ABOUT SOME REGULAR FEATURES OF CHANGE OF BEEF FAT FRACTIONAL COMPOSITION DURING FERMENTATIVE GLYCEROLYSIS

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Background

Beef fat having high level of saturated fatty acids has limited use in current food diets, because its frequent consumption can provoke occurrence of cancer. Therefore, the search for possible ways of incorporation of transformed beef fat into combined meat products with balanced fatty acid composition is an urgent problem. One way of resolving this problem is the creation of technology of enzymatic modification of beef fat in the mixture of lipids having high functional-technological properties that are needed in meat industry. Enzymatic glycerolysis changes the lipid composition of the fats, which leads to accumulation of fractions of mono- and diglycerides having wide use as food additives. It was interesting to study the kinetics of beef fat fractional composition change during enzymatic glycerolysis.

Objectives

The purpose of the work was to study kinetic regularities of accumulation and consumption of individual lipid fractions during glycerolysis of beef fat in the presence of enzymatic preparation Liposym TL IM.

Materials and methods

Sterilized melted beef fat, food grade glycerol, and enzymatic preparation Liposym TL IM (Novozymes company, Denmark) were used for investigations. Lyposym TL IM is a granulated enzymatic preparation of purified 1,3 specific lipase immobilized on silica gel from Thermomyces lanuginosus, being produced during deep cultivation of GM strain Aspergillus oryzae. The preparation is mechanically stable, is intended for use in technological processes with batch loading, and has an activity of 175 IUN/g. As a unit of interesterification Novo (Interesterification Unit Novo, IUN) was taken the amount of enzyme, transforming 0.01% mass of tristearin per min during interesterification of soy oil by completely hydrogenized soy oil in a ratio 73: 27 (mass.%) at 70ºC in the absence of solvents.

Glycerolysis was carried out in a thermostatted glass reactor provided with a three-blade stirrer with constant rotation speed. Molar ratio fat-glycerol varied from 1:1 to 1:10, whereas the enzyme-substrate ratio E:S was changed in the range from 1:100 to 1:5. During investigation of kinetic characteristics of the process of glycerolysis was carried out at 45, 50, 55, 60, as well as 70ºC, periodically taking out samples for analysis of fractional composition by the method of thin-layer chromatography. Plates with the developed spots were scanned with the help of the system GelVue 2, by the area of the spots' percentages of fractions of tri-, di monoglycerols and fatty acids in the mixture of lipids were calculated. Calibration curves were constructed according to commercial standards Sigma.

Results and discussion

From literature it is known that the ratio of triglycerides to glycerol is the determining factor in obtaining different fractional composition of lipids. The amount of glycerol supplied to the reactor significantly influenced the yield of target fractions of mono- and diglycerides, The highest yield of fractions of mono- and diglycerides in this case was observed with mole ratio fat-glycerol 1:5, and did not increase with further increase of glycerol fraction in reaction mixture, which allows to recommend this ratio as the optimum. The process of enzymatic glycerolysis should be carried out with enzyme-substrate ratio that allows the achievement of maximum yield of target fractions with minimum doses of enzyme. The calculated enzyme-substrate ratio constituted 1:40 (mass.).

To clarify the influence of temperature on the velocity of glycerolysis, the dependencies of concentration change of separate lipid fractions in time were studied [1,2].

Fig. 1a shows experimental kinetic curves of the destruction process of triglycerides. Graphical differentiation of the above dependencies allowed to construct differential kinetic curves in co-ordinates (dP/dt; t), where the value dP/dt reflects the rate of destruction of triglycerides at the given time period (Fig.1b). We could with high correlation coefficients to approximate the curves with broken lines containing two rectilinear areas: horizontal and inclined, which may be considered as areas characterizing two stages of the process of glycerolysis. Observing the constant rate of change of triglycerides concentration at the first stage we can suppose that the number of elementary acts of triglycerides interaction with the active centers of enzymes per unit of time is also the constant value. Therefore, one can conditionally call the first stage as the stage of the effective work of the enzyme. In this case the break on differential curves will correspond to the time of effective work of enzyme, and the value of (dP/dt) on the first stage can be interpreted as the effective velocity of triglycerides destruction. The values of effective lengths of glycerolysis process were obtained by extrapolation of differential kinetic curves on abscissa axis.

The view of dependencies of triglycerides concentration change from time allowed us to suppose that the process of their destruction can be described by the equation of reaction of pseudo-first order. To check this supposition kinetic curves of Fig. 1a were linearized in semi-logarithmic co-ordinates. Determination of the value of inclination angle tangent of obtained straight lines allowed us to calculate values of effective constants of triglycerides destruction rates (Table 1).

From Table 1 it follows that the largest time period of effective operation of enzyme was at 45ºC and constituted 50 min, however, this temperature did not ensure high reaction rate. Temperature increase up to 60ºC led to the increase of effective initial rate 10-fold, and the time of the effective work of enzyme decreased to 5 min. Further temperature growth caused decrease of the rate of change of triglycerides concentration. Comparing experimental and differential curves of triglycerides conversion at 60ºC (Fig.1a and b) one can suppose that after 10-15 min the process of triglycerides hydrolysis was balanced by the process of their synthesis that was also evidenced by stabilization of concentration of triglycerides at 60ºC at the level 30%, i.e. 1.3 –1.5 times higher than at 45-55ºC.

The above approach was applied for description of kinetic curves of change of free fatty acids fraction concentration. The time of effective work of enzyme as determined from analysis of dependencies of the change of free fatty acids accumulation velocity was 2 times higher, than in the case of triglycerides destruction (Table 1).

Kinetic dependencies of monoglycerides fraction accumulation are interesting for discussion. Since in the system simultaneously occurred the processes of glycerolysis and autolysis, then one can consider formation of monoglycerides, on the one hand, as a result of synthesis from glycerol and free fatty acids, and on the other hand, as a result of incomplete hydrolysis of tri- and diglycerides. Simultaneously with the accumulation of monoglycerides the process of their hydrolysis to glycerol and fatty acids could also occur, therefore, the description of monoglycerides formation mechanism and the calculation of kinetic parameters of processes of their synthesis and destruction is a complex problem.

Fig.2(a) shows dependencies of change of mass share of monoglycerides fraction from time at different temperatures. Unlike triglycerides and fatty acid fractions there are no clear and simple dependency of monoglycerides yield from temperature. Thus, at temperatures 45-55ºC during the first hour the concentration of monoglycerides increased, and during the second hour: at 45ºC – decreased, at 50ºC – stabilized and at 55ºC continued to rise. At 60ºC the increase in monoglycerides concentration took place only during first 20 minutes, after which the growth stopped and total yield did not exceed 4-5%. At 70ºC during first 15 minutes there was no monoglycerides fraction at all, but then their concentration began to increase slowly, reaching only as little as 3%.

The process of monoglycerides formation looks more vividly on differential kinetic curves, presented in Fig. 2b. It is interesting to note that effective time for enzyme work, during which there was observed constant velocity of accumulation of monoglycerides fractions actually did not depend upon the temperatures in the interval 45 – 60ºC and constituted 15-20 minutes. The most effective initial velocity of accumulation of monoglycerides was marked at 50ºC , the least - at 60ºC. The dependencies dP/dt = f(t) constructed at 50-60ºC (Fig.2b) were satisfactorily approximated by broken line from three lengths. The appearance of

medium length on differential curve was associated with the fact that after the stage with constant velocity of monoglycerides formation, with the above temperatures there was an abrupt decrease of the velocity by 2 or more times during 5-10 min. Further the velocity of monoglycerides formation decreased much slower, which was seen from the third area of differential curve.

The process of monoglycerides accumulation at 45ºC, 50ºC and 55ºC was satisfactorily described by the reaction equation of pseudo-first order. The values of effective constants of monoglycerides accumulation velocities are presented in Table.1.

The dependence of accumulation of diglycerides fraction from temperature was of more complex nature.. The highest yield of diglycerides was reached at 55ºC, in 2 hours. Comparatively high values of diglycerides concentrations, about 22%, and correspondingly low yield of monoglycerides at 70ºC, can, probably be explained by two predominating processes: triglycerides hydrolysis on the first stage, and also the synthesis of diglycerides from monoglycerides and fatty acids. The absence of clear correlation between the yield of diglycerides fraction and glycerolysis temperature, is probably associated with multiple elementary acts leading to their formation and destruction.

Conclusions

As a result of investigations were determined the conditions of glycerolysis of beef fat in the presence of preparations Lipozym TL IM which ensure production of modified fat with maximum content of fractions of mono- and diglycerides. Optimum conditions of glycerolysis: correlation fat-glycerol 1:5 (mol.), correlation enzyme-substrate 1:40 (mass.), at temperature of 53-58ºC. Under the above conditions modified beef fat was obtained with the fractional lipids composition as follows: monoglycerides – 27-32%, diglycerides – 26-31%, triglycerides – 25-30%, free fatty acids – 7-12%. Such composition of the product allows to speak about good prospects of use of modified fat as a component of a stabilizing functional additive for production of emulsified meat products.

References

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Table 1. Main kinetic characteristics of glycerolysis of beef fat

T, ^{0}C	Effective initial velocity of reaction (horizontal length), % mass/min		Time of effective work of enzyme, min.			Effective time length of the process, min			Effective constant of velocity, min.$^{-1}$		
	dP_{TG}/dt	dP_{MG}/dt	TG*	FA**	MG***	TG	FA	MG	TG	FA	MG
45	0.75	0.32	50	90	20	78	110	60	0.016	0.013	0.024
50	1.8	0.7	10	55	15	32	67	26	0.022	0.032	0.077
55	2.2	0.4	6	45	15	14	58	37	0.025	0.032	0.03
60	8	0.2	5	18	20	16	27	25	0.02	0.112	-
70	0.6	-	0	15	-	60	30	-	0.01	0.032	-

*TG – triglycerides; ** FA – fatty acids; ***MG – monoglycerides

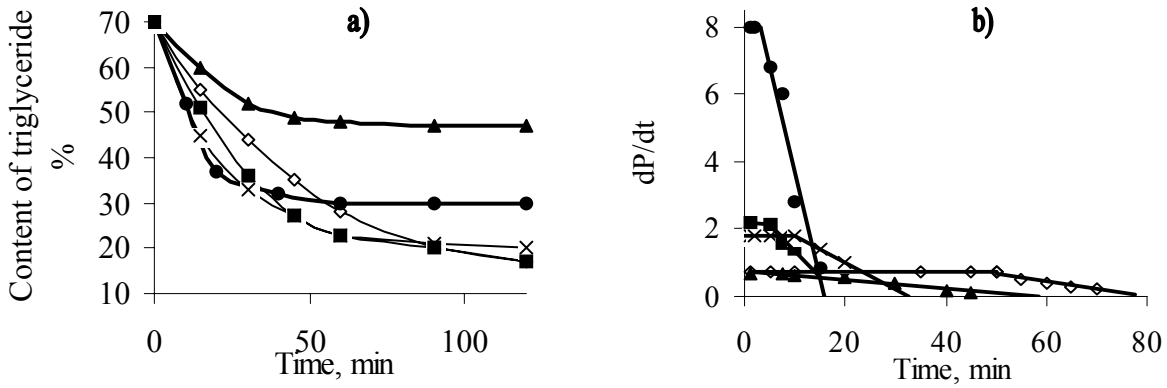

Figure1. Integral (a) and differential (b) kinetic dependencies of change of triglycerides mass share of fraction from time during glycerolysis of melted beef fat. (◊) 45º0C, (×) 50ºC, (■) 55ºC, (●) 60ºC, (▲) 70ºC.

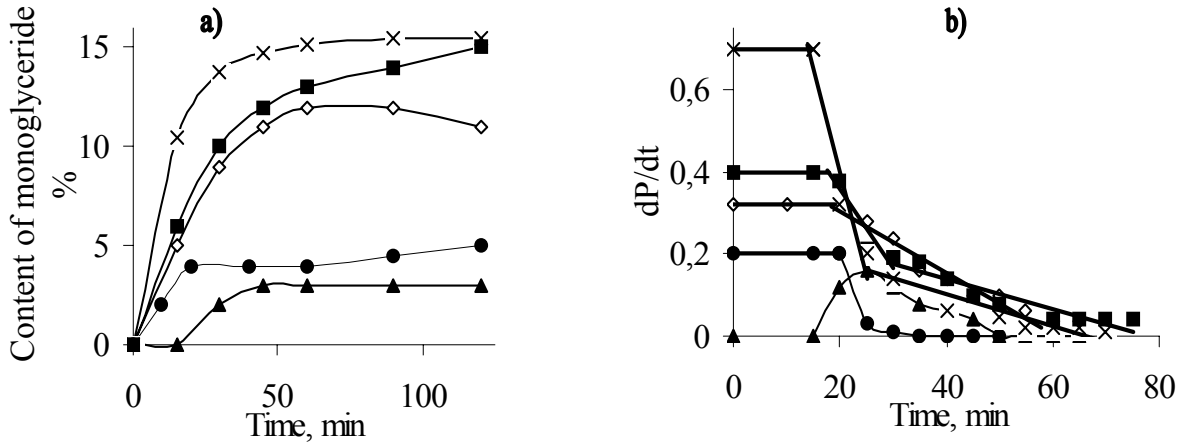

Figure 2. Integral (a) and differential (b) kinetic dependencies of change of mass fraction of monoglycerides mass share of fraction from time during glycerolysis of melted beef fat.
(◊) 45ºC, (×) 50ºC, (■) 55ºC, (●) 60ºC, (▲) 70ºC.