under the platforms.

Unaponifiables fraction: The fraction oil that could not be isaponified carried the name of unaponifiables and was constituted notably of tocopherols (E vitamin): antioxidant agent (Light 1992). It was determined by NF norm T 60 -205-1 application.

RESULTS AND DISCUSSION

We have shown on the occasion of the study of ageing during storage in two very different conditions of pumpkin seed oil that the conditions of preservation in Brazzaville facilitates the alteration of this oil. Indeed, there are two principal factors in these conditions namely temperature (Heat) and light (UV), then an always present third factor: oxygen (air). Accelerated ageing is going to consist therefore in keeping oil in the presence of these enumerated factors and in analyzing the behavior of this one in order to establish a number of interrelationships that will enable to bring some enlightenments in understanding the individual or conjugated action of these factors in oil.

Lightaction:

Analysis by physico-chemical indices: This action gives the results summarized in Table 1.

One notices that all the physico-chemical characteristic values indices increase except the content in unaponifiable (Fekarurhobo and Obomanu, 2009). The peroxide index increases considerably by a ratio of 37; this observation is in agreement with the freshness of the sample considered W02 (three months at the time of this study) as we already showed it during storage ageing. UV treatment accelerates exactly the increase of the rate of hydroperoxydes in oil. Indeed the ultraviolet radiances induce the photo-oxidization of oxygen and generate the oxygen singulet, very reactive in terms of initiation of the ipidic peroxydation l (Kubow, 1992). This expected result was actually observed. One notes that the sample acidity nearly doubled, which proves that the action of light facilitates FA release and shows that pumpkin seed oil must not be preserved in the presence of solar light. One can say therefore that the action of the UV favours on the one hand TAG cuts in order to free FA, thus the increase of the measured acid indices and on the other hand, an accumulation of hydroperoxydes in pumpkin seed oil, thus the strong values of the obtained peroxides indices.

Light, even in weak presence, facilitates the formation of hydroperoxydes which are the first deterioration compounds that appear in pumpkinseed oil. These formed hydroperoxydes can then play the role of self-catalysers in the accelerated reaction mainly providing some radical alkoxy RO* (Chan and Prescott, 1976). It is therefore an oil that doesn't resist light and UV like peanut oil as shown by (Bacon and Douville,

Table 1: Summary of physico-chemical indices during ambient oxygen action

Values	Acid	Peroxyde	Saponification	Un saponifiable	Réfraction	Density
Before UV	7.99	2.47	199.76	1.13	1.4713	0.9117
After UV	13.93	90.67	201.25	0.92	1.4772	0.9398
Ratio	1.74	36.71	1.01	0.82	1.0040	1.0308

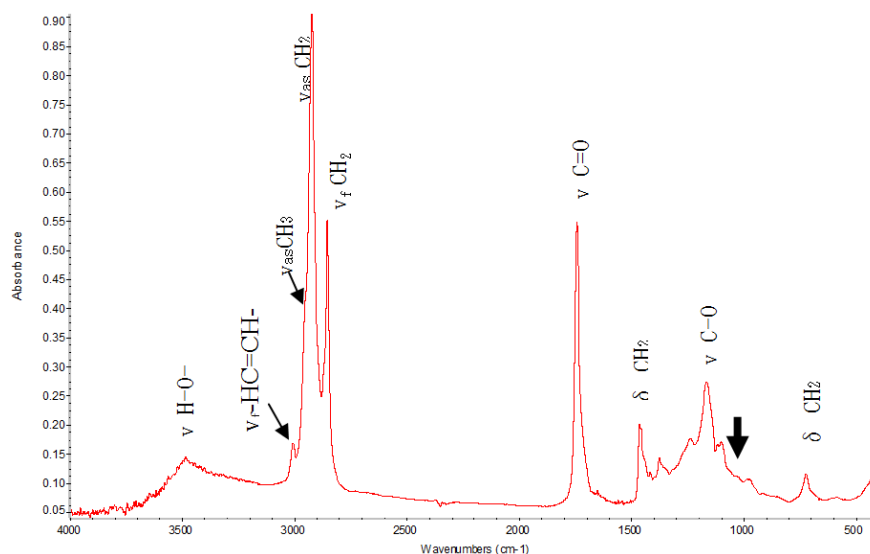


Fig. 1: W02 + UV. After 1440 min followed by a solvent evaporation

2006). The clear increase content in peroxides is the proof of big pumpkin oil oxidization. Indeed, the number of peroxides in oil is linked to the degree of its oxidization.

Table 1, gives us a nearly constant report of the variation of the saponification index (1, 01) which is very meaningful. One could think that the increase of free FA would facilitate the molecular rearrangements in order to get longer and longer FA carbon chains, which should result in a reduction of the saponification index. This observation allows thinking that the chains lengthening noticed in the study of ageing during storage are not certainly due to UV.

UV action reveals a decrease of content in unsaponifiable compounds. This result is contrary to the one obtained in ageing by storage, this action affects considerably unsaponifiable compounds. Indeed, a large part of the unsaponifiable compounds are unsaturated and aromatic molecules. Yet, these are very sensitive to UV, because the energy brought by UV acts on the double links and provokes its cutting or merely its relocation. This result is at the origin of the observed colour change during the manipulation of pumpkin seeds oil which then becomes whitish.

Physical indices globally have the same profile. Indeed the refractive index presents the same behavior as the density that means an increase.

This result allows to think that carbon chains underwent either lengthenings or cuts that had an action on the volumic mass and also on the oil capacity to reflect light, however the saponification index had not nearly varied. One can think therefore that this result is independent from the variation of the length of carbon chains or that the saponification index is not indicative

in this specific case of the real changes in pumpkin seed oil matrix.

Analyses by spectroscopic methods:

MIR: After 24 h the action of UV is meaningful enough. A band appears in the neighborhood of 3500 cm⁻¹ corresponding to the vibration of - OH groupings; This indicates an oil oxidization. Besides, the spectrum profile is the one of a long carbon chain FA (Cheikhousman *et al.*, 2005).

It is necessary to note that on the one hand the action of the UV seemed to have a great influence on the rate of oil insaturation (Fig. 1) and that on the other hand the 724 cm⁻¹ band is more intense in the sample before the beginning of UV action. However, this band is rather intense in unsaturated FA than in saturated FA (Cheikhousman *et al.*, 2005). This result is in agreement with the vibration near 3010 cm⁻¹ (face 2) corresponding to the « cis » lengthening vibration of the group = C-HS, this peak is not observable in « trans » isomers.

On the other hand, toward 970 cm⁻¹ the corresponding band to the vibration of distortion of the group = C-HS "trans" (arrow; Fig. 1) is also observed; this result is in agreement with the clear increase of the peroxide index. Indeed, the increase of the peroxides facilitated the "cis - trans" isomerization.

One notices that the corresponding band to the elongation vibration of the group = C-HS decreases in intensity (Fig. 2); this reduction is considerable during the first 4 h of the exhibition in UV. During this period the content of the unsaturated compounds "cis" decreases considerably in oil, a "cis-trans" balance seems to settle before this content starts to grow again in oil.

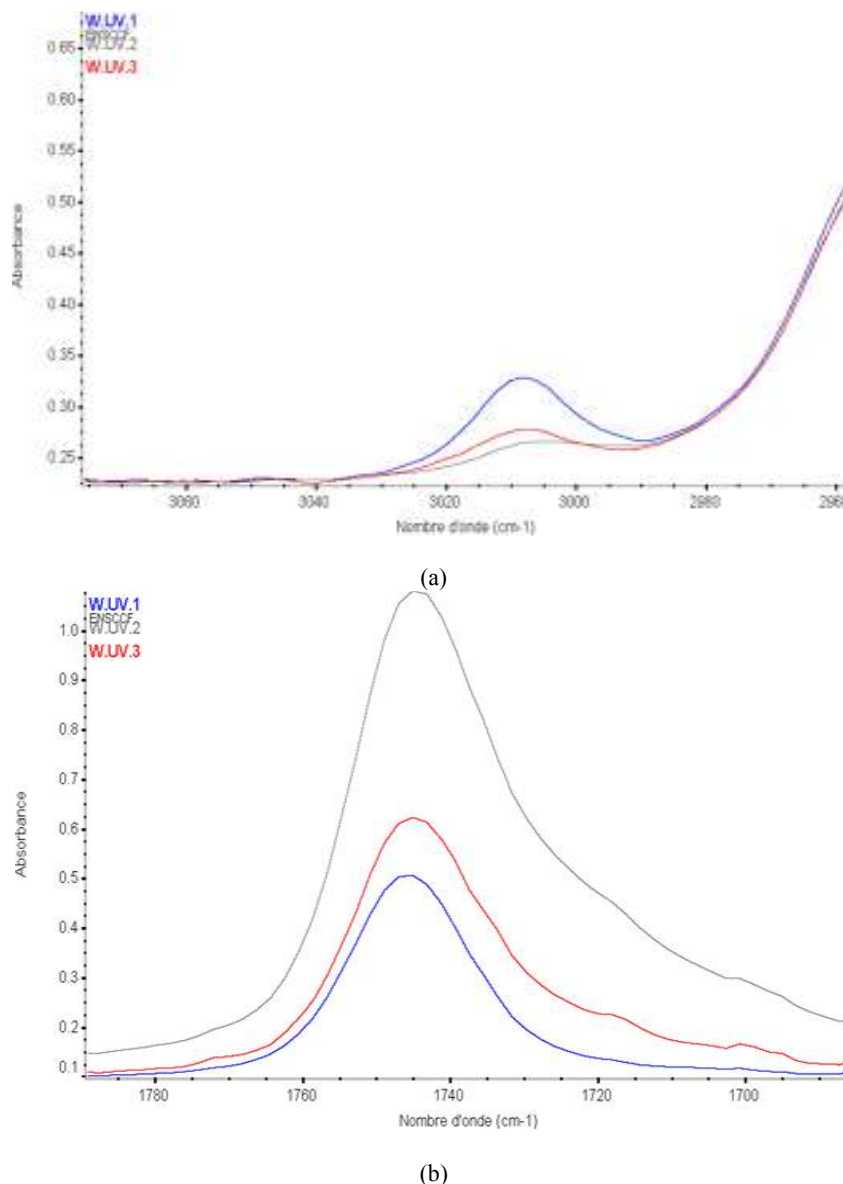


Fig. 2: Evolution of the grouping (a) and of esters (b) of FA during the action of UV (Blue: 60 min; Grey: 240 min; Red: 1440 min)

This result is in agreement with the apparition of the vibration band near 970 cm attributable to the vibration of distortion of the link = C-HS" trans ". Indeed, we have shown that the "cis" links turned into "trans" before these last disappeared during storage.

Considering Fig. 3, the oxidization rises then with the length of exhibition in UV up to a maximum and then decreases without ever descending below initial content.

Indeed, at 240 min the band corresponding to the esters vibration reached a maximum before decreasing again at the end a of 24 h exhibition. This result enables to say that the main UV action on pumpkin seed oil is

its oxidization. This oxidization happens in an increasing and progressive manner until a certain time before beginning to lower.

It is therefore during this period that all reactions bound to pumpkin seed oil ageing take place. This band, the most intense of spectra, is attributable to the vibration C = O group lengthening (Bertrand and Dufour, 2000), characteristic of esters (Fig. 2); the intensity of its peak is inversely proportional to the number of carbon atoms (Ahmed and Helal, 1977).

Indeed, at 240 min bands corresponding to the vibration of esters reached a maximum before decreasing again at the end 24 h exhibition.

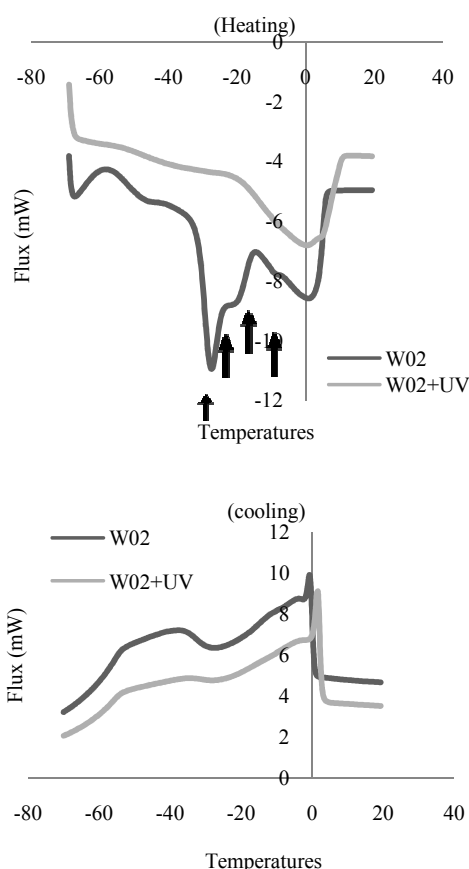


Fig. 3: Compared behavior compared between W02 sample having undergone UV action and W02 sample

This result also enables to say that the molecular rearrangement reactions take place in oil between the start and the 4th hour of exhibition.

The light action, therefore UV, confirms that pumpkin seed oil oxidation passes through an intermediate stage; this result was already highlighted at the time of the analysis of ageing during storage by DSC and by fluorescence spectroscopy. One can understand therefore why the number of reticulations increases at the start and decreases thereafter.

DSC: While analyzing the sample spectrum before UV action, one notices that during cooling, two peaks distinguish themselves, corresponding to the two major oil populations namely saturated FA (peak near -0.67°C) and unsaturated ones (large peak of -34.7° to -52.6°C). One sees very well In Fig. 3 that one of the two big populations of pumpkin seed oil disappears for the benefit of the other.

The profile is the same after UV action except that the enthalpies are notably extensively different during cooling; the first peak becomes more pointed

($+1.67^{\circ}\text{C}$), whereas the second is more flattened ($-32, 3^{\circ}\text{C}$ to $-52, 2^{\circ}\text{C}$). This result indicates two things:

- While ageing pumpkin seed oil requires less and less energy to solidify, that is to say that pumpkinseed oil solidifies more easily during cooling, it is the evidence that the number of reticulations increases in oil; there is formation of a more complex crystallin network. This result confirms the one already obtained at the time of the analysis of storage ageing. Therefore ramifications are created in the pumpkin seed oil matrix (Wright, 1986).
- The population of the saturated FA becomes more and more important in oil while the one of unsaturated FA decreases without disappearing. This result is in agreement with storage ageing study and also with the analysis of MIR spectra and confirms the disappearance of double "cis" links take place in pumpkin seed oil.
- So pumpkin seed oil that solidifies quickly or quickly enough can be considered as altered.

Examining heating, one realizes well that pumpkin seed oil actually presents two big populations; only, these populations are not homogeneous. Indeed the first peak -47°C (brown arrow Fig. 3) has a particularity, because it is the first to disappear during UV action. It is probably the C18:3. The second peak a lot more pointed marks an abundant enough population in pumpkin; it is about C18 :2; it appears at -28°C , its radical is consolidated by mesomer effect, the following peak near -22°C , less pointed, is the one of C18 :1

These last two peaks disappear during UV action and the immediate consequence is that the fusion is the widening of the two other peaks that are respectively those of C18:0 (-9°C) and C16:0 (1°C). One only peak appears after 1440 minutes of UV action, this observed behavior is in agreement with the analysis of oil composition in FA.

The energy of cooling (36.88 J.g-1s) is close enough to the one of heating (41.54 J.g-1s) which shows that a certain homogenization occurs in pumpkin seed oil.

The fact that the peaks move toward the positive temperatures proves that the reticulations increase and that the freedFA are used to form a more and more complex crystallin network that result in the formation of polymers.

Pumpkin seed oil must therefore be stored away from light. This piece of information is fundamental; in fact, in villages the peasants keep pumpkin seed oil in transparent small bottles and very often on a non-sheltered shelf from light.

Table 2: Summary of the chemical features during oxygen action

Values	Acid	Peroxyde	Saponification	Non saponifiable
Before oxyg	7.99	2.47	199.76	1.13
After oxyg	10.41	33.51	253.79	1.32
Ratio	1.30	13.57	1.27	1.16

Ambiant oxygen action:

Analyses by chemical indices: The action of oxygen results in two things:

- An increase of the formation of peroxides
- A systematic cut of FA carbon chains

In view of Table 2, oxygen is the determining factor of this study. In the presence of oxygen, pumpkin seed oil (80% of unsaturated FA) undergoes a much advanced change, the quantity of free FA in oil increases, which means that TAG undergo accelerated hydrolysis reactions.

FA carbon chains shorten during oxygen action on pumpkin seed oil. Indeed saponification indice increases considerably. This result is contrary to those obtained at the time of storage ageing study, but it is in agreement with the described reactions in the literature about the effects of oxygen that cause some cuts on double links. It is all the same what we learn from oxygen reactivity of and ozonisation with the formation of the compounds named epoxydes (Bourgeois *et al.*, 2001); the reaction can continue besides by epoxyde hydrolysis and can give a «trans» diol. Here, the reactions are radical, they result in a carbon-carbon link cut and an inversion of configuration; which explains the increase of the content of «trans» compounds in oil

and also the number of cuts of links therefore of carbon chains shortening.

The increase of content in unsaponifiable confirms the idea according to which saponifiable compounds at the start become unsaponifiable during biochemical reactions. Indeed, the unsaponifiable being in majority formed of aromatic and unsaturated compounds that that easily fix oxygen; it is besides the same phenomenon noted with the unsaturated FA that fix oxygen to give complex compounds. One can as well relativize this increase because the ratio is only 1.2 a very close value to 1; only such an approach would be far enough from reality since the presence of oxygen results in oxidization reactions. However the unsaponifiable consist to large extent of antioxidant compounds.

The noticed increase of saponification index and content in unsaponifiable is accompanied by the one of acid index. Indeed the oxygen in the air action also encourages TAG hydrolysis. However, the freedFA do not seem to have been re-used in the molecular rearrangement as we saw it previously. This approach seems quite difficult to understand.

It is necessary to note that peroxide index also increases, however this one is nearly three times less important than under UV action. This result enables to say that oxygen action does not encourage abundant formation of hydroperoxydes, but also that pumpkin seed oil resists the oxygen in the air actions when this one is not associated with UV.

Analysis by spectroscopic methods:

MIR: We note in Fig. 4 that, the blue curve and the purple curve are dominated in the first left of the

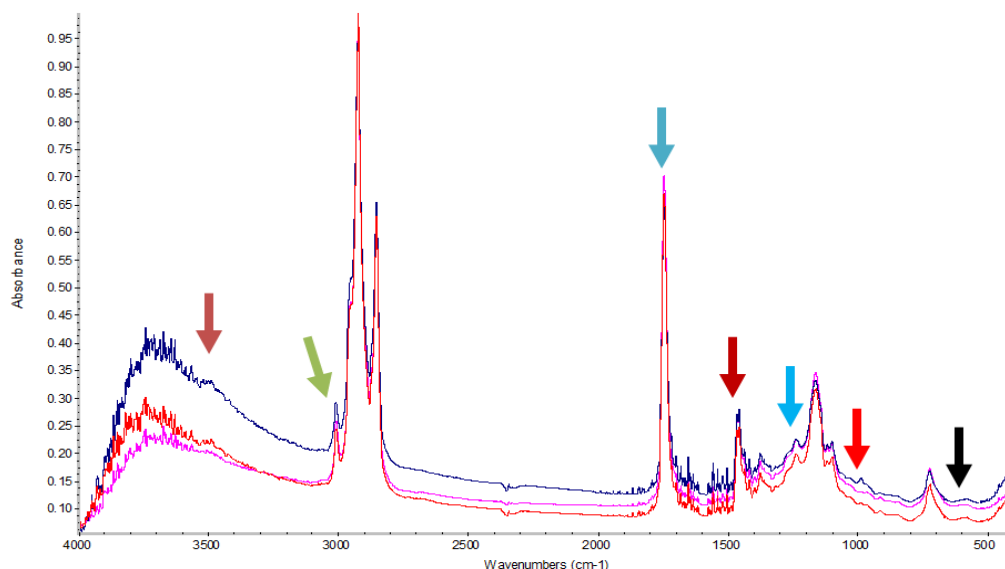
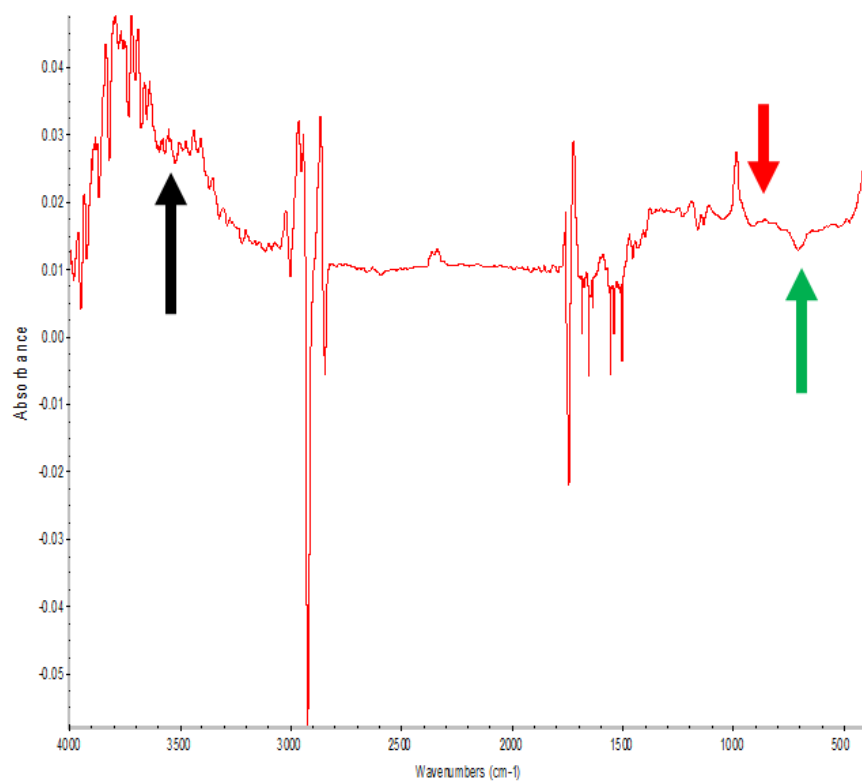
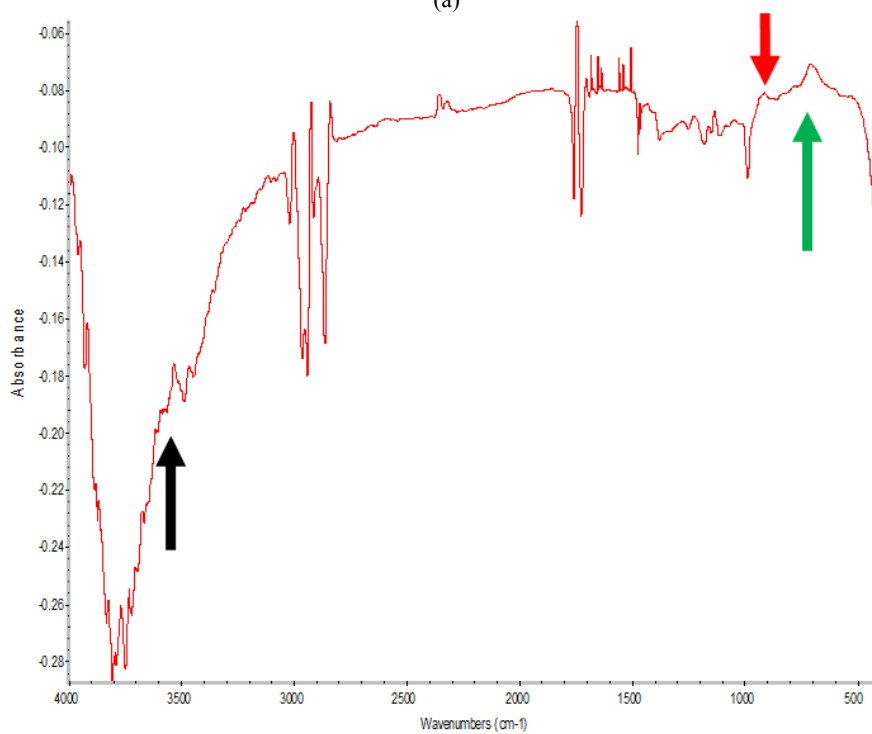


Fig. 4: Comparison of oxygen action on pumpkin seed oil; Red: 60 min; Blue: 240 min; Purple: 1440 min



(a)



(b)

Fig. 5: Subtraction of withdrawal spectra 240 and 60 (a), 1440 and 240 (b)

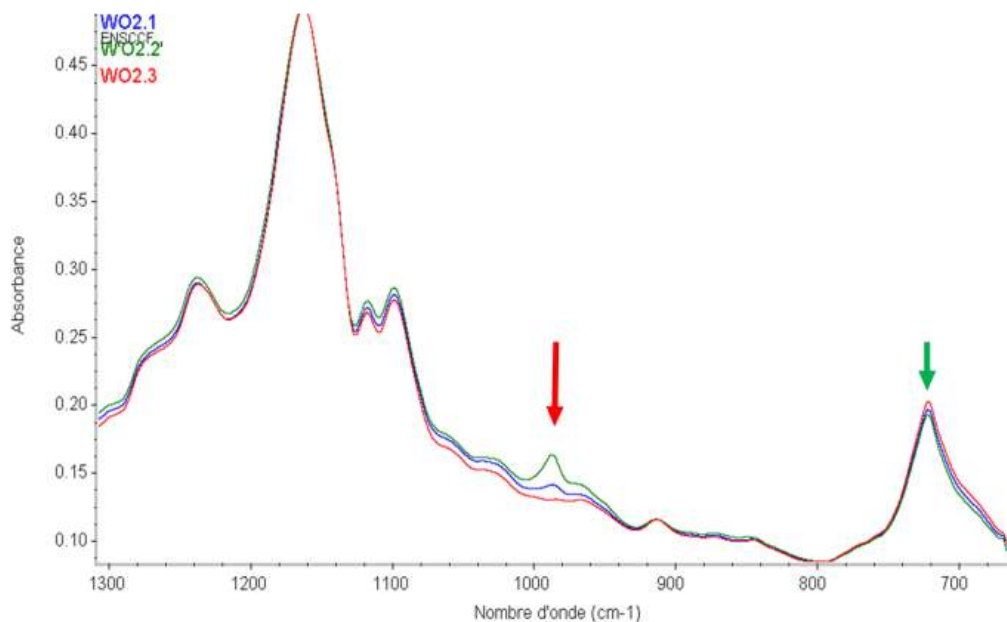


Fig. 6: Screen photo showing behavior variations of pumpkin seed oil matrix. (Blue: 60 min; green: 240 min; red: 1440 min)

spectrum by the red curve (2980 to 2850 cm^{-1}), which means that the vibrations due to them $-\text{CH}_3$ and $-\text{CH}_2$ are less important in the purple and blue curves.

This behavior is as reversed in the second part of the spectrum where the purple and blue curves take a real ascendant on the red curve (200 to 500 cm^{-1}). This profile is the one of short carbon chain FA (Ahlers *et al.*, 1953).

Indeed, Bertrand and Dufour (2000) showed that the absorbance intensity of vibration peaks of asymmetric and symmetrical elongations of $-\text{CH}_2$ toward 2924 and 2854 cm^{-1} increases with the carbon chain length. These observations confirm actually that the carbon chains become distinctly shorter after ambient oxygen action.

One notices comfortably (Fig. 5 on the right) that the band near 719 cm^{-1} (green arrow) is more important in the spectrum representing oil at the end of 1440 min of oxygen in the air action, yet the intensity of this band is inversely proportional to the carbon chains length.

The band attributable to stretching vibrations of the C-O group OH of esters near 1150 cm^{-1} is the same for the three withdrawals (Fig. 4); which does not give any other precisions because the intensity of this peak is also inversely proportional to the number of carbon atoms.

However, this result proves that pumpkin seed oil oxidization is a reaction that is independent from the duration of exhibition to oxygen. Indeed the three withdrawals nearly have notably the same degree of oxidization with regard to esters functions, however, $-\text{OH}$ grouping, increases very quickly between 60 and

240 min before decreasing during the action (Fig. 5; black arrow).

The vibration near 966 cm^{-1} is more marked on the green curve corresponding to 240 min. Conversely at the end of 1440 min (Fig. 6), there nearly is not any more peak; however this peak is due to the distortion vibration of the $=\text{C}-\text{HS}$ «trans» group. This result proves that oxygen in the air acts in the sense of the reduction of the rate of unsaturation oil.

Indeed the brown and blue arrows (Fig. 4) show that the degree of oil oxidization increases during this action by the formation of alcohols, the vibration band near 3500 cm^{-1} (brown arrow) proves it and so does esters formation : vibration band near 1450 cm^{-1} blue arrow) Fig. 4.

MIR spectra analysis results after ambient oxygen action on pumpkin seed oil are in agreement with the saponification indices, Indeed the determined saponification indices display a rise, which results in a relative reduction of the determined FA molar mass; in other words, by a decrease of FA carbon chains length.

Ambient oxygen has a specific action therefore on pumpkin seed oil, this one is characterized by a cut of FA carbon chains. This is in agreement with the high level of unsaturation (80% of pumpkin seed oilunsaturated FA). These results are identical to those found for peanut oil by Bacon and Douville (2006). The fact is that peanut oil has a very close overall composition to the one of pumpkin seed oil.

Oxygen in the air therefore has an action that facilitates FA carbon chains cuts followed by a relatively weak increase of contents in peroxides, in free fatty acid and in unsaponifiables.

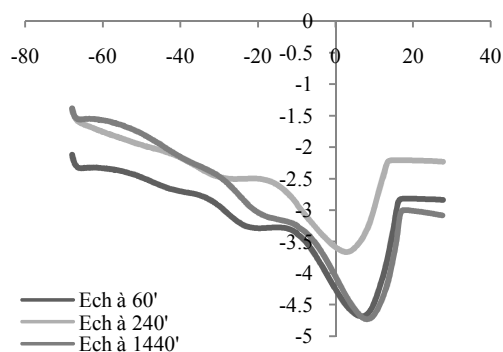


Fig. 7: Behavior of different AG families during air dioxygen action followed by DSC

Table 3: Fusion temperatures and enthalpies during ambient oxygen action

Sample withdrawals		W02		
		60 min	240 mn	1440 min
Peak n° 1	T en °C	-23.13	-29.78	-21.5
	ΔH en J/g	-1.38	-1.63	-2.97
Peak n°2	T en °C	7.19	3.10	6.16
	ΔH en J/g	-23.42	-26.60	-24.66

DSC : It enables us to notice:

- A decrease of reticulations during the first four h. Indeed, the red curve (240 min) presents only two peaks contrary to the one corresponding to 60 min of action (Fig. 7)
- Then reticulations increase beyond 4 h (green curve). Indeed the fusion temperatures increase, which means that the number intramolecular links increases and an organized crystallin network, forms itself. These observations are in agreement with the increase of peroxide and saponification indices.

One notices, two phases, the first marked by the decrease of temperature fusion between 60 and 240 min and the second marked by the increase of this temperature between 240 and 1440 min. However the fusion enthalpy increases notably during ambient oxygen action for peak n°1, the one that corresponds to unsaturated FA (Tan *et al.*, 2002a, b).

The decrease of fusion temperature indicates that the formation of reticulations is disfavored (Table 3). One can think therefore that during the first phase, the homolytic cut reactions are dominant. This result is in agreement with the rate of « trans » compounds (Fig. 7). On the other hand, during the second phase, the formation of reticulations becomes dominant, which explains the increase of fusion enthalpy. However, peak 2 displays a different behavior, the temperature and the

Table 4: Summary of physico-chemical indices during UV and oxygen accumulated action

VALUES	Acid	Peroxyde	Saponification	Non saponifiable	Refraction
Before	7.99	2.47	199.76	1.13	1.4744
Ox+UV					
After	19.08	98	231.83	1.21	1.4814
Ratio	2.39	39.68	1.16	1.07	1.0047

Table 5: Composition in FA evolution during accumulated action

Duration (h)	% C16:0	% C18:0	% C18:1	% C18:2	% C18:3	% C20:0
0	16.23	13.19	14.90	54.83	0.45	0.16
0,5	15.46	13.21	14.70	55.13	0.24	0.34
1	26.73	22.37	22.75	20.93	0.16	0.15
2	30.94	25.83	22.67	12.92	0.06	0.69
4	26.74	22.63	22.65	23.79	0.02	0.61
6	26.21	22.31	22.03	24.82	-	0.61
8	24.65	21.02	21.21	28.94	-	0.56
12	31.05	26.54	23.18	14.51	-	0.71
24	30.01	24.48	22.70	16.45	-	0.64

enthalpie of fusion evolves in an inverse sense. This behavior proves that other phenomena occur in pumpkin seed oil during ambient oxygenation.

Pumpkin seed oil is therefore very sensitive to ambient oxygen, which is in agreement with its composition in unsaturated FA.

UV+Oxygen action: In nature UV action is always done in the presence of ambient oxygen. We observed an identical behavior during UV and oxygen actions on pumpkinseed oil: the increase of content in peroxides and of acid index was observed. However the content in unsaponifiable presents an inverse evolution during the two actions. The study of the accumulated action of the two factors enables to understand the oil behavior during storage in solar light presence.

Analysis by physico-chemical indices: The results summarized in Table 4 show a big activity within pumpkin oil when the two factors act at the same time. Indeed, the content in unsaponifiable is almost steady; this is quite justified. We showed that the UV action decreases the content of the unsaponifiable part whereas ambient oxygen action increases it. So therefore the conjugated action of the two factors does not seem to have an important impact on unsaponifiable fraction.

The refractive index is almost steady, which means that the accumulated action of the two factors does not globally modify the pumpkin seed oil molecular disposition. This result is contrary to the one that gives the saponification index which grows by near 30 units during this double action, this result in a relative decrease of FA mass, therefore of carbon chains length (Table 4).

The values displayed for acid index are two times more; which shows the global degree of deterioration of

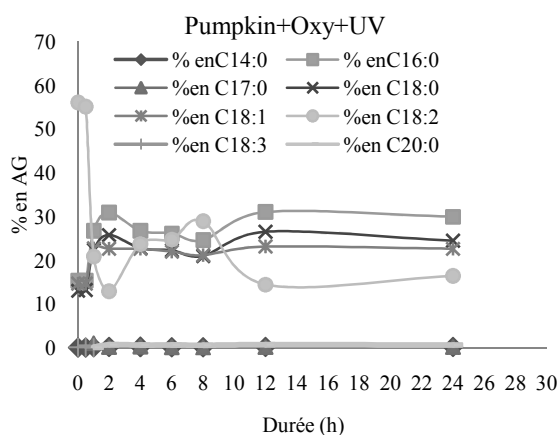


Fig. 8: FA contents behavior during accumulated action

pumpkin seed oil during this double action. The important number of free FA in oil proves that the deterioration of pumpkinseed oil is facilitated by this double action.

Pumpkin seed oil exposed to the action of the two factors undergoes a fast and very advanced deterioration. The value of peroxide index shows that the accumulated action of the two factors does not represent the good conditions to which one can submit pumpkin seed oil. The deterioration is influenced by the oxidization due to ambient oxygen which attacks double link molecules. This result is in agreement with those found by Bacon and Douville (2006) for olive and peanut oils. Which confirms that pumpkin seed oil does not resist light? Thus, this study enables to conclude that oils exposed to neglected storage conditions undergo an accentuated deterioration. Indeed, it is therefore interesting to note that an inadequate storage reduces meaningfully the pumpkin seed oil storage duration.

Composition in FA analysis: On looking at Table 5, the composition in FA varies greatly. The accumulated action of the two factors has a devastating effect on pumpkin seed oil, contents in FG considered. One notices an important decrease of content notably in C18:2 during the two first h of the exhibition, while the other FA contents especially C16:0, C18:0, C18:1 increase in the same period.

During this transformation several compounds of which content in percentage goes beyond 1% are formed, however, they disappear after 120 min. That is the case for the Butyrate of methyl whose percentage reaches 2.03% after 120 min before starting to disappear. Idem for methyl caproate. One must also note the unknown compounds of the used data base.

This behavior is contrary to the one displayed by the same AG during storage study. Two hour after the start of this action, the content in C18:2 increases, then decreases again after 8 h of exhibition. During this phase the contents of the other quoted FA display an inverse behavior.

All happens as if; the accumulated action of the two factors affects C18:2 a lot more. This result proves that a pumpkin seed oil poor in C18:2 is altered oil (Fig. 8). We can thus conclude that C18:2 is indeed the most stable fatty acid of pumpkin seed oil and that its decrease is one of the proofs of this oil change.

The increase of the content in C20:0 contradicts the molecular cuts indicated by the increase of the saponification index, something else therefore happens in the pumpkin seed oil matrix during this action.

Analysis by spectroscopic methods:

MIR: Given the fact that the saponification index increases considerably, it is therefore necessary to verify if AG carbon chains really undergo a substantial change.

The first information obtained thanks to the spectra is the one of oxidization that occurs since the 60th minute of exhibition. Indeed, the vibratin band near 3500 cm^{-1} (black arrow in Fig. 9) corresponding to the group -OH stretching vibration already appears on the green spectrum. The intensity of this band becomes very remarkable on the blue spectrum corresponding to 240 min exhibition.

This result that was not very clear at the time of separated actions becomes well visible. Kubow (1992) reported the same findings. Indeed, UV action facilitates that of ambient oxygen by production of very reactive singulet oxygen. UV thus enables oxygen atoms fixing on AG molecules.

The second information is the decrease of the number of unsaturated FA, indeed the vibration band of the group = C-HS « cis » near 3010 cm^{-1} (red arrow, Fig. 9) appears only on the green spectrum and not on the other spectra. It is an obvious proof of the change because the FA loses their double links « cis ». It is necessary to say that these double links do not all disappear in oil for in fact, when one observes these spectra, near 966 cm^{-1} (yellow arrow), a vibration band corresponding to the group = C-HS « trans » is notably respectively visible on the blue and red spectra within 240 min and 1440 min of exhibition.

Indeed, this band does not appear on the corresponding spectra of the 60 min withdrawal but it is well present on the two other with a bigger intensity in the case of the corresponding spectra to 240 min of exhibition (yellow arrow). Figure 10 (black arrow)

illustrates this state of fact. This result is the evidence that the double links do not immediately disappear during the double UV-ambient oxygenation but the « cis » undergo an action that transforms them into « trans » before disappearing. This is in agreement

with the geospecific reactivity of « trans » isomer. This result enables to propose an approach of explanation of the noted FA chains lengthenings, indeed the molecular rearrangements are chemically probable on « trans » than « cis » isomers.

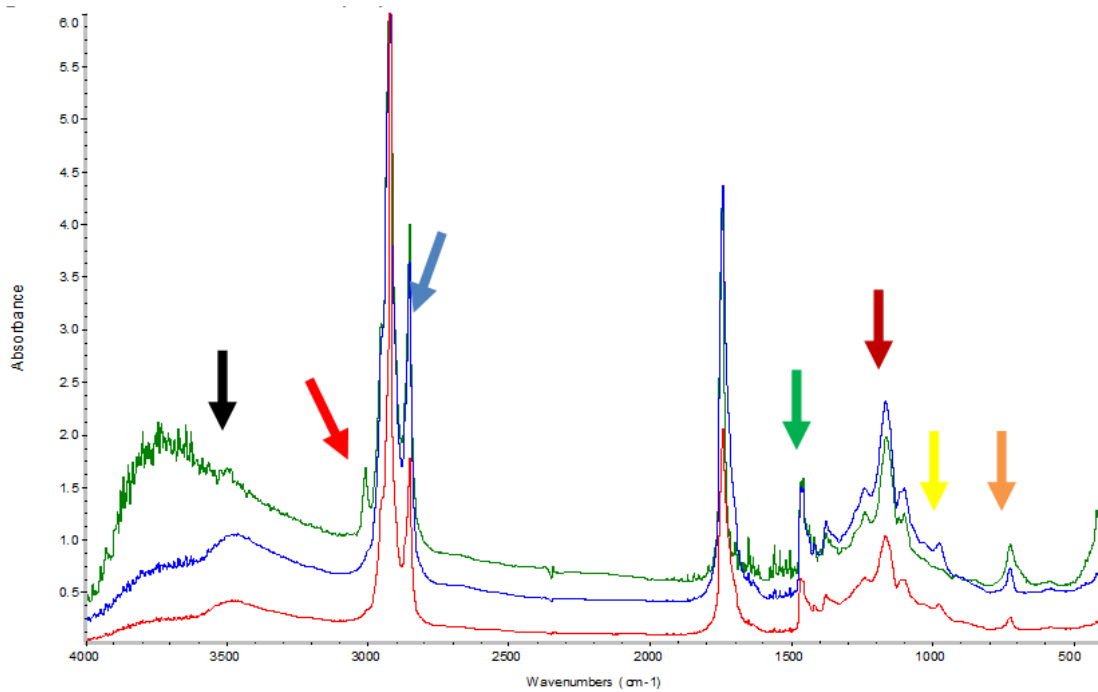
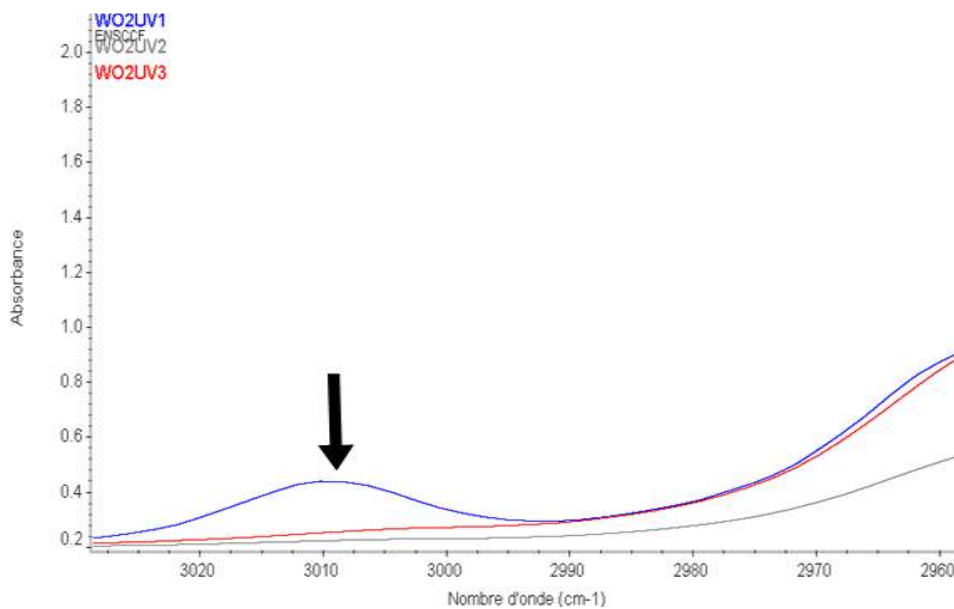
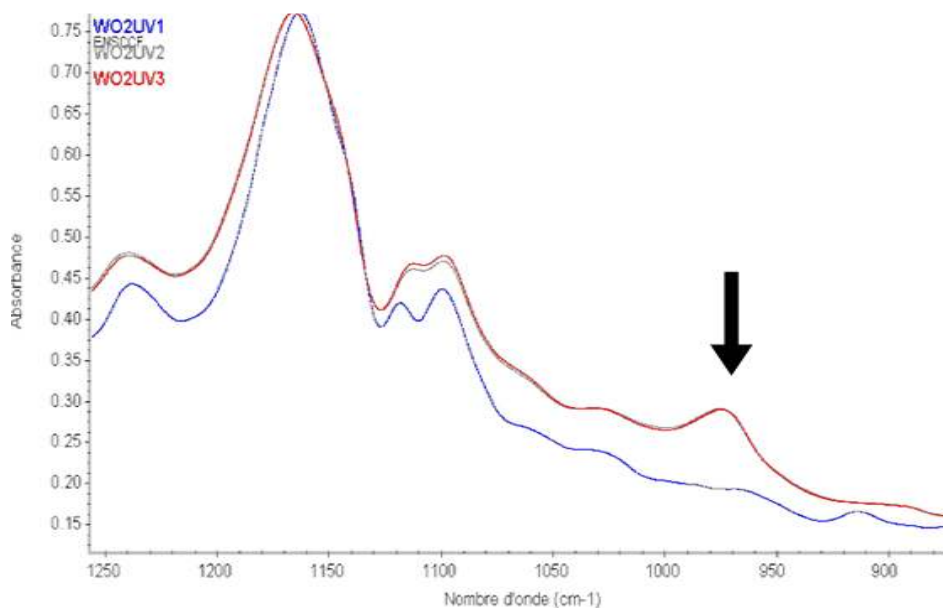


Fig. 9: Accumulated spectra of the 3 pumpkin seed oil withdrawals during double action. In green: 60 min; in blue: 240 min, in red: 1440 min



(a)



(b)

Fig. 10: (a) Group = C-HS « cis » vibration band, (b) « trans » in the neighborhood of 966 cm-1 in bleu: 60 min; in grey 240 min, in red: 1440 min

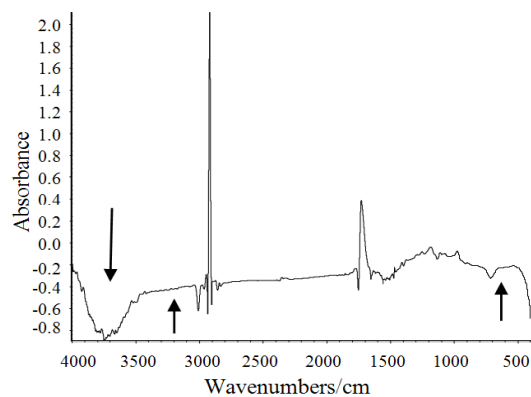
It is necessary to recall that natural FA is in predominantly « cis » and that the presence of the « trans » isomers in plant oil is a sign of a possible alteration. Indeed the oleic acid is "cis" its « trans » isomer that carries the name of elaidic acid exist only in plant oils having undergone a certain number of aggressive phenomena notably fryings, one knows that these are often done at temperatures ranging from 180° to 200°C.

Besides, the band near 3010 cm appears only on the spectrum corresponding to minimum deterioration (Fig. 10 and 11a: blue arrow). It disappears with the deterioration to the profit of the band near 966 cm.

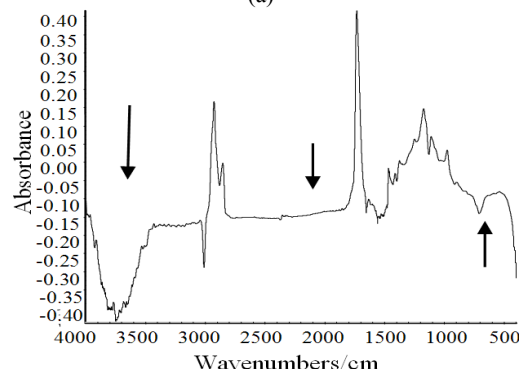
At 1150 cm one notices that the spectra at 1440 min present the weakest intensity. However this band attributable to the vibration of esters C-O group lengthenings is inversely proportional to chains length. While examining the zone from 1800 to 1400 cm corresponding to the vibration of esters C = O group lengthening, we notice that the spectrum at 240 min presents the most intense band vibration, which means that the degree of oxidization is maximal at 240 min of exhibition for pumpkin seed oil (Fig. 9).

Which proves that, the degree of oxidization does not increase anymore beyond four h of exhibition in accumulated action?

One notices, that the band near 716 cm (Fig. 9, 10 and 11: green arrow) is in favor of the 60 min withdrawal, this result proves that the carbon chains of this withdrawal is shorter because the intensity of this



(a)



(b)

Fig. 11: (a) Subtraction of 240 and 60 withdrawals spectra, (b) 1440 and 60

Table 6: Profile of FA families peaks in cooling phase during accumulated action

Sample Withdrawals		W02		
		60 min	240 min	1440 min
Peak n° 1	T en °C	-20.13	-24.71	-19.78
	ΔH en J.g ⁻¹	-1.61	-0.93	-1.30
Peak n°2	T en °C	8.33	5.84	9.60
	ΔH en J.g ⁻¹	-1., 62	-22 .19	-26.19

band is inversely proportional to the length of the carbon chains. This intensity decreases during the UV and the oxygen action, it is therefore the proof that the chains stretch out while oil deteriorates. This conclusion is contrary to the one obtained at the time of the analysis of the saponification index that increases of 30 units which results in a shortening of chains.

DSC: By DSC one notice contrary to all expectations that the different peaks are well present. However, at 240 min, the peaks are slightly displaced toward the left, which means that fusions temperatures decrease, yet when they decrease; it is a proof that the number of reticulations that means the number of links between matrix molecules decreases (Gloria and Aguilera, 1998), which facilitates the chains lengthenings.

All happens as if; the increase of the oxidization degree evidenced by the analysis of MIR spectra comes with a decrease of the number of reticulations in oil. After 24 h of exhibition, fusion temperatures increase, which shows that the number of links between oil matrix molecules increases, a crystallin network is, formed (Table 6).

This observation is in agreement with the decrease of the oxidization degree. So therefore an increase of the oxidization degree would result in a decrease of pumpkin seed oil fusion temperature and inversly, which has for immediate consequence the shortening of chains. We thus provide less energy to heat oxidized oil than non-oxidized oil.

Fusion temperature and enthalpy have the same behavior for peak n°1 (Table 6). A first phase revealing a decrease and a second phase during which the two parameters increase.

Their decrease implies an importance of carbon chains hemolytic cuts and their increase indicates that the number of reticulations becomes important in oil (Kowalski *et al.*, 1997).

However peak n°2 displays the same tendency at the fusion temperature level, but its fusion enthalpy increases all along ambient oxygen and UV accumulated action. This behavior suggests that at the end of 4 h of exhibition oil is in a phase of transition during which several reactions follow one another or continue, it is the case of « cis-trans » isomerization

forhomolytic cuts or reticulations formation. So therefore the conjugated UV-ambient oxygen action had for effect:

- An decrease of the unsaturations level that comes with an increase of peroxides
- An overall decrease of FA carbon chains length
- An increase of « Cic »/ »trans » isomerizationat FA level
- An increase of elasticity and viscosity that also reveals an advanced polymerization, oil
- Takes a varnish aspect and smell.
- a visible loss of the yellow color that characterizes pumpkin seed oil while becoming
- Whitish.

CONCLUSION

The UV and ambient oxygen present themselves as the most important factors that influence pumpkin seed oil ageing. From its composition in FA, more than 80% AGI, pumpkin seed oil is therefore naturally weakened (Murkovic and Pfannhanser, 2000). Indeed, we have showed during this study that UV action is mainly based on the double links favouring that of ambient oxygen. « Trans »isomers are created in the oil and these become the target of the molecular rearrangement reactions, which result in either a formation of a well organized crystallin network or incarbon chains length eningor cut within the oil matrix.

This study has showed among other that pumpkin seed oil must be kept in non-transparent containers and sheltered from solar light, which is not always the case in our countries.

The association of titrimetry and spectroscopyenabled us to highlight that an analysis merelybased on physico-chemicalvariation indices is important but not sufficient. Indeed, we noted an increase of 30 units of the saponification index which should result in a shortening of the length of carbon chains in the oil matrix, however, MIR spectroscopy gave us another reality of the molecular changes in pumpkin seed oil. There are probably other phenomena that occur within this matrix, that is why it is necessary, in prospects, to consider the temperature action on pumpkin seed oil and its photolysis.

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