# Oat Lipase

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Since the initial observations of the action of pancreatic lipase on neutral fat by Eberle in 1834 (see Sumner & Somers, 1943), lipases from various sources have been the subject of much detailed investigation. Most of this work has been directed at the elucidation of the mode of action of animal lipases, pancreatic lipase in particular, because of their obvious importance in the digestion and absorption of fat by the animal body. Among the plant lipases, that of the castor bean (*Ricinus*) has received most attention owing primarily to its relatively high activity. More recently, Fiore & Nord (1950) have published a comprehensive study on the lipase of *Fusarium lini*.

Cereal lipases have received relatively little attention. It was shown by Maestrini (1919, 1921*a*, *b*) that germinated barley and by Van Lear (1921) that malt extracts had lipase activity. Sullivan & Howe (1933) and Kretovitch, Sokolova & Ushakova (1940) have studied the lipase activity of wheats. More recently, Singer & Hofstee (1948) have made detailed studies on wheat-germ lipase.

In the present investigation oat lipase has been obtained in a highly purified condition relative to its activity in an oatmeal and the purified enzyme has been shown to split off, specifically, a single butyric acid radical from tributyrin. The latter observation has already been published in a preliminary note (Peers, 1953). Oat lipase is located in the outer pericarp layers of the groat and it is possible to remove this lipase from the groats by a 'wet-scrubbing' technique (Hutchinson, Martin & Moran, 1951). This paper is a more complete account of the results obtained in a general study of oat lipase, which appears to differ in certain properties from lipases from other sources.

## METHODS

*Wet-scrubbing technique for removing oat pericarp.* The apparatus (see Fig. 1) consists essentially of a test-tube brush (3 in. × 0·75 in.) mounted in the chuck of a stirrer motor ($\frac{1}{30}$ h.p.) revolving in a container made of two Quickfit adaptors (MF 18/5 and DA 59). The side arm of the lower adaptor contains a copper spiral (*C*) which retains the groats but allows the particles of pericarp in suspension to pass through a rubber tube which can be connected to a Büchner flask. The process is a 'batch' one, 10 g. of groats being scrubbed at a time in a total of 50 ml. of distilled water. The groats are placed in the container, 25 ml. of glass-distilled water added with the tube in position *A* and the brush revolved at approx. 2000 rev./min. for 2 min. Near the end of this period the tube is inserted in the Büchner flask and the liquor removed by suction (position *B*). With tube again in position *A* a further 25 ml. of water are added and scrubbing continued for a further 1 min. and then the liquor is drawn off. Approx. 95% of the water added is recovered as the liquor. The apparatus is conveniently cleaned out ready for the next batch by removing *S* and, with the brush slowly revolving, water is poured into the apparatus.

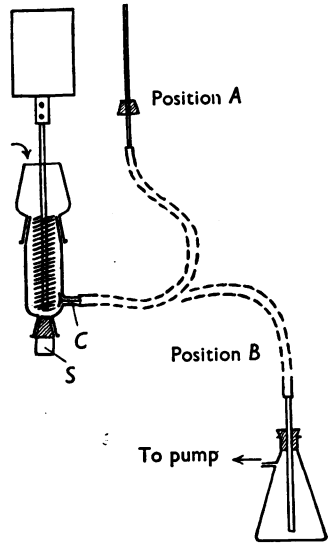


Fig. 1. Apparatus for scrubbing groats. For explanation see text.

*Dough method for measurement of lipase activity.* A modification of the method described by Hutchinson & Martin (1952) was used. Whole groats were inactivated with respect to lipase activity by immersion, in a sealed tube, in a boiling-water bath for 1 hr. The groats were then ground in a high-speed Christy-Norris disintegrator mill to pass through a sieve of aperture size 0·7 mm. The meal was defatted by two successive washings with light petroleum (40–60°) using approx. 50 ml./10 g., the solids being separated by centrifugation each time. The inactive, defatted oatmeal was spread out in a thin layer on filter paper and dried by exposure to the air. Samples (0·5 g.) were weighed into small test tubes (2 × 0·5 in.) and eight drops of olive oil,

equivalent to 149±6 mg. (B.P. grade: free fatty acid content <1%) were added from a standard pipette and mixed thoroughly with a glass rod. Water (0·2 ml.) or the solution (or suspension) under test was added at zero time and the mixture quickly worked into a dough with the glass rod. The tube was corked and immersed in a water bath at 37°. After an appropriate time interval the contents of the tube were washed into a 50 ml. centrifuge tube with light petroleum (40–60°) and after centrifuging at 3000 rev./min. for 15 min. the supernatant was carefully decanted into a tared 100 ml. extraction flask. The petroleum was distilled off, the flask dried in a vacuum desiccator over silica gel and then weighed to check the recovery of oil and fatty acid. The results were rejected if the net weight was outside the limits 149±6 mg.

Approximately 30 ml. of neutralized, boiling benzene: ethanol (1:1, v/v) were added and the sample titrated, whilst boiling on a hot-plate, with ethanolic 0·0354N-NaOH (1 ml. = 10 mg. free oleic acid). Olive oil is composed of mixed triglycerides with oleic acid as the predominant fatty acid (Hilditch, 1947). Calculations based upon the published constitution of olive oil show that the errors introduced by regarding it, for the purposes of lipase assay, as triolein, are negligible.

The lipase activity ($q_{oleic}$) is expressed as m-moles oleic acid released/hr./g. dry wt., and under these conditions 1 unit of lipase activity releases 1 m-mole oleic acid in 1 hr.

*Manometric assay of lipase activity.* This was based on the method of Rona & Lasnitski (1924). The activity of the lipase preparations was measured against tributyrin in the conventional Warburg apparatus at 37° using a bicarbonate: $CO_2$ buffer system at pH 7·4. Each flask contained 1·5 ml. 0·0246M-$NaHCO_3$, 0·5 ml. of a dispersion of tributyrin (4·0%, w/v) in 0·0148M-$NaHCO_3$ (in side bulb) and 1 ml. of enzyme solution or suspension. The manometers were gassed with 5% $CO_2$ + 95% $N_2$ and equilibrated for 10 min. in the bath at 37° prior to tipping the substrate. The manometers were shaken at 150 oscillations/min. The $CO_2$ evolution was measured over 30 min. and the rate of release of $CO_2$ over the initial 10 min. period (corrected for by appropriate blanks) was calculated. The activity ($q_{butyric}$) is expressed as m-moles $CO_2$ released/hr./g. dry wt.

*Saponification values.* These were determined on a semi-micro scale in boiling tubes fitted with reflux air condensers. Samples (100–150 mg.) of the glyceride were refluxed on a water bath with 10 ml. ethanolic 0·2N-KOH for 45 min. The tubes were then cooled, phenolphthalein added and the excess KOH back-titrated with 0·1N-HCl.

*Nitrogen determinations.* Determinations of total N were carried out in a Kjeldahl apparatus after 5 hr. digestion by the procedure described by Hiller, Plazin & Van Slyke (1948).

*Phosphorous determinations.* These were carried out according to Allen (1940). The colour densities were read on a Hilger 'Spekker' absorptiometer against a reagent blank using spectrum red (H 508) and heat-resistant filters. The amidol-bisulphite reagent was made up fresh daily and filtered before use.

## RESULTS

### *Evidence that the lipase is a true oat lipase*

As the lipase activity of oats is found mainly in the epidermal layers it is important to know that it is essentially a property of the seeds, and does not arise from subepidermal fungal mycelium, which is known to occur in some specimens of grain.

Observations on the subepidermal fungal contaminants of wheat grains made by Oxley & Jones (1944), Hyde (1950) and Hyde & Galleymore (1951) suggested that fungus-free oat grains might possibly be obtained by keeping them in a dry atmosphere for 3–4 weeks prior to harvesting. This was tested, with selected oat plants, by clamping a 3 kg. jar containing bags of silica gel over the heads, the stalks passing through a split cork. Control heads were grown in the same plot. The silica gel was regenerated every 2 days. In this way samples were obtained which varied in mycelial content and lipase activity. The mycelial content was determined by removing some epidermis from externally 'sterilized' groats and staining with aniline blue as described by Hyde & Galleymore (1951). These results are shown in Table 1.

Oats examined while at the 'milky' stage (i.e. at an early stage in the development of the grain) were free of mycelium but high in lipase activity.

Pieces of pericarp from groats which had been treated to sterilize the external surface, but which contained subepidermal mycelia, were plated out on 2% malt agar and incubated at 25° for 7 days. Such cultures indicated that two principal subepidermal contaminants of groats were an *Alternaria* species and an *Aspergillus* species. These two fungi, isolated from the groats, were grown on 2% malt agar at 25°, the mycelia were harvested after 4 days and their lipase activities determined by the dough method on a 'homogenized' suspension of the mycelium. The activities (m-moles oleic acid/hr./g. dry wt.) were 0·13 for the *Alternaria* mycelium and 0·19 for the

Table 1. *Lipase activity and sub-epidermal fungal contaminants in oats*

(The oat heads were kept in an atmosphere dried with silica-gel for 1 month prior to harvest; control oats were grown for each sample. The lipase activities were determined against olive oil.)

| Sample | Conditions of growth | $q_{oleic\ acid}$ (m-moles/hr./g. dry wt.) | Mycelial content |
|---|---|---|---|
| A | (1) Grown in dry atmosphere | 0·65 | Nil |
| | (2) Control | 0·42 | + + + + |
| B | (1) Grown in dry atmosphere | 0·54 | + |
| | (2) Control | 0·31 | + + + |

*Aspergillus* mycelium, compared with 0·31 for the whole oatmeal. Since the mycelial content of the groats constituted approximately 2 % by weight of the pericarp which in turn constituted approximately 2 % by weight of the whole groat it follows that the mycelial contamination could not possibly account for the total lipase activity.

*Distribution of lipase activity in fractions obtained by wet-scrubbing Victory groats*

The groats were scrubbed as described, the liquor centrifuged at 3000 rev./min. for 30 min. to separate the pericarp which had been scrubbed off, and the pericarp and the depericarped groats were spread out on filter paper and air dried. The three fractions were assayed for lipase activity and analysed for dry matter, nitrogen and phosphorus. Table 2 summarizes the results obtained.

*Purification and some properties of oat lipase*

The data given in Table 2 show that the lipase in the centrifuged extract obtained from scrubbed groats was considerably purified (approximately 300-fold on dry-matter basis: 350-fold on a nitrogen basis). The centrifuged aqueous extract was somewhat opalescent and could only be clarified by repeated filtration through the same filter paper. The clear, filtered extract had very little lipase activity and the activity was associated with the material causing the opalescence.

Precipitation occurred when the pH of the opalescent extract was adjusted to 5·5 with acetic acid with further precipitation at pH 4·8. Some lipase activity was found in the former fraction but analysis showed that over 80 % of the lipase had been inactivated by this procedure. Ethanol added up to a concentration of 75 % (v/v) to the cooled aqueous extract did not produce a precipitate. With ammonium sulphate added up to 80 % saturation a precipitate settled out after 30 min. at 0–4°, but both the precipitate and the supernatant were inactive. Both precipitate and supernatant were dialysed and retested separately and combined but no activity could be observed. The original extract after dialysis overnight against distilled water showed a 20 % reduction in activity, and with saturated ammonium sulphate added up to a concentration of 50 % saturation showed a 50 % loss in activity, although no precipitation occurred.

Lipase was adsorbed, from aqueous extracts, by calcium phosphate gel (prepared according to Singer & Kearney, 1950), kieselguhr and alumina C$\gamma$, but it was not possible to elute the lipase bound to these adsorbents although the adsorbent-lipase complex itself was lipolytically active. The calcium phosphate gel-lipase complex retained its activity for 10 days when stored at 0–4°. Extraction of the complex with *iso*butanol:water (1:1) caused the activity to disappear from the solid phase but no activity could be found in the water phase, in the *iso*butanol phase, in the slight interfacial precipitate which formed at the junction of the two liquid phases or in any combination of these fractions.

By repeating a cycle of freezing, and then thawing at about 17°, a precipitate formed which contained most of the original lipase activity. This precipitate was insoluble in a wide range of solvents including the ether:petroleum mixtures of Takamiya (1935) used for *Ricinus* lipase, the chloroform:methanol system of Folch & Lees (1951) used for proteolipids, and the $\alpha$-picoline-dioxan system of Takashima (1952) used for lipoprotein-chlorophyll complexes.

This complete insolubility of the enzyme after the freezing-thawing treatment ruled out the usual techniques for further purification, and accordingly the possibility was examined of removing inert material before preparing the opalescent extract. Extraction of the groats with organic solvents prior to scrubbing removed some inert material and so a continuous cold extractor was constructed of Quickfit apparatus in which the groats, contained in a Soxhlet thimble, were continuously extracted with a solvent for approximately 8 hr. A series of solvents were tried and after aerating off the solvent, 10 g. samples of the groats were scrubbed in 50 ml. of water and the centrifuged aqueous extracts examined for lipase activity. No loss of activity from the groats occurred with petroleum ether (40–60°) or benzene and ether, a partial loss occurred with acetone, *n*-butanol and *iso*butanol and complete loss with methanol, ethanol and chloroform: the ratio $q_{oleic}/q_{butyric}$ was between 2·45

Table 2. *Lipase activity in the various fractions obtained by scrubbing oats*

(The hulled oats (11·9 g.) were scrubbed in distilled water (100 ml.) and the suspension of pericarp centrifuged. All solid samples were air-dried to approximately 10 % moisture.)

| Fraction | Amount | Total solids (g.) | Total N (mg.) | Total P (mg.) | Lipase activity: $q_{oleic}$ (m-moles/hr./g. dry wt.) | Lipase activity: $q_{butyric}$ (m-moles/hr./g. dry wt.) |
|---|---|---|---|---|---|---|
| Wholemeal | 11·86 g. | 10·20 | 258·7 | 42·15 | 0·77 | 0·31 |
| Depericarped groats | 11·42 g. | 9·89 | 253·4 | 41·21 | 0·05 | 0·02 |
| Pericarp | 0·22 g. | 0·21 | 3·3 | 0·73 | 3·65 | 1·53 |
| Aqueous extract | 94 ml. | 0·04 | 0·9 | 0·17 | 234 | 94·50 |

and 2·56 in all cases. Ether was found to give the cleanest extract after centrifuging, and the following technique was finally adopted for the preparation of the partially purified oat lipase.

Groats which had been pre-extracted with ether were 'scrubbed' and the aqueous extract collected as described in the Methods section. This extract was centrifuged at 4500 rev./min. for 30 min. and the slightly opalescent solution so obtained frozen and thawed several times until precipitation had occurred. The suspension was then centrifuged and the precipitate resuspended in distilled water and washed several times; it was found necessary to freeze and thaw to avoid losses in the washing process. The material so obtained is the purified lipase which has been used for a study of some of its properties. The purification is some 2000-fold on a dry-matter basis compared with the activity in an oatmeal prepared from the same groats.

Nitrogen and phosphorus contents have been determined during this purification process and these figures are included in Table 3 which shows the activities, etc., at each stage. A number of preparations made by this procedure have been found to have similar nitrogen and overall purification figures. The lipase prepared in this manner is stable over a period of 10 days when kept at 0–4°, pH 7·4.

Samples of the preparation have been acid-hydrolysed (6N-hydrochloric acid) and the hydrolysate examined on two-dimensional paper chromatograms using phenol:$NH_3$ and *n*-butanol:acetic acid as irrigating solvents and ninhydrin as indicator. These chromatograms show all the commonly occurring amino acids and ethanolamine.

The centrifuged aqueous extract was examined by paper electrophoresis, using the technique of Gordon, Gross, O'Connor & Pitt-Rivers (1952). This technique showed the presence of at least three protein components. Two of these remained in solution after the freezing and thawing technique and the third, which was the major protein component, did not migrate in 0·05M-veronal buffer, pH 8·5, or in 50% (v/v) ethanol containing 5% (w/v) 0·880 ammonia.

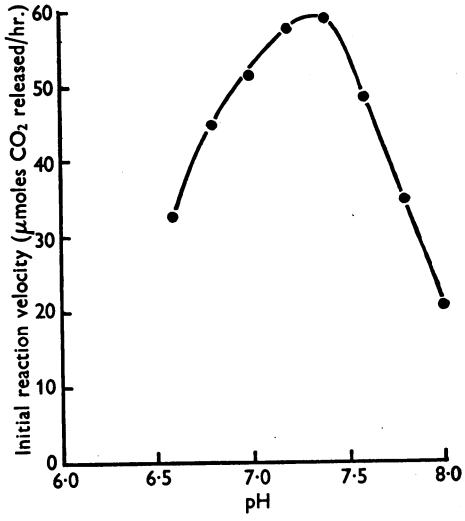


Fig. 2. Effect of pH on the activity of purified oat lipase. Initial reaction velocities (expressed as μmoles $CO_2$ released/hr.) are measured at 37° in $NaHCO_3$:$CO_2$ buffer systems in the presence of 20 mg. tributyrin and 0·21 purified lipase units/flask, total vol. 3 ml.

*Some kinetic properties of the purified lipase*

*Effect of* pH. Samples (1·0 ml.) of a suspension of the purified lipase (0·14 mg./ml. dry weight, 0·21 lipase units/ml.) were used in the manometric method to establish the pH-activity relationships at 37°. The manometer flasks contained 1·0 ml. enzyme, 0·5 ml. of a dispersion of tributyrin (4·0%, w/v) in appropriate strength sodium

Table 3. *Purification of oat lipase*

(20 g. of ether-extracted Victory groats were scrubbed in 100 ml. distilled water. The extract obtained was centrifuged at 4500 rev./min. for 30 min. and the supernatant obtained was alternatively frozen solid and thawed a total of six times. The precipitate formed was centrifuged off and washed with 3 × 10 ml. of distilled water. The solid samples were resuspended in water for assay purposes.)

| Stage of preparation | Amount | Dry matter (mg./ml.) | N (mg./g. dry wt.) | P (mg./g. dry wt.) | $q_{oleic}$ (m-moles/hr./g. dry wt.) | $q_{butyric}$ (m-moles/hr./g. dry wt.) | Purification on dry wt. | $\frac{q_{oleic}}{q_{butyric}}$ |
|---|---|---|---|---|---|---|---|---|
| (1) Groats | 20·0 g. | 87·8 | 25·4 | 4·13 | 0·78 | 0·31 | 1 | 2·52 |
| (2) Aqueous extract | 98 ml. | 3·78 | 19·2 | 4·15 | 47·6 | 19·3 | 61 | 2·47 |
| (3) Centrifuged extract | | | | | | | | |
| (*a*) Supernatant | 97 ml. | 0·60 | 36·8 | 7·93 | 281·0 | 114·0 | 361 | 2·47 |
| (*b*) Residue | 10 ml. | 31·0 | 16·0 | 3·45 | 4·5 | 1·8 | — | 2·50 |
| (4) Supernatant from (3) frozen and thawed | | | | | | | | |
| (*a*) Supernatant | 96 ml. | 0·53 | 32·2 | 8·25 | 106 | 42·0 | — | 2·52 |
| (*b*) Washed precipitate | 10 ml. | 0·70 | 66·0 | 5·20 | 1570 | 625 | 2000 | 2·51 |

bicarbonate and 1·5 ml. of sodium bicarbonate solutions varying from 0·0039M to 0·098M: the manometers were gassed with a nitrogen-carbon dioxide mixture (5%:95%) and the rates of release of carbon dioxide measured over the initial 10 min. period. The results are shown in Fig. 2.

*Effect of enzyme concentration.* Using the manometric technique with 0·0148M-sodium bicarbonate to maintain the pH at 7·4, varying amounts of purified lipase in suspension were assayed under the usual conditions. The results showed a linear response up to a rate of release of carbon dioxide of 45 $\mu$moles/hr. measured over the first 10 min.; at higher enzyme concentrations the initial reaction velocity falls off.

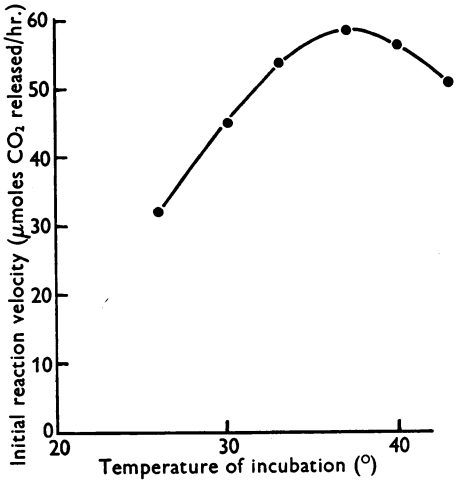


Fig. 3. Effect of temperature on the initial reaction velocity of purified lipase. Measured in 0·0148M-$NaHCO_3$ (pH 7·4) in the presence of 20 mg. tributyrin and 0·20 lipase units/flask, total vol. 3 ml.

*Effect of temperature of incubation.* Samples (1 ml.) of a purified lipase suspension (0·13 mg./ml. dry matter, 0·20 lipase units/ml.) were used to determine the initial reaction velocity against tributyrin over a range of incubation temperatures. The results obtained are shown in Fig. 4, and under these conditions indicate an optimum temperature of 37–38°.

*Effect of substrate concentration.* Using 1 ml. samples of the purified lipase (0·15 mg./ml. dry matter, 0·23 lipase unit per ml.) initial reaction velocities have been measured at initial tributyrin concentrations varying from $2{\cdot}2 \times 10^{-3}$ to $11 \times 10^{-3}$M. Reciprocal plots made as described by Lineweaver & Burk (1934) gave straight lines and from the intercepts on the axes the Michaelis constant ($K_m$) and the maximum initial reaction velocity ($V$) have been calculated as 0·0062M and 60·3 $\mu$moles carbon dioxide/hr. (means of three determinations) respectively under these conditions.

*Specificity and products of hydrolysis of tributyrin.* It was noticed that the hydrolysis of tributyrin ceased when one-third of the total possible carbon dioxide had been released. Typical progress curves for two different amounts of tributyrin (3·33 and 5·0 mg. per flask) are given in Fig. 4. This suggested

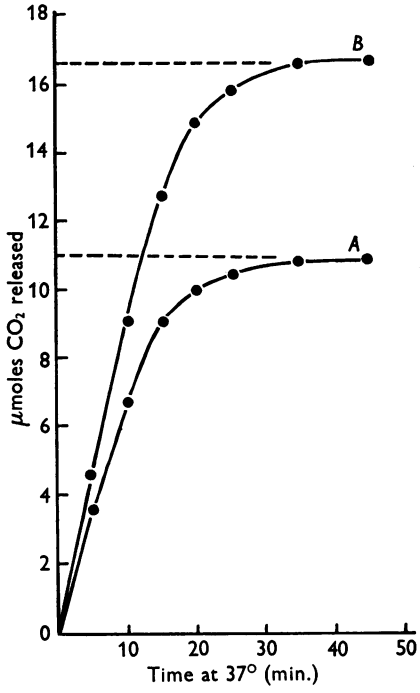


Fig. 4. Progress curves for purified oat lipase acting on tributyrin. Curves obtained using manometers (total vol. 3 ml.) containing 1·0 ml. lipase (0·13mg./ml. dry matter: 0·20 lipase units/ml.), 0·0148M-$NaHCO_3$. The amounts of tributyrin were 11·0 $\mu$moles ($A$) and 16·6 $\mu$moles ($B$). The dotted lines show the theoretical one-third total possible $CO_2$ evolutions.

that only one of the butyric acid radicals was split off by the purified enzyme leaving a dibutyrin as a reaction product. Krebs's pots containing 60 ml. 0·0245M-sodium bicarbonate, 20 ml. of enzyme preparation (0·18 mg./ml. dry matter, 0·26 lipase units/ml.) 20 ml. of water and 20 ml. of 2% tributyrin dispersed in 0·0148M-sodium bicarbonate were incubated at 37° with shaking for 2 hr. after gassing with a nitrogen-carbon dioxide mixture (95%:5%). The contents of the pots were then transferred to a separating funnel and extracted twice with 50 ml. dry ether. The ethereal extract was evaporated on a water bath leaving a faintly yellow oil. In five experiments yields of 311·7, 306·6, 310·3, 307·8 and 306·2 mg. of this oil were obtained (theoretical yield of dibutyrin, 307 mg.).

These samples were bulked and fractionally microdistilled at 26 mm. vacuum, when approximately 95% of the oil distilled at 184–187°. The product gave a negative periodate-fuchsin reaction. (Found: C, 56·7; H, 8·5%; saponification value, 478. $C_{11}H_{20}O_5$ requires C, 56·9; H, 8·7%, saponification value, 483.)

α-Monobutyrin was prepared by the method of Schuette & Hale (1930) as a light-yellow oil, b.p. 140–142°/5 mm. (Found: C, 51·5; H, 8·9%; saponification value, 349. Calc. for $C_7H_{14}O_4$: C, 51·8; H, 8·7%; saponification value, 346.) β-Monobutyrin was prepared as described by Daubert (1940) as an oil, b.p. 141–143°/5 mm. (Found: C, 51·7; H, 8·7%; saponification value, 343. Calc. for $C_7H_{14}O_4$: C, 51·8; H, 8·7%; saponification value, 346.)

The two dibutyrins were prepared from the corresponding dibromohydrins according to the methods of Güth (1903) as oils, b.p. 169–172/20 mm. (αβ-), and 177–9°/20 mm. (αγ-). (Found: for αβ-: C, 56·8; H, 8·7%; saponification value, 481; for αγ-: C, 56·9; H, 8·7%; saponification value, 479; calculated for $C_{11}H_{20}O_5$; C, 56·9; H, 8·7%; saponification value, 483.)

These four compounds, and the isolated product of reaction, were not hydrolysed by freshly prepared, purified lipase at pH 7·4, 37°. The purified lipase preparation hydrolysed the water-soluble triglyceride, triacetin, at roughly one-third of the rate it attacks tributyrin. It was inactive against 'Crill 6' (polyoxyethylene derivative of sorbitan laurate) by either method and the substrates tributyrin and olive oil were found not to be interchangeable in the two methods of assay.

*Possible activation.* Samples (0·5 ml.) of a purified lipase preparation (0·25 mg./ml. dry matter, 0·38 lipase units/ml.) were used to examine the influence of various substances upon the initial reaction velocity, with tributyrin as substrate. The addition of 0·5 ml. of 0·01 M-$CaCl_2$, $MgSO_4$ and $MnSO_4$ or 0·5 ml. of 0·5% (w/v) 'Crill 6', egg albumin, sodium oleate and sodium glycocholate caused neither activation nor inhibition.

## DISCUSSION

The difficulties of differentiating lipase and esterase activities and the choice of substrate for measuring lipolytic activity have recently been discussed by Desnuelle (1951). It is generally considered that tributyrin hydrolysis is effected by a true lipase and, in the case of milk, the 'tributyrinase' and lipase activities have definitely been correlated (Dunkley & Smith, 1951). Evidence is presented in this paper that the same enzyme is responsible for the hydrolysis of olive oil and tributyrin since the ratio of the activities of the enzyme, at various stages of purification, against these two substrates, measured by two different methods, remains the same. These two substrates are not, however, interchangeable in the two methods of assay described. Hutchinson & Martin (unpublished data) have shown that oat lipase activity against olive oil in a dough decreases with increased water (above 40%) until it is practically zero in a system which is predominantly aqueous: this would explain the non-hydrolysis of olive oil in the manometric system. Balls & Tucker (1938) have reported pancreatic lipase activity against both olive oil and tributyrin at low temperatures (−15 to −38°) where the system (which was predominantly aqueous) was solid.

This suggests that oat lipase differs from pancreatic lipase and a survey of the reported properties of lipases from various sources indicates that they may all differ from each other, judged by the criteria of solubility, specificity and kinetic properties. Thus wheat-germ lipase has been assayed against α-monobutyrin and 'Tween 20' (polyoxethylene sorbitan laurate) (Singer & Hofstee, 1948) and the lipase of *Fusarium lini* against triacetin (Fiore & Nord, 1950). Oat lipase has been shown to be completely inactive against the two former substrates and to hydrolyse triacetin at only one-third the rate it hydrolyses tributyrin although triacetin is water-soluble.

The rate of hydrolysis of tributyrin by the purified oat lipase is unaffected by the presence of dispersing agents such as 'Crill 6', egg albumin, glycocholate, sodium oleate, calcium, magnesium or manganese ions. In this respect it differs from the pancreatic lipase studied by Willstätter & Memmen (1924).

Differences between lipase preparations from different sources are reflected in their solubility relations. The only other lipase preparation reported to be water-insoluble is that from castor-bean but this is soluble in ether (Takamiya, 1935). The oat-lipase preparation described in this paper is insoluble in a wide range of solvents.

The kinetic properties of oat lipase resemble, more closely, those of the wheat-germ lipase described by Singer & Hofstee (1948) than any of the other lipases, but differ from the latter in solubility behaviour and in specificity towards different substrates.

Takamiya (1936) has demonstrated that *Ricinus* lipase requires an activator and Kraut & Pantschenko-Jurewicz (1934) and Schønheyder & Volquartz (1946) have shown that pancreatic lipase can be resolved into apoenzyme and coenzyme moieties. The inactivation of the oat enzyme by precipitation with ammonium sulphate and isoelectric precipitation at pH 5·5 might possibly be due to a dissociation of a coenzyme factor. However, experiments on the recombination of fractions provided no evidence for this suggestion. The increase observed in total lipase

activity in the fractions obtained by scrubbing the groats compared with that found in the original whole groats is thought to be due to differences in physical factors.

The purified preparation has a nitrogen content of 6·7 %. If the enzyme is an unconjugated protein the preparation is therefore far from pure. On the other hand, it is possible that the preparation does not contain as much inert material as the low nitrogen content would suggest but that, as the physical properties appear to indicate, oat lipase has an unusual constitution.

A high production of diglyceride has been observed with certain preparations of pancreatic lipase (Desnuelle, Naudet & Rouzier, 1948), and Frazer (see Desnuelle, 1951) has suggested that more than one enzyme may be involved in the complete hydrolysis of a triglyceride. Recently Mattson, Benedict, Martin & Beck (1952) have put forward evidence for selective hydrolysis by rat pancreatic lipase resulting in the formation of $\alpha\beta$-diglycerides and $\beta$-monoglycerides. In this paper it has been shown that the purified oat lipase will specifically split off one butyric acid radical from tributyrin and this fact would appear to give weight to the *in vivo* studies of Mattson *et al.* (1952) and the suggestion of Frazer.

## SUMMARY

1. The lipase causing lipolysis in oatmeals has been shown to be a true cereal lipase, not a product of the subepidermal fungal contaminants.

2. This lipase has been purified some 2000-fold on a dry-matter basis compared with the activity in the original oatmeal. The purified preparation contained 6·7 % nitrogen and 0·5 % phosphorus.

3. The purified preparation has an optimum pH of 7·4, optimum temperature 37–38°, and a Michaelis-Menten constant ($K_m$) of 0·006 M when tributyrin is the substrate.

4. The purified lipase splits off one butyric acid radical only from tributyrin and does not hydrolyse the various mono- and di-butyrins at pH 7·4 and 37°.

5. The oat lipase will not attack the polyoxyethylene derivative of sorbitan laurate nor is it 'activated' by the compensatory activators required, according to Willstätter, by pancreatic lipase.

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