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MECHANISMS OF SUNFLOWER OIL TRANSFORMING INTO FORCED THERMAL OXIDATION PROCESSES

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Abstract. This article investigates the processes that take place during the forced thermo-oxidation of sunflower oil. The results obtained in the study showed a major impact of thermo-oxidation. (90 ± 2 °C. supplied air with a speed of 8 ÷ 10 L·h^{-1} for 50 hours), on the physico-chemical indicators of the studied oil. The acidity index of fresh sunflower oil increased 13.7 times, with a value of 2.46 mg KOH·g^{-1} of fat for the thermo-oxidized oil compared to the initial value of 0.180 mg KOH·g^{-1} of fat. The thermo-oxidation of sunflower oil caused a significant decrease of the saponification index, which indicates a significant degree of polymerization and leads to viscosity increase of the studied sunflower oil. In order to highlight the impact of heat treatments, the analysis was performed by IR spectroscopy and the possible mechanisms of unsaturated fatty acids forced oxidation under the influence of thermal factor were analyzed. It was established that the applied treatment favoured both the formation of carbonyl secondary compounds and the simultaneous formation of hydroperoxides and triglycerides containing hydroxylated groups. The accumulation of hydroperoxides and triacylglycerides that have hydroxyl functions have facilitated the course of polymerization reactions, which are to increase the viscosity of thermo-oxidized studied sunflower oil. The formation during thermo-oxidation of the *trans*-isomers of poly-unsaturated acids led to the appearance of the *=CH* group deformation vibration band at 966 cm^{-1} in the spectrum of thermo-oxidized oil, simultaneous to a slight reduction in the intensity of the *=CH* group deformation vibration band at 1098 cm^{-1} at the respective *cis*-isomer of polyunsaturated acids.

Keywords: *sunflower oil, thermal oxidation, IR spectroscopy, peroxide index, acidity index, epoxides, trans- and cis- fatty acid isomers.*

Introduction

Lipid oxidation is one of the main problems of deterioration in food (edible oils and beverages) and cosmetic (skin cream) products, affecting their chemical, physical, and sensory properties [1]. In fact, lipid auto-oxidation and its inadequate storage contribute significantly to the deterioration of vegetable oils life causing changes in colour, texture, odor and aroma, as well as loss of constituent vitamins [2]. The understanding of lipid oxidation mechanisms in food and oils is deemed essential as we can construct effective prevention measures against the lipid oxidation in these commodities by identifying and targeting the responsible oxidation mechanisms. Oxidation of unsaturated lipids especially that of unsaturated fatty acids linoleic acid (LAOOH) and linoleic acid ethyl ester (ELAOOH), occurs according to three mechanisms: photo-oxidation, auto-oxidation and enzyme-oxidation, described in Figure 1.

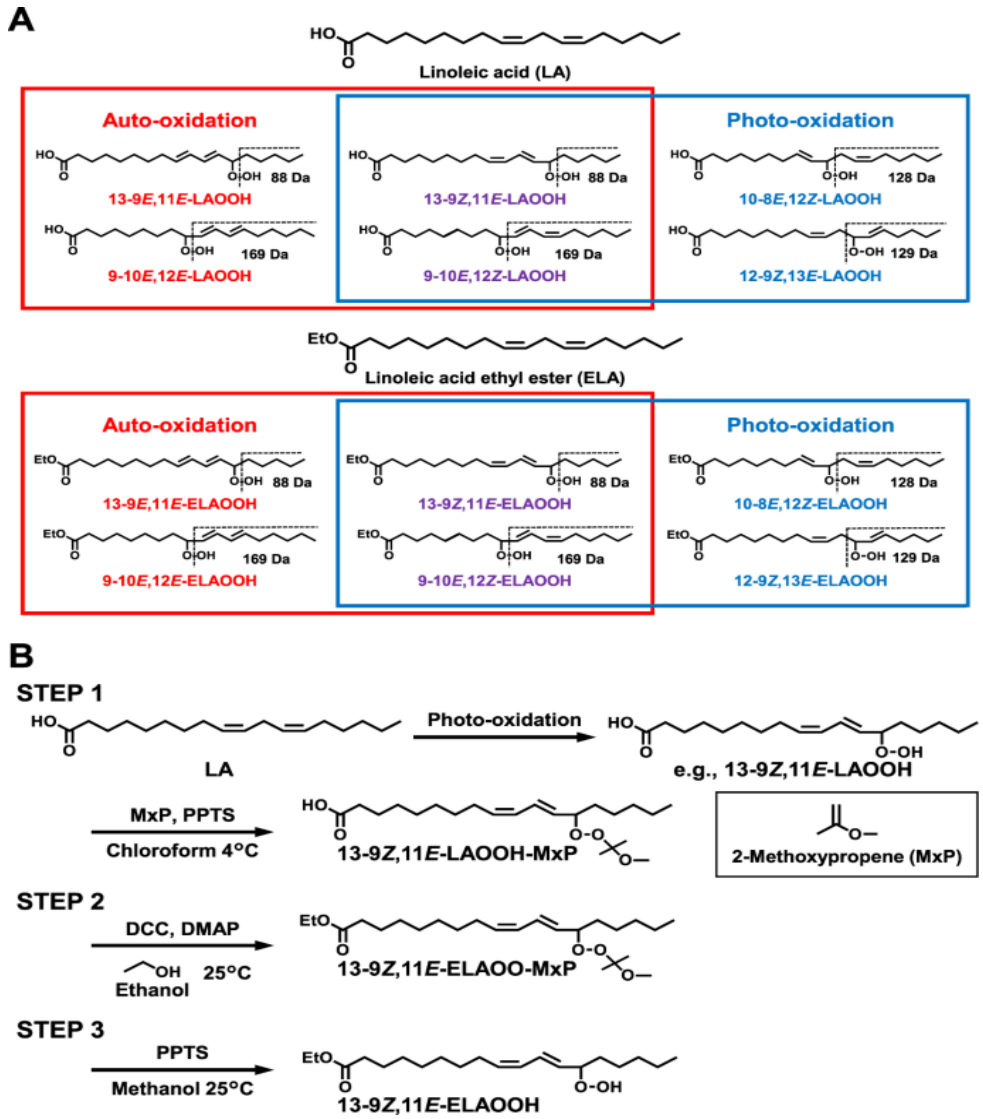


Figure 1. The chemical structures of six isomers of linoleic acid and linoleic acid ethyl ester depending on the type of oxidation (photo-oxidation or auto-oxidation) (A). The preparation schemas of linoleic acid ethyl isomers (B). (*13-9 E. 11 E-LAOOH; 9-10 E. 12 E-LAOOH; 13-9Z. 11 E-LAOOH; 9-10 E. 12 Z-LAOOH; 10-8 E. 12 Z-LAOOH and 12-9 Z. 13 E-LAOOH). and six isomers of ELAOOH (9-10 E. 12 Z-ELAOOH; 9-10 E. 12 E-ELAOOH; 10-8 E. 12 Z-ELAOOH; 12-9 Z. 13 E-ELAOOH; 13-9 Z. 11 E-ELAOOH and 13-9 E. 11 E-ELAOOH*).

The peroxide and acid indexes have become the most common indicators for lipid oxidation levels. These analysis methods do not provide enough information about the mechanisms of lipid oxidation.

In general, the mechanisms of lipid oxidation have been well known including photo-oxidation (singlet-oxygen induced oxidation) and auto-oxidation (radical oxidation), and it has been found that each mechanism forms different characteristic lipid hydroperoxides (LOOH) with different regio and geometric (*cis/trans*) isomers [3]. Photo-oxidation is initiated by UV light in the presence of photo-sensitizers (chlorophyll, riboflavin or hemoproteins) and catalyzed by singlet oxygen and enzymatic oxidation is initiated by yeast lipo-oxygenases.

Auto-oxidation is the most common fatty acids that can be subjected to this process both in free form and being combined (glycerol-lipids or glycol-lipids). Moreover, it has been observed that a poly-unsaturated fatty acid esterified in position *2* of glycerin molecule is better protected against oxidation than when it is esterified in position *1* of glycerin [4]. As well, lipids containing unsaturated fatty acids may undergo spontaneous peroxidation. Auto-oxidation is catalyzed by temperature, metal ions, free radicals, etc. This type of oxidation leads to the formation of numerous compounds, from unstable intermediate products of hydroperoxide type to stable final products (aldehydes, cyclic compounds, polar compounds and polymers). This spontaneous oxidation is an auto-catalytic reaction, which is responsible for the devaluation of food and the denaturation of tissues in living organisms.

The peroxidative change of unsaturated lipids can be caused by reactions triggered by free radical species such as peroxyl-radicals and non-radical species such as singlet oxygen. The latter is excited form of oxygen (O_2), very reactive from a chemical point of view, due to the presence of unpaired valence electrons. The formation of singlet oxygen is often related to the presence of a photo-sensitizer, which catalyzes the transmission of electrons excitation energy to molecular oxygen, with the formation of singlet oxygen, which is about 1500 times more reactive than triplet oxygen. Oxygen in its reactive form can attach directly to the double bonds of unsaturated fatty acids. In the absence of photo-sensitizers, an initiation step with free radicals is required to allow this fixation and formation of hydroperoxides [3].

Decomposition of hydroperoxides by heating or by the catalytic action of transition metal ions can produce peroxyl- and alkoxyl-radicals [5]. The formation of the peroxyl-radical is the main stage of the oxidation propagation stage during which free radicals "attack" fatty acids. Antioxidants can convert these radicals into an inactive, much more stable form, thus blocking this propagation reaction [6].

Hydroperoxide, as an unstable primary product, decomposes rapidly into secondary compounds, forming volatile compounds: aldehydes, acetones, organic acids, epoxides or polymers, which give a pungent and strong characteristic taste. This accelerates the oxidation of dyes, flavours and constituent vitamins, which makes the food containing oxidized unsaturated lipids completely rancid (accumulation of peroxyl-radicals) [7]. As well, reactive oxygen species (such as hydroxyl –OH or peroxyl-radicals –ROO•), formed in human tissue cells by endogenous agents and / or exogenous causes cause extensive oxidative damage, which in turn can contribute in aging, cancer and other human diseases [8, 9]. In order to control and reduce oxidative changes, nature uses several types of compounds, known as antioxidants, which react rapidly with free radicals at firsts stages of

the oxidative process, to delay or reduce the degree of oxidative damage of the constituent compounds [10, 11].

Experimental part

The study used 100 % native vegetable oil extracted from sunflower seeds species *Héliantalus Annus*. This oil contains a high proportion of linoleic acid in the form of fatty acid triglycerides (Table 1). A decrease in the proportion of this poly-unsaturated acid is used as an indicator of lipid oxidation.

Table 1

Chemical composition of sunflower oil used for thermo-oxidation (p <0.05)

Type of lipids	Contents, %
Total content	99.90
Triglyceride	99.20
β-sitosterol β-sitosterol	0.20
Fatty acid content of triglycerides as:	
- *saturated:*	10.82
C 16 : 0 Palmitic	6.46
C 18 : 0 Stearic	3.37
C 20 : 0 Arachidic	0.47
C 22 : 0 Behenic	0.52
- *mono-unsaturated:*	22.62
C 16 : 1 Palmitoleic	0.09
C 18 : 1 Oleic	22.38
C 20 : 1 Gadoleic	0.15
- *poly-unsaturated:*	66.41
C 18 : 2 Linoleic	66.41

The oxidation procedure of sunflower oil was performed according to the previously published method [12]. The experimental thermo-oxidation installation consists of a 1 L balloon capacity which is mounted on a hot plate, equipped with thermo-couple and temperature control. The air is supplied by a pump and the flow is regulated by a manometer. The device is equipped with a speed adjustable magnetic stirrer. Operationally, a volume of 500 ml of oil was introduced into the reactor, ensuring intense agitation. The oil temperature was maintained at 90 ± 2 °C and air was bubbled through the reaction mixture at a rate of 8 ÷ 10 $L \cdot h^{-1}$ for 50 hours.

Thermo-oxidation was performed under laboratory conditions that excluded the direct action of sunlight to exclude photo-oxidation of studied sunflower oil. At the end of the process, the thermo-oxidized oil samples were distributed in sealed 100 mL bottles and stored at 0 °C until used for analysis, and the fresh oil was stored under the same storage conditions. During the study, the following physico-chemical indicators of sunflower oil were assessed and evaluated dynamically:

- **Acidity.** The acidity of the oil was determined by the standard method [13, 14]. This indicator represents the amount of KOH (mg) needed to neutralize all the acids present in 100 mg of oil. The results are expressed in % oleic acid and calculated by the formula:

$$Ac = \frac{0.0282 \cdot V \cdot 100}{\mathrm{m}} \quad (1)$$

where: 0.0282 is the amount of oleic acid corresponding to 1 mL of 0.1N KOH, g;
V - volume of 0.1 N KOH used for titration, mL;
m - mass of fat taken into analysis, g.

- **Acidity index**. The acidity index expresses the amount of 0.1 N KOH (mg) required to neutralize the free fatty acids contained in 1 g of fat. This index certifies the duration and storage conditions. Fresh fats usually have a minimum content of acids, in vegetables fats this content is higher compared to that of animal fats. The acidity index of dietary fats must not exceed value of 3.5 and the calculation formula being:

$$I_a = \frac{5.611 \cdot V}{\mathrm{m}} \tag{2}$$

where: 5.611 is the 0.1 N KOH titre, mg;
V - volume of 0.1 N KOH used for titration, mL;
m - mass of fat sample taken into analysis, g.
Sometimes the acidity index is also expressed in degrees of acidity T (the degree of acidity is the number of mL 1 N alkaline solution, necessary to neutralize acids in 100 g of fat).

- **Saponification index**. The saponification index characterizes the molecular mass of the fatty acids that make up the fat. It is expressed by 0.5 N mg of HCl required to saponify 1 g of oil. This index was tested according to standard methods [15, 16]. and the value was calculated by the relation:

$$I_s = \frac{(\mathrm{a}-\mathrm{b}) \cdot 28.055}{\mathrm{m}} \tag{3}$$

where: a - the volume of 0.5 N HCl, used for the titration of the control sample, mL;
b - the volume of 0.5 N HCl, necessary for neutralization after saponification, mL;
m - mass of fat sample taken into analysis, g.

- **Iodine index**. The iodine index characterizes the amount of unsaturated fatty acids in the fat composition. This index expresses the amount of iodine (g) required to saturate the unsaturated fatty acids in 100 g of oil. The value of this index of sunflower oil does not differ considerably of vegetable oils. The standardized *Hanus* method was used to assess this indicator. The principle of the method consists in titrating the sample to be analyzed with 0.1 N sodium thiosulphate to the straw-yellow colour [17]. Then starch solution is added and the titration is continued until the blue colour has completely disappeared. This index is expressed in g $I_2 \cdot g^{-1}$ oil and is calculated by the formula:

$$I_{I_2} = \frac{0.1269 \cdot (V - V_1) \cdot \mathrm{n}}{\mathrm{m}} \tag{4}$$

where: 0.1269 - the corresponding iodine concentration at 1 cm^3 sodium thiosulfate $Na_2S_2O_3$, 0.1N;
V - volume of 0.1 N sodium thiosulphate solution ($Na_2S_2O_3$), used in the titration of the fat-free in control sample, mL;
V_1 - volume of 0.1 N sodium thiosulphate solution ($Na_2S_2O_3$), used in the titration of the fat analyzed sample, mL;
n - normality of the sodium thiosulphate solution used for titration;
m - mass of the analyzed sample, g.

▪ **Peroxide index**. Hydroperoxide is the main product of auto-oxidation of unsaturated lipids. The peroxide index directly measures the concentration of hydroperoxides formed in the initiation stage of the lipid oxidation process. This method of an iodometric titration, generally used for the measurement of hydroperoxides is recommended by the American Oil Chemist's Society as an official method [18]. The principle of this method is the titration by sodium thiosulphate the iodine (I_2) released after oxidation of potassium iodide by peroxides present in the studied oil. This standardized method [19] of analysis requires a relatively large amount of lipids and oxygen in the air, the presence of light and the absorption of iodine by poly-unsaturated acids can negatively influence the dosage. The advantage of the method is that it is simple and easy, and the peroxide content can be calculated directly and quickly by titration the released iodine by eq. 5 and 6. The equations of the reactions can be presented generally as follows:

$$ROOH + 2H^+ + 2KI \rightleftarrows ROH + I_2 + 2K^+ + H_2O \quad (5)$$

$$I_2 + 2Na_2S_2O_3 \rightleftarrows 2NaI + Na_2SO4 + SO_2 \quad (6)$$

▪ **The density and humidity** of the samples were determined by standard methods [20, 21]. The refractive index was measured using the Optika Abbe 2WAJ refractometer. The IR spectra of the analyzed oils samples were obtained at the Perkin Elmer Spectrum-2 FT-IR spectrometer, UK, wave numbers in the range 4000 ÷ 400 cm^{-1}.

Results and discussion

One of the objectives of this study was to investigate the forced oxidation of sunflower oil. For this, the thermal stability at 90°C was analyzed for a series of oil samples for a period of 50 hours and the following parameters were determined: the peroxide index: the acidity index, the oil acidity, the iodine index, the saponification index, refractive index, density and humidity (table 2).

Table 2

Value of physico-chemical indices of studied oil samples

Physico-chemical analyzed indices	Sunflower oil	Oxidized sunflower oil
Organoleptic characteristics	Fluid, straw yellow	Fluid, dark color and rancid odor
Peroxide index, *meq. O_2·kg $^{-1}$*	5.30 ± 0.70	144.5 ± 2.00
Acidity index, *mg KOH·g $^{-1}$*	0.18 ± 0.04	2.46 ± 0.10
Acidity, *degrees of acidity T*	0.09 ± 0.02	1.25 ± 0.06
Iodine index, *g I_2· 100 g $^{-1}$*	1.23 ± 1.20	0.80 ± 1.40
Saponification index, *mg KOH·g $^{-1}$*	192.60 ± 2.00	183.79 ± 1.30
Refractive index	1.463 ± 0.035	1.476 ± 0.025
Density at 20°C	0.912 ± 0.020	0.983 ± 0.020
Humidity, %	0.100 ± 0.02	2.00 ± 0.20

According to the values in Table 2, the peroxide index increased exponentially (27 times) fact that indicates a drastic forced oxidation of studied sunflower samples. In the case of heat treatments oxygen is the main oxidizing agent that regardless of how the oxidation reactions are initiated. In the normal state the oxygen is in triplet form as $3O_2$, where the last two electrons are on the π*2p (yz) orbitals and have parallel spins (S with value 1 of

total spin moment). Thus, the oxygen molecule in the ground state can easily interact with species that possess uncoupled (radical) electrons. But the activation energy of the reactions between molecular oxygen and molecules with coupled electrons is very high: between 146 and 273 kJ·mol^{-1} [22]. So, direct fixation of triplet oxygen by fatty acid chains is impossible. Among other electronic configurations of oxygen there are two forms: $^1\Delta$ g and $^1\Sigma$ g$^+$ which correspond to singlet states $^1O_2$. The first includes the two electrons with anti-parallel spins, located on the same anti-caking orbital and the second – on both anti-caking orbital.

The first is extremely electrophilic and reacts easily with the double bond and the second is unstable and turns into the first which can easily initiate oxidation (figure 2).

(7)

Figure 2. The mechanism of addition of singlet oxygen to the double bond [3].

Unstable intermediates are immediately converted to more stable hydroperoxides. The addition of singlet oxygen to linoleic acid occurs 1000-1500 times faster than the addition of triplet oxygen. The primary products have mixtures of conjugated and un-conjugated hydroperoxides.

Another mechanism for initiating lipid oxidation involves the thermal decomposition of hydroperoxides [23]. This endothermic reaction is favoured by high food processing temperatures:

$$2\ ROOH \rightarrow RO + H_2O + ROO\bullet \quad (8)$$

The alkyl radicals produced in the initiation step interact rapidly with molecular oxygen, forming ROO • peroxyl-radicals. These, being unstable quickly capture hydrogen atoms from unsaturated fatty acids in the vicinity, forming hydroperoxides and generating new lipid radicals:

$$R\bullet + O2 \rightarrow ROO\bullet \quad (9)$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet \quad (10)$$

In the case of poly-unsaturated acids, oxygen is fixed at one end of the conjugated double bond. Rupture of hydrogen from carbon 11 generates the formation of a delocalized pentadienyl radical in the case of linoleic acid. Fixation of oxygen to this radical in positions 9 or 13 leads to the formation of two radicals: 9 (Z), 11 (E) 13 peroxyl – and 10 (E), 12 (Z) 9 peroxyl-linoleic which lead, after the fixation of hydrogen at the formation of the respective hydroperoxides. Breaking the C-O bond in one of the intermediate hydroperoxides (β – fragmentation) generates the initial pentadienyl radical. If this rupture is followed by a rotation of the bond between the carbon atoms 12 and 13 a new radical with *trans* preferential geometric positioning is formed. The addition of hydrogen to carbon

9 will generate the formation of 10 (E), 12 (E) 9 linoleic hydroperoxide similar to the mechanisms described in the bibliographic sources [24, 25].

Completion of the reaction occurs at the time of interaction between two radical species, with the formation of stable compounds. As the number of present species and possible combinations is very large, various mechanisms may intervene as:

$$RO\bullet + R\bullet \rightarrow ROR \quad (11)$$

$$2\ R\bullet \rightarrow RR \quad (12)$$

Thus, compounds with higher molecular weight (dimers and oligomers) are formed, where the bonds are formed on the basis of ether or peroxide groups. They can have a significant impact on human metabolism. Polymerization reactions are especially favoured by high temperatures (for frying oils, for example).

The results obtained in the study showed a marked impact of lipid oxidation on the physico-chemical indicators of the oil (table 2). The acidity index of fresh sunflower oil increased 13.7 times, with a value of 2.46 mg KOH $\cdot g^{-1}$ oil for the thermo-oxidized samples compared to the initial value of 0.180 mg KOH $\cdot g^{-1}$ oil. The acidification of the oil samples were due to the hydrolysis reaction of glycerides, this process is favoured by the water contained in the oil even in the form of traces. The intensity of the hydrolysis reaction depends on various factors, including the temperature applied to the oil, as well as its water content. These two factors contribute to the hydrolysis of triglycerides to produce mono- and diglycerides (free fatty acids and possibly glycerin). Free fatty acids accumulate in the oil, thus increasing its acidity more than 10 times.

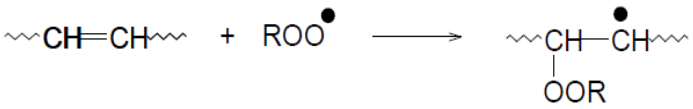
Thermo-oxidative treatment leads to a reduction in the total degree of unsaturation sunflower oil. This change was assessed by measuring the iodine index. This index makes it possible, in fact, to measure the degree of general unsaturation of oil. Our results show a significant decrease in the value of this index after the thermo-oxidation of sunflower oilsamples. The iodine index measured in fresh sunflower oil is estimated at 1.23 mg $I_2 \cdot g^{-1}$, while in thermo-oxidized sunflower oil it is only 0.80 mg $I \cdot g^{-1}$, fact that can be explained by the oxidation of double bonds and / or their involvement in active polymerization processes.

Increase of humidity of thermo-oxidized sunflower oil during the study, could be due to the formation of water and volatile products during oxidation reactions. In fact, water and CO_2 are some of the final products of the break-down of unsaturated fatty acid hydroperoxides. The humidity of the oil also increases due to the supply of water vapor from the air supplied through the system.

The thermo-oxidation of fresh sunflower oil caused a decrease in the value of the saponification index. In the thermo-oxidized oil, a value of 183.79 mg KOH$\cdot g^{-1}$ of oil was recorded. The saponification index provides information on the average molecular weight of fatty acids that are part of the glycerides of oil.

The decrease in the saponification index in the thermo-oxidized sunflower oil indicates a higher degree of polymerization acid. The increase in oil density is also explained by the thermo-oxidative polymerization. The formation of polymers can cause the viscosity to increase simultaneously with the increase of the chain length and decreases with the increase of the degree of oil unsaturation [26].

During the oxidation of alkenes epoxides can be formed by the addition of hydroperoxides to the double bonds, which contributes to the increase of samples saturation and viscosity degree according to the mechanisms described schematically by equations 13 and 14 (formation of epoxies by the addition of hydroperoxides to the double bonds).



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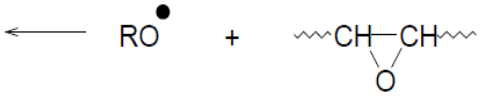

(14)

The formation of epoxides during thermo-oxidation can also occur in other ways described in Figure 3. These chemical reactions also justify the saturation of the fatty acid chains and the viscosity of the oil during thermo-oxidation.

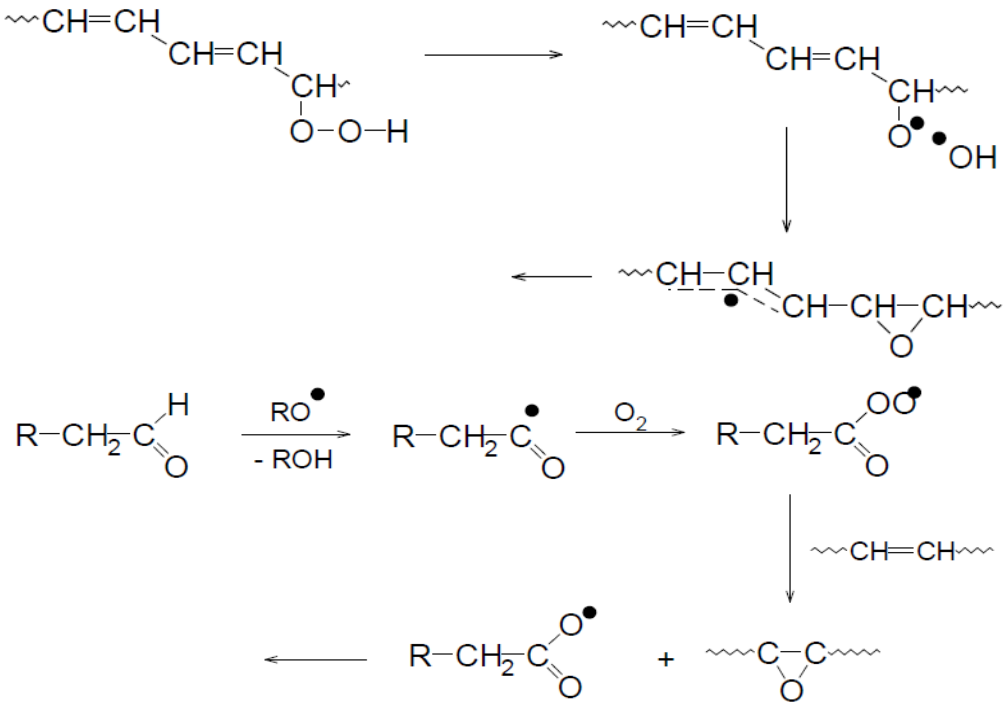

Figure 3. Possible ways of forming epoxies during thermo-oxidation.

Analyzing the results, a sharp increase in the value of the peroxide index was found simultaneously with the decrease of the iodine index, which demonstrates the existence of an intense oxidative process.

The oxygen present in the fresh sunflower oil and the one introduced experimentally by supplied air activates a series of reactions, which generate free radicals and hydroperoxides.

The peroxide index is an indicator of the presence of hydroperoxides and hydroxyperoxides in oil, called primary degradation products. Its value indicates on the degree of freshness of an oil, the smaller it is, the fresher the oil.

The peroxide index varies between 5 and 20 for crushed native oils and between 0 and 1 for refined oils.

The fresh sunflower oil used in the study had a peroxide index of 5.30 meq.O_2·kg^{-1}, while in thermo-oxidited oil this index increases and reaches the level of 144.5 meq.O_2·kg^{-1}. This value indicates a deep alteration (rancidity) of the heated oil. We attribute this dramatic increase in the peroxide index to the combined effects of high temperature and contact with air during prolonged heating of the oil, which induces the formation of peroxides. Similar studies on thermo-oxidized lipids suggest that an increase in oxidation temperature leads to the decomposition of hydroperoxides and therefore to a decrease in the peroxide index with the formation of oxidation by-products such as aldehydes [21]. These authors suggest that the activation energy of the decomposition reaction of primary oxidation products is significantly higher than that of their formation. In conclusion, being given the instability of peroxides, we suggest that the peroxide index cannot serve as a single parameter of the degree of oxidative degradation of oils. Therefore, other complementary analyses are needed, such as IR spectrometry, which allows the characterization of lipid thermal decomposition products. To highlight the impact of heat treatments the IR spectroscopy analysis of fresh oil and thermo-oxidited oil samples was performed (figure 4 (a) and (b)).

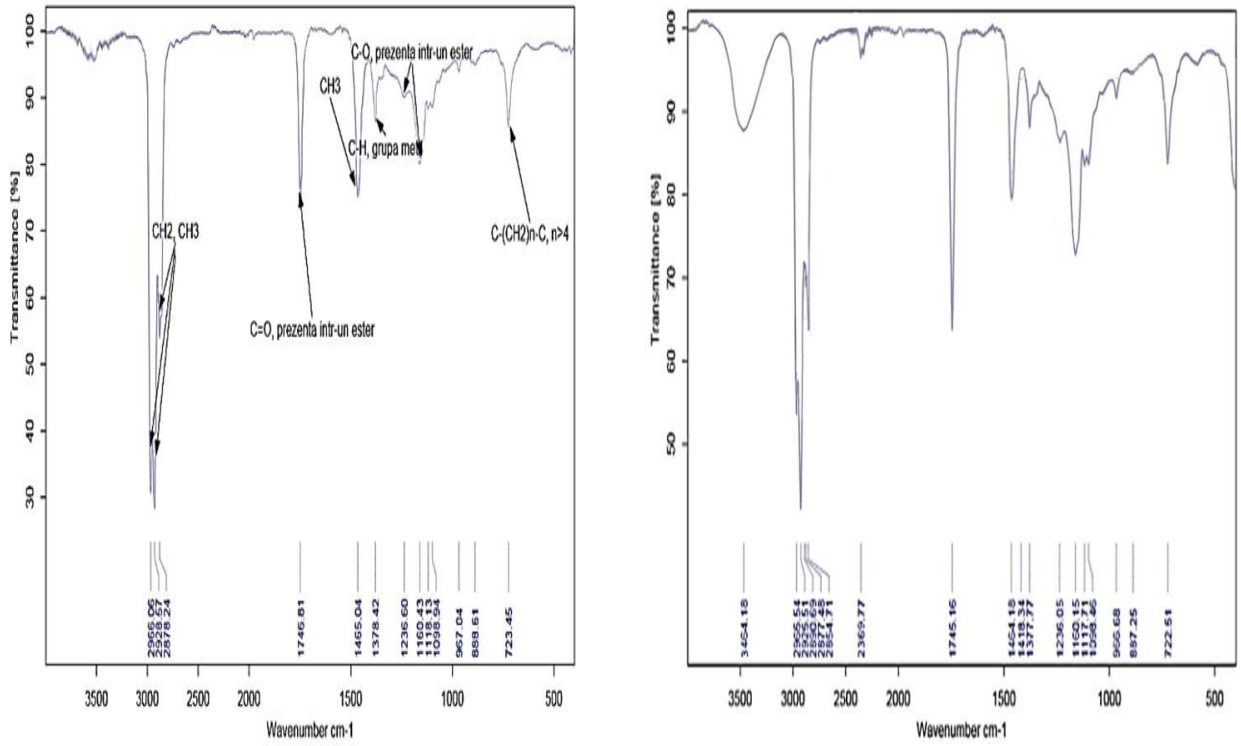

Figure 4. IR spectra of fresh (a) and thermo-oxidited (b) oil samples.

The freshness of the sunflower oil used in our study was confirmed by the low band intensities of 3500 cm^{-1} and the absence of intense absorption bands in the region of the assigned low frequencies (< 600 cm^{-1}), respectively, of primary and secondary oxidation products. This result confirms the low value of hydroperoxides in fresh oil. Auto-oxidation or oxidative rancidity is the main cause of quality loss in native and refined oils during storage [27].

The thermo-oxidative treatment applied to the oil caused the appearance of molecular and rotational vibrations in the initial structure of triglycerides and fatty acids, which explains the change in intensity or the appearance of new absorption bands in the low frequency region (at values less than 500 cm^{-1}) of infrared spectrum of sunflower thermo-oxidited oil [28]. In the IR spectrum (figure 4) of oxidized oil there is a marked increase in the intensity of the wide absorption band in the range 3600 ÷ 3200 cm^{-1} and maximum at 3465 cm^{-1}. Its occurrence is due to the elongation vibration of OH groups in hydroperoxides, water or triglycerides containing hydroxyl groups. Because in the conditions of accelerated thermal oxidation the hydrolytic process is favored, the increased absorption at these wavelengths can also be attributed to the accumulation of free fatty acids. From the comparative analysis of IR spectra it can be seen that the intensities of the peaks at 2928 cm^{-1} and 2878 cm^{-1} in the oxidized oil spectrum decreased, with the shortening of the hydrocarbon chains of fatty acids and the decrease in the total number of triglyceride double bonds. This result is confirmed by the decrease of the iodine index and supported by the increase in absorbance to 1745 cm^{-1}, attributed to the increase in the concentration of aldehydes, which are secondary oxidation compounds responsible for the rancidity effect.

Taking into account the increase in the viscosity of the thermo-oxidized sunflower oil, it can be stated that the thermo-oxidation conditions established in our study favoured the accumulation of products with high molecular weight. The absorption bands at 1464 cm^{-1} and 1377 cm^{-1} in the IR spectrum of thermo-oxidized oil are wider and have slightly higher intensities than those recorded at 1465 cm^{-1} and 1378 cm^{-1} in the IR spectrum of fresh sunflower oil. In addition to these bands, thermo-oxidation induced the appearance of new bands at 2369 cm^{-1} and 2335 cm^{-1} and corresponding to the vibration of – CH_2 groups and the asymmetric elongation vibration of groups of – CH atoms in triacylglycerides [28].

The formation during thermo-oxidation of the *trans*-isomers of poly-unsaturated acids led to the appearance of a band deformation vibration band =CH at 966 cm^{-1} in the spectrum of thermo-oxidized oil, parallel to a slight reduction in the intensity of the band deformation vibration, group =CH at 1098 cm^{-1} from the *cis*-isomer. This is a parameter that affects the quality of the oil. It would be necessary to evaluate the practical way to trigger the hydrolysis of oxidized triglycerides in the physiological environment [29].

Conclusions

In this study, the lipid oxidation mechanisms were determined that are most likely to be responsible for the deterioration of each samples. As a result, the thermo-oxidation was found to be the oxidation mechanism that occurred in the process of repeated frying, especially in oils. The process of forced oxidation of sunflower oil and the modification of the physico-chemical indices of the oil were investigated. It was found out, in particular, the increase of the peroxide index by 27 times and the decrease of the iodine index by 0.422 mg $I_2 \cdot g^{-1}$ which indicate the presence of an intense oxidative process.

Free poly-unsaturated fatty acids are the main promoters and substrates of oxidation reactions that occur when oil is maintained longer at high temperatures. The combined action of heat and oxygen generates primary and secondary oxidation products, and the nature of these products and their concentrations depend on the oxidative conditions applied. The possible mechanisms of forced oxidation under the influence of thermal factors of unsaturated fatty acids in sunflower oil were analyzed and established.

In order to highlight the impact of heat treatments, the analysis was performed by using IR spectroscopy. It was established that the applied treatment favoured both the formation of carbonyl secondary compounds, but also the simultaneous formation of hydroperoxides and triglycerides containing hydroxylated groups. The accumulation of hydroperoxides and triacylglycerides that have hydroxyl functions facilitated the course of polymerization reactions, which leads to the increase of thermo-oxidated sunflower oil viscosity.

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Bibliography:

1. Hrádková I., Merkl R., Šmidrkal J., Kyselka J. and Filip V. Antioxidant effect of mono- and dihydroxyphenols in sunflower oil with different levels of naturally present tocopherols. In: *European Journal of Lipid Science and Technology*, 2013, 115, pp. 747 – 755.
2. Schmidt. Š. and Pokorný. J. Potential application of oilseeds as sources of antioxidants for food lipids. In: *Czech Journal of Food Sciences*, 2006, 23, pp. 93 – 102.
3. Frankel E. Chemistry of free radical and singlet oxidation of lipids. In: *Progress in Lipid Research*, 2004, 23, pp. 197 – 221.
4. Wijesunder A. Docosahexaenoic acid is more stable to oxidation when located at the sn-2 position of triacylglycerol compared to sn-1(3). In: *Journal of the American Oil Chemists' Society*, 2008, 85, pp. 543 – 548.
5. Headlam H. and Davies M. Cell-mediated reduction of protein and peptide hydroperoxides to reactive free radicals. In: *Free Radical Biology Medicine*, 2003, 34(1), pp. 44 – 55.
6. Harry-O'Kuru R., Holser R., Abbott T. and Weisleder D. Synthesis and characteristics of polyhydroxy triglycerides from milkweed oil. In: *Industrial Crops and Products Journal*, 2002, 15(1), pp. 51 – 58.
7. Pignitter. M. et al. Regio-isomeric distribution of 9- and 13-hydroperoxyl linoleic acid in vegetable oils during storage and heating. In: *Journal of the Science of Food and Agriculture*, 2018, 98, pp. 1240 – 1247.
8. Aruoma I. Antioxidant action of plant foods. Use of oxidative DNA damage, as a tool for studying antioxidant efficacy. In: *Free Radical Research*, 1999, 30, pp. 419 – 427.
9. Reaven P, Witzum J. Oxidised LDL in atherogenesis. Role of dietary modification. In: *Annual Review of Nutrition*, 1996, 16, pp. 51 – 71.
10. Akoh C. and Min B. *Food lipid chemistry. Nutrition and Biotechnology*. New York, Marcel Dekker. Inc., 1997.
11. Yamamoto S. Enzymatic lipid peroxidation, reactions of mammalians lipoxyganases. In: *Free Radical Biology Medicine*, 1991, 10, pp. 149 – 159.
12. Drozdowski B. and Szukalska E. A rapid instrumental method for the evaluation of the stability of fats. In: *Journal of the American Oil Chemists' Society*, 1984, 64, pp. 1008-1011.
13. SM SR EN ISO 3596: 2002. Animal and vegetable fats and oils. Determination of acidity and acidity index.
14. Sturza R. *Modern principles of food analysis*. Chisinau, Tehnica-UTM, 2006, 310 p. [In Romanian].
15. STAS 145-67. *Determination of saponification index*. [In Romanian].
16. SM SR EN ISO 3557:2005. *Animal and vegetable fats. Determination of saponification index.* [In Romanian].
17. SM SR EN ISO 3961:2012. *Animal and vegetable oils and fats. Determination of iodine index.* [In Romanian].
18. AOCS. *Official and Recommended Practices of the American oil Chemists Society*. 5th eds., Champaign, I.L, 1989, pp. 48-62.
19. GOST 26593–85. *Vegetable oils. Method for determining the peroxide index.* [In Romanian].
20. SM SR EN ISO 661:2012. *Animal and vegetable fats and oils. Sample preparation for analysis*. [In Romanian].
21. SM SR EN ISO 662:2012. *Animal and vegetable fats and oils. Determination of water and volatile substances content.* [In Romanian].
22. Karlreskind P. *Manuel des corps gras*. Vol. I et II, Technique et documentation, PARIS, LAVOISIER, 1992.
23. Crapiste G., Brevedan M. and Carelli A. Oxidation of sunflower oil during storage. In: *Journal of the American Oil Chemists' Society*, 1999, 76, pp. 1437 – 1443.

24. Ito J. et al. Direct separation of the diastereomers of phosphatidylcholine hydroperoxide bearing 13-hydroperoxy – 9Z, 11 E-octadecadienoic acid using chiral stationary phase high-performance liquid chromatography. In: *Journal of Chromatography A*, 2015, 1386, PP. 53 – 61.
25. Belitz H., Grosch W. and Schieberle P. *Lipids. Food chemistry*. 3RD Edition, BERLIN, 2004, PP. 157 – 244.
26. Bastida S., Sánchez-Muniz F. Thermal oxidation of olive oil, sunflower oil and a mix of both oils during forty discontinuous domestic fryings of different foods. In: *Food Science and Technology International*, 2001, 7 (1), pp. 15 – 21.
27. Li J., Liu J., Sun X. and Liu Y. The mathematical prediction model for the oxidative stability of vegetable oils by the main fatty acids composition and thermogravimetric analysis. In: *Lebensmittel Wissenschaft and Technologie*, 2018, 96, pp. 51 – 57.
28. Malvis A., Simon P., Dubaj T. et al. Determination of the thermal oxidation stability and the kinetic parameters of commercial extra virgin olive oils from different varieties of Hindawi. In: *Journal of Chemistry*, 2019. Article ID 45679. 8 pages. https://doi.org/10.1155/2019/4567973.
29. Svetlana Haritonov, Rodica Sturza. In vitro bioavailability of sunflower oil fortified with iodine. In: Journal of Engineering Science, vol. xxv (4) 2018. DOI: 10.5281/zenodo.2576750. pp. 94-99.