

LIPOLYSIS OF OLIVE OIL AND TALLOW IN AN EMULSIFIER-FREE TWO-PHASE
SYSTEM BY THE LIPASE FROM OAT SEEDS (*AVENA SATIVA L.*)

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SUMMARY

Lipase activity from whole oat seeds is dependent upon calcium ion in a two phase olive oil-water system. No other tested ion can substitute for calcium. The lipolysis of melted tallow at 46°C was successfully conducted in the presence of calcium ion; the fatty acid fraction contained predominantly oleic acid.

INTRODUCTION

In industry, glycerides are cleaved by a high-temperature steam treatment. There is currently great interest in the use of lipase enzymes as catalysts to split glycerides under mild, less energy-demanding conditions (Wang et al, 1988). The fatty acid (FA) selectivity exhibited by some lipases might also be exploited in this procedure. For example, treatment of tallow with a lipase selective for monounsaturated FA would yield a FA fraction enriched in oleic acid, and this FA has several industrial uses because its double bond can be easily derivatized or cleaved. The remaining unreacted glycerides are enriched in stearic and palmitic acids. These can be used directly as bread softeners, as intermediates for the manufacture of alkyd resins, split completely with a nonspecific lipase, or subjected to transesterification with vegetable oil to yield a high-value cocoa butter-like product (Knight and Lehman, 1968; Boelhower, 1983). Unlike partially hydrogenated vegetable oil, this product would be free of the unnatural trans double bond isomer.

There is no commercially available lipase with a selectivity for monounsaturated FA. However, it was demonstrated that the lipase from oat seeds discriminates against stearic acid (Berner and Hammond, 1970). Using pure glycerides, we found that the oat lipase is selective for monounsaturated FA (Piazza and Bilyk, 1989). Since the oat seed lipase can be prepared very easily and inexpensively, we decided to explore its use for fat and oil breakdown in a simple reaction system where no emulsifier, surfactant, or organic solvent is utilized.

MATERIALS AND METHODS

Oat seeds. Regal race horse oats were from U.S. Grain Co. (Timonium, MD).

Chemicals and materials. Bleached tallow was from Chemol, Inc. (Greensboro, NC). Calcium chloride, dihydrate was from J.T. Baker Chemical Co. All other chemicals were from Sigma Chemical Co. (St. Louis, MO). Silica gel G thin layer plates were from Analtech (Newark, DE).

Lipase preparation. Oat seeds (5 g) were homogenized in 35 ml cold 10 mM Hepes (N-2-hydroxyethyl piperazine-N'-ethanesulfonic acid) buffer (pH 7.4) for 2 min using a Waring commercial blender fitted with a 110 ml stainless steel mini jar. The homogenate was filtered through two layers of Miracloth (Calbiochem) and centrifuged at 120 g for 15 min. The supernatant was used as the source of lipolytic activity. The protein content of the lipase preparation was determined using the Bio-Rad protein assay and calibrated with Sigma protein standard.

Activity titrations. The rate of olive oil lipolysis was measured using a Radiometer automatic titrator (Model ETS 822) to maintain pH 7.5. To the titrator cup was added 1.35 ml olive oil (Sigma), water containing any desired salts, and lipase (final volume 7.7 ml). The titrant was 0.1 N NaOH. Total reaction time varied but was usually 15 min for high-rate conditions and up to 1 hr for unfavorable conditions. The contribution of nonenzymatic hydrolysis to the reaction rate was negligible. In all cases, the reported rates were determined from the slope of the line drawn tangent to the steepest portion of the titration curve.

Tallow lipolysis. Reactions were conducted in glass-stoppered 125 ml Erlenmeyer flasks and contained tallow, tricine buffer, and lipase as indicated in the text. The flasks were placed in a Gyrotory water bath shaker (New Brunswick, Model G76) at 46°C. After reaction, the flasks were removed to a -20°C freezer for 10 min or until processed. The reaction products were collected on a 60 ml funnel with a coarse fritted glass disk. The products were washed with cold water (2 x 10 ml), transferred to a vial, and dried overnight under vacuum.

Analysis of tallow lipolysis products. A 50 mg sample of the hydrolyzed tallow was dissolved in 10 ml hexane-isopropanol (60:40 v/v), and a 20 µl aliquot was applied to a washed silica gel G thin layer chromatography (TLC) plate (20 cm, 250 micron). The TLC plate was

subjected to a two-stage development procedure: (A) benzene, ether, ethyl acetate, acetic acid (80:10:10:1 v/v/v/v); development finished when solvent was 10 cm above the origin; (B) hexane, ether, formic acid (80:20:2 v/v/v); development to the top of the TLC plate. After development, the TLC plate was sprayed with 60% aqueous sulfuric acid and charred. Estimation of the amount of each reaction product was made using a TLC scanner (Camag, Linomat IV, Model 27220) calibrated with standards of each class of glyceride or FA, each containing stearic acid. The degree of charring is very insensitive to the presence of one double bond in the FA.

For analysis of FA composition, the remaining 50 mg sample was applied to a TLC plate (500 micron). After development, the locations of the product bands were determined by examining the TLC plate under U.V. light. After extraction of the silica gel with ethyl acetate and methanol (80:20 v/v), FA methyl esters were prepared by transesterification using methanolic-KOH for the glycerides or by treatment of the free FA with diazomethane. The methyl esters were analyzed using a gas-liquid chromatograph (Perkin-Elmer, Model 3920) containing a J&W Scientific fused silica megabore column (DB-225-30-M) and equipped with a flame ionization detector.

RESULTS

Olive oil lipolysis. In our preliminary studies, oat lipase acted upon gum arabic-olive oil-water emulsions in the absence of added ions. However, when a two-phase mixture of olive oil and water was assayed in a stirred reaction vessel at room temperature, no lipolytic activity was observed. The addition of CaCl_2 promoted hydrolytic activity (Fig. 1), and optimal activity was obtained with the CaCl_2 concentration 40 mM or higher. The other salts that were tested (KCl , NaCl , MgCl_2 , CoCl_2 , ZnCl_2 , FeCl_3) did not stimulate lipolytic activity, except MnCl_2 , which gave only 8% of the activity seen with CaCl_2 .

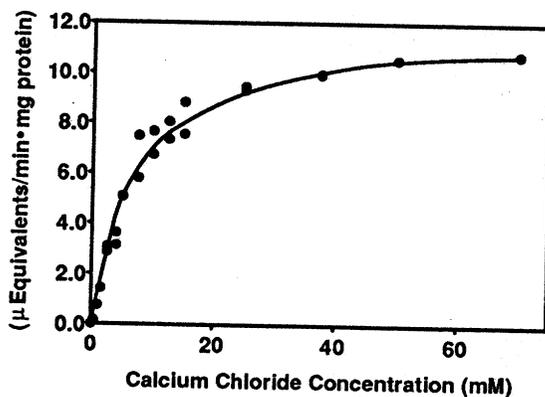


Fig. 1. Stimulation of olive oil lipolysis by CaCl_2 . Assays were performed at room temperature as described in Materials and Methods.

Tallow lipolysis. In the next experiment (Fig. 2), the breakdown of melted tallow at 46°C was followed by TLC. As previously shown

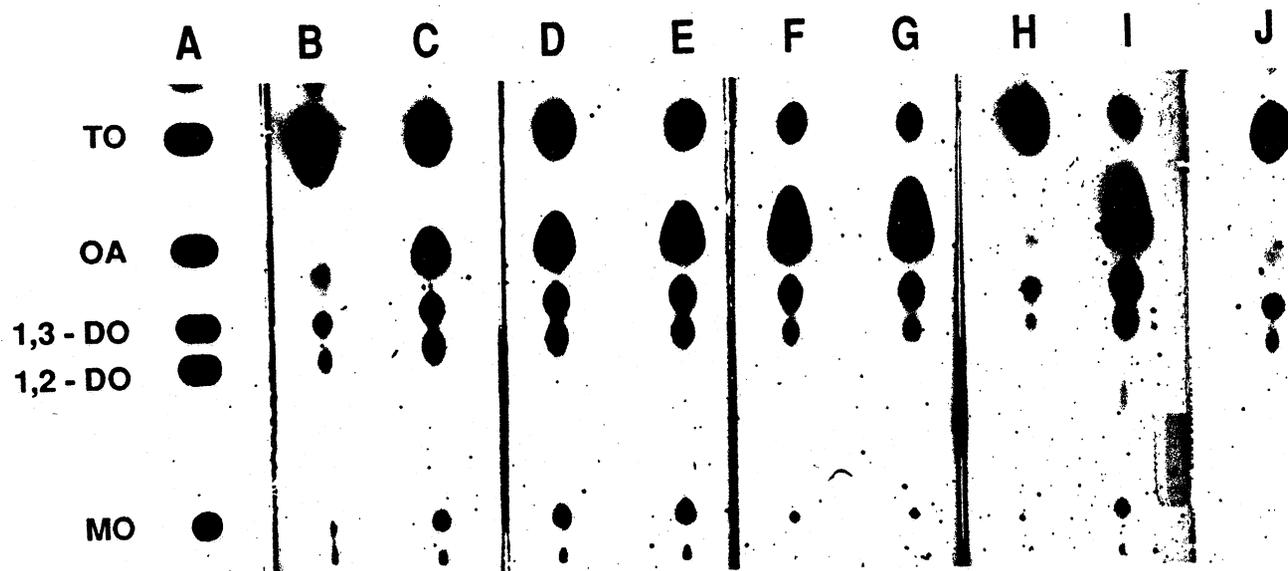


Fig. 2. TLC analysis of tallow lipolysis products.

Reactions contained 1 g tallow, 50 ml 0.1 M tricine (pH 9.0), and 4.5 ml oat lipase containing 7 mg protein. The reaction temperature was 46°C.

Lane A. Standards: TO, triolein; OA, oleic acid; 1,3-DO, 1,3-diolein; 1,2-DO, 1,2-diolein; MO, monoolein. Lanes B-G. Tallow plus lipase, 2 hr reaction time, CaCl₂ concentrations: 0, 5, 10, 15, 30, 45 mM. Lane H. Tallow without lipase, 30 mM CaCl₂, 2 hr reaction time. Lane I. Tallow plus lipase, 18 hr reaction time, 45 mM CaCl₂. Lane J. Untreated tallow.

Table I. Products of Oat Lipase Hydrolysis of Tallow

	Amount of Tallow			
	1 g	5 g	10 g	20 g
	Hydrolysis Products			
Monoglyceride	0.071	0.26	0.23	0.44
1,2-diglyceride	0.11 (USFA 19%) (SFA 81%)	0.60 (USFA 27%) (SFA 73%)	1.08	2.06 (USFA 27%) (SFA 73%)
1,3-diglyceride	0.041 (USFA 21%) (SFA 79%)	0.28 (USFA 29%) (SFA 71%)	0.46	0.76 (USFA 27%) (SFA 73%)
free acid	0.44 (USFA 86%) (SFA 14%)	1.79 (USFA 84%) (SFA 16%)	3.99	4.06 (USFA 83%) (SFA 17%)
triglyceride	0.31 (USFA 27%) (SFA 73%)	1.96 (USFA 36%) (SFA 64%)	3.92	12.45 (USFA 47%) (SFA 53%)
glycerol	0.028	0.11	0.32	0.23

Lipolysis was initiated with 3.5 ml of oat lipase containing 5 mg protein. Each reaction solution contained 5 mmol tricine (pH 9.0) and 1.6 mmol CaCl₂. Total volume (buffer, lipase, and tallow) was 55 ml. The reaction temperature was 46°C, and the reaction time was 2 hr. Glycerol production was estimated using the free fatty acid data with correction for the glycerol residing in the di- and monoglyceride fractions. The relative amounts of USFA and SFA in unreacted tallow are 51% and 49%, respectively.

with olive oil, the addition of CaCl_2 was necessary to promote good tallow breakdown. Increased CaCl_2 promoted breakdown up to a concentration of 45 mM. The addition of CaCl_2 alone with no lipase caused no breakdown of tallow. In addition, increasing the reaction time from 2 hr to 18 hr did not result in complete tallow breakdown, although it was found that oat lipase retains activity after 18 hr at 46°C . The nonspecific lipase from *Candida rugosa* can completely hydrolyze tallow under similar conditions (Linfield et al, 1984). In contrast, the oat lipase selectively acts upon oleic acid leaving highly saturated glycerides. This is shown in a second experiment in which the amount of tallow was varied, but the reaction volume was kept constant at 55 ml (Table I). The amount of lipase added to each reaction vessel was the same in each case. Total FA production as a percent of starting material remained about constant (~40% by weight) up to 10 g tallow but decreased for 20 g tallow (~20%). In every case, however, the unsaturated fatty acids (USFA) in the FA fraction greatly predominated over the saturated fatty acids (SFA). The predominant USFA was oleic acid (~80% of total USFA). In the diglyceride fraction, this pattern is reversed with the SFA predominant over the USFA. The two predominant SFA in these fractions were palmitic and stearic (~60% and 34%, respectively, of total SFA). Control experiments demonstrated that lipid levels in the oat lipase preparation itself were too low to influence these results.

In conclusion, the heat-resistant lipase from oat seeds can be utilized in a simple and inexpensive procedure for splitting tallow or other fats. This is the first time that a lipase from a higher plant has been applied to a biotechnological problem, and the methodology developed here should be usable for the production of monounsaturated fatty acids and diglycerides for food as well as for industrial uses.

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