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Enzymatic synthesis of alkylglycoside fatty acid esters catalyzed by an immobilized lipase

D. Coulon [a], A. Ismail [a], M. Girardin [b], M. Ghoul [a,*]

[a] *LSGC-ENSAIA, 2 avenue de la forêt de Haye, 54500 Vandoeuvre les Nancy, France*
[b] *LFBI, 2 avenue de la forêt de Haye, 54500 Vandoeuvre les Nancy, France*

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Abstract

An enzymatic process for lactose valorization has been established. Lactose was reacted in a first step with butanol in the presence of β-glucosidase and β-galactosidase to produce butylglycosides. Then, these alkylsugars were acylated in a second step by an immobilized lipase to synthesize alkylsugar fatty acid ester. The conversion rate was about 65%.

Keywords: Sugar esters; Acylation; Alkylation; Lipases; Glycosidase; Lactose; Enzymatic transesterification

1. Introduction

Sugar esters are non-ionic and biodegradable surfactants. They can be synthesized either chemically or enzymatically [1,2]. The chemical process presents a low selectivity and leads to a mixture of sugar esters, with different degrees of esterification [2]. Enzymatic synthesis can be realized in mild conditions [3] and as lipase acyls preferentially primary alcohols, it is considered as being highly regioselective [4]. However, enzymatic synthesis reactions are carried out in an organic medium to shift the equilibrium of the reaction towards ester bond synthesis. In such a medium, the difference of polarity between the two used substrates (sugar and fatty acid) is a serious limiting factor. In fact, there is no solvent compatible with enzyme activity that is both nontoxic and allows the solubilization of both substrates.

To perform enzymatic synthesis in an organic medium, several approaches have been investigated as the use of an intermediate polarity solvent [5], the complexation of sugar with boronic acid derivatives [6] or the alkylation of sugars [7]. Nevertheless, recent data have shown that phenyl boronic acid can inhibit lipase activities [8]. Thus, combining the use of alkylated sugars and solvent with an intermediate polarity seemed to be the most adapted solution for sugar ester synthesis. This is particularly true when the alkylation step is realized by enzymatic way.

The aim of this work is to develop a process combining enzymatic alkylation and acylation reactions. To highlight the pertinence of this approach, acylation of alkylated and non-al-

* Corresponding author.

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kylated sugars have been compared. Lactose, glucose, and galactose were used as raw materials.

2. Materials and methods

β-Glucosidase from bitter almonds (EC 3.2.1.21) (5 U/mg) and β-galactosidase from *Aspergillus oryzae* (EC 3.2.1.23) (4.4 U/mg) were purchased from Sigma. *Candida antarctica* lipase was immobilized on acrylic resin (a gift from Novo Nordisk). Lactose (ref. L-3750) was purchased from Sigma. Oleic acid methyl ester (Fluka, ref. 75163) was used as acyl donor. Butanol (Fluka, 19430) was used as alkyl donor. 2-methyl-2-butanol (Merck, ref. 806193) was used as a solvent for the acylation step.

2.1. Butylglycoside synthesis

In a typical experiment, lactose (317 mM) and enzyme (100 mg) were dissolved in 30-ml acetate buffer (10 mM, pH 4.5). Butanol (85 ml) and water (30 ml) were added to the medium. The incubation was carried out at 50°C in a 250-ml double jacket reactor, constantly shaken at a vigorous stirring rate. The production yield was calculated based on the glycosyl donor, which was the limiting reactant.

2.2. Butylglycosides purification

Butylglycosides were purified by liquid–liquid extraction, as described by Monsan et al. [9], with the product having a final purity above 95%.

2.3. Enzymatic synthesis of butylglycoside ester

Sugar (0.139 M) or alkylsugar were dissolved in 2-methyl-2-butanol. Oleic acid methyl ester was added at the same concentration. The final volume of the reaction medium was 1 l. Synthesis was catalyzed by 5 g/l Novozym and performed at 60°C at 200 mbar.

2.4. Analytical procedure

Products of the reaction were analyzed by thin-layer chromatography (TLC) [10].

3. Results and discussion

3.1. Synthesis of butylglycosides

Table 1 shows the results obtained for the enzymatic synthesis of butylglucoside and butylgalactoside. The concentration, yield, and reaction rate of butylgalactoside are higher than those of butylglucoside. This difference can be explained by the thermodynamic and kinetic aspects of the two reactions. Butylgalactoside is synthesized by a transfer of galactose moiety from lactose to butanol (transglycosylation reaction) while glucose is liberated and then condensed with butanol to give butylglucoside (reverse hydrolysis reaction). Synthesis of butylglucoside should be unfavourable because of the high water content in the medium (26% v/v) that shifts the thermodynamic equilibrium toward the hydrolysis of the glycosidic bond. The obtained results are higher than those reported by several authors [11,12].

3.2. Synthesis of butylglucoside and butylgalactoside oleic acid ester from purified butylglycosides

After the alkylation step, butylglucoside and butylgalactoside were purified as described above. Then, these molecules were acylated separately by oleic acid methyl ester, using an

Table 1
Concentrations and yields of butylglycosides obtained by enzymatic alkylation

Product	Butylglucoside	Butylgalactoside
Concentration (g/l)	15	20
Yield (%)	23	30
Time (h)	48	10

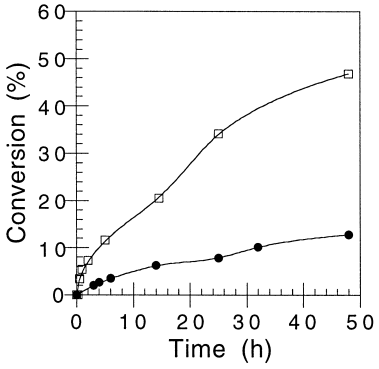

Fig. 1. Transesterification kinetics of galactose (●) and butylgalactoside (□) by oleic acid methyl ester catalyzed by *C. antarctica* lipase.

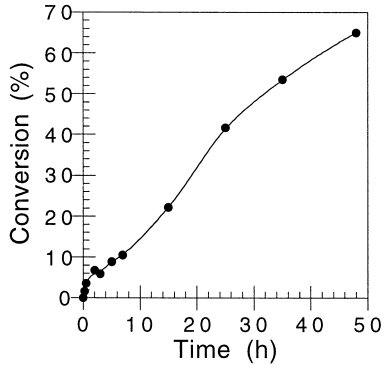

Fig. 3. Transesterification kinetics of butylglycosides synthesized by alkylation of lactose.

immobilized *C. antarctica* lipase. The performance of both alkylated and non-alkylated sugars for enzymatic acylation are summarized in Figs. 1 and 2. When glucose and galactose are used as substrates, dioleyl glycosides are synthesized in a minor extent (less than 5% conversion of sugar). With butylglycosides, only monoesters are produced. For both sugars, the alkylation step enhances the conversion rate, which is twofold higher than with non-alkylated sugars. These results are explained by the strong increase in solubility due to the presence of the butyl group. In fact, the solubility of glucose and galactose in 2-methyl-2-butanol at 60°C are, respectively, 1.5 g/l and 0.7 g/l. After the alkylation step, it rises to more than 25 g/l.

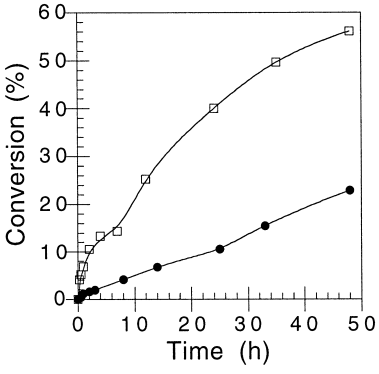

Fig. 2. Transesterification kinetics of glucose (●) and butylglucoside (□) by oleic acid methyl ester catalyzed by *C. antarctica* lipase.

3.3. Synthesis of butylglucoside and butylgalactoside oleic acid ester from lactose

To simplify the process, lactose was reacted in the presence of the two enzymes (β-glucosidase and β-galactosidase). Thus, a mixture of butyglucoside and butylgalactoside was produced. The acylation step was realized without separation of the two alkylated sugars. Only the excess of butanol and the enzymes were removed. The evolution of the conversion rate vs. time is described in Fig. 3. It appears that these experimental conditions lead to a 65% conversion of butylglycosides. This process seems to be a good alternative for valorizing lactose. Indeed, lactose was not acylated by *C. antarctica* lipase in 2-methyl-2-butanol.

4. Conclusion

The enzymatic alkylation of carbohydrates is a promising way for enhancing the production of sugar ester from sugars having a low solubility in organic medium. For lactose, this alkylation can be realized in one or two steps, depending on the end-use of the sugar ester produced. Further work will consist in the optimization of this process.

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