INFLUENCE OF SPECIFICITY OF LIPASES ON FRACTIONAL COMPOSITION OF BEEF FAT HYDROLYSATES

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Background

According to current concepts of nutrition science a human should consume within lipids composition 10-20% of polyunsaturated, 50-60% monounsaturated and 30% saturated fatty acids. The fatty acid composition of tallow, and particularly of beef fat, features increased content of saturated fatty acids, therefore their digestibility is lower, than that of vegetable oils. To enhance the biological value and increase the applications of tallows for foods production it is possible to use enzymatic biotransformation of fats using lipases of different position specificity.

Lipids hydrolysates, isolated from different sources, can be used for obtaining fats with optimized fatty acid composition. It seemed interesting to study the regular trends of biotransformation of lipids with the purpose to create the food ingredients with improved fatty-acid composition based on beef fat.

Objectives

The purpose was to determine the influence of a position specificity of lipases on fractional composition of beef fat hydrolysates, being the mixtures of free fatty acids, mono-, di- and triacylglycerines, as well as, to establish kinetic regularities of hydrolysis of lipids of beef fat with the lipase from *Candida rugosa.*

Materials and methods

The samples of raw beef fat with the acid number (AN) < 2.2 mg KOH/g were used. As the enzymic preparations were used the pancreatic lipase of Serva production (Germany) with the activity of 13 units/mg (1 unit of activity corresponds to the quantity of enzyme liberating 1 mcmol/min of oleic acid from a 50% emulsion of olive oil, as stabilized in the presence of 8mg/ml of sodium taurocholate at 37^0C in tris/NaCl – buffer with pH 9.2), as well as the lipase from *Candida rugosa* of Sigma production with the specific activity 1950 U/g. The isolation of lipids was performed by means of extractive homogenization of fatty tissue in the system of solvents chloroform-methanol in the ratio 2:1 and by redistribution of lipids in the system of solvents chloroform – methanol – water 8:4:3 (vol. %). After two times washing of the chloroform extract by 1% solution of KCl a fraction of neutral lipids was obtained, which at 98% was represented by triacylglycerines of different structure. The level of free fatty acids did not exceed 0.04 mmol/g in terms of stearic acid. Hydrolysis of each specimen was conducted separately in the reaction mixture volume not exceeding 0.5 ml, in triplicate. For this purpose the lipase solution in tris-buffer pH 7.5 and the solution of lipids in hexane in ratio 1:2 (mass.) were introduced in test tubes of type "Eppendorf" with 1.5 ml volume. Hydrolysis was carried out at enzyme-substrate ratio, optimum for each system. The test tubes were placed into thermomixer and kept for 15, 30, 60 minutes, and then removed them successively after each hour of hydrolysis for determination of fractional composition by the method of thin-layer chromatography, using hexane-diethyl ether-acetic acid in the ratio 7:3:1 (vol. %) as an elution system. Simultaneously in each specimen the acid number was determined.

Results and discussion

Study of the change of the fractional composition of lipids during hydrolysis by lipases of different specificity has shown that in case of hydrolysis with pancreatic lipase, which is specific for 1,3 – position, in the reaction mixture simultaneously with the reduction of the substrate (triacylglycerines) there was the accumulation mainly of 1,2 diacylglycerines and monoacylglycerines (Fig. 1a). Thus, after 24 hours of lipids hydrolysis with pancreatic lipase, the fractional composition was as follows: triacylglycerines (TG) –37%, monoacylglycerines (MG) – 12%, 1,2 diacylglycerines (1,2 –DG) – 19%, 1,3- diacylglycerines (1,3 – DG) – 5%, free fatty acids (FA) – 27%. The presence in the mixture of a small amount of 1,3 diacylglycerines can probably be explained by isomerization of corresponding 1,2- diacylglycerines, because the 1,3 form is more thermodynamically stable.

At the same time, in the hydrolysis with unspecific lipase from *Candida rugosa* there was observed the formation of all the mentioned fractions. It seemed interesting, that as long as hydrolysis proceeded, 1,3 diacylglycerines degraded faster to monoacylglycerines and further – to free fatty acids, than 1,2 diacylglycerines. The fractions of monoacylglycerines proved to be very reaction-capable, which can be judged from their low level during the whole period of hydrolysis (Fig. 1b). After 28 hours of hydrolysis of the 20% emulsion of lipids the fractional composition of the reaction mixture was as follows: triacylglycerines 29%, monoacylglycerines – 6%, 1,2- diacylglycerines – 15%, 1,3 diacylglycerines 7.5%, free fatty acids – 42.5%.

In the determination of kinetic regularities of beef fat lipids hydrolysis with the lipase from *Candida rugosa* it seemed interesting to calculate the macrokinetic constants of hydrolysis not only by the accumulation of reaction products, but also by the decrease of the substrate. As a result of the computation of initial velocities of hydrolysis and linearization of dependences of initial velocities from concentration of the substrate in Lineweaver-Burk coordinates the main kinetic constants of triacylglycerines hydrolysis have been determined. The effective values of the constant of Michaelis K_m and maximum observed velocity V, as calculated by the accumulation of products of reaction – free fatty acids – were 0.278 mol/l and $6.27*10^{-5}$ mol/l.s., respectively, while the same macrokinetic parameters as calculated by the loss of the substrate – triacylglycerines – constituted 0.236 mol/l and $2.54*10^{-5}$ mol/l.s , respectively. Thus, the values of Michaelis constant in both cases actually coincide, and the value of the maximum observed velocity, as calculated according to the accumulation of reaction products, almost 2.5 times exceeds the similar constant, calculated by the loss of the substrate. A higher value of V, as found by the accumulation of the products, can be explained by the fact that free fatty acids were formed not only as a result of hydrolysis of triacylglycerines, but also as a result of hydrolysis of intermediate products of reaction - mono- and diacylglycerines.

The study of kinetic regularities of lipids hydrolysis has shown that enzymatic hydrolysis of lipids differs significantly from other fermentative reactions, because it is a heterogeneous process. The lipases as used in the experiment, were solvable in water, and the substrate molecules were in emulsified condition. The enzyme-substrate interaction took place on the phases interface. It could be supposed that because of insolubility of the substrate the interaction with the active center should have difficulties, however, the experimental data show, that

molecular activity of lipolytic enzymes was not lower, than with other hydrolases, having an effect on water-soluble substrates. This is indicated by the data as obtained from the processing of kinetic regularities of beef fat hydrolysis by the lipase from *Candida rugosa,* especially, the possibility of their description by a classical equation of Michaelis-Menten in the field of not high initial concentrations of the substrate, as well as the values of the effective constants of Michaelis and maximum velocities of hydrolysis.

As is known, lipases are the enzymes of surface effect and become active, only being on the surface of the supersubstrate, insoluble in water. In this case the higher is the degree of dispersion of the substrate, the faster the lipolysis goes. Probably it is connected with the phenomenon of the sorption of the enzyme on the surface of the substrate. Therefore, in the field of high initial concentrations of the substrate with its insufficient emulsification the process of hydrolysis is limited by the sorption of the enzyme on the surface of phases interface, which is indicated by the deviations from the equation of Michaelis-Menten and impossibility of computation of the constant of the substrate inhibition.

Conclusions

Thus, when choosing the enzymic preparation one should take into consideration the agreement of its activity and specificity with the posed problem. Thus, if the target fractions of hydrolysates are the free fatty acids, which on the one hand, find wide application in the production of perfumery and cosmetics, and on the other hand, depending on their structure, may be the raw materials for carrying out the overetherification of triglycerides and correction of their fatty acid composition, then highly active non-specific microbe lipases should be preferred. For example, as the investigations have shown, for beef fat hydrolysis with the purpose of obtaining free fatty acids the best choice is lipase from *Candida rugosa,* which provides a high degree of hydrolysis (80-85%) and a high yield of the target product for comparatively not long time period (4-6 hours). On the other hand, if the task is to obtain the edible modified fats with favorable fatty acid composition, as solved by overetherification, as well as the task to obtain the mixtures of mono- and diacylglycerines, 1,3-specific lipases, especially, pancreatic lipases should be preferred.

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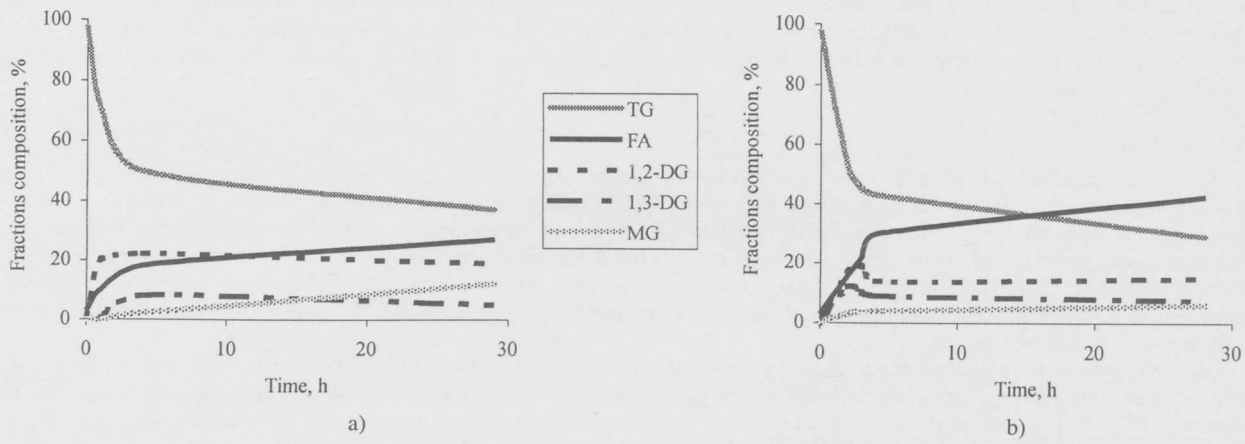

Fig.1. Dependence of fractional composition of beef fat hydrolysates from hydrolysis duration: a) with pancreatic lipase, b) with lipase from *Candida rugosa.*