Investigations concerning bioavailability *in vivo* of iodine from fortified lipid products

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Abstract
Sunflower oil takes up the biggest specific weight among edible fats, used in nutrition in the Republic of Moldova. Manufacturing and consumption of fortified sunflower oil with iodine, and derivatives products on it base is perspective direction on elimination alimentary dependent iodine deficiency disorders. With the aim of revelation of influence of process iodination on degree of merit of sunflower oil, and for determination of it oxidative stability were determined physicochemical properties of examined product. In consequence of studies was demonstrated high stability of fortified sunflower oil with iodine. Through *in vivo* study was demonstrated efficiency of fortification of lipid products with iodine under iodine status.

Keywords: iodine, Iodine Deficiency Disorders (IDDs), food fortification, sunflower oil, *in vivo* study.

1. Introduction

Iodine deficiency is one of the major public health problems often co-existing in many regions in the world and affect more than one third of the world's population, including the Republic of Moldova (WHO, 1996).

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 (Jaffiol *et al*., 1995; Delange, 1994).

The environment of the Republic of Moldova is characterized by a reduced values of the iodine content: 4,5-5,3mg/kg soil, 40mg/l water and 0,03-0,22mg/kg of vegetation, on a dry content of substances. Approximate 85% of populations of Moldova live in iodine deficient regions (UNICEF, 2002).

In order to eliminate the iodine deficiency disorders, the government of the Republic of Moldova adopted a National Program by the year 2004 (MO, 1998). However, all the measures taken in this direction, did not lead to significant improvement of the situation. Ministry of Health of Moldova with support of UNICEF studied nutritional statute of adults and children in Moldova in period from 1994 to 1997. Thus, 37% of the children in Moldova have goiter and only 32% of families consume adequate iodinated salt (R Moldova, 1996).

Three intervention strategies are available to prevent iodine deficiency. These are supplementation, dietary diversification, and both targeted and untargeted food fortification (Hurrell, 1999).The decrease of the consequences related to an insuficient iodine intake may be achieved through the application of a fortification strategy of different foodstuffs consummed by different population cathegories.

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.

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First, this would allow the easy incorporation of the iodine in the food fatty products. Secondly, the daily intake of lipids being limited, would alow an easy regulation of the iodine consumption, this being complementary with that from the iodinated salt and other products (John, 2003).

The purpose of present investigations consists in elucidation of efficiency of fortification with iodine of foodstuffs of lipidic origin.

2. Materials and methods

1.1. Sun flower oil fortification with iodine

In this study, double rafinated and deodorated oil was used (purchased from local stores), (STAS – 1129-93).

To obtain the iodinated oil, in sunflower oil chemicaly pure, cristaline iodine (I_2) (STAS – 4159-79) was administrated. After the establishement of the equilibrium, iodinated oil was used as sample for the present study.

1.2. Manufacturing of iodinated margarine

In proposed iodinated margarine a part of sun-flower oil is replaced by iodinated double refined and deodorated sun-flower oil with content of iodine 10μg I/cm^3.

Fatty basis constitutes 82.00–80.25% and includes following ingredients: double refined and deodorated sun-flower oil; iodinated sun-flower oil with content of iodine 10μg I/cm^3; extract of natural colour, obtained from carrot, on basis of double refined and deodorated sun-flower oil; refined maize oil; vegetable monoglycerides (emulsifiers) and soya lecithin (emulsifier). Liquid phase constitutes 19.75–18.00% and includes following ingredients: nonfat dry milk; bakery salt; potassium sorbate and water.

1.3. Physical and chemical indices of sun flower oil and margarine fortified with iodine

All the measurements were made according to the standard methods and standards STAS – 1129-93, STAS 240-85. Iodinated oil was analyzed at once and dinamicaly during three months (once a month).

1.5. Investigations in vivo

In 2006 in the laboratory of biochemistry of State University of Medicine and Pharmacology „N. Testemiteanu" was effectuated a cycle of investigations, which marked the preventive and treatment effect of products of lipidic origin fortified with iodine, elaborated in Technical University of Moldova, Faculty of Technology and Management in Food Industry.

For the purpose of elucidation of the influence of food regimes with different content of iodine on bioavailability of iodine of fortified lipidic products and the dynamics of evolution of experimental hypothyroidism were realized 2 series of experiments.

The experiment was realized with the 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 – 42 days. The animals were kept in individual cages, 5 heads in every cage.

The experiment provided 2 stages:

I stage – experimental reproduction of hypothyroidism with the help of mercazole for blocking of thyroid gland function (Teppermen and Teppermen, 1989). 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 (produce in the laboratory of Technical University of Moldova), with the purpose to exhaust the reserves of iodine of the organism.

II 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).

Scheme of experimental work with white laboratory rats is presented at the pic 1.

All the six groups of rats during the experiment got the following foodsuffs: well-milled frumenty welded on beef tea, so they got the lipidic products.

The frumenty was given daily, for dinner, on the assumption of daily consumption of 12g product/rat. After every stage of the experiment there were weighed the thyroid glands of animals and was determined the total content of iodine in them by spectrophotometer method.

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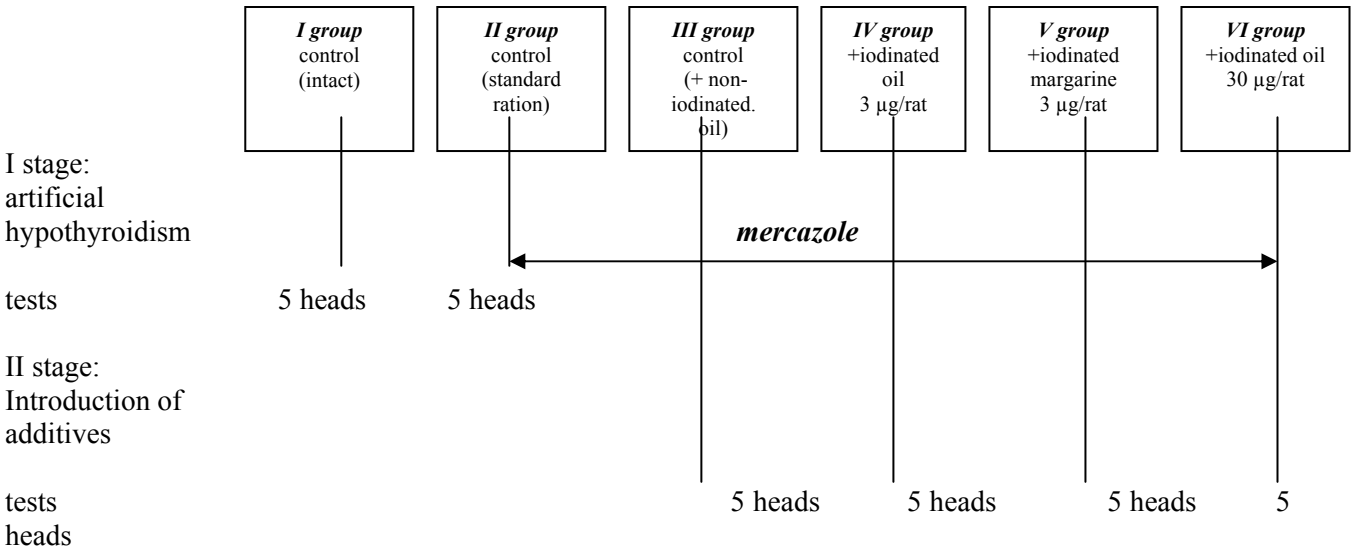

Figure 1. Scheme of experimental work with white laboratory rats

1.6. Analysis of iodine content in thyroid glands of investigated rats (ACTES SAR-2004)

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 carbone tetrachloride in presence of sodium nitrite in acidic medium, measurement of absorption of reaction products on wavelength 514 nm. Relative error of average result consists ±2,05%.

1.7. Determinations of errors and statistical analysis of obtained results

Investigations realized in triplication and processed statistically by the method of those small square with application of coefficient Student and determination of interval of investigation (Snedecor and Cochran, 1989)

3. Results and discussion

Sunflower oil is part of the vegetal oils group and has a high amount of mono- and polyunsaturated fatty acids.

Aiming the study of the influence of iodine administration in the sunflower oil, main indices have been evaluated and were referred to the product standards (table 1).

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

Physical and chemical indices	Reference sample	Iodinated oil, µg/ml		
		1	10	100
Iodine index	134±1	131±1	130±2	129±1
Refraction index, (20°C)	1,474± 0,001	1,475± 0,002	1,476± 0,001	1,476± 0,001
Saponification index, mg KOH/g oil	193±3	191±2	195±2	196±1
Free fatty acids content, % (oleic acid)	0,245± 0,005	0,245± 0,004	0,275± 0,003	0,285± 0,003
Peroxide index, méq/kg	10,0±0,2	8,9±0,1	9,8±0,2	10,9±0,1
Humidity and the volatile substance %	0,100± 0,005	0,055± 0,005	0,068± 0,005	0,100± 0,005

It was seen that the iodine indice varies little, so that even in the case of the sample with the highest iodine amount (100 $\mu g / ml$) 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*.

It is common knowledge, that halogens are capable of saturating double bonds present in the unsaturated lipids (Karlreskind, 1992). The addition of the active halogens to the double bonds is possible according to the mechanism of the nucleophile bimolecular substitution.

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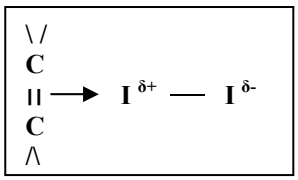
The speed of saturation depends on the:
- nature of halogen;
- number of double bonds;
- position of these double bonds in the chain of fatty acid;
- structure of triglycerides.
- It was established that, while the number of the carbon atoms between the carboxyl group – COO- and the double bond increases, the probability that the addition of the halogen reaction will take place decreases. Since fatty acids, present in sunflower oil have double bonds situated in the position -9=10- and -11=12- (linoleic acid), the probability that the iodine addition in these conditions will take place is very low.

It is obvious that, *during iodination of the studied sunflower oil the iodine addition cannot take place.* The activity of the double bonds is weaker when they are away from the carboxyl group. The growth of the carbon atoms in the acid chain decreases the activity of the double bonds and reduces the saturation speed.

At the same time verification of grade of widening of the product confirms the non-variability of connections number. There has its place the fixing of iodine molecules on double connection of the fatty acids, non-enriched by formation of the compounds of π type:



Formation of the compounds of π type is possible because of stabilization by resonance of excitation state that includes the both components, the fact established by analysis of the spectra IR of iodinated oil in correlation with the sample. In the field of UV/VIS has the place the displacement of absorption maximum characteristic for double bonds of the non-saturated acids A6 (figure 2).

Also, physical and chemical indices of iodinated margarine were determined in comparison with reference sample (table 2) according to the standard methods (STAS 240-85).

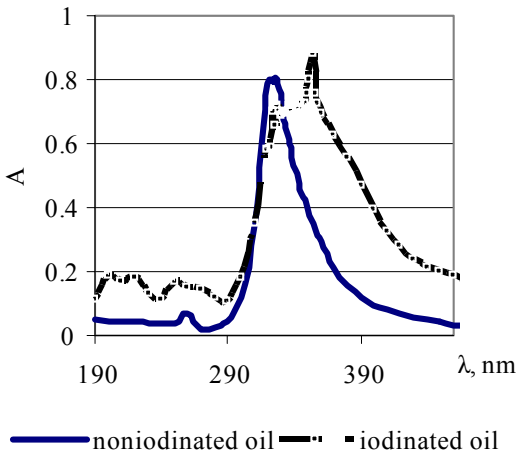


Figure 2. Spectrum of the sunflower oil before and after iodination in the UV/visible field

Table 2. Physical and chemical indices of iodinated margarine

Physical and chemical indices	Reference sample	Iodinated margarine (1μg I/g product)
Humidity and the volatile substance %, maximum	15.1±0.1	14.9±0.1
Melting tempetature, °C	32±1	32±1
Acid number (20^0C)	0.132±0.002	0.130±0.002
Content of NaCl, %	0.51±0.1	0.47±0.1

The physical and chemical properties of the iodinated margarine do not vary insignificantly in comparison with the reference sample.

Connection of iodine and vegetable oil gave the fixed organic connection with increased biological value, which is available for obtaining and does not require the creation of additional voluminous technologies.

But the problem of rise of biological availability of iodine from its connections with fats is studies not sufficiently and needs specification.

In the connection there were realized the investigation of study of influence of iodinated fats of different concentration of the capacity of iodine accumulation by thyroid gland of rats.

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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 (Alioshin, 1982).

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 (Dedov *et al.*, 1998).

For hypothyroidism confirmation we effectuated the determination of iodine content in thyroid glands of rats (table 3).

Table 3. 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%
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 ± 1,9	39,4 ± 5,7	28,0 ± 1,9

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

Earlier the similar investigations were effectuated by Berenstein (1966). It was established by him that addition to feed of iodine and of potassium iodic positively influence of function of thyroid gland. Iodic preparations assisted not only the improvement of thyroid gland functioning but also made better the use of feed by animals.

The obtained by us investigations results let us suppose that application of iodinated fats supplies the lack of iodine in animals' organism, and also it has not side effects.

Iodine content in thyroid glands characterizes the intensity and direction of iodine exchange of animals. Realized by us investigations on iodine accumulation in thyroid glands confirmed the positive influence of optimal iodine level (3 μg/rat) on organism of experimental animals. Obtained by us data on investigation of iodine content in thyroid glands agree with the works of Baranov (1970) and Seleatitskaia (1994).

Feeding of experimental animals by optimal iodine level (3 μg/rat) increased the functional activity of thyroid gland and iodine concentration in it. The obtained data agree with the investigation results of Fenchenco (2003) and Kashin (1990).

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 realized by us, proved that is on experimental hypothyroidism the iodine content of rats decreased from 4,8 to 1,2 mg% (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% (groups III and IV). On addition of considerable quantities of iodine (30 μg/rat) the iodine content also increased, but the capacity of thyroid gland to iodine accumulation decreased (fig. 3).

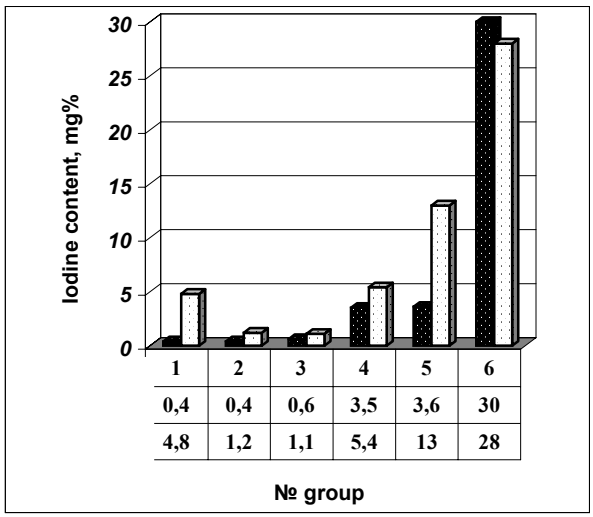

Figure 3. Influence of consumption by animals of iodinated fats on the process of its metabolism and the accumulation capacity by thyroid gland.

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 gives the possibility to

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mention the improvement of functioning and the capacity of iodine accumulation by thyroid gland.

Study of literary data and results of investigation effectuated by us on laboratory animals lets us judge concerning the safety, bioavailability and simplicity of use of organically connected iodine forms as iodinated fats (vegetable oil, margarine.

4. Conclusions

Connection of iodine and vegetable oil gave the fixed organic connection with increased biological value, which is available for obtaining and does not require the creation of additional voluminous technologies.

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.

Application of iodinated fats supplies the lack of iodine in organism, does not have side effects and they can be used in prevention of diseases, provoked by iodine deficiency.

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