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Lipid Composition of the New Functional Lyophilized Product "SB-Lyo"

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Abstract

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The rich content of fatty acids in the lyophilized symbiotic includes varied specter of short- and medium chain fatty acids, which determines its application range for treatment of the intestines, the pancreas, some forms of lipoproteinaemia and other morbid states, related to fat metabolism. The lipolysis, affected by the applied microbial lipase, results in a release of a considerable quantity of free fatty acids in the lactic acid product, especially those of short-chain spectrum. The comparatively large number of free fatty acids contributes to the increased absorption of available lipids and correspondingly – to the increase in the substrate, utilized by the useful micro-flora in the product. One the other hand, the large share of free fatty acids in the new symbiotic product "SB-Lyo" confirms the favorable effect of the double immobilization, as well as the role of the hydro-colloidal matrix for preserving the activity of the used microbial lipase during sublimation drying and storage of the lyophilized functional food.

Key words: lipid composition, free fatty acids, lyophilized functional food.

Introduction

The concentration of useful living microorganisms, entering into human organisms, mainly in the form of fermented products, determines their probiotic effect (Murgov et al., 2001). This effect leads to reduction of the body weight, regulation of metabolism, improvement of the endocrinium activity, increasing the immune resistance and many other parameters related to the human healthy status.

It is found the extended shelf life of probiotic foods is due to the freeze-drying. This technological method includes the combination of two ways of conservation: freezing and drying under vacuum at temperatures below the critical values, i.e. such temperatures not damaging the micro-and macrostructure of the product. The increased interest in probiotic lyophilized foods can be explained because of their completely preserved taste qualities, nutritional and biological value, along

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with the extended shelf life (Tsvetkov, 1979).

The objective of the investigations: Biochemical analysis and evaluation of the lipid composition of the new symbiotic product "SB – Lyo", containing four types of lactic acid bacteria complex, brewer's yeasts – *Saccharomyces cerevisiae 1248*; enzymatic complex of proteolytic and lipolytic enzymes (included in the hydrocolloid matrix), plant extracts, fructose, etc.

Materials and Methods

This new functional product is developed on the basis of highly-developed approaches:

Technological methods

- Immobilization of the cellular suspension on the hydrocolloid matrix and enzymatic complex on water insoluble carrier - brewer's yeasts.
- Fermentation
- Freeze-drying

Biochemical investigations

In the course of our experiments we conduct extraction of lipids, according to AOAC. The method described is based on the principle of Roese- Gottlieb (Jong et al., 1975) and provides full lipid extraction. After the evaporation of the organic solvents, the dried lipid extract obtained at temperature in a water bath (40 ^{0}C), preserves the intactness and nativity of the lipids. For the objective of our investigation, we conduct estherification of the available fatty acids using isopropyl alcohol. As a result we obtain isopropyl esters according to the method described by Robert Wolff (Wolff, 1994). Wolff substitutes the estherification with methyl alcohol and respectively the methyl esters are substituted with isopropyl ones and all this helps for providing more precise analytical result. The isopropyl esters have higher boiling point compared with the respective methyl ones that allows for determination (the short-chained components) the fatty acid spectrum (C4-C8). It provides a possibility as to obtain more precise quantitive result of the shortest-chained components.

The investigation of the fatty acid composition is conducted by means of a gas chromatograph "PYE Unicam" 304, produced by Philips. The conditions for chromatography are analogical to those applied by Robert Wolff (Wolff et al.**,** 1995) and modified in our laboratory with the following purpose: programming the conditions for optimal eluation of separate fatty acids, combined with high selectivity.

The column is capillary, length - 30m, section-0.32 mm and filling "Carbovax 20M". As to be achieved high selectivity in the gaschromatographic investigation, contributes the applied temperature program. This program includes two-phase change in programming in contrast with the temperature programming, adopted by Robert Wolff, aiming at most adequate conditions for separation of all constituent fatty acids to be acquired.

Results and Discussion

In order to accomplish our objective, we determine the fatty acid composition of the new functional product.

The application of investigations with high permissive ability (contemporary capillary gas chromatography) allows proving the availability of a large number of constituent fatty acids.

In Table 1 are presented and compared the results based on the chromatographic

Table 1

Fatty acid composition of "SB Lyo"

Fatty acids	Cow milk		"SB-Lyo"	
	Triglycerides	Free fatty acids (FFA)	Triglycerides	Free fatty acids (FFA)
4:00	5.24	0.78	2.21	0.8
6:00	3.49	0.42	1.21	0.81
8:00	2.15	78.67	2.66	49.06
10:00	4.69	0.81	2.92	1.98
10:01	0.56	0.22	0.38	0.13
11:00	0.04		0.06	
12:00	3.92	0.78	3.48	3.23
13:00	0.04	0.24	0.19	0.18
14:0 iso	0.22	0.18	0.24	0.03
14:00	15.42	2.19	12.31	5.17
14:01	2.6	0.06	1.19	0.7
15:0 iso	0.72	0.19	0.83	0.42
15:00	1.38	0.53	1.42	0.6
16:0 iso	0.62	0.62	0.44	0.15
16:00	28.82	6.53	26.3	7.39
16:1 D9	0.15	0.21	0.25	0.2
16:1 D7	1.61	0.37	0.78	0.57
17:0 iso	1.42	0.06	0.45	0.23
17:00	0.47	0.15	0.56	0.29
17:01	0.2	0.13	0.17	0.17
18:0 i		0.49		1.09
18:00	7.67	2.99	12.87	3.67
18:01	17.67	2.9	26.52	12.68
18:2 iso	0.19		0.72	
18:03	0.49	0.11	0.12	0.18
20:00	0.31		0.42	
18:02	0.74	0.76	1.12	1.49

investigations on the fatty acid composition of the new synbiotic lyophilized product – "SB Lyo" and the one of the cow milk. In the fatty acid spectra obtained are not included the components with comparatively low value.

The results presented prove, that the fatty acid spectrum of triglycerides of the "SB-Lyo" product, is very close to the one of the milk fat.

In Table 1 are presented the results of the fatty acid composition of the total number of lipids that contains in the investigated product. This composition is a mixture of short-, middle- and long-chained fatty acids with various quantitive proportions. The long-chained fatty acids - myristic (14:0), palmitic (16:0), stearic (18:0) and oleic (18:1) have the highest concentration whereas the middle- and short-chained fatty acids - butyric (4:0), caproic (6:0), caprylic (8:0), capric (10:0), and lauric (12:0) acids, despite their lower content, are important for the specific qualities of the milk fat. In our product, comparatively high is the content of the butyric acid, that on a molecular level is important to the histone hyperacetylation and hypermetilation of the DNA (DNA plays a main role during inactivation of the oncogenic expression). In the human colon the butyric acid, in spite of its function as being an energy source, is a protective factor against colon cancer. On the basis or the reasons stated we are able to trace the butyric acid in the milk fat that justifies our investigations aiming at its determination. In the new product we determine two unsaturated fatty acids-linoleic and linolenic acid not found in the yoghurt. Their presence in the product is probably due to the other components in the content.

The human organism is not able to synthesize linoleic and linolenic acids. The arahidonic acid is produced in insignificant quantity by the human organism. Therefore, these fatty acids are irreplaceable (essential) due to their important physiological role as to decrease the cholesterol level in blood and the body as a whole. The essential acids bond with cholesterol and as a result metabolic active esters are formed, that are transported to the liver and transformed with cholesterol extraction in the gallbladder (Williams, 1986). The content of the polyunsaturated fatty acids in the new type of functional food increases its biological value.

The hydrolysis of triglycerides to fatty acids may be either caused by the lipase, resulting from microorganisms' activity or consequence of the milk lipase. In both cases, the free fatty acids stimulate or suppress the development of various species of microbes. The hydrolysis in our product is a result of the action of the lypolitic enzymes in the microbial cells, and the exogenous microbial lipase with high lypolitic activity, involved into the enzymatic complex which is immobilized on water - insoluble carrier. The aim of the exogenously imported microbic lipase, produced by *Rhizopus arrizos* in our synbiotic food, is to regulate the intensity and hydrolysis direction of the milk fat. As a result increases the level of free fatty acids /FFA/ in the product.

The lipase is characterized by clearly expressed specificity relating to 9-cis unsaturated acids; releases with higher speed the short- fatty acids, hydrolyzes the ester bonds of all fatty acids, containing in the milk fat. As a result of the hydrolysis, under the influence of the microbial lipase, a comparatively large number of free fatty acids are formed. On one hand, this contributes to the increase both in the lipid absorption and the substrate assimilated by the micro flora from the other hand.

In Table 2 are presented the results based on the lipolytic activity. They show convincingly the availability of a considerable concentration of FFA that illustrates

Table 2

Content of free fatty acids – mmol

	Cow milk	"SB-Lyo"
In 1 g lipid	0.832	2.765
In 1 g product	0.031	0.411

a high lipolytic activity of *Rhizopus arrizos*, containing in the enzymatic product.

In the spectra of FFA are presented all other fatty acids, available in the triglycerides. It can be observed an extremely high content of capryl acid (8:0). A considerable number of them have a lower percentage content compared to their levels in the fatty spectrum of the triglycerides. This tendency is clearly expressed in the cases of myristic, palmitic, stearic, oleic and linoleic acids (Table1).

This is possible to be related to the specifications of the action of the *Rhizopus arrizos* lipase on the position distribution of the fatty acids in the molecules of the triglycerides, or the reason might be found in the influence of the selective use of separate FFA of the available microflora in the product. In this respect, influence undoubtedly has the fact that a predominant number of the quantity of the triglycerides molecules remains unaffected by the lypolitic action.

Conclusions

The fatty acid spectrum of the triglycerides in the new synbiotic product "SB-Lyo" is almost analogical to the one of the milk acid in the cow milk.

The fatty acid composition of the total number of lipids in the product contains short-, middle- and long-chained fatty acids in various quantitive proportions. This contributes to the increase in the absorption of the available lipids and respectively, to the increase in the substrate assimilation by the microflora in the product.

The applied lipolytic enzyme regulates the intensity of the milk fat hydrolysis and as a result the level of FFA increases. The high percent of the free fatty acids in "SB Lyo" confirms the favorable effect of the double immobilization, along with the maintenance of the hydrocolloid matrix for the activity of the microbial lipase during freeze drying and preservation of the lyophilized functional food.

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