

16. Biocatalytic Transformation of Fats and Oils^{1,2}

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Over the past several years our laboratory has been evaluating the application of biocatalysis to fats and oils. An example of the types of biocatalytic reactions studied include the regiospecific determination of triacylglycerol structures using 1,3-specific lipases. Other lipase reactions that have been successfully applied to fats and oils include the exploitation of the fatty acid selectivities of lipases for obtaining enriched erucic acid and γ -linolenic acid (GLA) fractions from rape seed oil and borage seed oil fatty acids, and for reducing the α -linolenic acid (ALA) content of soybean oil. Another biocatalytic process that we have studied employed the enzyme lipoxygenase for the positional and enantio selective introduction of the hydroperoxide functionality into polyunsaturated fatty acids (PUFA) and PUFA-containing glycerides. The unsaturated hydroperoxy derivatives obtained subsequently were reduced to hydroxy acids or enzymatically cleaved to oxo-fatty acids by the enzyme hydroperoxide lyase.

16.1 Introduction

The use of biocatalysts in transformations involving fats, oils, partial glycerides and fatty acids and their derivatives is well documented. One area where considerable effort is currently being expended is the study of the chemistry of lipases (triacylglycerol hydrolase, EC 3.1.13). More specifically, there has been a recent surge of interest in the application of lipases that exhibit either positional selectivity or fatty acid specificity. A second area of biocatalysis that has seen a resurgence of recent interest is the use of the enzyme lipoxygenase for the oxygenation of polyunsaturated fatty acids. It is the intent of this paper to provide an overview of this laboratory's recent and current research thrusts in the prospective applications of these enzymes to fats and oil chemistries.

16.1.1 Lipase analysis of triacylglycerols

Recently, two extremely useful procedures for determining lipase regioselectivity in reactions with homogeneous triacylglycerols (TAG) were reported (1,2) that also have utility for determining triglyceride structures. The methods require the partial hydrolysis of TAG with highly 1,3-specific lipases followed by the analyses of the partial acylglycerols formed (3). We have applied this lipase approach to the regiospecific determination of specific TAG structures. The procedure (5) is based on the partial hydrolysis of TAG in organic solvent, followed by chromatographic isolation of liberated free fatty acids (FFA) and diacylglycerols (DAG) and their subsequent analysis by gas chromatography (GLC). The results obtained with the symmetrical triglycerides PSP, OLO and OMO are given in Table 16.1. The lipases used included a commercial

TABLE 16.1 Fatty acid composition of free fatty acid (FFA) and 1,2 (2,3) diacylglycerol (DAG) fractions from partial hydrolysis of triacylglycerols (TAG) with 1,3-selective and acyl specific lipases³

TAG ⁴	Fatty acid ⁵	<i>M. miehei</i> ⁶		TAG	<i>R. oryzae</i> ⁷		<i>G. candidum</i> ⁸	
		FFA	DAG		FFA	DAG	FFA	DAG
PSP	C16:0	90	55	66	93	53		
	C18:1	10	45	34	7	47		
OLO	C18:1	95	52	67	96	51		
	C12:0	5	48	33	4	49		
OMO	C18:1	92	52	66	96	51		
	C14:0	8	48	34	4	49		
POS	C16:0			33			3	48
	C18:1			33			95	4
	C18:0			33			2	48

¹ Mention of brand or firm name does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

² Presented in part at the 21st World Congress of the International Society for Fat Research, The Hague, The Netherlands, October 1995.

³ Lipase reactions carried out to 12–15% hydrolysis with supported lipases in isooctane for 2 h; reaction temperature; PSP: 40°C; OLO, OMO and POS: 30°C. See reference 5 for experimental details.

⁴ TAG designation: PSP = 1,3-dipalmitoyl-2-stearoyl-*sn*-glycerol; OLO = 1,3-dioleoyl-2-lauroyl-*sn*-glycerol; OMO = 1,3-dioleoyl-2-myristoyl-*n*-glycerol; POS = 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol.

⁵ Fatty acid designations: C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 = oleic acid; C12:0 = lauric acid and C14:0 = myristic acid.

⁶ Lipozyme IM™.

⁷ *Rhizopus oryzae* lipase supported on silical gel (6).

⁸ *Geotrichum candidum* lipase supported on silica gel (7).

immobilized preparation of *M. miehei* (Lipozyme IM) and the silica supported lipases *R. oryzae* and *G. candidum* (6). Previous work on supported lipases has shown that the essential character of a given enzyme, as judged by fatty acid selectivity or stereoselectivity, is not impaired by immobilization onto this support (7). Each supported enzyme contained sufficient bound water (10%) to reach the degree of hydrolysis (>15%) needed to assess the regioselectivity of the lipases in organic solvent (isooctane). As shown in Table 16.1, for the 1,3-selective lipases both the FFA and DG distribution of fatty acids were predictive of the regioposition of acyl groups on the glycerol backbone for each TAG. In general, the data obtained with the supported *R. oryzae* lipase suggested that it was a more 1,3-selective lipase than was the *M. miehei* lipase but these differences were within experimental errors. Moreover, the data show that under the conditions used acyl migration was minimal. The apparent lower 1,3-selectivity of both lipases towards PSP, however, was consistently different with that observed for the other TAG, but this may be a temperature effect. In short, a simple but direct regiospecific determination of the triglyceride structures can be inferred by analysis of the FFA fractions alone. However, if a more rigorous proof of structure is needed, analysis of both the FFA and DAG fractions should be done. Also given in Table 16.1 are data obtained for the regioselective analysis of the triglyceride, POS using the lipase of *G. candidum*, a lipase that is selective for δ -9 unsaturated fatty acids (8,9). In this case characterization of this TAG structure required the isolation of the FFA and 1,3-diglyceride fractions.

16.1.2 Fatty acid selectivity

Recently, we have given considerable attention to the lipases of the fungus *Geotrichum candidum* because of the high substrate selectivity expressed by isozymes of this lipase for unsaturated fatty acids that contain a *cis*-9-double bond. We have compared the relative reactivity of several long chain fatty acids in esterification catalyzed by isozymes of the *G. candidum* lipase (9). Esterification reactions were selected because enhanced lipase selectivity was expected (10, 11). As shown in Table 16.2 there is a distinct preference in reactivity of the *G. candidum* lipase for oleic acid compared to the saturated acids, palmitic and stearic. As expected, the reactivities of linoleic (δ -9,12) and linolenic (δ -9,12,15) acids were similar to oleic acid, whereas petroselinic (δ -6) and γ -linolenic (δ -6,9,12) acids, which possess unsaturation sites closer to the carboxyl, were much slower reacting. Recent studies (11) have reported that other lipases,

including those from rape (*Brassica napus*), *M. miehei*, and *C. cylindracea*, also discriminate against fatty acids where the double bond is located near the carboxyl end of the chain (δ -4, 6 and 8). We also found that increasing the carbon chain length from C₁₈ to C₂₀, yet retaining the *cis*-9-unsaturation (9-docosenoic acid), Table 16.2 also resulted in decreased reactivity. More importantly, the data in Table 16.2 show that with either increasing fatty acid chain length (behenic acid), change in double bond position towards the terminus end of the carbon chain (erucic acid), or chain substitution (ricinoleic acid) results in a marked decrease in fatty acid reactivity with this lipase. These results indicate that *cis*-9 unsaturation is not a sufficient selectivity criterion for *G. candidum* lipase and that both chain length and/or substitution on the chain are important criteria when considering fatty acid selectivity.

We also examined the effect of alcohol structure on the esterification selectivity of the *G. candidum* lipase with palmitic and oleic acid used as substrates. The data in Table 16.2 show that compared to 1-butanol, when 2-methyl-1-butanol was employed, there was a twenty-fold increase in selectivity of oleic to palmitic acid. Similar but less pronounced effects in enhanced lipase selectivity were obtained with other branched alcohols. These observations suggest that one can further improve fatty acid selectivity in lipase catalyzed esterification reactions by judicious choice of the alcohol substrate.

16.1.3 Lipase harvesting of fatty acids

The expression of a lipase's selectivity for or against a given fatty acid structure can be exploited for the isolation of industrially or nutritionally important fatty acids from fats and oils. As stated above, the expression of a lipase's selectivity is more pronounced in the esterification mode than in the hydrolysis mode. Using this selectivity concept, we recently developed a two-step process for obtaining highly enriched erucic acid fractions from high erucic acid rapeseed (HEAR) oil (10). Erucic acid was a targeted fatty acid because it has several important industrial applications. The first step of the process was the total hydrolysis of HEAR oil using the lipase from *P. cepacia*. The latter lipase is a suitable catalyst for the total hydrolysis of triglycerides because the enzyme exhibits neither positional nor fatty acid selectivity. In the next step, the lipase of *G. candidum* was used to catalyze the esterification of the free fatty acids (FFA) of HEAR oil with 1-butanol. Because the *G. candidum* lipase strongly discriminates against certain fatty acids (see Table 16.2) the erucic acid was concentrated in the FFA fraction. The results are summarized in Table 16.3. In a reaction conducted up to 52% conversion, the ester fraction contained 12.5% butyl erucate and the residual FFA fraction contained 85.4% erucic acid. The HEAR oil used initially contained 47.5% erucic acid; therefore, the erucic acid content in the FFA fraction represented a total recovery of 86% of the amount originally present in the oil. This two-step process also was used for the enrichment of γ -linolenic acid (GLA), a (*n*-6)-polyunsaturated fatty acid, in borage oil FFA (9). Borage oil is an excellent source of this nutritionally important fatty acid, as GLA comprises 25% of the fatty acids of this oil. The data in Table 3 show that in this manner one can obtain an acid fraction that contains >70% GLA with a total recovery of 95% of the GLA in the oil. Similar enrichments in GLA from primrose oil have been obtained with *M. miehei* lipase, though in lower absolute amounts because of the lower GLA (10%) content in the oil (12).

TABLE 16.2 Relative reactivity of fatty acids in esterification with butanol catalyzed by *Geotrichum candidum* lipase¹

Acid	Designation	Relative reactivity to oleic acid ²
Palmitic	16:0	40
Stearic	18:0	2 ²
Petroselinic	18:1(<i>n</i> -12)	10
Oleic	18:1(<i>n</i> -9)	100
Linoleic	18:2(<i>n</i> -6)	100
Linolenic	18:3(<i>n</i> -3)	100
Ricinoleic	18:1(<i>n</i> -9,12-OH)	2
γ -Linolenic	18:3(<i>n</i> -6)	1
Behenic	22:0	2
9-Docosenoic acid	22:1(<i>n</i> -13)	3
Erucic	22:1(<i>n</i> -9)	1

¹Values are the average of 2-4 determinations (8). The lipase used was GC-4 from Amano Co.

In the harvesting of targeted fatty acid it is advantageous to use immobilized lipase preparations for practical considerations, such as enzyme reuse and ease of product isolation. To address

TABLE 16.3 Esterification of high erucic acid rapeseed (HEAR) oil and borage oil fatty acids by lipases¹

Substrate	Lipase ²	Conversion ³ (%)	Acid ⁴ (%)	Yield ⁵ (%)
HEAR oil	<i>G. candidum</i>	52E	12.5	86
		48A	85.4	
Borage oil	<i>G. candidum</i>	67E	1.8	95
		33A	71.8	
	<i>G. candidum</i> /Si	64E	7.5	80
		36A	55.5	
	<i>M. miehei</i>	59E	8.4	78
41A		47.2		

¹ HEAR oil and borage oil free fatty acids esterified with 1-butanol in hexane (7,10).

² *G. candidum*/Si is *G. candidum* lipase supported on silica (6); *M. miehei* lipase is Lipozyme.

³ Conversion expressed as % acids esterified to butyl esters (E); % unreacted fatty acids (A).

⁴ Wt% erucic acid or γ -linolenic acid in ester fraction and acid fraction, respectively.

⁵ Total wt% recovery of erucic acid or γ -linolenic acid originally present in HEAR oil (47.5%) and borage oil (24.9%), respectively.

As shown in Table 16.3, the selectivities of both the supported lipases were equally effective in concentrating the GLA of borage oil FFA in esterification reactions. For both supported enzymes, GLA was recovered to the extent of about 80% in the FFA fraction. Additionally, the supported lipases could be recycled and the recovery of GLA was about 70% after two additional re-uses.

Because the lipases of *G. candidum* have such a high selectivity for *cis*-9-unsaturated fatty acids, one can envision processes that are targeted to obtaining products enriched in such fatty acids. A recent example of this type application reported the selective hydrolysis of oleoyl-containing triglycerides, e.g., tallow and lard, for obtaining fatty acid fractions enriched in oleic acid (13). Moreover, polyunsaturated fatty acids that have a *cis*-9 double bond as the first site of unsaturation from the carbonyl group also are preferred by *G. candidum* lipases (Table 16.2). We have exploited this selectivity to reduce the α -linolenic (ALA) content in soy oil by studying the lipase-catalyzed interesterification of soy oil with oleic acid. Reduction of ALA content in soybean oil was sought as one possible way of improving the keeping quality of the oil. Table 16.4 gives the results obtained with a supported acyl-specific *G. candidum*

lipase, and with the supported 1,3-specific *R. oryzae* lipase. For comparative purposes, Table 16.4 also includes similar data obtained with *M. miehei* lipase (14). Briefly, the results obtained with the 1,3-specific lipases were comparable in that both enzymes gave reduction of ALA at 5°C from an initial content of 7.5% to 2.0%. On the other hand, the ALA content of soy oil was reduced to 2% by room temperature acidolysis with oleic acid using the *G. candidum* lipase. As expected, for all instances there was a concomitant decrease in linoleic acid and an increase in oleic acid content in the restructured oils.

16.2 Immobilized lipoxygenase

Lipoxygenase (LOX) from several sources has the ability to introduce the hydroperoxide moiety into a polyunsaturated fatty acids both with regioselectivity and stereospecificity, Scheme (linoleic acid to 13-HLA). The resulting chiral hydroperoxide derivatives have potential as useful synthons for further chemical transformations (15–18). For LOX to be a useful enzyme in synthetic reactions, it would be desirable to have a very stable immobilized preparation of the enzyme that could be used repeatedly. While the literature on lipase immobilization is extensive, there are few reports that describe ways to immobilize LOX (19–21). Recent work in our laboratory has focused on the immobilization of soybean LOX (Sigma, Lipoxidase, Type 1-B) on a commercially available carbonyldi-imidazole activated support (Pierce, Reacti-Gel 6X) (19). The urethane linkage that is formed when a protein is bound to this support is about 20-fold more stable than the N-substituted iso-urea linkage formed during protein immobilization onto cyanogen bromide-activated matrices (20). Our immobilized preparation of LOX was extremely stable, retaining 95% of its activity after 6 months storage at 5°C. However, at 15°C this immobilized LOX preparation had reduced stability, but its stability was still much greater than that of free LOX. For example, the half-life of free LOX was estimated to be 7 hours, whereas the half-life of immobilized LOX was estimated to be 75 hours by replotting the activity data on a logarithmic scale and using a linear least-squares regression fit (19).

The enzymatic activity toward linoleic acid of lipoxygenase immobilized on Reacti-Gel 6X was determined at 15°C in mixtures containing organic solvent and aqueous buffer in which air was the sole source of oxygen (17). In the absence of aqueous buffer, 21% of the linoleic acid was converted to hydroperoxyoctadecadienoic acid (13-HLA, Scheme) using the supported enzyme. Addition of aqueous buffer, up to 35% (v/v), gave a 3-fold increase in 13-HLA formation. Under these conditions, the rate of 13-HLA formation was maximal at

TABLE 16.4 Lipase-catalyzed acidolysis of soy oil oleic acid¹

Enzyme ²	Time (h)	T (°C)	Fatty acid composition (wt%) ³				
			C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}
	0		10.9	3.7	21.5	56.4	7.5
<i>G. candidum</i>	5	25	7.9	2.2	40.7	45.7	3.5
<i>G. candidum</i>	10	25	6.8	3.0	39.3	48.9	2.0
<i>R. oryzae</i>	8	10	6.9	2.0	38.2	49.6	3.3
<i>M. miehei</i> ⁴	4	5	6.9	1.0	48.7	41.5	2.0

¹ Reactions were carried out in hexane solution at 1:1 molar ratio of oleic acid to triglyceride at 200 rpm. Triglyceride fraction was isolated by preparative thin layer chromatography.

² *M. miehei* lipase is Lipozyme; *G. candidum* and *R. oryzae* lipases are supported on silica.

³ Values listed are average of 2–3 determinations as wt% methyl esters of triglyceride fraction as determined by gas-liquid chromatography.

pH 9–9.5. The effect of organic solvent on the lipoxygenase-catalyzed formation of 13-HLA also was examined. Best yields of 13-HLA were obtained when 1,1,2-trichlorotrifluoroethane was the solvent. Approximately equal amounts of 13-HLA were formed when the solvents hexane, heptane, octane and 2,2,4-trimethylpentane were used. Poor conversions of linoleic acid to 13-HLA were obtained in toluene and cyclohexane, and the poorest conversions of linoleic acid to 13-HLA were in reaction mixtures that contained diethyl- or di-isopropyl ether. No 13-HLA formation was detected in the solvents 2-butanone or 2-octanone. These results are consistent with other work that shows that in general polar organic solvents are detrimental to enzymatic activity (17).

16.3 Substrates of lipoxygenase

LOX from different sources and LOX isozymes from the same source showed different degrees of specificity with regard to whether the carboxylic acid moiety is free or esterified. The high pH form of LOX (lipoxygenase 1) from soybean is highly specific for free linoleic acid under most conditions, whereas LOXs from other sources are able to oxidize phospholipids that contain polyunsaturated fatty acids (PUFA). It is well documented that LOX from mammalian sources can oxidize PUFA-containing phospholipids (22), as well as membranes and lipoproteins that contain primarily esterified PUFA (23). However, some isozymes of LOX from plants can oxidize PUFA-containing membrane fractions and purified phospholipids (24, 25).

Early work on the high pH form of soybean LOX showed that in the presence of Tween 20, free linoleic acid was rapidly oxidized, but the methyl ester of linoleic acid and trilinolein were poor substrates for lipoxygenase (26). It was concluded that *in vivo*, lipoxygenase requires the prior action of a lipase or phospholipase to release free polyunsaturated fatty acid before lipoxygenase reacts. Modification of this view was required when it was discovered that soybean LOX had significant activity with phosphatidylcholine in the presence of bile salts, such as deoxycholate, cholate, and taurocholate (26). With the surfactants Tween 20, Triton X-100, Tween 80, sodium dodecyl sulfate (SDS), and octyl glucoside no oxidation occurred. Except for SDS, LOX inactivation by the detergents was eliminated as a reason for the lack of PUFA oxidation. In addition, it was shown that release of free fatty acid from phosphatidylcholine did not occur before oxidation.

This study (26) was extended to an examination of the structure of the oxidized arachidonyl and linoleoyl moieties in phosphatidylcholine that had been exposed to soybean LOX in the presence of deoxycholate (27), where it was shown that regio- and stereospecific hydroperoxide formation did occur. Additionally, when this oxidation was conducted in ^{18}O -labeled water there was no incorporation of ^{18}O in the product, eliminating the possibility of hydrolysis and reesterification during oxidation.

Recently the reaction of soybean LOX, in deoxycholate-containing media, with phospholipids, neutral glycerides and the methyl ester of linoleic acid has been investigated (26 and Piazza *et al.*, unpublished results). These studies were performed with free LOX. Presumably the advantages of using immobilized LOX on linoleic acid would also apply with esterified substrates, but this has yet to be demonstrated. Listed in Table 16.5 are the amounts of oxidation products formed by the action of LOX on several substrates in fifteen minute assays. Linoleic acid, methyl

TABLE 16.5 Relative amounts of oxidized linoleate by lipoxygenase (LOX)¹

Substrate	Oxidation (%) ²
Linoleic acid	100 ± 6
Methyl linoleate	90 ± 8
1-Monolinolein	126 ± 6
1,3-Dilinolein	55 ± 4
Trilinolein	3 ± 1
1-L,2-S,-3-P-choline ³	7 ± 3
1-P,2-L,-3-P-ethanolamine ^d	13 ± 2

¹Oxidations conducted at pH 8 in buffer containing deoxycholate as surfactant (26).

²Data are average of 3–5 determinations

³1-Linoleoyl-2-stearoyl-3-phosphatidylcholine

^d1-Palmitoyl-2-linoleoyl-3-phosphatidylethanolamine

one-half the rate of linoleic acid oxidation. Trilinolein, phosphatidylethanolamine and phosphatidylcholine were oxidized slowly. Methyl linoleate and trilinolein are approximately equally soluble in the assay buffer, and thus their highly different rates of oxidation by LOX must be due to steric constraints at the active site. The pH profiles for optimal reactions of soluble soybean LOX upon trilinolein and 1,3-dilinolein range between pH 8.0–9.0. The oxidation rate diminishes at higher and lower pH values, though action against trilinolein decreases more slowly than 1,3-dilinolein as the pH is reduced.

To better understand the oxidation of dilinolein and trilinolein by LOX, complete reaction time courses for these substrates were followed. These data showed that only 15% of available linoleoyl residues in trilinolein were oxidized, and increasing the amount of lipoxygenase did not increase the extent of oxidation. Although direct evidence is lacking, this result may be due to reaction inhibition by the oxidation product. In contrast to the results obtained with trilinolein, 67% of the available linoleate residues in dilinolein were converted to hydroperoxide by lipoxygenase. When dilinolein oxidation was monitored using HPLC, all the dilinolein was eventually oxidized, but besides mono- and dihydroperoxides, other, unidentified polar materials were formed. Although exact yields have not yet been determined, clearly both methyl linoleate and monolinolein are acceptable substrates for soybean lipoxygenase.

16.4 Hydroperoxide lyase

Hydroperoxyoctadecadienoate, 13-HLA, can be cleaved enzymatically to an oxo-carboxylic acid by hydroperoxide lyase (HPLS, Scheme). Membrane-bound HPLS from higher plants produces a twelve carbon oxo-carboxylic acid from 13-HLA (27), whereas the water soluble HPLS from the unicellular algae *Chlorella pyrenoidosa* and *Oscillatoria* sp. yields a thirteen carbon acid (Figure 16.1; references 27–29). The thirteen carbon oxo-acid can be easily oxidized to a dicarboxylic acid, which

TABLE 16.6 Purification of hydroperoxide lyase (HPLS) from *C. pyrenoidosa*

	Vol. (mL)	U ¹ /mL	Yield (U)	Specific activity ²
Homogenization	24.5	1.11	27.2	216
French press	14.0	3.60	50.3	265
Acetone powder	35.0	4.24	149.0	1,050

¹One unit (U) is defined as the loss of one nmol of substrate (13-HLA)

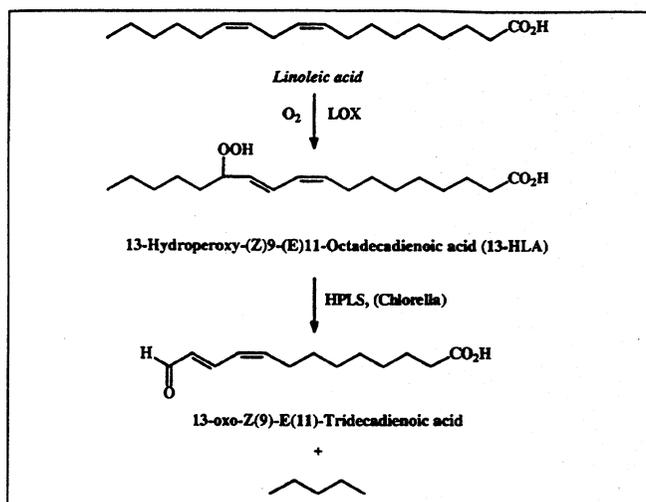


Fig. 16.1 Scheme for enzymatic cleavage of hydroperoxyoctadecadienoate using hydroperoxide lyase from *Chlorella pyrenoidosa*

then can be used to produce a polyamide similar to nylon 13,13, a polymer that has excellent dimensional stability, low affinity for moisture, high dielectric constant and good melt properties (30). The enzymatically assisted production of nylon 13,13 would be an environmentally safe, potentially cost-effective technology, compared to the production of nylon 13,13 from the ozonolysis of erucic acid (30).

Although algae are an attractive source for obtaining HPLS, algae cell walls are usually difficult to lyse, and the recovery of HPLS is poor. Because of these problems, we have developed an effective procedure for obtaining HPLS from algae and determined which strains of *Chlorella* contain significant amounts of this enzyme (27). Previous work with *C. pyrenoidosa* and *Oscillatoria sp.* used mechanical homogenization for the breakage of cells (28, 29). However, we found from microscopic inspection of the homogenized cultures of *C. pyrenoidosa* that only a small percentage of the cells were lysed by this method. Homogenization was particularly poor for the *C. vulgaris* strain. Accordingly, a more effective procedure was needed to improve the recovery of HPLS. We developed the alternative techniques where cells also were lysed by passing them through a French press or by preparing an "acetone powder" with cold acetone. The French press treatment increased both the total protein and HPLS activity compared to the homogenization method (Table 16.6). These results were consistent with microscopic observations that showed few intact cells remained after French press treatment. However, HPLS activity was found only in those fractions that eluted after the bulk of the total protein, possibly because protease or HPLS inhibitors also were liberated by the French press method.

The acetone powder method was found to be most effective for extraction of HPLS. Although the amount of extracted protein was similar to that obtained by the homogenization method, HPLS activity increased 5-fold, as can be seen from the increase of specific activity (U/mg of protein) in Table 16.6. Another benefit of the acetone powder preparation is that acetone removes the bulk of the chlorophyll, and the cellular extract can be assayed directly for HPLS activity without subjecting it to ion exchange chromatography, as was required for the other extraction procedures. Acetone powder extracts could be stored for several days at -10°C without significant loss in HPLS activity.

In earlier studies, it was found that *Chlorella pyrenoidosa* and *Oscillatoria sp.* contained a heat labile, water soluble HPLS enzyme (28, 29). No other strains of algae have been reported to

C. fusca and assayed them for the presence of HPLS using *C. pyrenoidosa* as a reference. The *C. vulgaris* and *C. sorokiniana* strains did not contain detectable levels of HPLS. In contrast, *C. fusca* contained HPLS levels comparable to those of *C. pyrenoidosa* after cycling the culture four times. After partial purification, HPLS isolated from *C. fusca* retained its activity up to six days at room temperature.

16.5 Conclusion

In this paper we have described several potential biotechnology applications for the preparation of value-added products from fats, oils and other lipids. The use of microbial lipases in hydrolyses and interesterification technology of lipids is now well documented with more recent studies being directed to exploiting the selectivities of lipases for lipid biotransformations. Now that lipoxygenase has been immobilized, the enzyme can be used as an efficient catalyst for the oxygenation of unsaturated fatty acids. Other results indicate that lipoxygenase has the potential to act upon a variety of structurally disparate molecules which should enhance the usage of lipoxygenase in biotechnological applications.

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