Hydrolysis of Fats and Oils with Moist Oat Caryopses

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To improve the economic feasibility of hydrolyzing fats and oils with moist oat caryopses, various factors affecting the efficiency of the process were studied. Caryopses produced with an impact-type dehuller exhibited greater lipase activity than those produced by a wringer-type dehuller. Abrasion of oat caryopses against each other in a fluidized bed released particles rich in lipase. Such lipase concentrates could be added to moist caryopsis reactors to speed fat hydrolysis. Beef tallow, lard, soybean oil and crambe oil were hydrolyzed more efficiently than corn oil, castor oil and milk fat. The poor hydrolysis of castor oil was attributed to the formation of esters with the hydroxy group of ricinoleic acid, and the hydrolysis of castor oil was increased by dilution of the substrate with hexane. Diglycerides inhibited the hydrolysis and accounted for the slower hydrolysis of corn oil. Hydrolysis of milk fat by moist oat caryopses resulted in preferential hydrolysis of C_6 to C_{10} acids. Erucic acid was released from crambe oil at significantly slower rates than the other acyl groups. High conversions of fats and oils to free fatty acids could be attained by (i) exposing the fats and oils to two to three lots of moist caryopses, (ii) the use of special oat varieties with elevated lipase content, (iii) the addition of oat lipase concentrates to moist caryopsis reactors, and (iv) dilution of the substrate with hexane. Estimates of the cost of producing free fatty acids with these processes indicated that the first three should be profitable. Growth of *Clostridium sporogenes* spores could not be demonstrated in caryopsis reactors. During the incubation of moist oat caryopses immersed in oil, the free fatty acid content of the internal caryopsis lipid increased only slightly, but there were changes in its fatty acid composition.

KEY WORDS: Fat hydrolysis, lipase, oats.

Currently, commercial hydrolysis of fats and oils to fatty acids and glycerol is accomplished by the energy- and capital-intensive Colgate-Emery process. Alternatively, fats and oils may be hydrolyzed with lipases under mild conditions and with simple equipment, but so far lipase hydrolysis has not been commercially competitive because of the cost of lipase and the difficulties of recycling, attaining complete reaction and isolating the reaction products (1).

Oat caryopses have long been known to be rich in lipase, and, typically, the first step in processing dehulled oats is a steam treatment to destroy lipase (2,3). Martin and Peers (4) first demonstrated that oat lipase occurred on the surface of caryopses and was capable of hydrolyzing both tributyrin and triolein. Urquart *et al.* (5) and Ekstrand *et al.* (6) verified that the lipase of oats occurs in the aleurone layer. Lee and Hammond (7,8) explored the possibility of using the oat caryopses as a lipase bioreactor for the hydrolysis of triglycerides, and Piazza *et al.* (9) studied the properties and substrate specificity of oat lipase, Parmar (10) reported the effect of variety and growth location on the lipase activity of oats.

Lee and Hammond (7) reported that when moist caryopses were immersed in oil, the lipase on the surface of the oats released fatty acids. The reaction slowed as glycerol accumulated in the moist oats and fatty acids accumulated in the oil phase. The reaction could be pushed to 90% completion by exposing the partly hydrolyzed oil to fresh moist caryopses after the reaction with the first lot of caryopses had slowed. Glycerol could be extracted from the spent caryopses with water, and the reaction ran well at 40°C. This paper further studies the hydrolysis of fats and oils by moist oat caryopses and examines various strategies for the practical exploitation of the process.

MATERIALS AND METHODS

Oats were procured from the Agronomy Department, Iowa State University (Ames, IA) and were stored at 4°C and 40% relative humidity. Unless otherwise noted, oats were dehulled by an impact-type dehuller (Wintersteiger G.m.b.H., Reid, Austria). A wringer-type dehuller was used for some studies (Quaker Oats Co., Chicago, IL).

Crambe oil was obtained from the Center for Crops Utilization Research at Iowa State University. Tallow was procured from Feed and Energy Co. (Des Moines, IA) or by rendering suet. Milk fat was prepared by centrifuging melted butter. Other fats and oils were obtained commercially. Randomization of soybean and corn oils was done according to Lau *et al.* (11).

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 a column containing 8 g alumina. The polar substances retained on the alumina were eluted with three 15-mL portions of distilled diethyl ether (12). The ether eluate was chromatographed on thin-layer plates, and the individual components were added back to 10 g of soybean oil to test their action on oat lipase.

Lipase hydrolysis was accomplished by two methods, unstirred reactors and circulating reactors. In the unstirred reactors, 10 g of oat caryopses were uniformly moistened with 20% water in a 50-mL conical flask. After 2 h of equilibration, the moistened oat caryopses were covered with 10 g of oil, and the reaction mixture was incubated at 40°C. For the circulating reactor, a 60 × 4.5-cm glass column was encased in a helix of 6.35-mm o.d. copper tubing through which water was circulated at 40°C unless otherwise specified (Fig. 1). The column was filled with 150 g of oat caryopses, moistened and equilibrated with 20% water. Next, 150 g of oil was added to the column, an amount that just covered the caryopses, and the oil was circulated by means of a peristaltic pump, usually at a rate of 0.017 $Lhr^{-1}cm^{-2}$. Samples were periodically withdrawn from both types of reactors, and free fatty acids were determined according to Lee and Hammond (7). The circulating reactor gave faster hydrolysis than the unstirred reactor during the early stages of reaction, but both methods tend to the same end point with time.

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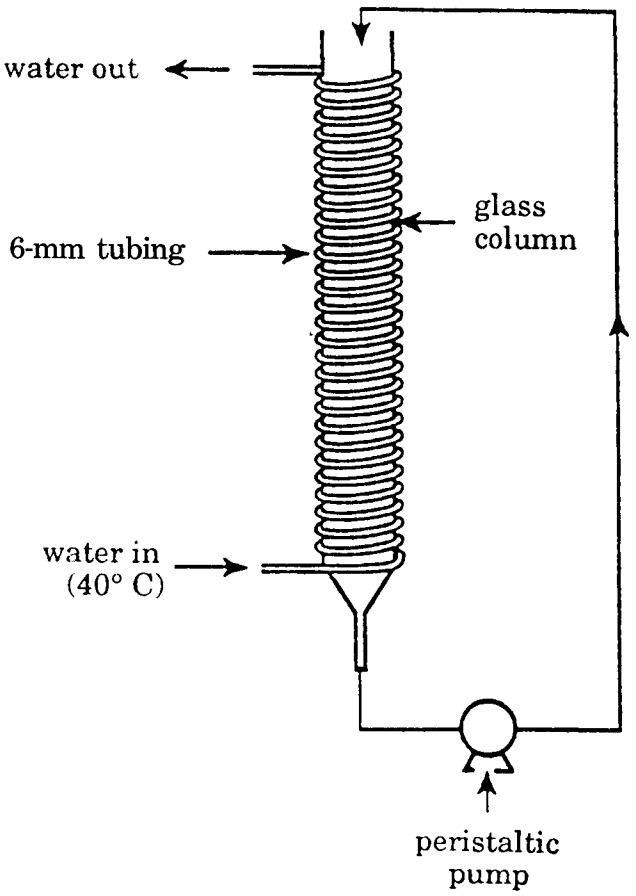

FIG. 1. Diagram of the apparatus for circulating fats and oils through a bed of moist oat caryopses.

Triglycerides and free fatty acids were separated on thin-layer plates of silica gel G by development in hexane/diethyl ether/acetic acid (84:15:1, vol/vol/vol). The plates were sprayed with 0.2% 2′7′-dichlorofluorescein in 95% ethanol and viewed under ultraviolet light. Bands were scraped from the plates, eluted with diethyl ether, and the solvent was evaporated under nitrogen. Except for milk fat, fatty acids and triglycerides were converted to methyl esters with methanolic sulfuric acid and analyzed by gas chromatography (7). Free fatty acids from milk fat were analyzed by the procedure of Vangtal and Hammond (13).

Lipase was removed from the surface of oat caryopses by abrasion in a fluidized bed (Fig. 2). The bed consisted of a 76 × 6.35-cm glass column through which compressed air was passed. The exit of the pipe was fitted with a filter, consisting of two layers of 25-mesh cheesecloth supported by a 40-mesh wire screen, and an air-flow meter (Meriam Instruments, Cleveland, OH). The column was loaded with 100 g of oat caryopses, and air was passed for various times and flow rates. Broken and intact caryopses were separated by sieving after the desired treatment. The abraded material (1 g) was added to caryopses (10 g) in which the native oat lipase had been inactivated by boiling in water for 6 min. The boiled caryopses were dried overnight at 60°C, and 20% moisture was added before use.

To recover glycerol from spent caryopses after hydrolysis of soybean oil in the circulating reactor, the oil was drained, the caryopses were washed with 250 mL hexane to remove the residual oil and 250 mL water was circulated at 0.29 $Lh^{-1}cm^{-2}$ for 12 h. The water was sampled hourly and analyzed for glycerol by the method of Molever (14).

To determine the effect of moist caryopsis reactors on the native caryopsis lipid, 10-g samples of caryopses were placed in a series of 50-mL conical flasks, moistened with 20% water, and contacted with 10 g of soybean oil or mineral oil. The flasks were incubated at 40°C, and every two days the oil was drained from one of the flasks, and the caryopses were washed with hexane to remove surface oils. The caryopses were ground, and the native oat lipids were extracted with chloroform–methanol (15).

To test the ability of clostridia to grow in moist caryopsis reactors, caryopses were inoculated with 1000 spores/g of *C. sporogenes (PA 3679)* by making a suspension of the spores in the water used to moisten the caryopses. The wet caryopses (100 g) moistened with 20% water were transferred to a 53 × 4.5-cm glass column and covered with 100 g soybean oil. The oil was drained after one week at room temperature. Samples (1 g) of ground wet, oily caryopses and the oil phase were diluted and plated on Bacto Brewer anaerobic agar (Difco, Detroit, MI), and the plates were incubated at 37°C for 48 h.

RESULTS AND DISCUSSION

To use moist oat caryopses to hydrolyze fats and oils economically, it is important to have caryopses with high lipase activity and to operate the bioreactors under optimum conditions. A number of factors affecting the lipase activity were examined.

Effect of dehuller type. There was about 20% more lipase activity on caryopses dehulled with an impact-type dehuller as compared with a wringer-type dehuller. In the impact dehuller, a rotating fan threw the oats against a wall, whereas the wringer dehuller squeezed the caryopses out of their glumes as they moved between two rollers. The wringer-type dehuller probably rubbed more of the surface lipase off the caryopses and onto the glumes.

Temperature. A careful comparison of reactions at 38, 40 and 42°C in the circulating reactor verified the previous studies that found the optimum temperature to be approximately 40°C.

Isolation of oat lipase concentrates. Previous work (7) showed that if external lipase was added to beds of moist oat caryopses, the lipase attached itself to the caryopses and could greatly increase the lipase activity of the caryopses. Previous methods of making oat lipase concentrates were not practical (4,7,9). We found that the lipase on the surface of oat caryopses could be abraded by rubbing caryopses against one another in a fluidized bed, and the abraded, lipase-rich particles could be collected with a filter (Fig. 2). An air flux of 0.067 $Ls^{-1}cm^{-2}$ was the minimum for producing a fluidized bed, and a flux greater than 0.1 $Ls^{-1}cm^{-2}$ transported the oats from the apparatus. A flux of 0.086 $Ls^{-1}cm^{-2}$ was considered optimum. Table 1 shows the effect of various residence times on the recovery of abraded material and the integrity of the caryopses. The losses could probably be significantly reduced by additional filters or an electrostatic precipitator.

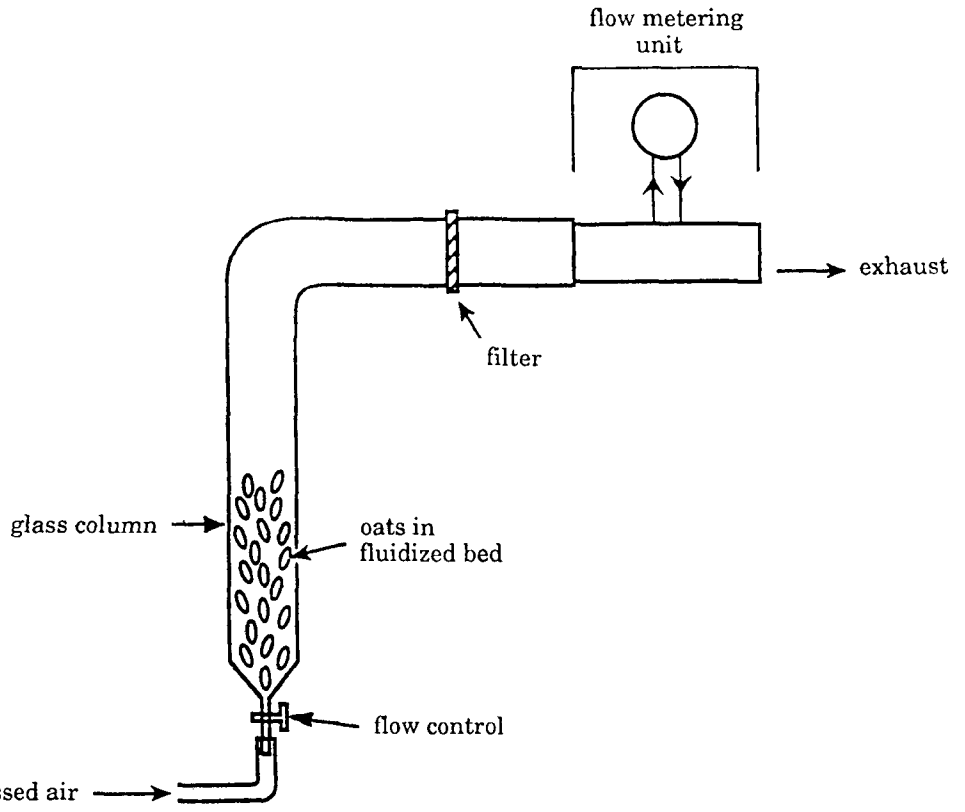

FIG. 2. Diagram of the apparatus for producing a lipase-rich concentrate from oat caryopses in a fluidized bed.

TABLE 1

Effect of Various Process Times on the Recovery of Caryopsis Fractions from a Fluidized Bed at an Air Flux of 0.086 $Ls^{-1}cm^{-2}$

Time (h)	Whole caryopses (g)	Broken caryopses (g)	Abraded particles (g)	Loss (g)
0.5	91.2	3.3	4.3	1.2
1.0	84.8	4.8	8.8	1.6
1.5	73.9	5.2	16.4	4.5

Figure 3 compares the lipase activity of caryopses abraded for 0, 0.5 and 1 h. As the time in the fluidized bed increased, the residual activity in the caryopses decreased, and about half of the lipase activity was recovered after 1 h. As time in the fluidized bed increased, the activity of the abraded particles decreased about 10% h^{-1}, indicating that lipase was removed first and inert material accumulated on further abrasion. About 1 h was regarded as the optimum residence time. The abraded particles had a lipase activity/unit weight about ten times greater than that of the original caryopses. The lipase activity of the abraded material could be increased further by removal of coarse material by sedimentation in hexane.

Effect of substrate. The percentage of hydrolysis attained for several fats and oils were compared by using caryopses from the oat variety B605-1085, a variety that has only a modest level of lipase. After four days in an unstirred reactor, beef tallow and lard attained over 70% hydrolysis (Table 2). At 40°C, the lard and tallow were just

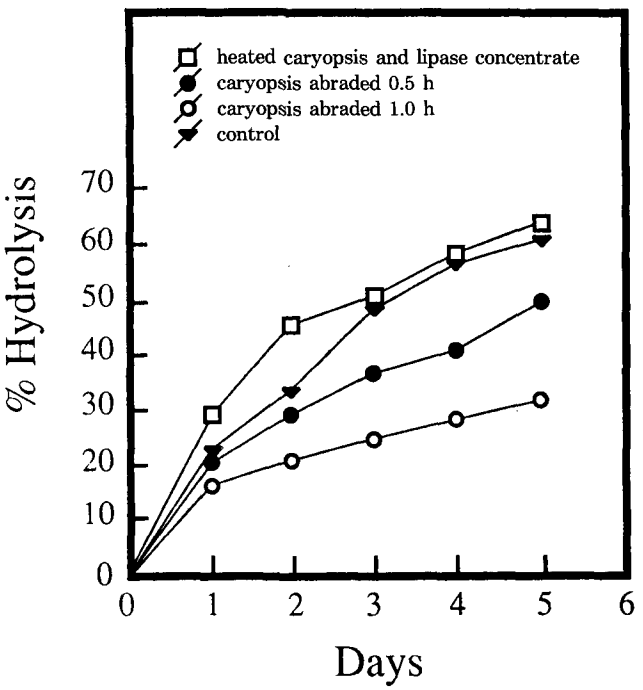

FIG. 3. Lipase activity of caryopses of B605-1085 oats subjected to a fluidized bed for 0.5 and 1 h and lipase activity of heat-inactivated caryopses, treated with 10% by weight of particles abraded for 0.5 h. Activity was measured in unstirred reactors.

TABLE 2

Percentage Hydrolysis with Time of Various Fats and Oils Contacted with Moist Oat Caryopses

	Unstirred reactor				Circulating reactor	
Days	Crambe	Lard	Milkfat	Tallow	Corn	Soybean
1	38	39	24	40	26	42
2	50	49	31	58	36	63
3	61	69	41	75	50	70
4	64	71	49	76	62	83

above their melting points. The hydrolysis of crambe oil was slightly depressed with respect to that of tallow and lard, but milk fat gave significantly less hydrolysis.

Crambe oil contains about 62% erucate, and analysis of released fatty acids showed that they contained only 56% erucic acid. Bias against the hydrolysis of erucate may account for the slightly depressed hydrolysis of crambe oil.

In unstirred reactors, milk fat gave only about 50% hydrolysis after four days. The cause of this low yield was not investigated, but it might be caused by the unique composition of milk fat. Analysis of released fatty acids of milk fat, however, revealed that C_6 to C_{12} fatty acids were released preferentially and accumulated in the free fatty acids in proportions significantly greater than their proportions in milk fat.

Corn oil gave much less hydrolysis than soybean oil in circulating reactors, in spite of the similarity of their fatty acid compositions and glyceride structures of the two oils (Table 2). Randomization of both corn and soybean oil with sodium methoxide did not affect their relative hydrolysis rates. The polar components in corn oil were concentrated on alumina and fractionated by thin-layer chromatography; the individual components were added to soybean oil to give concentrations comparable to their original concentration in corn oil, and the hydrolysis of the soybean oil was tested. The polar fraction from corn oil and the diglyceride fraction isolated from it inhibited hydrolysis of the soybean oil and accounted for the slower rate of hydrolysis of the corn oil. The addition of 5% 1,3-dilinolein to soybean oil also inhibited hydrolysis. It was surprising that intermediates of the hydrolysis reaction had such an inhibitory effect, but previous results have shown that mono- and diglycerides do not accumulate to a great extent in the hydrolyzed fat in the moist caryopses system (7).

In a circulating reactor, castor oil gave only 20% hydrolysis by titration and became very viscous. The percentage of free fatty acid did not increase more than 1% after the first day. Dilution of the castor oil with an equal amount of a 1:1 mixture of hexane and benzene more than doubled the extent of hydrolysis. We believe that the poor hydrolysis of castor oil resulted from the hydroxyl group on ricinoleate becoming esterified, and dilution of the substrate decreased this transesterification.

Conditions necessary for high conversion. For hydrolysis of fat and oil with oat caryopses to be an economically feasible method, it is important to get high conversion in the shortest possible time. The hydrolysis is slowed by product accumulation, but one can achieve good conversions by: (i) use of oats with high lipase content; (ii) the addition of extra lipase to the moist caryopses; (iii) use of more than one batch of caryopses to carry the charge of fat to complete hydrolysis; or (iv) adjustment of the fat/lipase ratio by dilution of the fat with solvent.

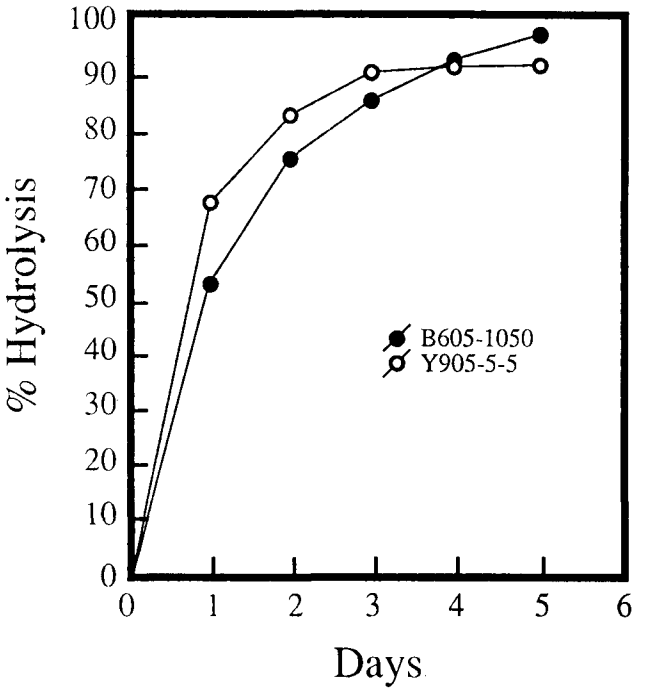

FIG. 4. Hydrolysis of soybean oil by caryopses from immature Y905-5-5 oats, a high-lipase variety (unstirred reactor), and by caryopses from B605-1085 oats, a medium-lipase variety, that were supplemented with a lipase concentrate produced in a fluidized bed (circulating reactor).

The caryopses used in most of the experiments reported here are from the oat variety B605-1085, a medium-lipase oat; its lipase content depends on its growth conditions (10). Y905-5-5, the oat variety with the highest lipase that we know of, has about 2–2.5-fold more lipase than B605-1085 (10). Figure 4 shows that Y905-5-5 (when harvested early, so that its caryopses had an optimum activity to weight ratio gave 93% hydrolysis in three days in an unstirred reactor. By plant breeding, it should be possible to produce lines with lipase activity as high as Y905-5-5 or higher, but there would be extra cost in producing such special lines and keeping them segregated.

One can enrich an oat bioreactor with oat lipase concentrates, obtained by using a fluidized bed of oats. When fines from the fluidized bed were enriched by sedimentation in hexane, and 10% of the fines were added to the caryopses of B605-1085 oats, 96% hydrolysis of soybean oil was achieved in five days (Fig. 4). The production of such lipase concentrates will require the handling of additional oats and the cost of abrading them in a fluidized bed. Other sources of lipase could be used, but oat lipase seems to be the cheapest source.

One also can achieve good conversion by exposing the oil charge to fresh lots of caryopses. Thus, exposure of soybean oil to three batches of moist caryopses from B605-1085, with average lipase activity, for two days each in the circulating reactor gave 95% hydrolysis.

Another way to achieve high conversion is to increase the ratio of oats to substrate by diluting the substrate with an inert solvent such as hexane. This results in the accumulation of lower concentrations of inhibitory end products. About 90% hydrolysis was achieved with 50 and 25% tallow in hexane in 4 and 7 d, respectively, in the circulating reactor. This system has the advantage that the oats can be rinsed free of oil with hexane at the end of the experiment, and not as much fatty acid is left clinging to the caryopses. 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 in which hexane is used would be more expensive to construct in compliance with safety regulations.

Economic analysis. The hydrolysis of fats and oils with moist oat caryopses is a simple process that can be accomplished with simple equipment, but the process requires handling large quantities of oats and has reaction times of several days. The cost of lipase has been an important barrier to its use in processes such as fat hydrolysis, and this has led to various recycling schemes (1). An advantage of the moist caryopses process is that oat lipase is regarded as having no value, and the hydrolysis process does little to decrease the value of the oats as feed. The moist, glycerol-laden caryopses that issue from the process may be subjected to microbial attack, but if the process is operated as part of a feed mill, it should be possible to stabilize the moist caryopses by mixing them with other dry feed ingredients. The glycerol in the caryopses and the fatty acids clinging to their surface will be valuable as feed ingredients.

Estimates of the cost of hydrolyzing fats and oils with variations of the moist caryopses process were made by using standard engineering methods (16) for a reactor unit holding 21,800 Kg of caryopses. It was assumed that the caryopses could be stabilized by mixing with other feed ingredients, and that the fatty acids and glycerol attached to the spent caryopses had the value in feed of tallow and starch, respectively. Free fatty acids were assumed to be worth +0.11/Kg more than the starting fat or oil. High-lipase oats were assumed to command a premium of $0.0034/Kg. The estimates are shown in Table 3. The cost of housing for the reactors and other equipment was not considered in the calculations.

Use of multiple lots of ordinary caryopses to hydrolyze fats appears to be economically feasible. Three reactors were necessary in this process, so the greater investment resulted in a lower return on investment. Even greater profits should be realized by optimizing the use of each lot of caryopses. The high costs of safety features for a plant that handles hexane, and the cost of removing hexane from the spent oats made this process unfeasible. High-lipase oats would decrease the profits/reactor slightly because of their additional cost, but they would increase return on investment because the process time would be reduced. The recovery of lipase concentrates from oats, by using a fluidized bed and adding the concentrate to a moist caryopsis reactor, increased profits and return on investment. The production of such concentrates may be a feasible process in itself.

Characteristics of the spent caryopses. For the hydrolysis of fats and oils to be an economically feasible process, it is important that the spent caryopses be suitable for feed. In some instances, moist, nonacid foods preserved by immersion in oil have been subject to the growth of *C. botulinum* (17). It is not clear that the high acidity of the lipid phase will protect the caryopses from the growth of clostridia. To test this, the caryopses in a lipase bioreactor were inoculated with spores of *C. sporogenes*, a nontoxin-producing *Clostridium* with growth characteristics similar to those of *C. botulinum* (18). After one week in the bioreactor, the caryopses were tested and no increase of *C. sporogenes* could be detected. It was tentatively concluded that no toxin would be produced under the conditions used.

TABLE 3

Economic Analysis of the Hydrolysis of Fats and Oils with Moist Oat Caryopses

Process	Profit/year 1000 ($)	Return on investment (%)
Ordinary oats		
Three-stage hydrolysis	192	68
Dilution with hexane[a]	−673	−191
High-lipase oats		
Alone	136	134
+Oat lipase concentrate	187	153

[a]Cost of special equipment estimated by Crown Iron Works (Minneapolis, MN).

The internal lipids present in oat caryopses were tested before and after storage of the moist caryopses in both soybean and mineral oil to see if this lipid also underwent hydrolysis and if there were changes in its fatty acid composition. The caryopsis lipid had a starting value of 2% free fatty acid, and after 8 d, free fatty acid increased to 4 and 8% for caryopses immersed in mineral and soybean oil, respectively. In both samples, the percentage of oleate in the lipid decreased significantly during storage, and the linoleate percentage increased.

Glycerol recovery is an important economic consideration in the Colgate-Emery process. In the moist caryopses process, over 90% of the glycerol is found inside the caryopses (7). If the oil is drained from the caryopses and replaced with sufficient water to just cover the caryopses, the glycerol can be leached from the caryopses. This process can be accelerated by gentle circulation of the water. Thus, in a circulating reactor where 83% hydrolysis of soybean oil had been achieved, the spent caryopses were subjected to circulating water, and after 6 h the amount of glycerol in the water became constant. From the circulating water, 66% of the theoretical amount of glycerol was recovered. The primary limitation on glycerol recovery was the proportion of water inside the caryopses, which will absorb a total of nearly 50% of their weight of moisture. The glycerol is recovered from the caryopses in a dilute solution, and attempts to increase recovery would likely require more water. The equipment and utility cost to evaporate the water will slightly exceed the value of the glycerol at current prices and result in a −0.15% return on investment.

Glycerol recovery efficiency could be increased if the caryopses could be reduced in size and extracted with several smaller portions of water, but this would also result in extraction of additional water-soluble components of the caryopses.

Reuse of the spent and extracted caryopses for further fat hydrolysis would require careful drying to the correct moisture level and results in caryopses with diminished hydrolytic capacity, possibly because of loss of surface lipase and residual glycerol content. This does not appear to be a practical process.

In conclusion, the use of moist oat caryopses to hydrolyze fats and oils is an attractive process that has potential to compete with current methods. The preparation of lipase concentrates by abrading caryopses in fluidized beds also appears to be competitive with other commercial lipase preparations. Increased costs of fossil fuels should make this process even more attractive relative to current methods of fat hydrolysis.

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