ENZYMATIC ISOLATION AND ENRICHMENT OF ERUCIC ACID FROM HEA SEED OILS: CURRENT STATUS

C. Tao, B. B. He

ABSTRACT. *Erucic acid (EA), a fatty acid of 22-carbon chain with one isolated double bond, has broad industrial applications. Currently, erucic acid is isolated from* Crucifereae *plant oils through steam splitting, namely the Colgate-Emery process, followed by fractional distillation. This two-step process involves drastic conditions, low energy efficiency, and extensive product degradation. Enzymatic approaches, on the other hand, have many advantages including mild operating conditions and high selectivities. To develop a more efficient alternative process, researchers have studied enzymatic approaches for EA isolation and enrichment for about two decades. Lipases have shown three types of specificities in catalyzing high-erucic-acid (HEA) oils, namely fatty-acid-specific, region-specific, and non-specific. With lipases of certain specificities, various processes of hydrolysis, esterification, interesterification, and transesterification have been studied. Research has also been conducted on investigating the effects of process parameters, including operating temperature, lipase content, and water content, on the process efficiency. The enzymatic approach has shown its potential in isolating and enriching EA from different* Crucifereae *seed oils. This article reviews the current status of the studies, especially the performance of different lipases and corresponding enzymatic reactions, for EA enrichment from* Crucifereae *plant oils.*

Keywords. *Enzymatic process, Erucic acid, Lipase specificity, Reaction selectivity, Seed oils.*

Erucic acid (EA) is the common name for *cis*-13-docosenoic acid, a typical example of a very-long-chain mono-unsaturated fatty acid (VLCMFA). EA has 22 carbon atoms in its straight-chain molecule and one double bond between C_{13} and C_{14} (fig. 1). EA is insoluble in water and highly soluble in ethers. Its solubility in ethanol and methanol are 175 g/100 mL and 160 g/100 mL, respectively. It has an iodine number of 74.98, a neutralization value of 165.72, and a melting point of 33.8°C. EA decomposes at 381.5°C under 101.3 kPa before reaching a boiling point (Windholz et al., 1976).

The major source of EA is the high-erucic-acid (HEA) seed oils of the *Crucifereae* family, which includes rapeseed, mustard, crambe, and wallflower, all containing about 45% to 60% EA (Sonntag, 1991). In these seed oils, EA exists as a member of the fatty acids (FA) in triacylglycerols (TAG), also known as triglycerides (TG). Chemically, almost all EA are present at only two positions, namely *sn*-1 and *sn*-3 (fig. 2), of the TAGs, although these two positions are not exclusively occupied by EA (Brockerhoff and Yurkowski, 1966; Grynberg and Szczepanska, 1966; Taylor et al., 1994).

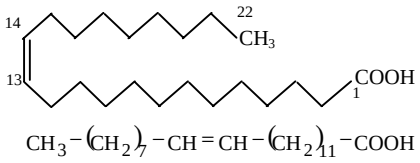

Figure 1. The structure of erucic acid.

In spite of the concern about its safety for human consumption, EA is a very important raw material in the oleochemical industry. Researchers have found that EA and its derivatives possess varieties of superior properties in slipping, softening, antifoaming, emulsifying, and corrosion inhibiting. All these properties offer EA and its derivatives wide applications in the production of pharmaceuticals, soaps, detergents, cosmetics, plastics, lubricants, rubbers, coatings, etc. (Carlson and Van Dyne, 1992; Erickson and Bassin, 1997). Sonntag (1991) summarized 214 applications of EA and its derivatives in various industries. It has been shown that EA has the potential to be one of the most important feedstocks for the chemical industry in the 21st century.

EA can be liberated from HEA seed oils by alkaline saponification, followed by acidification. A free fatty acid (FFA) mixture is then obtained for further separation (Hagemann et al., 1962; Vargas-Lopez et al., 1999). To separate EA from other FFAs and purify it, existing technologies, including low-temperature crystallization (Hagemann et al., 1962; Vargas-Lopez et al., 1999), acid soap crystallization combined with urea-complexes (Chobanov et

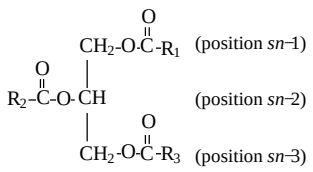

Figure 2. The structure of triglycerides (TGs).

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al., 1965), "silicalite" adsorption and aqueous surfactant separation (Sonntag, 1991), and chromatography (Breuer et al., 1987; Painuly and Grill, 1992; Wilson and Sargent, 2001), have been employed. In current industrial practice, EA is produced from HEA *Crucifereae* plant seed oils by the Colgate-Emery steam splitting process followed by fractional distillation. The Colgate-Emery process is very energy consuming because of its high operating temperature (250°C) and pressure (5 MPa) (Piazza and George, 1999). Furthermore, the drastic reaction conditions not only require expensive splitting facilities but also cause extensive degradation of the liberated FFAs. In fractional distillation, chain cleavage of unsaturated EA occurs when it is heated above ordinary distillation temperatures (Brady et al., 1988).

Alternative processes are of great interest to many researchers in exploring more effective and efficient EA preparation processes. With mild reaction conditions and high selectivities, enzymatic processes provide a potential for efficient EA isolation and enrichment. It is even more promising when combined with other processes such as biodiesel production, where the rest of the FAs resulting from enzymatic EA isolation can be the feedstock for biodiesel production. Triacylglycerol lipases (lipase or triglyceride lipase, E.C.3.1.1.3) catalyze numerous types of enzymatic reactions, including hydrolysis, esterification, transesterification, and interesterification, and have shown potential in processing HEA oils for EA isolation and enrichment (Diks and Bosley, 2000; Mukherjee, 1990; Neklyudov et al., 2002; Pandey et al., 1999). This article aims to review and summarize the studies on lipase specificities, lipase performance under special conditions, and different enzymatic processes in isolating and enriching EA from *Crucifereae* plant oils.

Lipase Specificities for EA Enrichment

Applications of lipases in modification and bioconversion of fats and other lipids were previously reviewed by Mukherjee (1990) and Neklyudov et al. (2002). In addition to the common capability for lipases to cleave ester bonds, some lipases hold special specificities, which are essentially important for EA isolation and enrichment (Piazza et al., 1992; Sonnet et al., 1993a; Stadler et al., 1995; Lee and Parkin, 2000).

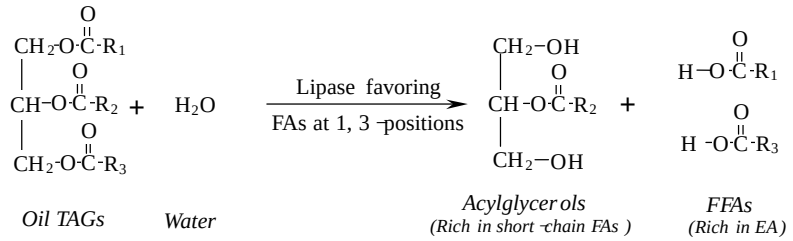
The mold *Geotrichum candidum* is a fungus that has been found growing on the surfaces of sour cream and cheese (Jensen, 1974). This fungus produces multiple extracellular lipases: lipases A and B from *G. candidum* CMICC 335426, and lipases I (major), II, III, and IV from *G. candidum* ATCC 34614 (Sidebottom et al., 1991; Sugihara et al., 1991). Among these *G. candidum* lipases (GCLs), GCL B and GCL I have shown a preference for *cis*-9 unsaturated FAs and discrimination against *trans*-9-containing structures and EA, although the structural basis is still obscure (Holmquist et al., 1997; Sidebottom et al., 1991; Charton and Macrae, 1992). Residues within GCL I sequence positions of 349-406 were considered to be essential in recognizing *cis*-9 fatty acyls (Holmquist et al., 1997; Holmquist, 1998).

In HEA seed oils, most short-chain FAs, including C18:1 (*cis*-9-octadecenoic acid), C18:2 (*cis*, *cis*-9, 12-octadecadienoic acid), C18:3 (*cis*, *cis*, *cis*-9, 12, 15-octadecatrienoic), and C16:1 (*cis*-9-hexadecenoic acid), contain *cis*-9 double bonds. Therefore, the above-mentioned *G. candidum* lipases show strong preference for short-chain FAs and discrimination against C22:1 EA (*cis*-13-docosenoic acid), which contains a single *cis*-13 double bond, in catalyzing HEA oil conversions.

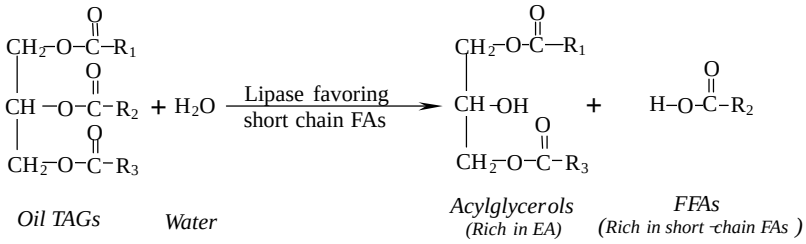
In addition, most EAs only appear at the primary positions (*sn*-1 and *sn*-3) of TAGs. Coincidently, some lipases preferentially attack FAs at certain positions only. This property is called positional specificity, or regiospecificity. Pancreatic lipases, a group of lipases from the overall pancreatic lipase gene family, are well known for their positional specificities in hydrolyzing fat and oil TAGs (Carriere et al., 1998). Only FAs at the primary positions are cleaved by these lipases. Among the pancreatic lipases, the 3-D structure of human pancreatic lipase (HPL) was first determined. The access of its active site was identified to be hindered by three loop structures: β5, β9, and one surface loop named the lip domain (Winkler et al., 1990). It was proposed that the highly hydrophobic β9 loop and the lip domain play major roles in the adsorption of the lipase HPL to lipidic matrix and in the orientation of *sn*-1, 3 acyl chains in the lipid TAGs along the β9 loop. Those acyl chains at the *sn*-1, 3 positions are therefore hydrolyzable. On the contrary, the *sn*-2 acyl chains remain at the center position and always in interaction with the lipidic matrix. As a result, the acyl chains at the *sn*-2 position are not hydrolyzable (Carriere et al., 1998). This strong positional specificity has been explored for the regiospecific analysis of TAGs, the production of structured lipids, and EA enrichment from HEA seed oils.

To isolate and enrich EA from HEA seed oils, various types of lipases have been explored. Although individual lipases may have different mechanisms, these lipases could be grouped into three major categories according to their catalysis preferences. Category I includes lipases from *Geotrichum candidum* and *Candida rugosa* (formerly *Candida cylindracea*), which favor short-chain FAs. Category II includes lipases from porcine pancreas, *Chromobacterium viscosum*, *Rhizopus arrhizus*, *Rhizomucor miehei*, *Mucor miehei*, etc., which favor FAs at the *sn*-1, 3 positions of oil TAGs. Category III includes lipases from *Pseudomonas cepacia*, *Penicillium* sp., *Candida antarctica*, etc., which have no specificity and can completely cleave all FAs in HEA TAGs (Mukherjee, 2000).

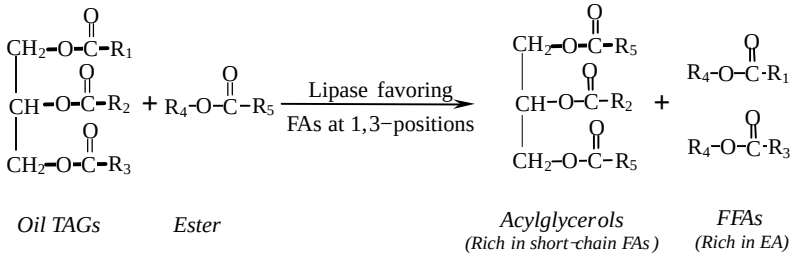
With their catalytic preference, lipases of the first two categories possess the potential for isolating and enriching EA from HEA oils. These lipases preferentially attack specific ester bonds in catalyzing the reactions of HEA oil TAGs and selectively cleave the FA residues in those TAGs. Two fractions result: a fraction of the uncleaved FAs remaining on the glycerol backbone, and the other of cleaved FAs combined with a new acyl acceptor, such as alcohol or water. EA remains untouched and is enriched in the former fraction when the reaction is catalyzed by a lipase from category I. By contrast, EA will be cleaved and enriched in the latter fraction when a lipase of category II is employed. Figure 3 shows the selective distributions of FAs in the two fractions through selected processes catalyzed by lipases from the two categories.



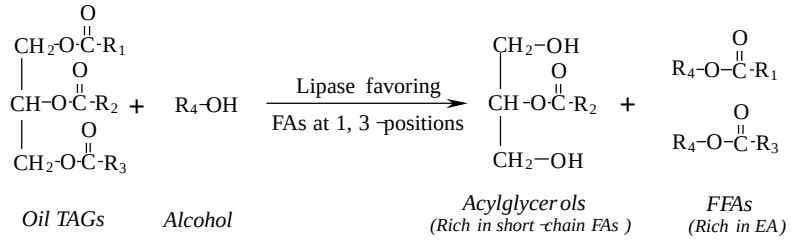
(a) Selective hydrolysis by lipases favoring FAs at 1, 3-positions of oil TAGs.



(b) Selective hydrolysis by lipases favoring short-chain FAs.



(c) Selective interesterification by lipases favoring FAs at 1, 3-positions of HEA oil TAGs.



(d) Selective alcoholysis by lipases favoring for FAs at 1, 3-positions of oil TAGs.

Figure 3. EA enrichment through processes catalyzed by different lipases.

PARTIAL HYDROLYSIS FOR EA ISOLATION

Enzymatic hydrolysis is the most direct enzymatic approach for isolating and enriching EA from HEA seed oils. With water as the acyl acceptor, TAGs can be selectively hydrolyzed by lipases from the above two categories. Two fractions, namely residual acylglycerol (AG) and FFA fractions, are produced. EA residues can be enriched in either fraction. Through selective hydrolysis by lipases favoring FAs at 1, 3-positions of TAGs, EA can be selectively liberated, and thus enriched in the FFA fraction (fig. 4). On the other hand, EA stays and enriches in the AG fraction (mainly *sn*-1, 3-dierucin) through selective hydrolysis if lipases favoring short-chain FAs are used (fig. 5).

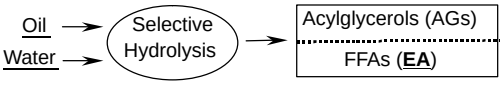


Figure 4. Enrichment of EA through selective hydrolysis by lipases favoring FAs at 1, 3-positions.

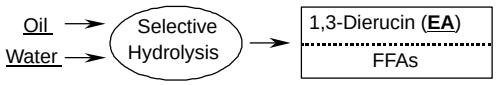


Figure 5. Enrichment of EA through selective hydrolysis by lipases favoring short-chain FAs.

Lipase Investigations

Mukherjee and Kiewitt (1996) compared the hydrolysis of HEA mustard oils by all three categories of lipases. They magnetically stirred mixtures of 2.42 g (2.5 mmol) TAGs and 2 mL water with lipases in sealed screw-capped tubes under nitrogen at ambient temperature (20˚C to 22˚C). Hydrolysis by a lipase either from *Penicillium* sp. or *Candida antarctica* produced only minor differences in the individual FA compositions in the FFA and AG fractions, which were also close to the original individual FA compositions in the oils, indicating less effectiveness in EA enrichment. In contrast, EA was increased from 39% (w/w of acyl moieties) of the starting composition of a white mustard seed oil to 64% and 55% in the AG fractions in 1.25 h by *Candida cylindracea* lipase and in 13 h by *Geotrichum candidum* lipase, respectively, and from 43% of the starting composition of a brown mustard seed oil to 68% in the AG fraction in 1.75 h by *Candida cylindracea* lipase. Correspondingly, lipase from porcine pancreas (pancreatin) enriched EA in FFA fractions to 50%, 65%, and 63% from 39%, 43%, and 44% of EA in the original oils, respectively. With the above two HEA mustard oils, *Rhizopus arrhizus* lipase enriched EA in the FFA fractions to 58% from the initial 39% after 14 h hydrolysis and to 61% from 43% after 38 h hydrolysis. In addition, EA was enriched in the FFA fraction to 50% from 39% in 3 h hydrolysis by *Chromobacterium viscosum* lipase. The degree of hydrolysis was not directly calculated, but data cited reported the proportion of the FFA and AG fractions to be around 45:55 (w/w) in the reaction mixture. The authors found that lipases of category I were as effective in enriching VLCMFA as lipases of category II.

McNeill and Sonnet (1995) and McNeill (1997) compared the hydrolysis of HEA rapeseed (HEAR) oil by three lipases. In their procedures, HEAR oil of 5 g was mixed with 3.5 mL of 50 mM pH 7 phosphate buffer and 100 mg lipase powder. The mixture was magnetically stirred at 600 rpm in a 3 × 5 cm stoppered flat-bottom glass tube, placed in a glass mantle with temperatures controlled by circulating water. Lipase from *Pseudomonas cepacia* showed a non-selective and almost complete hydrolysis. After 24 h hydrolysis at 35˚C, C22 (free EA) content was 47.4% (area) in the FFA fraction, accounting for 84.1% of total reaction mixture, and C44 (dierucin) was 8.2% and 17.1% in the residual AG fraction and diacylglycerol (DG) fraction, respectively. In contrast, *Candida rugosa* lipase released C22 much more slowly than C20 and C18. C22 reduced to 32.3% in the FFA fraction (72.1% in the total reaction mixture), and C44 increased to 49.8% and 78.1% in the AG and DG fractions, respectively. Under the same conditions, *Geotrichum candidum* lipase released the C20 and C22 FAs extremely slowly, and about 2.1% C22 was lost in the FFA fraction (38.9% in the reaction mixture). C44 was enriched to 42.9% and 58.2% in the AG and DG fractions, respectively. The degree of hydrolysis was not directly calculated, and it could only be estimated from the percentage of FFA fraction in the total reaction mixture. However, it was concluded that the last two enzymes could both enrich EA from HEAR oil.

Sonnet et al. (1993b) also compared the hydrolysis of HEAR oil by some lipases in shaken tubes. Mixtures of 2 g HEAR oil and 2 mL 0.05 M phosphate buffer at pH 7.00, containing 1 to 20 mg lipase, were shaken in tubes at 30˚C, with 0.1 M Tris at pH 8.0 and 10 mM calcium chloride as the buffer solution especially for *Geotrichum candidum* lipase. *Pseudomonas cepacia* lipase showed no specificity. Lipases from *G. candidum* and *Candida rugosa* showed the potential for cleaving long-chain (>C18) FAs for EA isolation from HEAR oil, but very slowly. For about 70% degree of hydrolysis, the EA compositions in the unhydrolyzed AGs were increased from 37.5% (wt) of that in the original oil to 58.9% and 78.8% by *C. rugosa* lipase and *G. candidum* lipase, respectively.

Ergan et al. (1992) obtained similar results from selective hydrolysis of HEAR oil by *C. rugosa* lipase. Mixtures of 40 g HEAR oil and 16 mL water were stirred in beakers at 25˚C, with addition of 6.4 mL *Candida rugosa* lipase solution of 0.01 g/mL. After 16 h hydrolysis, a maximum of 70% EA was present in the form of dierucin, and the FFA fraction contained only 10% EA. Dierucin of 75% purity was then separated and enriched with 85% recovery. No hydrolytic reaction occurred under the same conditions when HEAR oil was substituted with pure chemical dierucin. However, trierucin was completely hydrolyzed to EA by the *C. rugosa* lipase under the same conditions if HEAR oil was substituted with pure trierucin. No further explanations on the possible mechanism were presented in the paper.

Enzymatic isolation and enrichment of EA from vegetable oils was also explored with non-commercial lipases. Using self-prepared *Geotrichum candidum* NRRL Y-553 lipase, Baillargeon and Sonnet (1991) enriched EA in AGs to 66.2% (wt) from the initial 37.8% in starting HEAR oil. Parmar and Hammond (1994) even employed lipases on the surface of oat caryopses to hydrolyze crambe oil, and found that EA was slightly enriched in the AGs. However, no follow-up work has been reported.

Reaction Optimization

Most studies discussed above show the theoretical and technical feasibility of isolating and enriching EA from HEA seed oils through selective hydrolysis. To increase the lipase activities and reaction specificities, studies have also been performed on optimization of reaction parameters, including operating temperatures, lipase content, and water effect (e.g., Lee and Parkin, 2001).

The effect of temperature on HEAR oil hydrolysis was studied by McNeill and Sonnet (1995) and McNeill (1997). They found that the TAG disappearance rate was similar at all temperatures tested in the hydrolysis by *Candida rugosa* lipase. But temperature significantly affected the time courses of C44 (dierucin) and C22 (free EA) concentrations. C44 concentration increased at all temperatures during the first hour, but remained constant at 10˚C. Increasing temperature accelerated the decline of C44 concentration after its early increase, with the final concentrations in the reaction mixture reaching 30% (area), 27%, 14% and 5% at 10˚C, 15˚C, 20˚C and 35˚C, respectively, in 48 h reactions. Correspondingly, the final concentration of C22 increased with the increased temperatures. It was clear that high temperatures increased the decomposition of C44 and the loss of EA into the FFA fraction under these conditions. However, no composition difference was observed in the reaction mixtures hydrolyzed by *P. cepacia* lipase at 10˚C and 35˚C, indicating that the specificity was not affected by temperatures for this type of lipase.

McNeil and Sonnet (1995) and McNeill (1997) also investigated the effects of water and lipase contents on the hydrolysis of HEAR oil at 10˚C by *C. rugosa* lipase. Water

contents varying from 2% to 60% were tested. When the water content was 2% (wt of total reaction mixture), the hydrolysis process was incomplete, with a low concentration of C44 (dierucin) at about 20% (area). For other water contents higher than 2%, the hydrolysis was complete, and concentrations of C44 were approximately 30%. But C22 (free EA) concentration was lower when the water content was less than 30%, indicating that lower water content reduced the loss of EA into the FFA fraction. From the hydrolysis of 5 g oil at 10˚C by *C. rugosa* lipase of 10 to 400 mg, the rate of C44 production increased as the enzyme concentration increased. With high *C. rugosa* lipase concentrations of 200 mg and 400 mg, the C44 production rates were almost identical. A slight decrease in C44 concentration was observed at the higher enzyme concentrations during the late period of 24 to 48 h. Results showed that a very high enzyme concentration could not increase the reaction rate, but increased the loss of EA by contrast. Although the authors did not give a detailed explanation, the possible reason could be the saturation of the oil and water interface by the adsorbed enzyme. Furthermore, the hydrolysis of C44 might cause the decrease in C44 concentration and the increase in the EA loss at the higher enzyme contents.

The effect of water content on selective hydrolysis of HEA oils for EA isolation was also studied by Kaimal et al. (1993). Water of 10 μL was added to a mixture of 1 g HEA mustard oil and 100 mg *Candida cylindracea* lipase. After being stirred magnetically for 4 h, the EA content in the liberated FFA fraction decreased to 10.5% (wt), compared to 43.9% in the starting oil. Both hydrolysis extent and EA content in the liberated FFA fraction increased with the increase of water amount. However, neither was affected by the substitution of water with each buffer of pH 7.0 phosphate, pH 8.0 Tris, and pH 9.0 borate. To achieve a better water-limited condition, water was added intermittently at 3 mL/h for the first 13 h into the uniform mixture of 700 g oil, 300 mL *tert*-butanol, and 5 g *C. cylindracea* lipase. After a total of 50 h, the EA content in the FFA fraction decreased to approximately 8%, and most of the EA stayed in AGs. The EA content was about 84%, with an overall hydrolysis degree of 32.2%.

The above results show that both the reaction rate and the loss of EA into the FFA fraction for hydrolysis catalyzed by *Candida rugosa* were increased at higher temperatures. The use of high lipase doses led to a similar results. Low water content was helpful to reduce the EA loss and increase the hydrolysis selectivity by *Candida rugosa* lipase (or formerly *Candida cylindracea* lipase), but extremely low water content might cause the incompletion of reaction. Consequently, intermittent addition of water during the reactions course showed encouraging results.

Other Reactions for EA Isolation

In addition to hydrolysis, lipase also has catalytic capabilities and specificities in transesterification, interesterification, and esterification. Researchers have explored all these reactions in order to develop strategies for isolating and enriching EA and other VLCMFAs via kinetic resolution.

Transesterification

Transesterification reactions transfer acyl moieties from TAGs or other lipids to the alkyl of an alcohol or a hydroxide group of an organic fatty acid. The two reactions are called alcoholysis and acidolysis, respectively. Studies are available in the literature only on alcoholysis for EA isolation from HEA vegetable oils. Through selective enzymatic alcoholysis, two fractions are generated: a residual AG fraction containing untouched FAs, and a new ester fraction containing cleaved FAs. Depending on the specificity of the lipase used, EA can be enriched in either of the two generated fractions. After selective alcoholysis by a lipase favoring FAs at 1, 3-positions, EA is enriched in the fraction of esters (fig. 6).

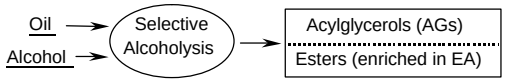


Figure 6. Enrichment of EA through selective alcoholysis by lipases favoring FAs at 1, 3-positions of HEA oil TAGs.

EA enrichment was investigated through selective alcoholysis of HEA white mustard seed oil and *n*-butanol catalyzed by *Rhizomucor miehei* lipase (Mukherjee and Kiewitt, 1996). In sealed screw-capped tubes under nitrogen at ambient temperature, 248 mg (0.25 mmol) of TAGs were magnetically stirred with 148 mg (2 mmol) *n*-butanol and 40 mg *R. miehei* (Lipozyme) lipase. As a result of the selective enzymatic catalysis, EA content in butyl esters (butyl erucate) was enriched from 48% (w/w of acyl moieties) to 56% and 51% after 8 h and 16 h, respectively, while the EA content in the AG fraction (containing minor FFAs) decreased to 27% and 19% after 8 h and 16 h, respectively. Alcoholysis showed a similar effect as hydrolysis for enrichment of VLCMFAs from HEA oils.

Interesterification

In interesterification reactions, an ester serves as the acyl acceptor and acyls are transferred between two ester bonds. The two fractions resulting after reaction are the new TAG fraction with new acyl moieties and the new ester fraction with FA residues transferred from the TAGs. If a lipase favoring FAs at 1, 3-positions is used, EA can be selectively transferred to the other ester (fig. 7). Consequently, EA is enriched in the fraction of the new esters.

Mukherjee and Kiewitt (1996) compared EA enrichment through hydrolysis, transesterification, and interesterification under the same conditions. They investigated selective interesterification of HEA white mustard seed oil with different alkyl acetates catalyzed by *Rhizomucor miehei* lipase, which favors fatty acids on *sn*-1, 3-positions and thus selectively cleaves EA from TAGs. Oil of 248 mg (0.25 mmol) reacted separately with 2 mmol each of ethyl, propyl, and butyl acetate, catalyzed by *R. miehei* (Lipozyme, 10%, w/w of the total mixture). After 4 h, the EA content in the alkyl ester fractions of ethyl, propyl, and butyl ester increased from 48% (wt of acyl moieties) of the starting oil to 54%, 55%, and 56%, respectively. It was shown that all these reaction methods had the similar extent of enrichment of VLCMFAs from HEA vegetable oils.

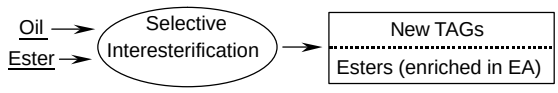


Figure 7. Enrichment of EA by selective interesterification by lipases favoring for FAs at 1, 3-positions.

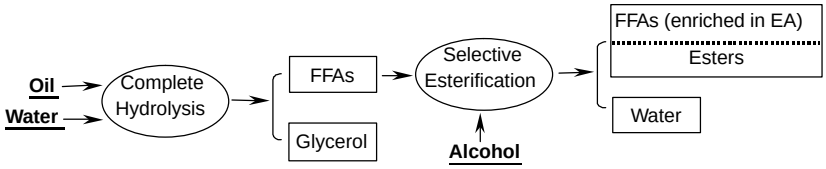


Figure 8. Enzymatic route of selective esterification by a lipase favoring short-chain FAs.

ESTERIFICATION

Esterification is a reaction between a fatty acid and an alcohol to form an ester. This type of reaction cannot be used directly for the purpose of EA enrichment from HEA seed oils, as all FAs are already bound to the glycerol backbone. FAs therefore need to be liberated first before esterification. Therefore, a multi-step process is necessary in order to enrich EA through esterification (fig. 8).

Sonnet et al. (1993b) studied the isolation of EA from HEAR oil through a two-step process, as shown in fig. 8. In the first step, 50 g HEAR oil was completely hydrolyzed by *Pseudomonas cepacia* lipase, and the resulting FFA fraction was separated from the aqueous phase containing glycerol by hexane extraction. In the second step, *Geotrichum candidum* lipase was used to selectively esterify *n*-butanol. After the undesired FAs were esterified into butyl esters, EA was enriched in the remaining FA fraction. Because the desired EA should remain unreacted after the esterification, the process was closely monitored so that EA would not react before other FAs were consumed. The hypothesis tested by the authors for this process was that to separate an FA, it was better to discriminate against rather than in favor of the desired FA. With the unoptimized conditions, 94% to 97% hydrolysis was obtained from the first step, and the residual FAs contained 85.4% (wt) EA with 51.9% conversion from the second step. However, the esters also contained 7.5% butyl erucate. It suggested that the lipase specificity in esterification and transesterification was better than that in hydrolysis. As discussed by the authors, one possible reason was that lipase catalysis discrimination might have "an added benefit" when the intermediate acylated enzymes react with larger alcohols, compared to water. The other possible reason was that the reversibility and equilibrium of hydrolysis might render the reaction less selective.

OTHER PROCESSES

As EA and its derivatives both have wide industrial applications, the products from HEA oils can take different forms. Through rearrangement of high erucic *Crucifereae* oils catalyzed by a 1, 3-specific lipase, Padley (1996) produced 1, 3-dibehenoyl triglycerides with good anti-blooming properties. Trani et al. (1993) developed a process to produce trierucin (fig. 9).

To get the final product of trierucin out of the HEAR oil, the authors conducted comprehensive research with different two-step reaction processes, along with necessary separation/purification operations. During the first step, dierucin was produced by stirring a mixture of 40 g oil, 16 mL water, and 6.4 mL of 0.01 g/mL *Candida rugosa* lipase solution for 18 h at room temperature. Approximately 85% of the EA in the original oil was enriched in the form of 1, 3-dierucin. After selective hydrolysis, the reaction mixture was extracted with 400 mL chloroform/water (1:1 v/v). The chloroform layer containing the oil fraction was dried by magnesium sulfate, filtered, and evaporated under vacuum. The resulting oil fraction from the chloroform layer was washed with ice-cold ethanol, and dierucin was thus solidified. FFAs were then removed by filtering. Dierucin of 73% purity was obtained, with an overall recovery rate of 53.6%.

Two methods were then investigated to produce trierucin from dierucin (Trani et al., 1993). In the first method, dierucin was treated at 60°C by Lipozyme IM-20, an immobilized 1, 3-specific *Mucor miehei* lipase, with various amounts of water. Water in the reacting mixture significantly impaired both the reaction rate and the conversion rate. Approximately 46% of the EA initially in dierucin was converted to trierucin after 264 h reaction with no water present. The authors described the transference of EA from one dierucin to another dierucin to produce trierucin as interesterification.

Because of the low conversion rate and relatively long reaction time in the above method, the authors tried an alternative way to produce trierucin from the dierucin (Trani et al., 1993). At first, free EA was produced from 4.164 g dierucin and 13 mL water, catalyzed by 100 μL of 0.01 g/mL 1, 3-specific *Rhizopus arrhizus* lipase solution. Free EA was separated through a similar procedure of chloroform/water extraction and cold ethanol washing, as described above. Then, equimolar free EA and dierucin were mixed to produce trierucin at 60°C in a jacketed beaker under vacuum, catalyzed by 0.5 g Lipozyme for each 5.5 mmol of EA, or 0.5g lipase SP 382, an immobilized nonspecific *Candida antarctica* lipase, for each 10 mmol of EA. Using lipase SP 382, a maximum of 95% EA was incorporated into trierucin after 72 h, with a comparison of 91.5% EA after 96 h for the Lipozyme. There was no explanation of why the 1, 3-specific Lipozyme could catalyze the incorporation of free EA into 1, 3-dierucin to produce trierucin with a high conversion.

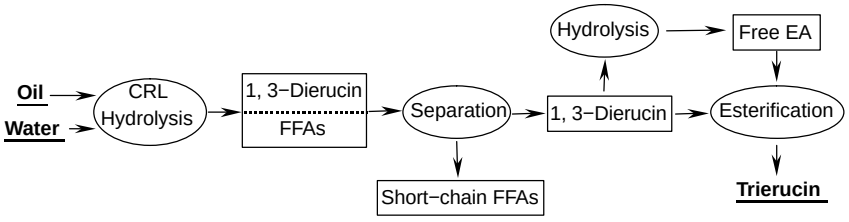


Figure 9. An integrated process to produce trierucin.

Table 1. Enrichment of EA from HEA oils by lipase-catalyzed reactions.

Lipase Source	Reaction	Performance	Reference
Fatty-acid-specificity (favoring short-chain FAs):			
Candida rugosa (formerly *Candida cylindracea*)	Hydrolysis of different HEA mustard oil	EA enriched in AGs to 64% (w/w) and 68% from 39%[a] and 43%, respectively	Mukherjee and Kiewitt, 1996
	Hydrolysis of HEA/HNA[b] *Lunaria annua* oil	EA enriched in AGs to 63% (w/w) from 44%	Mukherjee and Kiewitt, 1996
	Hydrolysis of HEAR oil	C22 free EA 32.3% area % in FFAs, and C44 dierucin 49.8% in AGs	McNeill and Sonnet, 1995; McNeill, 1997
	Hydrolysis of HEAR oil	EA enriched in AGs to 58.9% wt% from 37.5%	Sonnet et al., 1993b
	Hydrolysis of HEAR oil	10.6 g *sn*-1, 3-dierucin obtained from 40 g oil	Trani et al., 1993
	Hydrolysis of HEAR oil	EA enriched to 70% in AGs[c]	Ergan et al., 1992
	Hydrolysis of HEA mustard oil	EA enriched to about 84% wt% from 43.9%	Kaimal et al., 1993
Geotrichum candidum	Hydrolysis of HEA mustard oil	EA enriched in AGs to 55% w/w from 39%	Mukherjee and Kiewitt, 1996
	Hydrolysis of HEAR oil	C22 free EA 2.1% area % in FFAs, and C44 dierucin 42.9% in AGs	McNeill and Sonnet, 1995; McNeill, 1997
	Hydrolysis of HEAR oil	EA enriched in AGs to 78.8% wt% from 37.5%	Sonnet et al., 1993b
	Esterification of HEAR oil FAs and *n*-butanol	EA enriched in residual FFAs to 85.4% from 47.5%	Sonnet et al., 1993b
	Hydrolysis of HEAR oil	EA enriched in AGs to 66.2% from 37.8%[a]	Baillargeon and Sonnet, 1991
Regiospecificity favoring FAs at 1, 3-positions:			
Chromobacterium viscosum	Hydrolysis of HEA mustard oil	EA enriched in FAs to 50% w/w from 44%	Mukherjee and Kiewitt, 1996
Porcine pancreas	Hydrolysis of HEAR oil	EA reduced in AGs to 32.3% wt% from 37.5%	Sonnet et al., 1993b
	Hydrolysis of different HEA mustard oil	EA enriched in FAs to 50% w/w and 65% from 39% and 43%, respectively	Mukherjee and Kiewitt, 1996
	Hydrolysis of HEA/HNA[b] *Lunaria annua* oil	EA enriched in FAs to 63% w/w from 44%	Mukherjee and Kiewitt, 1996
Rhizomucor miehei	Hydrolysis of HEA mustard oil	EA enriched in FAs to 54% w/w from 43%	Mukherjee and Kiewitt, 1996
	Hydrolysis of HEA/HNA[b] *Lunaria annua* oil	EA enriched in FAs to 58% w/w from 44%	Mukherjee and Kiewitt, 1996
	Interesterification of HEA mustard oil with alkyl acetates	EA enriched in alkyl esters to 54% w/w, 55%, and 56% from 48% with ethyl, propyl, and butyl acetate, respectively	Mukherjee and Kiewitt, 1996
	Alcoholysis of HEA mustard oil with *n*-butanol	EA enriched in butyl esters to 56% w/w and 51% from 48% after 8 h and 16 h, respectively	Mukherjee and Kiewitt, 1996
	Hydrolysis of HEAR oil	No specificity	Sonnet et al., 1993b
Rhizopus arrhizus	Hydrolysis of HEA mustard oil	EA enriched in FAs to 58% w/w and 61% from 44% and 43%, respectively	Mukherjee and Kiewitt, 1996
	Hydrolysis of 1, 3-dierucin	Complete hydrolysis	Trani et al., 1993
Rhizopus delemar	Hydrolysis of HEAR oil	No specificity	Sonnet et al., 1993b

[a] Contents in original oils.
[b] High nervonic acid.
[c] The original EA content is not available.

SUMMARY

Different lipases were investigated to isolate and enrich EA through various reaction methods. Those lipases showed different specificities and effectiveness for EA isolation and enrichment (table 1). Esterification was found to be superior to hydrolysis for EA enrichment by *Geotrichum candidum* lipase (Sonnet et al., 1993b). As compared by Mukherjee and Kiewitt (1996), hydrolysis, transesterification, and interesterification were similarly effective for this purpose. However, the results of the EA enrichment were not satisfying, mostly below 80%, with two exceptions in which enriched EA content was above 80%.

The low reaction selectivity is not contradictory to the theory of lipase specificity on HEA TAGs, as introduced earlier in this review. The lipase specificity on different acyls of TAGs is actually the competition of those acyls for an enzyme active site (Rangheard et al., 1989). It can be regarded as a biocatalytic kinetic resolution (Sonnet et al., 1993b). Besides the inherent acyl-specificity of lipases, the observed relative reactivity of different acyls, namely reaction selectivity, may be affected by process parameters, such as the available concentration of different acyls. With the reaction proceeding, the content of the lipase-favored acyls decreases faster than that of the unfavored acyls. The lipase reactivity with those unfavored acyls may become increasingly significant, resulting in a decrease of the reaction selectivity.

In any of above discussed processes for isolating EA from HEA oils, there were two new fractions generated, the

residual AG fraction with untouched FAs, and the fraction with cleaved FAs. These two fractions were soluble in each other, although EA was enriched in one fraction with the remainder of the FAs in the other fraction. To obtain a final EA product, the two resulting fractions have to be further treated and separated through a suitable process. Previous research was mainly focused on the reactions, with little attention given to EA separation and purification. The only studies involved in the separation of EA were through *sn*-1, 3-dierucin solidification (Ergan et al., 1992; Trani et al., 1993). No studies were found that show how the free EA can be separated from the residual AGs if there is no *sn*-1, 3-dierucin generated when HEA oils are hydrolyzed by regiospecific lipases. Likewise, nothing was found on how the two ester fractions are to be separated when transesterification or interesterification is used.

Since EA can be used in different forms, the final product of EA may take different forms, such as free EA and trierucin. Therefore, different types of reactions can be employed. The practical application of any enzymatic approach for EA isolation and enrichment would be possible only if a cost-effective process of reaction and separation were well developed.

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NOMENCLATURE

AG	= acylglycerol
C16:1	= *cis*-9-hexadecenoic acid, palmitoleic acid
C18:1	= *cis*-9-octadecenoic acid, oleic acid
C18:2	= *cis*, *cis*-9, 12-octadecadienoic acid, linoleic acid
C18:3	= *cis*, *cis*, *cis*-9, 12, 15-octadecatrienoic, linolenic acid
C22	= C22:1, free erucic acid
C22:1	= *cis*-13-docosenoic acid, erucic acid
C44	= C44:1, dierucin
DG	= diacylglycerols
EA	= erucic acid or *cis*-13-docosenoic acid
FA	= fatty acid
FFA	= free fatty acid
GCL	= *Geotrichum candidum* lipases
HEA	= high erucic acid
HEAR	= high erucic acid rapeseed
HPL	= human pancreatic lipase
TAG	= triacylglycerols
TG	= triglycerides
VLCMFA	= very-long-chain mono-unsaturated fatty acids