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Iowa State University, 1992

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Hydrolysis of fats and oils by moist oat caryopses

by

Shantilal Parmar

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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GENERAL INTRODUCTION

Oleochemicals can compete successfully with petrochemicals and their derivatives by virtue of their economic and ecological advantages. This has led to a growing interest in the chemistry and biotechnology of fats and oils, and many new and interesting ideas for employing biotechnology to produce oleochemicals from fats have been investigated. Among the most promising routes to products of industrial interest are the hydrolysis, esterification, and interesterification of lipids by lipases. However the technology that has been developed so far is expensive, complicated and difficult to scale up.

Lipase catalyzes the hydrolysis of glycerol esters to yield free fatty acids. Various plant lipases have been isolated and characterized. It has been known for a long time that the lipase in oats is located almost exclusively on the surface of the caryopses. Prior research at Iowa State University has shown that when moist oats are immersed in oil, the lipase on the surface of the oat caryopses, releases free fatty acids. The reaction slows as the glycerol accumulates in the oats and fatty acids accumulate in the oil phase. The reaction can be pushed to > 90% completion by changing the oats after the reaction slows. Reuse of the oats, after

adjusting the moisture level results in significantly decreased rates of hydrolysis. The reaction runs well at 40°C. Although the process looked promising, a good deal of scale-up and development needs to be done.

Therefore, this study was conducted with the following objectives:

1. To optimize the hydrolysis of fats and oils with a moist oat bioreactor.
2. To attain the highest possible percentage of hydrolysis of soybean oil.
3. To study the effect on hydrolysis of diluting the fats with solvents.
4. To apply the technique to high-melting fats, such as tallow, and heat-sensitive oils, such as castor oil.
5. To remove the oat lipase from the caryopses and produce lipase concentrate.
6. To study the fate of native oat lipids during external hydrolysis of oils by moist oat caryopses.
7. To study the effect of oat variety and growing environment on the lipase activity.
8. To assess the microbial safety of the technique.
9. To analyze the economic feasibility of the technique.

Explanation of dissertation format

This dissertation consists of two major parts in the form of papers prepared for a professional journal. The first paper consists of "Effects of variety and growth location on the lipase activity of oats" and the second paper consists of "Hydrolysis of fats and oils with moist oat caryopses". The format of the two manuscripts followed that for the Journal of The American Oil Chemists' Society. These two papers are preceded by a General Introduction and a Review of Literature followed by General Summary, Literature Cited, Acknowledgment and Appendix. Literature cited in the entire dissertation is listed in alphabetical order of authors' names followed by the General Summary.

REVIEW OF LITERATURE

Acylglycerol acylhydrolases (EC 3.1.1.3) or lipases are enzymes that hydrolyze tri-, di-, and mono-glycerides present at an oil-water interface (Jensen et al., 1983). Although the reaction seldom goes to completion, hydrolysis of triglycerides by lipase can yield di- and mono-glycerides and free fatty acids. Currently, a diversity of lipases is being developed commercially, and attempts are being made to apply them as replacements for conventional chemical processes. Among the most promising chemical routes of industrial interest are the hydrolysis, ester synthesis, and interesterification reactions of lipids brought about by lipases (Werdelmann, 1974).

Occurrence of Lipases

Generally lipases have been divided into three groups: animal, plant and microbial. Berner and Hammond (1969) studied the characteristics of a number of animal and plant lipases. Among animal lipases, pancreatic and milk lipase have been studied extensively (Brockerhoff and Jensen, 1974).

In the past, interest in microbial lipases often resulted from investigations of foods spoilage, especially of dairy

products. Many other foods make use of microbial lipases, either formed in situ or added, to obtain desired flavors and textures. Production of lipases by microorganisms may assist in their classification and detection (Lawrence, 1967). Most microbial lipases are extracellular and relatively easy to isolate. Microbial lipases have diverse properties, and this diversity could be increased by screening microorganisms for new properties or by modifying lipases by chemical or genetic manipulations. Therefore, microbial lipases usually have been considered for commercial production of fats and oils (Stead, 1986). Efforts are being made to search for and to produce microbial lipases with useful properties for industrial applications such as high activity or substrate specificity (Chander and Klostermeyer, 1983; Huge-Jensen et al., 1988). Lawrence (1967) published a comprehensive review on microbial lipases and esterases. Lipases from various molds (*Geotrichum candidum, Rhizopus arrhizus, Aspergillus niger, Mucor pusillus, Puccinia graminis trictici, Candida cylindracea*) and bacteria (*Leptospirae, Pseudomonas, Staphylococcus, Corynebacterium, Propionibacterium, Achrombacter*) have been isolated and characterized (Tomizuka et al., 1966; Lawrence et al., 1967; Bjorklund, 1970).

In recent years, plants have begun to receive attention as potential sources of lipases. Seeds are generally rich in

triglycerides which serves as compact energy source for the newly emerging plant. Usually plant lipases are not detectable in ungerminated dry seeds, but activities increase dramatically on germination (Gavrichenkov et al., 1969; Sanders and Pattee, 1975). Grains and seeds are easy to grow and harvest, and crude lipase preparations can be obtained by simple processes. The activities and stabilities of such crude preparations are more than satisfactory according to Hassanien and Mukherjee (1986). Seed lipases are relatively specific for the native triacylglycerols or triglycerides of the same species (Huang et al., 1988). Crushing generally activates dormant lipase in seed, and the resulting accumulation of free fatty acids can cause an industrially important oil to become unacceptable or require additional processing to remove the acids. Seed lipases of castor bean (Ory, 1969), *Vernonia anthelmintica* (Krewson and Scott, 1964; Olney et al., 1968), oats (Martin and Peers, 1953; Berner and Hammond, 1970; Piazza and Bilyk, 1989; Lee and Hammond, 1990; Ekstrand et al., 1992), rice bran (Funastu et al., 1971; Shastry and Rao, 1971; Fujiki et al., 1978), french beans (Kermasha et al., 1986), faba beans (Dundas et al., 1978) and lupin seeds (Sanz and Olias, 1990) have been investigated.

Lipase Immobilization

Lipase immobilization refers to a localization or confinement of lipase that allows the lipase to be separated physically from a mixture of substrate and product for reuse (Shahani, 1975; Kilara and Shahani, 1977). Karube et al. (1977) described several characteristics that are useful for the selection of a carrier. These include mechanical strength, microbial resistance, thermal stability, chemical durability, chemical functionality, hydrophobic/hydrophilic character, ease of regeneration, loading capacity and cost. However, single substances rarely fulfill all these requirements.

Numerous methods for achieving immobilization of lipases are available. According to Bailey and Ollis (1986), these methods may be subdivided into two main categories: (i) chemical methods and (ii) physical methods. Chemical methods include lipase attachment to a matrix by covalent bonds and formation of a cross-linked lipase-containing matrix (Lieberman and Ollis, 1975; Tahoun, 1986). Physical methods include entrapment of the lipase within an insoluble gel matrix (Kubo et al., 1976; Marlot et al., 1985), containment of the lipase within porous hollow fibers or microcapsules

(Pronk et al., 1988; van der Padt et al., 1990) adsorption of the enzyme on a carrier (Kobayashi et al., 1980; Macrae, 1983; Omar et al., 1988), binding of the lipase to dried mycelia (Bell et al., 1981) or bacterial cell debris (Kosugi and Suzuki, 1973), and ion exchange between the enzyme and a support (Posorske et al., 1988; Jensen et al., 1988).

Support materials

Some procedures for immobilizing lipases are effective than others. Some of the insoluble support materials commonly employed are porous glass and its derivatives (Brockman et al., 1973; Kobayashi et al., 1980; Marlot et al., 1985), diatomaceous earth or kieselguhr (Kroll et al., 1980; Macrae, 1983; Kimura et al., 1983; Wisdom et al., 1984; Ison et al., 1988), Duolite (Kimura et al., 1983), cellulose and its derivatives (Horiuti and Imamura, 1978; Brady et al., 1988), silica and its derivatives (Wisdom et al., 1985; Brady et al., 1988), clay (Brady et al., 1988), alumina (Marlot et al., 1985), stainless steel (Lieberman and Ollis, 1975), nylon (Brady et al., 1988), polyethylene and its derivatives, polypropylene and its derivatives, (Kimura et al., 1983; Yokozeki et al., 1982), polystyrene (Kang and Rhee, 1989), polyacrylamide (Tahoun, 1986), polyurethane (Marlot et al.,

1985), sephadex (Kang and Rhee, 1988), gelatin (Schafer, 1975), alginate (Omar et al., 1988), Dowex (Kobayashi et al., 1980), collagen (Karube et al., 1977), polyethylene glycol attached to magnetite (Takahashi et al., 1987), and fragments of fungal mycelia (Bell et al., 1981) or bacterial cell walls (Kosugi and Suzuki, 1973). Synthetic materials have been manufactured or obtained in a number of forms and shapes, including membranes, fibers, granules, and powders.

Immobilized lipase reactors

The use of immobilized lipases for the modification of fats and oils is currently a subject of expanding interest because the use of immobilized lipase is more cost effective than use in a free form. Immobilized lipase facilitates the development of continuous, large scale commercial processes. The use of immobilized lipase reactors also decreases potential contamination of the product with residual lipase. Immobilization often increases the thermal and chemical stability of the lipase (Chibata and Tosa, 1976).

Several reactor configurations have been used in studies of immobilized lipases. Two types of phases are invariably present:a solid phase, a carrier on which the lipase is immobilized; and one or two liquid phases (feedstocks). If an

organic solvent is used to dissolve reactant and product species, then a single-liquid phase is present in the reactor. A typical configuration of the continuous solid phase corresponds is a membrane in either a flat sheet or a hollow fiber form.

Lipase acts upon substrates that are generally much more viscous than water at the same temperature. Hence, one way to overcome the difficulties associated with operating immobilized lipase reactors for processing these substrates is to use suitable solvents (Malcata et al., 1990). Solvents used to carry out reactions catalyzed by immobilized lipases include benzene (Miller et al., 1988), toluene (Takahashi et al., 1987), n-hexane (Schuch and Mukherjee, 1987), cyclohexane, n-heptane, octane, isooctane, nonane, decane, hexadecane, isopropylether, carbon tetrachloride, methyl chloride, ethyl acetate (Kang and Rhee, 1989), petroleum ether (Wisdom et al., 1985), and tetrahydrofuran, triacetin methylcyanide, dimethyl formamide, dioxane, (Miller et al., 1988). Kang and Rhee (1989) reported that the rates of enzymatic hydrolysis of oils by immobilized lipases are strongly affected by the polarity of the reaction solvents.

Membrane or diaphragm reactor These reactors have been employed in the presence of one (Karuba et al., 1977) and

two liquid phases (Hoq et al., 1985). In one reactor type, organic and aqueous phases are separated by a solid membrane on which the lipase is immobilized. Generally, two kinds of flow patterns have been employed: flow tangential to or normal to the membrane (van der Padt et al., 1990). The pressure drop along the reactor coordinate is small, and no bulk flow through the membrane is allowed. For normal flow through the membrane, one of the phases is pumped through the membrane, and the other phase remains stationary (Taylor et al., 1986).

Packed-bed or Fixed-bed reactor This reactor has traditionally been used for most large-scale catalytic reactors because of its high efficiency, low cost, and ease of construction and operation. This type is versatile and simple and provides more surface area for reaction than membrane reactors. The packed-bed reactor has been employed for lipase-catalyzed hydrolysis and interesterification reactions by a number of investigators (Layvayre and Baratti, 1982; Macrae, 1983; Wisdom et al., 1987; Brady et al., 1988). Granules of various sizes with lipase attached to them (Layvayre and Baratti, 1982; Macrae, 1983; Wisdom et al., 1987; Brady et al., 1988) or lipase-containing dried mycelia (Bell et al., 1981) are usually confined in a jacketed column. The single liquid phase is pumped upwards or downwards (Ison

et al., 1988). Solvents used in a single liquid phase include diisopropyl ether (Bell et al., 1981), petroleum ether (Macrae, 1985), and hexane (Wisdom et al., 1987). In the case of multi-phase liquid systems, the two streams may flow through the reactor in opposite directions with the denser phase flowing downwards, or in the same direction (Brady et al., 1988).

There are two operational constraints that must be considered when operating packed-bed reactors: i) intraparticle diffusion limitations on reaction rates; and ii) high pressure drops across the reactor packing.

Continuous stirred-tank reactor These reactors possess advantages over fixed bed-reactors, i.e., lower construction costs and efficient stirring which eliminates the temperature and concentration gradients. These reactors are larger than packed-bed reactors. Continuous stirred-tank reactors have been widely used for the hydrolysis of the olive oil triglycerides and for the synthesis of glycerides from fatty acids and glycerol dissolved in diisopropyl ether. To prevent the immobilized lipase leaving the continuous stirred-tank reactors, a microfilter or screen must be provided at the reactor outlet (Brady et al., 1988).

Fluidized-bed reactor These reactors have been employed to study the hydrolysis of glycerides. Lieberman and Ollis (1975) studied the hydrolysis of tributyrin in a fluidized lipase reactor. A differential fluidized-bed reactor has the following advantages over a fixed-bed reactor: a lower pressure drop at high flow rates, less channeling, reduced coalescence of the emulsion particles, and freedom from plugging by feed particulates. These reactors enable the study of flow rate and substrate concentration on conversion without resorting to integrated rate expression for analysis of experimental data.

Stirred-batch reactor This type of reactor is most commonly employed in bench-scale and industrial applications. Batch reactors are extremely versatile and easy to operate. Configurations include glass flasks stirred with magnetic bars (Brockman et al., 1973; Macrae, 1983; Kimura et al., 1983; Marlot et al., 1985; Miller et al., 1988), and vessels stirred by submerged impellers (Ison et al., 1988) as well as flasks mounted in reciprocating oscillators (Horiuti and Imamura, 1978; Kobayashi et al., 1980; Yokozeki et al., 1982). The mixing pattern avoids temperature and concentration gradients. Sampling can be accomplished at a single arbitrarily located point.

For interesterification reactions, stirred-batch reactors in which the lipase is immobilized on powdered supports have been used extensively. Stirred-batch reactors do not require pumping devices. Since these reactors can be made very small, heating and cooling can be achieved rapidly. To achieve measurable rates of interesterification, it is usually necessary to activate the catalyst particles by hydration with up to 10% of their weight of water. After the batch reaction is terminated, separation of the powdered lipase support from the reaction fluid can be accomplished by simple filtration or centrifugation.

Utilization of Lipases

The specificity of lipases towards triglyceride substrates with respect to the type and stereospecific position of fatty acid residues has prompted a number of special applications within the food field (Malcata et al., 1990)

Flavor modification

Enzymatically produced flavors are common to many types of foods. Flavors for consumption by both human and animals have been changed and/or enhanced by the partial hydrolysis of

triglycerides. Technology has been developed for the production of flavor systems via controlled enzyme modification of fats. Although butter churned from the lipolyzed cream can develop coarse, pungent flavors, certain type of cheeses, notably Italian and mold-ripened (Roquefort, blue) types, normally exhibit flavor notes that are associated with free fatty acids (Stead, 1986). Free acids that are short-chain steam volatile, and both water soluble and water insoluble are responsible for imparting the characteristics piquant or sharp flavor to Italian cheeses (Farnham, 1957). Compared to Italian and blue cheeses, Cheddar, Swiss and Dutch cheeses undergo very low levels of lipolysis (Kilara, 1985). Patented lipolyzed flavors and processes for their manufacture include lipolyzed milk compositions (Kempf et al., 1953), and lipolyzed milkfat products, such as butter flavors (Buhler et al., 1972), cultured cream flavors (Pangier, 1969), blue cheese flavors (Knight, 1963; Watts and Nelson, 1963) and cheese-like flavors (Johnson and Southworth 1973). Nelson (1972) researched the lipolysis of other types of natural fats. The most effective enzyme system for treatment of nonmilk fats combines esterase and lipase. Edible, unrefined animal fats show considerable promise as sources of useful flavors. Beef, chicken and pork fats, lipolyzed with mixed esterase-lipase systems yield flavor profiles that may be

useful in a variety of applications (Nelson, 1972). Haas and Lugay (1974) were granted a patent to improve the palatability of dog food by the partial hydrolysis of beef tallow with lipase.

Ester synthesis

Esterification reactions between polyhydric alcohols and free fatty acids are in essence the reverse of the hydrolysis reaction of the corresponding glyceride. The equilibrium between the forward and the reverse reactions is usually controlled by the water content of the reaction mixture. Examples of high-value chemicals obtained via use of lipases include the synthesis of oleic acid esters of primary and secondary aliphatic and terpenic alcohols (Okumura et al., 1979; Iwami et al., 1980) production of geranyl and menthyl esters of butyric acid and lauric acid (Marlot et al., 1985). Kodera et al. (1986) synthesized the esters of α-substituted carboxylic acids catalyzed by a polyethylene glycol-modified lipase from *Candida cylindracea* in benzene at 25°C. The esters synthesized were pentyl α-methylpentanate, methyl benzoate, and methyl retinoate. Inada et al. (1984) synthesized esters with lipase that had been modified by polyethylene glycol. The reaction was carried out in benzene. When very hydrophobic substrates such as lauryl alcohol and

stearic acid were used, the ester synthesis reaction proceeded efficiently in the transparent benzene solution with the maximum activity of 5.0 μmoles/min/mg protein. Miller et al. (1988) demonstrated that the commercial preparation, Lipozyme can be used to synthesize a very broad range of esters at an equally broad range of rates. The enzyme catalyzed ester synthesis with saturated, unsaturated and a variety of branched carboxylic acids. Kosugi et al. (1991) manufactured fatty acid esters from alkaline fatty acid solutions using lipase from *Pseudomonal mephitica lipolytica* immobilized on macroporous polystyrene membranes containing anion exchange residues. The esterification activity of *Candida cylindracae* lipase was enhanced by the modification of the enzyme with polyethylene glycol activated by p-nitrochloroformate (Basri et al., 1991). The rate of esterification of oleic was lower than that of butyric acid. Benzene was the best solvent. Seino et al. (1984) attempted to synthesize enzymatically carbohydrate esters of fatty acids in order to overcome the problems associated with chemical processes for the synthesis of commercial sucrose esters. The enzymes used were lipases from microorganisms. The fatty acids investigated were stearic, oleic and linoleic acids, and the alcohols were sucrose, glucose, fructose and sorbitol. The lipases from *Candida cylindracea* was the most active enzyme for the

synthesis of carbohydrate esters. The optimum conditions for its activity were as follows: molar ratio of carbohydrate to fatty acid: 0.05 M/L:0.2 M/L, amount of lipase, 4 g/L, pH of the reaction mixture, 5.4 in phosphate buffer, and reaction period, 72 h.

Interesterification

The term "interesterification" refers to the exchange of acyl radicals between an ester and an acid (acidolysis), an ester and alcohol (alcoholysis) or ester and another ester transesterification). This is a process which is used in the oils and fats industry to modify the properties of triglyceride mixtures. In this process, a chemical catalyst, such as sodium metal or sodium alkoxide, is used to promote acyl migration in glyceride mixtures so that the fatty acid acyl residues become randomly distributed among the glyceride molecules (Sreenivasan, 1978). The application of lipases for the modification of fats and oils by interesterification reactions at low water content is expected to become a parallel route to that involving the use of oil seed plants to produce oils and fats with desired characteristics (Nielsen, 1984).

The use of an immobilized lipase for interesterification allows retention of the lipase in batch or continuous-flow

reactor operation (Wisdom et al., 1984). The effects of reduced solvent levels in reaction mixtures for fat interesterification using lipase immobilized on diatomaceous earth have been examined by Ison et al. (1988). Equal rates of interesterification were achieved in a batch reactor with different solvent levels despite the increased viscosity of the fat mixtures. Triacylglycerols of ucuhuba (*Virola surinamensis*), that contain almost exclusively (90%) lauroyl and myristoyl esters, were interesterified with a variety of fatty acids, alcohols and esters using with an immobilized lipase from *Mucor miehei* (Schuch and Mukherjee, 1987). Exchange of acyl moieties occurred at the *sn*-1,3-positions of the triacylglycerols, and the rates of interesterification with various substances were of the order: long-chain alcohol > fatty acid > triacylglycerol > methyl ester > glycerol. The melting behavior of the triacylglycerols formed by interesterification revealed their potential use in food and dietetic products.

Hydrolysis of fats and oils for fatty acid production

Fatty acids are the major oleochemical feedstocks, and usually are produced by fat splitting. The current process, the Colgate-Emery process, uses steam to split fats and oils to produce fatty acids. Typical operating conditions are

250°C and 50 atm pressure. Maintaining these reaction conditions makes the splitting operation very energy-intensive. Furthermore, the capital investment for steam splitting is high because a special reactor is needed that will withstand the high temperature and pressure as well as the corrosiveness of the fatty acids. The merits of this process are high hydrolysis of fats and oils yielding 97% split in 2 h and the sweetwater stream contains about 12% glycerol (Brady et al., 1988).

An alternate approach to steam hydrolysis is enzymatic splitting at milder conditions, 30-45°C and atmospheric pressure. The advantages of this process are lower energy costs, lower capital investment and improved product quality. However, the disadvantage associated with this system is that it is slow and the reaction seldom goes to completion because the reaction is inhibited by the end products, fatty acids and glycerol. Therefore, to be competitive, this process must be able to produce yields comparable to or better than that of the steam process. The enzyme that catalyzes the hydrolysis of triglycerides to fatty acids is lipase. The ideal lipase for use in fat splitting would possess several characteristics. First, the enzyme should attack triglycerides randomly. It should not demonstrate position or chain-length selectivity. In other words, the lipase should

hydrolyze a variety of fats and oils to completion. Second, the lipase should be thermostable at 45 to 55°C. This condition may be necessary for the use of tallow as feedstock. Third, to match the energy costs associated with steam splitting, the price of the lipases should be comparable or the lipase should be reused.

Previous attempts to hydrolyze various fats and oils by lipases

Soybean oil

The hydrolysis of soybean oil by different lipases (lipase D from *Rhizopus delemar*, lipase G from *Penicillium* species and lipase N from *Rhizopus niveus*) was compared (Park et al., 1988), and the results demonstrated that lipase D, N and G hydrolyzed the oil to 44, 42 and 7.2% respectively, after a 10-h reaction. But the combined enzyme systems(lipase G + N and lipases G + D) hydrolyzed the oil to an extent of 95-98% after 10 h reaction.

Fatty acids were produced from soybean oil by hydrolysis with castor bean lipase (Khosla et al., 1985). The enzyme was most active at room temperature and pH 4.2 at an oil-water interface. The rate of hydrolysis of soybean oil increased with increasing surface area but was decreased by stirring. Under the proper conditions, hydrolysis was 100% in 24 h.

Lee and Hammond (1990) studied oat (*Avena sativa*) lipase.

The caryopses of oats, when moistened and immersed in oils, constituted a natural lipase bioreactor. Hydrolysis was monitored by titrating the free fatty acids released in the oil phase. The optimum amount of additional water was ~ 20% of the weight of the caryopses and the optimum temperature was ~40° C. The reaction was accelerated by gentle agitation, reducing the viscosity of the oil phase by addition of nonpolar solvents and increasing the amount of lipase on the caryopses. The reaction was inhibited by the accumulation of glycerol in the interior of the caryopses and of the free fatty acids in the oil phase. The lipase hydrolyzed all three positions of glycerol, and there was little accumulation of mono- or diglyceride in the nonaqueous phase. The time necessary to obtain 90% hydrolysis varied from a few days to several weeks. Greater degrees of hydrolysis could be obtained by replacing the caryopses when they became inhibited or by diluting the oil phase with hexane. The glycerol released could be recovered by washing the caryopses with water.

Olive oil

Yang and Rhee (1991) studied the continuous hydrolysis of lipid in a glass column by lipase from *Candida rugosa* immobilized on DEAE-Sephadex A50. Olive oil dissolved in

hydrophobic organic solvent(isooctane) was passed into the reactor with a concurrent stream of the aqueous buffer. The stability of the immobilized enzyme was greatly increased at higher substrate concentration.

Tsai et al. (1991) proposed a theoretical model for the lipase-catalyzed hydrolysis of high-concentration olive oil in biphasic isooctane-aqueous systems. Equilibrium conversions higher than 98% for 0.1 g/mL olive oil in isooctane were attained for systems with volume ratios up to one. Activity of the lipase showed no apparent change between 26 and 37° C, but decreased rapidly with temperatures above 43°C. The methodology presented in this work might be used to find kinetic parameters for reactor design and scale-up.

Hano et al. (1991) studied the kinetics of olive oil hydrolysis with lipase with various organic diluents in a stirred transfer cell. Among diluents investigated, isooctane gave the highest reaction rate. From adsorption and kinetic experiments, a new model of interfacial reaction between adsorbed lipase and olive oil was proposed. The effect of diluent on the reaction rate was explained not by the difference in adsorption characteristics of lipase or olive oil but by the difference in interfacial reaction rate constants.

Wang et al. (1988) studied the kinetics of lipase-

catalyzed oil hydrolysis by *Candida rugosa* in the absence of added emulsifier. Two optimal pH values, 5.0 and 7.0 were observed for lipase-catalyzed olive oil hydrolysis in a reaction mixture with high stirring speeds. The lipase activity at pH 5.0 was ~20% higher than at pH 7.0. In an oil-in-water system, the hydrolysis rate reached a maximum of 85 μmol/mg/min at a water to oil ratio of 1.0, and calcium ion increased the rate of olive oil hydrolysis. In a water-in-oil system, the amount of fatty acids released increased with increasing olive oil volume and reached a maximum of ~100 μmole/mg/min when the oil-water volume was >15.

A two-phase reaction system to hydrolyze olive oil was studied by Kwon and Rhee (1987). Kinetics of lipases in two-phase systems were investigated by determining the hydrolysis rate of triglycerides at various olive oil concentrations in isooctane using the microbial lipases from *Candida rugosa* and *Rhizopus arrhizus*. The results suggested that the olive oil concentration in isooctane affects the interfacial area. The dependency of the interfacial area on olive oil concentration is greater at lower olive oil concentration than at higher substrate concentration.

Lipases from *Candida rugosa* and *Rhizopus arrhizus* were investigated for the hydrolysis of olive oil in a 2-phase system by Kwon et al. (1987). Isooctane was used as an

organic solvent for both lipases. The lipid hydrolysis rate of olive oil by lipases in the two-phase system increased in proportion to the olive oil concentration up to 90%, whereas in emulsions the rate increased only to 5% olive oil and then decreased. The lipid hydrolysis rate in the two-phase system was maximum at pH 6.0 and 6.5 for the lipase from *Candida rugosa* and *Rhizopus arrhizus*, respectively. The optimum temperatures were 35 and 40°C for the *Candida* and *Rhizopus* lipases, respectively.

Fish oil

Ando et al. (1991) reported the use of marine bacterial lipases that were capable of hydrolyzing the n-3 polyunsaturated fatty acids of fish oils. Out of 45 strains of marine bacteria screened, 3, 6, and 13 strains from *Alteromonas*, *Pseudomonas*, and *Vibrio*, respectively, hydrolyzed Tween 80 or tributyrin. Hydrolysis by one strain of Vibrio, tentatively named VB-5 was enhanced by adding olive oil to the medium. The olive oil-induced lipase from the strain VB-5 was capable of liberating eicosapentaenoic and docosahexaenoic acids as free fatty acids along with palmitic and oleic acids from fish oils.

In order to investigate possible differences attributed to fatty acids during lumenal lipolysis and intestinal

absorption, the in-vitro hydrolysis of menhaden oil and its alkyl esters by pancreatic lipase was investigated by Yang et al. (1990). There was significant discrimination against the Δ^4- to Δ^7-unsaturated fatty acids of both medium and long chain lengths during the hydrolysis of menhaden oil and its fatty acid ethyl esters. In general, the ethyl esters were hydrolyzed 10-50 fold more slowly than the corresponding glyceryl esters, depending on the exact ratio of the two substrate types. None of the triacylglycerols or ethyl esters, however, was completely resistant to hydrolysis, and the treatment resulted in an eventual cleavage of all the alkyl esters and presumably all the primary ester bonds in the triacylglycerol molecule.

Castor oil

Juergen and Friedrich (1990) prepared hydroxy-monoglycerides (mainly monoricinolein) and ricinoleic acid by enzyme-catalyzed hydrolysis of castor oil. The castor oil was hydrolyzed in 0.1M phosphate buffer at room temperature by 1,3-specific *Rhizopus arrhizus* lipase in a stirred tank reactor. The pH was maintained at 7.5 by automatic titration with sodium hydroxide. A product containing 23% monoricinolein and 65% ricinoleic acid was obtained within 3 h. Monoricinolein content was increased to

65% by partial removal of ricinoleic acid by salt precipitation.

Lipase preparations from different microbial sources were used jointly for high levels of hydrolysis of castor oil (Yamaguchi et al., 1990). For example, lipase from *Penicillium cyclpium* was mixed with lipase from *Rhizopus javanicus* at the ratio of 1:10. At 25°C for 13-48 h, 82.4-92.9% of fatty acids esters in the castor oil were hydrolyzed.

Yamaguchi et al. (1989) studied castor oil hydrolysis in steps by using a lipase selectively hydrolyzing α-ester linkages in the first step and another lipase in the second step. In the first stage, 300 g castor oil shaken with a solution of 3 g of *Rhizopus japonicus* lipase in 300 g water at room temperature for 7 days to produce 270 g oil with 87% free fatty acids. In the second stage, the reaction mixture was added to a solution of 2.7 g of *Rhizopus delemar* lipase in 270 g water and shaken at room temperature for 2 days to produce an oil containing 98% free fatty acids.

Tallow

Vitro et al. (1991) reported that the lipase from *Candida rugosa* catalyzed the hydrolysis of inedible beef tallow in the presence of organic solvents at temperatures below the melting point of the fat. Reactions were carried out at 50% substrate

with 180 units/g of fat in a 2-L reactor. In the presence of isooctane (5-10%), beef tallow yielded 94% hydrolysis in 24 h both at 37 and 31° C.

Fats with high melting points such as beef tallow, were hydrolyzed at low temperature with nonstereospecific lipase in the presence of 10-20% (w/w) coconut oil (Tavss and Eigen, 1988). Yields were excellent (~98%) with no undesirable side products, and the process was economical due to the low temperature. Thus, a mixture 16.7g of beef tallow and coconut oil (83:13) and 1.67g lipase in 10 ml of 0.1N acetic acid was stirred for 24 h at 37°C. The fatty layer was removed and neutralized for soap manufacture.

Enzymic hydrolysis of bleachable fancy tallow was studied by Brady et al. (1988) as a low energy consuming alternative to the present steam (Colgate-Emery) process. Cost analysis of the enzymic process indicated that the use of immobilized lipase compared favorably with present steam processes. Hydrophobic microporous powders, membranes and fibers were found to give the best performance as supports since little of the lipase's activity was lost upon immobilization. Reactor designs studied include concurrent and counter-concurrent fixed beds, continuous stirred-tank reactors, and the diaphragm reactor. Productivities of the latter two reactor types were 1100 and 1700 kg fatty acid/kg immobilized lipase.

Serota (1986) was granted a US Patent for lipolytic splitting of fats and oils. A feedstock comprising 375 g tallow and 435 g of aqueous solution containing 623 mg of *Candida rugosa* lipase was incubated in an impeller-blade mixer at 40°C with constant stirring (900 rpm) for 72 h. Glycerol was recovered from the aqueous phase by multi-effect evaporation which removed the water.

Hydrolysis of beef tallow by lipase from *Candida cylindracea* in biphasic organic-aqueous reaction systems was investigated by Kobayashi et al. (1985). The addition of an appropriate organic solvent to the reaction system greatly stimulated the hydrolysis of tallow by lipase. Among the organic solvents tested, isooctane was the most suitable. The proportion of isooctane and phosphate buffer in the reaction mixture was best at the ratio of 1:9. An agitation speed > 500 rpm was sufficient for the reaction. Under the above conditions, the reaction rate in the biphasic isooctane-aqueous system was greatly enhanced compared to that in the organic-solvent-free system, and the percentage of hydrolysis reached almost 100%.

Commercial dry lipase from *Candida rugosa* was used to catalyze hydrolysis of tallow at 26-40°C (Linfield et al., 1984). A 95-98% hydrolysis was achieved in 72 h, requiring 15 units lipase/meq.

Milk fat

Milk-lipoprotein lipase hydrolyzed butterfat mainly to palmitic, oleic, stearic, myristic and lauric acids (Lebedev and Umanskii, 1981). No relation was found between the rate of hydrolysis and the chain length of the hydrolyzed fatty acids or their degree of saturation. When butterfat was incubated with lipoprotein lipase in the presence of coenzyme (blood serum), butyric acid accumulated in the mixture. The main lipid classes hydrolyzed were triglycerides and 1,3-diglycerides.

Umanskii et al. (1980) studied the hydrolysis of milk fat by lipase preparations of different origin. Lipase from *Oospora fragrans* preferentially liberated C14:1, C16:1 and C18:1 fatty acids from milk fat while pancreatic lipase and *Aspergillus awamori* lipase preferentially liberated butyric acid. All the enzymes, especially pancreatic lipase, were very active toward milk fat.

A lipase from *Aspergillus niger* immobilized by adsorption on a microporous, polypropylene flat-sheet membrane was used by Malcata et al. (1991) for continuous hydrolysis of the melted butterfat at 35°C. Under the reaction conditions of the experiment, a pseudo-zero order rate expression was used to model the kinetics of the overall hydrolysis. At pH 7.0, the maximum rate of release of each fatty acids depends on its

chain length. The relation between rate and chain length is accurately described by a skewed, bell-shaped distribution. Data taken at pH 5 were fitted assuming a Dixon-Webb diprotic model for the pH dependence of the reaction rate. Thermal deactivation of the immobilized lipase obeyed first order kinetics with a half-life of 19.9 days at 35°C. This model is useful for the predictions of the free fatty acids profiles of lipolysed butterfat, whereas the lumped-substrate model provides an estimation of the overall degree of hydrolysis as a function of reaction time.

A commercial lipase preparation was made by Garcia et al.(1991) from *A. niger*. Chromatographic analysis of the reaction products revealed that the short-chain fatty acids were preferentially released at pH 5, whereas the overall rate of hydrolysis for all major fatty acids showed a pH optima between 5 and 7, indicating the possibility of directing the selectivity of lipolysis in butteroil to enhance the production of the short-chain fatty acids associated with flavor development. The optimal temperature for this reaction was approximately 35° C.

Rapeseed oil

Lipolysis of rapeseed oil with lipases from *Candida lipolytica* and *Geotrichum* species was monitored by analysis of

the optimum fatty acids formed (Xie and Wu, 1987). The optimum conditions for the reaction were: total lipase activity, 90-100 units/g oil; water/oil=1:1; shaker rotation, 220 rpm; reaction temperature, 28°C; and reaction time, 20-24 h. A total lipase activity of 100 units/g oil is required to achieve 94.5% hydrolysis at a given condition. In the course of enzymic hydrolysis it was necessary to add neither buffer nor acid nor alkali to the reaction system.

Sunflower oil

Sunflower oil was 93.9% hydrolyzed in 5 h at 40°C, and lard was 98.1% hydrolyzed in 8 h at 40°C by a lipase preparation obtained by defatting castor beans with ethyl ether, grinding the meal and sieving to 0.25 mm diameter. Fatty acids and glycerol were obtained in 90.0 and 93.9% yield, respectively, in 5 h from a mixture of 100 g of sunflower oil, 10 g lipase preparation and 60 ml of 0.1N acetic acid (Meerov et al., 1976).

PAPER I.

EFFECTS OF VARIETY AND GROWTH LOCATION ON THE LIPASE ACTIVITY OF OATS

ABSTRACT

The effects of variety, growth locations, growing years, and early and late harvest on the lipase activity of oat caryopses have been studied. Nine early oat lines and 11 midseason lines grown at 6 and 12 locations in the US were examined for their lipase activity. Variety MN 87187 grown in Lafayette, IN gave the highest lipase activity in Uniform Early Oat Performance Nursery. Variety PA 8393-1500 grown in Fargo, ND gave the greatest lipase activity in the Uniform Midseason Oat Performance Nursery. Among early oat lines the means of lipase activity were significantly different at $P < 0.05$ for varieties and locations. The variety, growth location and the interaction of variety x location were significant at $P < 0.01$ for the lipase activity for oats in the Midseason Nursery. Yield, test weight and percentage of groat were positively correlated with lipase activity of some varieties of oats. When oats were harvested at various stages of development, caryopses from early harvested oats had more lipase activity/unit weight than normal harvested one. Variety Y907-5-5 had the most lipase activity of the varieties tested. Among the various growing years studied, 1988 was spectacularly better than other years. The oat caryopses from 1988, a very dry year, exhibited an unusually uniform golden color and were free from fungus attack.

INTRODUCTION

A knowledge of seed lipases is important in understanding their physiological roles, their action during storage, and their potential use in industry (1). Lipolytic enzymes are indispensable for the biological turnover of lipids (2) because lipases catalyze the initial steps of lipid mobilization, and, thus, may be rate controlling in germination and post-germinative growth. Depending on the plant species, lipase may be localized on the membranes of lipid bodies or in other subcellular compartments. Martin and Peers (3) first demonstrated that much of the lipase in oats is on the surface of the caryopses. The lipase activity in oat kernels at different pHs, at different stages of development, and in different parts of the oat kernels was studied (4). Frey and Hammond (5) reported a significant variation among varieties in lipase activity, and suggested that the lipase on the surface of the oat caryopses seems to be genetically controlled. The effect of growth conditions on lipase activity in oats has not been studied.

This paper reports the effect of variety, growing years early and late harvest and growth locations on the lipase activity of oat caryopses.

MATERIALS AND METHODS

Thirty-three early oat lines and 36 midseason lines that are grown each year by midwestern US oat breeders for systematic comparisons were examined. The lines examined were grown in Ames, IA in 1990 and were scanned for the lipase activity by the titrimetric method described by Lee and Hammond (6). The percentage of hydrolysis of soybean oil (Crisco brand, Proctor and Gamble Co., Cincinnati, OH) on the 5th day was taken as an index for strain selection. On this basis, nine early lines and 11 midseason lines were selected and samples grown in five locations for early lines and 11 locations from midseason lines were obtained (Table 1) through the cooperation of oat breeders.

The oat samples were dehulled with an impact type dehuller (Wintersteiger Gesellschaft, m.b.h & Co. A-4910, Reid, Austria). Percentage hydrolysis on the fifth day was determined (6) and yield (bushels/acre), test weight (lbs/bushel), % groat and % protein values were obtained from the report of Halstead and Rines (7). One hundred oat seeds were dehulled manually and weighed. Statistical analysis was done using the General Linear Model procedures of SAS 6.06 (8).

Oats of variety B605-1085 grown in Ames,IA during 1981, 1987 1988, 1989, and 1990 were stored at 4°C and 40% relative humidity. Oats of varieties B605-1085 and Y907-5-5 were planted in Ames, IA on March 30 and half of each variety was harvested on July 1 and 30, 1990, respectively.

Table 1. Locations contributing oat samples to the study

Location	Early	Midseason
Cornell Uni., Ithaca, NY	-	+
Iowa State Uni., Ames, IA	+	+
Michigan State Uni., East Lansing, MI	+	+
North Dakota State Uni., Fargo, ND	-	+
Ohio State Uni., Wooster, OH	+	+
Penn. State Uni., Uni. Park, PA	-	+
Purdue Uni., Lafayette, IN	+	+
South Dakota State Uni., Brookings, SD	-	+
Uni. of Illinois, Urbana, IL	+	+
Uni. of Minnesota, St. Paul, MN	-	+
Uni. of Missouri, Columbia, MO	+	+
Uni. Wisconsin, Madison, WI	-	+

RESULTS AND DISCUSSION

Table 2 shows the average percentage hydrolysis of soybean oil by different varieties of oats averaged across the six locations in the uniform early oat performance nursery. MN 87187 gave the highest hydrolysis while IL 86-1973 gave the lowest hydrolysis. Varieties of MN 87187, P8646B1-X-11-4, PA8598-8415, MN 87194 and PA 8393-15050 contained significantly ($P<0.05$) higher lipase than varieties of IL 83-7641-1, DON(CK), ANDREW(CK) and IL 86-1973. The extreme values differed by 1.8 fold.

Table 3 shows the average %hydrolysis of soybean oil by oats grown in various locations and averaged across the 9 varieties of oats selected from the uniform early oat performance nursery. The oats from Purdue University., Lafayette, IN were significantly ($P<0.05$) higher in lipase activity than the oats of other locations. There was no significant difference ($P<0.05$) in the oats from the oats of the other locations. An analysis of variance (Table 4) showed that the effect of variety, location and the interaction of variety x location, all were highly significant ($P<0.01$).

Table 2. Percent hydrolysis of soybean oil by various oat selections averaged for all locations in the Uniform Early Oat Performance Nursery

Variety	% Hydrolysis[a]
MN 87187	52.69[b]
P8646B1-X-11-4	48.98[b]
PA8598-8415	48.54[b]
MN 87194	48.18[b]
PA 8393-15050	47.60[b]
IL 83-7641-1	33.11[c]
DON(CK)	33.06[c]
ANDREW(CK)	32.05[c]
IL 86-1973	28.70[c]

[a]Average of 6 locations.

[bc]Means with the same superscript letter are not significantly different ($P<0.05$).

Table 5 shows the average %hydrolysis of soybean oil by different varieties of oats averaged across 12 locations in the uniform midseason oat performance nursery. PA 8393-1500 gave the highest hydrolysis while ND 8625585 gave the lowest hydrolysis. These extremes were significantly different and differed by 1.9 fold.

Table 3. Average % hydrolysis of soybean oil by oat varieties grown at various locations in the Uniform Early Oat Performance Nursery

Location	% Hydrolysis[a]
Purdue Uni., Lafayette, IN	51.79[b]
Michigan State Uni., East Lansing, MI	41.32[c]
Uni. of Illinois, Urbana, IL	40.89[c]
Iowa State Uni., Ames, IA	38.81[c]
Ohio State Uni., Wooster, OH	38.79[c]
Uni. of Missouri, Columbia, MO	37.01[c]

[a]Average of 9 varieties.

[bc]Means with the same superscript letter are not significantly different ($P<0.05$).

Average %hydrolysis of soybean oil by oats at locations and averaged across 11 varieties of oats are shown in Table 6. Oats from North Dakota State University., Fargo, ND gave significantly greater ($P<0.05$) hydrolysis than those from Cornell University., Ithaca, NY by 2.05 fold. Other locations in study were not significantly ($P<0.05$) different. An analysis of variance (Table 7) suggested a significant ($P<0.01$) difference in lipase activity for varieties, locations and their interaction of variety x locations.

Table 4. Anova for %hydrolysis of soybean oil by various variety of oats grown at various locations in Uniform Early Oat Performance Nursery

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Variety x Location	13	5512.99	424.07	12.86**	0.0001
Location	5	1269.22	253.84	7.70**	0.0001
Variety	8	4243.77	530.47	6.09**	0.0001
Error	40	1318.64	32.96		
Total	53	6831.63			

**Significant at 0.01 level.

The values of yield, test weight, %groat, %protein obtained from ther report of Halstead and Rines (7) and weight of 100 caryopses for the various varieties and locations in the uniform early oat performance nursery are shown in Table 8. The average yield, test weight, %groat, %protein and weight of 100 caryopses were 91.15 bushels/acre, 32.53 lbs/bushel, 70.48%, 17.69% and 2.33 grams for various varieties. There were similar values for locations also. The data for the uniform midseason oat performance nursery are shown in Table 9 for the varieties and Table 10 for the locations. The average yield, test weight, %groat, %protein

and weight of 100 caryopses were 97.75 bushels/acre, 32.73 lbs/bushel, 69.91%, 17.71% and 2.18 grams for various varieties. There were similar values for locations.

Table 5. Percent hydrolysis of soybean oil by various oat selections averaged for all locations in the Uniform Midseason Oat Performance Nursery

Variety	% Hydrolysis[a]
PA 8393-1500	61.30[b]
OH 1022	60.35[bc]
PA 8393-11138	60.03[bc]
PA 8494-11717	56.72[bcd]
OH 1007	56.28[cd]
P76134D3-27-3-5	52.58[de]
WI X5259-1	49.41[ef]
IA B605X	47.16[f]
ND 861246	37.02[g]
SD 85009	35.13[g]
ND 8625585	32.26[g]

[a]Average of 12 locations.

[bcdefg]Means with the same superscript letter are not significantly different ($P<0.05$).

Table 11 shows the correlation coefficients of percentage hydrolysis with yield, test weight, groat percentage, protein percentage and weight of 100 caryopses for varieties averaged across the four growing locations in the uniform early oat performance nursery for which data were available. Yield, test weight and groat percentage tended to be positively correlated with percentage hydrolysis while percentage of protein and weight of 100 caryopses were negatively correlated. There was a significant ($P<0.05$) correlation of test weight and %protein for variety IL 86-1973. However, the correlation coefficients averaged for all varieties were significant ($P<0.01$) for yield and weight of 100 caryopses.

When locations were averaged across varieties, only weight of 100 caryopses gave much correlation with lipase activity and these were negatively correlated (Table 12).

The correlation coefficients of percentage hydrolysis for varieties averaged across locations in the uniform midseason nursery with various parameters are shown in Table 13. Yield, test weight, groat percentage and protein percentage tend to be positively correlated with lipase

Table 6. Average %hydrolysis of soybean oil by oat varieties grown at various locations in the Uniform Midseason Oat Performance Nursery

Location	% Hydrolysis[a]
North Dakota State Uni., Fargo, ND	71.43[b]
Uni. of Minnesota, St. Paul, MN	60.29[c]
Purdue Uni., Lafayette, IN	59.50[c]
Uni. of Wisconsin, Madison, WI	54.38[c]
Uni. of Illinois, Urbana, IL	49.98[c]
Ohio State Uni., Wooster, OH	48.40[c]
Michigan State Uni., East Lansing, MI	46.69[c]
Iowa State Uni., Ames, IA	46.06[c]
South Dakota State Uni., Brookings, SD	43.76[c]
Penn. State Uni., Uni. Park, PA	42.45[c]
Uni. of Missouri, Columbia, MO	40.27[c]
Cornell Uni., Ithaca, NY	34.88[d]

[a]Average of 11 varieties.

[bcd]Means with the same superscript letter are not significantly different ($P<0.05$).

Table 7. Anova for %hydrolysis of soybean oil by various selection of oats grown at various locations in the Uniform Midseason oat Performance Nursery

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Variety x Location	21	26015.88	1238.85	33.29**	0.0001
Location	11	12349.82	1122.71	30.17**	0.0001
Variety	10	13666.05	1366.60	36.73**	0.0001
Error	110	4097.27	37.21		
Total	131	30113.15			

**Significant at 0.01 level.

activity while weight of 100 caryopses is negatively correlated. There was a significant ($P<0.05$) correlation for yield for varieties IA B605X, PA8393-11138 and PA8393-1500; test weight for varieties, PA8393-1500, PA76134C3-27 and SD 85009; groat percentage for varieties IA B605X, PA76134C3-27 and weight of 100 caryopses for varieties ND 862585. Yield, test weight, %groat and %protein were singficantly correlated when averaged for all varieties.

Table 8. Values for yield, test weight, %groat, %protein and weight of 100 caryopses for varieties and locations in the Uniform Early Oat Performance Nursery

Variety[a]	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt. of 100 caryopses (g)
ANDREW(CK)	79.34	30.75	71.40	18.25	2.35
DON(CK)	94.35	32.63	69.90	16.90	2.45
IL 83-7641-1	90.75	32.68	70.83	16.13	2.33
IL 86-1973	94.60	32.80	72.55	18.03	2.75
MN 87187	91.23	32.95	71.03	17.65	2.38
MN 87194	95.43	33.43	71.58	17.55	2.50
PA 8393-15050	90.28	35.20	69.40	19.10	2.07
PA 8598-8415	84.88	33.02	70.95	18.55	2.30
P8646B1-X-11-4	99.45	29.27	66.65	17.03	1.88
Average	91.15	32.53	70.48	17.69	2.33
Location[b]					
IA	118.61	33.12	70.25	16.98	2.39
IL	83.72	33.22	68.92	18.32	2.49
MO	44.58	30.32	69.17	16.86	2.28
IN	117.68	33.43	73.56	18.21	2.18
Average	91.15	32.52	70.48	17.59	2.34

[a]Average of 4 locations

[b]Average of 9 varieties

Table 9 Values for yield, test weight, %groat, %protein and weight of 100 caryopses for varieties in the Uniform Midseason Oat Performance Nursery.

Variety[a]	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt of 100 caryopses (g)
IA B605X	96.59	33.87	69.13	18.44	2.16
ND 861246	97.03	33.69	70.91	16.69	2.14
ND 862585	99.84	31.37	68.83	17.19	2.12
OH 1007	106.78	31.74	67.97	17.12	2.10
OH 1022	92.92	31.73	64.63	18.23	1.89
PA 8393-11138	91.69	34.93	72.73	18.23	2.20
PA 8393-1500	94.91	32.23	70.03	17.03	2.39
PA 8494-11717	91.41	33.52	70.09	17.80	2.09
PA76134C3-27	86.06	31.37	69.76	19.90	2.41
SD 85009	97.71	33.77	73.08	18.06	2.38
WI X5259-1	120.34	31.84	71.88	16.06	2.13
Average	97.75	32.73	69.91	17.71	2.18

[a]Average of 9 locations

Table 10. Values for yield, test weight, %groat, %protein and weight of 100 caryopses for locations in the Uniform Midseason Oat Performance Nursery.

Location[a]	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt. of 100 Caryopses (g)
IA	112.68	32.32	69.65	16.85	2.13
IL	93.19	31.68	68.86	18.72	2.46
IN	112.76	32.09	71.96	17.76	2.14
MN	122.03	38.64	75.22	17.62	2.21
MO	40.50	27.92	65.50	17.40	2.07
ND	157.86	39.56	75.65	18.21	2.20
NY	90.06	33.67	68.67	16.32	2.46
SD	92.67	31.76	69.29	17.98	2.31
WI	58.03	26.94	64.44	18.51	1.67
Average	97.75	32.73	69.92	17.71	2.18

[a]Average of 11 varieties

Table 11. Correlation of percentage hydrolysis with other parameters of oat varieties in the Uniform Early Oat Performance Nursery

Variety	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt. of 100 caryopses (g)
ANDREW(CK)	0.39	-0.02	0.89	-0.82	0.61
DON(CK)	0.65	0.44	0.88	0.73	-0.38
IL 83-7641-1	0.53	0.26	0.14	0.18	0.67
IL 86-1973	0.94	0.99**	0.58	-0.96*	-0.77
MN 87187	0.95	0.67	0.49	-0.06	0.35
MN 87194	0.96	0.72	-0.12	-0.42	-0.36
PA 8393-15050	0.83	0.26	0.07	-0.06	0.23
PA 8598-8415	0.91	0.43	0.08	0.04	-0.54
P8646B1-X-11-4	0.47	0.80	0.27	-0.61	0.52
Average for all varieties	0.44**	0.24	-0.09	0.11	-0.51**

*Significant at 0.05 level.

**Significant at 0.01 level.

Table 12. Correlation of percentage hydrolysis with other parameters of different locations in the Uniform Early Oat Performance Nursery

Location	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt. of 100 caryopses (g)
IA	-0.07	0.25	-0.66	-0.03	-0.53
IL	0.29	0.03	-0.62	0.30	-0.61
MO	0.06	-0.07	-0.59	0.16	-0.45
IN	0.03	0.09	-0.11	0.01	-0.76*

*Significant at 0.05 level.

The correlation coefficients for locations averaged across varieties for the midseason nursery (Table 14) were significant for yield in South Dakota and for test weight and %groat in Wisconsin.

In summary, in both the nurseries higher yield, higher test weight and higher groat percentage and lower weight of 100 caryopses tended to correlate with lipase activity for many varieties. In the early, but not the midseason nursery, low groat percentage and caryopses weight were correlated with lipase activity for several of the locations.

Table 13. Correlation of percentage hydrolysis with other parameters of oat varieties in the Uniform Midseason Oat Performance Nursery

Variety	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt. of 100 caryopses (g)
IA B605X	0.88**	0.68*	0.80**	0.53	-0.26
ND 861246	0.31	0.39	0.46	0.01	-0.12
ND 862585	0.10	0.10	0.15	0.38	-0.70*
OH 1007	0.58	0.34	0.14	0.44	-0.27
OH 1022	0.43	0.19	0.19	0.57	-0.23
PA 8393-11138	0.74*	0.54	0.51	0.16	0.44
PA 8393-1500	0.78*	0.73*	0.52	0.03	-0.27
PA 8494-11717	0.58	0.24	0.59	0.59	-0.02
PA76134C3-27	0.66	0.73*	0.75*	0.35	0.15
SD 85009	0.58	0.68*	0.44	0.17	-0.11
WI X5259-1	0.59	0.35	0.61	0.50	-0.13
Average for all varieties	0.39**	0.31**	0.21*	0.28*	-0.14

*Significant at 0.05 level.

**Significant at 0.01 level.

Table 14. Correlation coefficients of %hydrolysis with other parameters of different locations in the Uniform Midseason Oat Performance Nursery

Location	Yield (bu/ac)	Test wt. (lb/bu)	%Groat	%Protein	Wt. of 100 caryopses (g)
IA	-0.23	0.27	-0.43	0.09	-0.02
IL	-0.14	0.12	0.02	0.23	0.47
IN	0.44	0.37	0.02	0.10	0.02
MN	-0.11	-0.26	-0.25	0.36	0.28
MO	0.38	-0.23	0.12	-0.34	-0.42
ND	-0.14	-0.16	-0.26	0.27	0.12
NY	-0.12	-0.02	-0.06	0.07	0.06
SD	-0.66*	0.30	-0.20	0.40	-0.27
WI	-0.50	-0.79**	-0.78**	0.24	-0.44

*Significant at 0.05 level.

**Significant at 0.01 level.

Early and normal harvest

Fig 1 shows the effect of early harvest and normal harvest time on the lipase activity. Early oats were more active on a weight basis. These early oats were not fully developed and were light-weight oats. Since we determine the percentage hydrolysis on the basis of titer/gram of oats, it takes more early oats to make a gram than of those that are fully mature. Seemingly, the lipase is put on the surface early in the grain formation. The tendency of poorly developed caryopses to have more lipase activity/unit weight may explain some of the correlations seen in Tables 11, 12, 13 and 14 of lipase activity with low caryopses weight and in Table 14 with low groat percentage. On the other hand lipase activity seems to be positively correlated with varieties that gave good overall yields and test weights. This suggests that varieties and conditions that give many small caryopses produce more lipase than those that have fewer large caryopses. This is reasonable since the former would have more surface area, and the lipase is a surface enzyme.

With lipase-rich oats not only is the rate, which is measured by the slope of the line (Fig 1), faster, but the plateau that the reactions finally reaches is higher. This difference in plateau value is the result of enzyme inhibition by the end products. Thus, it is important in reducing the

time for this process and achieving a high degree of hydrolysis per treatment to have the lipase level and activity as high as possible. At both harvest times, the varieties, Y907-5-5 showed higher hydrolysis than B605-1085.

These results also suggest that the lipase level of oats might be raised significantly by plant breeding. Y907-5-5, the strain identified with the greatest lipase activity so far, is quite susceptible to oat crown blight disease. It is important to transfer high lipase to disease-resistant strains and produce high lipase levels for oats used to hydrolyze fats and oils. Economic analyses in the appendix show that this process will support a modest change for growing special oats.

Harvest Year

Fig 2 depicts activity of B605-1085 variety grown in Ames over sev eral years. The year 1988 was spectacularly better than other years. There was a severe drought in that year, and although the oat crop did very well, it was produced under very dry conditions. The grain was of unusually uniform color and free of marks of fungus attack. The storage conditions used were the optimum conditions for maintaining germination of oats. There may be a decline in lipase with long-term storage of oats. Lee (9) found that when the initial activity of newly produced oats were compared to the

oats stored 1 year, the former showed 40-60% higher activity than the latter. Presumably oat lipase is not completely stable under these storage conditions.

REFERENCES

1. Huang A. H. C.; Y. Lin, and S. Wang, *J. Am. Oil Chem. Soc. 65:*897 (1988).

2. Brockerhoff, H., and R. G. Jensen, 1974. *Lipolytic enzymes*, Academic Press, New York, 1974, pp. 108-129.

3. Martin, H. F. and F. G. Peers, *Biochem. J. 55:*523 (1953).

4. Ekstrand, B., I. Gangby, and G. Akesson, *Cereal Chem. 69:*379 (1992).

5. Frey, K. J., and E. G. Hammond, *J. Am. Oil Chem. Soc.52:*358 (1975).

6. Lee, I., and E. G. Hammond, *J. Am. Oil Chem. Soc. 67:*761 (1990).

7. Halstead, R. P. and H. W. Rines, H. W. *Report of the Cooperative Uniform Early and Midseason Nurseries for 1990.* ARS-USDA, St. Paul, MN (1990).

8. SAS Institute Inc. *SAS User's guide:basics.* SAS Institute Inc., Cary, NC (1991).

9. Lee, I. *Use of oat caryopses as a lipase bioreactor.* MS thesis, submitted to the Iowa State University, Ames, IA. (1989).

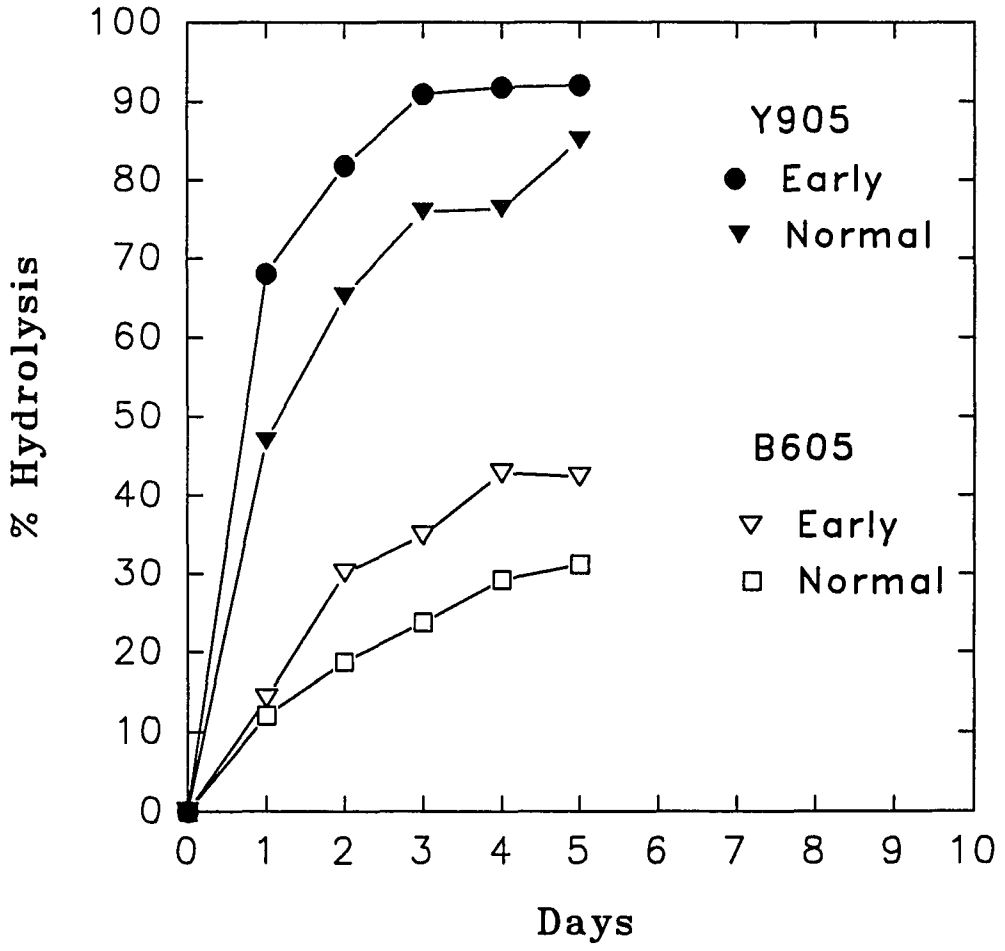

Figure 1. Effect of early and normal harvest of Y907-5-5 and B605-1050 oats on the ability of their caryopses to hydrolyze soybean oil at 40°C in an unagitated reactor

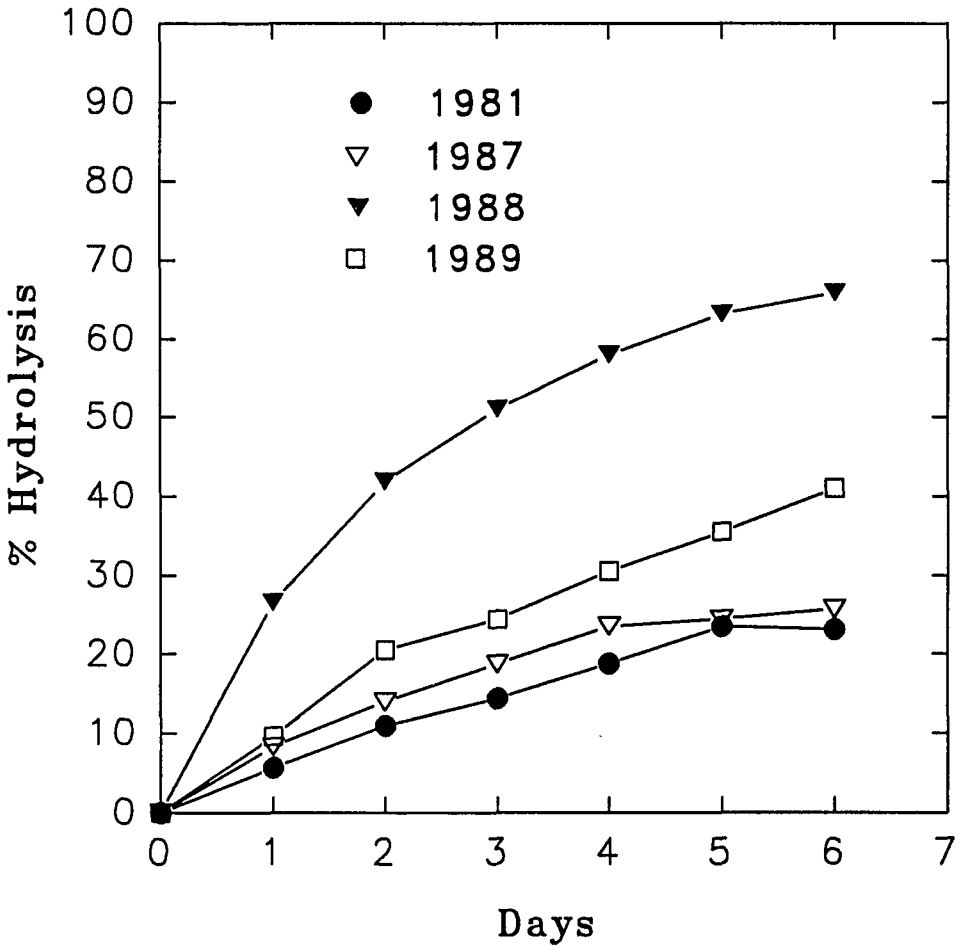

Figure 2. Effect of harvest year on the lipase activity of caryopses from B605-1050 oats at 40°C in an unagitated reactor

PAPER II.

HYDROLYSIS OF FATS AND OILS WITH MOIST OAT CARYOPSES

ABSTRACT

The hydrolysis of various fats and oils by moist oat caryopses was studied. Caryopses produced with an impact-type dehuller exhibited greater lipase activity than those produced by a wringer-type dehuller. Flow rates of 0.017 L/h/cms^2 of fat through a bed of oat caryopses was sufficient to give maximum hydrolysis rates. The activity of oat lipase at 38 and 42°C was lower than that at 40°C. Addition of 0.004% ethanolamine into the water used to moisten the caryopses, increased the lipase activity, but higher concentrations were inhibitory. Attrition in a fluidized bed of oat caryopses suspended in air partly removed the lipase. The lipase-rich particles from the fluidized bed were further fractionated by sedimentation in hexane. Such lipase concentrates could be used to speed fat hydrolysis. During the hydrolysis of soybean oil with moist oat caryopses, the free fatty acid content of the internal oat lipid increased only slightly, but there was continuing metabolism of the oats lipids during the incubation that resulted in an increase in linoleate and linolenate and a decrease in oleate and palmitate. During the hydrolysis of oil by moist oat caryopses, glycerol accumulates inside the caryopses. When the whole used caryopses were washed continuously with recirculating water, all the glycerol that

could be recovered was recovered in the wash water within the first 6 h. Hydrolysis of milkfat by moist oat caryopses resulted in preferential hydrolysis of caproic and caprylic acids. Erucic acid was released from crambe oil at significantly slower rates than the other acyl groups. Beef tallow and lard were hydrolyzed to 73% and 70% although the tallow was very near its melting point at the maximum operating temperature of 40°C. The hydrolysis of corn oil was slower than soybean oil. The inhibitory action of the diglyceride fraction of corn oil seemed to account for this effect. About 90% hydrolysis was achieved with 50 and 25% tallow dilution in hexane. Only 20% hydrolysis was achieved with neat castor oil, but dilution with equal weights of a hexane:benzene mixture resulted into 50% hydrolysis. After 1 week in a lipase bioreactor inoculated with the spores of *Clostridium sporogenes*, no growth of the spores could be detected. Estimates of the cost of producing free fatty acids with various processes using moist oat caryopses indicated that several of theses should be economically profitable.

INTRODUCTION

The current commercial process used to split fats and oils for fatty acids is known as Colgate-Emery process and uses steam hydrolysis. This process typically operates at 250°C and 50 atmospheres of pressure. These reaction conditions are energy intensive and require a large capital investment. This process typically gives 97% conversions to fatty acids and glycerol. An alternative approach to steam hydrolysis is enzymatic splitting under mild conditions (35-45°C and atmospheric pressure). The advantages associated with an enzymatic process are lower energy cost, low capital investment and improved product quality (1). To be competitive, enzymatic fat splitting must be able to produce yields comparable to or better than that of the steam process at reasonable cost.

Lipase (EC 3.1.1.3) catalyses the hydrolysis of glycerol esters to yield free fatty acids. Various plant lipases have been isolated and characterized (2). Lipases have been investigated in castor beans (3,4), rice bran (5, 6), faba beans (7), French beans (8), oats (9, 10, 11) and in a variety of bacteria, yeasts and molds (12). These lipases could be exploited for the hydrolysis, ester synthesis and interesterification reactions of fats and oils (13, 14).

Oats long have been known to be rich in lipase, and the first treatment applied to dehulled oats by oat processors typically is a steam treatment to destroy the enzyme (15). Martin and Peers (16) first demonstrated the presence of oat lipase activity on the surface of the caryopses that was capable of hydrolyzing both tributyrin and triolein. Lee and Hammond (10) explored the possibility of using the oat caryopses as a lipase reactor for hydrolysis of triglycerides and Piazza and Bilyk (9) studied the properties and substrate specificity of oat lipase.

Lee (17) standardized process parameters for a moist oat bioreactor. He found that when moist caryopses were immersed in oil, the lipase on the surface of the oats released fatty acids. The reaction slowed as the glycerol accumulated in the moist oats and fatty acids accumulated in the oil phase. The reaction was pushed to 90% completion by changing the oats after the reaction with the first lot of oats had slowed the hydrolysis. Glycerol could be extracted from the caryopses with water. Reuse of the oats, after extracting the glycerol and adjusting the moisture level resulted in significantly decreased rates of hydrolysis. The reaction ran well at 40°C.

This paper further describes studies of the hydrolysis of fats and oils by moist oat caryopses.

MATERIALS AND METHODS

Materials

Oats

Oats were procured from the Agronomy Department, Iowa State University, Ames, IA and stored at 4°C and 40% relative humidity after harvest. Generally, the oats were dehulled by an impact-type experimental dehuller (Wintersteiger Gesellschaft, m.b.h. & Co., A-4910, Reid, Austria). In studies of the effect dehuller type, a wringer-type dehuller also was used (Quaker Oats Co., Central Shop, Chicago, IL).

Fats and oils

Soybean oil (Crisco brand, Proctor & Gamble, Cincinnati, OH), corn oil (Wesson brand, Hunt-Wesson Inc., Fullerton, CA), lard, butterfat, beef tallow (all from the local market), castor oil (Aldrich Chemical Co., Milwaukee, WI) were purchased as required. The melted butter was fractionated by centrifugation into a top foam layer, a middle butter oil, and bottom serum portions. The butter oil layer was pipetted out after removing the foams. Beef tallow was prepared from beef suet. The suet was rendered by heating in boiling water. The fat phase was filtered and cooled. Crambe oil was obtained

from the Center for Crops Utilization Research, Iowa State University, Ames, IA. Tallow was procured from Feed and Energy Co., Des Moines, IA.

Experimental

Experiments in small flask

Ten g of dehulled oats were uniformly moistened with 20% water in a 50-ml conical flask and kept at room temperature for 2 h. Ten g of fats/oils was added to the moistened oats and incubated at 40°C. Samples were drawn periodically and the percentage of hydrolysis was monitored according to Lee and Hammond (10). In experiments with ethanolamine, 0.002 and 0.004 (w/w) aqueous solutions were used to moisten the caryopses.

Column experiment

The experimental apparatus consisted of a glass column (60 x 4.5 cm) which was encased in a helix of 1/4" copper tubing (Fig 1). The ends of the copper tubing were connected to a waterbath, and water was circulated to maintain the temperature of the column (40°C unless otherwise specified) throughout the experiment. The column was connected to a

peristaltic pump through norprene food-grade rubber tubing to circulate fats and oils. One-hundred fifty g of dehulled oats were uniformly moistened with 20% water and kept at room temperature for 2 h. The column was filled with moistened oats, and 150 g of fat/oil were added. The oil was circulated through the column at the rate of 0.017 L/h/cm^2 by the peristaltic pump. Periodically samples were drawn, and the percentage of hydrolysis was monitored according to Lee and Hammond (10). Tallow was diluted with hexane to 25, 50 and 75% concentrations (w/w) for circulation in the apparatus. Castor oil was diluted with a mixture of benzene:hexane (1:1, w/w) to 50% concentration (w/w).

Fatty acid analysis

Triglycerides and free fatty acids were separated on thin-layer plates of silica gel G developed in hexane/diethyl ether/acetic acid, 84:15:1, v/v/v. Fractions were detected by spraying with 0.2% 2'7'-dichlorofluorescein in 95% ethanol and by viewing under ultraviolet light. Bands were scraped off from the plates, eluted with diethyl ether and dried over a gentle stream of nitrogen. For fatty acid composition, the oils were esterified or transesterified by methanolic sulfuric acid (18) and analyzed by gas chromatography (17). Butterfat fatty acids were determined by the procedure of Vangtal and

Hammond (19). Randomization of soybean and corn oils was done as per the procedure of Lau et al. (20) using 0.5% sodium methoxide at 60°C and at a pressure of below 1 Torr for 12-16 h.

To explore the effect of minor corn oil constituents on the rate of hydrolysis, 10 g of corn oil was diluted with 10 ml hexane and fractionated on 8 g of alumina in a column, and the polar substances retained on the alumina were eluted with three 15-ml portions of distilled diethyl ether (21). The ether eluate was fractionated on thin-layer plates and the individual components were added back to 10 g of soybean oil to test their action on oat lipase.

Oat lipase enrichment

An apparatus was devised for producing a fluidized bed of oat caryopses in a glass column (76 x 6.35 cm) as shown in Fig 2. Compressed air (6.12 kg/cm^2 pressure) was passed into the bottom of the column which was filled with 100 g of oat caryopses. The top of the column was connected to a polyvinyl chloride (PVC) elbow and pipe. The pipe was cut into two parts and a cheese cloth filter (25 mesh) in two layers supported by a 40 mesh wire gauge was inserted in between the two parts to trap the surface debris from the oat caryopses. The exhaust outlet of the pipe was connected to an

air-flow meter (Meriam Instruments, Cleveland, OH).

The "fines" that were collected on the cheese-cloth filter, were recovered by dismantling the two halves of the pipe. The dry scrubbed oats and larger debris, which remained in the column, were separated by hand sieving (sieve #16, mesh size 14, Fisher Scientific Co., Pittsburgh, PA). Large pieces of caryopses which were not sieved were removed from the intact caryopses and termed "bran". The fines were further enriched by fractionating them in hexane. To 250 ml of hexane in a 250-ml cylinder, 5 g of fines were added and allowed to settle under gravity for 30 sec. The supernatant was decanted and hexane was evaporated in a rotary evaporator. Thus, the fines were fractionated into finest fines (supernatant) and coarse fines (bottom of the cylinder) fractions. Dry scrubbed oats, bran and fines, and finest fines were tested for their lipolytic activities on soybean oil by the method of Lee and Hammond (10). Bran and fines were incorporated at 1 g per 10 g of boiled caryopses in which the native oat lipase had been inactivated by boiling the caryopses in water for 6 min. The boiled caryopses were cooled and dried overnight at 60°C. Finest fines were incorporated at 10% of the caryopses weight in the apparatus in Fig 1 to test their effect on the hydrolysis of soybean oil.

Glycerol recovery

After hydrolysis of soybean oil in the column shown in Fig 1, the oil was drained and the caryopses were washed with 250 ml of hexane to remove the residual oil. Next, the caryopses were washed with 250 ml of water circulating at 0.29 L/h/cm^2 for 12 h. Every hour, a water sample was drawn and the glycerol content of the wash water was estimated by the method of Molever (22).

Fate of the native oat lipids

An experiment was conducted to elucidate the fate of the native lipids of caryopses during hydrolysis of external oils. In a series of 50-ml conical flasks, 10 g of oats was moistened with 20% water and 10 g of soybean oil was added. As a control, instead of soybean oil, 10 g of mineral oil was added. All the samples were incubated at 40°C. At the end of 0, 2, 4, 6 and 8 days, the oil and mineral oil were drained and the caryopses were washed with hexane to remove the oils from the surface. The native oat lipids were extracted as per the Folch procedure (23), and the fatty acids were transesterified (18) and estimated by gas chromatography (17). The free fatty acids in the oat lipids were estimated by the method of Lee and Hammond (10).

Safety evaluation of oats used in column

Oats were inoculated at 1000 spores/g with *Clostridium sporogenes (PA 3679)* per gram of oat by making a suspension of the spore in the water used to moisten the oat caryopses. The wet caryopses (100 g) moistened with 20% water were transferred to a glass column (53 x 4.5 cm) and 100 g of soybean oil was added in the ratio of 1:1 (w/w) with caryopses. The column was kept at room temperature and after 1 wk the oil was drained. One gram of wet oily oat caryopses was ground in a mortar and serial dilutions were made in sterile distilled water. Similarly, 1 ml of oil suspension was also diluted serially. The oats and oil suspensions were plated for the growth of spores on bacto brewer anaerobic agar (24) by incubating at 37°C for 48 h.

RESULTS AND DISCUSSION

Effect of dehullers type

There was a significant difference in the lipase activity of caryopses according to the methods used to dehull them (Fig 3). One dehuller was a wringer type in which the caryopses was squeezed out of the glume as it moved between two rollers. The second dehuller was an impact type in which a rotating fan threw the oats against a wall. There was at least a 20% difference in the percentage hydrolysis achieved by caryopses obtained with the two dehullers. Probably the wringer-type of dehuller rubs more of the surface lipase off the caryopses and onto the glume. In using the impact-type dehuller, we found that the lipase activity was increased slightly by drying the oats over phosphorus pentoxide before dehulling.

Effect pumping rate

Corn oil was pumped through a bed of oat caryopses as shown in Fig 1. The temperature was 40°C and pumping rate of 1.2, 3.6, 6.2 ml of oil/min were compared. The results were not significantly different, although slightly less hydrolysis occurred at lowest speed. In subsequent experiments, a flow rate of 3.6 ml/min was used. This is 0.017 L/h/cm^2.

Effect of temperature

All enzymes are proteins and have temperature optima. Lee and Hammond (10) studied the hydrolysis of soybean oil by moist oat caryopses at 30, 40, 50 and 60°C and reported that 40°C was the optimum. To determine the optimum temperature exactly, caryopses in the apparatus shown in Fig 1 were used to hydrolyze soybean oil at three temperatures, i.e. 38, 40 and 42°C. Fig 4 shows that the activity of the lipase at 38 and 42°C was less than at 40°C. Such temperature sensitivity is a disadvantage for hydrolysis of fats like tallow and lard which may not be completely liquid at 40°C.

Effect of ethanolamine

Lee (17) reported that alkaline conditions stimulated the oat lipase, but in general the rate of hydrolysis was depressed by the addition of buffer salts to the caryopses. Several amines have been reported to increase the activity of lipase-catalyzed triglyceride hydrolysis (25). We incorporated 0.004 and 0.002% ethanolamine in the water used for moistening the oats. Addition of 0.004% ethanolamine increased the activity compared to the control whereas 0.002% ethanolamine was not effective in improving the enzyme activity (Fig 5). Greater concentrations of ethanolamine than 0.004% inhibited the enzyme reaction.

Effect of added oat lipase

Lipase has been described as being located on the surface of the caryopses (11, 16, 26). The outer surface of the oat caryopses was abraded by rubbing caryopses against one another in a fluidized bed (Fig 2). Table 1 shows the effect of different air fluxes for 1 h on the recovery (grams) of fines, bran and dry scrubbed oats. An air flux of 0.067 L/sec/cm^2 was the minimum to create a fluidized bed, but the recovery of fines was very low. An air flux greater than 0.100 L/sec/cm^2 transported the oats out of the fluidized bed (Fig 2). An air flux of 0.086 L/sec/cm^2 was considered the optimum for the fluidized bed.

Table 1. Effect of different air fluxes for 1 h on the recovery(grams) of fines, bran and dry scrubbed oats

Air flux (L/sec/cm^2)	Dry scrubbed oats	Bran (g)	Fines (g)	Loss (g)
0.067	96.4	0.9	2.3	0.4
0.086	86.2	4.0	8.4	1.4
0.100	77.7	6.7	13.3	2.3

Table 2 shows that as the residence time increased, there is an increase in recovery of fines and bran, but the recovery of dry scrubbed oat decreased. Subsequently, the total loss of the material was also increased.

The oats obtained at each residence time were tested for their lipolytic activities. Fig 6 shows the effects of such processing on the lipolytic activity of the caryopses. As the residence time in the fluidized bed increased, the hydrolytic potency of the dry scrubbed oats decreased. As abrasion of oats in a fluidized bed is prolonged, more enzyme is removed from the surface of the oat caryopses.

Table 2. Effect of various time periods on the recovery (g) of fines, bran and dry scrubbed oats in a fluidized bed at an air flux of 0.086 L/sec/cm^2

Time(h)	Dry scrubbed oats (g)	Bran (g)	Fines (g)	Loss (g)
0.50	91.2	3.3	4.3	1.2
1.00	84.8	4.8	8.8	1.6
1.50	73.9	5.2	16.4	4.5

Fines and bran were incorporated into boiled caryopses to check their lipase activities (Fig 7). The result of their activities was: fines 0.5 h > fines 1.0 h > fines 1.5 h > bran. The lipase activity of these fines released 1500 μmoles of fatty acids/h/mg solids which was comparable to that of *Candida cylindrace* type VII lipase with 30% lactose as an extender (Sigma Chemical Co., St. Louis, MO). The bran had little activity. The lipase activity of the fines decreased with residence time in the fluidized bed, probably because the prolonged abrasion resulted in the removal of inactive material from the oat surface which accumulated in the fines. Residence time of 1 h and air flux of 0.086 L/sec/cm^2 were appropriate for a good fines preparation.

Thus, making a lipase concentrate by such a fluidized bed is an attractive process. The oat caryopses could be scalped for lipase and then processed as caryopses usually are, thereby increasing the economic value of oats, or when oat caryopses are transported in manufacturing facilities, the debris generated in such movement could be harvested for lipase. Lipase generated in this way also could be added to an oat bioreactor to increase rate and degree of conversion of fats and oils to improve the economics of this process.

Fate of native oat lipids

Oats are unique among the cereals for their high lipid content. An extensive survey of lipid contents among oat genotypes found a range in lipid concentration of 3.1-11.6% (27). The fate of the native oat lipids in the hydrolysis bioreactors described here is not documented. Fig 8 shows the fatty acyl composition of oats lipids after incubating wet caryopses in soybean oil at 40°C. There was not much change in stearate. There were an increases in linoleate and linolenate and a decrease in oleate and palmitate. A similar trend was also observed in the case of caryopses incubated in mineral oil (Fig 9). Mineral oil was used in control samples because it is a petrobase product containing a carbon chain length of C_{10}-C_{16}; therefore, it cannot be hydrolyzed or interesterified by fat splitting enzymes. The decrease in oleate was less than in caryopses incubated in soybean oil, and the increase in linolenate was less prominent. Thus, there was a continuous metabolism of the moist oats immersed in oil and, possibly, some transfer of acyl groups from the external oil during the hydrolysis process. The total oil contents and free fatty acid content of oat lipids also increased slightly with incubation time, and this change was greater in caryopses immersed in soybean oil than in mineral oil (Table 3).

Table 3. Percentage oil recovery and free fatty acid content of native oat caryopses incubated at 40°C in soybean and mineral oils for various time periods.

	Mineral oil		Soybean oil	
	% oil	% ffa	% oil	% ffa
Control	5.44	2.18	5.44	2.18
2 days	5.60	2.30	6.27	5.87
4 days	5.54	2.78	6.66	6.42
6 days	5.62	3.99	6.89	7.10
8 days	6.30	4.02	7.04	8.39

Glycerol recovery

The glycerol released during fat hydrolysis is a valuable by-product. In the Colgate-Emery process, the sweetwater stream contains nearly 12% glycerol (1). Fig 10 shows the extraction of glycerol from whole oats with water after 83% hydrolysis of soybean oil in the lipase bioreactor (Fig 1). About 66% of the glycerol was recovered in the wash water within first the 6 h, after which it remained constant. Lee and Hammond (10) reported that over 90% of the released glycerol was accumulated inside the caryopses and the rest was in the oil phase. Although there was 83% hydrolysis, some of the glycerol may not have been released and be present as mono- and diglycerides. Also the water in the caryopses

probably still contained glycerol in equilibrium with the external wash water.

Effect of substrate on hydrolysis rate

The application of this hydrolysis technique to several fats and oils is shown in Fig 11. Butterfat contains short-chain, water-soluble fatty acids, such as butyric and caprylic, which are very important in food flavor (28). With caryopses in unstirred flasks, we achieved about 50% hydrolysis. Analysis of released fatty acids of butterfat revealed that medium chain fatty acids were released at faster rate that other fatty acids (Table 4). After 4 days, caproic and caprylic acids were released in the greatest amount relative to their concentrations in the fat.

Crambe oil contains about 60% erucic acid which is an important industrial fatty acid (29). When we subjected crambe oil to the hydrolysis procedure, there was 65% hydrolysis in 4 days. Analysis of released fatty acids showed that erucic and linolenic acids were released at slightly slower rates than other acyl groups (Table 5).

Table 4. Methyl esters of fatty acids (%) of milkfat free fatty acids as hydrolyzed by the oat lipase for various time at 40°C

	Period						
Methyl esters	Original TG	30 sec	10 hr	1 day	2 day	3 day	4 day
butyrate	7.44	6.45	5.20	4.22	6.22	7.67	7.43
caproate	3.41	2.46	2.38	2.40	3.30	6.89	6.86
caprylate	1.66	5.16	3.78	3.51	3.56	6.18	6.26
caprate	4.61	4.76	4.91	5.18	5.08	6.50	6.25
laurate	3.58	4.12	4.19	4.94	4.16	4.36	4.16
myristate	11.66	15.37	15.37	13.16	12.72	11.91	11.29
palmitate	28.01	28.15	28.75	25.24	25.56	17.82	18.90
stearate	12.09	10.31	11.77	12.97	13.00	11.49	11.27
Oleate	27.54	23.23	23.65	28.38	26.40	27.18	27.59

Beef tallow (isolated from suet) and lard yielded 73 and 70% hydrolysis in four days respectively (Fig 11). This degree of hydrolysis is similar to that achieved for soybean oil although at 40°C the lard and tallow were very near their melting points.

Table 5. Composition of the free fatty acids freleased from crambe oil by wet oat caryopses at 40°C in an unagitated system

	Fatty acids %						
Day	C16:0	C18:0	C18:1	C18:2	C18:3	C:20:0	C22:1
0 TG	3.09	1.35	15.90	9.67	5.77	2.43	61.79
2	4.93	2.56	17.93	10.64	4.95	2.55	56.44
4	4.33	1.61	18.21	12.29	4.73	2.63	56.20

Corn oil vs soybean oil

Fig 12 shows hydrolysis of soybean and corn oils in the oat lipase bioreactor shown in Fig 1. For soybean oil, we were able to achieve 85% hydrolysis in 6 days but only 70% hydrolysis was achieved with corn oil over the same time period. This hydrolysis was done with good quality oats free from rust and fungus attack. Thus, the hydrolysis of corn oil was slower than that of soybean oil although the oils have similar acyl group compositions. To understand this phenomenon, both the oils were randomized with sodium

methoxide and it was observed that still the hydrolysis of randomized corn oil proceeded at a slower rate than that of randomized soybean oil. Thus, the effect cannot be attributed to glyceride structure. It seemed possible that some minor polar components present in corn oil might have an inhibitory effect. To explore this hypothesis the corn oil was passed through an alumina column using a technique for the purification of triglycerides (21). The alumina retains the polar materials such as phospholipids, diglycerides and sterols which after eluting with diethyl ether were separated on thin-layer plates. The yield of the corn oil polar material was 12.43%. The polar fraction and its individual components were added back to an appropriate amount of soybean oil and the hydrolysis of the soybean oil was tested. The unfractionated polar materials had an inhibitory action on the soybean oil hydrolysis. When individual components of the polar fractions were tested, the suppressive action of diglycerides was much stronger than that of sterols or triglycerides (Fig 13). The addition of 500 mg of 1,3-dilinolein to 10 g of soybean oil also inhibited the reaction, but seemingly not as much as the corn oil diglycerides. The fatty acid composition of the corn oil diglyceride fraction is shown in Table 6.

Table 6. Fatty acids composition of corn oil and diglyceride fraction from corn oil

Fatty acyl	Whole corn oil (%)	Diglyceride (%)
Palmitate	11.78	8.07
Stearate	1.69	1.51
Oleate	25.72	25.72
Linolate	60.21	63.22
Linolenate	1.22	1.48

Attempts to achieve higher conversions

Enrichment with lipase For oat bioreactors to be an attractive method of hydrolysis, the reaction must be essentially complete. The problem associated with such a system is that the inhibition of reaction by the end products formed, i.e. glycerol and free fatty acids. It is also important to reduce the time required for this process. One way to deal with this is to enrich the oat bioreactor with external oat lipase. The surface debris rich in lipase obtained by making a fluidized bed of oats (Fig 2) when added at 10% (w/w) to the oat column bioreactor (Fig 1), about 80% hydrolysis of corn oil was achieved (Fig 14) in 5 days. Without the added lipase the hydrolysis reached only 50% with B605-1085 oat caryopses, which are an average quality of oats

as far as lipase content is concerned. When fines from the fluidized bed were further enriched by sedimentation in hexane, and 10% of these finest fines were added to caryopses of B605-1085 year 1988 (which had higher than usual lipase activity), 96% hydrolysis of soybean oil was achieved in 5 days (Fig 15).

Dilution by solvents Another way to achieve high conversion in an oat bioreactor is to dilute the substrate rather than to increase the lipase activity. This results in lower concentration of inhibitory end products. Fig 16 shows the hydrolysis of a tallow sample diluted with various proportions of hexane. About 90% hydrolysis was achieved with 50 and 25% tallow in hexane. This sort of system has the advantage that the oats can be rinsed free of oil with hexane at the end of the experiment. The disadvantage is that the hexane would have to be purged from the oats before they could be used in food or feed. Also the dilution would require greater reactor capacity and a facility using hexane would be more expensive to construct in compliance with safety regulations.

In case of castor oil, no more than 20% hydrolysis was achieved (Fig 17). Vignolo and Naughton (30) reported that about 89-90% of the fatty acids of castor oil is ricinoleic

acid (12-hydroxyoleic acid). Upon treatment with lipase a linear polymer can form as the acidic groups esterify with the hydroxy groups on ricinoleic acid. This seemed to have happened when pure castor oil was used as the substrate because the oil in the reactor became very viscous. To increase the percentage of hydrolysis of castor oil, it was diluted with equal weights of hexane:benzene (1:1,w/w) mixture. This resulted in 50% hydrolysis when carried out in the column shown in Fig 1.

Economic analysis

The calculations detailed in the appendix indicate that several variations of the process might be profitable if moist oats can be used in mixed feeds without drying (Table 7). Use of multiple batches of ordinary oats or use of oats bred for high lipase activity appear feasible. Scalping oats for lipase is very attractive process, both for hydrolysis of fats and oil and lipase concentrate production. However, the dilution of oils with hexane is not feasible under present consideration because of the higher cost of equipment. Glycerol recovery from spent caryopses does not look promising.

Table 7. Economic analysis

Process	Profit/year ($)	Return on investment (%)
Three-stage hydrolysis with ordinary oats	192,026.00	68.04
High-lipse oats	135,603.00	134.42
Fines and high-lipase oats	186,731.00	152.68
Dilution with hexane	-672,729.00	-190.54
Lipase concentrate production	155,867,405.00	708,488.00
Glycerol recovery	-1,157.00	-0.15

Safety evaluation of oats

It is a concern that the moist oats immersed in oil might become anaerobic enough to support the growth of *Clostridium botulinum*. If this happened the oats would not be suitable for use as food or feed. To test this the oats in an oat lipase bioreactor were inoculated with spores of *Clostridium sporogenes*, a nontoxin-producing *Clostridium* with growth characteristics like that of *Clostridium botulinum*. After 1

wk in the bioreactor, the oats were tested and no growth of *Clostridium sporogenes* could be detected. Therefore, it is tentatively concluded that no toxin would be produced under the experimental conditions used.

Conclusion

The use of moist oat to hydrolyze fats and oils is an attractive process that has potential to compete with current methods of hydrolysis. Improvement in the rate and extent of conversion of such reactors has been demonstrated. There is a large commercial market for lipase. Currently, lipases represent about 3% of all enzymes used in industry (31). Increased costs of energy obtained from fossil fuels and increased demand for higher quality products coupled with very narrow purity specifications are likely to lead to new incentives for the biochemical transformation of fats and oils. Hence, such lipase bioreactor offers considerable potential for future development.

REFERENCES

1. Brady, C., L. Metcalfe, D. Slaboszewski, and D. Frank, *J. Am. Oil Chem. Soc. 65*:917 (1988).

2. Desnuelle, P. and P. Savary, *J lipid Res., 4*:369 (1963).

3. Ory, R. L., A. J. Angelo, and A. M. Altschul, *J. Lipid Res., 1*:208 (1960).

4. Ory, R. L., A. J. Angelo, and A. M. Altschul, *J. Lipid Res., 3*:99 (1962).

5. Aizono, Y.; Funatsu, M.; Sugano, M.; Hyashi, K. & Fujiki Y. *Agric. Biol. Chem. 37*:2031 (1973).

6. Fujiki, Y.; Y. Aizono, and M. Funatsu, *Agric. Biol. Chem., 42*:2401 (1978).

7. Dundas, D. G. A., H. M. Henderson and N. A. Eskin, *Food Chem., 3*:171 (1978).

8. Kermasha, S.; F. R. van de Voort, and M. Metche, *Can. Inst. Food Sci. Technol. 19* :23 (1986).

9. Piazza G. J. and A. Bilyk, *J. Am. Oil Chem. Soc., 66*:489 (1989).

10. Lee, I. and E. G. Hammond, *J. Am. Oil Chem. Soc. 67*:761 (1990).

11. Ekstrand, B., I. Gangby and G. Akesson, *Cereal Chem., 69*:379 (1992).

12. Severina, L. O., and N. A. Bashkatova, *Appl. Biochem. Microbiol., 16*:54 (1980).

13. Werdelmann, B. W., *Fette Seifen Anstrichm. 76*:1 (1974).

14. Malcata, F. X., H. R. Reyes, H. S. Garcia, C. G. Hill, and C. H. Amundson, *J. Am. Oil Chem. Soc. 67*:890 (1990).

15. Hammond, E. G. in *Lipids in Cereal Technology,* edited by P. Barnes, Academic Press, New York, 1983, P. 331.

16. Martin, H. F. and F. G. Peers, *Biochem. J. 55*:523 (1953).

17. Lee, I., *Use of oat caryopses as a lipase reactor.* MS thesis, submitted to the Iowa State University, Ames, IA, 1989.

18. Christie, W. W. Lipid analysis, Pergamon Press, New York, P. 82 (1982).

19. Vangtal, A. and E. G. Hammond, *J. Dairy Sci. 69*:2982 (1986).

20. Lau, F. Y., E. G. Hammond and P. F. Ross, *J. Am. Oil Chem. Soc. 59*:407 (1982).

21. Jensen, R. G., T. A. Marks, J. Sampugna, J. G. Quinn and D. L. Carpenter, *Lipids 1*:451 (1966).

22. Molever, K., *J. Am. Oil Chem. Soc. 64*:1356 (1987).

23. Folch, J. M., M. Lees and G. H. S. Sloane-Stanley, *J. Biol. Chem. 226*:497 (1957).

24. DIFCO Manual, Tenth Edition, DIFCO Labs, Detroit, MI, P. 165 (1986).

25. Bilyk, A., R. G. Bistline, M. J. Haas and S. H. Feairheller., *J. Am. Oil Chem. 68*:320 (1991).

26. Hutchinson, J. G., H. F. Martin and T. Moran, *Nature (London) 167*:758 (1951).

27. Brown, C. M. and D. J. Craddock, *Crop Sci. 12*:514 (1972).

28. Malcata, F. X., C. G. Charles, Jr. and C. H. Amundson, *Biotechnol. Bioeng. 38*:853 (1991).

29. Anonymous, *INFORM 2*:678 (1991).

30. Vignolo, R. and F. Naughton, *INFORM 2*:692 (1991).

31. Posrske, L. H., G. K. LeFebvre, C. A. Miller, T. T. Hansen, and B. L. Glenvig, *J. Am. Oil Chem. Soc. 65*:922 (1988).

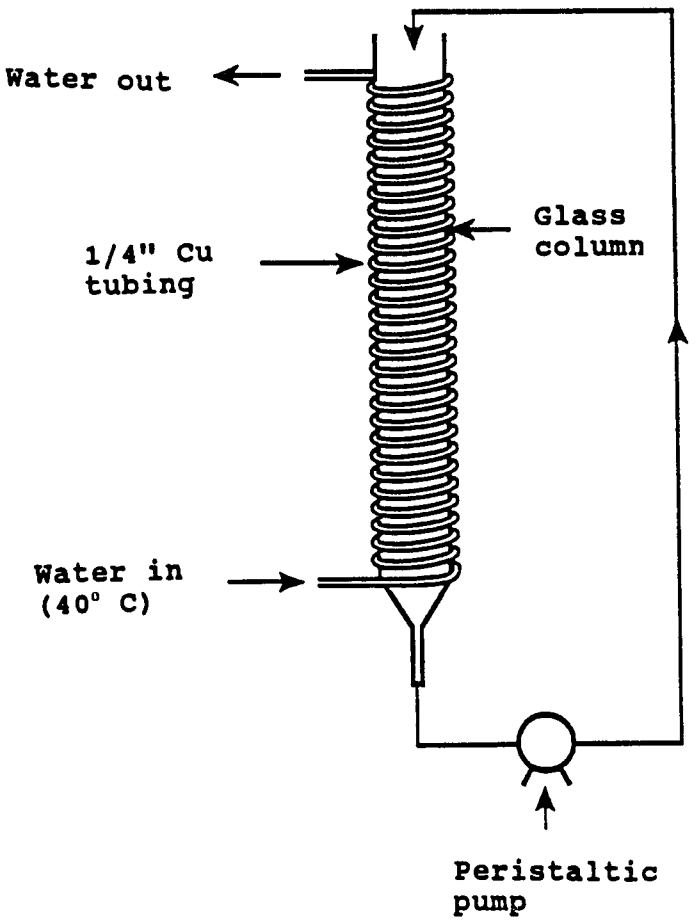

Figure 1. Schematic diagram of the experimental set up for circulating oil through a column of caryopses

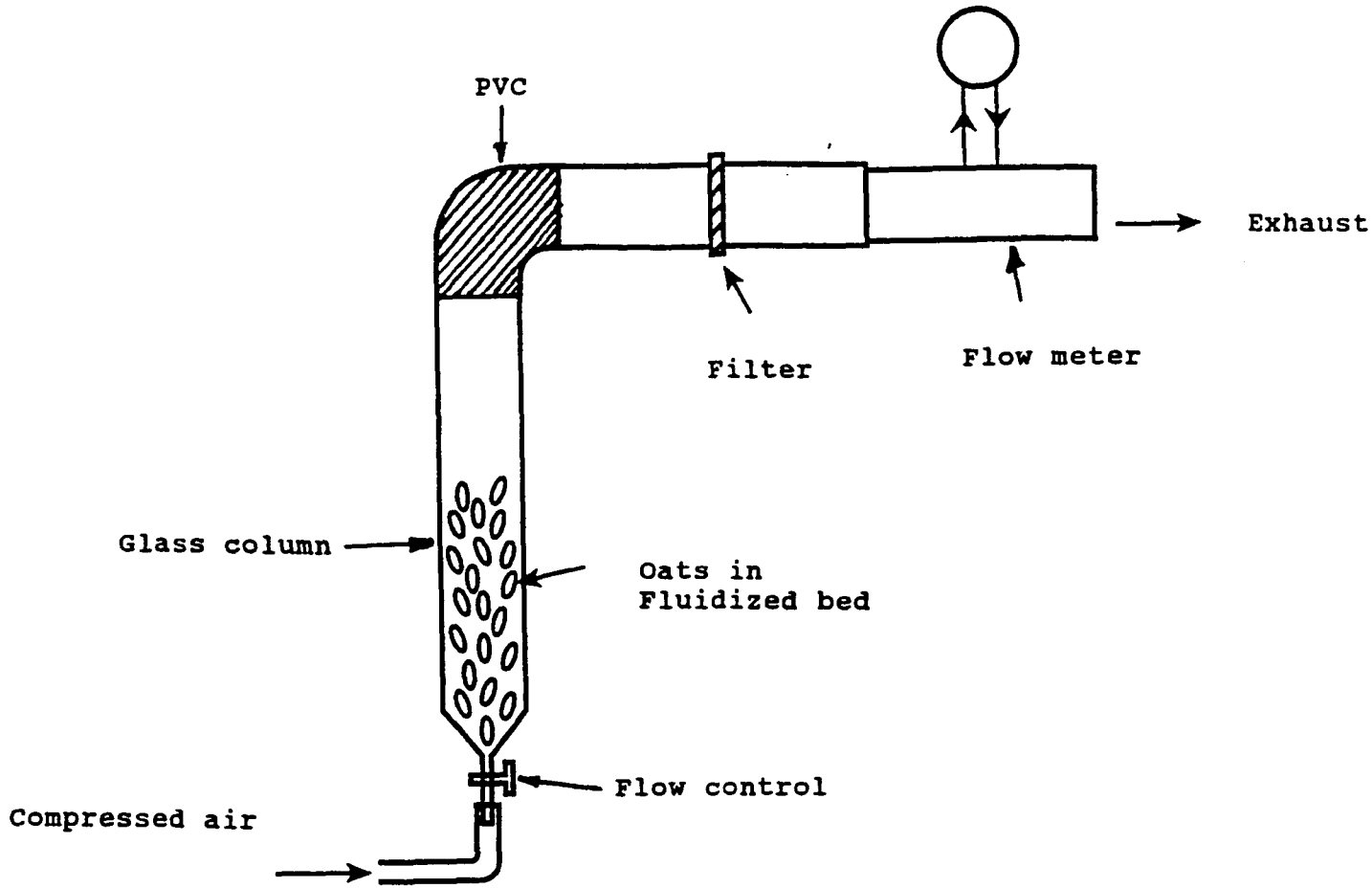

Figure 2. Schematic diagram of the fluidized bed of caryopses

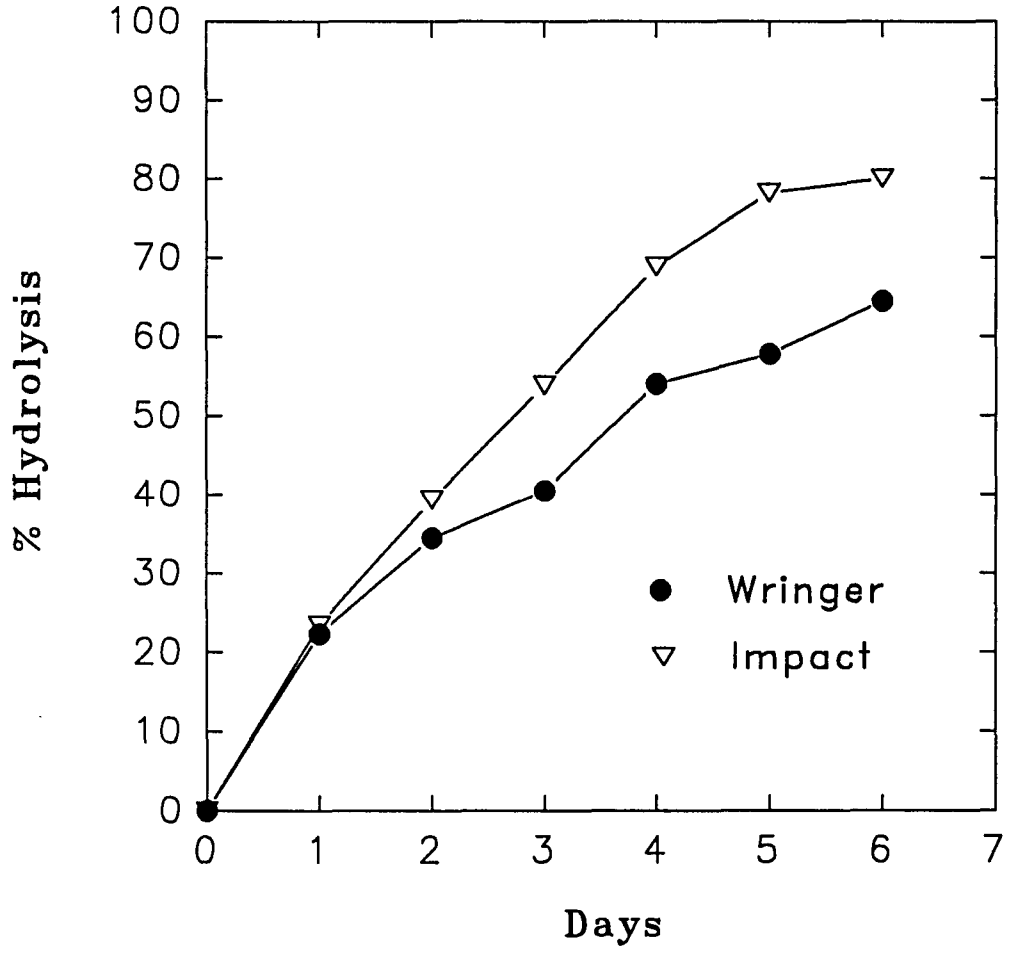

Figure 3. Comparison of the effect of dehuller type on the lipase activity of caryopses from B605-1050 oats at 40°C in an unagitated reactor

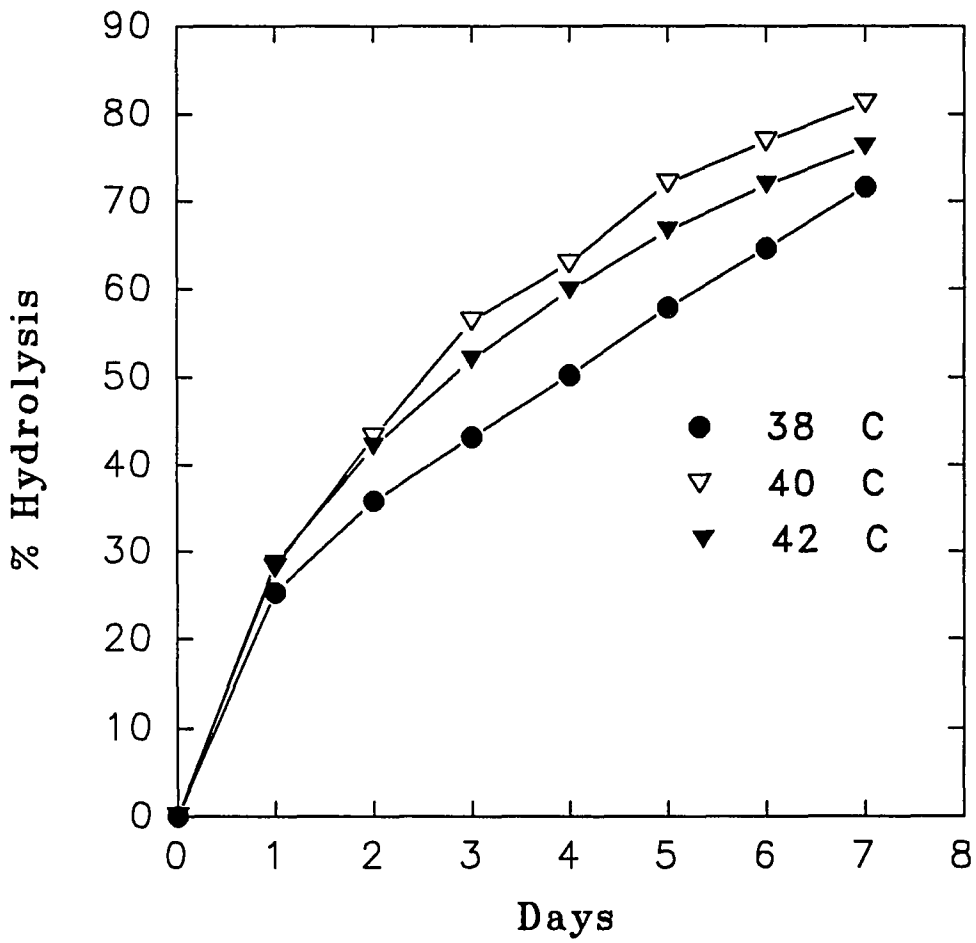

Figure 4. Hydrolysis of soybean oil by caryopses from B605-1050 oats at various temperatures while being circulated through a column at 0.017 L/h/cm^2

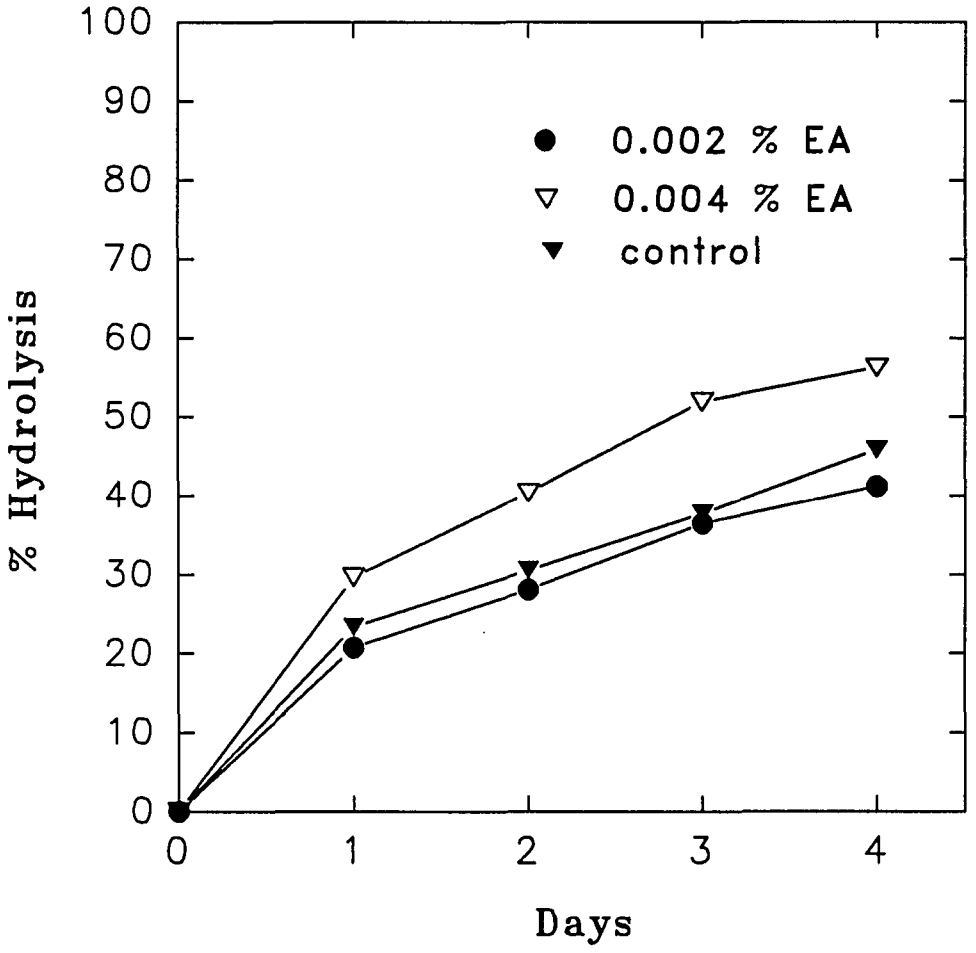

Figure 5. Effect of ethanolamine added to the aqueous phase on the lipase activity of the caryopses from B605-1050 oats at 40°C in an unagitated reactor

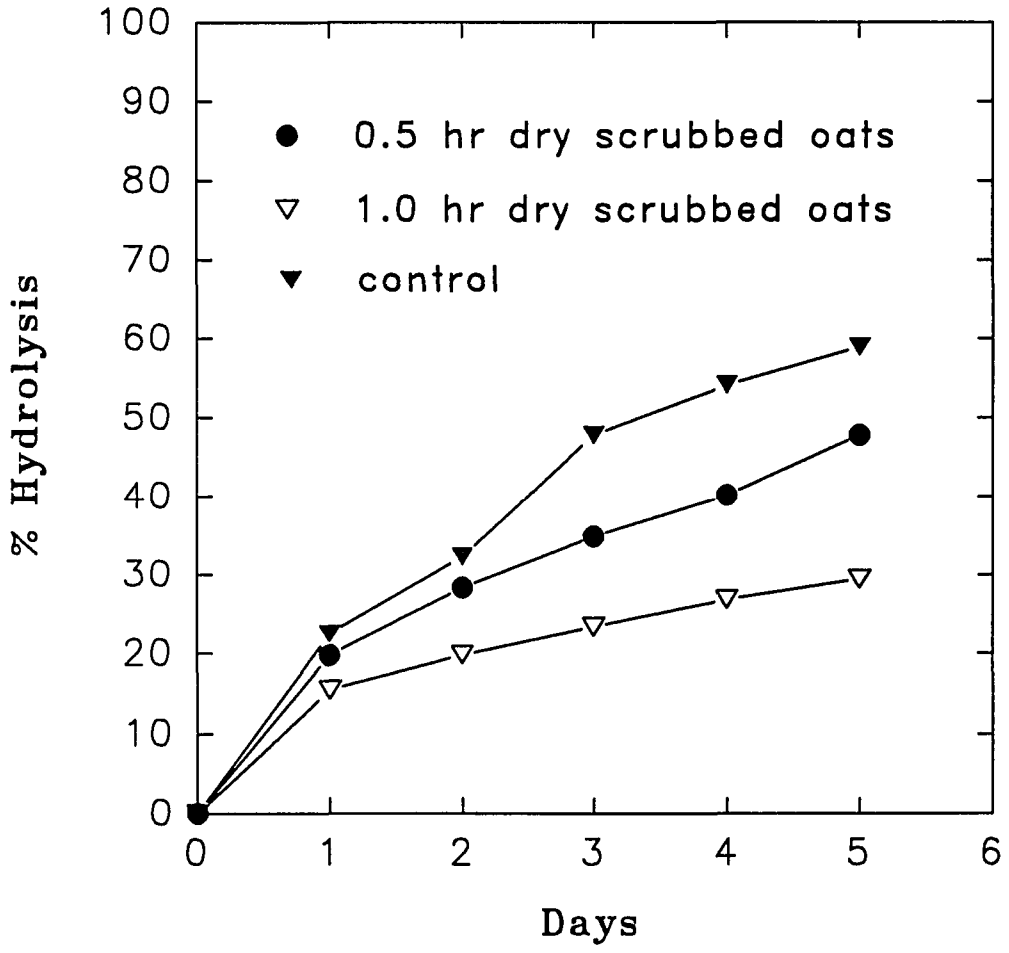

Figure 6. Lipase activity of caryopses at 40°C in an unagitated reactor. Caryopses were from B605-1050 oats subjected to a fluidized bed for various times

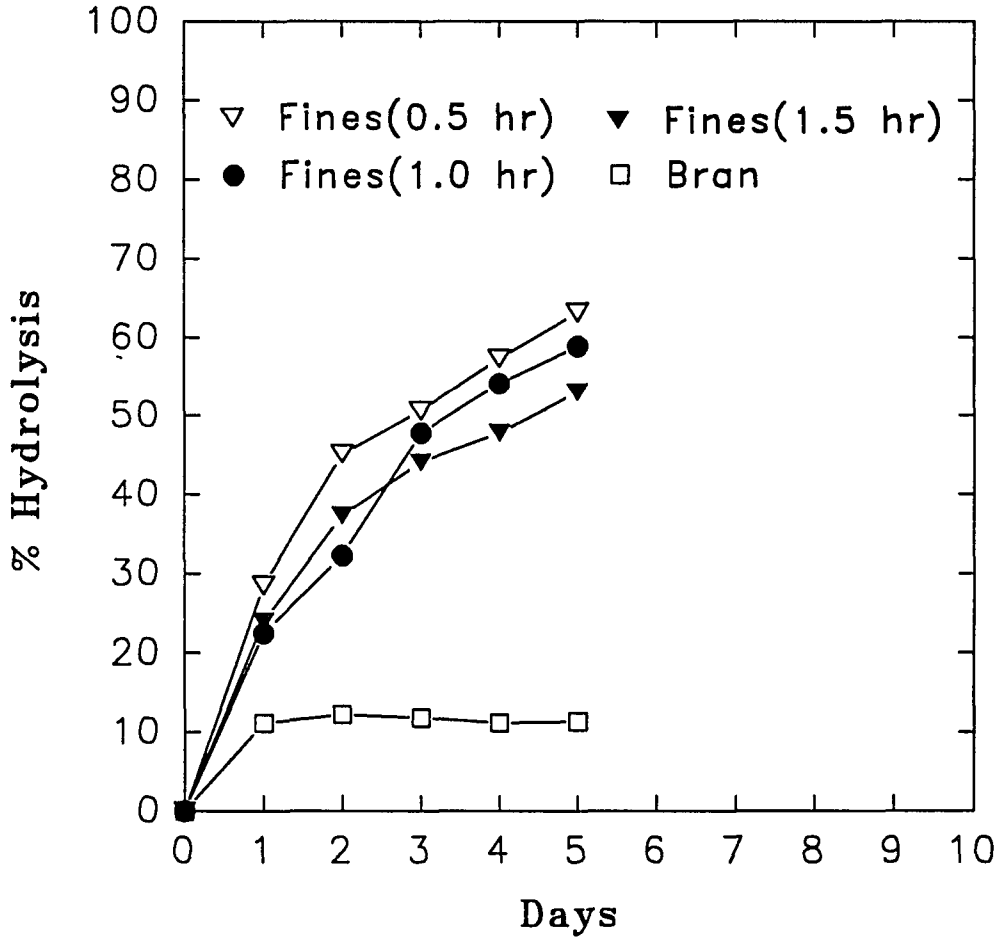

Figure 7. Effect of incorporation of 10% by weight of fines and bran on the lipase activity of boiled caryopses from B605-1050 oats at 40°C in an unagitated reactor

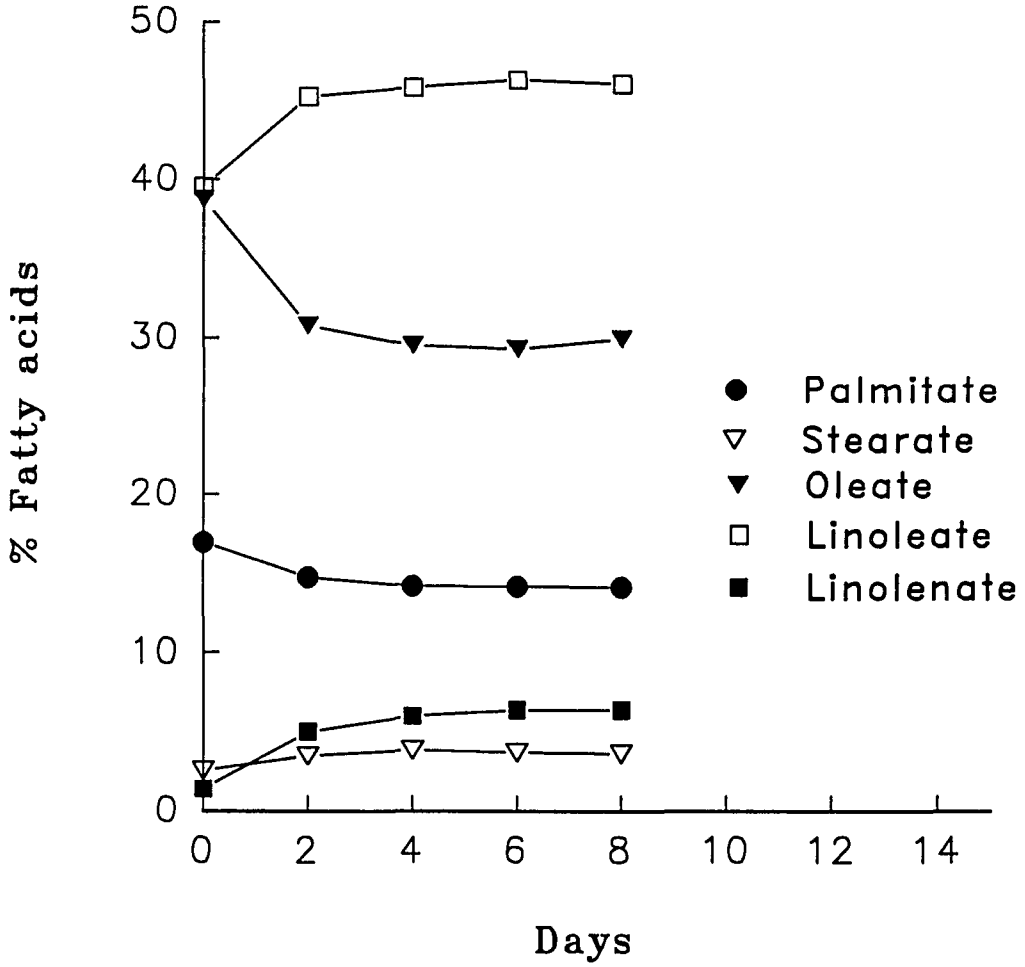

Figure 8. Fatty acid composition of B605-1050 oat lipids after incubating wet oats in soybean oil at 40°C in an unagitated reactor

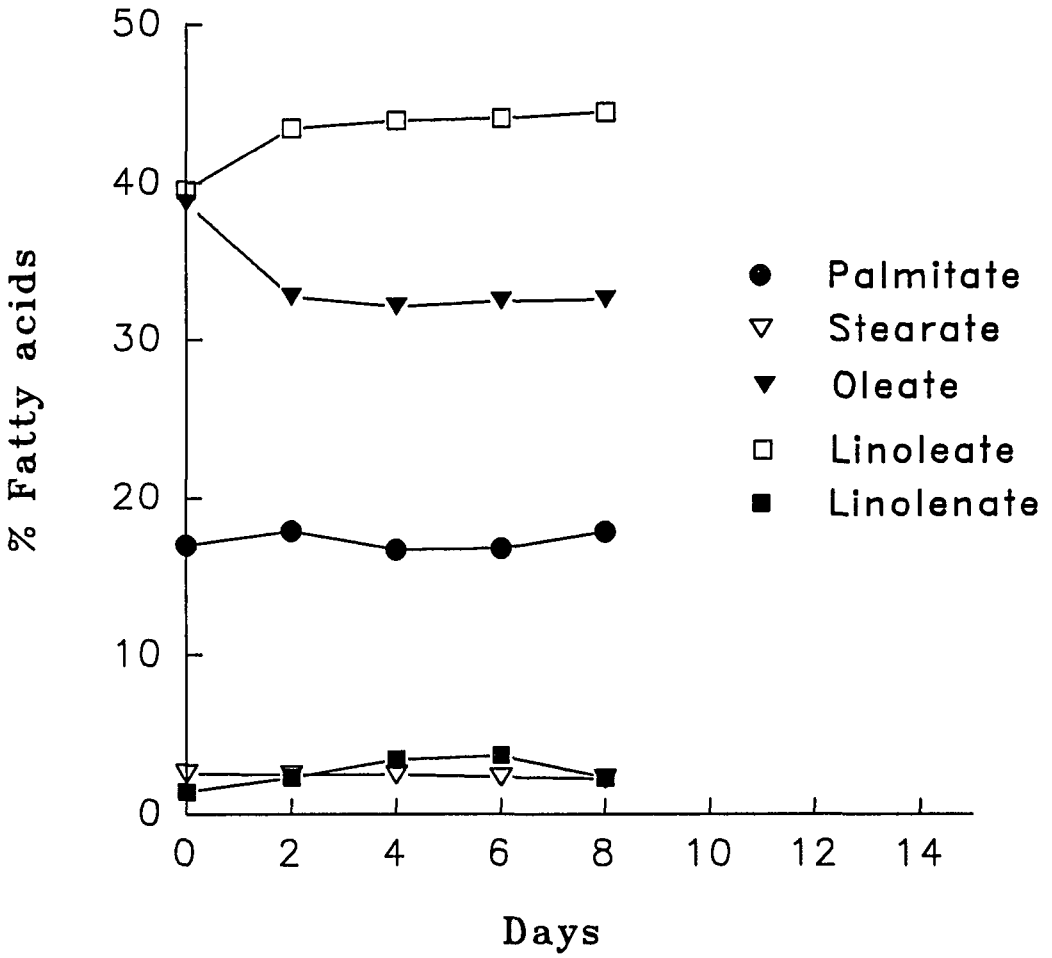

Figure 9. Fatty acid composition of B605-1050 oat lipids after incubating wet oats in mineral oil at 40°C in unagitated reactor

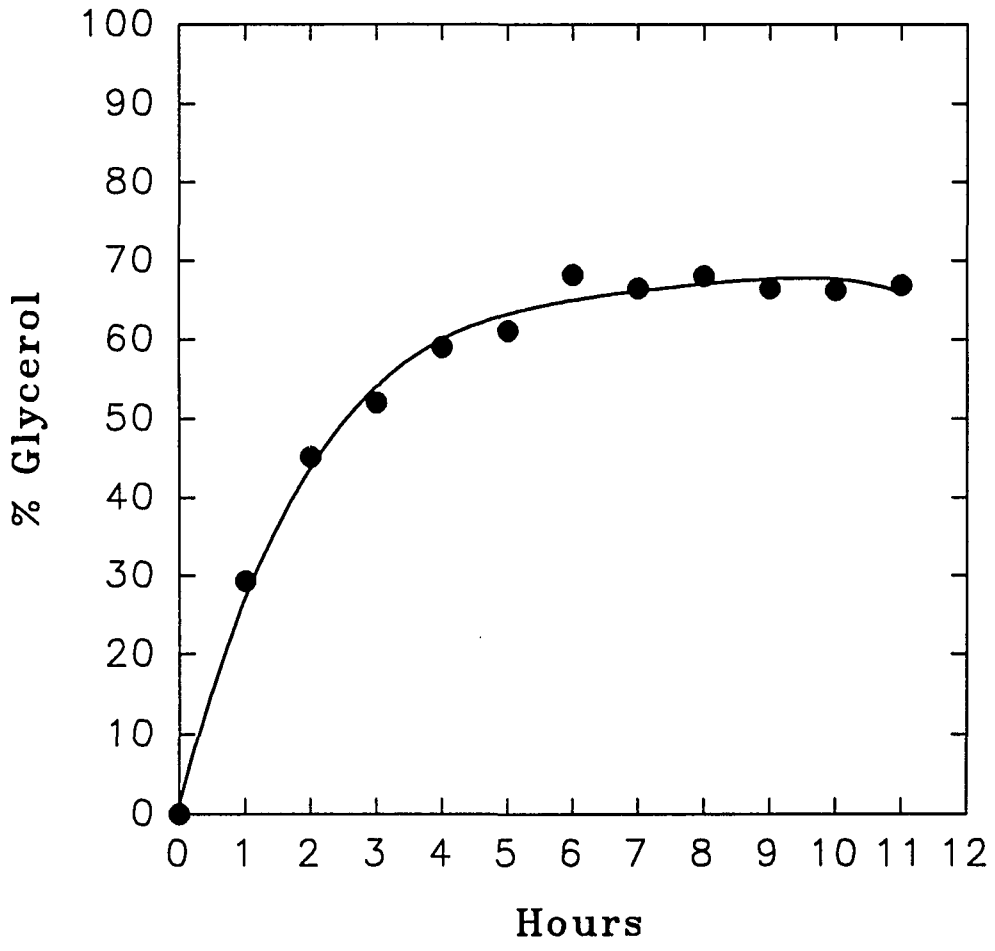

Figure 10. Percentage recovery of glycerol assuming 100% hydrolysis and release of glycerol from caryopses oats after hydrolysis of soybean oil (150 g) with 150 g of caryopses (B605-1050) for 4 days. 82.86% hydrolysis of soybean oil was achieved.

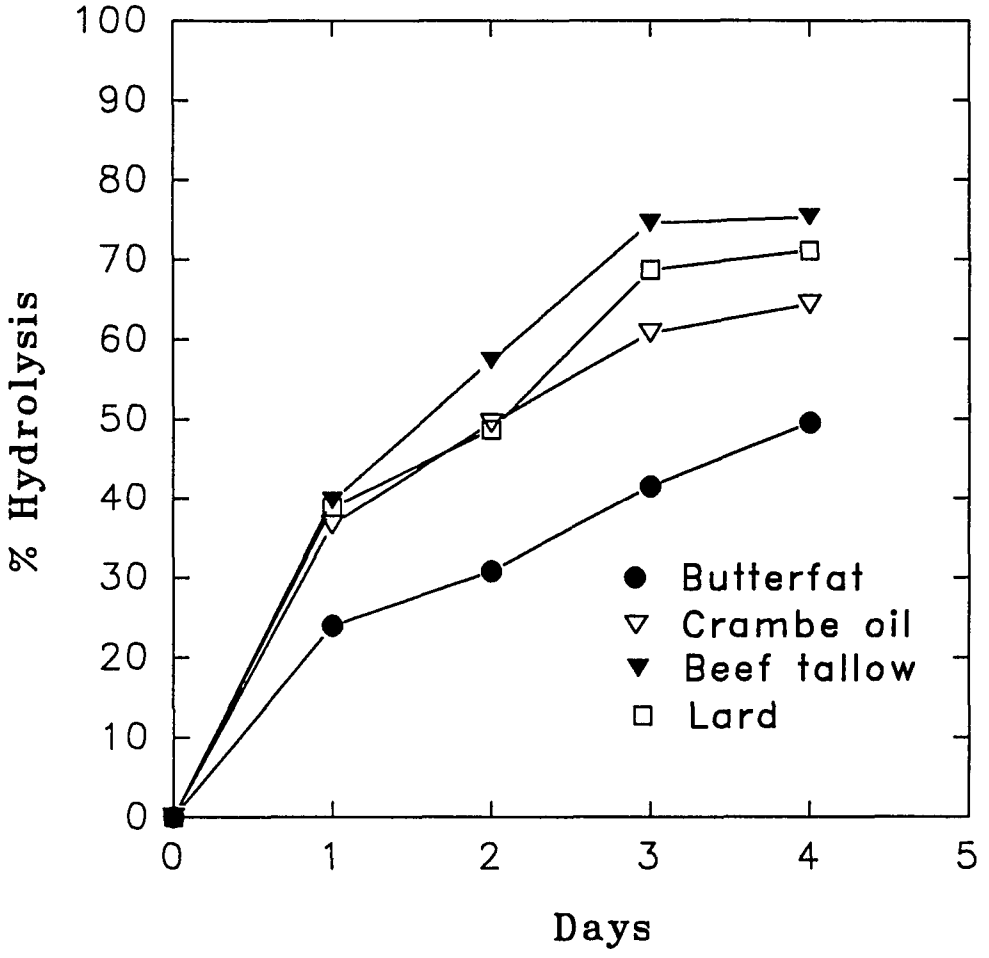

Figure 11. Hydrolysis of butterfat, crambe oil, beef tallow and lard by caryopses from B605-1050 oats at 40°C in an unagitated reactor

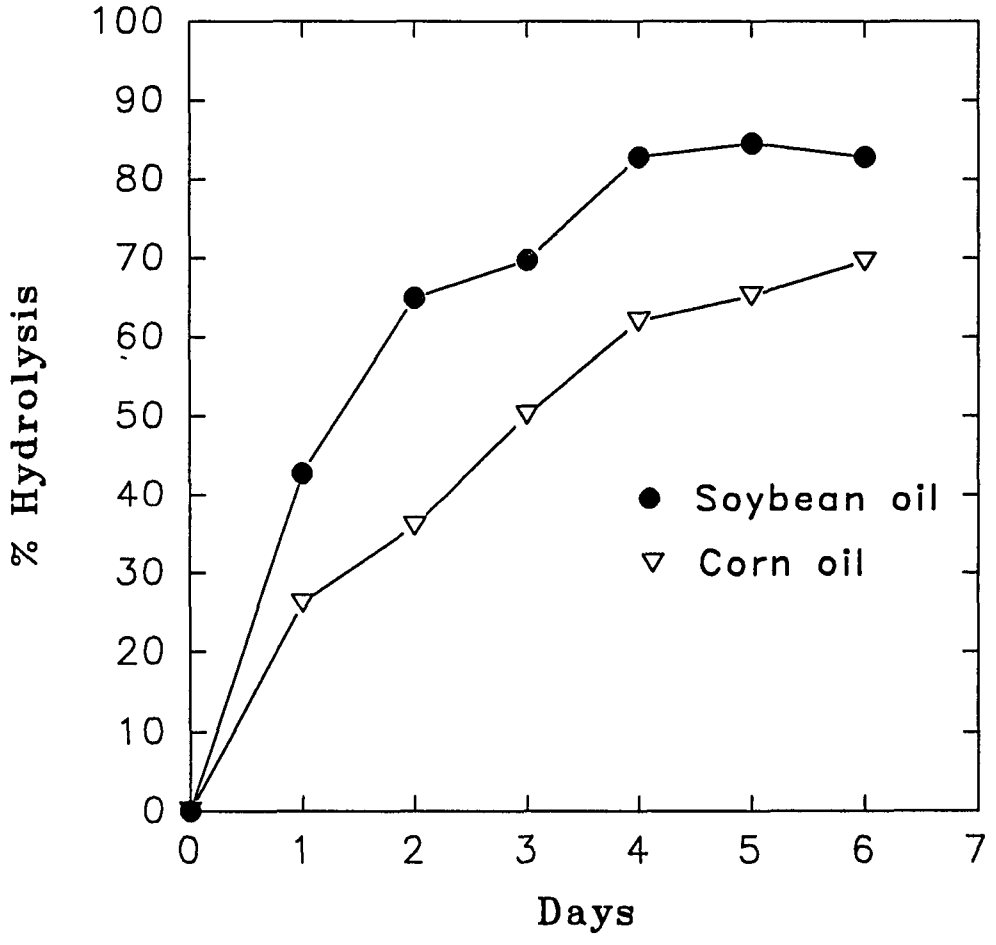

Figure 12. Hydrolysis of soybean and corn oil by caryopses from B605-1050 oats at 40°C while being circulated through a column at 0.017 L/h/cm^2

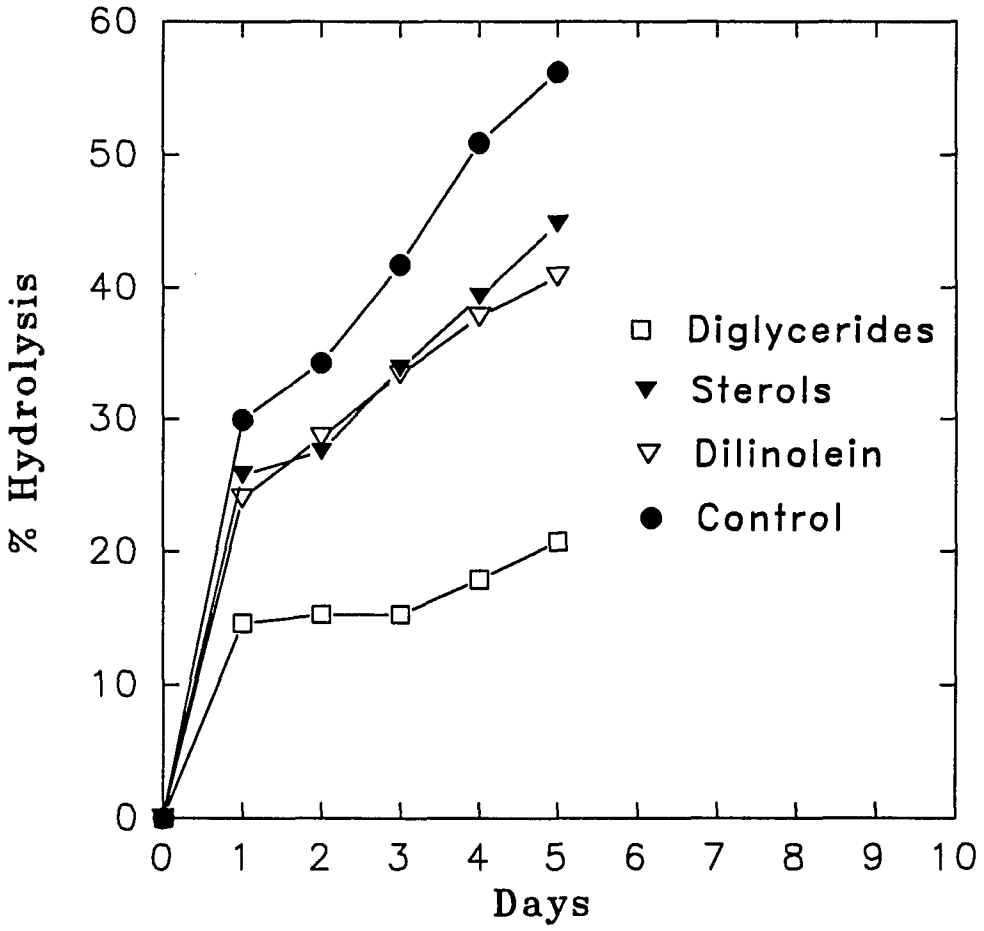

Figure 13. Effect of corn oil components on the hydrolysis of soybean oil by B605-1050 moist oat caryopses at 40°C in an unagitated reactor. 1,3-dilinolein was obtained from Sigma Chemical Co., St. Louis, MO

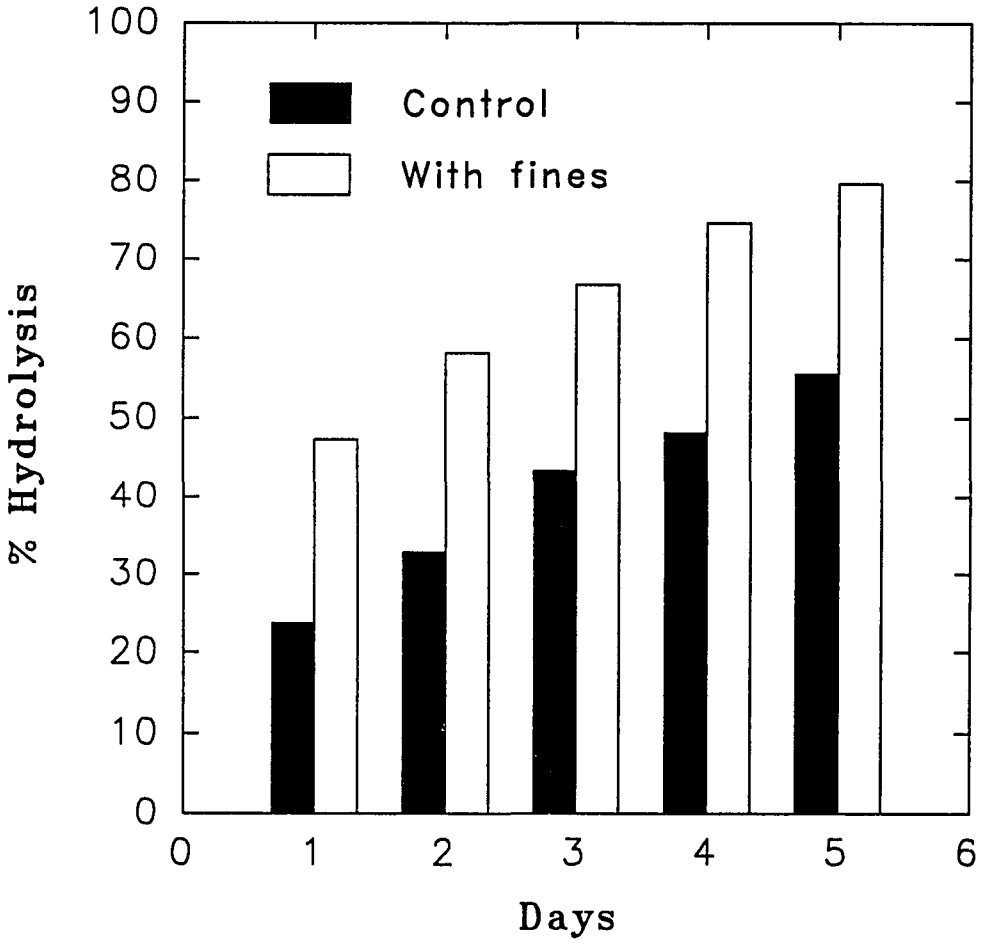

Figure 14. Effect of the incorporation of 10% (w/w) of fines on the hydrolysis of corn oil by b605-1050 caryopses at 40°C while being circulated through a column at 0.017 L/h/cm^2

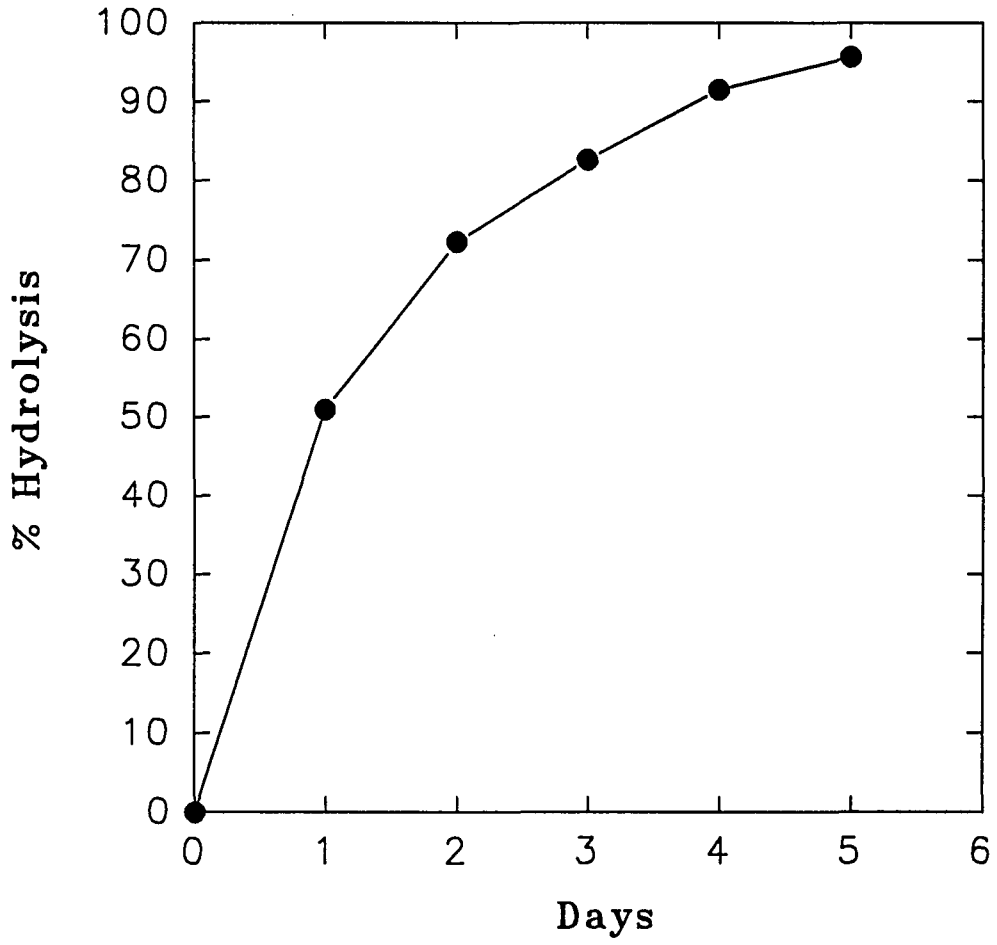

Figure 15. Effect of the incorporation of 10% (w/w) by weight of hexane fractionated-fines on the hydrolysis of soybean oil with 1988 B605-1050 caryopses at 40°C while being circulated though a column at 0.017 $L/h/cm^2$

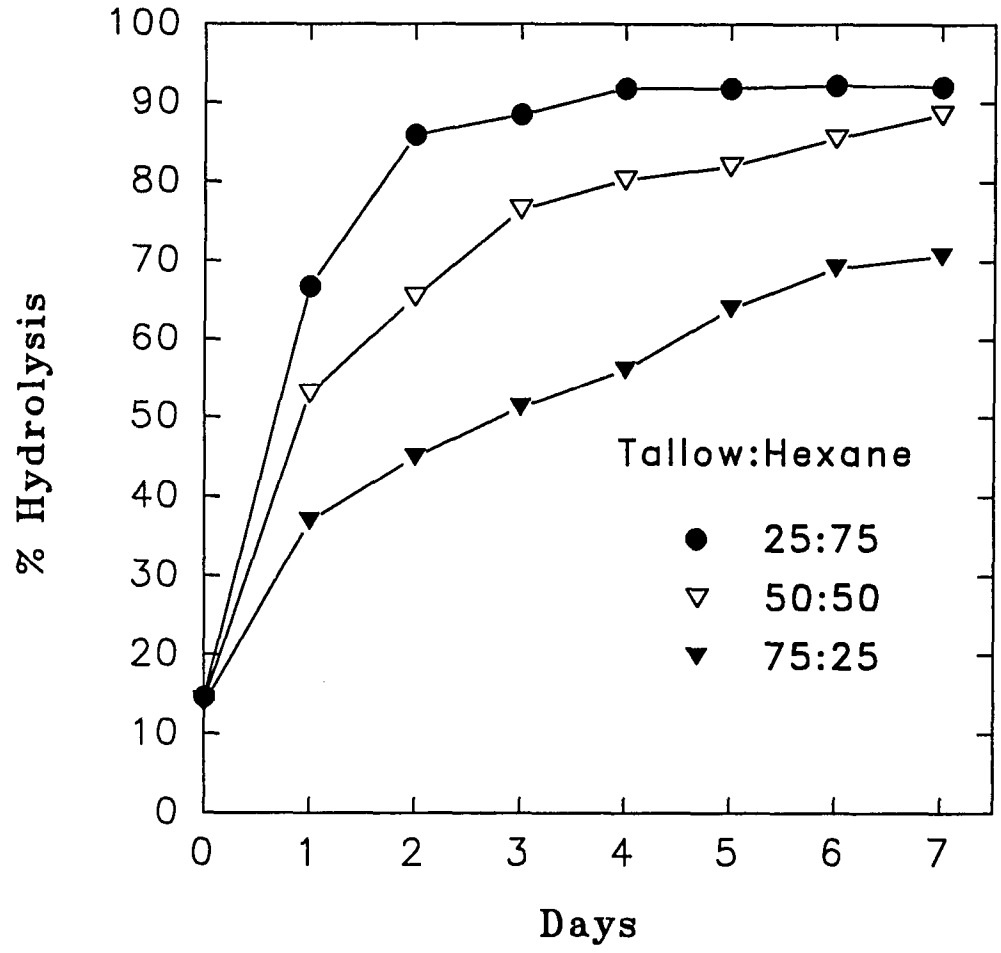

Figure 16. Hydrolysis of diluted tallow in hexane by caryopses from B605-1050 oats at 40°C while being circulated through a column at 0.017 L/h/cm^2

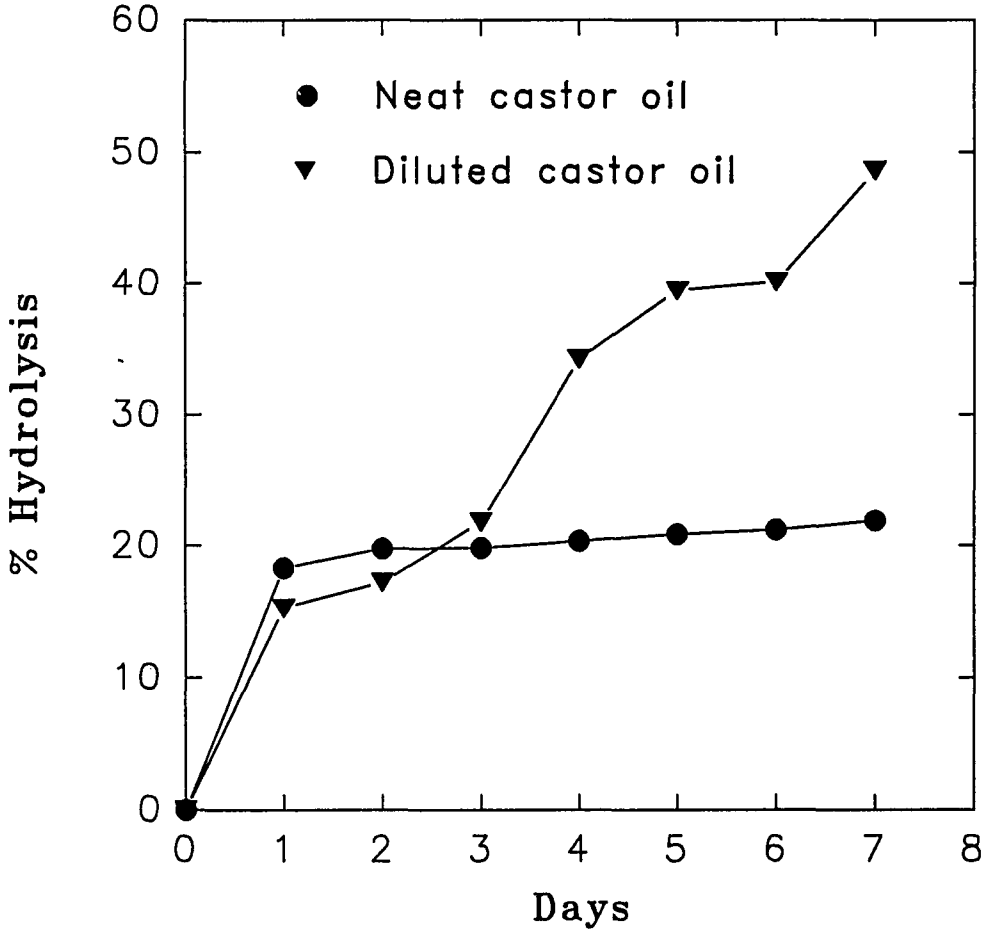

Figure 17. Hydrolysis of neat castor oil and castor oil diluted 1:1 with a 1:1 benzene:hexane mixture by caryopses from B605-1050 oats at 40°C while being circulated through a column at 0.017 L/h/cm^2

GENERAL SUMMARY

Earlier at Iowa State, the reaction conditions for the use of oat lipase bioreactors for the hydrolysis of fats and oils were standardized. This study further examined the hydrolysis of various fats and oils with moist oat caryopses using unagitated 50-ml conical flasks containing caryopses immersed in oil or by circulating oil through a glass column (60 x 4.5 cm) packed with caryopses. Various factors affecting the oat lipase activity were examined.

Nine early oat lines and 11 midseason lines grown at six and 12 US locations, respectively, were examined for their lipase activity. Variety MN 87187 grown in Lafayette, IN gave the highest lipase activity in the Uniform Early Oat Performance Nursery. Variety PA 8393-1500 grown in Fargo, ND gave the greatest lipase activity in the Uniform Midseason Oat Performance Nursery. The most active lines were 1.9 times more active than the least active. Analysis of variance of the data showed that the lipase activity varied significantly at $P < 0.01$ among variety and locations, and the interactions of variety x location were significant. Yield and test weight were positively correlated with the lipase activity of various oat varieties and, caryopses weight was negatively correlated. Seemingly, oats with many small caryopses had the most active

lipase. When a variety was harvested at various stages of development, early harvested caryopses had more lipase activity/unit weight than normal harvested caryopses. Variety Y907-5-5 has the most lipase activity of the varieties tested. Among the various growing years studied, 1988 gave oats with spectacularly more lipase than other years. The caryopses produced in 1988, a very dry year, were unusual for their uniform golden color and freedom from fungus attack.

There was a significant difference in the lipase activity of caryopses according to the method used for dehulling. The two dehullers used were a wringer-type and an impact-type. The impact-type dehuller gave at least 20% higher hydrolysis of soybean oil. This activity of the caryopses was slightly improved by drying the oats over phosphorus pentoxide before dehulling.

In an earlier temperature study of fat hydrolysis by moist oat caryopses 40°C was optimal, but this study was done using 10-degree increments. In the present study, narrow temperature increments, 38, 40 and 42°C, were compared. The activity of oat lipase at 38 and 42°C was lower than that at 40°C.

Alkaline conditions seemed to stimulate the lipase activity. Addition of 0.004% ethanolamine into the water used to moisten the caryopses increased the activity whereas 0.002%

ethanolamine was not effective in improving the enzyme activity. Percentages greater than 0.004% were inhibitory.

An apparatus was devised for producing a fluidized bed of caryopses in which the caryopses rubbed together and the surface lipase was partially removed. An air flux of 0.067 L/sec/cm^2 was the minimum to create a fluidized bed of caryopses, but the recovery of lipase-bearing fines was low. An air flux greater than 0.100 L/sec/cm^2 transported the oats out of the fluidized bed. An air flux of 0.086 L/sec/cm^2 was optimum for operating the fluidized bed of caryopses. Treatment of caryopses in the fluidized bed resulted in fines, bran (large pieces) and dry scrubbed caryopses fractions. As the residence time in the fluidized bed increased, the hydrolytic potency of the dry scrubbed caryopses decreased. The lipase activity of fines and bran for various residence period in a fluidized bed was in the order of: fines 0.5 h > fines 1.0 h > fines 1.5 h > bran. The lipase activity of fines was comparable to that of *Candida cylindrace* type VII of Sigma Chemical Co., St. Louis, MO. The bran had little activity. Thus residence time of 1 h and air flux of 0.086 L/sec/cm^2 were appropriate for active fines preparation.

The above fines could be further fractionated under gravity sedimentation with hexane. The finest fraction (81% of the total weight) was rich in lipase. About 95% hydrolysis

of soybean oil was obtained in five days after 10% enrichment of moist oat caryopses with the hexane-fractionated fines.

Caryopses were monitored for changes in their native oat lipids when they were used for the hydrolysis of soybean oil. There was not much change in stearate. Linoleate and linolenate increased and oleate and palmitate decreased, suggesting a continuing metabolism of the moist oats immersed in oil and, possibly, some transfer of acyl groups from the external oil during the hydrolysis process. The total oil and free fatty acid content of oats also increased slightly with increased incubation time.

During the hydrolysis of oil, glycerol accumulates in the caryopses. When whole caryopses were washed by continuous recirculation of water, maximum glycerol recovery was achieved in 6 h.

Moistened oats were used to hydrolyze various fats and oils including milkfat, crambe oil, soybean oil, corn oil, tallow, lard, beef tallow and castor oil. Caproic and caprylic acids were released preferentially from milkfat. Erucic acid was released from crambe oil significantly more slowly than the other acyl groups. Beef tallow and lard were readily hydrolyzed although the tallow was just melted at the 40°C temperature limit for the oat enzyme.

The hydrolysis of corn oil was slower than soybean oil although they have similar acyl groups composition. The unfractionated polar materials (yield 12.43%) from corn oil had inhibitory action on the soybean oil hydrolysis. The inhibitory action of the diglyceride fraction of corn oil was much greater than that of sterols or triglycerides.

The hydrolysis of tallow diluted with various proportions of hexane (25, 50 and 75%) was studied. About 90% hydrolysis was achieved with 50 and 25% tallow in hexane. In the case of castor oil, no more than 20% hydrolysis was achieved and considerable estolide formation seemed to occur. To increase the percentage hydrolysis of castor oil, it was diluted with equal weights of a hexane:benzene mixtures. This resulted into 50% hydrolysis.

The safety of the oats caryopses in the bioreactor was evaluated for food/feed purposes by inoculating the moist oats caryopses with the spores of *Clostridium sporogenes*, a nontoxin-producing *Clostridium* with growth characteristics like that of *Clostridium botulinum*. After 1 wk in the bioreactor, the caryopses were tested, and no growth of *Clostridium sporogenes* could be demonstrated.

An economic analysis of several possible variations of the process shows that several variations appear to be quite profitable if the moist caryopses can be used in feed formulations.

The major conclusions of this work are:

1. Oat lipase bioreactors are technically and economically feasible.
2. The optimum temperature for the oat lipase bioreactor is 40°C.
3. The method of dehulling the oats has a significant effect on the lipase activity of the oat caryopses.
4. With a fluidized bed of caryopses, lipase can be removed without significantly affecting the nutritional quality of the oats.
5. Both variety and growing conditions significantly affected lipase activity.
6. No growth of *Clostridium* spores were detected in moist oats immersed in oil.
7. Hydrolysis of soybean oil was faster than that of corn oil, seemingly because of high levels of diglycerides in the corn oil.
8. Hydrolysis with moist oat caryopses was successfully applied to a number of fats and oils including milkfat, corn oil, soybean oil, tallow, lard, beef tallow, crambe oil and castor oil.
9. There is continuing metabolism of the moist oats immersed in oil, and possibly some transfer of acyl groups from the external oil during the use of moist caryopses for hydrolysis of oils.

LITERATURE CITED

Aizono, Y., M. Funatsu, M. Sugano, K. Hyashi, and Y. Fujiki. 1973. Enzymatic properties of rice bran lipase. Agric. Biol. Chem. 37:2031-2036.

Anderson, G. 1981. Energy use in solvent extraction. Oil Mill Gaz. 86:20-23.

Ando, S., A. Yoshida, K. Nakajima, and M. Hatano. 1991. Hydrolysis of fish oil by marine bacterial lipase. Nippon Suisan Gakkaishi 57:1351-1353.

Anonymous. 1991. Work continues on new oils for industrial use. INFORM 2:678-691.

Bailey, J. E., and D. F. Ollis. 1986. Biochemical engineering fundamentals, McGraw-Hill Book Co., New York. 180 pp.

Basri, M., A. B. Salleh, K. Ampon, W. M. Yunus, and C. N. A. Razak. 1991. Modification of lipase by polyethylene glycol. Biocatalysis 4:313-317.

Bell, G., J. R. Todd, J. A. Blain, J. D. E. Patterson, and C. E. Shaw. 1981. Hydrolysis of triglycerides by solid phase lipolytic enzymes of *Rhizopus arrhizus* in continuous reactor systems. Biotechnol. Bioeng. 23:1703-1719.

Berner D. L., and E. G. Hammond. 1969. Specificity of lipase from several seeds and *Leptospira pomona*. Lipids 5:572-575.

Berner, D. L., and E. G. Hammond. 1970. Phylogeny of lipase specificity. Lipids 5:558-562.

Bilyk, A., R. G. Bistline, M. J. Haas, and S. H. Feairheller. 1991. Lipase catalyzed triglyceride hydrolysis in organic solvent. J. Am. Oil Chem. 68:320-323.

Bjorklund, A. 1970. Some Lipolytic Psychrophilic *Pseudomonas* bacteria and their hydrolysis of edible fats. State Inst. Tech. Res., Finland, Julkaisu 156. Publ., Helsinki, Finland.

Brady, C., L. Metcalfe, D. R. Slaboszewski, and D. Frank. 1988. Lipase immobilized on a hydrophobic, microporous support for the hydrolysis of fats. J. Am. Oil Chem. Soc. 65:917-921.

Brockerhoff, H., and R. G. Jensen. 1974. Lipolytic enzymes. Academic Press, New York. 108 pp.

Brockman, H. L., J. H. Law, and F. J. Kezdy. 1973. The hydrolysis of tripropionin by pancreatic lipase adsorbed to silicanized glass beads. J. Biol. Chem. 248:4965-4970.

Brown, C. M., and D. J. Craddock. 1972. Oil content and groat weight of entries in the world oat collection. Crop Sci. 12:514-515.

Buhler, A. C., E. Engel, J. H. Nelson, and C. H. Amundson. 1972. Butter flavored for additive concentrate. US Patent # 3,653,921.

Chander, H., and H. Klostermeyer. 1983. Production of lipase by *Geotrichum candidum* under various growth conditions. Milchwissen. 38:410-412.

Chibata, I., and T. Tosa. 1976. Industrial applications of immobilized enzymes and immobilized microbial cells. Pages 329-357 *in* L. B. Wingard, E. Katchalski-Katzir, and L. Goldstein, eds. Immobilizd enzyme principles, Academic Press, New York.

Christie, W. W. 1982. Lipid analysis. Pergamon Press, New York. P. 52.

Desnuelle, P., and P. Savary. 1963. Specificities of lipases. J Lipid Res., 4: 369-384.

DIFCO Manual. 1986. DIFCO Labs., Tenth Edition, Detroit, MI. PP. 165.

Dundas, D. G. A., H. M. Henderson, and N. A. Eskin. 1978. Lipase from *Vicia faba minor*. Food Chem. 3:171-178.

Ekstrand, B., I. Gangby, and G. Akesson. 1992. Lipase activity in oats-Distribution, pH dependence, and heat inactivation. Cereal Chem. 69:379-381.

Farnham, M. G. 1957. Enzyme-containing powder and enzyme modified product thereof. US Patent # 2,794,743.

Frey, K. J., and E. G. Hammond. 1975. Genetic characteristics and utilization of oil in caryopses of oat species. J. Oil Chem. Soc. 52:358-362.

Folch, J. M., M. Lees, and G. H. S. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226:497-509.

Fujiki, Y. Y. Aizono, and M. Funatsu. 1978. Chemical properties of major subunits of rice bran lipase. Agric. Biol. Chem. 42:559-606.

Fujiki, Y.; Y. Aizono, and M. Funatsu. 1978. Characterization of minor subunit of rice bran lipase. Agric. Biol. Chem., 42:2401-2402.

Funatsu, M., Y. Aizono, K. Hayashi, M. Watanabe, and M. Eto. 1971. Biochemical studies on rice bran lipase. Part I. Purification and physical properties. Agri. Biol. Chem. 35:734-742.

Garcia, H. S., C. H. Amundson, and C. G. Hill, Jr. 1991. Partial characterization of the action of an *Aspergillus niger* lipase on butteroil emulsions. J. Food Sci. 56:1233-1237.

Gavrichenkov, Y. D., E. D. Kazakov, and A. I. Starodubtseva. 1969. Lipase activity in soybean seeds. Prikl. Biokhimiya 5:324-328.

Haas, G. J., and J. C. Lugay. 1974. Method for preparing animal food of increased palatibility. US Patent # 3,857,968.

Halstead, R. P., and H. W. Rines. 1990. Report of the Cooperative Uniform Early and Midseason Nurseries for 1990. ARS-USDA, St. Paul, MN.

Hammond, E. G. 1983. Oat lipids. Pages 331-352 *in* P. J. Barnes, eds. Lipids in cereal technology, Academic Press, New York.

Hano, T., M. Matsumoto, T. Ohtake, and F. Hori. 1991. Kinetic study of olive oil hydrolysis with lipase. Kagasu Kogaku Ronbunshu 17:449-454.

Hassanien, F. R., and K. D.. Mukherjee. 1986. Isolation of lipase from germinating oilseeds for biotechnological processes. J. Am. Oil Chem. Soc. 63:893-897.

Hoq, M. M., T. Yamane, S. Shimizu, T. Funada, and S. Ishida. 1985. Continuous hydrolysis of olive oil by lipase in microporous hydrophobic membrane bioreactor. J. Am. Oil Chem. Soc. 62:1016-1021.

Horiuti, Y., and S. Imamura. 1978. Stimulation of *Chromobacterium* lipase activity and prevention of its adsorption to palmitoyl cellulose by hydrophobic binding of fatty acids. J. Biochem. 83:1381-1385.

Huang, A. H. C., Y. Lin, and S. Wang. 1988. Characteristics and biosynthesis of seed lipases in maize and other plant species. J. Am. Oil Chem. Soc. 65:897-899.

Huge-Jensen, B., D. R. Galluzzo, and R. G. Jensen. 1988. Studies on free and immobilized lipases from *Mucor meihei*. J. Am. Oil Chem. Soc. 65:905-910.

Hutchinson, J. G., H. F. Martin, and T. Moran. 1951. Location and destruction of lipase in oats. Nature (London) 167:758-759.

Inada, Y., H. Nishimura, K. Takahashi, T. Yoshimoto, and A. R. Saha, and Y. Saito. 1984. Ester synthesis catalyzed by polyethylene glycol-modified lipase in benzene. Biochem. Biophys. Res. Com. 122:845-850.

Ison, A. P., P. Dunhill, and M. D. Lilly. 1988. Effect of solvent concentration on enzyme catalyzed interesterifiation of fats. Enzyme Microb. Technol. 10:47-51.

Iwami, M., S. Okumura, and Y. Tsujisaka. 1980. Synthesis of terpene alcohol esters by lipase. Agric. Biol. Chem. 44:2731-2732.

Jensen, B. H., D. R. Glluzzo, and R. G. Jensen. 1988. Studies on free and immobilized lipase from *Mucor miehei*. J. Am. Oil Chem. Soc. 65:905-916.

Jensen, R. G., F. A. DeJong, and R. M. Clark. 1983. Determination of lipase specificity. Lipids 18:239-252.

Jensen, R. G., T. A. Marks, J. Sampugna, J. G. Quinn, and D. L. Carpenter. 1966. Purification of triglycerides with an alumina column. Lipids 1:451-452.

Johnson, J. D., and D. L. Southworth. 1973. Method for imparting cheese-like flavor to proteinaceous materials. US Patent # 3,780,182.

Juergen, F., and S. Friedrich. 1990. Hydroxy-monoglycerides by lipase-catalyzed partial hydrolysis of castor oil. DECHEMA Biotechnol. Conf., 4:139-142, Muenster, Federal Republic of Germany.

Kang, S. T., and J. S. Rhee. 1988. Effect of water on hydrolysis of olive oil by immobilized lipase in reverse phase system. Biotechnol. Lett. 10:341-346.

Kang, S. T., and J. S. Rhee. 1989. Effects of solvents on hydrolysis of olive oil by immobilized lipase in reverse phase system. Biotechnol Lett. 11:37-42.

Karube, I., Y. Yugeta, and S. Suzuki. 1977. Electric field control of lipase membrane activity. Biotech. Bioeng. 19:1493-1501.

Kempf, C. A, J. S. Niedlinger, A. P. Stewart, and P. F. Sharp. 1953. Milk product containing milk fat, and process of producing the same. US Patent # 2,638,418.

Kermasha, S., F. R. van de Voort, and M. Metche. 1986. Characterization of french bean (*Phaseolus vulgaris*) seed lipase. Can. Inst. Food Sci. Technol. J. 19:23-27.

Khosla, C., A. P. Kudchadker and A. K. Lala. 1985. Enzymatic fat hydrolysis using castor bean lipase. Proceedings of Dept. of Atomic Energy Symposium: Newer approaches for biological applications. Bhabha Atomic Research Center, Bombay, India. P. 93-97.

Kilara, A. 1985. Enzyme-modified lipid food ingredients. Process Biochem. 20:35-44.

Kilara, A., and K. M. Shahani. 1977. The use of immobilized enzymes in the food industry-A review. C.R.C. Rev. Food Sci. Nutr. 12:161-198.

Kimura, Y. A., Tanaka, K. Sonomoto, T. Nihira, and S. Fukui. 1983. Application of immobilized lipase to hydrolysis of triglycerides. Eur. J. Appl. Microbiol. Biotechnol. 17:107-112.

Knight, S. G. 1963. Process for preparing flavoring compositions. US Patent # 3,100,153.

Kobayashi, T., I. Kato, K. Ohmiya, and S. Shimizu. 1980. Recovery of foam stability of yolk-contaminated egg white by immobilized lipase. Agric. Biol. Chem. 44:413-418.

Kobayashi, T., S. Mukataka, H. Kataoka, and J. Takahashi. 1985. Enzymic hydrolysis of fats in biphasic organic-aqueous systems. Hakko Kogaku Kaishi. 63:439-444.

Kodera, Y., K. Takahashi, H. Nishimura, A. Matsushima, Y. saito, and Y. Inada. 1986. Ester synthesis from α-substituted carboxylic acid catalyzed by polyethylene glycol-modified lipase from *Candida cylindracea* in benzene. Biotechnol. Lett. 8: 881-884.

Kosugi, Y., M. Shiraki, T. Kunieda, and H. Murase. 1991. Manufacture of fatty acid esters with immobilized lipase (in Japanese). Japanese Patent # 03127992.

Kosugi, Y., and H. Suzuki. 1973. Fixation of cell-bound lipase and properties of the fixed lipase as an immobilized enzyme. J. Ferment. Technol. 51:895-903.

Krewson C. F., and W. F. Scott. 1964. *Vernonia anthelmintica* (L.) wild. Extraction of oil or trivernolin from seed. J. Am. Oil Chem. Soc. 41:422-426

Kroll, J., F. R. Hassanien, E. Glapinska, and C. Franzke. 1980. Herstellung und eigenschaften der immobilisicrten lipase von *Geotrichum candidum*. Die Nahrung 24:215-225.

Kubo, M., I. Karube, and S. Suzuki. 1976. Electrical control of enzyme membrane activity. Biochem. Biophys. Res. Commun. 69:731-736.

Kwon, D. Y., and J. S. Rhee. 1987. Kinetics of lipase reactions in two phase system. Sanop Misaengmul Hakhechi. 15:98-103.

Lau, F. Y., E. G. Hammond, and P. F. Ross. 1982. Effect of randomization on the oxidation of corn oil. J. Am. Oil Chem. Soc. 59:407-411.

Layvayre, J., and J. Baratti. 1982. Preparation and properties of immobilized lipases. Biotechnol. Bioeng. 24:1007-1013.

Lawrence, R. C. 1967. Microbial lipase and related esterases. Part I. Detection, distribution and production of microbial lipases. Dairy Science Abstr. 29:1-8.

Lawrence, R. C., T. F. Fryer, and B. Reiter. 1967. The production and characterization of lipases from a *Micrococcus* and a *Pseudomonad*. J. General Microbiol. 48:401-418.

Lebedev, A. B., and M. S. Umanskii. 1981. Study of the hydrolysis of butterfat by milk lipoprotein lipase. Molochn. Promst. 9:13-14.

Lee, I. 1989. Use of oat caryopses as a lipase reactor. MS thesis, submitted to the Iowa State University, Ames, IA.

Lee, I., and E. G. Hammond. 1990. Oat (*Avena sativa*) caryopses as a natural lipase bioreactor. J. Am. Oil Chem. Soc. 67:761-765.

Lieberman, R. B., and D. F. Ollis. 1975. Hydrolysis of paticulate tributyrin in a fluidized lipase reactor. Biotechnol. Bioeng. 17:1401-1419.

Linfield, W. M., D. J. O'Brien, S. Serota, and R. A. Barauskas. 1984. Lipid-lipase interactions: I. Fat splitting with lipase from *Candida rugosa*. J. Am. Oil Chem. Soc. 61:1067-1071.

Macrae, A. R. 1983. Lipase-catalyzed interesterification of oils and fats. J. Am. Oil Chem. Soc. 60:291-294.

Macrae, A. R. 1985. Interesterification of fats and oils. Pages 195-208 *in* J. Tramper, H. C. van der Plaas, and P. Linko, eds. Biocatalysts in organic syntheses. Elsevier Applied Science Publishers, Amsterdam.

Malcata, F. X., C. G. Charles, Jr., and C. H. Amundson. 1991. Use of lipase immobilized in a membrane reactor to hydrolyze the glycerides of butteroil. Biotechnol. Bioeng. 38:853-868.

Malcata, F. X., H. R. Reyes, H. S. Garcia, C. G. Hill Jr., and C. H. Amundson. 1990. Immobilized lipase reactors for modification of fats and oils-a review. J. Am. Oil Chem. Soc. 67:890-910.

Marlot, C., G. Landgrand, C. Triantaphylides and J. Baratti. 1985. Ester synthesis in organic solvent catalyzed by lipases immobilized on hydrophilic supports. Biotechnol. Lett. 7:647-650.

Martin, H. F., and F. G. Peers. 1953. Oat lipase. Biochem. J. 55:523-529.

Meerov, G. I., U. I. Tros'ko, K. G. Mikhal'skaya, and S. N. Norkina. 1976. Use of specially defatted castor plant seed kernel as a lipase preparation for fat hydrolysis. Parikl. Biochim. Microbiol. 12:934-939.

Miller, C., H. Austin, L. Posorske, and J. Gonzalez. 1988. Characteristics of an immobilized lipase for the commercial synthesis of esters. J. Am. Oil Chem. Soc. 65:927-931.

Molever, K. J. 1987. Quantitative determination of glycerin in soap by gas chromatograph. J. Am. Oil Chem. Soc. 64:1356-1357.

Nelson, J. H. 1972. Enzymatically produced flavors for fatty systems. J. Am. Oil Chem. Soc. 49:559-562.

Nielsen, T. 1984. Industrial application possibilities for lipases. Fette Seifen Anstrichm. 87:15-19.

Okumura, S., M. Iwami, and Y. Tsujisaka. 1979. Synthesis of various kinds of esters by four microbial lipases. Biochim. Biophys. Acta 575:156-165.

Olney, C. E., R. G. Jensen, J. Sampugna, and J. G. Quinn. 1968. The purification and specificity of a lipase from *Vernonia anthelmintica* seed. Lipids 3:498-502.

Omar, I. C., H. Saeki, N. Nishio, and S. Nagai. 1988. Hydrolysis of triglycerides by immobilized thermostable lipase from *Humicola lanuginosa*. Agric. Biol. Chem. 52:99-105.

Ory, R. L. 1969. Acid lipase of castor bean. Lipids 4:177-185.

Ory, R. L., A. J. Angelo, and A. M. Altschul. 1960. Castor bean lipase:action on its endogenous substrate. J. Lipid Res. 1:208-213.

Ory, R. L., A. J. Angelo, and A. M. Altschul. 1962. The acid lipase of castor bean. Properties and substrate specificty. J. Lipid Res. 3:99.

Pangier, D. J. 1969. Lipolyzed milk fat products. US Patent # 3,469,993.

Park, Y. K., G. M. Pastore, and M. M. Almedia. 1988. Hydrolysis of soybean oil by a combined lipase system. J. Am. Oil Chem. Soc. 65:252-254.

Piazza, G. J., and A. Bilyk. 1989. Properties and substrate specificity of lipase activity from oat (*Avena sativa*) seeds. J. Am. Oil Chem. Soc. 66:489 (Abstr.)

Posorske, L. H., G. K. LeFebvre, C. A. Miller, T. T. Hansen, and B. L. Glenvig. 1988. Process considerations of continuous fat modification with an immobilized lipase. J. Am. Oil Chem. Soc. 65:922-926.

Pronk, W., P. J. A. M. Kerkhof, C. van Helden, and K. van't Riet. 1988. The hydrolysis of triglycerides by immobilized lipase in a hydrophilic membrane reactor. Biotechnol. Bioeng. 32:512-518.

Sanders, T. H., and H. E. Pattee. 1975. Peanut alkaline lipase. Lipids 10:50-54.

Sanz, L. C., and J. M. Olias. 1990. Characterization of lupin seed lipase. Food Chem. 37:221-228.

SAS Institute Inc. 1991. SAS User's guide:Basics. SAS Institute Inc., Cary, NC.

Schafer, H. W. 1975. Increasing the recovery of milk proteins in cheese made by direct acidification and encapsulation of enzymes for acceleration of cheese ripening. Dissertation Abstr. Int. 36:1127B.

Schuch, R., and K. M. Mukherjee. 1987. Interesterification of lipids using an immobilized *sn*-1,3 specific triacylglycerol lipase. J. Agric. Food Chem. 35:1005-1008.

Seino, H., T. Uchibori, T. Nishitani, and S. Inamasu. 1984. Enzymatic synthesis of carbohydrate esters of fatty acid (1). Esterification of sucrose, glucose, fructose, and sorbitol. J. Am. Oil Chem. 61:1761-1765.

Severina, L. O., and N. A. Bashkatova. 1980. Isolation and properties of exolipase from *Serratia marcescens 343*. Appl. Biochem. Microbiol. 16:54-60.

Serota, S. 1986. Lipolytic splitting of fats and oils. US Patent # 86,882,103.

Shahani, K. M. 1975. Lipases and esterases. Pages 181-217 *in* G. Reed, ed. Enzymes in food processing. Academic Press, New York.

Shastry, B. S., and M. R. Rao. 1971. Studies on rice bran lipase. Indian J. Biochem. Biophys. 8:327-332.

Sreenivasan, B. 1978. Interesterification of fats. J. Am. Oil Chem. Soc. 55:796-805.

Stead, D. 1986. Microbial lipases: Their characteristics, role in food spoilage and industrial uses. J. Dairy Res. 53:481-505.

Tahoun, M. K. 1986. Large agarose-lipase beads for the hydrolysis of triglycerides. Food chem. 22:297-303.

Takahashi, K., Y. Tamaura, Y. Kodera, T. Mihama, Y. Saito, and Y. Inada. 1987. Magnetic lipase active in organic solvents. Biochem. Biophys. Res. Comm. 142:291-296.

Taylor, F., C. C. Panzer, J. C. Craig, and D. J. O'Brien. 1986. Continuous hydrolysis of tallow with immobilized lipase in a microporous membrane. Biotechnol. Lett. 28:1318-1322.

Tavss, E. A., and E. Eigen. 1988. Vegetable oils in enzymic hydrolysis of fats at low temprature for manufacture of soap (in French). French Patent # 2603900.

Tomizuka, N., Y. Ota and K. Yamada. 1966. Studies on lipase from *Candida cylindracae*. Part I. Purification and properties. Agri. Biol. Chem. 30:576-584.

Tsai, S. W., G. H. Wu, and C. L. Chiang. 1991. Kinetics of enzymic hydrolysis of olive oil in biphasic organic-aqueous system. Biotechnol. Bioeng. 38:761-766.

Ulrich, G. D. 1984. A guide to chemical engineering process design and economics. John Wiley & Bros., New York. 331 pp.

Umanskii, M. S., Y. A. Borovkova, and A. I. Chibotareva. 1980. Hydorlysis of milk fat by lipase preparations of different origin. Parikl. Biokhim. Microbiol. 16:784-787.

van der Padt, A., M. J. Edema, J. J. W. Sewalt, and K. van't Riet. 1990. Enzymatic acylglycerol synthesis in a membrane bioreactor. J. Am. Oil Chem. Soc. 67:347-352.

Vangtal, A. and E. G. Hammond. 1986. Correlation of the flavor characteristics of Swiss type cheeses with chemical parameters. J. Dairy Sci. 69:2982-2993.

Vignolo, R., and F. Naughton. 1991. Castor: a new sense of direction. INFORM 2:692-699.

Vitro, M. D., J. M. Lascaray, R. Solozabal, and M. De Renobales. 1991. Enzymatic hydrolysis of animal fats in organic solvents at temperatures below their melting points. J. Am. Chem. Soc. 68:324-327.

Wang, Y. J., J. Y. Sheu, F. F. Wang, and J. W. Shaw. 1988. Lipase-catalyzed oil hydrolysis in the absence of added emulsifier. Biotechnol. Bioeng. 31:628-633.

Watts , J. C., and J. H. Nelson. 1963. Cheese flavoring process. US Patent # 3,072,488.

Werdelmann, B. W. 1974. Fat and oil chemistry-a vital obligation. Fette Seifen Anstrichm. 76:1-7.

Wisdom, R. A., P. Dunhill, and M. D. Lilly. 1985. Enzymic interesterification of fats:the effect of non-lipase material on immobilized enzyme activity. Enzyme Microb. Technol. 7:567-572.

Wisdom, R. A., P. Dunnill, and M. D. Lilly. 1987. Enzymic interestierification of fats:Laboratory and pilot-scale studies with imobilized lipase from *Rhizopus arrhizus*. Biotechnol. Bioeng. 29:1081.

Wisdom, R. A., P. Dunhill, M. D. Lilly, and A. Macrae. 1984. Enzymic interesterification of fats:factors influencing the choice of support for immobilized lipase. Enzyme Microb. Technol. 6:443-446.

Xie, S., and Q. Wu. 1987. Hydrolysis of rapeseed oil by two lipases. Shengwu Gongchemg Xuebao 1:53-55.

Yamaguchi, C., A. Masatsugu, S. Asaoka, and F. Osada. 1989. Two-step enzymic manufacture of castor oil fatty acids. Japanese Patent # 01016593.

Yamaguchi, C., A. Toyana, S. Asaoka, and F. Ogada. 1990. Hydrolysis of castor oil by lipase. Japanese Patent # 02013389.

Yang, D., and J. S. Rhee. 1991. Stability of the lipase immobilized on DEAE-Sephadex for continous lipid hydrolysis in organic solvent. Biotechnol. Lett. 13:553-558.

Yang, L. Y., A. Kuksis, and J. J. Myher. 1990. Lipolysis of menhaden oil triacylglycerols and the corresponding fatty acid alkyl esters by pancreatic lipase in vitro:a reexamination. J. Lipid Res. 31:137-148.

Yokozeki, K., S. Yamanaka, K. Takimani, Y. Hirose, A. Tanaka, K. Sonomoto, and S. Kukui. 1982. Application of imobilized lipase to regiospecific interesterification of triglyceride in organic solvent. Eur. J. Appl. Microbiol. Biotechnol. 14:1-5.

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APPENDIX

ECONOMIC ANALYSIS

Case 1. Three stage hydrolysis process with ordinary oats

This process could be carried out with presently-available varieties of oats. We assume the process is connected with a feed mill that will use the wet oily caryopses in feed after one use in the hydrolysis process. We assume that 1) the caryopses suffer no loss of feed value, 2) that the fatty acids clinging to the caryopses have the same value as unhydrolyzed oil in feed, 3) that the glycerol absorbed by the caryopses has the same feed value as comparable weight of starch, 4) that the wet oily oats do not need to be dried because they can be stabilized to an acceptable water activity by mixture with drier feed ingredients, and 5) that there is room in the feed mill to add the needed equipment.

The value of whole oats often seems to be greater than can be accounted for by their content of protein and starch. Seemingly this is because they often are sold for food purposes or in nitch markets, such as horse feed, where they command a high price. For these calculations we have assumed the value of the caryopses in a feed mixture is the same as that for whole oats.

Assumptions:

1. 32 lbs/bushel of oats (buying rate)
2. 70 % recovery of groats on dehulling
3. Cost of oats: $ 2.00/bushel for ordinary oats
4. Cost of tallow $ 0.15/lb
4. It will take 5 days to achieve 95 % hydrolysis
5. Molecular weight of tallow = 870
6 Glycerol in feed is sold at the rate of corn starch i.e. $ 0.12/lb
7. Hulls have no economic value
8. Calculations are based on one truckload i.e. 48000 lbs of caryopses
9. Three reactor tanks with oil moving to fresh caryopses after 2 days
10. At least 175 batches will be processed per year
11. Labor cost will be 30 hrs/batch @ $ 20/hr
12. 95% hydrolysis adds $ 0.05/lb to the value of fat

A. Calculation for Reactor capacity:

48000 lbs of caryopses = 1866 ft^3 oats
Reactor capacity = 2053 ft^3
Tallow solution fits into the spaces between the oats

Reaction tank dimension: 8′ diameter, 45° cone and 67.2 feet total height, 57.2′ straight height measurement.

Material: Mild steel

B. Insulation cost:

Surface area for insulation: $2\pi rL + \pi r\{(r^2 + h^2)^{1/2}\}$ where
$r = 4'$, $h = 10'$ and $L = 57.2'$
$= 1437.59 + 135.34$
$= 1572.93\ ft^2$

Surface area of 1572.93 ft^2 is to be covered at a cost of $ 2.75/ft^2[a]

Therefore the cost of insulation will be $ 4,325.00

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[a]Values supplied by Mr. Robert Riley, Feed and Energy Co. Des Moines, IA

C. Accessories:[a]

a.	Controller(40°C), President Series	$ 450.00
b.	Flow monitor, Hersey or equivalent	$ 1,500.00
c.	Recirculation pump, mild steel, 20 gal/min	$ 1,200.00
d.	Basket filter (Ronneger- Peter type, dual)	$ 1,200.00
f.	Initial storage tank	$ 12,000.00
g.	Fatty acid storage tank(mild steel)	$ 18,000.00
h.	Reaction tank foundation	$ 10,000.00
i.	Conveyor from reaction tank	$ 10,650.00
	Subtotal.....	$ 55,600.00

D.. Labor to install all necessary equipment[a]

(2 men, 2 wks, 50 hrs/wk, 200 man hrs @ $ 35/hr)	$ 7000.00
Crane rental	$ 500.00
Miscellaneous	$ 1500.00
Subtotal.....	$ 9000.00

A. FIXED COST:

1. Reactor	3 x 20,000[a]	=	$ 60,000.00
2. Insulation	3 X 4,300[a]	=	$ 12,900.00
3. Accessories	3 x 55,600[a]	=	$ 166,800.00
4. Dehuller	1 x 10,000[a]	=	$ 10,000.00
5. Construction	3 x 9,000[a]	=	$ 27,000.00
	Fixed cost		$ 276,700.00
Insurance and Tax (2% of fixed cost = Ulrich, 1984)			$ 5,534.00
			$ 282,234.00

Amortize the fixed cost over 10 years, therefore,

the fixed cost per year	=	$ 28,223.00
Interest(6%) on $ 282,234.00	=	$ 16,934.00
Total fixed cost	=	$ 45,157.00

[a]Values supplied by Mr. Robert Riley, Feed and Energy Co. Des Moines, IA

Materials:

a. Oats:

For the reactor, 48000 lbs of oats= 68572 lbs of undehulled oats = 2143 bushels and $ 2.00/bushel will cost $ 4,286.00

b. Tallow: 48000 lbs, Tallow at $ 0.15/lb would cost $ 7,200.00

c. Water: We need 9600 lbs of water = 154 ft^3. Water costs $ 0.95/100 ft^3 ~ $ 2.00

B. OPERATING COST:

We will process about 175 batches per year

1.	Oats	$ 4,286.00
2.	Tallow	$ 7,200.00
3.	Water	$ 2.00
3.	Labor (30 hr/batch @ $20/hr)	$ 625.00
		$ 12,113.00 per batch
		x 175 batch = $ 2,119,775
4.	Maintenance(3% of capital[a])	$ 8,467.00

Total operating cost = $ 2,128,242.00

Total cost = Fixed cost + Operating cost

= 45,157.00 + 2,218,242.00
= $ 2,263,399.00

Returns:

A. Value of wet oily oat = Value of oats + Value of tallow clinging to tallow(1.5%) + Value of glycerol at value of starch
= 48000 lbs of oats + 720 lbs of tallow + 4822 lbs of glycerol

$ value respectively = 4,286 + 108 + 579
= $ 4,973.00

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[a]Ulrich, 1984

B. Value of fatty acids (95% split).
48000 lbs tallow - 4822 lbs of glycerol - 720 lbs clinging to oats + 2830 lbs water incorporated
= 45288 lbs x 20 cents/lb = $ 9,058.00

C. Total returns = 4,973 + 9,058 = $ 14,031.00/batch x 175 batch
= $ 2,455,425.00

D. Credits = $ 2,455,425.00 - 2,263,399.00 = $ 192,026.00 profit

E. Tallow processed: 48000 x 175 batch = 8400000
Profit/lb of tallow = $ 0.023

F. Return on investment = 192,026.00/2,82,234 x 100 = 68.04%

Since the oats and tallow are assumed to lose no value when used in this process, the chief expenses are equipment and labor. Profitability depends on the number of batches that can be processed/year. Probably after a batch of caryopses is used to process the second or third stage of the hydrolysis, they will still not be strongly inhibited by glycerol accumulation and can be used to process additional oil. This should increase the profits even more, but the optimum use of partially spent oats will depend on their particular lipase level.

Case 2. Use of high lipase oats

Assume we obtain oats with specially high lipase activity by plant breeding or other means. We suppose that we have to pay a premium of $ 0.05/bushel to have these grown but that the used caryopses do not have any increased value when used in feed mixture.

Note that the equipment estimates are the same as before.

COSTING:

A. FIXED COST

Reactor tank	$ 20,000.00
Dehuller	$ 10,000.00
Insulation	$ 4,300.00
Accessories	$ 55,600.00
Construction	$ 9,000.00
total.....	$ 98,900.00

Insurance & Tax (2 % of fixed cost[a]) = $ 1,978.00

Total fixed cost = $ 100,878.00

Amortize fixed costs over 10 years. Therefore, the fixed cost per year = 100,878.00/10 = $ 10,088.00 + $ 6053.00 (6 % interest on $ 100,878.00
Fixed cost = $ 16,141.00

Materials:

a. Oats:

For the reactor, 48000 lbs of oats= 68572 lbs of undehulled oats = 2143 bushels and $ 2.05/bushel will cost $ 4393.00

b. Tallow: 48000 lbs, Tallow at $ 0.15/lb would cost $ 7,200.00

c. Water: We need 9600 lbs of water = 154 ft^3. Water costs $ 0.95/100 ft^3
~ $ 2.00

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[a]Ulrich, 1984

C. OPERATING COST

oats	$ 4,393.00
water	$ 2.00
tallow	$ 7,200.00
labor	$ 225.00
	$ 11,820.00 per batch x 70 batch
	$ 827,400.00 +
maintenance	(3% of capital) = $ 3,026

Total operating cost = $ 830,426.00

Total cost = fixed cost per year + operating cost

= 16,141.00 + 830,426.00

= $ 846,557.00

Returns:

A. Value of wet oily oats= Value of oats + Weight of fatty acids clinging to oats (1.5%) + weight of glycerol at value of starch

Weight of oily oats= 48000 lbs of oats + 720 lbs fatty acids + 4822 lbs of glycerol

Dollar amount respectively= $ 4,286.00 + 108.00 + 579.00
= $ 4,973.00

B. Value of fatty acids (95 % split)

Weight of fatty acids = Weight of tallow - Weight of glycerol + 3 moles of water

= 48000 lbs - 720 lbs to oats - 4822 lbs(glycerol) + 2830 lbs(water)
= 45288

Value of tallow fatty acids = 45288 x 20 cents/lb
= $ 9,058.00

C. Total returns = Value of wet oily oats + value of fatty acids
= 4,973.00 + 9,058.00
= 14,031 per batch, x 70
= $ 982,170.00/yr

D. Credits:

Profit = $ 982,170 - 846,557
$ 135,603.00

Tallow processed 48000 lbs x 70 = 3360000 lbs
Profit per lb = 135,603.00/3360000 = $ 0.040

E. Return on investment = 135,603.000/100,878 x 100 = 134.42% return on investment.

Case 3. Use of fines and high lipase oats

Oat caryopses can be "scalped" in a fluidized be for extra lipase. This can be used to accelerate hydrolysis. From Fig 14 and 15, we can infer that this process will take only 2.5 days to achieve 95% hydrolysis.

COSTING:

A. FIXED COST

Reactor tank	$ 20,000.00
Dehuller	$ 10,000.00
Insulation	$ 4,300.00
3,Fluidized bed vessel[a] (@ $ 5000 each)	$ 15,000.00
3, Fans(@ $1000 each)[a]	$ 3,000.00
3, Basket filter for fines(@ $ 1200 each)[a]	$ 3,600.00
Accessories	$ 55,000.00
Labor (construction)	$ 9,000.00
total.....	$119,900.00

Insurance & Tax (2 % of fixed cost[a]) = $ 2398.00

Total fixed cost = $ 122,298.00

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[a]Ulrich, 1984

Amortize fixed costs over 10 years. Therefore, the fixed cost per year = 122,298.00/10 = $ 12,230.00 + $ 7,338.00 (6 % interest on $ 122,298.00

Fixed cost = $ 19,568.00

Materials:

a. Oats:

For the reactor, 48000 lbs of oats= 68572 lbs of undehulled oats = 2143 bushels and $ 2.05/bushel will cost $ 4393.00
For producing 4800 lbs of fines = 54,549 lbs of oats = 77,927 lbs of undehulled oats = 2435 bushels will cost $ 4,992.00

Therefore, total cost on oats = $ 4,393.00 + $ 4,992.00
= $ 9,385.00

b. Tallow: 52800 lbs, Tallow at $ 0.15/lb would cost $ 7,920.00

c. Water:

We need 10560 lbs of water = 169 ft^3. Water costs $ 0.95/100 ft^3

~ $ 2.00

d. Scalping the oats for fines

From each 100 gram of oats, we will get 84.8 gram dry scrubbed oats, 4.8 gram bran and 8.8 gram fines.

Therefore, we need 48000 lbs x 0.10 (10% incorporation)
= 4800 lbs fines=2182 kg fines

For each 0.088 kg of fines we need 1 kg of caryopses
Therefore 2182 kg of fines =24,795 kg of caryopses = 54,549 lbs of caryopses

54,549 lbs caryopses- 800 lb fines = 49,749 lb scrubbed oats + bran

e. Calculation for the cost of air:

Oats in grams to be processed:

$$\frac{54549 \text{ lbs} \times 1000 \text{ gram/kg}}{2.2 \text{ lbs/kg}} = 24795000 \text{ grams oats to be processed}$$

$$\text{Requirement of air} = \frac{2.4795 \times 10^6 \text{ grams oats} \times 2.73 \text{ Lit/sec}}{100 \text{ grams of oats}}$$

$$= 676904 \text{ lit/sec}$$

$$= 678 \text{ cubic meter/sec}$$

Assume we run 100 1-h batches in three fluidized bed
Therefore, we need approximately 6.78 m^3/sec/bed
Temperature correction : 6.78 x 273/298 = 6.21 m^3/sec/

$/cubic meter = $5x10^{-5}xq^{-0.30}$Ln p (for process module from Ulrich, 1984). Where q=cubic meter/sec and p = bar pressure

$5x10^{-5}$ x $6.21^{-0.30}$ x Ln 2

= $5x10^{-5}$ x 0.5782 x 0.6931

$/cubic meter/sec = 2.00^{-5}
= 7.21 $/ 100 hr

B. OPERATING COST

oats	$	9,385.00
water	$	2.00
tallow	$	7,920.00
air blow...	$	7.00
labor	$	1,200.00 (33.3 hrs @ $ 36/hr)
		18,514.00/batch x 140 batches
	$	= 2,591,960.00 operating cost + $ 3669.00 Maintenance (3 % of capital cost)

Total ...$ 2,595,629.00

Total cost = fixed cost per year + operating cost

= 19,568.00 + 2,595,629.00

= 2,615,197.00

Returns:

A. Value of oats= Value of dry oats + Fines + value of dry scrubbed oats + Weight of fatty acids clinging to oats (1.5%) + weight of glycerol at value of starch

Weight of oats= 48000 lbs of oats + 4800 lbs of fines + value of 49749 lbs of dry scrubbed oats + 792 lbs fatty acids + 5304 lbs of glycerol

Dollar amount respectively= $ 4,714.00 (oats) + $ 4,442.00
+ $ 119.00 + $ 636.00
= $ 9,911.00

B. Value of fatty acids (95 % split)

Weight of fatty acids = Weight of tallow - Weight of glycerol + 3 moles of water

= 52800 lbs - 792 lbs to oats - 5304 lbs (glycerol) + 3113 lbs(water)
= 49817 lbs

Value of tallow fatty acids = 49817 x .20 cents/lb
= $ 9,963.00

C. Total returns = Value of oats + value of fatty acids
= 9,911.00 + 9,963.00
= 19,874.00 per batch, x 140
= $ 2,782,360.00/yr

D. Credits:

Profit = $ 2,782,360.00 - 2,595,629.00
$ 186,731.00

Tallow processed 52800 lbs x 140 = 7392000 lbs
Profit per lb = 186,731/7392000 = $ 0.025

E. Return on investment = 186,731/122,298 x 100 = 152.68% return on investment.

To produce lipase concentrate

Capital costs

Fluidized bed	$ 15,000.00
Fans	$ 3,000.00
Basket filter	$ 3,600.00
Total	$ 21,600.00
Tax and Insurance	$ 432.00
	~$ 22,000.00

Amortize over 10 years = $ 2,200 + $ 1320 interest =
$ 3,520/year

Operating costs

Oats	$ 4,992.00
Air	$ 7.00
Labor	$ 1,200.00

$ 6,199.00/batch x 210 batch/yr = $ 1,301,790.00

Total costs = Capital + Operating + Maintenance
$ 3520.00 + $ 1,301,790.00 + $ 106.00
= $ 1,305,416.00

Credits:
1. 49,749 lbs of dry scrubbed caryopses = $ 4,442.00/batch
= $ 932,820.00/year

Loss = 1,305,416.00 - 932,820.00
= $ 372,595.00

Therefore, to break even our lipase should sell,

$$\frac{372,595}{4800 \text{ lbs fines x } 210 \text{ batches}}$$

= $ 0.36/lb

Sigma Chemical Co., St. Louis, MO sells *Candida cylindracae* type VII lipase of similar activity at $ 382/lb

We will produce 1,008,000 lbs of enzyme/year (4800 lbs x 210 batches). Assume, we get $ 155/lb, our return would be $ 156,240,000.00 - 372,595.00 loss

Net return = $ 155,867,405.00

Return on investment = 708,488 %

Case 4. The dilution of tallow with hexane

The cost of this process depends strongly on the cost of equipment to recover hexane from the fatty acids and oat.

COSTING:

A. FIXED COST

Reactor tank	$ 20,000.00
Dehuller	$ 10,000.00
Insulation	$ 4,326.00
Fluidized bed vessel	$ 5,000.00
Fan	$ 1,000.00
Basket filter for fines	$ 1,200.00
Desolventizer-toaster[a]	$ 240,000.00
Accessories	$ 55,600.00
Labor(construction)	$ 9,000.00
total.....	$ 346,126.00

Insurance & Tax (2 % of fixed cost[b]) = $ 6,923.00

Total fixed cost = $ 353,049.00

Amortize fixed costs over 10 years. Therefore, the fixed cost per year = 353,049.00/10 = $ 35,305.00 + $ 21,183.00 (6 % interest on $ 353,049.00)
Fixed cost = $ 56,488.00

Materials:

a. Oats:

For the reactor, 48000 lbs of oats= 68572 lbs of undehulled oats = 2143 bushels will cost $ 4286.00
For producing 4800 lbs of fines = 53933 lbs of oats = 77047 lbs of undehulled oats = 2408 bushels will cost $ 4,816.00

Therefore, total cost on oats = $ 4,286.00 + $ 4,816.00
= $ 9,102.00

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[a]Crown Iron Works Company, Minneapolis, MN
[b]Ulrich, 1984

b. Tallow with hexane dilution:Tallow : hexane (50:50). For 1:1 ratio of oats to mixture(tallow:hexane), we need 48000 lbs of oats + 4800 lbs of fines = 52800 lbs of mixture, of which 26400 lbs of tallow and 26400 lbs (or 4792 gallons) of hexane (sp. gr.= 0.660)

Tallow at $ 0.15/lb would cost $ 3960.00
Hexane at $ 1.09/gallon would cost $ 5223.00

c. Water:

We need 10560 lbs of water = 169 ft^3. Water costs $ 0.95/100 ft^3

~ $ 2.00

d. Cost of air

$/cubic meter = $5x10^{-5}xq^{-0.30}$Ln p (for process module from Ulrich, 1984). Where q=cubic meter/sec and p = bar pressure

$5x10^{-5}$ x $676^{-0.30}$ x In 2

= $5x10^{-5}$ x 0.1420 x 0.6931

$/cubic meter = 4.9^{-6}

We have 676 cubic meters/sec

Therefore the cost would be 4.9^{-6} x 676
= 0.33 cents/sec
= 19.80 cents/min
= 11.88 $/hr
= 12.00 $/hr

B. OPERATING COST

Operating cost for desolventizer toaster:

The desolventizer-toaster system operates with 50% hexane and 50% tallow at a rate of 600 lbs/hr[a]. The extracted oat groats leaves the system with 2% residual oil at a rate of 600 lbs/hr. The energy efficiency is 70%[b]. Heat capacity of hexane is 6.896 kcal/mol and molecular weight of hexane is 86.

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[a]Crown Iron Works Company, Minneapolis, Minn.

[b]Anderson, 1981

Energy required for 600 lbs with 50% miscella

$$= \frac{6.896 \text{ kcal/mol} \times 456 \text{ g/lb} \times 600 \text{ lbs}}{86 \text{ g/mol} \times 2}$$

$$= 10982 \text{ kcals/hr}$$

Seemingly for oats, hexane and tallow with 2% residual oil the energy requirement would be 439 kcal/hr.

Assume the heat capacity of oat is 0.5 cal/g and the oat is heated from 25°C to 60°C.

To heat 600 lbs of oats, the energy requirement would be 0.5 cal/g x 600 lbs x 456 g/lb x 35 (temp. difference) =4788000 cal/hr = 4788 Kcal/hr

Total energy requirement = 10982 + 439 + 4788 = 16209 kcal/hr. With 70% efficiency, we need 16209/0.7 = 23156 kcal/hr = 23156 kcal/hr x 41840 J/Kcal = 968847040 Joules/hr.

One giga (billion) joules energy costs $ 5.5[a]
There 968847040 joules will cost $ 5.30/hr
Assume the plant runs for 88 hrs/batch, therefore the energy cost will be $ 470

oats	$	9,102.00
water	$	2.00
tallow	$	3,960.00
hexane	$	5,223.00
energy costs	$	470.00
air blowing	$	12.00
labor	$	9,000.00
Total.....	$	27,769.00 X 70 batches

= $ 1,943,830.00 operating cost + $ 10,600.00 maintenance (3% on capital cost)

= $ 1,954,430.00

Total cost = fixed cost per year + operating cost

= 56,488.00 + 1,954,430.00

= 2,010,918.00

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[a]Ulrich, 1984

Returns:

A. Value of wet oily oats= Value of dry oats + Fines + add weight of glycerol at value of starch + value of tallow clingging to oats

Weight of oily oats= 48000 lbs of oats + 4800 lbs of fines + 2652 lbs of glycerol + 528 lbs fatty acids clingging to oats

Dollar amount respectively= $ 9,102.00(oats) + $ 318.00 + $ 47.00
= $ 9,467.00

B. Value of fatty acids (95 % split)

Weight of fatty acids = Weight of tallow - Weight of glycerol + 3 moles of water

= 26400 lbs - 2652 lbs(glycerol) - 528 lbs clingging to oat + 1557 lbs(water)

= 24777 lbs

Value of tallow fatty acids = 24777 x 20 cents/lb = $ 4,955.00

C. Recovery of hexane (90%)

4792 gallons x 0.90 = 4313 gallons x $ 1.09/gal = $ 4,702

Total returns = Value of wet oily oats + value of fatty acids + value of recovered hexane
9,467.00 + 4,955.00 + 4,702.00
= 19,124.00 per batch, x 70 = $ 1,338,680.00/yr

D.

Loss = $ Total cost - return
2,010,918.00 - 1,338,680.00
$ 672,238.00/yr

Return on investment = - 672,238/353,049 = - 190.41%

Glycerol recovery

Glycerol can be recovered from the used caryopses by circulating water through the bed of caryopses for 4-6 hrs. We assume that the caryopses will absorb a maximum of approximately 47% water. We extract with a volume of water equal to that of the oil.

$$\frac{48{,}000 \text{ lbs oil}}{870 \text{ lbs/mol}} = 55.17 \text{ mols} = 5076 \text{ lbs glycerol}$$

At 95% hydrolysis about 1.5 x 5% of the glycerol is still combined in mono- and diglyceride =	380 lbs
	4686 lbs free glycerol

Wash water = 48,000 x 1.1 (density correction)	=	52,800 lbs
20% x 48, 000(added water)		9,600 lbs
12% x 48,000 (moisture in the field dry oats		5,760 lbs
		68,160 lbs
55.17 mols x 3 x 18 lbs/mol (moisture used up in hydrolysis)	=	2,979 lbs
At 95% hydrolysis	=	2,830 lbs
		65,330 lbs

Water in caryopses = 48,000 x 0.47 = 22,560 lbs

$$\frac{22{,}560}{65{,}330} = 34.5\ \%$$ of water and glycerol in caryopses

Total glycerol extracted = 4,696(1.00 -0.345) = 3,076 lbs
Price = $ 0.5/lb = 0.5 x 3,076 = $ 1,538.00

Assume wet oats can be absorbed in dry feed without further drying, therefore, 42,770 lbs of water must be evaporated.

Cost justification for glycerol-water evaporator

$$\frac{42{,}770 \text{ lbs of water to evaporate} \times 1000 \text{ g/kg}}{2.2 \text{ lbs/kg} \times 18 \text{ g/mol}} = 1{,}080{,}50 \text{ mols}$$

$1{,}080{,}050 \text{ mols} \times 9717 \text{ cal/mol} = 1.0495 \times 10^{10} \text{ cal}$

$1.0495 \times 10^{10} \text{ cal} \times 4.184 \text{ J/cal} = 4.3910 \times 10^{10} \text{ J}$

Heat transfer coefficient U in $J/s.m^2.K$ estimated at 1000[a]

Assume 108 hrs to evaporate the water

$$\frac{4.3910 \times 10^{10}}{108 \times 60 \times 60} = 112937 \text{ J/sec}$$

$$\text{Heat transfer Area} = \frac{112{,}937}{1000 \times \Delta T} = \text{Approximately } 11.3 \text{ m}^2 \text{ if } \Delta T=10.$$

Evaporator cost approximately $\$ 2.10^5 \times 2.9 \times 1.3 = \$ 754{,}000$ (Ulrich, 1984)

Amortize $\$ 754{,}000/10 = \$ 75{,}400/\text{yr} + \$ 45{,}240$ interest
$= \$ 120{,}640/\text{yr}$

Assume natural gas at $\$ 5/10^9$ J and 70% efficiency

$$\frac{4.391 \times 10^{10} \text{ J} \times \$ 5.0}{10^9 \times J \times 0.7} = \$ 314$$

Cost = equipment + operating + loss of reactor time + loss of glycerol credit in feed
= $ 1,723/batch + $ 314 + 0.05 x $ 1,937 + $ 579
= $ 2,713

Loss/batch = $ 2,713 - $ 1,538
= $ 1, 157
Return on investment = -0.15%

This process does not appear feasible except in a plant with excess evaporator capacity.

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[a]Ulrich, 1984