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Enhancing catalytic activity of lipases from *Candida cylindracea* and *Pancreatic porc* for glucose laurate synthesis

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ABSTRACT

Sugar esters are widely used nonionic and nontoxic bio-surfactants. Their enzymatic synthesis has important advantages over the conventional chemical synthesis. This article is concerned with modified lipases catalyzed synthesis of glucose fatty acid ester in organic solvent. Sorbitan monostearate and porous celite were used as support to modify lipases from *Candida cylindracea* (CCL) and *Pancreatic porc* (PPL).The biocatalysts prepared were tested in the synthesis of 6-O-Dodecanoyl-D-Glucopyranose by esterification of glucose and lauric acid in ethylmethylketone medium. The effects associated with the specific nature of the lipase catalyst used are discussed. Optimal conversion was around 45%, with surfactant coated PPL and with immobilized PPL and CCL after 72 hours of reaction.

Keywords: *coated lipase, immobilized lipase, D-Glucose, esterification.*

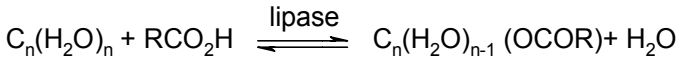
1. INTRODUCTION

Sugar fatty acids are known to be tasteless and odorless nonionic surfactants. They are biodegradable, nontoxic and nonirritant and have various applications [1]. Their synthesis by enzymatic route is currently widespread [2]. Enzymatic esterification in organic solvents [3] has recently received much attention by bioindustries.

Enzymes such as lipase often exhibit innovative catalytic functions in organic solvents as improvement the solubility of hydrophobic substrates and facilitation of product recovery [4]. Modification of the surface of lipase to protect it from denaturation in an organic solvent has widely been achieved. Surfactant-coated lipase [5] and lipase immobilization [6] appear to be the most promising methods although some other proposed methods for protecting lipases [7-9].

Lipases present a specific catalytic mechanism of action, existing in two structural forms, the closed one, where a lid formed by a polypeptide chain, isolates the active site from the medium, and the open form, where this lid moves and the active center is exposed [10]. This equilibrium is shifted towards the open form in the presence of hydrophobic surfaces like celite or sorbitan monostearate [11].

This article is therefore focused on the use of modified lipases for the synthesis of sugar nesters. Sugar fatty acid esters are amphiphilic molecules produced by an esterification between sugars and fatty acids (scheme 1), with a promising structural modification to enhance the better the therapeutic, cosmetic or food potential [12-14].



$$C_n(H_2O)_n + RCO_2H \xrightleftharpoons{\text{lipase}} C_n(H_2O)_{n-1}(OCOR) + H_2O$$

Scheme 1. Lipase catalyzed synthesis of sugar fatty esters.

Although reactions of esterification are generally catalyzed by one specific lipase preparation [15], the concept of "modified lipase" could be better explored for the production of sugar esters [16, 17].

To our knowledge, the reaction of glucose and lauric acid being catalyzed by modified PPL and CCL has not been reported so far.

In this context, lipases from *Candida cylindracea* (CCL) and *Pancreatic porc* (PPL) were adsorbed onto the surfactant sorbitan monostearate (Span 80) and the specific activities were compared in ethylmethylketone (EMK) to crude powder (used straight from the bottle). In further comparisons CCL and PPL were adsorbed on porous celite support (Accurel EP-100).

In our earlier report, we demonstrated the enhanced synthesis of xylose fatty acid esters using immobilized *Pancreatic porc* lipase on celite in organic solvents [18]. In present work we are reporting the improved synthesis of 6-*0*-Dodecanoyl-D-Glucopyranose by enzymatic esterification of α-(+)-D-Glucose. The efficiency of different preparations of lipases from *Pancreatic porc* and *Candida cylindracea* was tested and evaluated in this synthesis.

Moreover we have included in this study an immobilized commercial lipase from *Candida antarctica* B (CAL B) [19], which is the most frequently used lipase in sugar esters synthesis [20].

2. EXPERIMENTAL SECTION

Candida cylindracea and *Pancreatic porc* lipase (Type II) were purchased from Sigma Co. (USA) and were used as received, without further purification. *Candida antarctica* type B lipase immobilized on acrylic resin was provided by Novo Nordisk (Bagsvaerd, Denmark). Glucose (Sigma-Aldrich) and lauric acid (Merck) were tested as starting materials. All other chemicals used in this work were of analytical grade and used without further purification.

2.1. Lipase immobilization.

Celite (60 mg) was added to 10mL of 0,1 M phosphate buffer (pH = 8) containing the lipase (100U/mL). The reaction was then stirred with a magnetic bar at 4 ºC and 100 rpm for 0.5 h. 20 mL of cold acetone were then added. After 2 h, the suspension was filtered. The immobilized enzyme was washed with acetone, dried in a vacuum desiccator and then stored at -18ºC [21].

2.2 Preparation of surfactant-coated lipase.

Lipase (100U/mL) in 0.1 M phosphate buffer solution (250 ml, pH 6.9) and 0.5 g of a surfactant (span 80 dissolved in 10 ml ethanol)) were mixed and sonicated in an ultrasonic bath for 30 min(8-10°C). After incubation for 4 h at 5°C, the translucent solution was collected by centrifugation and dried under reduced pressure. A white powder was obtained and the yield of enzymes was about 35% [22].

2.3. Glucose ester synthesis.

D-glucose (180 mg, 1mmol) was first dissolved in the solvent for one night. After that, lauric acid (200 mg, 1mmol) was added, the mixture equilibrated for 15 min, the biocatalyst and molecular sieves finally incorporated.

Aliquots were removed at intervals, filtered and analyzed qualitatively by thin layer chromatography and quantitatively by volumetric titration. At the end of the esterification reaction, lipases were removed by filtration using filter paper with a pore-size of 60- mm. After filtration, the solvent was evaporated to dryness under reduced pressure. The residue was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH: 90/10 as eluent.

2.4. Analysis.

The sugar content was quantified by calculating the residual fatty acid amount in the reaction mixture. Samples were analyzed by volumetric method: 0.1 g of sample of reaction mixture was diluted in 20 mL of 0.1 wt % phenolphthalein solution in absolute ethanol and then titrated with standardized sodium hydroxide solution of 0.1 M in water [23].

3. RESULTS SECTION

In this work, lipolytic esterification of glucose and lauric acid in an organic solvent (EMK) was investigated. CCL and PPL interacted with the surfactant span 80 in a buffer solution of pH 6.9 to form surfactant-coated lipases. The same enzymes were immobilized on celite witch is a micro-porous support. The interaction between the two provides a new lipase with specific chemical, biochemical, mechanical and kinetic properties. Immobilized lipases from *Candida cylindrecea* and *Pancreatic porc* (CCL I and PPL I) and surfactant-coated lipases from *Candida cylindrecea* and *Pancreatic porc* (CCL C and PPL C) were designed to enhance the esterification.

The esterification yields for 6-*O*-Dodecanoyl-D-Glucopyranose synthesis are given in table 1.

Table 1. Yields for glucose ester synthesis according to the source of the lipase.

Lipase source	T (°C)	Yield(%)
CCL	40	11
CCL I	60	43
CCL C	60	09
PPL	40	12
PPL I	60	44
PPL C	60	39
CAL B	60	44

Figures 1 and 2 show the time courses of the conversion to glucose laurate using surfactant-coated and immobilized CCL and PPL prepared by the usual procedure. It was found that the catalytic behaviour of modified CCL (Fig.1) and PPL (Fig.2) was greatly affected by the origin of the enzyme and lipase modification method.

With surfactant-coated PPL and CCL, the conversion at 72 h reached 48% and 07%, respectively.

The PPL-coated lipase was solubilised completely in EMK, whereas the CCL I showed little solubility and remained suspended in the reaction media.

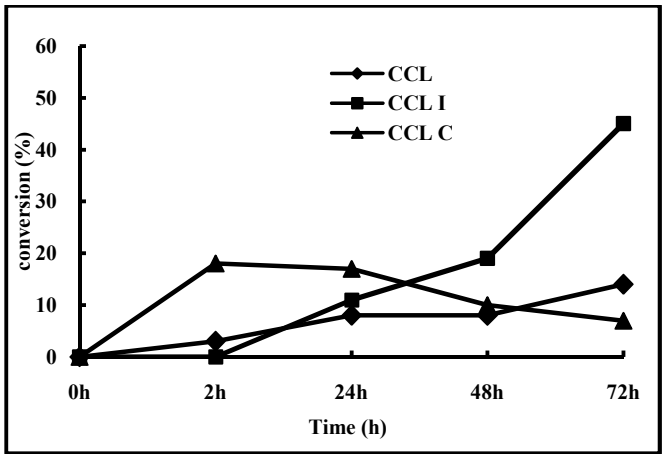


Figure 1. Reaction kinetics for glucose laurate production with modified CCL. **Reaction conditions:** 1 mmol D-glucose, 1 mmol lauric acid, 5 mL ethyl methyl ketone, 30 mg (CCL I, CCL C) at 60°C, 50 mg (CCL) at 40 ºC, molecular sieves (4Aº) in equimolar quantity to the biocatalyst, 250 rpm.

The catalytic site is often buried in the molecule, surrounded by hydrophobic residues. A helical polypeptide structure acts as a cover, making the site inaccessible to solvents and substrates. It may also prevent proteolytic activity of the catalytic triad, protecting the enzyme. The side of the lid structure facing the catalytic site is composed mainly of aliphatic hydrophobic side chains. The opposite face is hydrophilic, stabilized by protein surface interactions.

According to this hypothesis, surfactant binds to hydrophobic areas surrounding the active site of the lipase, causing displacement of peptide lid covering the active site and promoting the interfacial activation of the enzyme.

This may be a likely reason for higher activity of coated PPL in comparison to uncoated lipase. These results show that PPL coated with span 80 was protected from denaturation in the organic solvent and exhibited higher catalytic performance.

In case of immobilized *Candida cylindracea* CCL I (Figure1) and *Pancreatic porc* lipase PPL I (Figure 2) catalyzed esterification of glucose and lauric acid, a maximum conversion of 45% was achieved at 72 h. It was shown that CCL and PPL have a surface with a very high hydrophobicity [24] and hydrophobic

interactions have probably a significant role in the adsorption process. These enzymes appear to be adsorbed easily on the porous support in order to restrict their mobility which makes them accessible to reagents in solution continuously.

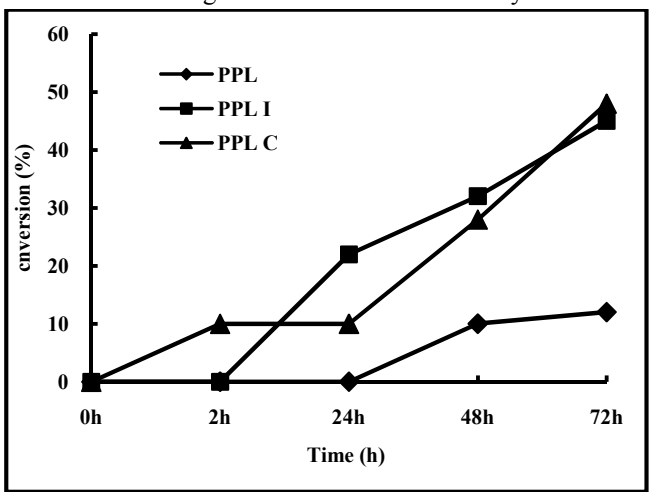


Figure 2. Reaction kinetics for glucose laurate production with modified PPL. **Reaction conditions**: 1 mmol D-glucose, 1 mmol lauric acid, 5 mL ethyl methyl ketone, 30 mg (PPL I, PPL C) at 60°C, 50 mg (PPL) at 40 ºC, molecular sieves (4Aº) in equimolar quantity to the biocatalyst, 250 rpm.

The immobilized catalyst facilitates mass transfer by spreading the enzyme on a larger surface area and by preventing the enzyme particles from aggregation [25].

On using free PPL and CCL, a maximum conversion of glucose of 12% and 14% was obtained, respectively, at 40°C.In addition, there is a clear differential behaviour of the enzyme at 60°C.Lipases did not lose their catalytic activities and modification of CCL and PPL has increased their thermal stability. These results invariably demonstrate that modified lipases work better than the free ones [26, 27].

The commercially available *Candida antartica* B (CAL B) was also used for further comparison.

To date, numerous publications have appeared on catalytic estertrification of sugars with *Candida antartica* lipase (CAL B) [28].

In an effort to make PPL and CCL more attractive catalysts from a synthetic standpoint, we applied our method to make them more soluble in organic solvents and therefore more active.

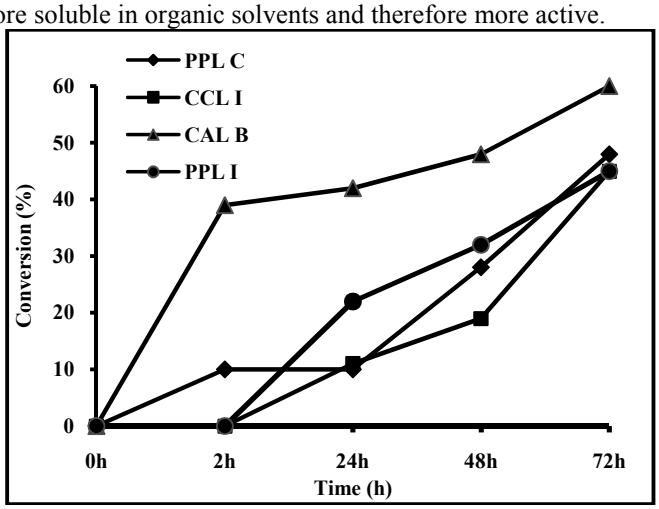


Figure 3. Reaction kinetics for glucose laurate production with PPL C, PPL I, CCL I and CAL B. **Reaction conditions**: 1 mmol D-glucose, 1 mmol lauric acid, 5 mL ethyl methyl ketone, 30 mg (PPL C, CCL I, CAL B) at 60 ºC, molecular sieves (4Aº) in equimolar quantity to the biocatalyst, 250 rpm.

Figure 3 represents reaction kinetics for glucose laurate production with the most active lipases: PPL I, CCL I, PPL C and CAL B.

It can be seen that after 72 of reaction at 60°C, 58 % of the CAL B activity was expressed. Comparison of the esterification activities of the modified lipases from *Candida cylindracea* and *Pancreatic porc*, the most active lipases showed similar activities to that observed with commercial *Candida antartica* B. These lipases exhibit comparable catalytic activity with CAL B.

In addition, PPL, and CCL are cheaper than most of the commercially available enzymes. Therefore, under their modified forms they can be used for the synthesis of sugar esters at a large scale.

4. CONCLUSIONS

Surfactant-coated and immobilized lipases from *Candida cylindrecea* and *Pancreatic porc* were tested in the synthesis of 6-*O*-Dodecanoyl-D-Glucopyranose by esterification of glucose and lauric acid in organic medium. Their specific activities were compared with that of *Candiada antartica* B lipase. Highest specific activities were obtained when lipases from *Candida cylindracea* and *Pancreatic porc* were immobilized on celite and when *Pancreatic porc* lipase coated-span 80 was employed. As shown in this paper the crude powder is not an effective catalyst for the synthesis. In conclusion, our results suggested that modified lipases from *Candida cylindracea* and *Pancreatic porc* are suitable catalysts for the production of glucose monolaurate. CCL I, PPL I, PPL C and CAL B are similarly useful and their activities are almost comparable. These modified enzymes demonstrate better activity and high thermal stability. On another hand, a lipase protection system for PPL and CCL was developed to minimize the direct contact of organic solvent and substrates with lipase and to facilitate the lipolytic esterification of sugars. The high activities obtained were probably due to an improved dispersion of the catalyst in the organic media

5. REFERENCES

[1] Piccicuto S., Blecker C., Brohée JC., Mbampara A., Lognay G., Deroanne C., Paquot M., Marlier M., Les esters de sucres : voies de synthèse et potentialités d'utilisation,*Biotechnologie, Agronomie, Société et Environnement*, 5, 209–220, **2001.**

[2] Walsh M.K., Bombyk R.A., Wagh A., Bingham A., Berreau LM., Synthesis of lactose monolaurate as influenced by various lipases and solvents, *Journa*l *of Molecular Catalysis B: Enzymatic*, 60,171–177, **2009.**

[3] Klibanov A.M., Enzymatic catalysis in anhydrous organic solvents,*Trends in Biotechnology*, 14,141–144, **1989.**

[4] Faber K., *Biotransformations in Organic Chemistry*, 2nd ed, Springer, New York, **1995.**

[5] Okahata Y., Mori T.,Lipid-coated enzymes as efficient catalysts in organic media, *Trends in Biotechnology*, 15,2, 50-54,**1997.**

[6]Petkar M., Lali A., Caimi P., Daminati M., Immobilization of lipases for non-aqueous synthesis, *Journal of Molecular Catalysis B: Enzymatic*, 39, 83–90,**2006.**

[7] Persson M., Mladenoska I., Wehtje E., Adlercreutz P.,Preparation of lipases for use in organic solvents, *Enzyme and Microbial Technology,* 31, 833–841, **2002.**

[8] Gitlesen T., Bauer M., Adlercreutz P., Adsorption of lipase on polypropylene powder,*Biochim Biophys Acta*, 1345, 188–96, **1997.**
[9] Persson M., Wehtje E., Adlercreutz P., Immobilisation of lipases by adsorption and deposition: high protein loading gives lower water activity optimum,*Biotechnol Lett*, 22, 1571–5, **2000.**
[10] Brady L., Brzozowski A.M., Derewenda Z.S., Dodson E., Dodson G., Tolley S., Turkenburg JP., Christiansen L,Huge-Jensen B., Norskov L., A serine protease triad forms the catalytic centre of a triacylglycerol lipase,*Nature*, 343:767–70, **1990.**
[11] Bastida A., Sabuquillo P., Armisen P., Fernàndez-Lafuente R., Huguet J.,Guisàn JM., A single step purification, immobilization, and hyperactivation of lipases via interfacial adsorption on strongly hydrophobic supports., *Biotechnology Bioengineering*, 58, 486–93, **1998.**
[12] Van Kempen S. E.H.J., Boeriu C.G., Schols HA.,de Waard P., Van der Linden E., Sagis LMC., Novel surface-active oligofructose fatty acid mono-esters by enzymatic esterification, *Food Chemistry*, 138, 1884–1891, **2013.**
[13] An D., Zhao X., Ye Z., Enzymatic synthesis and characterization of galactosyl monoesters, *Carbohydrate Research*, 414, 32-38, **2015.**
[14] Zhang X., Nie K., Zheng Y., Wang F., Deng L.,Tan T., Enzymatic production and functional characterization of D-sorbitol monoesters with various fatty acids, *Catalysis Communications*, 72, 138–141**, 2015.**
[15] Yahya A.R.M., Anderson W.A., Moo-Young M., Ester synthesis in lipase-catalyzed reactions, *Enzyme and Microbial Technology*, 23, 438 – 450, **1998.**
[16] Huang SY., Chang HL., Goto M., Preparation of surfactant-coated lipasefor the esterification of geraniol andacetic acid in organic solvents, *Enzyme and Microbial Technology,* 22, 552–557, **1998.**
[17] Palomo JM., Muñoz G., Fernández-Lorente G., Mateo C., Fernández-Lafuente R., Guisán JM., Interfacial adsorption of lipases on very hydrophobic support(octadecyl–Sepabeads): immobilization, hyperactivationand stabilization of the open form of lipases, *Journal of Molecular Catalysis B: Enzymatic*, 19–20, 279–286, **2002.**
[18] Bidjou-Haiour C., Klai N., Lipase Catalyzed Synthesis of Fatty Acid Xylose Esters and their Surfactant Properties, *Asian Journal of Chemistry*, 25, 8, 4347-4350, **2013.**
[19] AndersonEM., Larsson KM., KirkO., One Biocatalyst–Many Applications: The Use of Candida Antarctica B Lipase in Organic Synthesis**,***Biocatalysis and Biotransformation*, 16, 3, 181-204, **1989.**
[20]Ferrer M., Soliveri J., Plou FJ., López-Cortés N., Reyes-Duarte D., Christensen M., Copa-Patiño JL., Ballesteros A, Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties, *Enzyme and Microbial Technology*, 36, 391-398, **2005.**
[21] KhareSK., Nakajima M., Immobilization of*Rhizopus japonicus*lipase on celite and its application for enrichment of docosahexaenoic acid in soybean oil, *Food Chemistry*, 68, 2,153–157, **2000.**
[22] Kamiya N., Goto M., Nakashio, F.,Surfactant-coated lipase suitable for the enzymatic resolution of menthol as a biocatalyst in organic media, *Biotechnology Progress*, 11**,** 270-275, **1995.**
[23] Leitgeb M., Knez Z., The influence of water on the synthesis of n-butyl oleate by immobilized Mucor miehei lipase, *Journal of the American Oil Chemists' Society*, 67, 775-778, **1990.**
[24] Sugimura Y., Fukunaga K., Matsuno T., Nakao K., Goto M., Nakashio F., A study on the surface hydrophobicity of lipases,*Biochemical Engineering Journal,* 5, 123–128, **2000**.
[25] Gitlesen T., Bauer M., Adlercreutz P., Adsorption of lipase on polypropylene powder,*Biochimica and Biophysica Acta*, 1345, 188–196, **1997.**
[26] Thakar A., Madamwar D., Enhanced ethyl butyrate production by surfactantcoated lipase immobilized on silica, *Process Biochemistry*, 40, 3263–3266, **2005.**
[27] Lopez C., Guerra NP., Rua L., Purification and characterization of two iso-forms from *Candida rugosa* lipase B, *Biotechnology Letters*, 22, 1291–1294, **2000.**
[28] Coulon D., Girardin M.,Rovel B.,Ghoul M., Comparison of direct esterification and transesterification of fructose by*Candida antartica*lipase, *Biotechnology Letters*, 17, 2, 183-186, **1995.**

6. ACKNOWLEDGEMENTS

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