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ACTION OF A COMPLEX LIPASE PREPARATION FROM *Oospora lactis* ON TRIGLYCERIDES

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In a study of microbial enzymes it has been established that some of them exhibit a specific action on primary ester groupings [1] and others catalyze the splitting off mainly of oleic acid, regardless of its position in the triglyceride molecule [2].

We have studied the action on cottonseed oil triglycerides of a microbial lipase isolated from the culture liquid of the fungus *Oospora lactis*, strain UzLM-2. The activity of the lipase was 2000 units/g. For comparison, in parallel we performed the hydrolysis of the triglycerides with pancreatic lipase isolated from porcine pancreatic gland. As is well known, the latter splits off the fatty acids from positions 1 and 3 of triglycerides.

After separation by the TLC method, the hydrolysis products were saponified, the fatty acids were isolated, and they were esterified with diazomethane and their composition was determined by the GLC method. The results of the analysis are given in Table 1.

It can be seen from Table 1 that the microbial lipase does not possess position specificity with respect to fatty acids. It is known that unsaturated liquid fatty acids mainly occupy position 2 in the triglyceride molecule [3]. The high content (more than 30%) of palmitic in the monoglyceride fractions shows that the microbial lipase splits off fatty-acid radicals regardless of their positions.

We determined the optimum concentration of the microbial lipase necessary for the hydrolysis of triglycerides. All the experiments were performed by a time of 60 min. Below we give information on the influence of the amount of enzyme on the completeness of the hydrolysis of 1 g of oil:

Amount of enzyme, g	0.02	0.04	0.06	0.1	0.15
Acid No. of the hydrolyzate, mg of KOH	58.3	84.6	91.5	110.7	103.5

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TABLE 1. Fatty-Acid Compositions of the Initial and Hydrolyzed Triglycerides

Component	Fatty acid					
	14:0	16:0	18:0	16:1	18:1	18:2
Initial unhydrolyzed triglycerides	0,5	24,4	2,0	1,8	16,1	55,2
Triglycerides hydrolyzed by the complex preparation (CP)	2,7	48,6	1,7	1,2	12,8	33,0
Triglycerides hydrolyzed by pancreatic lipase (PL)	—	38,2	2,4	4,2	18,8	36,4
Diglycerides (CP)	2,2	50,1	2,0	0,7	11,9	33,1
(PL)	1,6	36,5	0,7	1,2	16,5	43,5
Monoglycerides (CP)	1,9	32,3	6,5	1,5	17,0	40,8
(PL)	—	3,7	—	—	19,0	77,3
Fatty acids (CP)	—	19,2	—	0,5	20,0	60,3
(PL)	2,0	52,3	2,3	1,0	12,7	29,7

Consequently, the hydrolysis process accelerates only up to the addition of a certain amount of enzyme to the reaction mixture, which agrees with literature information [4]. When hydrolysis was carried on for 2 h with 0.1 g of the enzyme, the acid No. of the hydrolyzate rose to 174-180 mg of KOH/g. Such a far-reaching cleavage of the triglyceride by the enzyme of *Oospora lactis* can be used to isolate the fatty acids in the native state, since in existing methods of saponification [5] an alkaline medium is used which gives rise to position and geometric isomerization of the acids. This can be seen from the results of the analysis of the acids that we obtained by enzymatic hydrolysis and also by cold and hot saponification, which were checked for the presence of position and geometric isomers. It was found that the UV spectrum of the acid obtained by hot saponification gave an absorption in the 234 nm region which is characteristic for a —CH=CH—CH=CH group, indicating the position isomerization of the double bonds of the acids obtained by hot saponification in an alkaline medium. No absorption band at 234 nm was observed in the UV spectrum of the acids obtained by enzymatic hydrolysis. The IR spectrum showed no absorption band at 968 cm^{-1} characteristic for the geometric isomers of the acids.

EXPERIMENTAL

The triglycerides were isolated in the pure state by the method of Kuinlen [3], and the pancreatic lipase was isolated from procine pancreatic gland by a method described previously [6]. The hydrolysis products were isolated on silica gel with the addition of 5% of gypsum using the diethyl ether—petroleum ether (3:7) solvent system. The methyl esters of the fatty acids isolated were investigated on a UKh-2 gas chromatograph. The support used was Chromaton N-AW-DMCS, impregnated with 15% of Reoplex.

Isolation of the Complex Lipase Preparation. The lipase enzyme preparation was obtained from the culture liquid of the fungus *Oospora lactis*, strain UzLM-2, grown under the optimum conditions in a fermenter on a bench apparatus with a nutrient medium of the following composition (%): KH_2PO_4, 0.2; $(NH_4)_2SO_4$, 0.3; $CaCl_2$, 0.1; NaCl, 0.1; $MgSO_4$, 0.1; yeast autolyzate, 0.7; cottonseed oil, 1.

The preparation was isolated from the filtrate of the culture liquid by means of isopropanol in a ratio of 1:6 v/v at a pH of the culture liquid of 6.0.

The lipase activity of the preparation obtained was determined by a modification of the method of Ota and Yamada [7] and was expressed in arbitrary units — milliliters of 0.05 N KOH per 1 g of preparation at 37°C in 1 h.

Hydrolysis of the Triglycerides. The hydrolysis of 1 g of oil with 0.1 g of enzyme was performed at 37°C with stirring; phosphate-citrate buffer with pH 7.5 was added to the reaction medium. Poly(vinyl alcohol) was used as emulsifying agent. The time of hydrolysis was 1 h.

The hydrolysis of cottonseed oil with pancreatic lipase was performed by the method described previously [6].

The IR spectra were taken on a UR-10 instrument and the UV spectra on a Hitachi instrument.

SUMMARY

It has been established that a complex lipase preparation from *Oospora lactis* possesses a high activity, causes no position and geometric isomerization of the fatty acids isolated with its aid, possesses no specificity of action, and splits off the fatty acids from all three positions of the triglyceride molecule.

The high activity of the complex lipase preparation permit its recommendation for the hydrolysis of fat in order to isolate the total fatty acids in the native state, which is important in the study of their chemical structure and also in the splitting of fats in industry.

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METABOLITES OF THE PATHOGENIC FUNGUS *Verticillium dahliae*.

IV. INFLUENCE OF THE SOURCE OF CARBON ON THE LIPID COMPOSITION OF THE FUNGUS *Verticillium dahliae*

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The dependence of the metabolism of the fungus *Verticillium dahliae* Kleb. on the external conditions is one of the reasons for the limited nature of information on its lipid metabolism.

There is information in the literature on the influence of the source of carbon-containing nutrient on the growth and metabolism of *V. dahliae* [1-3], but nothing is known about how the source of carbon affects its lipid composition. Continuing investigations of the lipid metabolism of *V. dahliae* [4-6], we have studied the influence of the source of carbon-containing nutriment and fermentation on its lipid composition.

We used as nutrient media: 1) starch (biomass — dark gray powder); the yield of lipid fraction amounted to 5.9%; 2) sucrose (biomass — gray powder); 13.6%; 3) glucose (biomass —

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