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IN VITRO BIOAVAILABILITY OF SUNFLOWER OIL FORTIFIED WITH IODINE

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Abstract. The use of iodized sunflower oil as an additive is an affordable and inexpensive method. In addition, iodine is a liposoluble element, which facilitates its incorporation into oil. But the incorporation of iodine in oil is a complex phenomenon, accompanied by a change in the physicochemical properties of the finished product, and therefore the evolution of the composition of triglycerides in iodinated oil has been investigated, depending on the amount of iodine administered. Free fatty acids (FFAs) are subjected to a different metabolic mechanism depending on the length of their chain (their molar mass). Thus, long chain fatty acids after re-esterification are deposited in the adipose tissue in the form of triglyceride-rich lipoproteins, while medium chain FFAs are usually oxidized in the liver. The structure of triglycerides (the distribution of fatty acids in the triglyceride molecule) can also influence the digestibility of this product. The purpose of the researches is to determine the influence of sunflower oil iodine on the digestibility of triglycerides and the evolution of the iodine content in the system during the enzymatic hydrolysis of the product. The kinetics of MGs, DGs and FFAs accumulation and the percentage of iodine recovered from the digestate were investigated during in vitro pancreatic digestion of iodinated sunflower oil.

Keywords: *iodine, triglycerides, oil, free fatty acids, pancreatic digestion in vitro.*

Introduction

The problem of food iodine deficiency is one of the most common cases of nutritional diseases on the earth [1, 2]. In the last 10 years in Moldova the incidence of endemic diseases of the thyroid gland increased by 8-10 times. The percentage of children and adolescents with endemic hyperplasia of the thyroid gland is 33-47%; 2.8-5.7% of the population has a goiter, which is often expressed by the appearance of nodules, while 1.5-4.2% of people suffer from hypothyroidism [3, 4].

Iodine is a mineral essential to the body, being essential for the synthesis of thyroid hormones. As a liposoluble element, its use in products of lipid origin is of particular interest. Firstly, it allows easy incorporation of iodine into food. Secondly, because the daily intake of fat is limited, the iodine intake can be easily adjusted. The advantage of this method of regulating iodine intake is that the absorption of iodine from foods of lipid origin occurs gradually, depending on the needs of the organism [5, 6].

Sunflower oil is a current consumer product for the Republic of Moldova, so that the manufacture of iodine fortified oil would constitute a considerable iodine supplement (40-50μg / day) which, associated with the iodine intake of kitchen salt, would contribute to the eradication of iodine deficiency [7,8]. Of particular interest is the incorporation of iodine oil into different food groups in order to broaden the spectrum of products with adequate iodine content [9].

The objective of this paper is to develop the process of fortification of sunflower oil and to evaluate the quality index (of the physicochemical properties) of the iodine oil.

Materials and methods

The advantage of this research lies in usage of inland products for fortification, products that are characteristic to this area.

Vegetable oils in the commercial, nutritional and medicinal planes are of particular importance, since a number of methods for analyzing the digestibility of triglycerides have been developed. Some of these studies are performed using pancreatic lipase, but most involve enzymes of industrial products of microbial origin [10, 11]. All currently known methods can be classified into two categories: in vivo methods and in vitro methods.

In vivo methods are the most reliable, but due to the many uncontrollable factors present in real systems that can directly influence processes, the study of triglyceride hydrolysis is technically impossible. Verifiable results can only be obtained with the use of radioactive labeling (isotope analysis). In addition, the duration of in vivo testing is, in these cases, quite long.

The advantage of in vitro methods is the possibility of isolation of the studied parameter - free fatty acids (FFAs), mono-, bi- and triglycerides [12,13]. This is an important quality parameter in fat and oil processing. However, the practical way to trigger hydrolysis of triglycerides essentially influences the results, which can not be reproduced in the physiological environment. Thus, in vitro investigations are performed with pancreatic lipases in an aqueous medium, in the presence of a buffer solution, an emulsifier and cofactors. The need to provide a stable interface complicates the in vitro research of the kinetics of triglyceride hydrolysis. Since enzyme activity is optimal only in organic media, the nature of the solvents used also influences the results obtained.

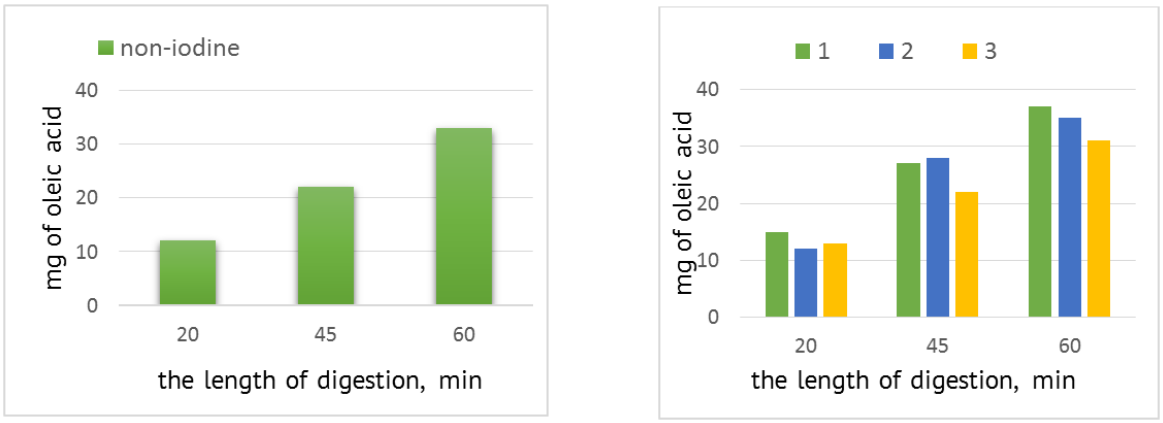
Results and discussions

The investigations carried out aimed to determine the influence of sunflower oil iodine on the digestibility of triglycerides. Also, the evolution of iodine content in the system during the enzymatic hydrolysis of the product was analyzed [14, 15].

FFAs dosing after pancreatic digestion was performed at 20, 45 and 60 minute intervals. The results obtained for the control sample (unedged oil) are shown in Figure 1a.

It is observed that hydrolysis of triglycerides in sunflower oil occurs during in vitro pancreatic digestion, which confirms the correctness of the experiment. Testing of the FFAs content in the iodine oil samples containing 1, 10 and 100 μg iodine/ml of oil was then performed. The results obtained are shown in Figure 1b.

It is noted that at low concentrations of iodine the content of FFAs, expressed in mg of oleic acid, does not vary significantly from the control sample. Only at the concentration of 100 μg iodine/ml of oil is a decrease of up to 10%, the rate of enzymatic hydrolysis reaction of triglycerides and the accumulation of FFAs in the digestate.



a) b)

Figure 1. Evolution of the FFAs content, expressed in mg of oleic acid, during the in vitro pancreatic digestion of sunflower oil: a) non-iodine (control sample); b) iodate: 1 – 1 μg iodine/ml oil; 2-10 μg iodine/ml oil; 3 – 100 μg iodine/ml oil.

In the digestate samples taken at the same time intervals, the total iodine content was analyzed after extraction in chloroform. The results obtained are shown in Figure 2.

It is found that the percentage of iodine recovered from the digestate (in relation to the administered content) varies between 57 and 92%, being lower for the maximum iodine content (100 μg iodine/ml of oil).

This is due to the more significant iodine losses during digestion when such a large amount of iodine is administered. However, such a recovery rate demonstrates that iodine from the iodine sunflower oil could serve as an important source of this deficient micronutrient for the organism because it is mostly preserved in the digestive medium.

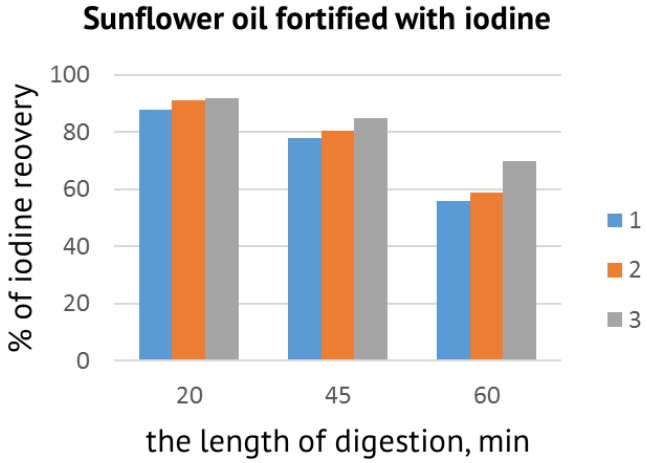


Figure 2. Percentage of iodine recovered during pancreatic digestion of iodized sunflower oil with an iodine content of: 1 – 1 μg iodine/ml oil; 2-10 μg iodine/mL oil; 3 - 100 μg iodine ml of oil. Duration of digestion: 20, 45 and 60 minute.

Analysis of lipid digestion products. To determine the kinetics of hydrolysis of triglycerides in sunflower oil, a pre-test with pure glycerides was performed.

The purpose of this experiment was to determine the yield of enzyme dosing of glycerin from standard solutions. The results obtained are shown in Table 1.

Hydrolysis of glycerides does not occur completely, even in the case of the maximum duration of pancreatic digestion (6 hours). The recovery percentage is in the average at 80%. This was taken into account in the analysis of the data obtained for the iodine sunflower oil.

Table 1.

Yield of enzyme dosing of glycerol from standard solutions of triolein, diolein and monoolein *

Glyceride	% recovery of glycerol **		
	0,5 g/l	1,0 g/l	1,5 g/l
TGs	92,7 ± 6,8***	77,1 ± 4,2	76,7 ± 7,8
DGs	78,4 ± 5,7	79,8 ± 5,9	81,7 ± 7,2
MGs	81,6 ± 7,2	80,9 ± 5,2	83,1 ± 4,5

* solvent - absolute ethanol; ** - duration of pancreatic digestion - 6 hours.
*** - average and dispersion of results from 3 parallel trials.

Figure 3 shows the kinetics of triglyceride hydrolysis in iodine sunflower oil during 6 hours of pancreatic digestion. In all cases, the residual amount of triglycerides is very low and does not exceed 15% of the initial amount. Thus, it is evident that administration of iodine in sunflower oil does not influence the degree of pancreatic digestion of triglycerides, demonstrating that the biological value of the product is not impaired.

Of particular interest is the analysis of the structure of products of pancreatic digestion of triglycerides (TGs) from iodine sunflower oil [16,17]. In the paper, the kinetics of the accumulation of monoglycerides (MGs), diglycerides (DGs) and FFAs during the in vitro pancreatic digestion of the iodine sunflower oil and the control sample were investigated. It is noted that the maximum level of MGs and DGs is already reached after 2 hours of pancreatic digestion (Figure 4). For DGs, the maximum obtained corresponds to only 14,7% of the total glyceride, while the MGs peak corresponds to about 37% of the total glyceride content. Over the next 4 hours an insignificant decrease in MGs and DGs content occurs, and a significant increase in FFAs content. There was not a significant difference between kinetics of in vitro pancreatic hydrolysis and the structure of products obtained during iodine oil hydrolysis as compared to the control sample.

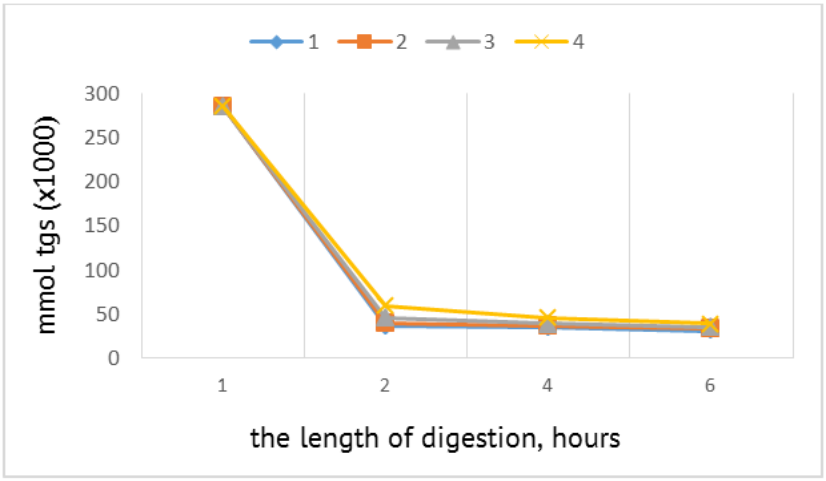
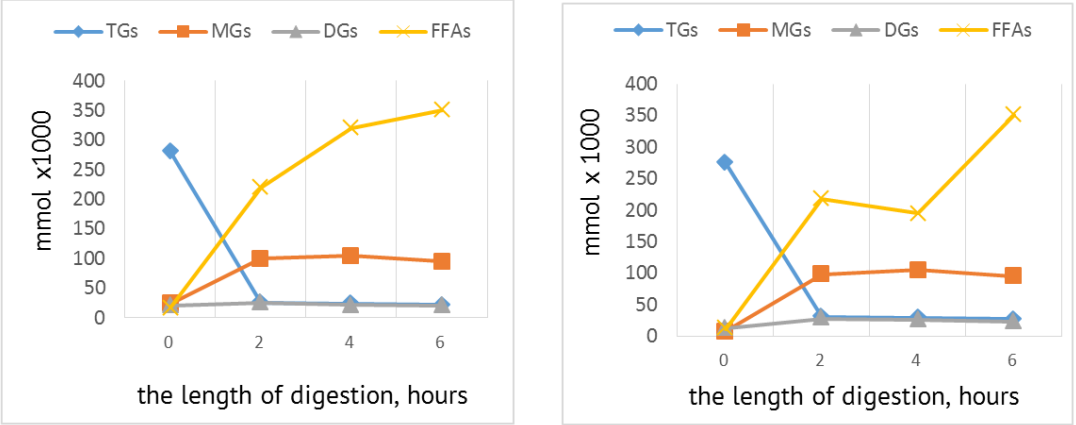


Figure 3. Kinetics of pancreatic hydrolysis of TGs from iodine sunflower oil: 1 - control sample (unrendered oil); 2 – 1 μg iodine ml oil; 3 - 10 μg iodine/ml oil; 4 – 100 μg iodine/ml of oil.

The disappearance of TGs during hydrolysis is a global indicator of the digestibility of a lipid source. DGs shows an intermediate substrate because they generate MGs after release of an FFAs molecule under the influence of pancreatic lipase. Analyzing the distribution of digestive products of a lipid source resulting from hydrolysis allows to highlight the difference between the biological value of these sources. In the examined case no significant

difference was found between the distribution of the iodine sunflower oil hydrolysis products compared to the control sample. This is due, first of all, to the advanced degree of hydrolysis of the substrate in the first two hours.



a) b)

Figure 4. Kinetics of the appearance of pancreatic digestion products (in vitro) of sunflower oil (a) and iodized sunflower oil (b) with an iodine content of 10 μg iodine/ml of oil.

When taking into account that the final products of hydrolysis are glycerine and FFAs, hydrolysis reactions may be described schematically as follows:

$$TGs \rightarrow DGs_1 + FFAs_1$$

$$DGs_2 \rightarrow MGs_2 + FFAs_2$$

$$MGs_3 \rightarrow glycerine + FFAs_3$$

DGs1 is the amount of diglycerides formed by hydrolysis of triglycerides, and DGs2 is the amount of diglycerides remaining after the formation of monoglycerides (MGs2) and FFAs2. Hydrolysis of a triglyceride generates a DGs and releases an FFAs, etc. If the content of TGs, glycerine and FFAs is known in the given environment, the FFAs content formed at DGs hydrolysis can be calculated:

$$FFAs_2 = FFAs_{total} - FFAs_1 - FFAs_3$$

Subsequently, the amounts of DGs and MGs remaining in the reaction medium can be calculated:

$$DGs_2 = FFAs_1 - FFAs_2; \quad MGs_3 = FFAs_2 - FFAs_3$$

These calculations can be carried out without necessity of determining the rate of hydrolysis of the intermediate reactions involved, only by final testing of the glycerin content and FFAs, which reduces the number of experiments to be performed [18,19]. Obviously, the presence of other substances in the reaction medium, which could affect to some extent the digestibility of lipids, was not taken into account. In particular, it is about Ca^{2+} proteins and ions, which, according to bibliographic sources, can significantly alter the rate of lipid hydrolysis.

Conclusions

Research on pancreatic (in vitro) digestion of iodinated sunflower oil and control sample showed that at low concentrations of iodine the content of FFAs, expressed in mg of oleic acid, did not vary significantly from the control sample. Only at the concentration of 100 μg iodine/ml of oil is a decrease of up to 10% of the rate of enzymatic hydrolysis reaction

of triglycerides and accumulation of FFAs in the digestate. The percentage of iodine recovered from the digestate (in relation to the administered content) varies between 57 and 92%, which shows that iodine from the iodine sunflower oil could serve as an important source of this deficient micronutrient for the body.

The kinetics of triglyceride hydrolysis of iodine sunflower oil were investigated during 6 hours of in vitro pancreatic digestion. In all examined cases (iodine content ranges from 1-100 μg iodine/ml of oil, the remaining amount of triglycerides is very low and does not exceed 15% of the initial amount). Iodine administration in sunflower oil does not influence the degree of pancreatic digestion of triglycerides. The kinetics of MGs, DGs and FFAs accumulation were investigated during in vitro pancreatic digestion of iodinated sunflower oil (10 μg iodine/ml of oil) and in the control sample. It has been established that the maximum level of MGs and DGs is already reached after 2 hours of pancreatic digestion. For DGs, the maximum obtained corresponds to only 14,7% of the total glyceride, while the MGs peak corresponds to about 37% of the total glyceride content. There was no significant difference between the kinetics of in vitro pancreatic hydrolysis and the structure of the products obtained during the iodine oil hydrolysis as compared to the control sample, which demonstrates that the biological value of sunflower oil is not affected by iodine administration.

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