ABOUT HYDROLYTIC BIOTRANSFORMATION OF BEEF FAT IN THE PRESENCE OF PANCREATIC LIPASES

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Background

The amount of necessary fats in the daily diets of a human should be not less than 30%. Not all the fats, however, are equally assimilated. Beef fat has the least degree of utilization by live organisms (80-90%) due to the presence of saturated higher fatty acids in it (stearic and palmitinic – more than 40%). Partial hydrolysis of natural fats helps to their easier assimilation by human's organism. It seemed interesting to study the kinetics of bioconversion *in vitro* of beef fat by pancreatic lipase.

Objectives

The objective of the work was to find the method of selective splitting of not easily assimilated beef fat with subsequent release of fatty acids unfavorable for food purposes.

Materials and methods

Samples of beef fat with the acid number (AN) < 4 mg KOH/g obtained together with the OAO "Meat packing plant Ramensky" were used in the experiment. Pancreatic lipase produced by Serva (Germany) with the activity of 13 units/mg (I unit of activity corresponds to the amount of enzyme releasing 1 μmol/min of oleic acid from a 50% emulsion of olive oil , as stabilized in the presence of 8 mg/ml of sodium taurocholate at 37°C in tris/NaCl-buffer with pH 9.2) was used as an enzymic preparation. Hydrolytic decomposition of fat was carried out in a reactor with an agitator in aqueous medium, where a mixture of beef fat with an organic solvent – hexane - was introduced as a 50% emulsion, as well as a needed amount of enzyme. The calculation of the degree of conversion of fat was evaluated, determining the value of AN in the aliquot of the specimen, taken with little time intervals. The value of AN was determined according to the formula: $AN = 5.611 \cdot V K / g$ where 5.611 – titre of 0.1 M solution of KOH, V – amount of KOH solution, used for titration, ml, K correction to the titre, G – fat specimen, g.

Results and discussion

To evaluate the conditions influencing the pancreatic hydrolysis of animal beef fat, the influence of temperature, enzyme substrate ratio, concentration of the substrate and other admixtures, and type of the substrate on the value of maximum velocity of the controlled biodegradation of triglyceride, which was the initial fat leading to the release of free fatty acids were studied.

The treatment of kinetic data shows that with the enzyme (lipase)-substrate (beef fat) ratio E:S = 1:20 at 40°C, the value of the purposeful conversion of fat into free fatty acids is 50% for the first 200 min of hydrolysis process, and at 50°C and 60°C the value of purposeful conversion into free fatty acids is less, being only 25% for the first 200 min. of hydrolysis process, which may be the indication of possible going out of the process from the temperature optimum, which under the conditions of the experiment was 45°C. The conversion value was 25-50% basing on the theoretical value AN 175 - 200 mgKOH/g of fat.

The study of the dependence of hydrolysis velocity value from the temperature of reaction medium at stationary parts of the curves allows to determine effective values of hydrolysis velocity (v) at that temperature and find effective energies of activation as well. For this purpose hydrolysis of beef fat with the use of pancreatic lipase at temperatures close to the temperature optimum of the enzyme, i.e. at 30, 40, 50 and 60°C , determining the values of velocities (v). was carried out. These data are presented in the Table.

At all temperatures values the long process of treatment of beef fat with pancreatic lipase led at the beginning to the increase of AN which characterized the presence of increasing amount of free fatty acids in the treated system, and then, as a rule, when the time was more than 24 hours, the value of AN fell, which could indicate the decrease of lipase activity in the system of development of reverse processes of transesterification , i.e. reverse binding of released free fatty acids into the animal fat composition.

Comparison of velocities of hydrolytic splitting of beef and pork fats in the presence of pancreatic lipase shows the absence of marked influence of the type of the studied fats on velocity of free fatty acids release A similar trend is observed in the case of use of vegetable fat for hydrolysis under comparable conditions in the presence of pancreatic lipase which may indicate the factor of adequate homogeneity of the system and carrying out the measurement in practically "ideal" enzyme-substrate saturation at sufficiently strong dilution of the substrate at the level of (1.5 – 5% mass.).

The dependence (v) from T in the studied temperature interval in semi-logarithmic co-ordinates is approximated by a straight line, i.e. at this temperature interval the Arrhenius equation is fulfilled. The value E_a - activation energy of catalytic reaction , the value of which was determined graphically as a slope of the curve in Arrhenius coordinates of velocity logarithm [10 + ln(v)] as a function of the reverse temperature [(I/T) 10^3], proved equal to: $E_a = 19.1 + 1.1$. kJ/mol.

From the data of kinetic investigations it was found that optimum ratio of the enzyme to the fat substrate was about 0.05 mass. parts to 1 mass part of fat, which appeared to be determined by the conditions of the experiment, because it is known that in real live organism the composition of lipids, particularly in the pancreas, takes place at considerably less concentrations of the enzyme.

The study of the influence of reagents concentrations has shown that the increase in fat concentration in the system of type water/oil with 1.5%mass to experimentally acceptable concentration of 10% mass practically led to the 3-fold decrease of equilibrium level of free fatty acids. Further increase in substrate concentration in the system leads to even more considerable fall of the degree of hydrolytic decomposition of beef fat in the presence of pancreatic lipase.

Table 1. Values of elementary velocities of hydrolysis of beef fat (v) under the influence of pancreatic lipase as a function of temperature T (fat 1.5%)

t °C	T, K	$(1/T) \cdot 10^3$, K^{-1}	V, (mg of fat/(ml min)·	10 + ln (v)
60	333	3.0	0.308	8.82
50	323	3.1	0.256	8.64
40	313	3.2	0.205	8.41
30	303	3.3	0.154	8.,13

The general kinetic scheme of beef fat biotransformation process was carried out according to a classical version on the basis of the equation of Michaelis-Menten $v= V_{max}[S]_o/(K_m+[S]_o]$ where v – current velocity of bioconversion process, V_{max} - instantaneous maximum velocity, K_M^H empirical constant of Michaelis, $[S]_o$ initial concentration or animal fat. The results of the investigations have shown that the classical equation of Michaelis-Menten, describing hydrolysis of substrate in the presence of lytic enzymes is suitable for obtaining positive values of the constants only at low concentrations of fat (1.5 – 3)%). To determine the constant of the substrate retardation (K'_s) a relationship of the reverse value of velocity (1/v) from $[S]_0$, was used which quantitatively could be described based on the assumption about formation of the triple complex substrate-enzyme-substrate.

The results of the determination of the main kinetic constants value of the process of biodegradation of animal fats in the presence of pancreatic lipase: maximum value of velocity of biodegradation V_{max} = (1.25 ±0.1) (mg of fat/min); the constant of Michaelis – $\mathbf{K}^H_m$ =(100±12), mg fat/ml.); the constant of substrate inhibition (retardation) – K'_S = (10.0 ± 0.8), mg fat/ml, as well as the constant of reversibility, coinciding with the value of the constant of equilibrium – K_P = (277 ±170), mg fat/ml. It is shown that the constant of equilibrium (K_P) which is more that 25 times exceeds $\mathbf{K'}_S$ and more than 2.5 times - $\mathbf{K}^H_m$ makes the most considerable contribution into the fall of conversion of the substrate (X) depending upon its initial concentration. $[S]_0$.

Conclusions

Thus, as a result of the investigations the main kinetic constants of hydrolysis of beef fat in the presence of pancreatic lipase are determined. It is shown that under the conditions of the experiment the effective release of maximum amount of free fatty acids took place at 40°C during first three hours with subsequent predomination of processes of transesterification. The used kinetic approach can serve as the basis for the development of a real process of biotransformation of low-value and not easily assimilable fats into the products with improved biological properties.

Pertinent literature

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