FOOD SUPPLEMENT BASED ON CHITIN WITH ENHANCED LIPID-LOWERING AND SORPTION PROPERTIES

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Abstract

Method for evaluating lipid-binding qualities of enterosorbent was described. Mathod takes into account peculiarities of food digestion. A number of lipid binding agents was tested.

The researches showed that biologically active additive to food "Chizitel" extracted via electrochemical method has the highest sorption properties towards triglycerides and fatty acids in comparison with well-known enterosorbents such as chitosan and microcrystalline cellulose (MCC) in the investigated PH-range.

Key words: *enterosorbents, lipid-binding agents, chitin-mineral complex, chizitel,*

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1. Introduction

The problem of recycling waste from crustaceans is a relevant problem in today's fishing industry. Shellfish processing can result in acquiring up to 50% of waste. This waste pollutes the environment, but at the same time, it contains such components as valuable proteins, lipids and biologically active polysaccharide chitin.

Biological activity of this natural polysaccharide is based on its immunoadjuvant properties, biocompatibility with animal and human tissues, sorption of heavy and transitional metals, radionuclides, and antimicrobial activity against a number of pathogens. Particularly interesting is its ability to bind to polysaccharides of various lipids, which opens the possibility of its use as lipid-binding dietary supplements[1,2].

It is known that an increase of stress on the human body is often accompanied by metabolic disturbances, hyperlipidemia, an increase of cardiovascular diseases, and accumulation of toxic contaminants (metal ions radionuclides and others) in the body.

One of the fastest growing fields in application of chitin and its derivatives is the production of preventive dietary supplements for weight normalization [3]. This is due to the unique properties of these biopolymers, including sorption of lipids. They are very efficient in binding of lipids (especially the "bad" low-density cholesterol). Chitin-containing materials are 5 - 10 times more efficient in binding fat compared to other polysaccharides. Therefore, the use of chitin and its derivatives as a preventive agent for excess fat is growing.

Studies have shown that chitin and chitosan protected mice against peanut-induced anaphylaxis reactions[4]. Chitin-based networks of sponge origin are useful for effective uranium adsorption[5]. The parameters of high-fat diet-induced rats indicated that chitosan and its two derivatives not only have low cytotoxicity but can control overnutrition by fat and achieve insulin resistance therapy[6]. It was shown that there is a direct correlation between lowering of serum cholesterol with chitosan and inhibition of atherogenesis, and suggests that the agent could be used to inhibit the development of atherosclerosis in individuals with hypercholesterolaemia[7].

Currently on the international market of supplements there are many preparations based on chitin and its deacetylated derivative chitosan.

Studies in in vivo experiments demonstrated that chitin-containing materials substantially exceed other polysaccharides (e.g. MCC) (5-10 times) in efficiency of fat binding, they lower the level of triglycerides and LDL cholesterol and increase HDL cholesterol concentration in the blood.

However, in vivo studies are expensive and require significant time. The lack of a standard method for evaluating properties of lipid binding agents in vitro makes it difficult to develop and implement new lipid binding agents.

Chitosan is the most widely used as a lipid-binding dietary supplement. It has significantly higher sorption properties in relation to metal ions, triglycerides and fatty acids than chitin.

However, due to the solubility of chitosan in the stomach there is a danger of the of its low molecular weight fraction (molecular weight <5000 Da) getting through the wall of the gastrointestinal tract (GIT). In addition, it has a negative effect on human natural intestinal microflora and increases gastric pH, which makes it suitable only in case of excess acidity.

The aim of this study was to develop a food supplement based on chitin that will be insoluble in the gastrointestinal tract, will have high sorption properties towards lipids, to develop a method of evaluation of the sorption properties of lipid-binding agents in vitro in relation to triglycerides, fatty acids and heavy metals.

2. Materials and Methods

The samples for the study were used chelators - food supplements, belonging to the group of natural dietary fiber: chitincontaining materials and cellulose.

Chitinel – chitin produced by electrochemical demineralization and deproteinization freshwater crustacean Gammarus pulex.

Chizitel – Chitin-mineral complex obtained from freshwater crustacean Gammarus pulex by electrochemical deprotonation of their shells[8,9].

As reference samples following substances were taken due to being available on the market as lipid sorption agents:

- Microcrystalline cellulose (MCC). Manufactured by JSC "Ekvalon" .
- Chitan - chitosan from crustacean shells, manufactured by JSC “Bioprogress”, Moscow.
- Polyphepan - hydrolyzed lignin. Manufactured by LLC "Ecosphere", Veliky Novgorod.

We proposed to conduct evaluation of lipid-binding capacity by the number of adsorbed lipids rather than a change in their concentration in the supernatant. Method for evaluating lipid-binding ability in vitro included the following:

1. Creating conditions simulating the digestion of food in the stomach in vivo. creating conditions simulating digestion of food in the stomach in vivo. To do this, a sample of lipid-binding agent was added to a solution of hydrochloric acid (0.25 M), which will ensure the pH = 2,4 taking into account that the acid-base and buffer properties of enterosorbents may significantly change the pH of the system. Then the analyzed lipid was added to the mixture of hydrochloric acid and enterosorbent (triglyceride and fatty acid). The resulting mixture was incubated at 37C with constant stirring with a frequency of 32 rpm for two hours. Despite the fact that food stays in the stomach in 4-6 hours incubation time can be limited to two hours. This temporal limitation of the experiment was chosen as the optimum, since a further increase in the incubation time had no effect on the final result of the sorption-lipid.
2. Creating conditions simulating digestion of food in the intestine in vivo. Neutralization of the acid with phosphate buffer to pH = 6.5-7.0 was carried out, and the mixture was incubated at a temperature of 37C with constant stirring at 200 rpm for three hours.

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3. To reduce the error in determining the lipid binding ability of enterosorbents calculation should be carried out by the number of lipid adsorbed by the agent and not from the supernatant. To do this the suspension should be centrifuged at 6000 rpm for 40 minutes. Supernatant was drained and the residue was dried at constant temperature of 50 ° C and then weighed.
4. Calculations should be based on the formula for determining the value of lipid binding ability to fatty acids and triglycerides:

$$E_L = (m_1 - m_0) / m_0,$$

where: E_L- value of lipid-binding ability, [g/g]; m_0 - sample weight of dry material, [g]; m_1 - weight of residue after centrifugation and drying, [g].

For the final result is an arithmetic mean of three parallel experiments, the relative discrepancy between which shall not exceed 5%, with a confidence level of 0.95-tive.
In accordance with established method lipid-binding properties of enterosorbents were defined in respect of triglycerides and fatty acids, the results are shown in Table 1.

3. Results and Discussion

Table 1. Evaluated lipid-binding capacity of enterosorbents

Sample	Lipid-binding capacity, g/g					
	Towards triglycerides (linen oil)			Towards fatty acids (linolenic acid)		
	pH=2	pH=4	pH=6 - 7	pH=2	pH=4	pH=6 - 7
Chizitel	7.67±0.08	4.70±0.05	4.68±0.09	4.46±0.09	3.58±0.07	3.27±0.07
Chitan	3.50±0.10	3.41±0.10	3.26±0.10	2.20±0.09	4.44±0.13	7.22±0.14
MCC	0.57±0.01	-	-	1.83±0.09	-	-
Chitinel	-	-	2.20±0.64	-	-	1.64±0.03
Polyphepan	3.93±0.05	-	-	2.08±0.04	-	-

In result of this work, we have developed a technology for producing dietary supplement “Chizitel”, which is a chitin-mineral complex obtained from chitin-containing raw materials by deproteinization of chitin using electrochemical method.

Dietary supplement "Chizitel" is produced by a novel technology without the use of alkali. Deep deproteinization of raw materials is achieved by the action of reducing agents and OH^- ions produced by the electrolysis of water.

In contrast to chitosan, "Chizitel" is insoluble in the gastrointestinal tract and, it has sorption properties at the level of chitosan due to developed inner surface and availability of the reaction centers.

A method for evaluating the ability of a dietary supplement to bind triglycerides and fatty acids in vitro (taking into account the acid-base properties of the sorbents and their influence on the pH of the gastrointestinal tract) was developed. A comparative analysis of the current lipid-binding dietary supplements was carried out. Results are shown in Table 1.

It was established that dietary supplement "Chizitel" has sorptive capacity towards fatty acids at the level of chitosan, depending on the pH it varies from 3.2 to 4.5 g/g (for linolenic acid). At the same time "Chizitel" substantially exceeds chitosan in sorption towards triglycerides. Depending on the pH, it is varies 7.67 to 4.68 g/g for Chizitel and from 3.5 to 3.2 g/g for chitosan. Total exchange sorption capacity with respect to universal sorbate methylene blue for "Chizitel" is about 18.8 mg/g. As for the ions of heavy and transitional metals, sorption of Cu^{2+}, Pb^{2+}, Sn^{2+}, Cr^{3} +, Fe^{2} + for "Chizitel" is at the same level as for chitosan and constitutes from 1.5 to 2.5 mmol/g.

4. Conclusions

It was shown that dietary supplement "Chizitel" is suitable for use as a lipid-binding agent, has the ability to remove lipids from the body, as well as heavy and transition metals, it is a significantly cheaper and more effective alternative to existing dietary supplements from chitin-containing materials on the market.

Using the method that takes into account peculiarities of food digestion in digestive tract there was carried out researches in vitro lipid-binding properties of chitin-containing absorbents.

The study showed that biologically active additive to food "Chizitel extracted via electrochemical method has the most absorbing properties to triglycerides and fatty acids in comparison with well-known chitin-containing fat absorbents in whole investigated PH-range.

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