ISOLATION OF TWO FORMS OF TRIACETINASE FROM COTTON SEEDS

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UDC 577.15.08+577.153

We have investigated the total fraction of alkaline lipases [1] to determine their enzymatic activity at pH 9.5. At this pH there is an intensive hydrolysis of cottonseed oil with an activity of 69 units, of tributyrin with 37 units, of triacetin with 1200 units, and of methyl butyrate with 160 units, but not of ethyl butyrate. As can be seen, the greatest activity at pH 9.5 relates to triacetin.

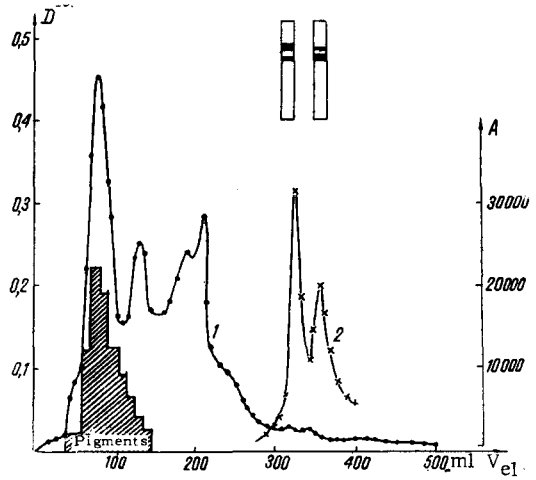

Fig. 1. Results of the separation of the two forms of triacetinase with pH 9.5 on a column of Sephadex G-50 and of the disc electrophoresis of the active fractions: 1) protein; 2) lipase activity.

TABLE 1. Results of the Purification of the Triacetinase from Cotton Seeds

Fraction	Protein content	Activity		Yield, %		Deg. of purification
		specific, $\frac{\mu mole}{min \cdot mg}$	total, $\frac{\mu mole}{min}$	protein	activity	
Suspension in triphosphate buffer	5000	40	200 000	100	100	1
Supernatant at 187,000 g	1280	140	180 000	25,6	90	3,5
Acetone fraction	780	190	150 000	15,6	75	4,8
Triacetinase I (first peak from a column of G-50)	3,7	30 600	110 000	0,074	55,5	765
Triacetinase II (second peak from a column of G-50)	2,0	19 300	36 600	0,04	19	485
Triacetinase I after rechromatography)	1,8	37 900	76 000	0,036	38,0	948
Triacetinase II after rechromatography)	1,6	22 600	36 000	0,032	18	565

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 135-136, January-February, 1972. Original article submitted October 4, 1971.

Below we give the results on the isolation of enzymes catalyzing the hydrolysis of triacetin. Undefatted cottonseed kernels (15 g) were ground in a mortar with quartz sand in 60 ml of 0.1 M phosphate buffer, pH 7.4. The mixture was centrifuged at 18,000×g for 30 min and then at 148,000×g for 30 min. The precipitate and the top fatty layer were rejected, and the supernatant (48 ml) was treated with two volumes of cold (−7°C) acetone. The white precipitate was separated off by centrifuging at 6000×g and was dissolved in a glass-Teflon homogenizer to obtain a homogeneous suspension. Centrifuging at 18,000×g for 60 min was repeated. The insoluble precipitate was rejected, and the clear supernatant was deposited on a column of Sephadex G-50 (coarse).

The triacetinase activity of the fractions was determined by the titrimetric method [1] at pH 9.5. This showed the presence of two enzymes (triacetinases I and II, Fig. 1) in the fractions. The yields of the fractions and the changes in activity over the stages of purification are given in Table 1.

The rechromatography of triacetinases I and II on a column of Sephadex G-100 in 0.1 M phosphate buffer, pH 7.4 increased their specific activities. On disc electrophoresis [2], one band was obtained for each enzyme.

LITERATURE CITED

1. M. M. Rakhimov, N. R. Dzhanbaeva, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 6, 642 (1970).
2. C. J. Fielding, Biochim. Biophys. Acta, 178, 499 (1969).