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Solvent-free lipase-catalyzed preparation of diglycerides from co-products of vegetable oil refining

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RESUMEN

Obtención de diglicéridos a partir de subproductos del refinado de aceites vegetales mediante la esterificación catalizada por lipasas en ausencia de disolventes.

Subproductos del refinado de los aceites vegetales tales como el destilado obtenido en el desodorizador al refinar distintos aceites vegetales, el destilado crudo resultante de la refinación física del aceite de coco, y mezclas comerciales de los ácidos grasos obtenidos en la destilación de aceites de girasol y coco fueron utilizados como materiales de partida para la preparación enzimática de diglicéridos. Se estudiaron las condiciones de reacción (temperatura, presión, relación molar) para la formación de diglicéridos mediante esterificación/transesterificación catalizada por lipasas usando la mezcla obtenida del desodorizador y glicerol como materiales de partida. Los mejores resultados se obtuvieron con lipasa B inmovilizada de *Candida antarctica* (Novozym 435) a vacío y 60 °C obteniéndose una concentración moderada (~52%) de diglicéridos. La proporción de diglicéridos aumentó cuando los aciglicéridos residuales de los subproductos de la refinación de los aceites vegetales fueron hidrolizados como paso previo a la esterificación. Así, la esterificación de subproductos hidrolizados del refinado de aceites vegetales con glicerol produjo una alta (62-72%) formación de diglicéridos. La posterior destilación a vacío de los productos de esta esterificación produjo destilados conteniendo del 70 al 94% de diglicéridos. Las proporciones de ácidos grasos y monoglicéridos en estos destilados fueron bastante bajas (≤1% y 1-3.9%, respectivamente). Las lipasas inmovilizadas de *Rhizomucor miehei* y *Thermomyces lanuginosus* fueron menos activas como biocatalizadores de esterificación.

PALABRAS-CLAVE: Coproductos del refinado de aceites vegetales – Diglicéridos – Destilado de ácidos grasos – Esterificación – Lipasas microbianas inmovilizadas – Transesterificación.

SUMMARY

Solvent-free lipase-catalyzed preparation of diglycerides from co-products of vegetable oil refining.

Co-products of vegetable oil refining such as a mixed deodorizer distillate resulting from the refining of various vegetable oils, a crude distillate resulting from the physical refining of coconut oil and commercial mixtures of distilled sunflower and coconut fatty acids were used as starting materials for the enzymatic preparation of diglycerides. Reaction conditions (temperature, pressure, molar ratio) for the formation of diglycerides by lipase-catalyzed esterification/transesterification were studied using the mixed deodorizer distillate and glycerol as starting materials. The best results were obtained with the immobilized lipase B from *Candida antarctica* (Novozym 435) in vacuo at 60 °C leading to moderate proportions (~52%) of diglycerides. The proportion of diglycerides increased when residual acylglycerides of the co-products of vegetable oil refining were hydrolyzed prior to esterification. Thus, the esterification of hydrolyzed co-products of vegetable oil refining with glycerol led to high formation (62-72%) of diglycerides. Short-path vacuum distillation of the esterification products yielded distillation residues containing from 70% to 94% diglycerides. The proportions of fatty acids and monoglycerides in the distilled residues were quite low (≤1% and 1 to 3.9%, respectively). Immobilized lipases from *Rhizomucor miehei* and *Thermomyces lanuginosus* were less active as esterification biocatalysts.

KEY-WORDS: Co-products of vegetable oil refining – Diglycerides – Distilled fatty acids – Esterification – Immobilized microbial lipases – Transesterification.

1. INTRODUCTION

Edible fats and oils mainly consist of triglycerides (triacylglycerols). Diglycerides (diacylglycerols, DAG) are naturally occurring minor constituents of fats and oils which are formed by enzymatic and non-enzymatic hydrolysis of triglycerides. The fairly high water-retaining ability of diglycerides which have higher lipophilicity than monoglycerides (monoacylglycerols) makes them suitable as emulsifiers for special purposes (Nakajima, 2004). They are utilized –mostly together with mono- or triglycerides– as ingredients for foods and feeds, cosmetics, and toiletteries as well as pharmaceutical preparations and technical products (Gunstone, 1999; Yamada *et al.*, 2005). For example, mixtures of mono- and diglycerides are widely used as emulsifiers in industrially processed foods. Moreover, it has been shown that particularly 1,3-diglycerides have beneficial effects in preventing obesity and lipemia despite their similar energy value to that of triglycerides (Maki *et al.*, 2002; Tada and Yoshida, 2003; Yamada *et al.*, 2005). Diglycerides are offered as "Food for Specified Health Use" (FOSHU) in Japan and a cooking oil containing around 80% diglycerides ("DAG oil") as well as mayonnaise containing such an oil are on the market. DAG oil has received the status "Generally Recognized as Safe" (GRAS) by the US

Food and Drug Adminstration (Sakaguchi, 2001). Several chemical methods are available for the preparation of individual and stereochemically pure diglycerides. Lipase-catalyzed preparation of diglycerides and monoglycerides by partial hydrolysis of triglycerides of fats and oils, esterification of fatty acids with glycerol, and transesterification of triglycerides with glycerol (glycerolysis) are also known (Mukherjee, 1990; Bornscheuer, 1995; Gunstone, 1999; Yamada *et al*., 2005). Mixtures of diglycerides and triglycerides for food use have been prepared by esterification of fatty acids with glycerol and transesterification of acyl moieties from triglycerides to monoglycerides or glycerol, catalyzed by lipases from *Candida antarctica* (lipase B) and *Rhizomucor miehei* (Rosu et al., 1999; Watanabe *et al*., 2003; Weber and Mukherjee, 2004).

Little is known, however, about the preparation of diglycerides by lipase-catalyzed esterification and/or transesterification of glycerol with co-products of the refining of fats and oils such as deodorizer distillates, crude fatty acid mixtures resulting from physical refining or fatty acid fractions obtained by vacuum distillation from co-products of vegetable oil refining or fat splitting (Ko *et al*., 2004a; Ko *et al*., 2004b; Ko *et al*., 2004c; Nandi *et al*., 2005). Presently, co-products of oil refining mostly serve as low-value additives in animal feed production or in the soap and detergent industry. Using biotechnological methods, these low-value or waste materials may have the potential of being processed to value-added multi-purpose products. The aim of the present work was to develop a simple and environmentally friendly method for the preparation of diglycerides from co-products of vegetable oil refining such as deodorizer distillates, crude distillates of physical refining or fractions of distilled fatty acids by esterification/transesterification with glycerol using commercial immobilized lipases as biocatalysts. The esterification/transesterification reactions were performed at moderate temperatures without solvents or drying agents using reduced pressure to remove reaction water.

2. MATERIALS AND METHODS

2.1. Chemicals

A mixed deodorizer distillate from the refining of soybean, sunflower, rice bran and corn germ oil was obtained from the Kamolkij Group of Companies, Bangkok, Thailand. A crude fatty acid distillate from the physical refining of coconut oil was a gift from Walter Rau, Neuss, Germany and mixtures of distilled fatty acids from sunflower and coconut oil were products of Cognis Deutschland GmbH, Düsseldorf, Germany. The composition of the various co-products of vegetable oil refining is given in Table 1. Glycerol, Silica Gel H, Silica Gel 60 and 1-methyl-imidazol were products of E. Merck-VWR International, Darmstadt, Germany. Trimethyl sulfonium hydroxide (TMSH) reagent (0.2 M solution of TMSH in methanol) and *N*-methyl-*N*-

Table 1
Lipid and fatty acid composition of various co-products of vegetable oil refining as well as mixtures of distilled fatty acids

Composition[a] of	Co-product from deodorization of various vegetable oils (mixed deodorizer distillate)	Co-product from physical refining of coconut oil	Mixture of distilled fatty acids from coconut oil	Mixture of distilled fatty acids from sunflower oil
Lipid classes	**Lipid composition (wt %)**			
FFA	61.0	53.1	100	100
MG	2.3	4.1	n.d.	n.d.
DG	10.5	17.1	n.d.	n.d.
TG	26.2	25.7	n.d.	n.d.
Fatty acids	**Fatty acid composition of total lipids (wt %)**			
8:0	n.d	1.9	1.1	n.d.
10:0	n.d.	3.0	4.7	n.d.
12:0	n.d.	33.9	53.6	n.d.
14:0	tr	15.6	21.2	tr
16:0	29.5	20.0	9.8	6.1
18:0	4.0	12.4	1.3	5.0
18:1ω9	37.2	10.3	6.0	22.1
18:2ω6	27.6	4.0	2.4	66.8
18:3ω3	0.8	n.d.	n.d.	n.d.

[a] Abbreviations: DG, diglycerides; FFA, unesterified fatty acids; MG, monoglycerides; TG, triglycerides. Fatty acids are designated by number of carbon atoms : number of double bonds; wx indicates the position of the first double-bond counted from the methyl end. n.d. = not detected; tr = trace (<0.5 %).

trimethylsilylheptafluoro-butyramide (MSHFBA) were purchased from Macherey-Nagel, Düren, Germany. The immobilized lipase preparations from *Candida antarctica* (lipase B, Novozym 435®), *Rhizomucor miehei* (Lipozyme RM IM®) and *Thermomyces lanuginosus* (Lipozyme TL IM®) were kindly provided by Novozyme, Bagsvaerd, Denmark. Various lipid standards including fatty acid methyl esters as well as lipase from *Candida rugosa* (Type VII) were purchased from Sigma-Aldrich, Deisenhofen, Germany. A solution of diazomethane in diethyl ether was prepared from the reaction of an etherial solution of *N*-methyl-*N*-nitroso-*p*-toluolsulfonamide (Aldrich) with potassium hydroxide.

2.2. Lipase-catalyzed hydrolysis

Co-products of vegetable oil refining containing varying proportions of mono-, di- and triglycerides such as mixed deodorizer distillate or a crude distillate of fatty acids from the physical refining of coconut oil were hydrolyzed using microbial lipases. As an example, 15.0g mixed deodorizer distillate, 15.0g distilled water and 500 mg lipase from *C. rugosa* were stirred at 40 °C for 2 h in order to hydrolyze the residual proportions of the various glycerides (cf. Table 2). Finally, the lipase was separated by filtration under vacuum and the oily phase was used for esterification experiments.

2.3. Lipase-catalyzed esterification/transesterification reactions

The resulting mixture of fatty acids was esterified with glycerol at 60 °C under stirring (900 rpm) using Novozym 435-lipase as biocatalyst. Residual water was removed under reduced pressure (0.5 kPa) for 10 min using a vacuum pump with vacuum controller. The standard reaction mixture consisted of 10g of the respective co-product fatty acids, 1.8g glycerol and 0.209g Novozym 435. The molar ratio of the fatty acids to glycerol was ~2.5 : 1 and the proportion of Novozym 435 was ~1.8 wt%. The reaction was started by adding the immobilized lipase preparation. Reaction water was removed by evaporation at 0.5 to 1.5 kPa and trapping in the gas phase using potassium hydroxide pellets. At several time intervals 30 µL aliquots of total lipids were withdrawn from the reaction mixture and kept at −40 °C prior to analysis. Similarly, a crude fatty acid distillate from the physical refining of coconut oil and the corresponding hydrolysates as well as mixtures of distilled fatty acids from sunflower and coconut oil were used for the preparation of diglycerides. Under similar conditions, esterification experiments were performed using Lipozyme RM IM and Lipozyme TL IM as biocatalysts.

2.4. Thin layer chromatography (TLC)

Aliquots of total lipids were withdrawn from the reaction mixtures at different times and the conversion was checked by TLC on 0.3 mm layers of Silica Gel H. The plates were predeveloped with diethyl ether (~4 cm) and then developed in *i*-hexane - diethyl ether - acetic acid (60:40:1, v/v); spots were located by iodine staining. Alternatively, the TLC plates were sprayed with 30% aqueous sulfuric acid and heated in an oven kept at 200 °C.

Similarly, aliquots of total lipids were fractionated by TLC on 0.5 mm layers of Silica Gel 60 G impregnated with boric acid (5 g boric acid/100g silica gel). The plates were predeveloped with diethyl ether (~4 cm) and then developed in *i*-hexane - diethylether (3:2, v/v). The following fractions were isolated for fatty acid analysis: triglycerides, 1(3)2-diglycerides, 1,3- diglycerides, monoglycerides and unesterified fatty acids.

2.5. Lipid analyses

The reaction mixtures contained tri-, di- and monoglycerides µL) of total lipids were removed from the reaction mixture, dissolved in 6 mL methyl-*t*-butylether and filtered through a 1.0 µm syringe filter to separate the immobilized enzymes. Around 2 mL of the filtrate (~10 mg of total lipids) were concentrated in a stream of nitrogen at ~20 °C. Unesterified fatty acids were converted to the corresponding methyl esters by treating with an ethereal solution of diazomethane containing small proportions of methanol. The solvent was removed from the reaction mixture in a stream of nitrogen and these methylated lipid mixtures were finally silylated with 100 µL MSHFBA in the presence of 5 µL 1-methyl-imidazol at 110 °C for 60 min. After cooling, the reagents were removed in a stream of nitrogen at 60 °C and the reaction mixture was dissolved in diethyl ether for GC injection.

The fractions of fatty acid methyl esters, silylated mono- and diglycerides as well as triglycerides were analyzed by high-temperature gas chromatography (HT-GC) using a 12 m HT5 AQ (SGE, Darmstadt, Germany) fused silica capillary column (0.22 mm i.d., 0.1 mm film thickness). Hydrogen was used as carrier gas (column head pressure 80 kPa) at a split ratio of 1:10. Temperature was programmed from 100 °C (2 min) at 10 °C/min to 420 °C (3 min). Injector and FID temperatures were set at 420 °C. Peaks were integrated using a Hewlett Packard GC ChemStation software. Response factors of flame ionization detector (FID) were determined for, e.g., fatty acid methyl esters, silylated mono- and diglycerides as well as triglycerides using purified compounds.

2.6. Fatty acid analyses

Fatty acid composition of total lipids from various co-products of vegetable oil refining were studied after conversion to the methyl esters. Aliquots of total lipids (around 2 mg, each) were dissolved in 40 µL methyl-*t*-butylether and treated with 20 µL TMSH reagent in order to prepare methyl esters of their

constituent fatty acids. The mixtures were shaken vigorously and kept at 75 °C for 15 min prior to direct injection onto the gas chromatograph. Unesterified distilled fatty acids from coconut oil were converted to the corresponding methyl esters by treating with an ethereal solution of diazomethane.

Fatty acid methyl esters were analyzed by gas chromatography on a 40 m DB-23 (methyl/50% cyanopropyl silicone; J&W, ASS-Chem, Bad Homburg, Germany) fused silica capillary column (0.18 mm i.d., 0.2 mm film thickness). Hydrogen was used as carrier gas (column head pressure 142 kPa) at a split ratio of 1:10. Temperature was programmed from 160 °C (2 min) at 1 °C/min to 178 °C, then at 8 °C/min to 225 °C (2 min) and finally at 10 °C/min to 250 °C (10 min). Injector and FID temperatures were set at 280 °C. Peaks were integrated as described above.

2.7. Vacuum distillation

Unesterified fatty acids and, in part, monoglycerides were removed from the reaction mixtures (2-3 g) by short-path distillation in a 50 mL round-bottom flask. Optimum conditions for the enrichment of diglycerides in the distillation residue were determined by variation of heating temperatures (140-200 °C) and distillation times (5 to 12 min). The pressure was in the range of 0.01 to 0.05 kPa.

3. Results and Discussion

3.1. Optimization of esterification conditions

Diglycerides were prepared by lipase-catalyzed esterification/transesterification using fatty acid-containing co-products of vegetable oil refining and glycerol as the starting materials. As an example, mixed deodorizer distillate containing around 61% unesterified fatty acids and 26% triglycerides (Table 1) was reacted with glycerol using Novozym 435 as biocatalyst in order to determine the optimum conditions of diglyceride formation. The following reaction parameters were studied: molar ratio of substrates, type of enzyme, temperature, pressure and time. The amount of enzyme was kept constant at ~1.9 wt% of the reaction mixture consisting of mixed deodorizer distillate and glycerol.

A typical time-course of the esterification of the mixed deodorizer distillate with glycerol in a solvent-free system is presented in Figure 1A. At the beginning of the reaction the substrates were separated into two phases. In the course of the esterification process, however, mono- and diglycerides were formed which were effective as emulsifiers leading to the formation of a homogeneous phase. The concentration of unesterified fatty acids decreased continuously with increasing reaction time. Since the esterification of fatty acids with glycerol is a reaction consisting of three consecutive acylation steps the concentrations of monoglycerides and diglycerides pass through a maximum with increasing reaction time. Above 100 min the concentration of triglycerides steadily increased, most probably by the transesterification reactions (disproportionation) of diglycerides into monoglycerides and triglycerides (Yamada *et al.*, 1999). For the preparation of diglycerides the reaction was stopped as soon as the diglyceride concentration reached its maximum (46% at 180 min).

Figure 1B demonstrates the effect of the molar ratio of the substrates such as unesterified fatty acids from mixed deodorizer distillate to glycerol on the formation of diglycerides using Novozym 435 as biocatalyst. The molar ratio of the mixed deodorizer distillate to glycerol varied in the range of 1 : 0.11 to 1 : 5.15. Under the conditions described, the maximum diglyceride formation depended only moderately on the proportion of the unesterified fatty acids. This may result, in part, from concurrent transesterification reactions of both diglycerides (~10%) and triglycerides (~26%) which were also present in the starting material (Table 1). Figure 1B shows, however, that the maximum concentration of diglycerides was reached at a molar ratio of ~2.5 : 1 which is consistent with the results of others (Kristensen *et al.*, 2005a). This molar ratio of starting materials was used in all further experiments.

The time course of the formation of diglycerides by esterification/transesterification of mixed deodorizer distillate with glycerol catalyzed by Novozym 435 at different temperatures is demonstrated in Figure 1C. Increasing the reaction temperature may lower viscosity, enhance solubility of the substrates and raise interfacial area and diffusion coefficients. Therefore, the diglyceride formation depended on the reaction temperature as is obvious from Figure 1C; the maximum formation was observed at 60 °C. It is known that thermal stability of Novozym 435 is quite high at this temperature which is of particular interest for industrial applications.

The equilibrium of the enzyme-catalyzed esterification/transesterification can be directed to the formation of glycerol esters by continuous evaporation of the reaction water under reduced pressure. The influence of reaction pressure on the Novozym 435-catalyzed diglyceride formation by esterification/transesterification of mixed deodorizer distillate with glycerol is presented in Figure 1D. A distinct increase in the maximum diglyceride concentration from 46 to 52% was observed by reducing the pressure from 1.5 to 0.5 kPa which is consistent with findings by others (Kristensen *et al.*, 2005a).

It is evident from the results described above that the optimum reaction conditions determined for the preparation of diglycerides from mixed deodorizer distillate by Novozym 435-catalyzed esterification/transesterification with glycerol are as follows: (i) molar ratio of unesterified fatty acids to glycerol, ~2.5 : 1; (ii) temperature, 60 °C; (iii) pressure, $<$1.5 kPa. These reaction conditions were

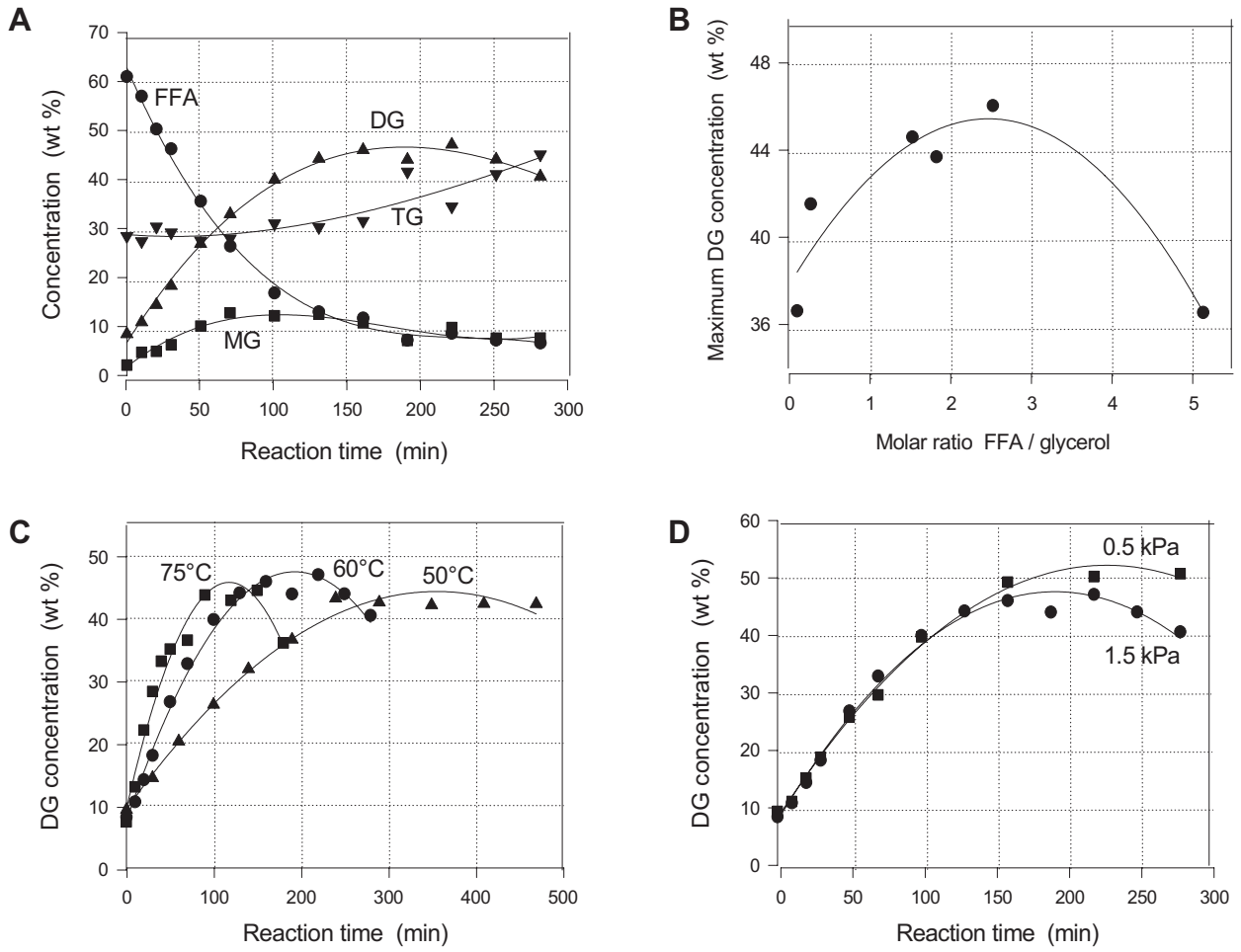

Figure 1
Formation of diglycerides by enzyme-catalyzed esterification of mixed deodorizer distillate with glycerol using Novozym 435 as biocatalyst. **(A)** Time course; **(B)** Effect of molar ratios; **(C)** Effect of temperature; **(D)** Effect of pressure. Abbreviations: FFA, unesterified fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides.

also used in experiments with other co-products of vegetable oil refining such as hydrolyzed distillate from the physical refining of coconut oil and distilled coconut fatty acids.

3.2. Partial hydrolysis of co-products of vegetable oil refining

Mixed deodorizer distillate and a crude fatty acid distillate from the physical refining of coconut oil (Table 1) were enzymatically hydrolyzed using *C. rugosa* lipase in order to increase the concentration of unesterified fatty acids (Figure 2A) because a high initial concentration of unesterified fatty acids reportedly increased the maximum concentration of diglycerides in the final product (Yamada *et al.*, 1999). The reaction conditions of hydrolysis and the lipid composition of the hydrolyzed co-products are presented in Table 2. It was found that the various acylglycerides (mono-, di-, and triglycerides) of both starting materials were almost completely hydrolyzed to unesterified fatty acids within 1-2 h at 40 °C in the presence of water using *C. rugosa* lipase as biocatalyst. Similar results were described for the hydrolysis of soybean oil using sol-gel entrapped *C. rugosa* lipase (Noureddini *et al.*, 2003). Experiments on the partial hydrolysis of mixed deodorizer distillate using Novozym 435 failed because of extremely long reaction times (Figure 2B).

3.3. Preparation of diglycerides from co-products of vegetable oil refining

The (partially) hydrolyzed co-products, i.e., mixed deodorizer distillate and a crude distillate from the physical refining of coconut oil (Table 2), as well as mixtures of distilled fatty acids from sunflower and coconut oil (Table 1) were used for the preparation of diglycerides by esterification under optimized processing conditions. The results of these kinetic studies as given in Figure 3A–D and Table 3 show that maximum diglyceride concentrations obtained by esterification/transesterification of the various co-products of vegetable oil refining with glycerol catalyzed by Novozym 435 varied in the range of 61-72%. By comparing the results from Table 3 with those from Figure 1A, it is obvious that a high concentration of unesterified fatty acids in the starting materials generally increases the formation

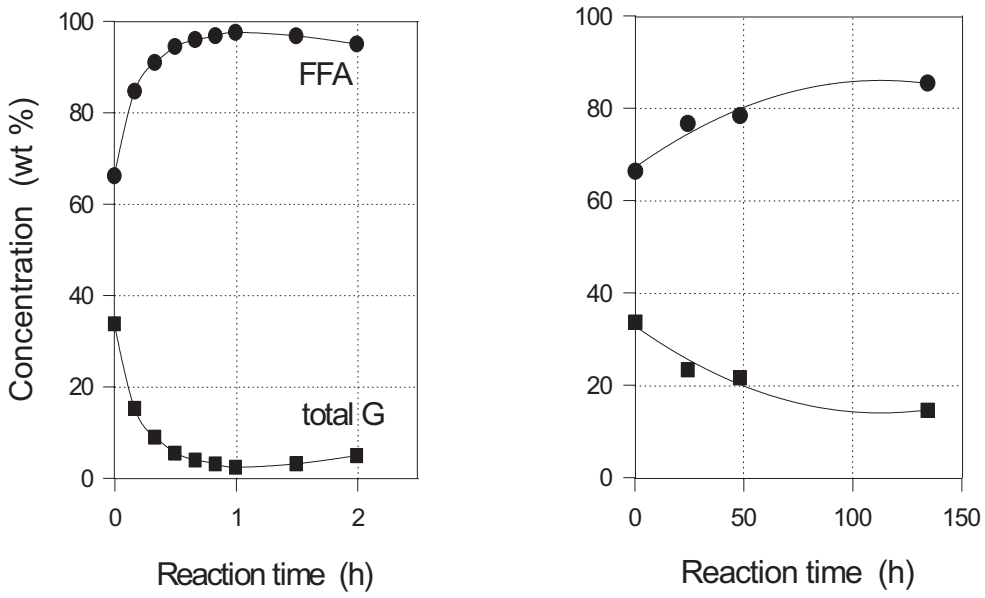

Figure 2
Time-course of the lipase-catalyzed hydrolysis of total glycerides (total G) of mixed deodorizer distillate using **(A)** lipase from C. *rugosa* and **(B)** Novozym 435-lipase. Abbreviations: as given in Figure 1.

of diglycerides. For example, starting with a co-product having a concentration of unesterified fatty acids of 61%, as was found in the mixed deodorizer distillate, a fairly high diglyceride concentration of 46-52% was expected (Figure 1D). If the reaction was carried out, however, with co-products containing >95% of unesterified fatty acids as starting materials (Table 3) the concentration of diglycerides in the final reaction mixture was substantially higher (up to 72%). Similar kinetic esterification studies were performed with Lipozyme RM IM and Lipozyme TL IM as biocatalysts. From the results of these experiments it follows that the esterification activity of Lipozyme RM IM is clearly lower (48.7% diglycerides in the reaction products after 460 min) than that of Novozym 435, whereas poor esterification activity was found for Lipozyme TL IM (data not shown).

3.4. Enrichment of diglycerides by vacuum distillation

Diglycerides were concentrated by short-path vacuum distillation which was particularly used to remove unesterified fatty acids and

Table 2
Reaction conditions of the hydrolysis of various co-products of vegetable oil refining and lipid composition of the hydrolyzed co-products.

	Reaction conditions for the hydrolysis[a] of		
	Co-product from deodorization of various vegetable oils (mixed deodorizer distillate)	**Co-product from deodorization of various vegetable oils (mixed deodorizer distillate)**	**Co-product from physical refining of coconut oil**
Type of lipase	Novozym 435	*Candida rugosa*	*Candida rugosa*
Amount of lipase (g)	0.21	0.50	0.66
Co-product oil (g)	10.0	15.0	20.0
Water (g)	2.5	15.0	20.0
Time (h)	134	2	2
Temperature (°C)	60	40	40
Lipid classes	**Lipid composition (wt %) after hydrolysis[b]**		
FFA	85.6	96.9	99.2
MG	2.1	1.8	n.d.
DG	1.3	0.4	0.8
TG	11.0	0.9	n.d.

[a] Reaction conditions are summarized in Materials and Methods.
[b] Abbreviations: as given in Table 1.

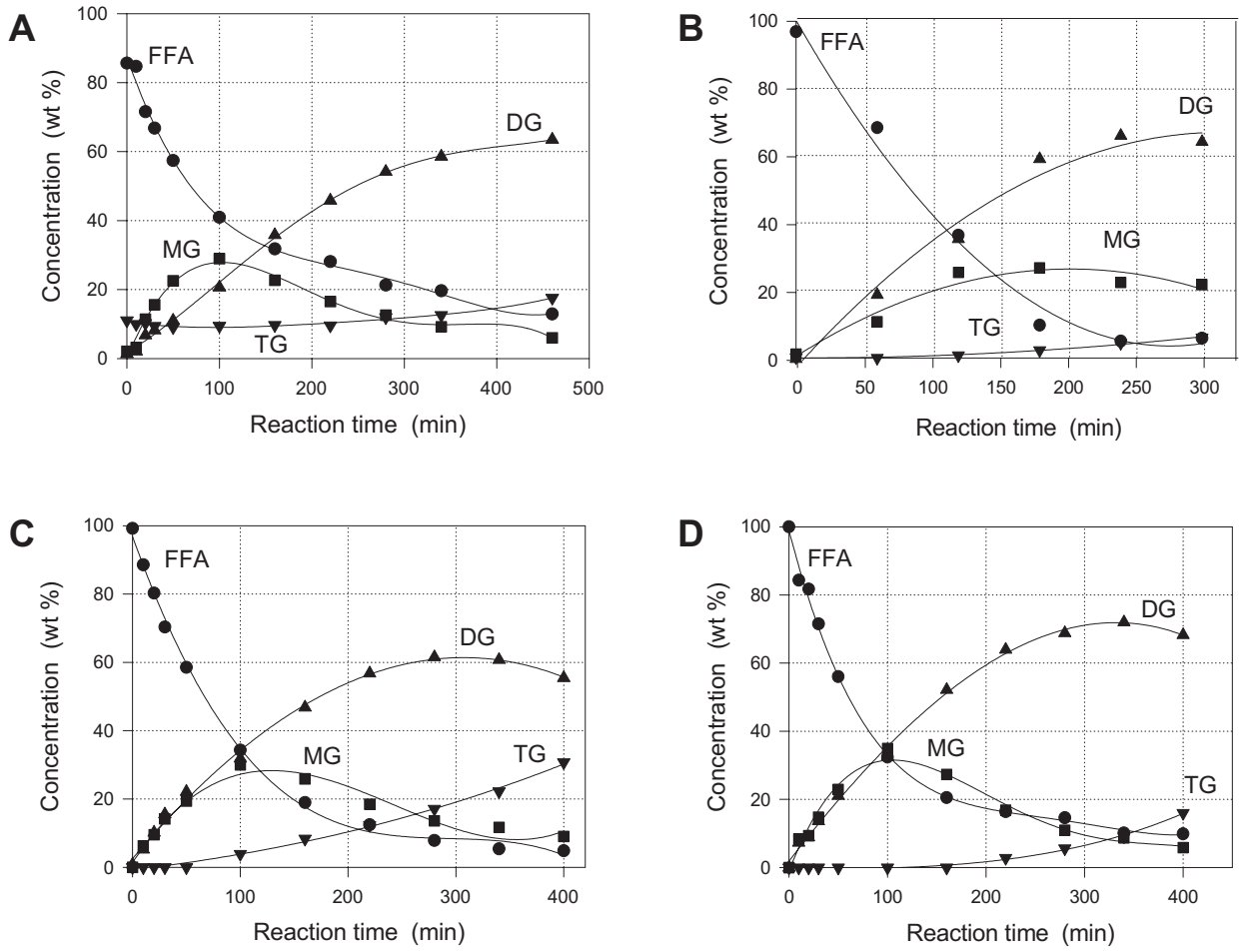

Figure 3
Time course of the formation of diglycerides by Novozym 435-catalyzed esterification of hydrolyzed co-products of vegetable oil refining and of distilled fatty acids from coconut oil with glycerol: **(A)** partially hydrolyzed mixed deodorizer distillate; **(B)** totally hydrolyzed mixed deodorizer distillate; **(C)** totally hydrolyzed distillate from the physical refining of coconut oil; **(D)** distilled fatty acids from coconut oil. Abbreviations: as given in Figure 1.

Table 3
Lipid composition of products of the Novozym 435-catalyzed esterification of fatty acids from three hydrolyzed co-products of vegetable oil refining or of distilled fatty acids from sunflower and coconut oil with glycerol at the maximum diglyceride concentration

Starting materials[a]	Esterification time (min)	Lipid composition (%) of esterification products[b]			
		FFA	MG	DG	TG
Partially hydrolyzed mixed deodorizer distillate	460	12.9	6.0	63.4	17.6
Totally hydrolyzed mixed deodorizer distillate	300	5.0	21.2	66.8	7.0
Hydrolyzed co-product from physically refined coconut oil	300	8.3	11.3	61.5	18.9
Distilled fatty acids from sunflower oil	400	17.7	3.8	72.9	5.6
Distilled fatty acids from coconut oil	340	11.1	7.7	72.0	9.2

[a] The lipid composition of (partially) hydrolyzed co-products used as starting materials is given in Table 2.
[b] Abbreviations: as given in Table 1.

monoglycerides from the reaction mixtures. Under the conditions described the reaction products were separated into a distillate consisting of unesterified fatty acids plus monoglycerides and a distillation residue containing diglycerides and triglycerides which was almost free of unesterified fatty acids and monoglycerides (≤1% and 0.9-3.9%, respectively). A separation of triglycerides from diglycerides is completely unnecessary, particularly for products that may be used as fortified foods, emulsifiers for food and feed or as ingredients for cosmetics and toiletteries or pharmaceutical preparations. The results of various distillation experiments starting with the esterification products of various co-products of vegetable oil refining such as mixed deodorizer distillate, partially and totally hydrolyzed mixed deodorizer distillate and distilled fatty acids from sunflower and coconut oil are shown in Table 4. The reaction conditions were optimized with regard to distillation temperature and time.

Distillation pressure was kept constant at approximately 0.01-0.05 kPa in all experiments. The results show that unesterified fatty acids and monoglycerides were almost completely separated from the distillation residue which consisted of diglycerides plus triglycerides. It is worth noting that the ratio of diglycerides to triglycerides significantly decreases at high distillation temperatures which may result from thermally induced acyl migration. Aliquots of diglycerides prepared from the distilled fatty acids of coconut oil and purified by distillation (160 °C; 0.01 kPa; 8 min) were separated by thin-layer chromatography on boric acid impregnated silica plates in order to determine the ratio of 1,3- and 1,2(2,3)-diglycerides in the reaction mixture. The concentrations of 1,3-diglycerides and 1,2(2,3)-diglycerides were found to be 74.4 and 25.6%, respectively, corresponding well with the known equilibrium composition (Rosu et al., 1999; Kristensen et al., 2005b).

4. CONCLUSION

The main parameters affecting the solvent-free lipase-catalyzed preparation of diglycerides by esterification of co-products of vegetable oil refining with glycerol have been studied, i.e., (i) the molar ratio of co-product to glycerol, (ii) type of enzyme, (iii) temperature, and (iv) pressure. High concentrations of unesterified fatty acids in the starting materials such as deodorizer distillates, crude distillates from physical refining or the corresponding hydrolysates and distilled fatty acids generally increase the concentration of diglycerides in the esterification products. Esterification activity of Novozym 435 was higher than that of Lipozyme RM IM and, particularly, of Lipozyme TL IM. Other reaction parameters such as temperature strongly increased the rate of esterification, whereas the influence of pressure on esterification was only moderate. Enrichment of diglycerides in the final products by short-path vacuum distillation led to concentrates containing up to 94 % diglycerides and around 5% triglycerides which were practically free of unesterified fatty acids and monoglycerides. These results demonstrate that co-products of vegetable oil refining are useful starting materials for the lipase-catalyzed production of diglycerides for food and feed, cosmetics and toiletteries as well as pharmaceutical and technical applications.

Table 4
Lipid composition of distillation residues of diglyceride preparation from different co-products of vegetable oil refining and from distilled fatty acids of sunflower and coconut oil after short-path vacuum distillation

Co-products[a]	Lipid composition (%)[b] of residues after distillation			
	FFA	MG	DG	TG
Mixed deodorizer distillate[c]	<0.1	1.8	51.7	46.5
Partially hydrolyzed mixed deodorizer distillate[c]	0.2	0.9	70.0	28.9
Totally hydrolyzed mixed deodorizer distillate[d]	n.d.	3.9	74.4	21.7
Distilled fatty acids of sunflower oil[e]	<0.1	0.9	81.3	17.9
Distilled fatty acids of coconut oil[f]	0.3	1.0	94.0	4.7

[a] Abbreviations: as given in Table 1.
[b] The lipid compostion of the diglyceride reaction mixtures prior to distillation are given in Tables 1 and 3.
[c] Distillation conditions: 10 min, 200 °C, 0.05 kPa.
[d] Distillation conditions: 5 min, 200 °C, 0.05 kPa.
[e] Distillation conditions: 12 min, 200 °C, 0.05 kPa.
[f] Distillation conditions: 8 min, 160 °C, 0.01 kPa.

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