Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 45 (2007) 45–49
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Convenient enzymatic preparation of conjugated linoleic acid alkyl esters with C6–C22 alcohols

Csilla Dianóczki [a], Katalin Recseg [a,*], Katalin Kővári [a], László Poppe [b,**]

[a] *Bunge Europe, Research and Development Center, H-1095 Kvassay J. u. 1, Hungary*
[b] *Institute for Organic Chemistry and Research Group for Alkaloid Chemistry, Budapest University of Technology and Economics, H-1111 Budapest, Gellért tér 4, Hungary*

Received 13 September 2006; received in revised form 12 November 2006; accepted 15 November 2006
Available online 18 December 2006

Abstract

A fast and effective enzymatic process was developed for preparing CLA C6–C22 alkyl esters using immobilized *Candida antarctica* lipase B (Novozym 435). The reaction parameters (temperature, amount of starting materials and enzyme, effect of water removal during the reaction) were optimized. The re-use study showed that the enzyme can be used at least ten times without significant loss in its activity. Reactions between equimolar amounts of starting materials with 8.9% Novozym 435 (w/w_{CLA}) at 65 °C in vacuum (20 mbar) for 1.5 h resulted in almost quantitatively (>95%) wax esters needing no further treatment. At room temperature, CLA esters with long chain (≥18 °C) saturated alcohols are solids.

Keywords: Conjugated linoleic acid ester; CLA ester; Lipase; Esterification; Solvent free condition

1. Introduction

Conjugated linoleic acid is a collective term for positional and geometrical isomers of linoleic acid. CLA is a physiologically active lipid with wide range of health benefit, such as reduction of the risk of developing cancer [1], reduction of the body fat content and increasing the lean muscle mass [2,3]. In addition, it was found that CLA could reduce the risk of developing coronary heart disease [4]. Furthermore, CLA had positive effect on the milk fat content and on the CLA content of milk in lactating cows compared to control group [5]. *In vitro* experiments showed that the 80–90% of CLA decomposed due to the biohydrogenation under rumen conditions, therefore CLA should be protected from this process [6]. The CLA can be protected from the biohydrogenation by encapsulating in protein matrix or by converting it to a solid derivative, which remains solid at rumen temperature. CLA wax esters may cover this requirement.

Enzymatic preparations of CLA esters with long chain (C8 or C12) alcohols were investigated predominantly for separation of the two most important biological active isomers (9*Z*,11*E*-CLA and 10*E*,12*Z*-CLA). In these cases, however, the main goal was to find the most selective enzymes for discrimination between the two isomers [7–9]. The CLA-containing structured lipids represent another class of CLA esters which were prepared mostly by enzyme-catalyzed reactions [10–19].

Candida antarctica lipase B (Novozym 435) proved to be an exceptionally versatile biocatalyst [20]. It is very efficient in catalyzing different esterification reactions, such as direct esterification of fatty acids and alcohols [21–24], transesterification of fatty acid esters (alkyl esters or glycerides) and alcohols [21,25–30]. Novozym 435 can be applied as catalyst in production of *biodiesel* as fatty acid methyl esters from used frying oil [31]. In addition to the usual carboxylic ester formation, this enzyme proved to be effective catalyst in thioester formation as well [32]. It can catalyze reactions between a wide range of starting compounds resulting in various products, for example, steryl esters [21], terpene esters [33] and wax esters [22,28].

Long chain wax esters have been obtained in high yield using Novozym 435 as biocatalyst. In reactions starting from stoichiometric amounts of starting compounds and performed under vacuum, the highest conversion was observed in 4–6 h [22]. Similar conditions seemed to be optimal for direct esterification of lauric acid with diglycerol [27]. Oleyl oleate

* Corresponding author. Tel.: +36 1 4763610; fax: +36 1 2175241.
** Corresponding author. Tel.: +36 1 4632229; fax: +36 1 4633297.
E-mail addresses: katalin.recseg@bunge.com (K. Recseg), poppe@mail.bme.hu (L. Poppe).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.11.005

was prepared applying Novozym 435 as biocatalyst in organic solvent at 37 °C [23] or without solvent at higher temperature [23].

Our aim was to develop and optimize an enzymatic esterification process for producing solid alkyl esters of CLA and long chain alcohols, where the product is pure, free from any residual starting material or solvent.

2. Experimental

2.1. Materials and methods

Alcohols (1-hexanol (C6-OH), 1-octanol (C8-OH), 1-decanol (C10-OH), 1-dodecanol (C12-OH), 1-tetradecanol (C14-OH), 1-hexadecanol (C16-OH), 1-octadecanol (C18-OH), 1-eicosanol (C20-OH), 1-docosanol (C22-OH)) and solvents (hexane, tetrahydrofurane) were purchased from Sigma–Aldrich Co. (Steinheim, Germany). The CLA free fatty acid (CLA-FFA) was a kind gift of Loders and Croklaan Lipid Nutrition Co. (Wormerveer, Netherlands). It had a CLA content of 79.7% [the two main isomers 9*Z*,11*E* (48.6%) and 10*E*,12*Z* (48.5%) along with minor amounts of *E,E*-CLA isomers (2.1%) and *Z,Z*-CLA isomers (0.8%), all normalized to the total CLA content. The enzyme (immobilized *Candida antarctica* lipase A, Novozym 435) was a kind gift of Novozyme A/S (Bagsvaerd, Denmark). Molecular sieve 0.4 nm was the product of Merck (Darmstadt, Germany).

The conversion of the CLA esterification was measured on Agilent 6890 GC equipped with FID detector and HP-1 column (25 m × 200 μm i.d. × 0.11 μm film thickness, Agilent Technologies) using H_2 as carrier gas at a flow of 2.1 mL/min. Oven temperature was programmed as follows: 50 °C for 2 min, 50–300 °C at 25 °C/min, 300 °C for 8 min. The injector and detector were operated at 320 °C. These GC conditions allowed simultaneous determination of the starting materials and the products without any derivatization prior to the analysis. Conversions were calculated from the area of the product and the residual CLA-FFA peaks using response factors determined by calibration with standards containing equimolar mixture of the CLA-FFA and the corresponding CLA-ester.

The purity of the CLA esters were further checked by NMR analysis using the most specific signals in the ^{1}H-NMR and ^{13}C-NMR spectra. The NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-500 spectrometer (at 500 MHz for ^{1}H and 125 MHz for ^{13}C spectra) and are reported in ppm on the δ scale. The following ^{1}H-NMR signals were used for conversion analysis and purity check: the olefinic C**H** signals at 5.3 (m), 5.6 (m), 5.9 (t) and 6.3 (t); the CO-O-C**H**$_2$ group at 4.1 (t). In addition, signals for the residual acid [COO**H** (>10 ppm)] and alcohol [C**H**$_2$-O at 3.6 (t) and -O**H** at ∼2.3 (∼t)] were also monitored. In the ^{13}C-NMR spectrums the following signals were characteristic for the CLA esters: eight signals for the conjugated **C**H groups in the range 125–134 (s, each) and the signals of the **C**O-O-**C**H$_2$ group at 173.6 (s) and 64.4 (s).

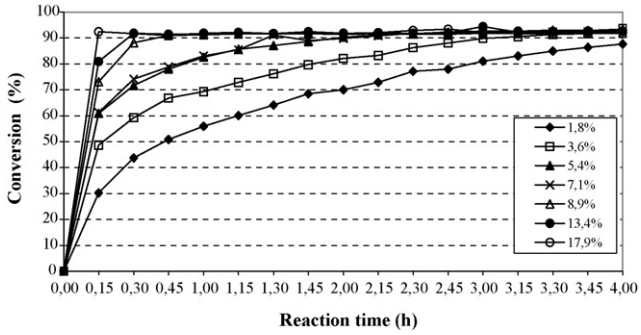

Fig. 1. Effect of enzyme amount on the esterification of CLA-FFA and C18-OH. Reaction conditions: CLA-FFA: 20 mmol; C18-OH: 20 mmol; shaking at 65 °C. The different curves represent reactions with different amounts (w/w$_{CLA\text{-}FFA}$%) of Novozyme 435 catalyst.

2.2. Novozym 435-catalyzed esterification of CLA with long chain alcohols

2.2.1. Preliminary test of Novozym 435-catalyzed esterification of CLA

Different reaction conditions with and without solvents were tested to find optimal conditions for Novozym 435-catalyzed esterification of CLA with 1-eicosanol (C20-OH) as alcohol component (Table 1). The reaction mixtures were shaken at 250 rpm in sealed glass vials for 24 h using horizontal waterbath shaker (Memmert). At 1, 2, 4 and 24 h, samples (7.5 μL) were taken from the reactions. For GC analysis, the samples were dissolved in hexane (1 mL).

2.2.2. Effect of enzyme amount on enzymatic esterification of CLA

The effect of enzyme dosage (1.8–17.9 w/w$_{FFA}$% Novozym 435) on the conversion was studied in the esterification reaction between CLA-FFA (20 mmol) and 1-octadecanol (C18-OH, 20 mmol) (Fig. 1). The reaction mixtures were shaken at 250 rpm in sealed glass vials for 4 h at 65 °C using horizontal waterbath shaker (Memmert). At every fifteen minutes samples were taken from the reactions. For GC analysis, the samples (7.5 μL) were dissolved in hexane (1 mL).

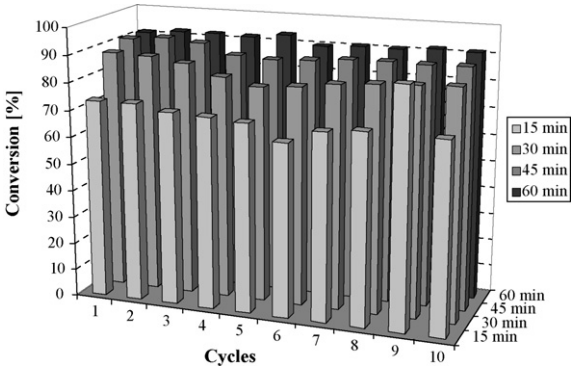

Fig. 2. Re-usability of Novozym 435 catalyst in esterification of CLA-FFA and C18-OH. Reaction conditions: CLA-FFA: 20 mmol; C18-OH: 20 mmol; Novozym 435: 500 mg; shaking at 65 °C. Conversions of subsequent reactions catalyzed with the same portion of immobilized enzyme are shown at four different times.

Table 1
Preliminary test of Novozym 435-catalyzed esterification of CLA

Entry	Starting compounds (mmol)		Enzyme (mg)	Solvent[b] (mL)	Temp. (°C)	Time (h)	Conv. (%)
	FFA[a]	C20-OH					
1	2	2	50	5	25	24	30
2	2	2	100	5	25	24	30
3	10	2	100	10	25	24	28
4	2	2	50	–	65	2	98
5	20	20	500	–	65	2	91

[a] FFA mixture containing CLA (77.9%).
[b] Hexane/THF 1:1 (v/v).

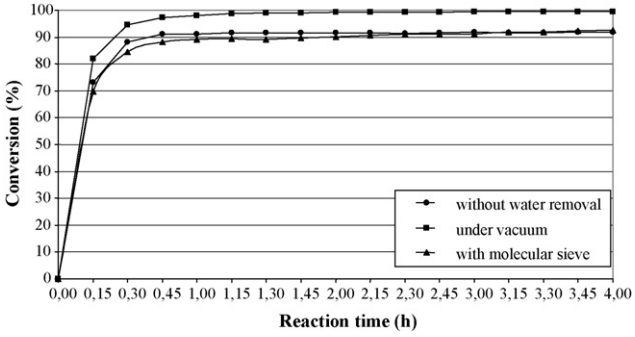

Fig. 3. Effect of water removal on the conversion of enzymatic CLA-FFA esterification with C18-OH. Reaction conditions: CLA-FFA: 20 mmol; C18-OH: 20 mmol; Novozym 435: 500 mg; shaking at 65 °C. Water removal by vacuum (20 mbar) or with molecular sieve (0.4 nm, 500 mg) or without water removal.

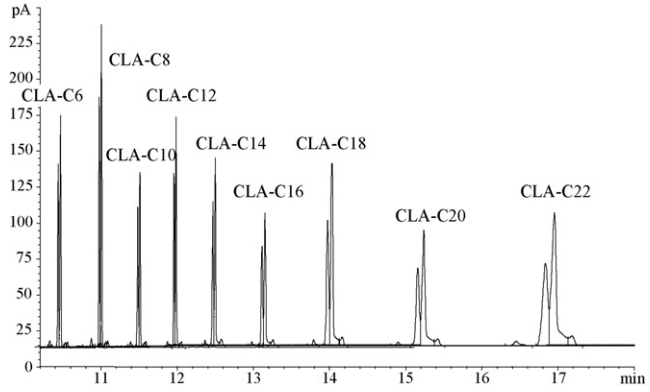

Fig. 4. GC chromatogram of equimolar mixture of CLA-C_n esters prepared by the solvent-free enzymatic method from CLA-FFA with alcohols C6-OH to C22-OH. GC was performed on a HP-1 column (25 m × 200 μm × 0.11 μm, Agilent Technologies). For more details see Section 2.1 in Experimental.

2.2.3. *Testing the enzyme reusability and effect of water removal in enzymatic esterification of CLA*

The re-use study of the Novozym 435 catalyst (500 mg) was carried out by reacting CLA-FFA (20 mmol) and C18-OH (20 mmol) as described in Section 2.2.2 (65 °C, 4 h) but using the same portion of the enzyme in ten subsequent reactions (Fig. 2). The immobilized lipase was recovered after each reactions, it was washed with hexane (3 × 5 mL), dried at room temperature (>6 h) and used for the next batch. Conversions in each reactions were analyzed by GC as described in Section 2.2.2.

For study of the effect of water removal on the rate of conversion, the reactions [CLA-FFA (20 mmol), C18-OH (20 mmol), Novozym 435 (500 mg), shaking at 65 °C for 4 h] were carried out in the presence of an effective drying agent (0.4 nm molecular sieve, 500 mg), under vacuum (20 mbar) and without any water removal (Fig. 3).

2.2.4. *Novozym 435-catalyzed esterification of CLA with long chain alcohols*

Preparation of solid CLA wax esters (CLA-C6–CLA-C22) were performed by Novozym 435 (500 mg) catalyzed esterification reactions between equimolar amounts (20 mmol, each) CLA-FFA and alcohols having even-numbered carbon chains between six (C6-OH) and twenty-two (C22-OH) at 65 °C under solvent-free conditions for 2 h under vacuum (20 mbar). At the end of the reactions, the catalyst was filtered off from the hot reaction mixture to leave without any further treatment virtually pure CLA alkyl esters in almost quantitative yield.

The CLA-C6–CLA-C16 esters are viscous oils. CLA-C18: mp. 25–27 °C; CLA-C20: mp. 25–27 °C; CLA-C22: mp. 40 °C. All CLA-C_n esters were fully characterized by GC (see Fig. 4) and by ^{1}H- and ^{13}C-NMR spectra. As a typical example, NMR data of the CLA hexyl ester [CLA-C6; as a mixture of (9*Z*,11*E*)-hexyl octadeca-9,11-dienoate and (10*E*,12*Z*)-hexyl octadeca-10,12-dienoate in about 1:1 ratio] is given below.

^{1}H-NMR (δ_H, integrals are normalized to the 1′-C**H$_2$**-O as 2H): 0.87 (*m*, 6H, 18-C**H$_3$** and 6′-C**H$_3$**); 1.24–1.40 (*m*, ∼28H, 11 × C**H$_2$** for CLA-C6 ester and ∼6H for saturated ester impurities); 1.60 (*m*, 4H, 3-C**H$_2$** and 2′-C**H$_2$**); 2.07 (*m*, 2H, 9-C**H$_2$** (10*E*,12*Z*) and 14-C**H$_2$** (9*Z*,11*E*)); 2.14 (*m*, 2H, 9-C**H$_2$** (9*Z*,11*E*) and 14-C**H$_2$** (10*E*,12*Z*)); 2.30 (*t,* 2H, 2-C**H$_2$**); 4.04 (*s*, 2H, 1′-C**H$_2$**-O); 5.29 (*m*, ∼0.9H, 9-C**H** (9*Z*,11*E*) and 13-C**H** (10*E*,12*Z*)); 5.65 (*m*, ∼0.9H, 10-C**H** (10*E*,12*Z*) and 12-C**H** (9*Z*,11*E*)); 5.94 (*t*, ∼0.9H, 10-C**H** (9*Z*,11*E*) and 12-C**H** (10*E*,12*Z*)); 6.29 (*t*, ∼0.9H, 11-C**H** (9*Z*,11*E*) and 11-C**H** (10*E*,12*Z*)). The ^{1}H-NMR spectra of the other CLA-C_n esters differ in the integral of the 1.24–1.40 signals (*m*, ∼(16 + 2*n*)H, (*n* + 5) × C**H$_2$** for CLA-C_n and ∼6–8H for saturated ester impurities).

^{13}C-NMR (δ_C): 14.20 and 14.24 (18-**C** for (9*Z*,11*E*) and (10*E*,12*Z*)); 14.26 (6′-**C**); 22.7–34.5 (multiple signals, aliphatic **C**'s); 64.39 (1′-**C**); 125.41 (11-**C** (9*Z*,11*E*)); 125.50 (11-**C** (10*E*,12*Z*)); 128.41 (12-**C** (10*E*,12*Z*)); 128.52 (10-**C** (9*Z*,11*E*)); 129.68 (9-**C** (9*Z*,11*E*)); 129.89 (13-**C** (10*E*,12*Z*)); 134.33 (10-**C** (10*E*,12*Z*)); 134.51 (12-**C** (9*Z*,11*E*)); 174.75 (1-**C**). The

^{13}C-NMR spectra of the other CLA-C$_n$ esters differ in the number of the 22.7–34.5 signals.

3. Results and discussion

3.1. Preliminary test of Novozym 435-catalyzed esterification of CLA

Esterification reactions between CLA-FFA and 1-eicosanol (C20-OH) as alcohol performed at room temperature in organic solvent (hexane–tetrahydrofurane 1:1) did not result in good conversions (Table 1). Increasing the amount of enzyme (Entry 2, Table 1) or applying excess free acid (Entry 3, Table 1) did not improved the conversion under these conditions. On the other hand, esterification between stoichiometric amounts of starting materials at 65 °C under solvent free conditions resulted in high (>90%) conversion (Entries 4 and 5, Table 1.). This temperature is required not only to enhance the rate of the enzymatic reaction but also for melting the solid long-chain alcohols.

3.2. Effect of the enzyme amount on the esterification of CLA

The effect of enzyme loading on the rate and conversion was studied in esterification reactions between equimolar amount of CLA-FFA and 1-octadecanol (C18-OH) (Fig. 1). In this reaction series our aim was to find the lowest enzyme amount of which can provide the maximum conversion within 2 h. Most of the reactions with variable doses of enzyme reached the same degree of conversion (91–92% which seems to be the equilibrium conversion of this enzyme-catalyzed esterification under this conditions) but in different reaction times (Fig. 1). Our results indicated that reactions with enzyme loadings lower than 5 w/w$_{CLA\text{-}FFA}$% did not reach the equilibrium conversion within 2 h, whereas enzyme loadings over 8.9 w/w$_{CLA\text{-}FFA}$% could achieve the maximum conversion within 60 min. Due to the solvent free conditions, proper mixing became more difficult at higher amounts of enzyme (>10 w/w$_{CLA\text{-}FFA}$%). Taking these aspects into consideration, 8.9 w/w$_{CLA\text{-}FFA}$% enzyme loading has been chosen for the further applications.

3.3. Testing the enzyme reusability in enzymatic esterification of CLA

The stability and reusability of the enzyme was studied in ten repeated esterification reactions between equimolar amount of CLA-FFA and 1-octadecanol (C18-OH) at 65 °C for 2 h using 8.9 w/w$_{CLA\text{-}FFA}$% Novozym 435 catalyst (Fig. 2). In spite of the relatively long reaction time at elevated temperature, the enzyme retained its high activity even after repeating the esterification reaction ten times. In all repeated reactions, the equilibrium conversion was reached within 60 min. This is due to the known heat stability of this immobilized lipase [20]. Only small differences in the initial conversion values were observed which did not significantly influenced the final conversion rate (Fig. 2). These differences in the initial rate might be due to mechanical damage of the immobilized biocatalyst resulting in different (decreasing) particle size distribution. The effect of enzyme amount and the reusability of Novozym 435 in the esterification of oleic acid with oleyl alcohol at lower temperature (37 °C) have also been studied [23]. In these studies about five times more enzyme was used as in our reactions at elevated temperature. The Novozyme 435 catalyst was found to be very stable but in contrast with our results they observed a slight decrease in the enzyme activity after ten times use [23].

3.4. Shifting the equilibrium conversion by water removal in enzymatic esterification of CLA

Our previous results indicated that the solvent free direct esterification of CLA-FFA with long chain alcohols catalyzed by Novozym 435 has an equilibrium conversion of around 91%. Similar degree of equilibrium conversion has been found in Novozym 435-catalyzed solvent free direct esterification between stoichiometric amounts of oleic acid and oleyl alcohol [23]. Because our aim was to develop a high yield – low cost process, separation of the CLA wax from the starting compound would be too costly. Our next step was, therefore, to shift the equilibrium conversion of the esterification to completeness by water removal. Two possible solutions were tried: *in situ* drying with molecular sieve and reaction conducted under vacuum (Fig. 3). Application of molecular sieve did not significantly shift the equilibrium conversion compared to the reaction without any water removal. The method for synthesis of long chain wax esters using Novozym 435 as biocatalyst showed that high yield could be achieved in reactions starting from stoichiometric amounts of substances under vacuum at ambient temperature in 4–6 h [22]. However, this water removal method which is efficient for shifting the equilibrium conversion to completeness was not combined with the benefit of the high temperature stability of the catalyst. Our results indicated that the reaction performed at 65 °C under vacuum (20 mbar) can result in practically complete reaction (conversion >98%) within 2 h (Fig. 3). After a relatively short reaction time (2 h) and simple filtration of the Novozym 435 catalyst, this method provided the virtually pure CLA wax product without further treatment.

3.5. Novozym 435-catalyzed esterification of CLA with long-chain alcohols

As biohydrogenation of accessible CLA in the rumen can diminish the beneficial biological effects of these compounds, our primary goal was to find CLA derivatives which are solid at the rumen temperature and thus less accessible for biodegradation. To find CLA waxes which are solid at room temperature, CLA-FFA was esterified with various alcohols having chain length of six to twenty-two carbon atoms under the optimized conditions [equimolar amounts of starting compounds, 8.9 w/w$_{CLA\text{-}FFA}$% Novozym 435, 65 °C, 20 mbar, 2 h]. The final purities of the CLA esters were checked by GC (Fig. 3) and NMR. In the ^{1}H-NMR spectra of the products the characteristic CO-O-C$\underline{\mathbf{H}}_2$ signal at 4.1 (t) was always indicative for the ester moiety, whereas no traces of COO$\underline{\mathbf{H}}$ signals (>10 ppm) were observed. In several cases small amounts (<2%) of

alcohols [C**H**$_2$-O at 3.6 (t) and -O**H** at ∼2.3 (∼t)] could also be detected.

We have found that CLA-octadecyl ester is semi-solid (mp. 25–27 °C), whereas CLA-C20 and CLA-C22 alkyl esters are real solids at room temperature (mp.'s are 34 °C and 40 °C, respectively). Taking the rumen temperature into account, the CLA-C22 wax ester could be a potential rumen protected CLA derivative.

4. Conclusions

An effective and fast enzymatic reaction was developed for preparing CLA alkyl esters, especially preparation of solid CLA wax esters. Under the optimized solvent free reaction conditions [equimolar amounts of starting materials, 8.9 w/w$_{\text{CLA-FFA}}$% Novozym 435 catalyst, 65 °C, 2 h, under vacuum (20 mbar)] the catalyst retained its full activity even after ten times reuse. After a simple catalyst filtration, the CLA wax products prepared by this method were virtually pure (>95%) without any further treatment or purification. CLA wax esters of alcohols having carbon chain length of above 18 are solid at room temperature. As the melting point of CLA C22 wax ester is above the rumen temperature it can have further application in animal feeding.

Acknowledgements

L.P. thanks OTKA for financial support (T-48854).

References

[1] C. Ip, S.F. Chin, J.A. Scimeca, M.W. Pariza, Cancer Res. 51 (1991) 6118–6124.
[2] J. Park, K.J. Albright, W. Liu, J.M. Storkson, M.E. Cook, M.W. Pariza, Lipids 32 (1997) 853–858.
[3] E. Ostrowska, M. Muralitharan, R.F. Cross, D.E. Bauman, F.R. Dunshea, J. Nutr. 129 (1999) 2037–2042.
[4] M. Pfeuffer, J. Scherezenmeir, Br. J. Nutr. 84 (2000) 155–159.
[5] J.W. Perfield II, G. Bernal-Santos, T.R. Overton, D.E. Bauman, J. Dairy Sci. 85 (2002) 2609–2617.
[6] S.K. Gulati, S.M. Kitessa, J.R. Ashes, E. Fleck, E.B. Byers, Y.G. Byers, T.W. Scott, Anim. Feed Sci. Technol. 86 (2000) 139–148.
[7] M.J. Haas, J.K.G. Kramer, G.P. McNeill, K. Scott, T.A. Foglia, N. Sehat, J. Fritsche, M.M. Mossoba, M.P. Yurawecz, Lipids 34 (1999) 979–987.
[8] T. Nagao, Y. Shimada, Y. Yamauchi-Sato, T. Yamamoto, M. Kasai, K. Sugihara, Y. Tominaga, J. Am. Oil Chem. Soc. 79 (2002) 303–308.
[9] T. Nagao, Y. Yamauchi-Sato, A. Sugihara, T. Iwata, K. Nagao, T. Yanagita, S. Adachi, Y. Shimada, Biosci. Biotechnol. Biochem. 67 (2003) 1429–1433.
[10] (a) F. Duane, PCT Int. Appl. (2001) A2, WO 2001078531;
(b) F. Duane, Chem. Abstr. 135 (2001) 303122.
[11] G.G. Haraldsson, A. Saebo, C. Skarie, D. Jerome, PCT Int. Appl. (2000) WO2000018944.
[12] (a) L.C. Lievense, G.W. Meijer, G.P. McNeill, Eur. Pat. Appl. (1997) A1, EP 0779033;
(b) L.C. Lievense, G.W. Meijer, G.P. McNeill, Chem. Abstr. 127 (1997) 175701.
[13] K. In-Hwan, Y. Chil-Surk, C. Soo-Hee, L. Kwang-Won, C. Soo-Hyun, T. Beom-Seok, J. Am. Oil Chem. Soc. 78 (2001) 547–551.
[14] K. In-Hwan, C. Soo-Hyun, J. Jae Hong, Y. Chil Surk, U.S. Pat. Appl. (2003) A1, US 2003032672.
[15] (a) B. Scott, K.P. Rakowsky, V. Taran, Eur. Pat. Appl. (2002) A2, EP 1167340;
(b) B. Scott, K.P. Rakowsky, V. Taran, Chem. Abstr. 136 (2002) 69113.
[16] Y. Watanabe, Y. Yamauchi-Sato, T. Nagao, T. Yamamoto, K. Tsutsumi, A. Sugihara, Y. Shimada, J. Am. Oil Chem. Soc. 80 (2003) 909–914.
[17] C.F. Torres, F. Munir, L.P. Lessard, C.G. Hill Jr., J. Am. Oil Chem. Soc. 79 (2002) 655–661.
[18] C.F. Torres, H.S. Garcia, J.J. Ries, C.G. Hill Jr., J. Am. Oil Chem. Soc. 78 (2001) 1093–1098.
[19] P.S. Sehanputri, C.G. Hill Jr., Biotechnol. Bioeng. 83 (2003) 608–617.
[20] P.D. de María, C. Carboni-Oerlemans, B. Tuin, G. Bargeman, A. van der Meer, R. van Gemert, J. Mol. Catal. B: Enzym. 37 (2005) 36–46.
[21] N. Weber, P. Weitkamp, K.D. Mukherjee, J. Agric. Food Chem. 49 (2001) 5210–5216.
[22] G. Steinke, P. Weitkamp, E. Klein, K.D. Mukherjee, J. Agric. Food Chem. 49 (2001) 647–651.
[23] S.M. Radzi, M. Basri, A.B. Salleh, A. Ariff, R. Mohammad, M.B.A. Rahman, R.N.Z.R.A. Rahman, Electr. J. Biotechnol. 8 (2005) 291–298.
[24] S.M. Radzi, M. Basri, A.B. Salleh, A. Ariff, R. Mohammad, M.B.A. Rahman, R.N.Z.R.A. Rahman, J. Chem. Technol. Biotechnol. 81 (2006) 374–380.
[25] K. Kobata, M. Kawaguchi, T. Watanabe, Biosci. Biotechnol. Biochem. 66 (2002) 319–327.
[26] R. Irimescu, K. Furihata, K. Hata, Y. Iwasaki, J. Am. Oil Chem. Soc. 78 (2001) 743–748.
[27] E. Garcia, F. Ferrari, T. Garcia, M. Martinez, J. Aracil, J. Surf. Deterg. 4 (2001) 257–262.
[28] A. Salis, V. Solinas, M. Monduzzi, J. Mol. Catal. B: Enzym. 21 (2003) 167–174.
[29] C.F. Torres Jr., C.G. Hill, C. Otero, Biotechn. Progr. 20 (2004) 756–763.
[30] O. Kose, M. Tuter, H.A. Aksoy, Bioresour. Technol. 83 (2002) 125–129.
[31] A. Coteron, G. Vincente, M. Martinez, J. Aracil, Recent Res. Dev. Oil Chem. 1 (1997) 104–119.
[32] N. Weber, E. Klein, K.D. Mukherjee, Appl. Microbiol. Biotechnol. 51 (1999) 401–404.
[33] G.D. Yadav, P.S. Lathy, J. Mol. Catal. B: Enzym. 27 (2004) 113–119.