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THEORETICAL AND PRACTICAL ACHIEVEMENTS IN THE IODINE FORTIFICATION OF THE FOOD PRODUCTS

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Abstract: The main objective of this study was the investigation of the principles of food fortification with iodine. It was researched the opportunity of molecular iodine incorporation in vegetable oil, physical and chemical properties of iodized vegetable oil and other food products fortified with iodized oil. By physical and chemical methods it was established the presence of *π iodine-triglyceride* compounds in iodized oil, formed by fixing the molecular iodine at the double bound of the unsaturated fatty acids. The results of the investigations of the physical-chemical, microbiological and organoleptic properties, the oxidation stability of iodine fortified products compared to the control samples, indicate the absence of sensitive difference during the maturation and storage processes. The iodine bioavailability in fortified lipid products has been investigated *in vitro* and *in vivo* conditions. It was established, that the recovery percent of iodine represents 57-92% (*in vitro*). *In vivo* researches have proved that iodized lipid products are influencing the metabolic processes by accumulation of iodine in animal's body, as a result of an efficient digestion and a high iodine bioavailability from the present complexes.

Keywords: *iodinated sunflower oil, oil iodination, food lipids, π compounds iodine – triglycerides*

INTRODUCTION

Iodine is a trace element necessary for the synthesis of the thyroidal hormones, acceleration of the body metabolism, of the process of growth and development particularly of the fetus brain. The iodine deficiencies at the small child can provoke cretinism [1]. The WHO estimates that there is in the world 43 million individuals suffering in varying degrees from cerebral lesions which they would have been able to prevent, among which 11 millions attained by profound cretinism. The goiter affects about 760 million persons. Iodine deficiency leads to slowing down of the body functions, but an iodine excess brings about tiredness and weight gain [2]. When the physiological needs in iodine are not covered, functional anomalies can appear, depending on the intensity: endemic goiter, abnormal mental development, fertility decrease, increase of prenatal and infantile mortality [3]. Accidents in nuclear power stations caused the throw of great amounts of radioactive iodine in atmosphere. It is one of the reasons of the increase of the thyroid cancer rate after the Chernobyl accident [4]. Researches of WHO and UNICEF on the territory of the Republic of Moldova have demonstrated that prevalence rate of endemic goiter among children and teenagers makes up 37% [5-8].

Daily intake must compensate the physiological losses of the body; they vary according to the age of subject and its state of health. The WHO established the desirable minimal daily intake for an adult to 125 μg [9]. The iodine intake lower than 25 μg/day quasi systematic involves a symptomatology of hypothyroids. If insufficient intake is assured by food products, a supplementation adapted to the intensity of deficiency and to ways of life is often brought to populations. In countries at small risk of deficiency, an intake is systematically offered, basically in form of salt of common use enriched in iodides and iodates of potassium or sodium, oily iodized products [10].

There are used different methods of administration of the iodine: administration of iodine by oral or injectable way, iodation of the consumption or irrigation water, basic iodized food products [11]. The fortification of the food with micronutrients can help by overcoming the problems of insufficiency. The main problems involved in elaboration and production of the fortified foods include the identification of the appropriate vehicles, the choice of the appropriate fortification compound, the determination of technologies to be used in the process of fortification and the execution of the mechanisms of surveillance appropriated to determine if purposes of program are accomplished [12].

The most appropriate way of fighting with the iodine deficiency is considered the production of foodstuff for functional purposes which contain stable forms of iodine [13-19]. In order to eliminate the iodine deficiency disorders, on 1st of June 2007 was approved Moldavian Government Decision nr. 585 “Decision regarding the approving of national system of eradication of disorders caused by iodine deficit till the 2010”. It was adopted the Law regarding foodstuffs that provide for the fortification of foodstuffs with insufficient food [7, 8].

Sunflower oil takes up the biggest specific weight among edible fats used in nutrition in Moldova. Iodine administration in products with a lipid origin represents a remarkable interest. Firstly, 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 [19, 20].
The main objective of this study was the investigation of the principles of food fortification with iodine. It was researched the opportunity of molecular iodine incorporation in vegetable oil, physical and chemical properties of iodized vegetable oil and other food products fortified with iodized oil.

STUDY ABOUT IODINE INCORPORATION IN SUN FLOWER OIL

Sunflower oil is part of the vegetal oils group and has a high amount of mono- and polyunsaturated fatty acids. Saturated fatty acids constitutes just 11.3-11.6%, the iodine index of the oil varies from 119 till 135 [21]. Normally, unsaturated fatty acids from the vegetal oil are situated in the 2nd position of the glycerin molecule. Linoleic acid that prevails in the oil is concentrated in this position. Oleic acid has the first position and the saturated acids the 3rd position (Figure 1).

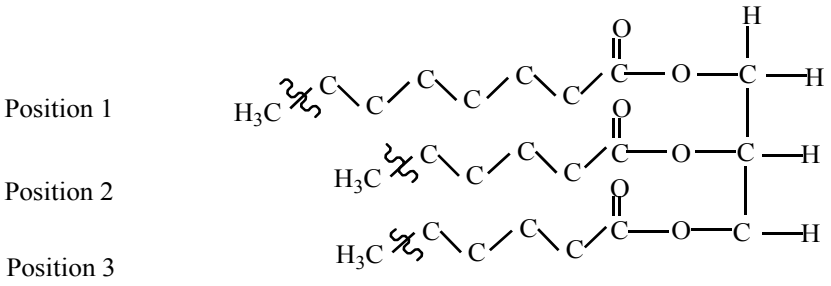


Figure 1. *Fatty acids positioning in the triglyceride molecule*

This kind of positioning as well as the "fourchette" (fork), shape that is representative for the triglycerides facilitates the attack from the molecular iodine and its fixation in the position 2, predominantly occupied by the linoleic acid (Figure 2).

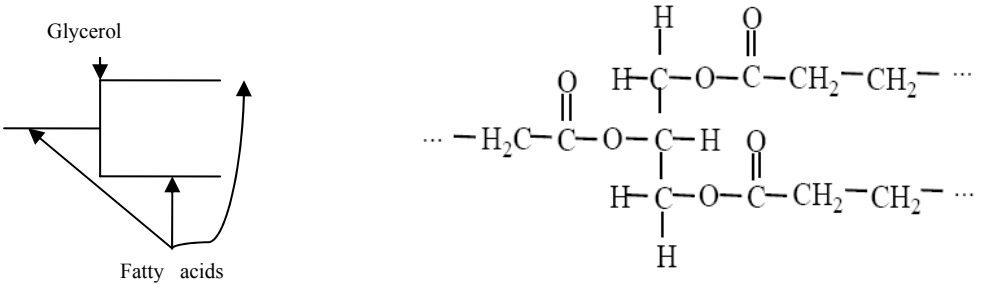


Figure 2. *"Fourchette" (fork) shape of a triglyceride molecul*

Studying the influence of iodine administration in the sunflower oil, main indexes have been evaluated and were referred to the product standards. Physical-chemical indexes of the iodinated oil are presented in Table 1. It was observed that the iodine value varies little, so that even in the case of the sample with the highest iodine amount (1000 µg I/mL) its value does not surpass the allowed limits. This indisputably certifies the fact 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 µg I/mL. Just for the samples with 1000 µg I/mL the presence of the free iodine could be certified.

It is well known the fact that halogens are capable to saturate double bonds present in the fats. In the case of the active halogens such as fluoride and chloride the addition 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 [21]:

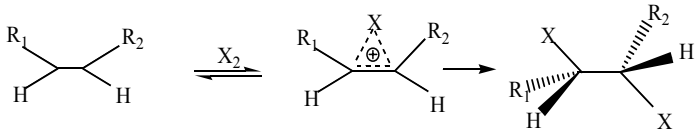


Table 1. *Physical and chemical properties of the iodinated oil*

Physical and chemical indexes	Reference sample	Iodinated oil, µg/mL				Maximum allowed
		1	10	100	1000	
Iodine index	134 ± 1	131 ± 1	130 ± 2	129 ± 1	127 ± 2	119 - 135
Refraction index	1.474 ± 0.001	1.475 ± 0.002	1.476 ± 0.001	1.476 ± 0.001	n/a	1.472 – 1.476
Saponification index, mg KOH/g	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	0.4
Peroxide index, mEq/kg	10.0 ± 0.2	8.9 ± 0.1	9.8 ± 0.2	10.9 ± 0.1	23.0 ± 0.3	12
Humidity and volatile substances, %, maximum	0.100 ± 0.005	0.055 ± 0.005	0.068 ± 0.005	0.100 ± 0.005	0.098 ± 0.005	0.100

The addition of the iodine through this mechanism does not take place because the reaction activation energy is high. However the electrophile 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 addition of the iodine takes place fast and it is a good modality to establish the degree of the instauration of the triglycerides.
Also, the value of the iodine index depends greatly on the position of the double bond comparing with the carboxyl group ─COO─. Thus, the experimental values of the obtained iodine index depend on the positioning of the double bond in the oleic acid molecule and varies considerably depending on the distance between the double bond and carboxyl group (Table 2).

Table 2. *Experimental values of the iodine index obtained for different oleic acid isomers [21]*

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

It was seen that as the number of the carbon atoms between group ─COO─ and double bond increases the probability of the addition reaction of the halogen decreases [17]. Since fatty acids, present in the sunflower oil have double bond situated in the positions ─9=10─ and ─11=12─ (linoleic acid), results that the iodine addition is almost not probable in these conditions.

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 carboxyl 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 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).

Table 3. *The composition of fatty acids in sun flower oil fortified with molecular iodine*

Type of oil	Iodine conc. [µg/mL]	Concentration, %					
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{22:0}$
Reference	-	6.4610	3.3749	22.3761	66.4074	0.4675	0.5185
1:1000	1	6.4232	3.3858	22.3793	66.7023	0.5674	0.5421
1:100	10	6.4209	3.3772	22.2911	66.5791	0.7521	0.5796
1:10	100	6.4205	3.3772	22.3376	66.6163	0.6349	0.5261
1:1	1000	6.4106	3.3369	22.2941	66.7722	0.6717	0.5145

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 (1 – 1000 µg I_2/mL) the spectra were analyzed for two specific wavelengths for fats: 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.

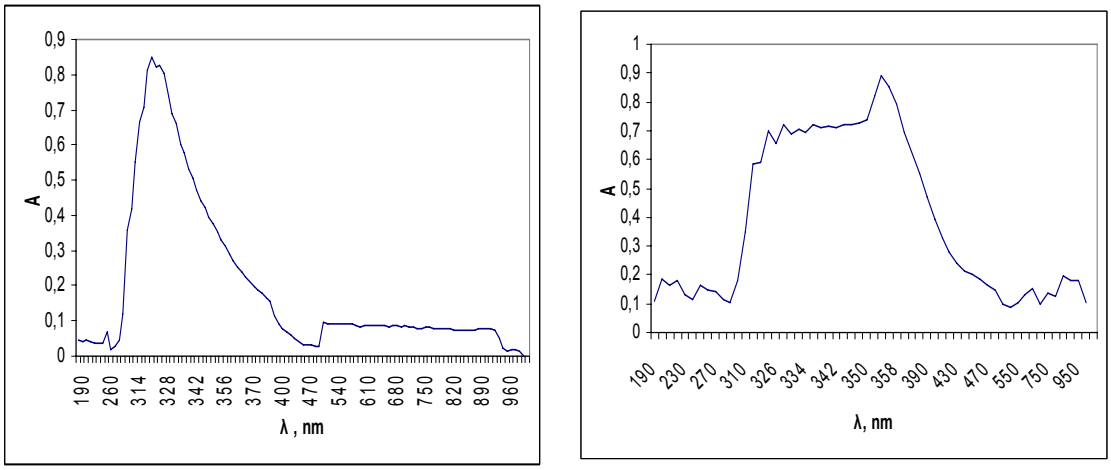
According to the experimental data, following were established:

I. 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 nucleophyle bimolecular substitution mechanism, characteristic for other halogens. This was established certainly through the estimation of the composition of fatty acids from the triglycerides in the oil in a large range of iodine (1 – 1000 µg I_2/mL). The insaturation degree of the product (iodine index) confirms as well the invariability of the number of double bonds in the triglyceride molecule;

II. In the infrared region of the electromagnetic spectrum the fat absorbs the radiant energy at 2 specific wavelengths in the medium infrared: λ_{max} = 3.45 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 with the fat amount that contains these groups.

In this study the spectrum from the UV/visible field, for the natural and iodinated

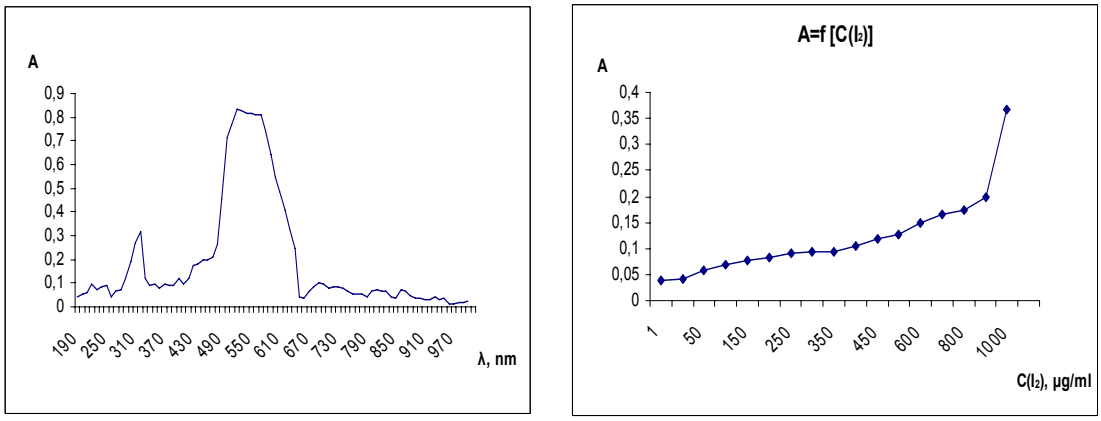
sunflower oil and for the alcoholic solution of iodine, has been investigated (190 – 990 nm). The comparison of the spectrum for the oil without additive and the one with iodine shows a great difference (Figure 3).



(a) (b)

Figure 3. *UV - Spectrum of the non iodinated (a) and iodinated (b) sunflower oil with 10 µg I_2/mL*

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 absorption was observed at 354 nm, indicating the formation of the π complexes. The absorption of the non iodinated and iodinated sunflower oil spectra were compared with the absorption spectrum of the alcoholic iodine solution (Figure 4a).



(a) (b)

Figure 4. *Molecular absorption spectrum of the iodine (a) (1% alcoholic solution); the influence of iodine concentration on the absorbance of the sunflower oil (b) ; (λ_{max} = 520 nm)*

It was established that the iodine in the alcohol solution presents an important absorption maximum in the visible field of the spectrum (λ_{max} = 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:

$$I_3^- \leftrightarrow I_2 + I^-$$

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

The lack of the maximum absorption 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 oil 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. In the compounds type π the link between electrons acceptor (iodine) and 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 that formed 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 that 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 displacement of the double bonds.

In order to be able to investigate the fixation capacity of the iodine to the sunflower oil, the dependence of the absorption was analyzed versus administrated iodine amount in the maximum absorption field of the iodine (λ_{max} = 520 nm). It was established that in the concentration range 1 – 400 μg I/mL the increase of the iodine concentration does not lead to an essential increase of the absorbance (Figure 4b). Further an insignificant increase of the slope was noticed (450 – 900 μg/mL). After 950 μg I/mL a steep increase of the absorbance was noticed which is due to the fact that the 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 π type compounds is 950 μg I/mL, this amount corresponds to ≈ 3.74 μmol/mL, or 3.74 mmol/L oil. If this characteristic is compared with the iodine index of the oil (127 – 131 g iodine/100 g oil, namely about 5 mol/kg oil), it is obvious that only a small part of the double bonds present in the sunflower oil are able 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 Å), towards the double bonds from the triglycerides.

STUDY OF THE KINETICS OF π-COMPLEXES FORMATION IN THE OIL –IODINE SYSTEM

We consider the reaction (1):

$$\text{Triglyceride} + \nu I_2 \leftrightarrow \pi - \text{complex} \quad (1)$$

or:	A	+	νB	↔	C
initial concentration:	*a*		*b*		-
equilibrium concentration:	*(a – bx)*		*(b – bx)*		*bx*

Because the stoechiometry of the reaction (1) is not known, for the kinetics examination of this reaction the notion of reaction promotion was used which can be determined from the equation (2):

$$\frac{dn_i}{\nu_i} = d\xi \quad (2)$$

where ξ means the reaction promotion, which means its evolution towards the equilibrium state and ν_i represents the stoechiometry coefficient of the *i* component. The *i* component is the component of which amount variation can be analyzed. By integration of the differential equation (2) we obtain:

$$\frac{1}{\nu_i}\int_{n_0}^{n_i} dn_i = \int_0^{\xi} d\xi \quad (3)$$

where:

$$\frac{n_i - n_0}{\nu_i} = \xi \text{ , or } n_i = n_0 + \nu_i \cdot \xi \quad (4)$$

Therefore, the issue regarding reaction rate description is defined through the unique reaction rate with variation speed of the reaction promotion:

$$\nu = \frac{1}{\nu_i}\cdot\frac{dn_i}{dt} = \frac{d\xi}{dt} \quad (5)$$

The reaction rate can be defined according to the law of mass effect through the relation:

$$\nu = k\prod[R_i]^{\alpha_i} \quad (6)$$

where *k* is the reaction constant; $[R_i]$ - reactants concentration, and α_i - partial order of the reaction compared to the respective reactant. It is obvious that the algebraic sum of the partial orders represent the global order of the reaction $n = \alpha_1 + \alpha_2 + \ldots$. For the examined case (1), the expression for the reaction rate can be written as follows:

$$\nu = k\cdot[\text{Triglyceride}]^{\alpha_1}\cdot[I_2]^{\alpha_2} \quad (7)$$

But the triglyceride concentration is higher that iodine concentration and during reaction process till the equilibrium state is reached this varies no significantly (Table 4).
Due to this, in the kinetic equation of the reaction, reaction promotion compared to the triglyceride concentration can be neglected which means that the rate expression will be given by the following relation:

$$\nu = \frac{d\xi}{dt} = k\cdot([A]_0 - \xi)^{\alpha_1}\cdot[B]_0^{\alpha_2} \quad (8)$$

or:

$$\frac{d\xi}{dt} = k_1 \cdot \left([\mathrm{A}]_0 - \xi\right)^{\alpha_1}, \text{ where: } k_1 = k \cdot [\mathrm{B}]_0^{\alpha_2} \quad (9)$$

Table 4. *Kinetic parameters of the reaction iodine-triglycerides* from the sunflower oil depending on the temperature*

Temperature [K]	Reaction evolution, ξ [mol/L]	Reaction rate, v [mol· L^{-1}·h^{-1}]	Rate constant, k [h^{-1}]	Arrhenius parameters	
				Activation energy, E_a [kJ·mol^{-1}]	Frequency factor, A [h^{-1}]
298	$1.9\cdot10^{-4}$	$7.9\cdot10^{-6}$	$2.12\cdot10^{-2}$		94.7
310	$2.5\cdot10^{-4}$	$12.1\cdot10^{-6}$	$2.84\cdot10^{-2}$		89.6
320	$3.2\cdot10^{-4}$	$13.3\cdot10^{-6}$	$3.72\cdot10^{-2}$	20.735	91.4
330	$4.1\cdot10^{-4}$	$17.1\cdot10^{-6}$	$4.79\cdot10^{-2}$		91.5
340	$5.2\cdot10^{-4}$	$21.7\cdot10^{-6}$	$6.14\cdot10^{-2}$		95.3

* Initial concentration $[I_2]_0 = 3.9\times10^{-3}$ mol·L^{-1}

In this case, the reaction order is considered to be degenerated and the reaction constant k_1 also known as pseudo-constant will depend on the excess component concentration. During this study the reaction order variation depending on the molecular iodine which is considered as the component, whose initial concentration is noted as $[A]_0$, and the reaction order is α_1. In order to establish the reaction order during this work we studied the evolution of the iodine concentration in time. It was established that this relationship has an exponential form (Figure 5). A similar variation of the reactive concentration is characteristic for the first order reaction.

For the first order reaction the reaction speed can be expressed by the following relation:

$$v = -\frac{d[\mathrm{A}]}{dt} = \frac{d\xi}{dt} = k \cdot [\mathrm{A}] \quad (10)$$

After integration we obtain:

$$\ln\frac{[\mathrm{A}]_0}{[\mathrm{A}]} = k \cdot t, \text{ or: } [\mathrm{A}] = [\mathrm{A}]_0 \cdot e^{k\cdot t} \quad (11)$$

Based on the equation (9) we can calculate the value of the speed constant considering the reaction progression:

$$\ln\frac{[\mathrm{A}]_0}{[\mathrm{A}]_0 - \xi} = k \cdot t \quad (12)$$

The results are presented in figure 6. It was established that tg $\alpha = 2.12\times10^{-2}$ h^{-1}. But tg $\alpha = k$, thus the rate constant of the reaction (1) has the value: $k = 2.12\times10^{-2}$ h^{-1} (at $T =$ 298 K). If the order and the rate constant of the reaction are known, the half-time of the reaction can be established. For a first order reaction the following relation can be used:

$$\ln\frac{[\mathrm{A}]_0}{[\mathrm{A}]_0/2} = k \cdot t_{1/2} = \ln 2 \quad (13)$$

which leads to the relation:

$$t_{1/2} = \frac{0.693}{k} \quad (14)$$

Therefore, the half-time reaction of the triglycerides iodination process from the sunflower oil is: $t_{1/2}$ = 32.69 h (for T = 298 K).

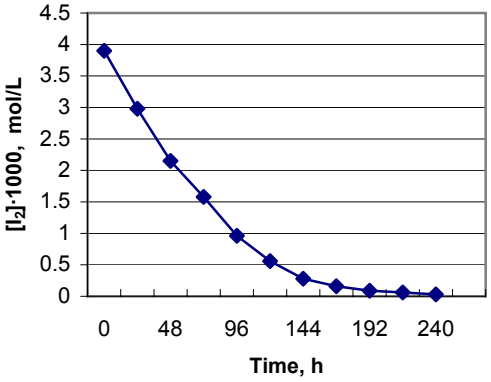


Figure 5. *Iodine concentration evolution during iodination of the sunflower oil; ($[I_2]$=3.9×10^{-3} mol.L^{-1}; t = 25 ºC)*

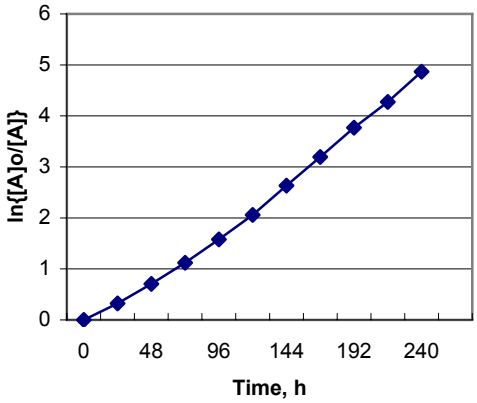


Figure 6. *Relation ln {$[I_2]_0$/$[I_2]$} = f(t) for the iodination reaction of the triglycerides from the sunflower oil ($[I_2]_0$ =3.9×10^{-3}mol.L^{-1}, t = 25 ºC)*

Elucidating the influence of the temperature on the reaction rate, it was established for the temperature range 298 – 340 K a reaction progressing and the reaction rate and the rate constant were calculated (the reaction rate was examined at different temperatures). The results are presented in Table 4.

Although the reaction speed increases when temperature increases, the thermal coefficient is not too high: $\frac{k_{T+10}}{k_T} \approx 1.28$. This proves that the reaction is limited not by the kinetic factors but by the diffusion factors. These are caused by the high viscosity of the system as well as by the steric factors which hinders the molecular iodine penetration in the sites assigned for the π compounds formation. It is obvious that the settlement of the electronic density requires energy and time outlay.

In order to establish the Arrhenius parameters of the iodine-triglyceride reaction from the sunflower oil the diagram ln k in terms of 1/T was drawn. The relationship: ln k = f(1/T) represents a straight line which proves that the examined process may be described using Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{R \cdot T} \tag{15}$$

From the diagram, the slope was established:

$$-\ln k = f\left(\frac{1}{T}\right) = \frac{E_a}{R} = 2.494 \tag{16}$$

The activation energy of the examined reaction is 20,735 J/mol, or 20.735 kJ/mol. The calculations made through analytical method based on the relation:

$$\ln \frac{k_2}{k_1} = -\frac{E_a}{R} \cdot \left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{17}$$

proved that the average of the obtained values for the activation energy of the triglyceride iodination process of the sunflower oil are: $E_a \approx 20.74\times10^3$ J/mol. Subsequently, the values of the frequency factor (A) were calculated based on the Arrhenius equation and the average value of the activation energy:

$$\ln A = \ln k + \frac{E_a}{R \cdot T} \tag{18}$$

Obtained values are indicated in Table 4. The average value of the frequency factor is: $A = 92.5\ h^{-1}$. The obtained results show that the reaction involved in the π compounds triglycerides-iodine formation are characterized by a relatively low activation energy – $E_a \approx 20.74\times10^3$ J/mol, but the frequency factor is extremely low which explains the low speed of the π complex formation reaction iodine – triglyceride from the sunflower oil.

EVOLUTION OF THE PHYSICAL-CHEMICAL PROPERTIES OF THE IODINATED OIL DURING STORAGE

Lipids represent an easily alterable food product fraction, thus the period and preservation conditions of these ones 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. Due to this it is very important to investigate the evolution of the physical-chemical properties of the iodinated oil during storage.
In order to reveal the influence of the iodination process on the indices of sunflower oil quality, and to determine its oxidative stability we investigated physical and chemical parameters of the product in dynamics (Table 5).
It was seen that main quality indexes of the iodinated oil do not vary essentially during storage (3 months). Saponification indexes vary insignificantly and remain 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 indexes and free fatty acids.

Table 5. *Change of the physical and chemical indexes of iodinated oil during 3 months of storage*

Physical and chemical indexes	**After 1 month of storage**				
	Reference sample	**Iodinated oil, μg/mL**			
		1	**10**	**100**	**1000**
Saponification number, mg KOH/g oil	193 ± 1	191 ± 4	195 ± 2	196 ± 3	198 ± 5
Free fatty acids content, % oleic acid	0.250 ± 0.005	0.250 ± 0.005	0.255 ± 0.007	0.285 ± 0.008	0.365 ± 0.006
Iodine index	132 ± 2	131 ± 1	130 ± 1	128 ± 2	127 ± 1
Refraction index (20 °C)	1.475 ± 0.002	1.475 ± 0.002	1.476 ± 0.002	1.477 ± 0.002	n/a
Peroxide index, mEq/kg	10.0 ± 0.2	9.9 ± 0.2	10.1 ± 0.3	10.9 ± 0.2	23.0 ± 0.5
Humidity and volatiles, %	0.100 ± 0.007	0.050 ± 0.005	0.060 ± 0.005	0.095 ± 0.005	0.090 ± 0.008

Physical and chemical indexes	After 3 months of storage				
	Reference sample	Iodinated oil, µg/mL			
		1	10	100	1000
Saponification number, mg KOH/g oil	193 ± 1	194 ± 1	196 ± 3	197 ± 2	200 ± 3
Free fatty acids content, % oleic acid	0.250 ± 0.005	0.245 ± 0.007	0.330 ± 0.005	0.370 ± 0.004	0.410 ± 0.008
Iodine index	132 ± 2	130 ± 2	130 ± 2	127 ± 3	125 ± 3
Refraction index (20 °C)	1.475 ± 0.002	1.475 ± 0.002	1.477 ± 0.002	1.478 ± 0.002	n/a
Peroxide index, mEq/kg	10.0 ± 0.2	8.1 ± 0.2	8.5 ± 0.2	9.3 ± 0.3	22.7 ± 0.6
Humidity and volatiles, %	0.100 ± 0.007	0.089 ± 0.005	0.105 ± 0.003	0.099 ± 0.009	0.105 ± 0.011

Iodine indexes and refraction indexes vary insignificantly for each concentration of the iodinated oil that is analyzed. Peroxide indexes vary little 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 of the peroxide indexes in comparison with product characteristics immediately after iodination is observed. The humidity and the amount of volatile compounds vary insignificantly for all analyzed samples during evaluation of the physical-chemical properties of the products.
The performed study permits to establish that physical and chemical properties of the iodinated sunflower oil vary insignificantly with regard to the reference sample during iodination and storage (3 months). In case of sunflower oil iodination, fixation of molecular iodine to double bond takes place with formation of π - type compounds, without breaking of double bond from molecules of unsaturated fatty acids. The implementation of studies indicates that lipids present an important vehicle for food fortification with iodine.

THE FORTIFICATION TECHNOLOGIES WITH IODINE OF THE LIPIDIC PRODUCTS USING THE IODINATED SUNFLOWER OIL

Carried out researches have shown that lipids represent a suitable matrix-carrier for iodine fortification of food products. It was elaborated the scientific basis and the technology of fabrication of lactic acid products, margarine and food emulsions iodine fortified by the addition of iodized oil.

Lacto-acidic products fortified with iodine

In this study it was researched the influence of the iodine fortification process on the technological process of fabrication of lactic acids, on their time of preservation, on their organoleptical, microbiological, physical-chemical indexes of the products. The

iodine had been administrated as iodinated oil. The iodinated oil was added in the proportion of 0.5% to the volume of the milk (50 μg iodine/mL oil). The oil was incorporated in the second stage of the homogenizing (the sixth technological operation). After the incorporation of the iodinated oil, it is necessary the thoroughly mixing of the product.
We compared the chemical composition of the reference sample and the fortified with iodine sample. We observed an unimportant influence of the 0.5% iodinated oil at 100 g of the product (Table 6). The proportion of the macro- and microcomponents barely changes. The content of the iodine varies, which in the reference product, represents 5 – 6 μg/100 g of the product. The content of the iodine in the iodinated kefir is 30 – 32 μg/100 g of the product. Thus, if we consume 250 mL of this product, we will get the half of the necessary daily dose of this essential oligoelement.

Table 6. *The chemical composition of the reference lacto-acidic products and fortified with iodine lacto-acidic products*

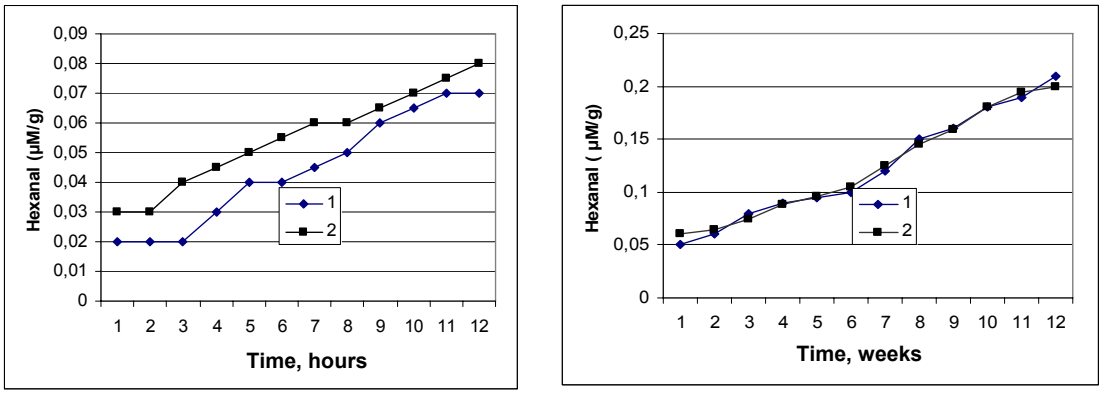
Product	Dry substance (%)	Proteines (%)	Fats (%)	Glucides (%)	Mineral substances (mg/100 g of product)				Iodine, (μg/100 mg product)
					Ca	P	Fe	Mg	
Kefir (1%)	9.5	3.0	1.0	4.05	114.2	91.5	0.2	14.6	6.5 ± 2.0
Non-fat kefir	8.6	3.0	0.05	3.80	126.0	95.1	0.1	15.2	5.5 ± 1.8
Iodinated kefir (G 1%)	9.5	3.0	1.05	4.05	114.2	91.5	0.2	14.6	2.5 ± 3.0
Non-fat iodinated kefir	8.6	3.0	0.10	3.80	126.0	95.1	0.1	15.2	30.0 ± 2.5

In the reference kefir's microbiota prevail the *Streptococcus* and *Lactobacillus* species. The additive has been administrated and after the process of the fermentation and maturation had taken place, the alien microbiota has not been observed. During the lacto-acidic fermentation process, the acid pH and the iodinated oil have an inhibitory effect on the alien microbiota. In the reference kefir the microorganisms grow faster, than in the iodinated kefir. The iodine inhibits the fermentation process of the kefir, this fact is proven by the small value of the acidity in the fortified samples.

Iodinated margarine

The iodinated margarine was made at the enterprise S.A. "DRANCOR", which afterwards bought the license. The process of the fabrication of the margarine consists in the preparation of the water/oil emulsions, using the technique that emulsifies, solidifies, and plasticizes continuously. The iodinated sunflower oil, that contains 10 μg iodine/cm^3, it is included in the fat phase, in this way, the content of the iodine in the final product will be 1 – 2 μg iodine/g of product. The organoleptical, physical-chemical indexes of the iodinated margarine are practical the same with the indexes of the normal margarine. This demonstrates that the incorporated iodine doesn't have a bad influence.
During the stocking, the principal cause of the deterioration of the food products is the

oxidation of the lipids. It was decided to research the oxidative stability of the iodinated margarine and the oxidative stability of the reference sample, during the stocking (3 months, at –17 ± 1 °C) and exhibiting the products (24 hours, 22 °C). It was analyzed the content of the primary products of the lipidic oxidation – monohydroperoxides, and the content of the secondary products of the oxidation, expressed as hexanal.
The dynamic of the hydroperoxides accumulation in the two types of examined margarine, hasn't any difference (Figure 7).



(a) (b)

Figure 7. *The evolution of the content of hexanal: a) margarine was exhibited (12 hours, 22 °C); b) margarine was stocked (3months, -17 °C): 1- reference sample; 2- iodinated margarine (1 µg iodine/g product)*

During the stocking this variation is unessential. The accumulation kinetics of the volatile compounds, expressed as hexanal, presents a small difference, when the iodinated margarine and the reference sample were exhibited, while stocking the iodinated margarine; the kinetic curves have the same shape.
The fortification of the margarine with iodinated oil doesn't change the oxidation speed of the lipids. This is due to the fact that iodine is fixing on the delocalized π connection; this is provoked by the decrease the oxidative capacity and by the presence of the antioxidants in the margarine. The physical-chemical and microbiological characteristics of the lacto-acidic products and of the margarine, which were fortified with iodine allows to industrialize and commercialise this food products.

Food emulsions

The basic objective is the study of the enrichment possibilities of food emulsions type o/w, as mayonnaise, in iodine as a method of improvement and prevention of iodine deficiency. There have been proposed the following study objectives:

I. Analysis of the factors susceptible to influence the efficiency of iodine fortification of emulsions (pH, ionic strength, parameters of formulation and composition);
II. Study of oxidative stability of food emulsions of type oil in water enriched in iodine.

The droplets size distribution of the obtained mayonnaise is almost monomodal. The agglomerates of microparticles are observed in the volume. This fact is due to the

formation of three-dimensional structures. The appearance of these three-dimensional structures is owed to the conglomeration of fat globules, thanks to the presence of two types of surfactants: macromolecular (proteins, the main representative of which in that case is casein), and CREMODAN, which represents a mixture of mono- and diglycerides.

No difference was disclosed between the distribution of the fat droplets in the case of mayonnaise witness and the iodine enriched mayonnaise samples (1µg iodine/mL oil). For all studied emulsions, the average diameters in surface are included in the intervals from 0.98 ± 0.07 µm to 1.25 ± 0.10 µm. The dimensions of the agglomerates of the fat droplets vary from 7.76 ± 0.16 to 17.86 ± 0.23 µm. It was established that the addition of iodized oil in the mayonnaise formulation does not bring on any detectable change of the droplets distribution in the mayonnaise enriched in iodine. The droplets size distribution of the mayonnaise witness and iodine enriched mayonnaise samples are presented in Figure 8.

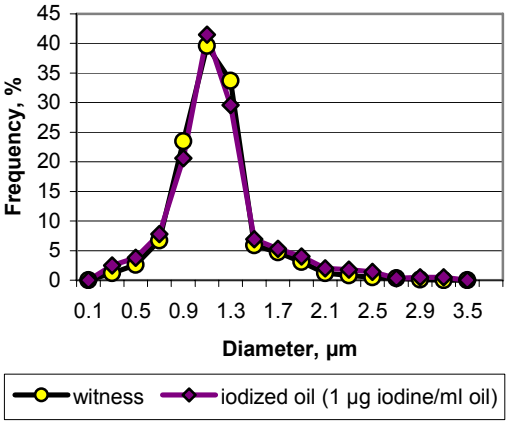


Figure 8. *Droplets size distribution*

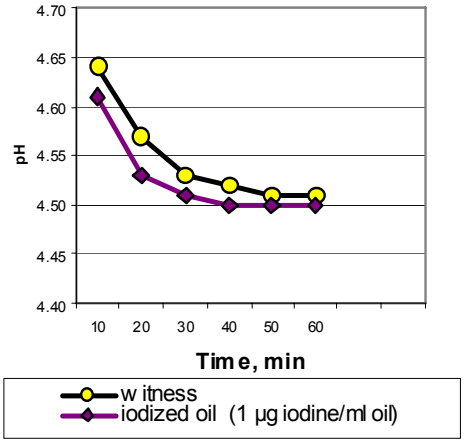


Figure 9. *pH variation after mayonnaise fabrication*

Experimental data allowed to calculate the specific surface of droplets S, expressed in m^2/cm^3 with the aid of following expression:

$$S_{sp} = 6/D_{[3,2]} \tag{19}$$

where $D_{[3,2]}$ [µm] is the average diameter of the sphere which has the same surface as the particles. The fat droplets have an almost perfectly spherical form.

It was determined that the specific surface of mayonnaise is 5.263 m^2/cm^3. Experimental data denote, by consequence, that obtained emulsions have a well developed specific surface, stabilized by the common action of the surfactant of weak molecular weight and by caseinate.

The protein surface load of emulsions represents an important characteristic of the emulsified systems. The protein surface load can be calculated from the droplet specific surface according to following expression:

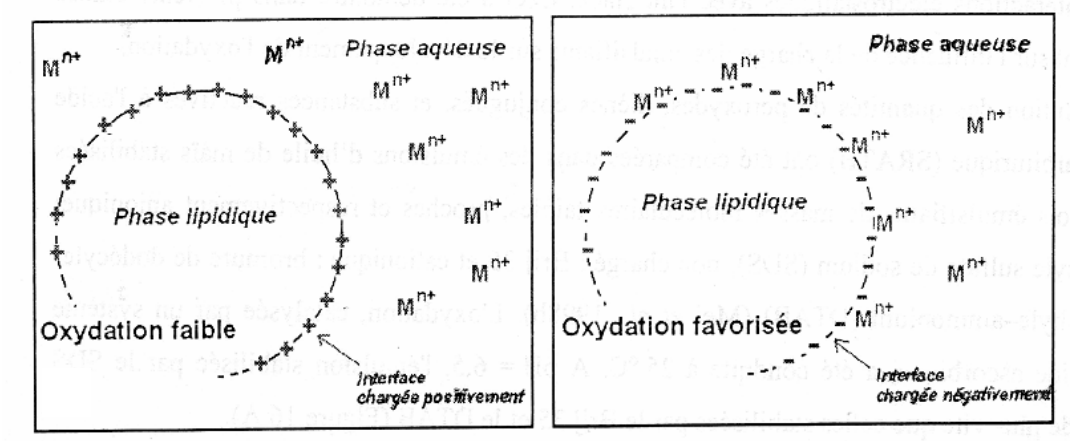
$$\Gamma = \frac{\%P_{ads} \cdot [P]}{S_{sp} \cdot [H] \cdot \rho_{oil}} \tag{20}$$

where: $\%P_{ads}$ – percentage of adsorbed proteins; $[P]$ - rate of proteins in emulsion;

S_{sp} - the specific surface; [H] - rate of oil in mayonnaise; ρ_{oil} - oil density (0.9 g/cm^3).
Given the low concentration of casein in examined case (0.5 %), it can be considered that proteins are completely adsorbed at the interfaces, $\%P_{ads}$ = 1 (for mayonnaise - witness).

$$\Gamma = \frac{\%P_{ads} \cdot [P]}{S_{sp} \cdot [H] \cdot \rho_{oil}} = \frac{1 \cdot 0.5 \cdot 10^{-3}}{5.263 \cdot 77 \cdot 0.9} = 1.371 \text{ mg protein/m}^2$$

Therefore, the protein surface load will be 1.371 mg protein/m^2. This value of the protein surface load is considerably lower compared to the known values for casein allowing stabilizing permanently an emulsion – about 3 mg protein/m^2. But in examined case a competition between the surfactant of low molecular weight and casein takes place, consequently decreasing the interfacial area, occupied by the protein. On the other hand, this fact contributes to the development of a three-dimensional structure by the formation of agglomerates.
Food emulsions have a comparatively complex water phase containing a great number of ingredients with elements susceptible to exercise pro- or anti-oxidizing effect. Besides its composition, water phase is characterized by different physicochemical parameters such as the pH that can interfere in the oxidation development. The pH interferes at the same time on several characteristics of emulsion with opposite effects sometimes on the sequence of lipid oxidation. To exercise its catalytic activity over unsaturated fatty acids or hydro peroxides already formed, iron or other catalytic metals have to approach lipid phase. Metals in the ionic state being positively charged, this approach is strongly dependent on electrostatic interactions with interface.
In the case of emulsions stabilized by proteins, the interface load is determined by the pH of water phase and the isoelectric point (I_p) of the protein. In the present study it was examined the pH changes after emulsification. When the pH of water phase was initially adjusted to 4.5, after emulsification it was lightly higher (Figure 9). A very small difference between the witness sample and the iodized sample of mayonnaise was certified the first time after the emulsification. But at the end of 30 min, it becomes stable around the initial value of 4.5.
When the pH is lower than the I_p of the protein, this one is positively loaded. The interactions of metals with interface would then be minimized due to electrostatic repulsion, what would restrict the development of oxidation (Figure 10a). In the opposite, when the pH is superior to the I_p of the protein, the droplets area is negatively loaded, interactions with metals could take place and oxidation would be favoured (Figure 10b).
Lipid oxidation is one of the main potential reasons of deterioration of the quality of food. It is a chemical phenomenon consequences of which go well beyond the deterioration of some particularly fragile molecules. Among these negative consequences, the easiest to be detected and the most repulsive is the appearance of a smell, often described to be rancid, but sometimes also of "old" or "metallic". This smell changes sensory characteristics of the food, therefore its evaluation, and diminishes its acceptability. The ingestion of products of lipid oxidation could on long term to participate in the development of degenerative pathologies such as arteriosclerosis, carcinogenesis, diabetes, etc.



(a) (b)

Figure 10. *Schematic representation of the effect of metallic cations (M^{n+}) on the oxidation of emulsions stabilized by a cationic (a) or anionic (b) emulsifier*

The more a food is rich in polyunsaturated fatty acids, the more risks of oxidation is high, and therefore, more risks than it contains oxidized lipids, potentially toxic, are increased. For these reasons, but also to guarantee an optimum sensory quality in food, it is necessary to know how to control the lipid oxidation in complex products and to understand its mechanisms and the factors interfering in polyphase systems. The kinetics of emulsified fats oxidation differs from that of continuous lipid phase because of the features of the emulsion droplets interface and the distribution of prooxidizers, antioxidizers and oxidable substrates between the oil phase, water phase and the emulsion interface. In emulsified medium, the kinetics of oxidation is often more accelerated than in the case of continuous lipid phase.

The *p*-anisidine interacts with aldehydes compounds of oils and fats, but the intensity of the yellowish colour of the reaction products depends not only on present aldehydes amount, but also of their structure. The colour intensity in the case of double links in the carbonic chain, conjugated with carbonyl double links leads to the increase of the molar absorbance of 5 times. The measurements of 2-alkenales and of dienes have the maximum contribution in the total absorbance of the system. It has been measured the time variation of the *p*-anisidine index in iodine enriched mayonnaise in comparison with the witness sample. Experimental data are presented in Figure 11a.

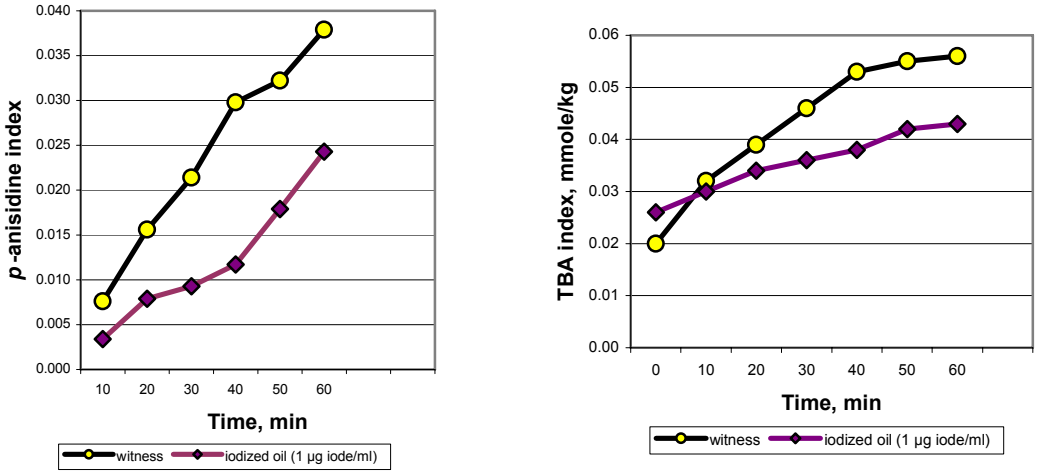
It was established that the *p*-anisidine index – the expression of the non-saturated aldehydes amount formed during oxidation, varies of the same manner for both types of mayonnaises. However, it was determined a more important rate of *p*-anisidine index for the witness sample.

The thiobarbituric acid test is the most popular method of evaluation of the oxidation secondary products. The principle of method is the following:

$$\text{TBA + malonaldehyde} \rightarrow \text{coloured complex } (\lambda_{530})$$

The secondary products of the lipids oxidation, like ketones and aldehydes, unlike peroxides, are accumulated in grease and they cannot be eliminated by simple heating. This is the reason why the thiobarbituric acid test represents an adequate index of the oxidative rancidity of lipids. Experimental data are shown in Figure 11b. It was established that the mayonnaise oxidation degree is very low – the rate of substances sensitive to the TBA test is very low. The paths of both curves are the same, meaning

that oxidation occurs in the same way. However, the rate of the secondary products of the lipid oxidation is lower in the iodine enriched mayonnaise.



(a) (b)

Figure 11. *The influence of the mayonnaise enrichment in iodine on the oxidation degree, examined by the p-anisidine index (a) and by TBA index (b)*

As tests in *p*-anisidine and in thiobarbituric acid (TBA) are supplementary, it follows, that iodine enrichment of mayonnaise (1 µg iodine/mL oil) allows fabricating a stable product considering the oxidative stability.

STUDY OF THE SAFETY AND BIOAVAILABILITY OF ORGANICALLY BONDED IODINE FORMS FROM IODIZED FATS

The aim of the research consisted in examination of metabolic displacement in the animals' organism at the correction of experimental and spontaneous thyroid pathology by means of iodinated food products (sunflower oil, margarine). The experiment was realized with a lot of white rats line Wistar with the mass 180 – 210 g. The feed was realized on standard ration with free access to water. Duration of the experiment was of 42 days. The animals were kept in individual cages, 5 heads in every cage.
The experiment provided 2 stages (figure 12):

1st stage – experimental reproduction of hypothyroidism with the help of mercazole for blocking of thyroid gland function. Daily (14 days) the rats were given to drink water with mercazole. At the same time they were fed by bread without addition of iodinated salt, with the purpose to exhaust the reserves of iodine of the organism.

2nd stage – feed of animals with experimental hypothyroidism (28 days) by standard ration, without addition of iodine (group II); with additive of sunflower non-iodinated oil (group III); with addition of iodinated oil with iodine content 3 µg/rat (group IV); with addition of iodinated margarine with iodine content 3 µg/rat (group V); with addition of iodinated oil with iodine content 30 µg/rat (group VI).

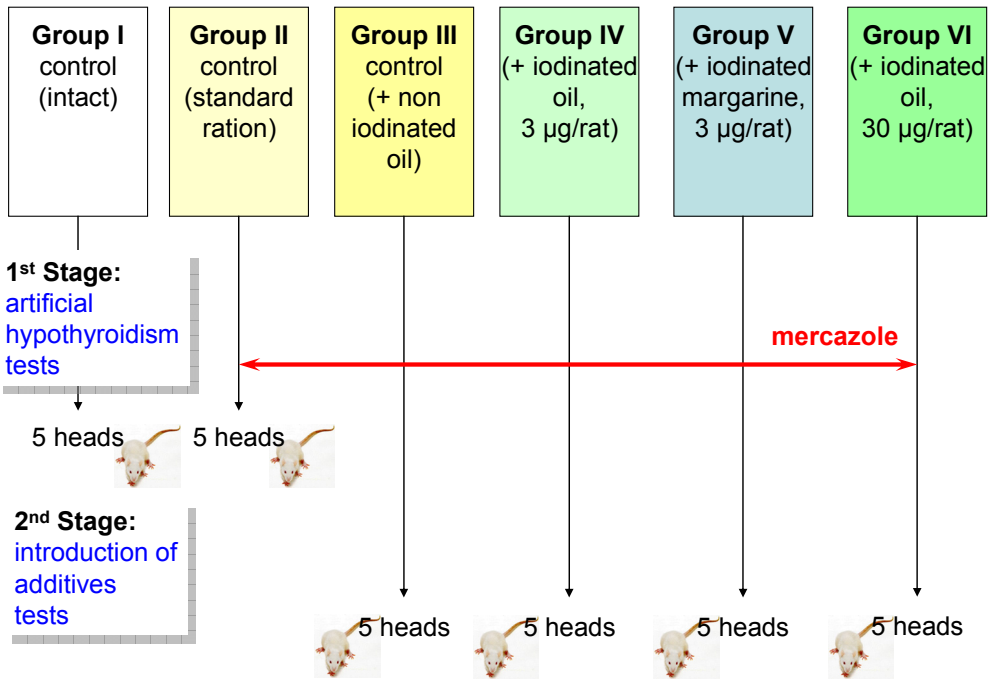


Figure 12. *Scheme of experimental work with white laboratory rats*

Analysis of Total Triiodothyronine hormone (T_3) in the serum of investigated rats

The procedure follows the basic principle of enzyme immunoassay where there is competition between an unlabeled antigen and an enzyme-labeled antigen for a fixed number of antibody binding sites. The amount of enzyme-labeled antigen bound to the antibody is inversely proportional to the concentration of the unlabeled analyte present. Unbound materials are removed by decanting and washing the wells. The absorbance measured is inversely proportional to the concentration of T_3 present in the serum. A set of T_3 Standards is used to plot a standard curve of absorbance versus T_3 concentration from which the T_3 concentrations in the unknowns was calculated.

Analysis of the Thyroxine hormone (T_4) in the serum of investigated rats

The principle of the Thyroxine (T_4) analysis in the serum of investigated rats is the same. A set of T_4 Standards is used to plot a standard curve of absorbance versus T_4 concentration from which the T_4 concentrations in the unknowns was calculated.

Analysis of the Thyroid-stimulating hormone (TSH, thyrotropin) in the serum of investigated rats

The TSH analysis is an enzymatically amplified "one-step" sandwich-type immunoassay. In the assay, standards, controls and unknown serum samples are incubated in microtitration wells which have been coated with anti-hTSH antibody in the presence of another anti-hTSH detection antibody labeled with the enzyme horseradish peroxidase (HRP). After incubation and washing, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual

wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of TSH in the sample. A set of TSH standards was used to plot a standard curve of absorbance versus TSH concentration from which the TSH concentrations in the unknown samples were calculated.

Analysis of iodine content in thyroid glands of investigated rats

For analysis of iodine content in thyroid glands of investigated rats was used spectrophotometer method of iodine determination. The method consists in mineralization of the sample with the following extraction of iodine with carbon tetrachloride in presence of sodium nitrite in acidic medium, measurement of absorption of reaction products on wavelength 514 nm. Relative error of average result is ± 2.05%.

The level of thyroid hormones of rats during the examination of iodinated sunflower oil and margarine

For getting of the model of artificial hypothyroidism there was used mercazole for blocking of thyroid gland function. Peroxidase catalyzes the oxidation reactions. It is known that the activity of oxidation ferments decreases on hypothyroidism and increases on hyperthyroid states. Mercazole depresses the ferment activity of iodineperoxidase – the ferment which provides the iodination of α – thyroxine, because in the content of thyroxine being the obligatory ingredient is iodine that provokes hypothyroidism.

Biological activity of examined iodinated products was evaluated according to indexes reflected the functional state of animals' thyroid gland. Pursuing this aim, it was determined the level of thyroid gland hormones such as total Triiodothyronine (T_3), Thyroxine (T_4) in the experimental rats' blood serum. The condition of hypophysial-thyroid system was judged according to the content of serum Thryroid-stimulating hormone (TSH, Thyrotropin). The obtained data are presented in Table 7.

Table 7. *Content of thyroid hormones in the rats' serum*

Group of rats	Total Triiodothyronine hormone (T_3) [ng/dL]	Thyroxine hormone (T_4) [nmol/L]	Thyroid-stimulating hormone (TSH, thyrotropin) [mUI/L]
I	92.65 ± 19.12	114.1 ± 16.49	0.894 ± 0.032
II	84.65 ± 14.54	93.1 ± 14.54	1.272 ± 0.037
III	89.94 ± 18.52	95.81 ± 19.53	0.966 ± 0.025
IV	90.26 ± 13.39	107.42 ± 15.02	0.856 ± 0.099
V	91.15 ± 16.76	119.37 ± 14.43	0.814 ± 0.034
VI	73.00 ± 19.94	92.83 ± 14.48	1.257 ± 0.027

Data regarding blood serum immune-enzyme analysis witnesses the decrease of thyroid glands functional activity of rats that were introduced in the condition of experimental hypothyroidism (II^{nd} and III^{rd} group). Introduction of mercazole called experimental hypothyroidism condition that was accompanied by morphological functional displacement in the thyroid system, expressed in T_4 level decrease and TSH concentration increase in the controlled group (group I) (Figure 13).

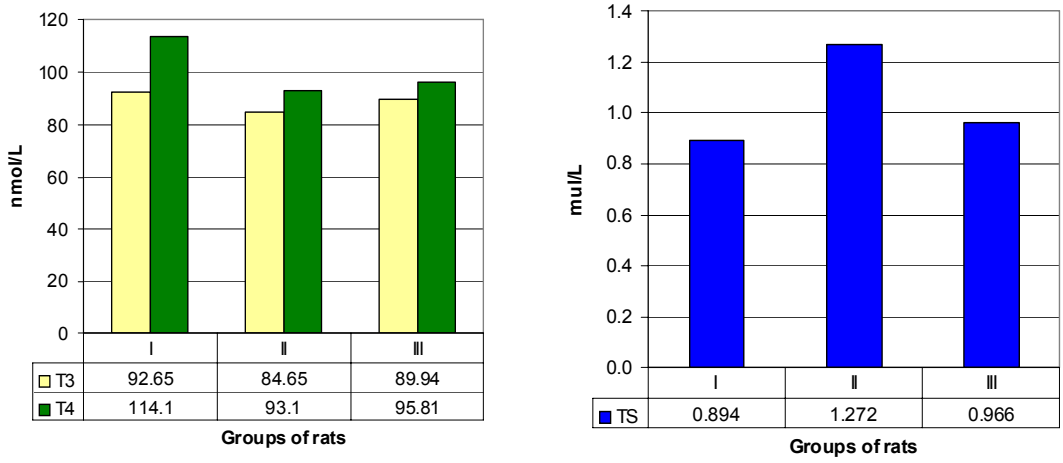
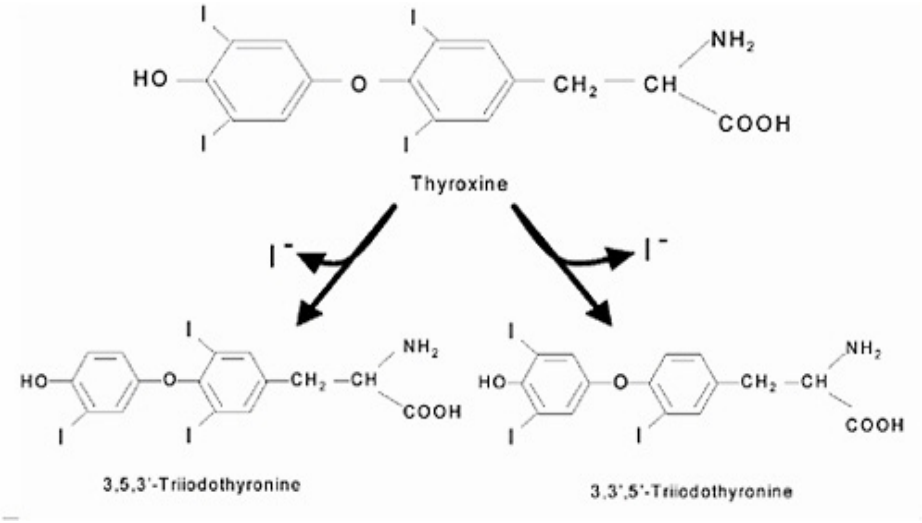


Figure 13. *Content of thyroid hormones in the serum of rats belonging to I, II, III groups*

Thus, T_4 concentration in the hypothyroid rats serum (IInd group) decreased and become 93.1 ± 14.54 nmol/L, in the IIIrd group – 95.81 ± 19.53 nmol/L against 114.1 ± 16.49 nmol/L of the control group. At the same time while hypoterioses increases thyrotrophic hormone (TSH) with 0.894 ± 0.032 mUI/L (control group) till 1.272 ± 0.037 mUI/L (IInd group) and 0.966 ± 0.025 mUI/L (IIIrd group).

Thereby, experimental rats, introduced in the condition of mercazole hypothyroids, show expressed destructive-degenerative processes in the thyroid glands in comparison with the controlled group. In the thyroid gland it is determined the lack of colloid in follicles in the result of termination of thyreoglobulin thyrocotis synthesis.

It is necessary to mark, that the T_3 concentration at the hypothyroid rats (IInd and IIIrd group) did not decreased significantly that can be explained as activation of deiodination T_3 in T_4 processes:



The present regularity is observed after analyzing the protective-compensatory reaction of the animal in the condition of blocking the tyrosine in the structure of thyreoglobuline. In the course of further research the evaluation of efficiency and safety

of examined iodine comprising complexes in the five compound groups.
The analysis of the received data confirms that research organically connected forms of iodine assisted in the increase of thyroid glands functional activity. Thus, rats of IVth and Vth groups had the significant bigger serum T_4 level than animals of IInd and IIIrd group (Figure 14).

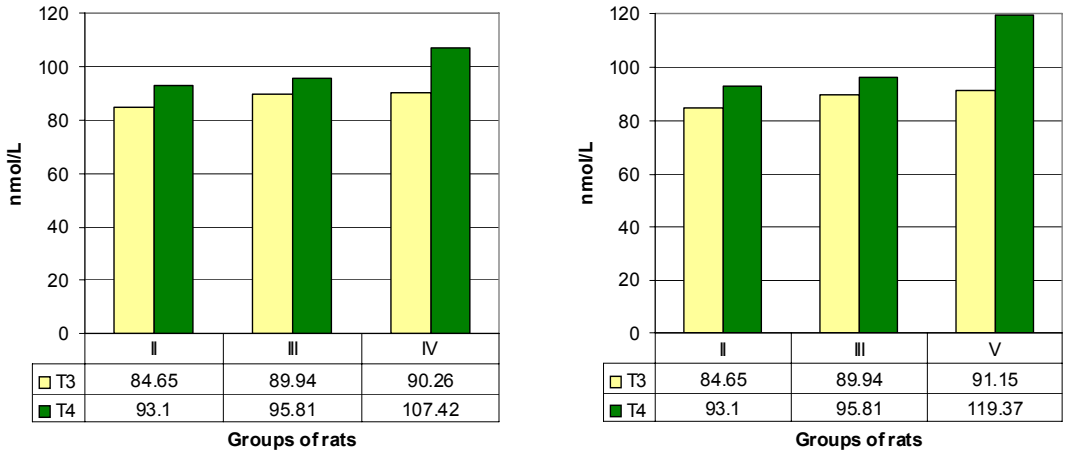


Figure 14. *Content of thyroid hormones in the serum of rats belonging to II, III, IV and V groups*

At the same time, T_4 concentration in the IVth group made up 107.42 ± 15.02 nmol/L, in the Vth one 119.37 ± 14.43 nmol/L against 93.1 ± 14.54 nmol/L in the IInd one and 95.81 ± 19.53 nmol/L in the IIIrd one. Concentration of T_3 in the compared groups does not differ in a significant way, remaining in limits between 90.26 ± 13.39 ng/dL (IVth group) and 91.15 ± 16.76 ng/dL (Vth group). The relatively high level of T_3 of the IIIrd group of rats (98.94 ± 18.52 ng/dL) is explained by the activation of T_4 deiodation processes in the condition of iodine deficit.
All animals who received additionally every day iodinated sunflower oil and margarine, increased level of T_4 secretion was accompanied by rather low TSH level (Figure 15).

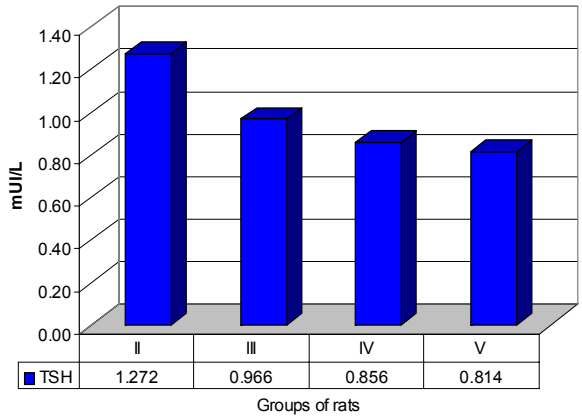


Figure 15. *Influence of iodinated food products over the rat thyroid-stimulating hormone synthesis*

Hereby, if animals of the IInd and IIIrd group have TSH concentration of 1.272 ± 0.037 mUI/L and 0.966 ± 0.025 mUI/L correspondingly, rats of the IVth and Vth groups' identical indexes reflected 0.856 ± 0.099 mUI/L and 0.814 ± 0.034 mUI/L. It is the evidence of stimulating influence of examining iodine products over the functional activity of rats' thyroid glands.

Use of iodine sunflower oil and margarine in the rats ration attributed to the gradual restoration of tyrocytes functional activity with the formation of colloid in follicles. All tested iodinated products have the homogeneous actions in the view of tyrocytes activity restoration. When the main components of the tyroglobuline-iodine in the combination with fat acids come into the organism, the synthesis of thyroid hormones in the tyrocytes is restarted. Consequently, regeneration possibilities and differentiation of thyroid glands tyrocytes are high and used by us substances assist to it. Besides of that, we carried out the research in evaluation of iodinated sunflower oil safety, which included examination of chronic toxicity as well. The toxicity was tested on the animals (of IVth group) that received daily during the whole experiment period 10-fold iodine dose. The value of tested iodine dose efficiency and safety over the rat organism held in two compared groups: IVth – introduction of 1-fold iodine dose in iodine sunflower oil and VIth group. The data analyses show that tested organic bonded iodine form is not toxic for the experimental animals. Thus, the rats of VIth group had the following T_3 and T_4 concentration levels 73.00 ± 19.94 ng/dL and 92.83 ± 14.48 nmol/L against 90.26 ± 13.39 ng/dL and 107.42 ± 15.02 nmol/L in the IVth group correspondingly. While the TSH level of the rats from the VIth group increased and constitute 1.257 ± 0.027 mUI/L in comparison with TSH concentration of rats from IVth group – 0.856 ± 0.099 mUI/L (Figure 16).

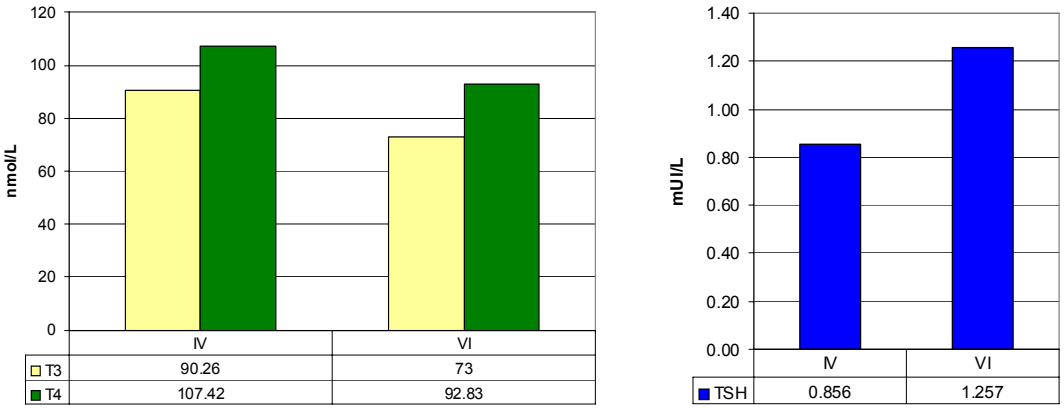


Figure 16. *Influence of 10-fold iodine dose in the structure of iodinated food products over the synthesis of rat thyroid hormones*

Data received in the result of executed research certify that the consumption of iodine in large quantities leads to decrease of T_3 and T_4 hormone secretion and increase of TSH concentration in comparison with the group of rats that have received 1-fold iodine dose. It can be explained by the decrease of thyroid gland ability to accumulate iodine and large quantities of iodine are taken away by the organism through kidneys. It is necessary to mark, that iodinated sunflower oil and margarine contributed to better use of feedstuff for rats.

Effect of iodine intake on iodine content of the thyroid gland

Iodine content in thyroid glands characterizes the intensity and direction of iodine exchange of animals. The investigations that we realized on iodine accumulation in thyroid glands confirmed the positive influence of optimal iodine level (3 μg/rat) on organism of experimental animals.

Feeding of experimental animals by optimal iodine level (3 μg/rat) increased the functional activity of thyroid gland and iodine concentration in it (Table 8).

Table 7. *Effect of iodine intake on iodine content of thyroid gland*

Group of rats	Iodine content of diet [μg/rat]	Weight of thyroid gland [mg]	Thyroid iodine [mg/100 g]
I	0.4 ± 0.1	25.8 ± 1.5	4.8 ± 0.9
II	0.4 ± 0.1	34.2 ± 1.7	1.2 ± 0.7
III	0.6 ± 0.2	18.2 ± 0.9	1.1 ± 0.6
IV	3.5 ± 0.8	24.8 ± 2.2	5.4 ± 0.7
V	3.6 ± 0.7	31.4 ± 3.8	13.0 ± 1.5
VI	30.0 ± 1.9	39.4 ± 5.7	28.0 ± 1.9

*average daily quantity of feed for rats – 12 ± 4 g

The investigation data indicate that iodinated fats influence on metabolism processes to the accumulation by animals' organism of the iodine, as a result of more effective digestion and assimilability of iodine from present connections.

In whole, the investigations of thyroid gland that we realized, proved that on experimental hypothyroidism the iodine content of rats decreased from 4.8 to 1.2 mg/100 g (groups I and II), so on addition of iodinated fats with iodine content (3 μg/rat) the iodine quantity in thyroid gland increased from 5.4 to 13.0 mg/100 g (groups III and IV). At addition of considerable quantities of iodine (30 μg/rat) the iodine content also increased, but the capacity of thyroid gland to iodine accumulation decreased.

Analysis of iodine content in thyroid glands, which was obtained from rats after correction of iodine-critical state, at the expense of introduction in their ration of iodinated fats showed the possibility to mention the improvement of functioning and the capacity of iodine accumulation by thyroid gland.

The study of literary data and the results of the investigation, that we effectuated on laboratory animals allow concerning the safety, bioavailability and simplicity of use of organically bonded iodine forms as iodinated fats (vegetable oil, margarine).

CONCLUSIONS

By physical and chemical methods it was established the presence of π *iodine-triglyceride* compounds in iodized oil, formed by fixing the molecular iodine at the double bound of the unsaturated fatty acids, with the participation of the π electrons of the donor group (the double bound of the unsaturated fatty acid). There were established the thermodynamic and kinetic parameters of the reaction of formation of the π *iodine-triglyceride* compounds. It was studied the stability and the validity term of storage and technological treatments. The researches about the iodine fixing capacity of the sunflower oil as π *iodine-triglyceride* compounds allowed generalizing the

incorporation technology of a significant amount of iodine (1 – 100 μg/mL), without sensitive changes of the product's physico-chemical properties.

The performed study permits to establish that physical and chemical properties of the iodinated sunflower oil vary insignificantly with regard to the reference sample during iodination and storage. In case of sunflower oil iodination, fixation of molecular iodine to double bond takes place with formation of π - type compounds, without breaking of the double bond from molecules of unsaturated fatty acids.

Carried out researches have shown that lipids represent a suitable matrix-carrier for iodine fortification of food products. It was elaborated the scientific basis and the technology of fabrication of lactic acid products, margarine and mayonnaise iodine fortified by the addition of iodized oil. The results of the investigations of the physical-chemical, microbiological and organoleptic properties, the oxidation stability of iodine fortified products compared to the control samples, indicate the absence of sensitive difference during the maturation and storage processes.

The iodine bioavailability in fortified lipid products has been investigated *in vitro* and *in vivo* (on Wistar white rats) conditions. *In vivo* researches have proved that iodized lipid products are influencing the metabolic processes by accumulation of iodine in animal's body, as a result of an efficient digestion and a high iodine bioavailability from the present complexes. After the introduction of iodinated fat products in the ration of hypothyroid animals, they manifest biological activity. Iodinated fat products contribute to restoration of such thyroid hormones levels as T_3, T_4 and TSH, i.e. functional activity of thyroid glands. Investigational data indicates that iodinated fats influence on metabolism processes and contribute to the accumulation of the iodine by animal organism, as a result of more effective digestion and assimilability of iodine from present connections. Application of iodinated fats supplies the lack of iodine in organism, does not have side effects and can be used in prevention of diseases, provoked by iodine deficiency.

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