METHOD FOR ACCELERATING THE ENZYMATIC HYDROLYSIS OF TRIACYLGLYCEROLS

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The basic possibility of lowering the concentration of pancreatic lipase in the hydrolysis of triacylglycerols (TAGs) deposited on a solid sorbent has been shown. The method of preparing the initial TAGs for hydrolysis that has been developed permits not only a 10-fold reduction in the amount of ligase but also the retention of a short time of hydrolysis with a high yield of reaction products.

At the present time, lipase hydrolysis is used to establish the nature of the distribution of fatty acids in positions 2 of triacylglycerols (TAGs). The preparation of highly purified type A pancreatic lipases is associated with a number of technological difficulties, because of which its output is extremely limited, and type B lipases are more frequently used. Type B pancreatic lipase has a lower activity, as a result of which it is necessary to increase either the amount of lipase or the time of performance of hydrolysis. It is known that an increase in the time of hydrolysis lowers the possibility of obtaining representative diacylglycerols (DAG) because of an intensification of transesterification [1]. Type B pancreatic lipase also contains a larger amount of various impurities which may have an influence on the course of hydrolysis and somewhat distort the final results, particularly, if a very large amount of lipase is added. The fact that pancreatic lipase is an expensive reagent is also, probably, not unimportant.

The aim of the present investigation was to reduce the amount of pancreatic lipase in this analysis. This has been achieved by depositing the TAGs on a solid support with a developed surface.

Pancreatic lipase is a highly soluble enzyme working at the boundary of separation of a fat and a liquid phase [2]. The deposition of the fat on a solid support increases the surface accessible to the lipase and thereby raises the efficiency of its action, which permits a considerable reduction in its amount in this analysis with the retention of a short time of hydrolysis.

As the solid sorbent we used type L silica gel (Chemapol, Czechoslovakia). The TAGs of sunflowerseed oil, deposited on silica gel, were hydrolyzed with the aid of pancreatic lipase, as a result of which the monoacylglycerols with fatty acids in the 2-position (2-MAGs), the sum of the sn-1,2- and Sn-2,3-diacylglycerols (DAGs), the free fatty acids (FFAs), and uncleaved TAGs were obtained. After isolation by preparative TLC, the hydrolysis products were analyzed for their fatty acid compositions (mole%):

Sample	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
Initial TAGs	8.2 ± 0.1	5.2 ± 0.2	21.8 ± 0.1	64.8 ± 0.4
Uncleaved TAGs	8.0 ± 0.1	4.9 ± 0.3	21.8 ± 0.1	65.3 ± 1.0
2-MAGs	1.7 ± 0.1	0.7 ± 0.1	18.8 ± 0.5	78.8 ± 0.6
DAGs	9.0 ± 0.6	4.9 ± 0.3	24.1 ± 0.5	62.0 ± 1.5
FFAs	13.5 ± 1.0	7.8 ± 0.1	26.2 ± 0.4	52.5 ± 1.2

The set of acids in position 2 of the TAGs was judged from the fatty acid composition of the 2-MAGs. It agreed with that taken from literature sources [3], which enables us to speak of the possibility of using this method for the analysis of the structures of TAGs. The agreement of the compositions of the acids of the uncleaved and the initial TAGs, and also the absence of 1,3-diacylglycerols served as a proof of the absence of isomerization of the DSGs in the course of the lipolysis.

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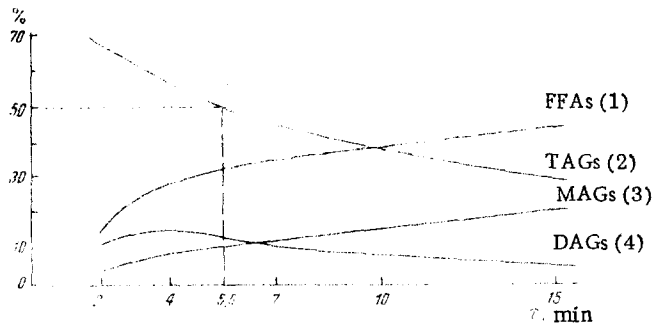

Fig. 1. Dynamics of the change in the triacylglycerols and the hydrolysis products with 20% of pancreatic pipase on the weight of the initial TAGs: 1) free fatty acids; 2) triacylglycerols; 3) monoacylglycerols; 4) diacylglycerols.

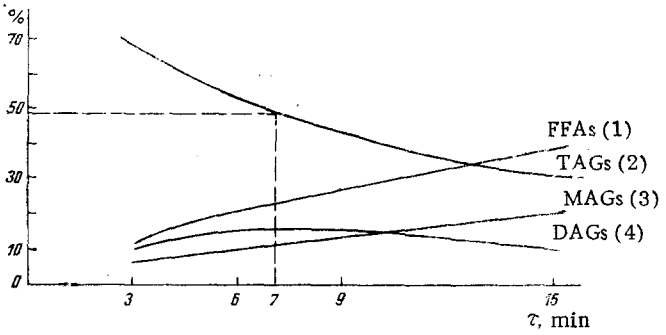

Fig. 2. Dynamics of the change in the triacylglycerols and the hydrolysis products with 10% of pancreatic lipase on weight of the initial TAGs. Symbols as in Fig. 1.

We studied the dynamics of hydrolysis with amounts of lipase of 20 and 10% on the weight of the initial TAGs. For the TAGs deposited on silica gel, the amount of lipase used in this experiment did not exceed 2%. The other conditions of hydrolysis were the same in all three cases. As a result of the investigations performed, the dependences of the yields of the 2-MAGs, DAGs, and the FFAs and of the residual amount of TAGs on the time of hydrolysis were obtained (Figs. 1-3). It can be seen from the graphs presented that when 20% of lipase on the weight of the initial TAGs was used a 50% degree of hydrolysis was reached after 5 min 30', the maximum amount of DAGs was obtained 3-4 min from the beginning of hydrolysis, and the degree of hydrolysis then amounted to 38-43%. Lowering the amount of lipase used from 20 to 10% of the weight of the initial TAGs led to an increase in the time necessary for the performance of hydrolysis with a high yield of final products. Thus, the 50% degree of hydolysis was reached only after 7 min, and the maximum amount of DAGs was obtained 8-9 min from the beginning of hydrolysis, the degree of hydrolysis than being 53-57%.

These results permit the statement that a further lowering of the amount of lipase taken for hydrolysis leads to an increase in the time of hydrolysis and, as a consequence of this, to an intensification of the process of isomerization and to a distortion of the results obtained.

When the hydrolysis of the TAGs deposited on silica gel was performed with 1.78% of lipase on the weight of the initial TAGs, a 50% degree of hydrolysis was reached after only 3 min 15' from the beginning of hydrolysis. The maximum yield of DAGs was reached after 4-5 min, and the degree of hydrolysis was then 53-57%.

The results obtained indicated the high efficiency of the use of this method of preparing the initial lipids for hydrolysis. The procedure developed not only permits the amount of lipase used to be reduced more than 10-fold but also enables a short time of hydrolysis to be retained with a high yield of DAGs and MAGs.

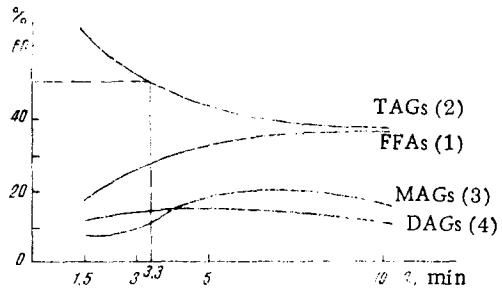

Fig. 3. Dynamics of the change in the triethylglycerols and the hydrolysis products with 1.78% of pancreatic lipase on the weight of the initial TAGs. The TAGs were first deposited on a solid sorbent. Symbols the same as in Fig. 1.

EXPERIMENTAL

Type L silica gel with particle dimensions of 40/100 μm was used. The sample of sunflower seed oil TAGs was prepared for hydrolysis in the following way: 445 mg of silica gel was added to a flask containing 150 g of TAGs dissolved in 5-10 ml of diethyl ether. The ether was distilled off in a rotary evaporator in vacuum (0.2 atm).

Hydrolysis with pancreatic lipase was performed under the conditions described by Ozerinina et al. [4], using 2.7 mg (1.78%) of type A pancreatic lipase (Olaine chemical reagents factory) at 40°C. The hydrolysis products were separated by preparative TLC in the hexane–diethyl ether–formic acid (50:45:1) system and were identified by comparison with markers.

The fatty acid compositions of the products obtained were determined by analyzing the corresponding methyl esters by the GLC method on an LKhM-8MD chromatograph with a flame-ionization detector, using packed stainless steel columns 2 m long and 4 mm in internal diameter. The support was Inerton AW-HMDS and the stationary phase diethyleneglycol succinate (20%). The temperature of the detector was 200°C, that of the column thermostat 170-190°C, and that of the evaporator 230°C. The rate of flow of the carrier gas, helium, was 30-40 cm^3/min, that of hydrogen 40 cm^3/min, and of air 400 cm^3/min.

The quantitative TLC of the hydrolysis products were performed as described by Malkhas'yan et al. [5].

SUMMARY

The deposition of the initial TAGs on silica gel permits: a more than 10-fold increase in the amount of expensive lipase; the retention of a short time of hydrolysis and, thereby, the exclusion of a possible isomerization of the initial TAGs; and the production of a higher yield of DAGs with the same time of hydrolysis.

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