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STUDY OF THE INCORPORATION OF IODINE IN VEGETAL OILS

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ABSTRACT

The aim of this work is the study of means to obtain iodized oil. Iodine consumption in Moldova is of an average of 40-60 μg I/a day and 37 % of the Moldavian children have goiter. Moldavian National Program for iodized salt from 1998 has been effective in preventing congenital hypothyroidism and the associated mental retardation by the year 2004. However, all the measures taken in this direction, did not lead to significant improvement of the situation. Sunflower oil takes up the biggest specific weight among food fats used in nutrition in the Republic Moldova. Manufacturing and consumption of sunflower oil fortified with iodine is a perspective direction for elimination of alimentary dependent iodine deficiency disorders. In order to reveal the influence of iodination process on the indexes of quality of sunflower oil, and for determination of its oxidative stability there were determined physical and chemical parameters of studied product.

During present study a high stability of sunflower oil fortified with iodine during storage was demonstrated.

Keywords: iodine, Iodine Deficiency Disorders (IDDs), food fortification, sunflower oil.

INDRODUCTION

Iodine deficiency is one of the major public health problems often co-existing in many regions in the world and affecting more than one third of the world's population, including Republic of Moldova [1]. Iodine deficiency impairs growth and neurological development, which can lead to the damage of brain. Depending on its severity and stage of development at which it occurs, iodine deficiency can lead to a wide spectrum of health problems, ranging from mild intellectual impairment to severe mental retardation, growth stunting, apathy, and impaired movement, speech or hearing [2]. Cretinism, in which many of these abnormalities occur, represents the extreme of early iodine deficiency [3].

The environment of Republic of Moldova is characterized by a reduced values of the iodine content: 4,5-5,3 mg/kg soil, 40 mg/l water and 0,03-0,22 mg/kg of vegetation, on a dry content of substances. Approximately 85 % of the population of Moldova lives in iodine deficient regions [4]. In order to prevent the iodine deficiency disorders, the government of Republic of Moldova adopted a National Program by the year 2004 [5]. However, all the measures taken in this direction, did not lead to a significant improvement of the situation. The Ministry of Health of Moldova with the support of UNICEF studied the nutritional status of adults and children in Moldova in the period from 1994 to 1997. Thus, 37 % of the children in Moldova have goiter and only 32 % of the families consume adequate iodinated salt [6].

Three intervention strategies are available to prevent iodine deficiency. These are supplementation, dietary diversification, and both targeted and untargeted food fortification [7]. The decrease of the consequences related to an insufficient iodine intake may be achieved through the application of a fortification strategy of different foodstuffs consumed by different population categories.

Sunflower oil takes up the biggest specific weight among edible fats used in nutrition in the Republic of Moldova. Iodine administration in products with a lipid origin represents a remarkable interest. First, this would allow the easy incorporation of the iodine in the food fatty products. Secondly, the daily intake of lipids being limited would allow an easy regulation of the iodine consumption, this being complementary with that from the iodinated salt and other products [8].

EXPERIMENTAL

Obtaining of the iodinated sunflower oil

Double refined and deodorized sunflower oil, purchased from local stores, was used (STAS – 1129-93) [9]. To obtain the iodinated sunflower oil in one liter of oil 1g of chemically pure, crystalline iodine (I_2) (STAS – 4159- 79) was administrated. The oil with the total iodine content 1000 µg/ml, was diluted, obtaining products with iodine content of 100 µg/ml, 10 µg/ml, 1 µg/ml. After the establishment of the equilibrium, iodinated oils were used as samples for the present study.

Determination of physical and chemical indexes of iodinated sun flower oil

All the measurements were made according to the standard methods STAS – 1129-93. Iodinated sunflower oil was analyzed at the beginning of the study and dynamically during three months (once a month).

Chromatography analysis of fatty acids content in the sunflower oil

The analysis of the fatty acids in the samples with iodinated oil was performed by gas chromatography with flame ionization detector, using gas chromatograph (helium) HPCHEM 1 FID1 A, equipped with a database and an autosampler. Fatty acids were separated depending on the length of the chain and depending on their degree of unsaturation. The concentrations were determined from the picks area using the standard curve of the authentic oil and the database.

p –anisidine index

p-anisidine index establishes the amount of unsaturated aldehydes (2,4-dienale, 2-alchenale) in the products of an animal origin, vegetal origin and oils through the reaction of the unsaturated aldehyde in the sample with p-anisidine and then absorption determination at 350 nm. A sample of 0,5-4,0 g i is then placed in a 25ml marked vial and it is dissolved with isooctane. Since isooctane is inflammable, many times it is replaced with n-hexane.

5.0 ml solution of fatty material with solvent is placed in a test-tube. In another test-tube 5.0 ml of solvent is also placed. 1ml of p-anisidine is added (0.25 g p-anisidine/100ml concentrated acetic acid). After 10 minutes the absorbance of the sample is measured using solvent solution as a reference. The test with p-anisidine is representative and correlates well with the results from the sensorial measurement of the lipid oxidation [9].

RESULTS AND DISCUSSION

Sunflower oil is part of the vegetal oils group and has a high amount of mono- and polyunsaturated fatty acids. Saturated fatty acids constitute just 11.3-11.6 %, the iodine indice of the oil varies from 119 till 135 [10]. Normally, unsaturated fatty acids from the vegetal oil are situated in the 2nd position of the glycerine molecule. Linoleic acid that prevails in the oil is concentrated in this position. Oleic acid occupies first position, and the saturated acids occupy 3rd position (Fig. 1).

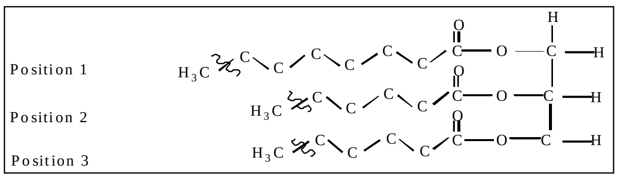


Fig. 1. Fatty acids positioning in the triglyceride molecule.

This kind of positioning as well as the « fourchette » shape that is representative for the triglycerides facilitates the attack from the molecular iodine and its fixation at the 2nd position, predominantly occupied by the linoleic acid (Fig. 2).

Aiming the study of the influence of iodine administration in the sunflower oil, main indexes have been evaluated and were referred to the product standards.

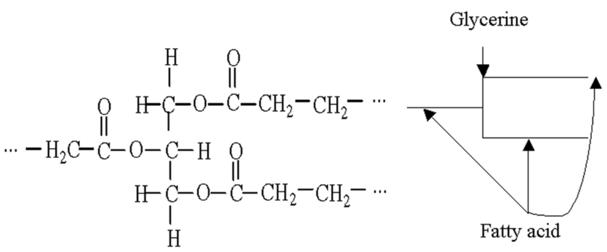


Fig. 2. Shape "fourchette" of a triglyceride molecule.

that administrated iodine does not settle to the double bond through covalent bonds.

Refraction index varies insignificantly, which disputes the free iodine presence in the samples with 1-100 iodine. Just for the samples with 1000 iodine the presence of the free iodine could be certified.

It is well known that halogens are capable to saturate double bonds in the fats. In the case of the active halogens such as fluorine and chlorine the binding to the double bond takes place according to the mechanism that involves the formation of an ion type halonium, as a result of the bimolecular nucleophyle [10]:

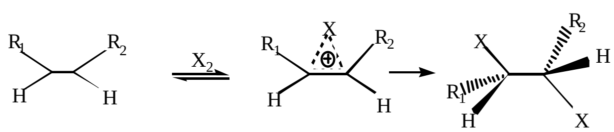


Table 1. Physical and chemical properties of the iodinated oil.

Physical and chemical indexes	Reference sample	Iodinated oil, $\mu g / ml$				Maximum allowable
		1,0	10,0	100,0	1000,0	
Iodine index	134 ± 1	131 ± 1	130 ± 2	129 ± 1	127 ± 2	119-135
Refraction index (20°C)	1,474 ± 0,001	1,475 ± 0,002	1,476 ± 0,001	1,476 ± 0,001	1,479 ± 0,002	1,472 – 1,476
Saponification index,mg KOH/g oil	193 ± 3	191 ± 2	195 ± 2	196 ± 1	198 ± 2	181 - 198
Free fatty acids content, % oleic acid	0,245 ± 0,005	0,245 ± 0,004	0,275 ± 0,003	0,285 ± 0,003	0,360 ± 0,005	Maximum 0,4
Peroxid index, méq/kg,	10,0 ± 0,2	8,9 ± 0,1	9,8 ± 0,2	10,9 ± 0,1	23,0 ± 0,3	Maximum 12
Humidity and the volatile substance %, maximum	0,100 ± 0,005	0,055 ± 0,005	0,068 ± 0,005	0,100 ± 0,005	0,098 ± 0,005	0,100

Physical-chemical indexes of the iodinated oil are presented in Table 1.

It was seen that the iodine indice varies a little, so that even in the case of the sample with the highest iodine amount (1000 $\mu g / ml$) its value does not surpass the allowed limits. This indisputably certifies the fact

The binding of the iodine through this mechanism does not take place because the reaction activation energy is high. However the electrophilic attack of the iodine is frequently used for mixed halogens. Thus the measurement of the iodine index is made through the Wijs – ICI reactive. The iodine binding

Table 2. Experimental values of the iodine index obtained for different oleic acid isomers *.

Double bond positioning	Theoretical values	Experimental values
-2=3-	89,7	9,04
-3=4-	89,7	16,27
-4=5-	89,7	26,96
-6=7-	89,7	89,7

* [10]

takes place fast and it is a good modality to establish the degree of the unsaturation of the triglycerides.

Also the value of the iodine index depends greatly on the position of the double bond comparing with the carboxy group %COO%. Thus the experimental values of the iodine index obtained depend on the positioning of the double bond in the oleic acid molecule and vary considerably depending on the distance between the double bond and the carboxy group (Table 2).

It was seen that when the number of the carbon atoms between the carboxy group –COO- and the double bond increases the probability for the halogenation decreases [10]. Since the fatty acids in the sunflower oil have double bond situated in the positions -9 = 10- and -11 = 12- (linoleic acid), the iodine attachment is almost impossible.

It is obvious that in conditions of the iodination of the sunflower oil the addition of the iodine cannot take place. The activity of the double bonds decreases if the distance between them and the carboxy group increases. The increase of the carbon atoms in the acid chain decreases the activity of the double bonds and reduces the saturation speed.

Thus it was certainly established that the amount of the main fatty acids in the sunflower oil (oleic and linoleic acids) almost does not vary. Also, the amount of the corresponding saturated fatty acids does not vary (Table 3).

In order to elucidate the influence of the molecular iodine incorporation in the sunflower oil the infrared spectrum of the iodinated oil was analyzed comparing with the non iodinated one. For the reference sample as well as for the iodinated oil (0,1 - 1000) the spectra were analyzed at two specific for fats wavelengths: 1724 cm^{-1} for the carbonyl group C=O of the unsaturated acids (based on the inductive –I effect the length of the bond and increases the intensity of the absorption band) and 1230 cm^{-1} (resonance band) with 2 harmonic bands at 1110 cm^{-1} and 1163 cm^{-1} specific for the group C–O. It was established that the intensity of the absorption bands of the light for these wavelengths almost does not vary regardless the concentration of iodine used and corresponds to the literature data for the sunflower [11, 12].

According to the experimental data, was established as following:

• Incorporation of the molecular iodine in the sunflower oil does not lead to the bursting of the double bond and the addition of the iodine according nucleophylic bimolecular substitution mechanism, characteristic for other halogens. This was established certainly through the estimation of the composition of the fatty acids from the triglycerides in the oil in a large range of iodine (1-1000**)**. The unsaturation degree of the product (iodine index) confirms as well the invariability of the number of double bonds in the triglyceride molecule;

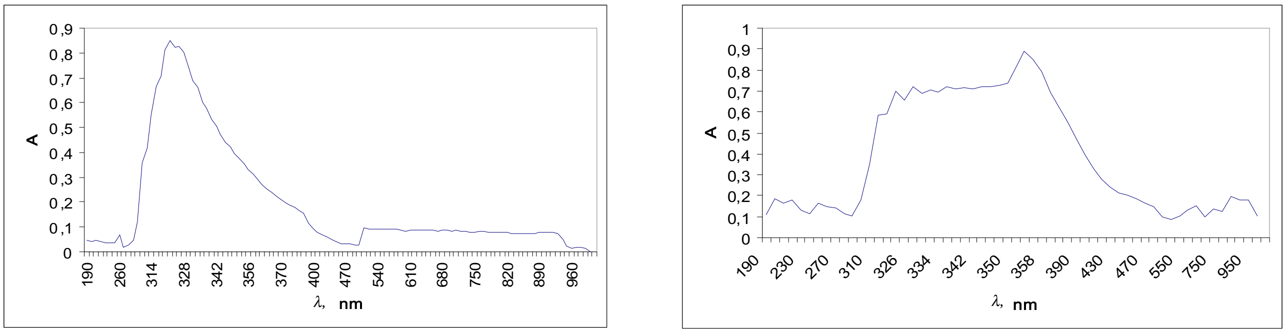
• In the infrared region of the electromagnetic spectrum the fat absorbs the radiant energy at 2 specific wavelengths in the medium infrared - μm and 5,73 μm; and 2 specific wavelengths in the near infrared - 1724 cm^{-1} and 1230 cm^{-1}. The vibration of the characteristic groups for the lipids for these wavelengths causes an important variation of the optic density, which is related directly to the fat amount containing these groups [13].

In this study the UV/Vis spectra for the natural and iodinated sunflower oil and for the alcoholic solution of iodine have been investigated (190-990 nm). The comparison of the spectrum for the oil without additive and the one with iodine shows a great difference (Fig. 3). Maximum absorption (320 nm) characteristic for the unsaturated fatty acids from the oil is essentially out of place (320-380 nm). This indicates the displacement of the electronic density caused by the formation of the π-complexes. Maximum

Table 3. The composition of the fatty acids in the iodinated sunflower oil*.

№	Type of oil	Conc. of iodine μg/ml	Concentration, %									
			$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$
1	Reference sample	-	0,0846	0,07189	6,4610	0,0915	3,3749	22,3761	66,4074	0,4675	0,1466	0,5185
2	1:1000	1	-	-	6,4232	-	3,3858	22,3793	66,7023	0,5674	-	0,5421
3	1:100	10	-	-	6,4209	-	3,3772	22,2911	66,5791	0,7521	-	0,5796
4	1:10	100	0,0874	-	6,4205	-	3,3772	22,3376	66,6163	0,6349	-	0,5261
5	1:1	1000	-	-	6,4106	-	3,3369	22,2941	66,7722	0,6717	-	0,5145

* t=18°C



a) b)

Fig. 3. UV/Vis spectra of the non iodinated (a) and iodinated (b) sunflower oil 10 μ g/ml.

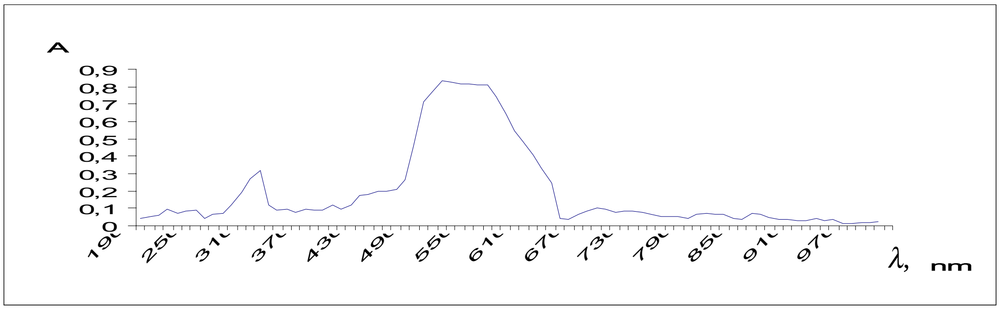


Fig. 4. Molecular absorption spectrum of the iodine (1% alcoholic solution).

absorption seen at 354 nm, indicates the formation of the π-complexes.

The absorption of the non iodinated and iodinated sunflower oil spectrum were compared with the absorption spectrum of the alcoholic iodine solution (Fig. 4).

It was established that the iodine in the alcohol solution presents an important absorption maximum in the visible field of the spectrum (= 520-530 nm). Also, a less important maximum was observed in the ultraviolet field (370 nm). The spectrum of the iodine solution in alcohol reflects the following equilibrium:

Maximum absorption in the visible field characterizes the molecular iodine, and that from the UV field – the presence of the ion . Obviously, in the case of the iodinated oil the presence of the ions was not observed.

The lack of the absorption maximum typical for the molecular iodine in the iodinated oil demonstrates the lack of superposition of the maximum absorption of the oil (non iodinated and iodinated) with maximum absorption of the molecular iodine, thus the lack of the free iodine in the iodinated oil (for the investigated concentrations). In the case of the sunflower oil the fixation of the molecular iodine takes place to the double bond of the unsaturated fatty acids, thus forming type π complexes, without breaking the double bond of the acid molecules [14-16]. In the π-type compounds the link between the electrons acceptor (iodine) and the electrons donor (unsaturated fatty acids) is formed with the participation of the electrons from the π bond of the donor group (double bond of the unsaturated fatty acids).

In the compounds thus formed a displacement of the double bond takes place with the displacement of the electronic density towards the iodine molecule which is more electronegative and this ensures the stability of the complex thus formed. Administrated iodine is fixed but not through covalent bonds, that occur as a result of the molecular iodine addition and disruption of the double bonds from the triglyceride molecules but through the formation of the molecular complexes due to the displacement of the double bonds.

In order to investigate the fixation capacity of the iodine to the sunflower oil, the dependence of the absorption was analyzed versus administrated iodine

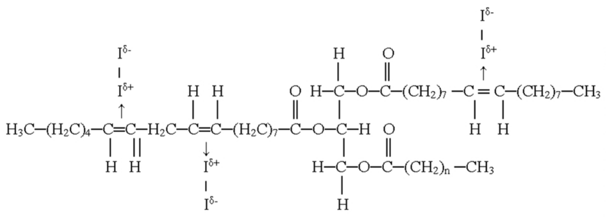


Fig. 5. Type π compounds.

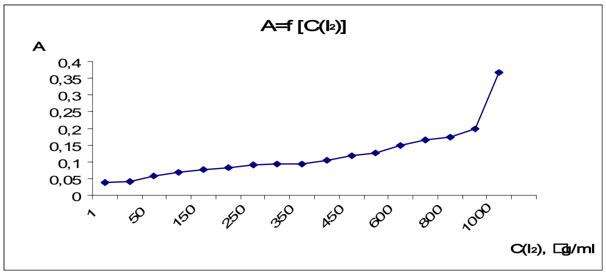


Fig. 6. The influence of the iodine concentration on the absorbance of the sunflower oil (λ_{max} = 520 nm).

amount in the maximum absorption field of the iodine (= 520 nm). It was established that in the concentration range 1- 400 g/ml the increase of the iodine concentration does not lead to an essential increase of the absorbance (Fig. 6).

Further an insignificant increase of the slope was noticed (450-900 g/ml). After 950 g iodine/ml a steep increase in the absorbance was noticed which is due to the fact that a free iodine appears.

This sudden variation of the absorbance demonstrates that the fixation capacity of the molecular iodine to the oil takes place in a certain concentration range. Thus, if the maximum fixated iodine concentration as -type compounds is 950 g iodine/ml oil, this amount corresponds to 3,74 mol/ml, or 3,74 mmol/l oil. If this characteristic is compared with the iodine index of the oil (127-131g iodine/100 g oil, namely 5 mol/1kg oil), it is evident that only a small part of the double bonds present in the sunflower oil are capable to fix molecular iodine without breaking the double bond.

This phenomenon is confirmed by the variation of the refraction index of the oil with the variation of the administered iodine amount.

Limited fixation of the iodine by the sunflower oil is caused probably by the steric factors which restrict the access of the molecular iodine with important molecular dimensions (the length of the bond – 2,74 E), towards the double bonds from the triglycerides.

Lipids represent an easily alterable food product fraction, thus the period and preservation conditions depend on their amount and constitution.

Stability of the fatty material during storage raises many problems for the food producers and the commercialization chain of these products. So, it is very important to investigate the evolution of the physical-chemical properties of the iodinated oil during storage.

It was seen that the main quality indexes of the iodinated oil do not vary essentially during storage (3 months). Saponification index varies insignificantly and remains within admissible limits for this particular product. Just in case of the maximum concentration of the iodine (1000 μg/ml) a slow overtaking of the maximum allowed limit could be seen for the saponification index and the free fatty acids.

Iodine index and refraction index vary insignificantly for each concentration of the analyzed iodinated oil. In the case of the maximum iodine concentration (1000 μg/ml), where the presence of the free iodine have been noticed, the refraction index remains undetermined during whole storage period of the product, phenomenon demonstrated also by the intensive colour of the product.

Peroxide index varies slightly even for the samples with iodine content of 1-100 μg/ml and remains within the range of allowed values for the sunflower oil. In the case of the limit concentration of administered iodine (1000 μg/ml) a slow decrease in the peroxide index was observed in comparison with product characteristics immediately after iodination. The humidity and the amount of the volatile compounds vary insignificantly for all analyzed samples during the evaluation of the physical-chemical properties of the products.

The more rich in polyunsaturated fatty acids product is considered to be more valuable from the nutritional point of view, but also more sensitive to the oxidative processes. Taking into account the toxicological risks and nutritional losses associated with this phenomenon as well as the necessity to ensure the optimal sensorial qualities, it is absolutely necessary to investigate the evolution of the oxidation processes in the food products during storage and treatment.

p-anisidine indiex allows to establish the amount of the unsaturated aldehydes (2,4-dienale, 2-alchenale) in the animal and vegetal products and oils through the reaction in the acid solution with acetic acid of the

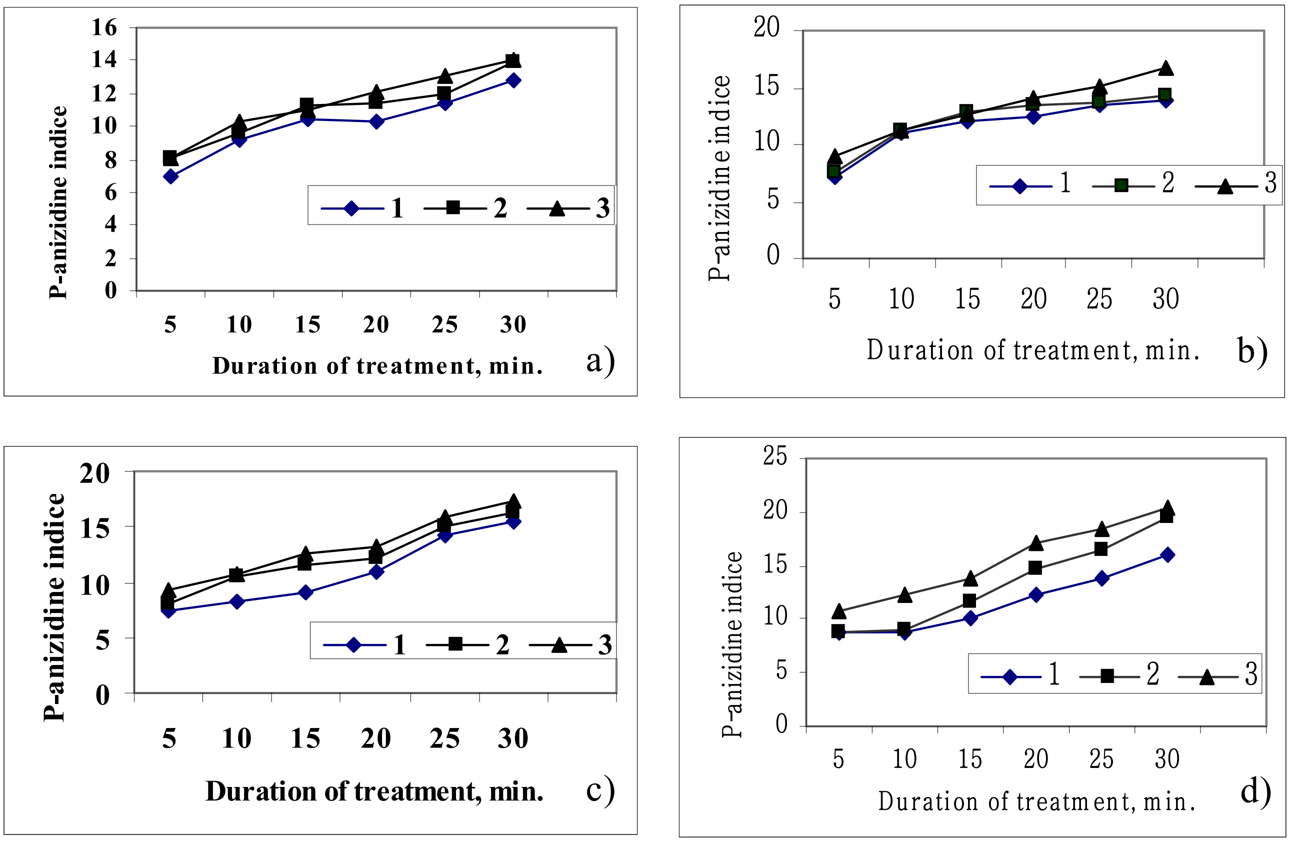


Fig. 7. Variation of the p-anizidine index for the iodinated oil (a - 1 $\mu g/ml$; b – 10 ; c -100 ; d – 1000) depending on the duration and termal treatment temperature: 1- 110°C; 2 – 140°C; 3 – 170°C.

unsaturated aldehydes from the fatty material with *p*-anisidine and determination of the absorbance at 350 nm. *p*-anizidine interactions with aldehyde compounds from the oils and fats, but the intensity of the yellowish colour of the reaction products formed does not depend only on the amount of the aldehyde compounds present but also depends on their structure. The intensity of the color in the case of the double bonds in the carbon chain, concerted with double carbonyl bonds leads to the increase of the absorbance up to 5 folds.

Thus p-anizidine index is an important index for the characterization of the oxidation degree of the fats especially for the evaluation of the formation of the secondary stable products due to fatty oxidation in food products.

During this study the evolution of the p-anisidine index for the iodinated oils in comparison with the reference sample depending on the technological factors which may interfere during usage of the iodinated oil was investigated. It was established that for the non iodinated oil the p-anizidine indice increases during thermal treatment of the oil (Fig. 7). Also, in the case of the iodinated oil the p-anizidine index increases with the severity of the applied thermal treatment – temperature and duration of the treatment (Fig. 7).

It has not been seen any sensitive difference between the evolution of the p-anizidine index for the non iodinated and iodinated oil, which confirms the fact that the mechanism for the fatty oxidation in both cases is the same. An inessential increase of the p-anizidine index for the maximum concentration of the iodine (1000 μg/ml) was anyway registered. This phenomenon is probably due to the presence in this sample of a certain amount of free iodine, which demonstrates an oxidative effect on the triglycerides.

CONCLUSIONS

The method and capacity of incorporation of the molecular iodine in the sunflower oil of native production was investigated. It was established that in the case of the oil iodination the addition of the iodine does not take place according to the bimolecular nucleophylic substitution mechanism, but the molecular iodine is fixed to the double bond with the formation of the type -compounds, without breaking the double bond from the unsaturated fatty acids. This allows the incorporation of a considerable amount of iodine (1-100 μg/ml) without modifying essentially the physical-chemical properties of the product.

Through physical-chemical methods (UV-Vis, refractometry) the absence of essential changes in the composition of the fatty acids was established in the samples from the iodinated and non iodinated oils without thermal treatment as well as for those submitted to thermal treatment. The intensity of the absorption bands for the triglyceride specific wavelengths (1724 cm^{-1} and 1230 cm^{-1} (resonance band) with 2 harmonic bands at 1110 and 1163 cm^{-1}) almost does not vary regardless the iodine concentration and corresponds to the literature data for the sunflower oils. These experimental results certify the lack of essential changes in the fatty acids from the triglycerides capable to affect the biological value of the products.

It was established that the main quality parameters of the iodinated oils (1 - 100 μg/ml) do not vary essentially during thermal treatment and during storage in comparison with the reference sample. In the samples with the highest iodine concentration (1000 μg/ml), where the presence of free iodine could be noticed, the quality indexes surpass the allowed limit value.

The accumulation process of the oxidation products has been investigated (amount of the unsaturated aldehydes 2,4-dienale and 2-alchenale) from the iodinated and non iodinated sunflower oil in comparison with the reference sample and as a function of the iodine concentration as well as technological factors of oil treatment (temperature 110, 140 and 170°C and applied thermal treatment duration – 5-30 min). It was observed that for the reference sample as well as for the iodinated oil, the amount of the oxidation products from the product increases with the severity of the thermal treatment. There was no sensitive difference between the evolution of the p-anizidine indice for the non iodinated and iodinated oil which confirms that the oxidation mechanism in both cases is similar. An insignificant increase in the p-anizidine index has been recorded for the highest iodine concentration (1000), which is due to the presence of the free iodine in the sample that demonstrates an insignificant oxidative effect on the triglycerides.

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