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Optimization of lipase-catalyzed rosin acid starch synthesis by response surface methodology

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ABSTRACT

Rosin acid, the major component of natural resin, was a useful reagent for polymers modification. In this work, the enzymatic synthesis of rosin acid starch was carried out by esterifying cassava starch with rosin acid directly using DMSO as solvent. Response surface methodology (RSM) based on a three-factor-three-level Box–Behnken central composite design was applied to evaluate the effects of synthesis conditions, namely reaction temperature, reaction time and enzyme amount. The optimal condition for achieving high degree of substitution (DS) of the esterified product was reaction time 4.11 h, temperature 48.18 °C, immobilized catalyst dosage 15.47% (by weight of starch), and a molar ratio of rosin acid/anhydrous glucose unit 2:1. The experimental DS value of 0.106 matched well with the predicted value of 0.11. The structural changes between native starch and rosin acid starch were investigated by Fourier transform infrared analysis (FTIR) and Scanning electron microscopy (SEM).

1. Introduction

Due to the challenges of fossil resource depletion and the environmental problems, development of bio-based polymers to replace the traditional petrochemical polymers has attracted much attention in the past decades [1,2]. Among the biopolymers, starch is most interesting because of its low cost, availability, biocompatibility and biodegradability. However, due to some inherent drawbacks including the poor surface properties, high hydrophilicity, poor mechanical and thermal properties, the application of native starch in industrial was limited. To improve the properties of starch, a variety of physical or chemical modifications on native starch have been developed [3]. Esterification is one of the common and effective methods to tailor properties of this renewable material. Starch esters with low molecular weight fatty acid (C1–C4) and higher carboxylic acid (C4–C18) have been successfully synthesized [4], and showed a wide variety of applications in biodegradable plastics, adhesives, and auxiliaries [5]. Considering the carboxylic acid is one of the major factors affect the properties of starch esters, much more acid candidates should be explored for the starch ester synthesis.

Rosin, also called colophony, is a kind of renewable natural resin obtained from pines and other conifers. More than 1 million metric tons rosin is produced each year and widely used in the manufacture of adhesives, paper sizing agents, printing inks, solders and fluxes, and surface coatings [6]. Gum rosin mainly consists of different rosin acid, such as abietic, levopimaric, and pimaric acids which have a characteristic bulky hydrophenanthrene rings structure with the molecular formula $C_{19}H_{29}COOH$ [7]. The special structure and property of rosin acid make it a useful reagent for polymers modification [8]. The reactive carboxyl group of rosin acid lets it to be integrated into polymers as backbone or side chains, the bulky hydrophenanthrene group of rosin acid can dedicate hydrophobicity to the attached polymers and alter their thermal properties. In addition, the rosin acid can be converted to a large number of downstream derivatives easily, which means rosin acid integrated polymers can be further grafted and modified, and that would lead to novel products with new functionalities [9,10]. Moreover, rosin acid shows excellent biodegradability and biocompatibility. Satturwar reported that rosin film implanted in rats did not cause necrosis or abscess in the surrounding tissues, and was completely degraded in 90 days in vivo [11]. Rosin and rosin-derived esters are permitted to be used as food additives approved by the U.S. Food and

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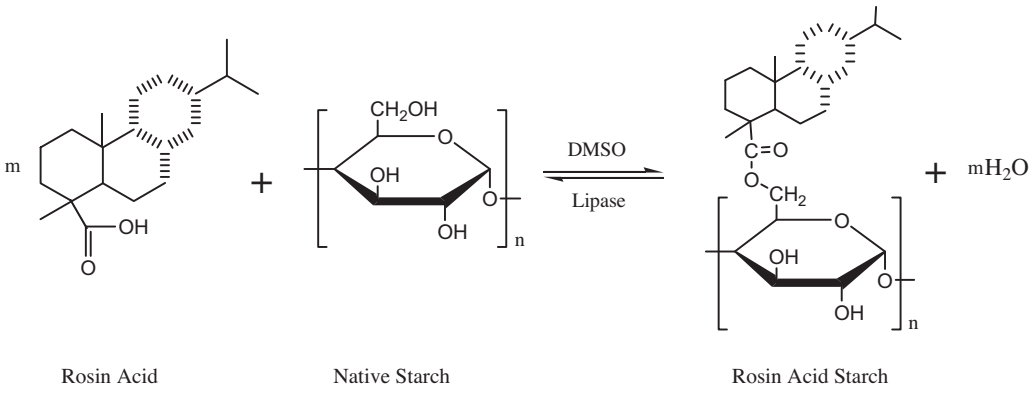

Fig. 1. Esterified starch with rosin acid catalyzed by lipase. Although in the figure the substitution was represented only on the OH in position 6, it also could occur on hydroxyl groups located at positions 2 or 3.

Drug Administration [12]. Several chemical methods dealing with integrating rosin moiety to starch molecule have been reported [10,13,14]. The rosin acid starch has improved its thermal stability and hydrophobicity obviously, and finds potential applications in waterproof coating and plastic.

Lipases are an important group of biotechnological catalysts which catalyze the hydrolysis of triglycerides into free fatty acids and glycerol. Besides, they can also catalyze esterification, acidolysis, interesterification, alcoholysis and aminolysis, and have been widely applied in food, dairy, detergent and pharmaceutical industries [15,16]. Lipases have been used as effective catalyst for starch esterification [17]. However, to the best of our knowledge, there has been no report about enzymatic synthesis of rosin acid starch until now.

Considering the advantages of milder reaction condition, higher reaction selectivity and fewer by-products in enzymatic esterification of starch [17,18], a lipase-catalyzed synthesis of rosin acid starch method (Fig. 1) has been developed in our group recently. In order to optimize the enzymatic synthesis process of rosin acid starch, response surface methodology (RSM) is employed in this work. RSM is a collection of mathematical and statistical techniques, which can be used in experiments designing, models building and the effects analyzing of multiple parameters [19]. RSM is helpful in reducing number of experimental trials that are needed to evaluate multiple parameters and their interactions. Moreover, RSM is helpful in determining the target value based on investigation of the individual and interactive effects of the parameters. RSM provides an effective tool, which can help optimize the process if there are many parameters and interactions in the experiment [20].

In this study, the mutual effect of immobilized catalyst dosage, reaction temperature, reaction time on the enzymatic synthesis of rosin acid starch was investigated, using Box–Behnken design in response surface methodology (RSM) by Design Expert Version 8.0.6.1 (Stat Ease, USA).

2. Materials and methods

2.1. Materials

Gum rosin was kindly supplied by Guangxi Wuming Chaoyan Rosin Plant, China, and was used directly for esterification reaction. Cassava starch (approximately 17% amylose and 83% amylopectin) was purchased from Guangxi Cenxishi Sanjiao Food Scuffled. Novozym 435 with activity of 10 unit/mg was purchased from Novo Industries, Denmark. DMSO, methanol and acetone were analytical grade purchased from Chengdu Kelong Chemical Reagent Co., China.

2.2. Esterification of cassava starch with rosin

2.2.1. Pretreatment of cassava starch

In order to improve its solubility for the subsequent reaction, the cassava starch was pretreated according to the literature [21]. 4%(w/v) cassava starch was dissolved in NaOH/urea solution (6 g NaOH and 3 g urea in 100 ml deionized water) completely, then neutralized with HCl. After precipitated and washed with 95% of ethanol, the resulting precipitate was dried at 70 °C for 24 h.

2.2.2. Esterification reaction

The esterification reaction was performed in a 150 ml round flask with a magnetic stirrer as an agitator. Temperature was controlled by thermostatic water bath. The pretreated cassava starch and different amounts of rosin were placed in the reactor followed by the addition of DMSO to a working volume of 50 ml. Different amounts of immobilized lipase (Novozym 435) were subsequently added to initiate the reaction. The esterification process was carried out for different time at different temperature as experimental design with initial molar ratio of rosin/anhydrous glucose unit (AGU) fixed at 2 and the agitator speed set to 200 rpm. Subsequently, the immobilized lipase was removed by filtration to stop the reaction. The reaction products were collected by methanol precipitation and washed by methanol three times. The esterified starches were dried at 70 °C for 24 h and their degree of substitution (DS) were analyzed by titration method [22]. Control experiments without enzyme were run in parallel under the same conditions, and titration result showed the DS of the controls was about 0.

2.3. Experimental design and statistical analysis

In the synthesis procedure of rosin acid starch, the effects of reaction condition on DS value including the molar ratio of rosin acid/AGU, reaction time, reaction temperature, and immobilized catalyst dosage had been investigated by the single factor method (Unpublished results). Based on the results of the previous experiments, although the DS value increased with the molar ratio of rosin acid/AUG rose during the enzymatic synthesis of rosin acid starch, it showed the conversion rate of rosin acid was highest when the molar ratio of rosin acid/AGU was 2:1. Hence, the molar ratio of rosin acid/AUG was not generally considered as a variable in the case of experimental designs and was fixed at 2:1.

In the present study, the Box–Behnken method-3^3 (three factors each, at three levels) experimental design model were used. Actual values of the factors were selected at three levels, coded as −1, 0 and +1 for low, middle and high values respectively (Table 1). The software of design expert 8.0.6.1 (Stat-Ease, USA) was used for the design of experiment. Table 2 showed the actual experiments