Preparation of Soybean Oil Corn Starch Ester by using Immobilized Lipase

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Abstract. The soybean oil starch ester was synthesized by using Novozym435 lipase as biocatalyst, corn starch and soybean oil as substrate in solvent-free system. In order to improve the reaction activity of starch, the corn starch was pretreated using NaOH/Urea/H₂O solution. The optimum pretreatment reaction conditions of enzymatic esterification of soybean oil with pretreatment starch were water activity<0.01, reaction temperature was 60°C, reaction time was 30h, the ratio of soybean oil to pretreatment starch was 6 (w/w) and the amount of lipase was 7%. Under this condition the degree of substitute of soybean oil with pretreatment starch was 0.0302. The solubility, emulsifying and freeze-thawing stability of soybean oil starch ester increased with the increase of its degree of substitute, but the transparency of soybean oil starch ester decreased with its degree of substitute. The transparency of soybean oil starch ester was improved in the presence of sucrose, but decreased in the presence of sodium chloride and citric acid.

Introduction

The importance of polyunsaturated fatty acids (PUFAs) in human nutrition and disease prevention has been and still is one of the major issues for the nutraceutical and pharmaceutical industries [1]. Over the last few decades, starch has been modified by various methods to achieve functionalities suitable for various industrial applications. Basically there are four kinds of modifications; chemical, physical, enzymatical and genetical[2]. Selected edible oils, such as flaxseed and fish liver oils contain polyunsaturated fatty acids(PUFAs), which have important functional and physiological roles in human diets. PUFAs are widely recognized in the modulation of risk of a variety of diseases including cardiovascular, immunological, cancer, visual impairment and memory loss [3]. However, due to their high content of PUFAs, these oils are susceptible to oxidation producing off-flavors and potentially harmful radical products [4]. It is well known that enzyme-catalyzed ester synthesis is thermodynamically unfavorable in conventional aqueous media. A lot of methods have been developed to facilitate the formation of ester bonds over their hydrolysis. One approach is to replace water with an organic solvent to favor synthesis under restricted water conditions [5]. Over the last few decades, starch has been modified by various methods to achieve functionalities suitable for various industrial applications. Basically there are four kinds of modifications; chemical, physical, enzymatical and genetical. A number of review articles on the subject of starch modification are available [6-8].

Materials and methods

Preparation of Soybean Oil Corn Starch Ester

Synthesis of Soybean Oil Corn Starch Ester. The pretreatment of 1G starch (Gan Ji), soybean oil and lipase had been placed in the sealed container with 3 molecular sieve in 2 days

Dried flask had been placed under 60 °C oil bath, added soybean oil, with magnetic stirring, to which add pretreated starch, and still had been stirring. Then we had get mixed homogeneous slurry, and adding lipase to esterification reaction at a constant temperature.

After a certain period of time, the 75 °C heat ethanol had been washed two times and placed in an oven whose temperature is 75 °C to dry and crush. Then we had get the finished product and placed it in the sealed bag in 105 °C after constant weight

Single Factor Experiment of the Preparation of Soybean Oil from Corn Starch

Effect of enzyme addition on the degree of substitution. 1g pretreated starch (dry basis) had been dealed with 3A molecular.

Sieve to balance Water activity. The reaction temperature had been 60 °C and the reaction time had been 12h. The quality ratio of pretreated corn starch and soybean oil had been 1:5. 0.6 g of 3A molecular sieve had been added when they are reacted. Under this reaction conditions, the effect of the addition of different lipase (1%, 3%, 5%, 7%) on the degree of substitution of starch ester had been investigated.

Effect of reaction temperature on the degree of substitution. With the certained added amount of the most suitable lipase, 1g of pretreated starch (dry basis) had been added with 3A molecular sieve to balance water activity. The reaction time had been 12h. The quality ratio of pretreated corn starch and soybean oil had been 1:5. 0.6 g 3A molecular Sieve had been added when they are reacted. Under this reaction conditions, the effect of different temperature (55 °C, 60 °C, 65 °C, 70 °C) on the degree of substitution of starch ester had been investigated.

Effect of quality ratio of pretreated corn starch and soybean oil on the degree of substitution. With the certained added amount of the most suitable lipase and the most suitable reaction temperature, 1g pretreated starch (dry basis) had been added with 3A molecular sieve to balance water activity. The reaction time had been 12h. 0.6 g of 3A molecular sieve had been added when they are reacted. Under this reaction conditions, the effect of different quality ratio of pretreated corn starch and soybean oil (1:4, 1:5, 1:6, 1:7, 1:8) on the degree of substitution of starch ester had been investigated.

Effect of reaction time on the degree of substitution. With the certained quality ratio of pretreated corn starch and soybean oil, the added amount of the most suitable lipase and the most suitable reaction temperature, 1g pretreated starch (dry basis) had been added with 3A molecular sieve to balance water activity. 0.6 g of 3A molecular sieve had been added when they are reacted. Under this reaction conditions, the effect of different reaction time (12h, 24h, 36h, 48h) on the degree of substitution of starch ester had been investigated.

Characterization and Analysis Methods

Determination of the degree of substitution. Take 0.03g dried to constant weight of soybean oil corn starch ester sample in 50mL of triangular reaction bottle, to which is added with 0.5mL of DMSO. The sample was fully dissolved, then added to the reaction flask with 1mL of 0.07mol/L sodium methoxide methanol solution. The reaction flask is placed in a constant temperature oil bath of 70 °C and the reflux 1h is condensed. After the end of the reaction, cooled to room temperature, added 1mL of deionized water and 1mL n-heptane and placed on a magnetic stirrer, stirred vigorously 1min. Still stratification, mixed fatty acid methyl esters in the extraction of the supernatant. Take supernatant liquid of 1μL sample was analyzed by gas chromatographic external standard method[5].

External standard: 3.373×10^{-4} mol/L of oleic acid methyl ester.

Chromatographic column: silica capillary column (30m×0.32mm×0.25μm), the column temperature is 220 °C, the inlet temperature is 250 °C, the detector temperature is 260 °C [6].

Degree of substitution: $DS = 162n / m - (m1 - mH2O)$

Type : DS-The number of polysaccharide monomers substituted carboxyl average; 162-The molecular weight of the glucopyranose residues, 162; n-The amount of starch, which is replaced by fatty acid in soybean oil, mol; m-The sample quality, 0.03g; m1-The starch quality replaced by fatty acid, g; mH2O-Replace the quality of off water, g.

SEM analysis. Dissolved the powder sample in deionized water and applied to the sample holder. The sample holder is placed in an ion sputtering apparatus, and a layer of 10nm thick gold film is deposited on the surface of the sample. The electron microscope was used to observe and photograph under different magnification.

infrared spectra analysis. Take 1g sample (dry basis), into the 100mL beaker, add 50mL 60 °C volume fraction of 95% ethanol halved 30min, then moved to NO.3 sand core funnel, with 60 °C volume fraction of 95% ethanol rinsed thoroughly 24h after drying at 50 °C. The treated samples and potassium bromide were placed in a dryer with a certain proportion to be mixed with a certain proportion, and the changes of starch functional groups before and after esterification were investigated by Fourier transform infrared spectroscopy.

Results

Results and Analysis of Soybean Oil Corn Starch Ester Prepared Single Factor Experiments.

Influence of Enzyme Addition on the Degree of Substitution. The lipase addition was 1%, 3%, 5%, 7%, respectively, reaction temperature was 60 °C, reaction time was 12h. The mass ratio of pre-starch and soybean oil was 1:5. 0.6g of 3Å molecular sieves were added to balance the water activity

Fig 1 showed that the degree of substitution (DS = 0.0017) was maximum when the enzyme addition was 5%. After enzyme addition exceeded 5%, the substitution degree was gradually decreased, which might be the occurrence of lipase agglomeration which influenced the contact of substrate and lipase activity center. Also, with the amount of enzyme used increasing, the viscosity of the reaction system was increasing accordingly. Increasing shear agitation caused the destruction of immobilized enzyme morphology, which influenced the enzyme activity. So the degree of substitution was reduced. Therefore the optimum enzyme addition was 5%.

Figure 1. The effect of enzyme addition on the degree of substitution of starch ester

Influence of Reaction Temperature on the Degree of Substitution. The reaction temperature was 55°C, 60°C, 65°C, 70°C, respectively, reaction time was 12h. The mass ratio of pre-starch and soybean oil was 1:5. 0.6g of 3Å molecular sieves were added to balance the water activity.

Figure 2. Effect of reaction temperature on the degree of substitution of starch ester

Fig. 2 showed that the degree of substitution ($DS = 0.0024$) was maximum when the reaction temperature was 60°C, exceeded this temperature the degree of substitution decreased. Immobilized lipase Novozym435 used in this test had a high reactivity in the range of 55~75°C, which might be overheated and partial inactivation of the enzyme. At the same time the high temperature made the reaction equilibrium move to reverse direction, hydrolyze the generated starch ester again. So the selected reaction temperature was 60°C in the following reaction.

Influence of the Ratio of the Pre-corn Starch and Soybean Oil on the Degree of Substitution. The ratio of pre-corn starch and soybean oil added was 1: 5, 1:4, 1:6, 1:7, respectively, reaction time was 12h, reaction temperature was 60 °C, the lipase addition was 5%, 0.6g of 3Å molecular sieves were added to balance the water activity. Fig. 3 showed, that the degree of substitution ($DS = 0.003$) was maximum when the ratio of pre-starch and soybean oil mass was 1:6. after the mass ratio exceeded 1:6, the degree of substitution decreased obviously. In the absence of solvent system, soybean oil was not only a reaction substrate, but also played a role as solvent. Reduced the viscosity of the reaction system greatly, was beneficial to the movement of esterification reaction direction. But when the amount of soybean oil used was too high, the contact chance of hydroxyl groups on starch and lipase activity center was reduced, which caused the decrease of the degree of substitution. Therefore the selected mass ratio of starch and soybean oil ratio was 1:6.

Figure 3. Effect of pre treated starch and soybean oil quality on the degree of substitution of starch ester

Influence of the Reaction Temperature on the Degree of Substitution. Reaction time was 6h, 12h, 18h, 24h, 30h, reaction temperature was 60 °C, the lipase addition was 5%, the mass ratio of pre-corn starch and soybean oil was 1: 6, 0.6g of 3Å molecular sieves were added to balance the water activity

Fig. 4 shows that the degree of substitution (DS = 0.017) was maximum when reaction time was 24h. The degree of substitution of product decreased with the time extending. This was because during the reactor operating, prolonged agitation shearing made the immobilized enzyme come off from the carrier, and abrasion caused reduction of particle size which affected the stability of the immobilized enzyme, leading to the enzyme catalytic efficiency reduce, even enzyme inactivation. With the reaction time extending, the generated product was partially hydrolyzed, resulting in the degree of substitution decreasing. In summary, the best reaction time should be 24h.

Figure 4. Effect of reaction time on the degree of substitution of starch ester

Scanning Electron Microscope Analysis. From the Fig. 5, it is clear that the pre-processing starch particle diameter in 500 ~ 1000nm and particle surface is uneven. Appearance of high oil corn starch ester is granular, but the particles become smaller, the average particle diameter is in 300 ~ 800 nm, but because of fatty acids in soybean oil is mainly long chain fatty acid, the infiltration of long chain fatty acid chains to starch granules reduce the agglomeration of starch granules. So the esters of starch granule surface will appear uneven.

Figure 5. Scanning electron microscope of pretreated starch (A) and soybean oil corn starch (B)

Infrared Spectrum Analysis. Infrared spectrum analysis is an important tool for analysis of starch and other organic polymer material, which uses the selective absorption of organic functional groups in the mid-infrared region, it can qualitative analyze the organic compounds, in particular functional groups. Infrared spectrogram of corn starch and pre-starch were basically similar, but its -OH stretching vibration peak was wider than the original starch significantly. Soybean oil starch esters had typical ester bond characteristic absorption peak at 1725 ~ 1750 cm^{-1} , which explained that the degree of substitution of starch esters prepared were already within the scope of the equipment testing, thus proved that soybean oil fatty acid substituted starch hydroxyl groups.

Summary

From the various factors that affected the degree of substitution of soybean oil corn starch ester showed that, the influence of reaction temperature on the esterification was biggest, followed was the amount of enzyme addition. When water activity <0.01 , optimal condition of soybean starch esters preparation was 7% of enzyme addition, reaction time was 30h, substrate ratio was 1:6, temperature

was 60 °C. The degree of substitution of soybean oil starch ester prepared under these conditions was 0.0302.

Appearance of soybean oil corn starch ester remained granular shape, and the shape was similar to corn starch. Starch particle size was smaller than the pre-treatment (500 ~ 1000nm) relatively, was 300 ~ 800nm. Starch esters particle surface appeared uneven. Infrared spectrogram showed that pre-corn starch and corn starch were basically similar, but its hydroxyl stretching vibration peak wider than the original starch significantly, which indicated that hydroxyl groups were increased; and soybean oil starch ester had typical ester bonding characteristic absorption peak at 1725 ~ 1750cm⁻¹, which proved that soybean oil fatty acid substituted starch hydroxyl groups due to the esterification..

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