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ORIGINAL PAPER

# Separation of Acylglycerides Obtained by Enzymatic Esterification Using Solvent Extraction

**Daniel Alberto Sánchez · Gabriela Marta Tonetto · María Luján Ferreira**

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**Abstract** New avenues to add value to glycerol are currently being explored. One of them is the synthesis of structured lipids through glycerol esterification. In this work we have analyzed the recovery and purification of dicaprin obtained by esterification of glycerol with capric acid (C) in heptane, mediated by Lipozyme RM IM. This is an intermediate step to obtain lipids MLM. In the first stage, the diglyceride synthesis MGM (being G a central HC–OH) was carried out. When M = C, the diglyceride is CGC. Recovery of the diglyceride CGC is required to carry out the esterification of the *sn*-2 position with palmitic acid (P), thus obtaining the triglyceride CPC. Different solvents were evaluated using Ecofac 1.0 (a molecular design software solvent) through a theoretical approach to explore the best solvents for the acylglycerides separation. Then, the performance of the selected solvents to separate dicaprin from mono and tricaprin was experimentally studied in a liquid–liquid extraction process. Previously, the remaining fatty acid had been neutralized. With liquid–liquid extraction in three simple steps, using ethanol/water, 94 % of the dicaprin obtained by enzymatic esterification was recovered with a purity of 89 % (wt%). It was also possible to obtain dicaprin with a purity of 97 % but with a yield of 56 %.

**Keywords** Separation · Fatty acids · Acylglycerides · Extraction · Liquid–liquid

D. A. Sánchez · G. M. Tonetto · M. L. Ferreira (✉)
Planta Piloto de Ingeniería Química (PLAPIQUI), Universidad Nacional del Sur, CONICET, Camino La Carrindanga Km 7, CC 717, 8000 Bahía Blanca, Argentina
e-mail: mlferreira@plapiqui.edu.ar

## Introduction

Structured lipids (SL) containing a mixture of medium-chain fatty acids (MCFA) and long-chain fatty acids (LCFA) in the same molecule have been designed for medical and nutritional purposes. The placement of a long (L) chain fatty acid at the *sn*-2 position of glyceryl carbons, particularly if it is saturated, increases the digestibility of the MLM lipid, being in the *sn*-1 and *sn*-3 positions a medium (M) chain fatty acid. These SL are reported to be helpful to the immune system and to improve lipid clearance, having application in the prevention of obesity and its associated medical conditions [1–3].

The most studied process for obtaining MLM is acidolysis between long-chain triacylglycerols (TAG) and MCFA in a one-step reaction. The most selective catalysts are some 1,3-specific lipases [4–9]. However, this method requires an important post-reaction purification because the desired MLM triglyceride is obtained with other secondary and undesired products, such as mono and diglycerides, fatty acids and other triglycerides. Hydrolysis is a secondary reaction very difficult to avoid using commercial lipases. In addition, acyl migration is also present, with the generation of minor amounts of glycerol and MMM triglyceride.

Several authors have reported a two-step process [10–13]. In the first step, 2-monoacylglycerols (2-MAG) are obtained (from vegetable oils or TAG with the desired fatty acids) by enzymatic alcoholysis using 1,3-specific lipases. In the second step, MCFA are added in the extreme positions of 2-MAG by enzymatic esterification with 1,3-specific lipases. This step can also be carried out with a novel heterogeneous catalyst.

The recovery of the reaction intermediate has also been studied. Esteban et al. [14] described a process of

extraction. However, they searched for the recovery of monoglycerides, separating the ethyl esters. A similar procedure was described by Irimescu et al. [15] but using a highly toxic solvent. Hita et al. [9] included capric acid neutralization as a first stage. However, they did not study the appropriate concentration of the KOH solution because the evaluated sample contained only triglycerides.

The enzymatic synthesis of 1,3-diacylglycerols (1,3-DAG) can be the first step to the desired MLM structured triglyceride. The metabolic characteristics of DAG are interesting too, and we recently researched them. Results of clinical studies showed that DAG can be considered to be effective in preventing obesity [16, 17] and reduces the postprandial increase in serum chylomicron TAG levels [18, 19].

In this work we studied the separation of the reaction products of the synthesis of 1,3-diacylglycerol, specifically 1,3-dicaprin (CGC). The same was carried out by enzymatic esterification of glycerol with capric acid (C) using as a biocatalyst an immobilized form of a lipase from *Rhizomucor miehei,* specific for *sn*-1 and *sn*-3 positions (Lipozyme RM IM). However, to maximize obtaining a specific triglyceride, it is necessary to purify the reaction intermediate, since in the first stage, besides specific generated diglyceride monocaprin, nonspecific diglycerides (generated by acyl migration) and tricaprin (CCC) are also obtained.

Different solvents were evaluated using a computer program to select those solvents that allow the dicaprin separation from the mixture, but are also of low toxicity. The purpose is the synthesis of products for nutritional purposes therefore toxic solvents must be avoided. Once the solvents were selected, the separation of acylglycerols present in the reaction products was carried out, previously performing the neutralization of un-reacted capric acid with a solution of KOH.

## Experimental

### Materials

Lipozyme RM IM, which is a commercial form of the 1,3-specific lipase from *R. miehei* immobilized by adsorption on a macroporous anion exchange phenolic resin Duolite 586-A, was kindly donated by Novo Nordisk A/S (Brasil). Glycerol, *n*-heptane, acetone, isopropyl ether and silica gel were supplied by Cicarelli Laboratorios. Capric acid, 1,2,4-butanetriol and silylation reagents were obtained from Fluka. Monocaprin, dipalmitin, trilaurin and tricaprin were supplied by Sigma-Aldrich. Absolute ethanol was supplied by Dorwil and phenolphthalein and pyridine were provided by Anedra S.A. All products were of analytical grade.

### Adsorption of Glycerol on Silica gel

One gram of glycerol and 2 g of silica gel were mechanically mixed until total adsorption on the solid.

### Lipase-Catalyzed Esterification

Esterification of glycerol was performed in 10-mL flasks which were kept in a thermostatic bath with temperature control and magnetic stirring. The reaction time was 6 h. One hundred and ten milligrams of capric acid was dissolved in 3 mL of *n*-heptane, then 250 mg of glycerol adsorbed onto 500 mg of silica were added. This silica acted as a water-removing surface. When the reaction mixture reached 60 °C, 50 % of the total amount of enzyme to use (time 0) was added. The remaining 50 % of the biocatalyst was introduced at 3 h of reaction. The total enzyme dosage was 40 mg.

In all reactions, once the set time was reached the flask was subjected to 15 min of ultrasound with the aim of desorbing capric acid and reaction products that could be adsorbed on the silica. Then the content of the flask was filtered and the solid material separated. From recovered liquid phase 100 μL of sample was taken and stored at −20 °C to analyze its composition by gas chromatography. The remaining volume was used to examine the separation of the acylglycerides.

### Evaluation of Solvents

To study the feasibility of separation of acylglycerols nine solvents were evaluated using the software Ecofac 1.0 (software of solvent molecular design) based on UNIFAC interaction parameters, designed by Cismondi and Brignole [20–22].

The log P is the log of the solubility of the un-ionized compound in octanol vs in un-ionized water and known as the distribution ratio. *n*-Heptane, chloroform, diethyl ether, *t*-butanol, propanol, acetic acid, acetone, ethanol and methanol (ordered in their log P values) were evaluated in a process of liquid–liquid extraction at 300 K. Being glycerol polar and capric acid non-polar their solubilities in different solvents are very different. This step was an attempt to explore and confirm the best solvent for the glycerol esterification reaction considering mainly the solubility of the compounds present in the reaction media.

The Unifac (UNIversal Function Activity Coefficient) group contribution method [23] and the corresponding parameters were used for the calculation. This group contribution approach enables one to assume that the mixture does not consist in molecules but in limited functional groups of which the interaction parameters are known [24].

The software enables the performance evaluation of solvents for the separation of two components, one of which is extracted with the solvent and the other one is the main component.

The separation mixtures analyzed were:

- Monocaprin extraction with solvent (from dicaprin and tricaprin mixtures respectively),
- Dicaprin extraction with solvent (from monocaprin and tricaprin mixtures respectively), and
- Tricaprin extraction with solvent (being dicaprin or monocaprin the main component in the solution).

The parameters that allowed us to evaluate the performance of the solvents using Ecofac were selectivity, solvent power and solute distribution coefficient.

Selectivity is defined by the following equation:

$$\beta = \frac{\gamma^{\infty}_{B,S}}{\gamma^{\infty}_{A,S}} \frac{MW_A}{MW_B} \quad (1)$$

Solvent power is defined as:

$$S_p = \frac{1}{\gamma^{\infty}_{A,S}} \frac{MW_A}{MW_B} \quad (2)$$

Solute distribution coefficient is defined using the folowing equation:

$$m = \frac{\gamma^{\infty}_{A,B}}{\gamma^{\infty}_{A,S}} \frac{MW_B}{MW_S} \quad (3)$$

Where

$MW_A$ is the molecular weight of the compound A (to be extracted with the solvent)

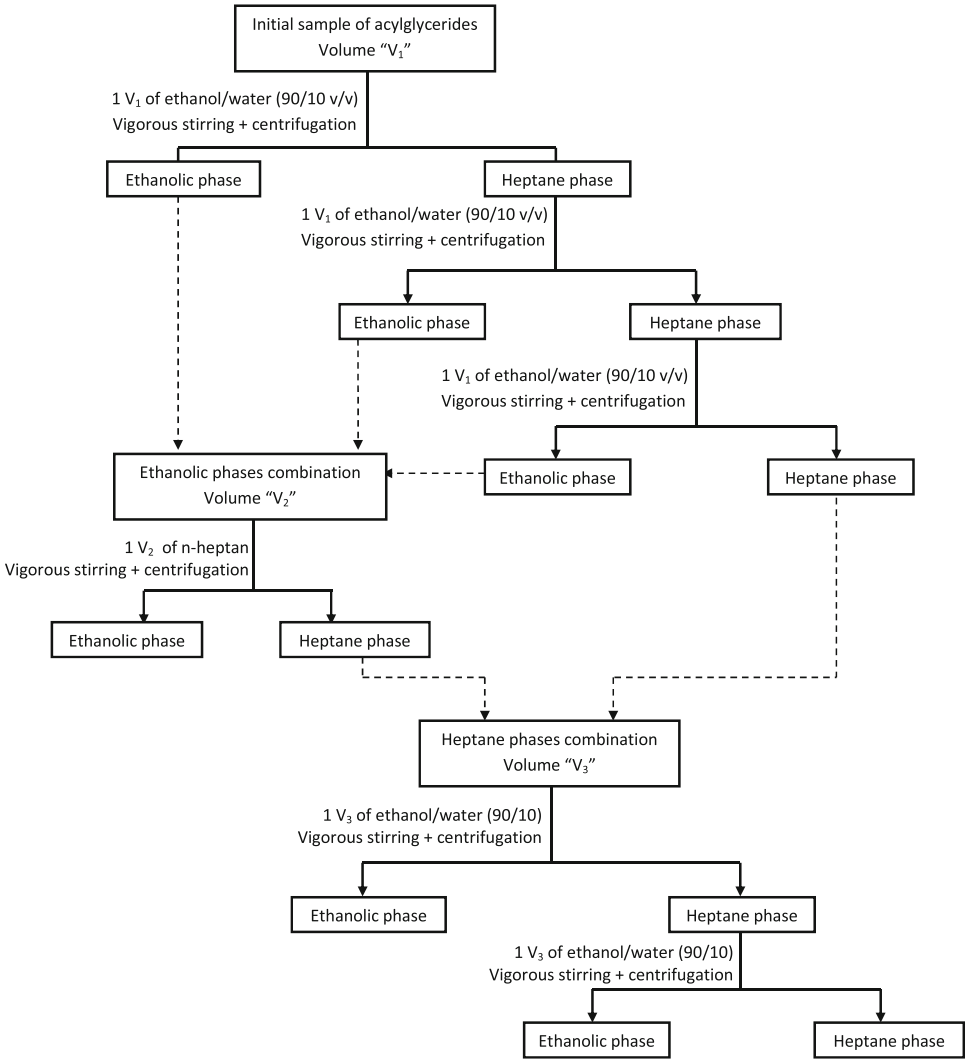


**Fig. 1** Separation stages for liquid–liquid extraction

$MW_B$

**Table 1** Liquid–liquid extraction

| Solvent | Selectivity | Solvent power | Distribution coefficient |
|---|---|---|---|
| Methanol | 2.65 | 3,595.36 | 120.28 |
| Acetic acid | 1.48 | 3,139.38 | 105.02 |
| Ethanol | 2.61 | 1,183.92 | 39.61 |
| Propanol | 1.63 | 806.76 | 26.99 |
| Acetone | 0.25 | 730.83 | 24.45 |
| *tert*-Butanol | 1.25 | 513.71 | 17.19 |
| Chloroform | 0.01 | 240.40 | 8.04 |
| Diethyl ether | 0.03 | 181.18 | 6.06 |
| *n*-Heptane | 0.01 | 0.77 | 0.03 |

Compound to be recovered: monocaprin; major component in the residual mixture: dicaprin

**Table 2** Liquid–liquid extraction

| Solvent | Selectivity | Solvent power | Distribution coefficient |
|---|---|---|---|
| Methanol | 26.05 | 3,595.36 | 620.19 |
| Acetic acid | 6.58 | 3,139.38 | 541.54 |
| Ethanol | 20.95 | 1,183.92 | 204.22 |
| Diethyl ether | 0.00 | 941.32 | 162.38 |
| Propanol | 8.50 | 806.76 | 139.17 |
| Acetone | 0.23 | 730.83 | 126.07 |
| *tert*-Butanol | 5.15 | 513.71 | 88.61 |
| Chloroform | 0.00 | 240.40 | 41.47 |
| *n*-Heptane | 0.00 | 0.77 | 0.13 |

Compound to be recovered: monocaprin; major component in the residual mixture: tricaprin

**Table 3** Liquid–liquid extraction

| Solvent | Selectivity | Solvent power | Distribution coefficient |
|---|---|---|---|
| Chloroform | 108.48 | 26,078.22 | 383.22 |
| Diethyl ether | 34.16 | 6,189.71 | 90.96 |
| Acetone | 3.99 | 2,918.95 | 42.89 |
| Acetic acid | 0.67 | 2,116.78 | 31.11 |
| Methanol | 0.38 | 1,356.12 | 19.93 |
| Propanol | 0.61 | 493.70 | 7.25 |
| Ethanol | 0.38 | 454.20 | 6.67 |
| *tert*-Butanol | 0.80 | 409.83 | 6.02 |
| *n*-Heptane | 81.01 | 62.44 | 0.92 |

Compound to be recovered: dicaprin; major component in the residual mixture: monocaprin

**Table 4** Liquid–liquid extraction

| Solvent | Selectivity | Solvent power | Distribution coefficient |
|---|---|---|---|
| Chloroform | 0.03 | 26,078.22 | 533.59 |
| Diethyl ether | 0.11 | 6,189.71 | 126.65 |
| Acetone | 0.91 | 2,918.95 | 59.72 |
| Acetic acid | 4.44 | 2,116.78 | 43.31 |
| Methanol | 9.83 | 1,356.12 | 27.75 |
| Propanol | 5.20 | 493.70 | 10.10 |
| Ethanol | 8.04 | 454.20 | 9.29 |
| *tert*-Butanol | 4.11 | 409.83 | 8.39 |
| *n*-Heptane | 0.06 | 62.44 | 1.28 |

Compound to be recovered: dicaprin; major component in the residual mixture: tricaprin

is the molecular weight of the compound B (main compound of the residual mixture product)

$MW_S$ is the molecular weight of the solvent

$\gamma^{\infty}_{A,S}$ is the infinite dilution activity coefficient of compound A in the solvent

$\gamma^{\infty}_{B,S}$ is the infinite dilution activity coefficient of compound B in the solvent

$\gamma^{\infty}_{A,B}$ is the infinite dilution activity coefficient of compound A in the compound B

Purity (P) was calculated by the following equation:

$$P = \frac{\text{DAG}}{(\text{DAG} + \text{TAG} + \text{MAG} + \text{FA})} \times 100 \quad (4)$$

where

DAG is dicaprin in the ethanolic phase

DAG + TAG + MAG + FA is total DAG, tricaprin (TAG), monocaprin (MAG) and fatty acid (FA) in the ethanolic phase

Yield is defined using

$$Y_{\text{DAG}} = \frac{\text{DAG}_i}{\text{DAG}_f} \times 100 \quad (5)$$

where is

$Y_{\text{DAG}}$ is yield in dicaprin (Recovered) related to the initial amount of dicaprin

$\text{DAG}_i$ is initial dicaprin

$\text{DAG}_f$ is recovered dicaprin

## Capric Acid Neutralization

After filtering the contents of the flask and obtaining the sample for chromatographic analysis, non-reacted capric acid was neutralized. A volume was withdrawn (3 mL) and an equal volume of an aqueous solution of KOH was added. Different KOH concentrations (1, 0.5, 0.25, 0.1 and 0.05 M) were studied to obtain one basic solution that allows the total acid neutralization but minimizes the

removal of the compound of interest, in this case, dicaprin.

After adding the appropriate volume of alkaline solution, the sample was subjected to intense agitation and then centrifuged at 8,000 rpm for 15 min. The heptane phase was recovered and 100 μL were withdrawn to analyze by gas chromatography. The remaining volume of sample was used for the separation of the acylglycerides.

### Liquid–Liquid Extraction

To the solution of *n*-heptane containing acylglycerides, an equal volume of an ethanol/water (90/10 v/v) is added. The small proportion of water allowed phase separation, which would otherwise not occur. The sample was subjected to intense agitation allowing contact between the phases and then centrifuged for 20 min at 8,000 rpm.

The heptane phase was extracted and 200 μL of this was taken for gas chromatographic analysis. The alcohol phase is maintained at −20 °C. This procedure with the heptanes phase was performed 3× as shown in Fig. 1.

Then, the three recovered ethanolic phases (Fig. 1) were combined and subjected to an extraction with an equal volume of *n*-heptane which eliminated the triglyceride fraction.

Finally, the two heptane phases were combined and subjected to a new extraction process with ethanol/water solution as shown in Fig. 1.

### Gas Chromatography Analysis

Samples were diluted with pyridine and silylated with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA). The analysis of samples was performed in a PerkinElmer AutoSystem XL gas chromatograph equipped with on-column injection, a flame ionization detector (FID) and a high temperature capillary column ZB-5HT Inferno (15 m × 0.32 mm, with an ID of 0.10 μm), using $H_2$ as gas carrier. The detector temperature was maintained at 380 °C. The initial column temperature was held at 50 °C for 1 min, elevated to 180 °C at a rate of 15 °C/min, then the temperature was increased to 230 °C at a rate of 7 °C/min, further increased up to 370 °C at a rate of 10 °C/min, and finally held there for 5 min. This method allowed the detection of capric acid and of all the acylglycerides present in the sample in <25 min [25]. Results are the average of two injections with an average relative error lower than 2 %. The determination of elution times of reactants and products was performed with high purity standards.

### Statistical Analysis of Experimental Error

The percentage error was calculated as.

$$\text{Error } \% = \frac{\text{Average of different measures} - \text{Individual values}}{\text{Average value}} \times 100$$

The average error is the summation of all errors (from replicates) divided by the total number of experiments.

Reactions were performed in triplicate. The average error found in the acid titration step was 2.3 %. The average error between all the chromatographic injections, including the calibration step, was 1.6 %. All the injections were performed in duplicate. The neutralization step was performed in duplicate. The average error in the determination of the remnant acid in the heptane phase was 3.4 %. The amount of capric acid that remains after the neutralization step in the organic phase is very low (and this low concentration increases the relative error of the titration step).

**Table 5** Liquid–liquid extraction

| Solvent | Selectivity | Solvent power | Distribution coefficient |
|---|---|---|---|
| Chloroform | 3,842.57 | – | – |
| Diethyl ether | 301.43 | 54,612.23 | 3,968.04 |
| Acetone | 4.37 | 3,194.51 | 232.11 |
| *n*-Heptane | 1,434.83 | 1,106.07 | 80.36 |
| Acetic acid | 0.15 | 477.13 | 34.67 |
| Methanol | 0.04 | 138.02 | 10.03 |
| *tert*-Butanol | 0.19 | 99.73 | 7.25 |
| Propanol | 0.12 | 94.96 | 6.90 |
| Ethanol | 0.05 | 56.51 | 4.11 |

Compound to be recovered: tricaprin; major component in the residual mixture: monocaprin

**Table 6** Liquid–liquid extraction

| Solvent | Selectivity | Solvent power | Distribution coefficient |
|---|---|---|---|
| Chloroform | 35.42 | – | – |
| Diethyl ether | 8.82 | 54,612.23 | 617.73 |
| Acetone | 1.09 | 3,194.51 | 36.13 |
| *n*-Heptane | 17.71 | 1,106.07 | 12.51 |
| Acetic acid | 0.23 | 477.13 | 5.40 |
| Methanol | 0.10 | 138.02 | 1.56 |
| *tert*-Butanol | 0.24 | 99.73 | 1.13 |
| Propanol | 0.19 | 94.96 | 1.07 |
| Ethanol | 0.12 | 56.51 | 0.64 |

Compound to be recovered: tricaprin; major component in the residual mixture: dicaprin

The overall separation process was performed by duplicate at similar conditions. The average error of the first, second and third step were 1.8, 1.3 and 2.1 % (with a global error for a whole procedure including the three steps of 5.2 %).

## Results and Discussion

### Solvent Selection

Using Ecofac 1.0, for selectivity, solvent power, and solute distribution coefficient values were obtained for each separation and proposed solvent. Based on these results, we selected the most suitable combination of solvents to maximize separation.

Table 1 shows the results obtained by the evaluation of the separation between monocaprin and dicaprin, performing solvent extraction of the monoglyceride. Solvents marked with shadow gray would perform an adequate liquid–liquid extraction. The resulting mixture or residual mixture solution is identified as "residual mixture" from here thereafter.

Table 2 shows the results of the evaluation of solvents that are suitable for the separation of mono- and tricaprin. Again, solvents highlighted with shadow gray are the best candidates for this separation. Methanol, acetic acid, ethanol and propanol are the best solvents for extraction of monocaprin, separating it of remaining acylglycerides. Of these solvents, ethanol would be most appropriate due to its low toxicity.

Tables 3, and 4 show the results obtained when evaluating the solvent extraction of dicaprin from mono- and tricaprin, respectively.

Among the tested solvents there is not a single solvent that allows the extraction of the dicaprin, separating it from other acylglycerols.

Tables 5, and 6 present the theoretical results obtained with Ecofac 1.0 to evaluate the performance of solvents for extraction of tricaprin from monocaprin and dicaprin. Chloroform, diethyl ether, acetone and *n*-heptane are presented as possible solvents to extract the triglyceride, *n*-

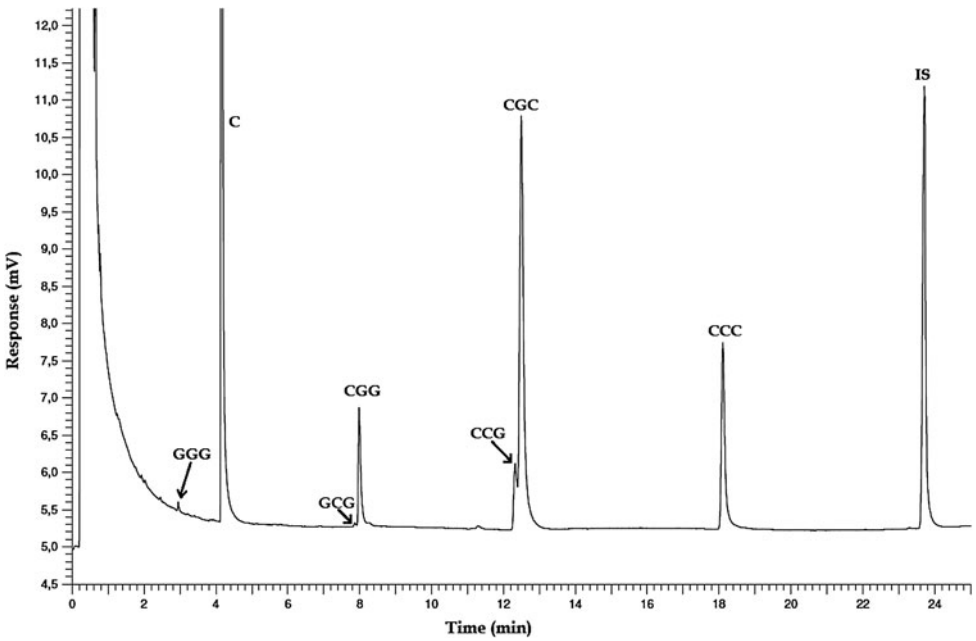


**Fig. 2** Typical chromatogram of the reaction products and unconsumed reactants in enzymatic esterification of glycerol and capric acid using Lipozyme RM IM. *IS* Internal calibration standards. Experimental conditions: glycerol: 250 mg (adsorbed on silica gel), temperature: 60 °C and enzyme: 40 mg, reaction time: 6 h

**Table 7** Performance of different KOH solutions for neutralization of capric acid and extraction of monocaprin

| KOH concentration | Acid neutralization (%) | Monocaprin extraction (%) | Dicaprin loss (%) | Tricaprin loss (%) |
|---|---|---|---|---|
| 1 M | 100 | 99 | 53 | 38 |
| 0.5 M | 99 | 97 | 7 | 5 |
| 0.25 M | 98 | 92 | 5 | 2 |
| 0.1 M | 93 | 82 | 3 | 0 |
| 0.05 M | 40 | 38 | 0 | 0 |

heptane being the most convenient because of its low toxicity.

According to the results obtained with the software the extraction procedure was designed using combinations of *n*-heptane and ethanol.

With *n*-heptane, triglyceride can be extracted and acylglycerides remain into the ethanolic phase. After the separation of the tricaprin, di- and monocaprin have to be separated using these same solvents. In the following sections the proposed separation is studied to verify the solvent-extraction using only heptane and ethanol. Relative volumes of sample and solvents and number of stages of treatment will be analyzed as important parameters in the general procedure.

## Identification of Products

The products of the enzymatic esterification of glycerol (GGG) with capric acid (C) are monocaprin (CGG) and 1,3-dicaprin (CGC), but also products with capric acid in the *sn*-2 position are found. Acyl migration makes it possible to obtain two additional dicaproylglycerol isomeric configurations: 1,2-dicaprin (CCG) and 2,3-dicaprin (GCC), which are undesirable reaction products. When these are not desirable, the dicaproylglycerols are esterified, tricaprin (CCC) is generated as the product. The used capillary column differentiates positional isomers. Based on the method for determination of mono- and diglycerides by gas chromatography reported by Bruschweiler and Dieffenbacher [26], their identification in the reaction media was possible.

A typical chromatogram of the reaction products and unreacted substrates is shown in Fig. 2.

## Acid Capric Neutralization

The final esterification mixture contains monocaprin, dicaprin, tricaprin and unreacted capric acid. All these compounds are dissolved in the reaction solvent, *n*-heptane. Purification of dicaprin, obtained as the product of esterification was performed by extraction with solvents. Unreacted acid is previously neutralized with an aqueous solution of KOH. Table 7 shows the experimental performance of the solutions of different concentrations.

As shown in Table 7, the hydroxide solution not only allows the neutralization of the acid and the removal of it from the heptane phase but it also allows the removal of the monoglyceride.

The KOH solution should be able to neutralize all the acid, and to minimize losses of interest products, in this case the diglyceride. With this objective, two washing steps were performed with an alkaline solution of concentration 0.1 M. The first stage allowed the neutralization and separation of 93 % of acid and 82 % monoglyceride with 3 % loss of diglyceride and no triglyceride extraction. The second stage allowed the total removal of 99.5 % acid and 95 % of monocaprin. In this neutralization process 4.5 % of dicaprin and 4 % of tricaprin were lost.

Figure 3 shows the chromatograms of the initial sample and the sample after the neutralization with 0.1 M aqueous solution of KOH.

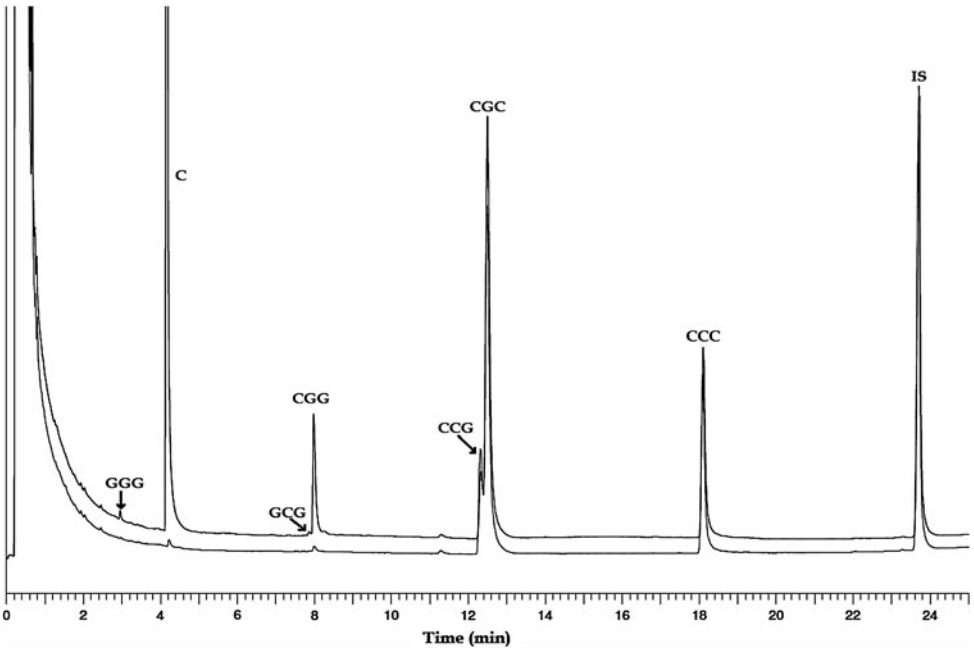


**Fig. 3** Overlay chromatograms comparing esterification reaction sample and the sample after two-stage neutralization with KOH solution with a concentration of 0.1 M. The initial sample contained 52 mg of capric acid, 4 mg of monocaprin, 28 mg of dicaprin and 12 mg tricaprin

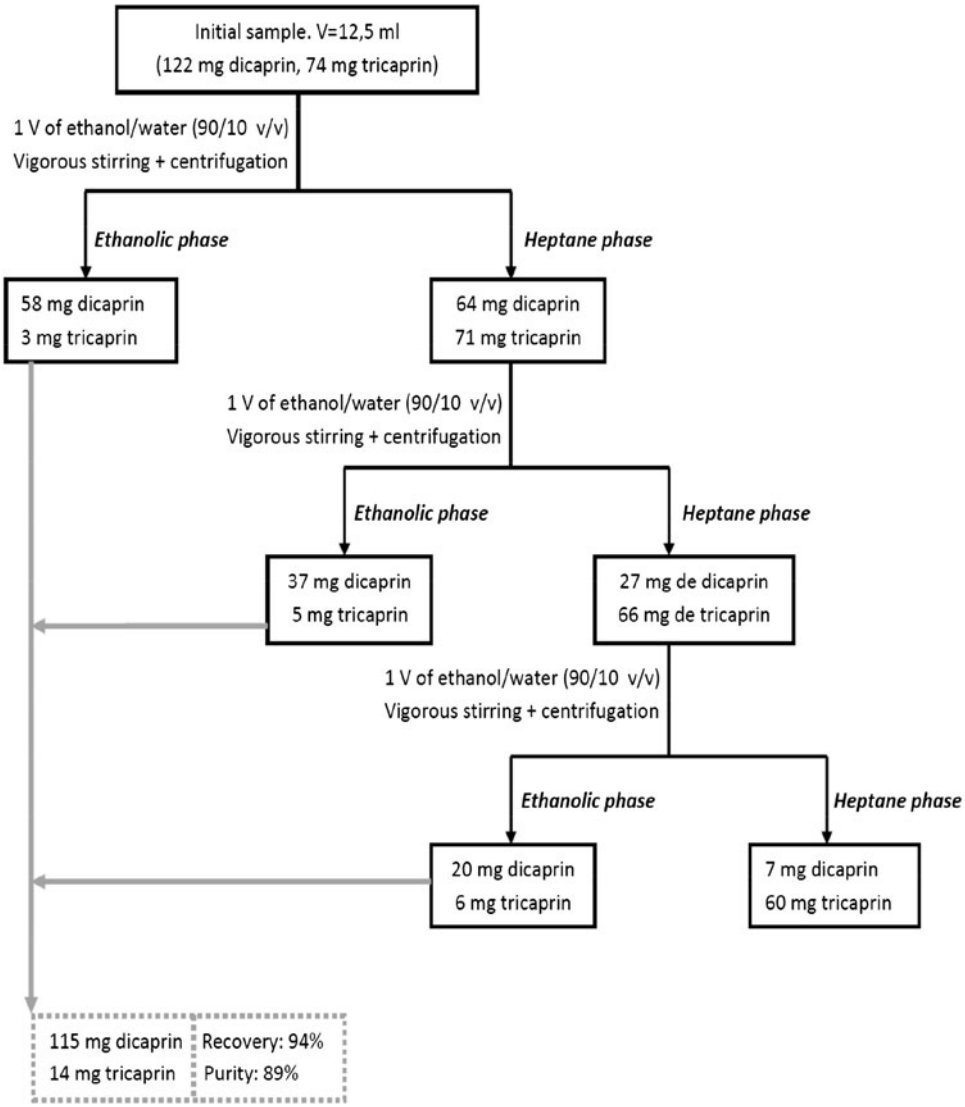


**Fig. 4** Results for the first extraction stage

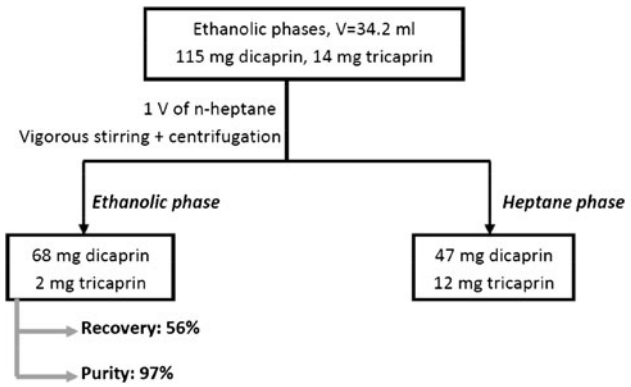


**Fig. 5** Results for the second extraction stage

## Liquid–Liquid Extraction

An initial sample containing 122 mg of dicaprin and 74 mg of tricaprin, in a total volume of 12.5 mL, was subjected to the separation process described in "Liquid–Liquid Extraction".

In the first extraction step 94 % of the initial dicaprin (115 mg) with a purity of 89 % was recovered. To increase the purity of the extracted diglyceride, the ethanolic phases obtained were combined, and washed with an equal volume of *n*-heptane. At this stage, 47 mg of dicaprin was lost, but its purity had increased to 97 %.

A third separation step was carried out with the aim of recovering a higher proportion of dicaprin, and with high purity. At this stage 45 mg of dicaprin was recovered, however, the purity was only 80 %. Using the overall procedure, 113 mg of the diglyceride was recovered with 89 % purity. This experimental finding does not represent any improvement comparing it to the process in a single step.

Figures 4, 5, and 6 show the performance of each of the separation steps.

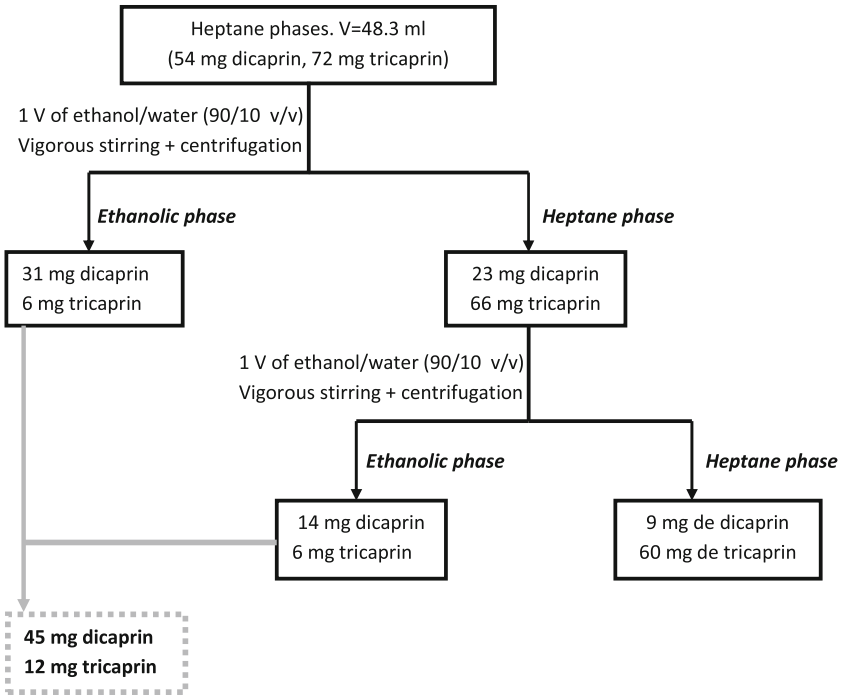


**Fig. 6** Results for the third extraction stage

There is a close relationship between the covered dicaprin fraction and the dicaprin purity. Carrying out only the first stage it was possible to recover 94 % of the dicaprin present in the sample after neutralization with a purity of 89 %. When looking for a higher purity product, two separation steps allowed a recovery of 56 % of the dicaprin with a purity of 97 %. A product with 93 % purity is obtained at the third extraction stage but only 81 % of the dicaprin present at the start of the extraction is recovered.

## Conclusions

Separation of acylglycerols obtained as a product of the esterification of glycerol with capric acid mediated by Lipozyme RM IM with a simple process using solvents of low toxicity has been carried out. Dicaprin recovery was possible, separating it from mono- and tricaprin.

A preliminary selection of solvents was made with Ecofac 1.0 and the solvents chosen performed well for liquid–liquid extraction. A preliminary step of the extraction was the neutralization of unreacted capric acid. A two-stage process was applied with a 0.1 M KOH solution, removing 99.5 % of the acid and 95 % of the monocaprin, with losses of 4.5 and 4 % of di-and tricaprin, respectively.

With a separation procedure in three simple steps, it is possible to recover 94 % of the dicaprin with 89 % purity. If higher purity is required, an extra step can increase it to 97 % but with a considerable decrease in dicaprin yield. An intermediate condition is achieved by performing one step of the third stage, in which 81 % of the dicaprin is recovered with a purity of 93 %. An extra step did not improve purity or yield. Considering the overall process, performing neutralization and only the first extraction stage it was possible to recover 90 % of the initial dicaprin with 89 % purity.

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