Enzymatic Synthesis of Sugar Fatty Acid Esters

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Abstract: Enzymatic synthesis of sugar fatty acid esters was performed in an organic solvent using an immobilized Candida antartica lipase and various sugars and oleic acid as substrates. A high solubility of the sugar in the solvent was needed for a high sugar ester yield. Among the sugars and solvents tested in this study, the highest yield (98 %) was obtained when xylitol and 2-methyl 2-butanol were used as the sugar and solvent, respectively. The optimal sugar-to-fatty acid ratio was in the range from 2:1 to 3:1 for a high conversion and minimization of the residual fatty acid concentration. The water content in the reaction medium was critical and had to be kept below 0.15 % to obtain a high sugar ester yield. The half life cycle of the enzyme used in this study was observed after 9-repeated uses. In a pilot reaction test, water formed during the reaction was effectively removed by recycling the reaction medium through a molecular sieve column outside the reactor; thus, a high sugar ester yield was obtained.

Keywords: sugar ester, Candida antartica lipase, solubility, water content, enzyme stability

Introduction

Sugar fatty acid esters, usually called sugar esters, are non-ionic and biodegradable surfactants that have very good emulsifying, stabilizing, or conditioning effects. They are widely used in the food, cosmetic, pharmaceutical, and detergent industries. Sugar esters are synthesized by esterification of sugars or sugar alcohols with fatty acids [1]. Synthesis of the esters can be carried out either chemically or enzymatically. The chemical process occurs with a low selectivity and leads to a mixture of sugar esters with different degrees of esterification. It requires toxic organic solvents and is carried out at high temperatures, which causes coloration of the final products. These problems can be overcome by the use of a biological catalyst, such as lipase, for the synthesis of sugar esters. The main advantage of enzymatic synthesis is that its high regioselectivity leads mainly to monoester production. In addition, the enzymatic method can be performed under mild reaction conditions; thus, denaturation of substrate and/or products can be avoided [2].

Direct enzymatic esterification of sugars with fatty acids in aqueous media was attempted in the early eighties, but the products formed in low yield [3]. More recently, enzymatic reactions have been carried out in organic media. In this case, a major problem is the low solubility of the sugars in the organic solvents. To solve this problem, activated fatty acids in polar solvents [4], or activated sugars in apolar solvents [5], have been used. However, these methods require substrate derivatization steps that increase production costs. As another approach, the partial solubilization of both substrates in intermediate-polarity solvents was reported to be effective for sugar ester synthesis [6,7].

Enzymatic sugar ester synthesis is based on esterification reactions catalyzed by hydrolases. Because esterification is a reversible reaction, the esterification reaction products such as water in the media should be removed to shift the equilibrium of the reaction away from hydrolysis to obtain a maximum yield of sugar ester [8]. Furthermore, the enzyme activity and/or stability are negatively affected by a high concentration of water [9]. In addition, the particles of an immobilized enzyme can be covered by a water layer preventing a lipophilic substrate (i.e., a fatty acid) access to the enzyme [10]. To remove the water liberated by the reaction, evaporation under reduced pressure [11] and azeotropic distillation [12] during the reaction were performed, with the condensed and water free solvents being

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Figure 1. Reactor system used for sugar ester synthesis.

returned continuously to the reactor. In these methods, however, continuous removal of water from the reaction mixture is rather difficult because the boiling points of most organic solvents are lower than that of water. Moreover, energy for vaporization can be costly.

In this study, the key parameters of enzymatic sugar ester synthesis catalyzed by an immobilized lipase were investigated, including the solubility, water content, molar ratio of sugar/acyl donor, and enzyme stability. A new reaction system for large-scale production was also investigated. In this system, the water liberated from the enzymatic sugar ester synthesis was removed by circulating the reaction media through an external column packed with molecular sieves.

### Experimental

**Materials**

A commercial lipase, Novozym 435 (immobilized lipase from *Candida antarctica* onto macroporous acrylic resin, Novo Nordisk), was used as biocatalyst for the sugar ester synthesis. The enzyme had an activity of 7000 PLU/g (propyl laurate unit) at 60 °C, as specified by the manufacturer. The acyl donor was oleic acid (Sigma, 90 %). Fructose (Yakuri), sucrose (Yakuri), xylitol (Aldrich), sorbitol (Duksan), and methyl-glucoside (Fluka) were used as acyl acceptors. Acetone and 2-methyl-2-propanol (t-butanol) were used as solvents for the esterification reaction. Molecular sieves (4 Å, 1/16" pellet, Yakuri) were used as a water removal adsorbent.

**Synthesis of Sugar Ester**

Reactions were carried out by mixing the desired amounts of sugar or sugar alcohol and oleic acid with the chosen solvent in stoppered glass bottles, which were shaken at 200 rpm in a thermoconstant shaker. Activated molecular sieves were added into the reaction medium, and esterification was initiated by adding the enzyme. A typical reaction mixture consisted of 6 mmol of sugar, 2 mmol of oleic acid, 0.3 g of enzyme, and 30 mL of solvent. The conversional yield of the sugar ester was calculated from the decrease in the concentration of oleic acid.

Large-scale sugar ester production was tested in a 5-L pilot-scale reactor, in which the water liberated during the enzymatic sugar ester synthesis was removed by circulation of the reaction media through an external column packed with molecular sieves, as shown in Figure 1.

**Determination of Solubilities of Sugars or Sugar Alcohols**

Saturated solutions of sugars or sugar alcohols in t-butanol or acetone at a given temperature were centrifuged. The supernatant was evaporated to remove the solvent. The residual sugar or sugar alcohol was dissolved in water. The concentration of each compound was then determined by HPLC.

**Analysis**

The analysis of the reaction medium was performed by HPLC (Waters 2690 Chromatography System). Fatty acid analysis was performed on an Alltech Nucleosil 100 C18 (250 mm × 4.6 mm) column with a refractive index detector. Elution was conducted with acetone/methanol/water (65/15/20). For the analysis of sugars or sugar alcohols, an Aminex HPX-87H column (Biorad) was used with a mobile phase of 0.005M H2SO4 aqueous solution. The moisture content in the reaction medium was determined using a Karl Fisher system (Model AF7).

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### Results and Discussion

**Effect of Solubility on Esterification**

The relationship between the solubility of a sugar in the organic solvent reaction medium and the sugar ester yield was investigated. Table 1 shows the solubilities of various sugars or sugar alcohols in acetone and t-butanol at 60 °C.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Acetone</th>
<th>t-Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylitol</td>
<td>3.1</td>
<td>17.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>3.1</td>
<td>15.6</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.73</td>
<td>10.6</td>
</tr>
<tr>
<td>Methyl-glucoside</td>
<td>2.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.36</td>
<td>2.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.21</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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Enzymatic Synthesis of Sugar Fatty Acid Esters

Figure 2. Typical time courses for the enzymatic esterifications of various sugars with oleic acid in t-butanol.

Figure 3. Typical time courses for the enzymatic esterifications of various sugars with oleic acid in acetone.

sugars t-butanol and acetone. Most sugars tested were more soluble in t-butanol than in acetone. Each sugar showed different solubilities in both solvents. In t-butanol, xylitol was most soluble (17.5 g/L), and sucrose was least soluble (1.1 g/L). A similar trend of the sugar solubility was observed in acetone. Figures 2 and 3 show the time courses of the enzymatic esterification reactions of six sugar compounds: xylitol, fructose, methylglucoside, sorbitol, glucose, and sucrose. The sugar ester yields achieved with these sugars were closely related to their solubilities. As shown in Figures 2 and 3, higher conversions were obtained when t-butanol was used as a solvent than when acetone was used. In addition, when t-butanol was used as the solvent, the conversion with xylitol, which was most soluble, was highest (98 %), and the yield with sucrose, which was least soluble, was lowest (41 %), as shown in Figure 2. Similar results were observed when acetone was used as a solvent, as shown in Figure 3.

The sugar ester yields with xylitol and sucrose in acetone were 70 and 11 %, respectively. This result indicates that a suitable organic solvent should dissolve enough substrate to allow the lipase-catalyzed esterification. Therefore, the use of water-miscible or polar organic solvents could have the advantage that hydrophilic substrates, such as saccharides, are solubilized to some extent to facilitate the esterification of the substrates.

Effect of the Molar Ratio of Sugar/Acyl Donor
The enzymatic sugar ester synthesis was studied at different molar ratios of sugar to fatty acid. As shown in Figure 4, the conversion of oleic acid increased initially with increasing the excess of the sugar. However, when the sugar/oleic acid ratio was above 2 ∼ 3, the conversion effectively remained constant. In the case of xylitol, the conversions were 64 and 98 % for xylitol/oleic acid ratios of 1:1 and 3:1, respectively. The equilibrium constant of the esterification reaction depends on the concentrations of both reactants. Therefore, an excess of sugar favored the conversion of oleic acid. Moreover, the presence of solid sugar in the reaction medium could affect the performance of the enzyme. Because sugars are lyoprotectant compounds, they can reduce the water activity of the medium [13].

The final ratio used in a commercial process should be decided on a cost basis, depending mainly on the ease of product separation. The residual fatty acid usually causes difficulties with product separation.

Effect of the Quantity of Lipase
The effect of the enzyme (Novozym 435) dosage in the sugar ester synthesis was investigated over the range from 5 to 100 % (g enzyme/g sorbitol). Figure 5 shows the time courses of sorbitol oleate synthesis with different Novo-
zym dosages. It indicates that the enzyme quantity did not affect significantly the equilibrium state of the reaction, but it lead to an increase of the initial rate. Except when using 5 % Novozym, the equilibrium state was reached within 10 h. A larger quantity of enzyme would allow the reaction to reach the equilibrium state more rapidly. However, the cost of the enzyme should be taken into account to optimize the process.

Stability of the Enzyme
The stability of the immobilized *Canadia antarctica* lipase (Novozym 435) used in this study was evaluated by comparing the enzyme activity after repeated uses. After each reaction at 60 °C in t-butanol, the enzyme was filtered and reused as a biocatalyst for a new reaction. The relative activity and the final conversion were observed for each reaction. The relative activity was defined as the ratio of conversion after 3 h of reaction with each used enzyme to that using the fresh enzyme. Total reaction time of each reaction was 12 h. The results are given in Figure 6. At 60 °C, the enzyme showed good stability in t-butyl alcohol. Only a ca. 17 % reduction of the final conversion of each reaction occurred after 9 reaction cycles. The relative activity of the enzyme decreased in half after 12 runs. The half-life of the enzyme was calculated from a linear extrapolation of nine experimental data points of the relative activity versus the number of runs, as shown in Figure 6.

Effect of Water Content
To obtain a maximum yield of sugar ester by shifting the equilibrium of the reaction away from hydrolysis, the esterification reaction products, such as water, in the media should be removed. For this reason, the addition of a desiccant to a reaction system or performing a reaction under reduced pressure has been adopted previously. However, there is yet no criterion for the water content level to achieve the desired conversion. In this study, the effect of the water content on the sugar ester synthesis was investigated by varying the amount of molecular sieves added in the reaction system. Figure 7(a) shows the time course of the sugar ester synthesis (conversion of xylitol and oleic acid to xylitol oleate in t-butanol) in the presence of various amounts of molecular sieves. The conversion was over 90 % in the presence of large amounts of molecular sieves. Without the addition of molecular sieves, the conversion was as low as 48 %. Figure 7(b) shows the water content in the reaction medium as a function of reaction time. Under the conditions of this study, the water content was ca. 0.3 % when no molecular sieves were added. Addition of larger amounts of molecular sieves resulted in lower water contents, as expected. From the results shown in Figures 7(a) and 7(b), we found that the water content in the reaction medium should be controlled to less than 0.15 % to obtain conversions higher than 90 %.

A Production Scale Reaction System
A new reaction system for large-scale production was investigated. In this system, the water liberated from the enzymatic sugar ester synthesis was removed by circulating the reaction medium through an external column packed with molecular sieves. Based on the optimum reaction conditions found from the flask test, a 5-L scale pilot test was performed. Figure 8 shows the good performance of the reaction system proposed in this study. The conversion obtained in a flask test (working volume: 30 mL) was similar to the level of conversion observed in the proposed reaction system (working volume: 4 L). Therefore, the use of an external molecular sieve (or any
Figure 7. Effect of the addition of molecular sieves on the (a) conversion and (b) moisture content of the enzymatic esterification of xylitol with oleic acid.

Figure 8. Time courses for enzymatic esterifications of various sugars with oleic acid performed in a 5-L pilot reaction system.

desiccant) column appears to be very efficient for water removal during the reaction for large-scale sugar ester production. This reaction system could also be used when conducting various enzymatic reactions in organic solvents that require the efficient removal of water.

Conclusion

In this study, we determined the effects of several parameters affecting the enzymatic synthesis of sugar esters. The influences of the solubility, molar ratio of sugar/acyl donor, enzyme stability, and water content were each investigated. It appeared that a suitable organic solvent should dissolve enough of the substrate to allow the lipase-catalyzed esterification. Among the sugars and solvents tested in this study, the highest yield (98%) was obtained when xylitol and t-butanol were used as the sugar and solvent, respectively. The optimal sugar-to-fatty acid ratio was in the range from 2:1 to 3:1 for a high conversion and minimization of the residual fatty acid concentration. The water content in the reaction medium was critical; it should be kept below 0.15% to obtain a high sugar conversion. The half-life of the enzyme used in this study was observed after 9-repeated uses. In a pilot reaction test, the water formed during the reaction was effectively removed by recycling the reaction medium through an external molecular sieve column; thus, a high sugar ester conversion was obtained. This reaction system could also be used for conducting various enzymatic reactions in organic solvents that require the efficient removal of water.

Acknowledgment

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References