Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 23 (2003) 3–8
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Enzymatic synthesis of partial glycerol caprate in solvent-free media

Y.M. Xia [a,*], Y. Fang [a], K.C. Zhang [b], G.Y. Shi [b], J.J. Brown [c]

[a] *School of Chemical and Material Engineering, Southern Yangtze University, 170# Huihe Road, Wuxi 214036, China*
[b] *School of Biotechnology Engineering, Southern Yangtze University, 170# Huihe Road, Wuxi 214036, China*
[c] *Department of Chemical Engineering, University of Michigan, 3085 H.H. Dow, Ann Arbor, MI 48109, USA*

Received 18 January 2003; received in revised form 3 March 2003; accepted 3 March 2003

Abstract

Enzymatic synthesis of partial glycerol caprate from capric acid and glycerol was investigated by using lipase from *Candida antarctica* (CAL) in a solvent-free microaqueous media. In a closed reactor, equilibrium conversion reached 65.6% in 9 h under optimal reaction conditions which were: temperature, 60 °C; initial water content in glycerol, 12% (w/w); lipase dosage, 100 U/g of capric acid; molar ratio of glycerol:capric acid, 1:1. Initial reaction mixture phase states were investigated by using a flat plate cooling-quenching method with a micrograph system. Regional capric acid concentration surrounding the immobilized lipase surface is higher than that in the bulk phase. CAL did not express 1,3-position specificity in the final product. Mechanical fray partly denatured CAL in batch reactor. Capric acid conversion as high as 96.9% was obtained in 6 h with several modified reaction procedures in a batch reactor.

Keywords: Esterification; Lipase; Microaqueous; Glyceride; Solvent-free

1. Introduction

Partial glycerol caprates are functional additives in medicine, cosmetics and food [1,2]. Glycerol caprates are produced industrially by reacting extra glycerol with capric acid or capric/caprylic acid mixture at 150 °C catalyzed by an inorganic acid. This is followed by phase separation, rinsing with alkaline solution, steam refining, filtration, deodorization, decoloration and even molecular distillation. Recently, enzymatic synthesis of glycerol caprates or medium chain glycerides has been reported [1–7]. The most economical enzymatic synthesis technique is the reaction in solvent-free media, but until now only several kinds of lipase have been investigated. Little attention has been paid to optimizing reaction parameters or to reaction phase state. Most of reported results were based on a large lipase amount and long reaction time [1–4]. To optimize reaction parameters, the effect of initial reaction phase state, method of dehydration from reaction system, method of feeding substrates, homogenization of reaction system, mechanical stability of immobi-

* Corresponding author. Present address: Department of Chemical Engineering, University of Michigan, 3085 H.H. Dow, Ann Arbor, MI 48109, USA. Tel.: +1-734-764-0111; fax: +1-734-764-0459.
E-mail address: ymxia@umich.edu (Y.M. Xia).

1381-1177/03/$ – see front matter
doi:10.1016/S1381-1177(03)00039-0

lized lipase and size of lipase granule on the reaction were investigated with a commercial lipase CAL.

2. Experimental

2.1. Chemicals

Lipase from *Candida antarctica* (CAL) with olive oil hydrolysis activity 7000 U/g, was a gift from Novo Nordisk Co. Capric acid (containing 0.1% H_2O) and glycerol (containing 1.14% H_2O) were purchased from Shanghai Chemicals Co., China. Monocaprin and caprin were purchased from Sigma. All other reagents are of analytical grade. Reactants unless otherwise stated were dehydrated with 4A zeolite before use.

2.2. Analysis

Water content was determined by Karl Fischer titration. Conversion of capric acid was defined as the percentage of consumed capric acid. Initial rate was defined as the millimoles of monoglycerides produced from 1 g of capric acid in 1 min, but calculated based on the amount of consumed capric acid in the first 5 min. A lower lipase amount was applied as 20 U/g capric acid where initial rate was investigated. HPLC proved that under the reaction conditions, only monoglyceride was produced and the reaction rates are linear in the first 5 min (data not shown). After removing unreacted glycerol from samples, the composition of caprate was determined by HPLC. The conditions for the HPLC were as follows: C_{18} column, column temperature was 40 °C, mobile phase was methanol with flow rate 1.0 ml/min, and UV detector detecting at 215 nm.

2.3. Enzymatic esterification

Capric acid (0.15 mol), glycerol (0.15 mol), water (12% in glycerol (w/w)) and lipase (100 U/g of capric acid, note as 100 U/g capric acid as abbreviation) unless otherwise stated were mixed with a magnetic stirrer at 400 min^{-1} in a 50 ml closed glass reactor submerged in a water bath. The reaction temperature was 60 °C unless otherwise stated. The reactor was opened or vacuum was applied to enhance dehydration after the esterification equilibrium was reached.

2.4. Initial reaction phase state

A flat plate cooling-quenching method was designed to observe the initial phase state of reaction systems. One or two drops of reaction mixture were spread well on a clean, dry, and cold (10–15 °C) microslide in 2–3 s, thus the reaction and phase state were fixed as if they were "quenched" quickly as soon as the mixture contacted the cold glass surface. The capric acid and lipase phases were opaque and flat-like while the glycerol phase was transparent so that each phase could be easy to identify through images. The slides were observed under a microscope, which was connected with a homemade skin surface scanner. Images of the slides were then scanned and processed on the connecting computer.

2.5. Stability of CAL

The esterification was carried out for 10 h at optimal reaction conditions. After each batch, the lipase was filtrated out from the mixture. The lipase was washed with 4 ml × 4 ml of cold acetone and dried under vacuum for 2 h before being used in next batch.

3. Results and discussion

3.1. Effect of temperature

As shown in Fig. 1, the initial reaction rate increased with increasing reaction temperature from 55 to 65 °C, while the equilibrium conversion reached an optimal at 60 °C. During the first 2 h the highest conversion reached at 65 °C, but at 10 h the highest conversion was obtained at 62 °C. It might be thermo-denaturing that the equilibrium conversion decreased when the temperature was higher than 62 °C.

3.2. Effect of molar ratio of capric acid to glycerol

The equilibrium conversion increased with increasing glycerol amount as in Fig. 2. There is no evident relationship between initial reaction rate and the reactants molar ratio. A possible explanation is that the molar ratio at the interface, where reaction is carried out, was not changed while the molar ratio in the heterogeneous phases system was changed within a

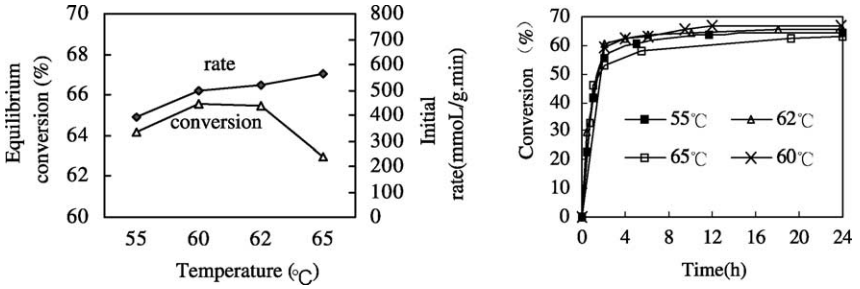

Fig. 1. Effect of reaction temperature on esterification. Conditions: closed reactor, 400 min^{-1}, 0.15 mol capric acid, 0.15 mol glycerol, 12% of water in glycerol (w/w), 100 U/g capric acid of lipase.

range. To confirm this and to learn how it was influenced, we designed a flat plate cooling-quenching method to observe the phase state of reaction system. The dispersing states in reaction systems at different molar ratios can be summarized mainly as three distinct types as in Fig. 3.

(a) Glycerol is dispersed in capric acid when glycerol:capric acid < 1:3 (mol:mol).
(b) Larger nonuniform capric acid droplets are dispersed in glycerol when the molar ratios are 1:3 (mol:mol) > glycerol:capric acid > 1:0.2 (mol:mol).
(c) Small uniform capric acid droplets are dispersed in glycerol phase when glycerol:capric acid > 1:0.2 (mol:mol). This agrees with the explanation mentioned above.

Thus the mass transfer between phases is related to the reactants dispersal states. Additionally, the regional capric acid concentration surrounding the immobilized lipase surface is higher than that in bulk phase (Fig. 3d). This implies that the extra capric acid may allow a pseudo-inhabitation to the reaction. This has been confirmed in a further study [8].

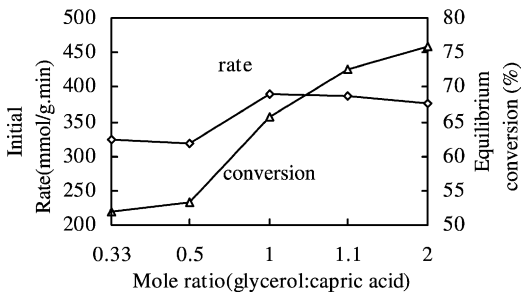

Fig. 2. Effect of molar ratio (glycerol:capric acid) on the esterification. Conditions: closed reactor, 400 min^{-1}, 60 °C, 0.15 mol capric acid, 12% of water in glycerol (w/w).

3.3. Effect of lipase dosage

Catalyst amount does not affect equilibrium conversion but affects the reaction rate. Relative initial rates and equilibrium conversion at different lipase dosages are shown in Fig. 4. The dosage of 20 U/g capric acid was taken as the control. A dosage of 100 U/g capric acid was proved to be the optimal lipase dosage based on relative initial rate.

3.4. Effect of water and initial water dosage

It is well known that water is essential in enzymatic synthesis. Most researchers have investigated the

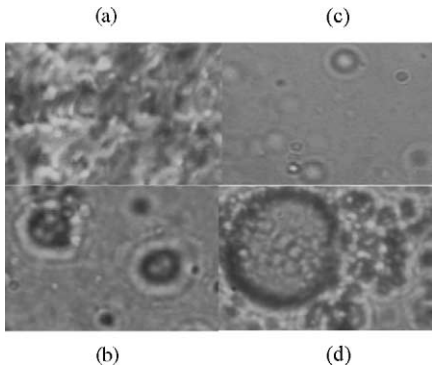

Fig. 3. Initial reaction system phase states image (1 × 1500) (a) glycerol:capric acid < 1:3 (mol:mol); (b) 1:3 (mol:mol) > glycerol:capric acid > 1:0.2 (mol:mol); (c) glycerol:capric acid > 1:0.2 (mol:mol); (d) lipase granule in system c.

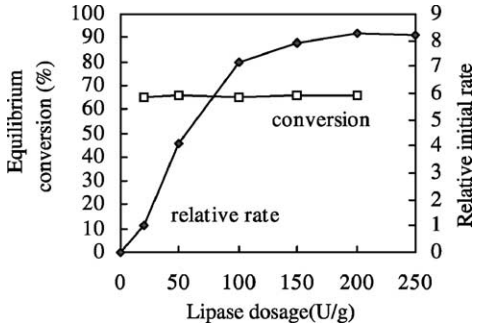

Fig. 4. Effect of lipase dosage on the esterification. Conditions: closed reactor, 400 min^{-1}, 60 °C, 0.15 mol capric acid, 0.15 mol glycerol, 12% of water in glycerol (w/w).

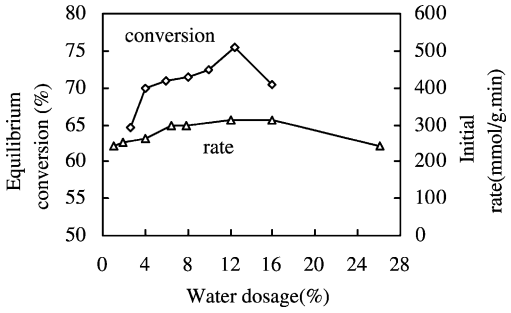

Fig. 5. Effect of initial water content in glycerol on the esterification. Conditions: closed reactor, 400 min^{-1}, 60 °C, 0.15 mol capric acid, 0.15 mol glycerol, 100 U/g capric acid of lipase.

effect of water activity a_w on enzymatic esterification by using different hydrated salts to get different a_w [9]. Unfortunately, they often overlooked the salt effect on lipase activity. Most investigators think that the salts are not in ionic status in nonaqueous media, so the salts would not affect the lipase activity. But in fact, the salts do affect the lipase activity even in nonaqueous media (like hexane) while these salts were used to control a_w of the reaction mixture [9]. A possible explanation to this effect is, although the reaction was applied in nonaqueous media, there was always a microaqueous phase or micro w/o interface, where the enzymatic reaction occurred and allowed the salt being ionized. Because of this reason it is better to consider this kind of solvent-free system as a microaqueous instead of nonaqueous. On the other hand, our previous works on synthesis of some glycerides showed that if the reaction systems were exactly the same, except the lipase, the optimal initial water content in hydrophilic phase would be almost same for different lipases [8]. Thus, in this paper, we chose to investigate initial water content in glycerol instead of a_w.

Fig. 5 shows that the initial reaction rate increased with increasing initial water content when the content was below 12%, probably the hydrolysis effect was started then, since the water produced at reaction equilibrium (the conversion was around 65.5%) was about 13% in glycerol.

As mentioned above, the optimal initial water dosage was 12% in glycerol. This is a large amount compared to other cases of glycerides synthesis, where the water dosage was <4% in solvent-free system [10]. If less water can be used at the reaction beginning without increasing reaction time or decreasing yield, it will be of benefit to the subsequent dehydration step. Zaks [11] reported that some compounds like DMF and DMSO, which can easily form hydrogen bonds with water, could be used as mimics of water in the organic phase. So if glycerol could be used as mimic water in a solvent-free system, that means if applying commercial capric acid (containing 0.1% H_2O) and extra glycerol (containing 1.14% H_2O) without pre-dehydration, we may avoid either the required initial water amount or the usage of a zeolite. As illustrated in Figs. 6 and 7, excess glycerol resulted in high reaction rates although the optimal water dosages were same in spite of the different molar ratios. Additionally, the two curves in Fig. 6 are almost parallel so this implies that the net donation to initial rate contributed by extra glycerol is almost independent of the initial water content. Similar initial reaction rate were observed at 0% initial water content (at 10:1 molar ratio) and at 2.7% initial water

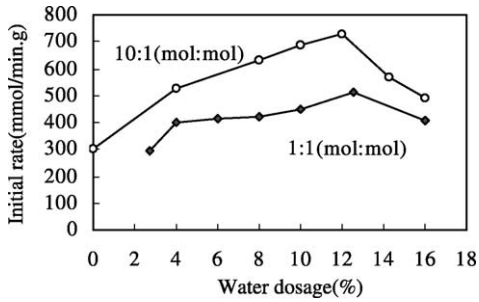

Fig. 6. The role of water in the esterification at different molar ratio (glycerol:capric acid). Conditions: closed reactor, 400 min^{-1}, 60 °C, 0.15 mol capric acid, 100 U/g capric acid of lipase.

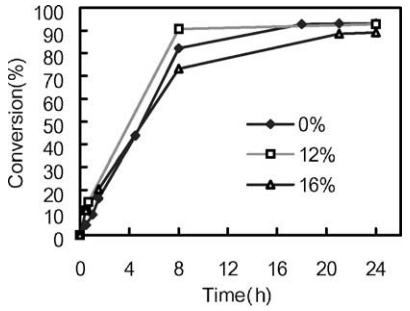

Fig. 7. Esterification at different initial water content when glycerol:capric acid = 10:1 (mol:mol). Conditions: closed reactor, 400 min^{-1}, 60 °C, 0.15 mol capric acid, 100 U/g capric acid of lipase.

content (at 1:1 molar ratio), this means that glycerol can be mimic water here.

Thus several procedures were designed as Fig. 8, an opened reactor or dehydration under vacuum was applied to enhance dehydration in some procedures. Glycerol (containing 1.14% H_2O) and capric acid (containing 0.1% H_2O) were used in these procedures without pre-dehydration; lipase dosage was 100 U/g capric acid. For each step, equilibrium time and conversion was pre-investigated under same reaction

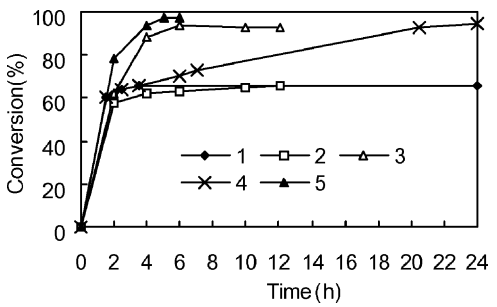

Fig. 8. Different esterification procedures (400 min^{-1}, 60 °C, 0.15 mol capric acid, 100 U/g capric acid of lipase, glycerol:capric acid (mol:mol) = 1:1 in curve 1–4, 2:1 in curve 5). (1) Feeding capric acid dropwisely within the first 1 h, closed reactor; initial water content 1.14% in glycerol; (2) feeding capric acid at beginning, closed reactor; initial water content 12% in glycerol; (3) feeding capric acid at beginning, opened reactor; initial water content 12% in glycerol; (4) feeding capric acid dropwisely within the first 1 h, closed reactor; after 3 h switched to an opened reactor; initial water content 1.14% in glycerol; (5) feeding 4/5 of capric acid dropwisely within the first 1 h to a closed reactor containing 1/5 of capric acid, then switched to an opened reactor for 2 h; followed by reaction at 5 mmHg, 2 h; initial water content 1.14% in glycerol.

conditions in other experiments (data not shown) so that based on the conversions, we can decide the time to switch reaction status from closing to opening or vacuuming.

As curve 1, feeding capric acid dropwisely to glycerol in a closed reactor within the first 1 h, the reaction rate was similar as in curve 2, where total capric acid, glycerol containing 12% of water were fed together to a closed reactor, the molar ratio were same in both cases. This means extra “dry” glycerol could be used to replace initial exogenous water. In curve 4, the reaction was in a closed reactor at first, and then when reaction equilibrium reached (4 h), the reaction mixture was switched to an opened reactor. After 4 h the reaction rate in curve 4 was less than that of curve 3, and the equilibrium time in curve 4 is much longer than that in curve 3. This implies that large extra amounts of glycerol might partly denature the lipase. The same phenomenon did not show in curve 1 and 2. Since there the highest conversions were not as high as that in curve 3 and 4, so even if the lipase was partly denatured in case of curve 1 and 2, the rest of lipase activity might be enough to allow the low conversion. Hoq [12] also reported that when the glycerol concentration was 97%, some kinds of lipase denatured quickly when glycerol was reacted with oleic acid at 40 °C, and the yields were very low.

Based on the results from curve 1–4, procedure as in curve 5 was designed. To a closed reactor the following was added: lipase, 0.3 mol of glycerol, 1/5 of total capric acid (glycerol:capric acid = 2 : 1 (mol:mol)), and the rest of the 4/5 capric acid was fed dropwisely within the first 1 h. The conversion reached equilibrium after the first 1 h, according to a pre-measurement based on same closed reaction system (data not shown); after 1 h the system was switched to an opened system to dehydrate until a second equilibrium was reached, and then reacted under vacuum or applying less vacuum since the first equilibrium, but should maintain enough water content to get high reaction rate. For this system, the final conversion reached 96.9% in 6 h. The excess glycerol can be easily removed after reaction.

3.5. Stability of CAL

Reusing CAL in five batches of reaction. With the first batch as a control, the relative esterification

activity after reacting 10 h for each batch was: 100, 100.87, 94.93, 92.88 and 91.24% in order of testing. After each batch the size of CAL was decreased. So the denaturation may be related to the mechanical fray or caused by the size of lipase under the reaction conditions.

Then the effect of particle size and mechanical stability of CAL were investigated. Reaction conditions were same as described in experimental procedures. The lipase was sieved to different particle size ranges before use. The six sizes were 20, 60, 80, 100, 120 and 200 meshes. With the case of 20 meshes as a control, the relative esterification activity of the lipases after reacting 10 h was: 100, 107, 121, 106, 99 and 82% in order of the increasing meshes.

So we tried to decrease the fray as described below. When the system was homogenized, lowering the stirring speed from 400 to 100 min^{-1} could reduce the fray, and 96.4% of catalyst activity was recovered after five batches. Also, applying the reaction in a column reactor could reduce the fray more [8].

Another method was applied to reduce the mechanical fray by lowering the stirring speed and adding an emulsifier. Tween 80 (TW80) was selected as emulsifier because of its HLB, security to food and cosmetics, cost, its gentle nature to lipase and less effect on product quality (compatibility to partial glycerides). When 1.5% of TW80 and a stirring speed of 100 or 400 min^{-1} were applied, the conversions were almost same when the equilibrium was reached (65.8% at 100 min^{-1} and 65.9% at 400 min^{-1}).

3.6. The specificity of CAL

CAL is known to be 1,3-specific in the hydrolysis of triglycerides. When a molar ratio of 1.5:1 (glycerol:capric acid) was applied in an opened reactor, the equilibrium product shows that the glycerides composition was: 46.74% monoglycerides, 53.25% diglycerides and 0.015% triglycerides. There was no dramatic difference between the contents of relative isomers, so the 1,3-position specificity was not expressed in the final product.

4. Conclusion

Based on investigation results about the optimal reaction conditions as well as the effect of the substrates on the enzymatic activity, by a lipase dosage as lower as 100 U/g of capric acid, a final capric acid conversion of 96.9% was obtained in 6 h in a batch reactor. So by optimizing the reactor parameters, choosing a larger lipase dosage, if we apply the reaction in a continuous packed bed reactor system, and connect the reactor to a molecular sieve column to dehydrate with an in situ water content analysis, a high yield can be expected in a short time. Furthermore, compared to chemical synthesis, because the enzymatic synthesis produces higher content of monoglycerides, it provides the possibility that a considerable high content of monoglycerides product can be obtained without expensive molecular distillation.

References

[1] S.M. Kim, J.S. Rhee, J. Am. Oil Chem. Soc. 68 (1991) 499.
[2] W.C. Wong, M. Basri, C.N.A. Razak, A.B. Salleh, J. Am. Oil Chem. Soc. 77 (2000) 85.
[3] A.M. Fureby, P. Adlercreutz, B. Mattiasson, J. Am. Oil Chem. Soc. 73 (1996) 1489.
[4] S. Ghosh, D.K. Bhattacharyya, J. Am. Oil Chem. Soc. 74 (1997) 593.
[5] D.Y. Kwon, H.N. Song, S.H. Yoon, J. Am. Oil Chem. Soc. 73 (1996) 1521.
[6] A. Marta, P. Langone, E.A. Melissa, J.C.R. Michelle, G.L. Sant'Anna Jr., Appl. Biochem. Biotechnol. 98–100 (2002) 987.
[7] A. Marta, P. Langone, G.L. Sant'Anna Jr., Appl. Biochem. Biotechnol. 98–100 (2002) 997.
[8] Y.M. Xia, Ph.D. Thesis, Wuxi University of Light Industry, 2000.
[9] Y. Xu, Ph.D. Thesis, Wuxi University of Light Industry, 1998.
[10] Y.M. Xia, K.C. Zhang, Chin. J. Biotechnol. 16 (2000) 235.
[11] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 8017.
[12] M.M. Hoq, H. Tagami, T. Yamane, S. Shimizu, Agric. Biol. Chem. 49 (1985) 335.