Iodine food fortification: biological effects and safety aspects

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Abstract: Food fortification can be an important compliment to food-based approaches, and iodine fortification of foods as one of the strategies for the control of iodine deficiency. During this study there were elaborated new technologies for iodine fortification of proposed fatty foods. Investigation data indicate that iodine fortified fats influence on the processes of metabolism and contribute to the accumulation of the iodine by animal organism, as a result of more effective digestion and assimilability of iodine from fortified foods. Application of iodine fortified 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.

Key words: iodine deficiency, food fortification, food safety.

1. Introduction

Iodine deficiency (ID) continues to be the most prevalent nutritional deficiency disorder in the world, affecting an estimated two billion people, in both industrialized and developing countries. ID 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, 5].

Three intervention strategies are available to prevent ID. These are supplementation, dietary diversification, and both targeted and untargeted food fortification. Food fortification increases micronutrient supply to reduce nutritional deficiencies in the population by taking advantage of existing delivery mechanisms for industry-manufactured products. Population coverage depends on the food vehicle, but impact is contingent on the additional micronutrient intake and the nutrient gap. The fortification level is often limited by the criteria of safety, technological compatibility, and cost. Nevertheless, knowledge of the dietary characteristics of the population is still necessary to select the fortification condition with the highest effectiveness potential. Successful programs require reliable food enforcement and monitoring systems; selecting efficacious products is not enough [1, 3].

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 iodine fortified salt and other products. The purpose of present investigations consists in elucidation of efficiency and safety of fortification with iodine of foods of lipid origin.

2. Materials and methods

2.1. Technology of samples preparation.

Double refined and deodorized sunflower oil was used (purchased from local stores). To obtain the iodine fortified sunflower oil, chemically pure, crystalline iodine (I_2) was used. After the establishment of the equilibrium, iodine fortified oil was used as sample for the present study. To obtain iodine fortified margarine, a part of sun flower oil was replaced by iodine fortified sunflower oil with iodine content of 10 μg I/ml.

2.2. Investigations in vivo.

The experiment was realized with the lot of white rats of the Wistar line with masses in the range 180-210 g. The feed was a standard ration with free access to water. Duration of the experiment was of 42 days. The animals were kept in individual cages, 5 animals in every cage. The experiment had 2 stages:

Stage I – experimental reproduction of hypothyroidism with the help of mercazole for blocking of thyroid gland function. Daily (for 14 days) the rats were given water to drink with mercazole added. At the same time they were fed by bread without addition of iodine fortified salt (produced in the laboratory of Technical University of Moldova), with the purpose to exhaust the reserves of iodine of the organism.

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

All the six groups of rats during the experiment received the following foods: whole-grain wheat porridge prepared on meat broth, so they got the lipid products. The porridge was given daily, for dinner, on the assumption of daily consumption of 12g product/rat.

2.3. Analysis of Total Triiodothyronine hormone (T_3).

The procedure followed 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 was inversely proportional to the concentration of the unlabeled analyte present. Unbound materials were removed by decanting and washing the wells. The absorbance measured was inversely proportional to the concentration of T_3 presented in the serum. A set of T_3 Standards was used to plot a standard curve of absorbance versus T_3 concentration from which the T_3 concentrations in the unknowns was calculated.

2.4. Analysis of the Thyroxine hormone (T_4).

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

2.5. Analysis of the Thyroid-stimulating hormone (TSH, thyrotropin).

The TSH analysis was an enzymatically amplified “one-step” sandwich-type immunoassay. In the assay, standards, controls and unknown serum samples were 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 were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured was 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.

2.6. Analysis of iodine content in thyroid glands.

For analysis of iodine content in thyroid glands of rats was used a spectrophotometric method of iodine determination. The method consisted in mineralization of the sample with the following extraction of iodine with carbon tetrachloride in presence of sodium nitrite in acidic medium. Measurement of reaction products absorption was performed by dual wavelength absorbance measurement at 514 nm.

2.7. Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student and Microsoft Office Excel program version 2007. Differences were considered statistically significant if probability was greater than 95% (p-value <0.05). All assays were performed by triplicate at room temperature 20 ± 1 ºC. Experimental results are expressed as average ± SD (standard deviation).

3. Results and discussion

Biological activity and safety of examined iodine fortified products was evaluated according to indexes that reflect the functional state of animals' thyroid gland. After every stage of the experiment there was determined the total content of iodine in thyroid glands and content of thyroid hormones (T_3, T_4 and TSH) in the serum of investigated rats (table 1).

Table 1. Effect of iodine fortified sunflower oil and margarine intake on iodine content in thyroid gland and thyroid hormones level in the serum of rats

Group of rats	Iodine content of diet, [μg/rat]	Weight of thyroid gland, [mg]	Thyroid iodine, [mg%]	Content of thyroid hormones in the serum of rats		
				Total Triiodothyronine hormone (T_3), [ng/dl]	Thyroxine hormone (T_4), [nmol/l]	Thyroid-stimulating hormone (TSH), [muI/l]
I	0.4 ± 0.1	25.8 ± 1.5	4.8 ± 0.9	92.65±1.91	114.1±1.64	0.894±0.032
II	0.4 ± 0.1	34.2 ± 1.7	1.2 ± 0.7	84.65±1.45	93.1±1.45	1.272±0.037
III	0.6 ± 0.2	18.2 ± 0.9	1.1 ± 0.6	89.94±1.85	95.81±1.95	0.966±0.025
IV	3.5 ± 0.8	24.8 ± 2.2	5.4 ± 0.7	90.26±1.33	107.42±1.50	0.856±0.099
V	3.6 ± 0.7	31.4 ± 3.8	13.0 ± 1.5	91.15±1.67	119.37±1.44	0.814±0.034
VI	30 ± 1.9	39.4 ± 5.7	28.0 ± 1.9	73.00±1.99	92.83±1.44	1.257±0.027

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

Data regarding blood serum immune-enzyme analysis witnesses the decrease of thyroid gland functional activity in rats that were in the condition of experimental hypothyroidism (II and III 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 reference group (group I).

Thus, T_4 concentration in the hypothyroid rats serum (II^{nd} group) decreased and become 93.1±14.54 nmol/l, in the III^{rd} group – 95,81±19.53 nmol/l against 114.1±16.49 nmol/l of the reference group. At the same time hypothyroidism contributes to the increase of thyrotrophic hormone (TSH) with 0.894±0.032 mul/l (reference group) till 1.272±0.037 mul/l (II^{nd} group) and 0.966±0.025 mul/l (III^{rd} group).

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

All thyroxine (T_4) and some triiodothyronine (T_3) are produced by the thyroid gland, and their production there is stimulated by thyroid-stimulating hormone (TSH), a product of the anterior pituitary gland. Some T_4 is converted to T_3 in other tissues, including the pituitary gland and the hypothalamus. T_3 inhibits pituitary secretion of TSH, and hypothalamic secretion of thyrotropin-releasing hormone (TRH), which stimulates TSH secretion. The interplay between T_3 and TSH maintains thyroid hormone production within a narrow range.

It is necessary to note, that the T_3 concentration at the hypothyroid rats (II^{nd} and III^{rd} group) did not decrease significantly, that can be explained as activation of deiodination T_3 in T_4 processes. The present regularity is observed as the account of protective-compensatory animal reaction in the condition of iodination blocking of remaining tyrosine as a component of thyreoglobulin:

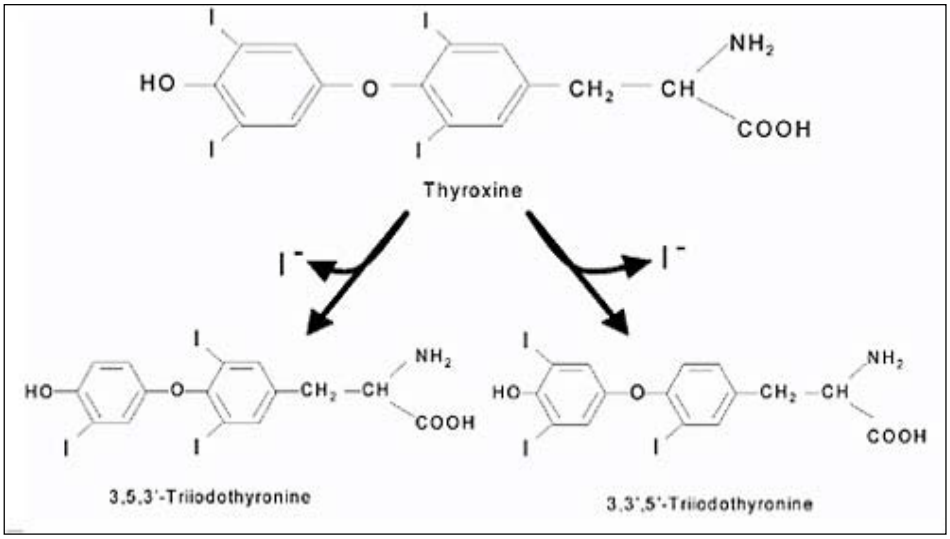

Figure 1. Formation of Triiodothyronine from Thyroxine

In the course of further research the evaluation of efficacy and safety of examined iodine complexes was performed in the five groups. The analysis of the received data confirms that organically connected forms of iodine contributed to the increase of functional activity of thyroid gland. Thus, rats of IV^{th} and V^{th} groups had significantly higher serum T_4 level then animals of II^{nd} and III^{rd} groups (Figure 2).

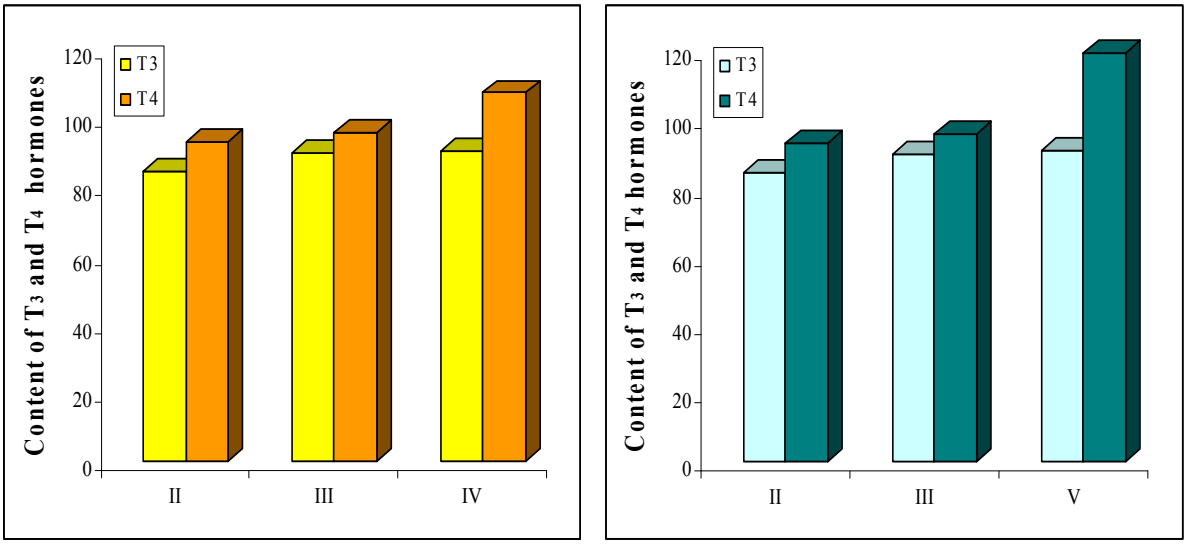

Figure 2. Content of T_3 and T_4 hormones in the serum of rats (II, III, IV и V groups).

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 of 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 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 daily iodine fortified sunflower oil and margarine, showed increased level of T_4 secretion accompanied by rather low TSH level (Figure 3).

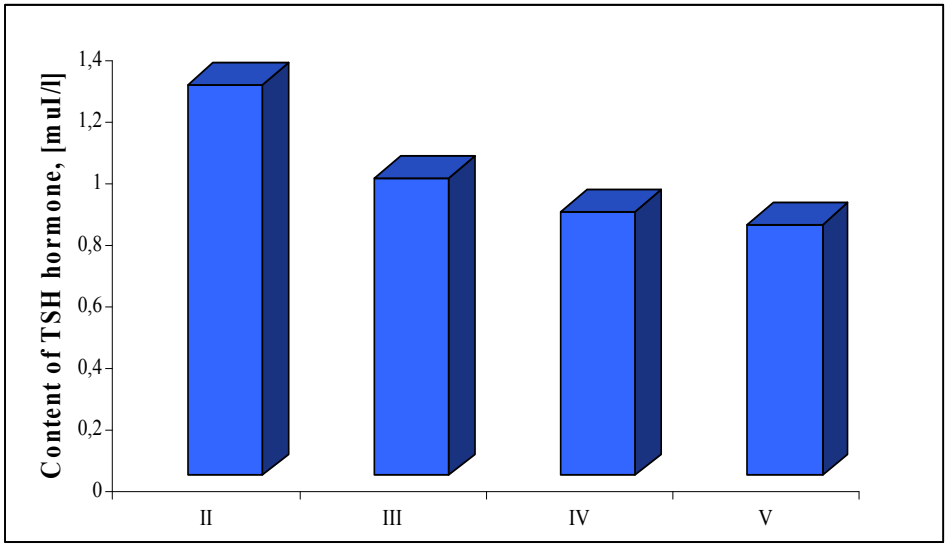

Figure 3. Influence of iodine fortified food products over the rat TSH hormone synthesis.

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, in 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 contributed to the gradual restoration of tyrocyte functional activity with the formation of colloid in follicles. All tested iodine fortified products have the homogeneous actions in the view of tyrocytes activity restoration.

When the main components of the tyroglobuline-iodine in the combination with fatty acids enter the organism, the synthesis of thyroid hormones in tyrocytes restarts. Consequently, regeneration possibilities and differentiation of thyroid glands tyrocytes are high and used substances assist in it. Besides of that we carried out the research in evaluation of iodine fortified sunflower oil safety that 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: IV – introduction of 1-fold iodine dose in iodine sunflower oil and VI group. The analysed data show that tested organic connected iodine form is not toxic for the experimental animals. Thus, the rats of VI group had the following T_3 and T_4 concentration level 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 IV group respectively. The TSH level of the rats from the VIth group increased and constituted 1.257±0.027 muI/l in comparison with TSH concentration of rats from IV group – 0.856±0/099 muI/l.

Data received in the result of our 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 received 1-fold iodine dose. It can be explained by decrease of thyroid gland ability to accumulate iodine and large quantities of iodine removed from the organism through kidneys. It is necessary to mark, that iodine fortified sunflower oil and margarine contributed to better use of feedstuff for rats.

Iodine content in thyroid glands characterizes the intensity and direction of iodine metabolism of animals. Our investigations on iodine accumulation in thyroid glands confirmed the positive influence of optimal iodine level (3 µg I/rat) on organism of experimental animals. Feeding of experimental animals with optimal iodine level (3 µg I/rat) increased the functional activity of thyroid gland and iodine concentration in it (figure 4).

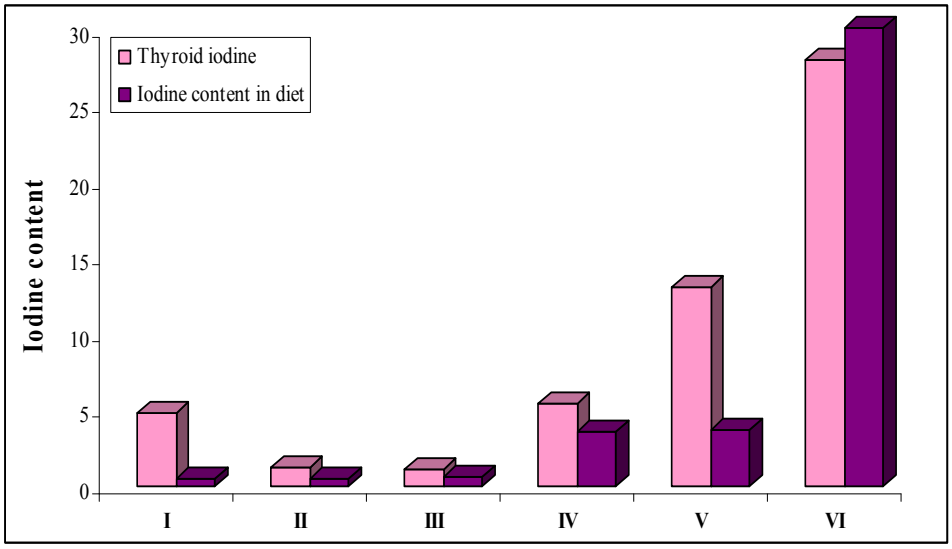

Figure 4. Influence of consumption by animals of iodine fortified fats on the process of iodine metabolism and accumulation capacity by thyroid gland.

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

The investigations based on estimation of iodine content in the thyroid glands of experimental animals, proved that in experimental hypothyroidism the iodine content of rats decreased from 4.8 to 1.2 mg% (groups I and II), and on addition of iodine fortified fats with iodine content (3 μg I/rat) the iodine quantity in thyroid gland increased from 5. to 13.0 mg% (groups III and IV). On addition of considerable amounts of iodine (30 μg I/rat) the iodine content also increased, but the capacity of thyroid gland for iodine accumulation is 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 iodine fortified fats gives the possibility to underline the improvement of functioning and the capacity of iodine accumulation by thyroid gland.

4. Conclusions

Food manufacturing industry is actively involved in fortifying processed/semi-processed foods that are targeted toward like particular segments of the population. The efficacy of iodine fortification depends not only on the appropriate identification of the vehicle, but also on the stability of the form used for fortification, packaging, storage and the methodologies of quality assurance. Literature data and the results of proper research on laboratory animals lets us conclude concerning the safety, bioavailability and simplicity of use of organically connected iodine forms as iodine fortified fats (sunflower oil, margarine).

In the present work were examined morphological changes in the thyroid system of rats at the experimental mercazole-induced hypothyroidism. As well it was determined the influence of iodine fortified oil and margarine on the thyroid system of rats. It specified the safe value of iodine fortified oil and margarine for rats. *In vivo* study demonstrated the efficacy and safety of fortification of lipid products with iodine under iodine deficiency status.

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