Synthesis, Characterization of Inulin Propionate Ester, and Evaluation of its In Vitro Effect on SCFA Production

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In this study, the authors developed a method for the synthesis of inulin propionate ester as dietary fiber carrier to increase the amounts of short chain fatty acids (SCFA). The propionylated inulin showed an appreciable effect for increasing SCFA compared with control inulin. The structure characteristics of inulin propionate ester are established based on FT-IR, 1H NMR, and 13C NMR spectra. Meanwhile, response surface methodology (RSM) is used to optimize the synthesis conditions and investigate the effect of three parameters on the degree of substitution (DS). The optimal conditions are as follows: the ratio of anhydride to inulin of 4.5:1, the concentration of 30%, the temperature of 40°C. The results indicated that the ratio of anhydride to inulin and the concentration has a significant effect on DS. Under these conditions, the experimental DS was 2.86. In a 48 h in vitro fermentation experiment, the results of propionylated inulin had a good potential for enhancing propionate ratio compared to control inulin. This experiment provides a novel carrier molecule whereby SCFA is chemically bound by an ester bond to inulin which would be beneficial for delivering high concentration of SCFA and influencing intestinal health.

1. Introduction

Growing evidence demonstrated that short chain fatty acids (SCFA), principally acetate, propionate, and butyrate, play an important role in improving intestinal health and gut microbiota composition. Their effects include immunity, energy metabolism, and stimulate the release of the anorectic gut hormones. Among the SCFA, acetate can be metabolized in the liver and it also can be used as energy source for hepatic. Furthermore, as propionate can promote the secretion of gastrointestinal hormones, such as peptide YY (PYY) and glucagon like peptide-1 (GLP-1), it could regulate appetite and body weight. Due to the anti-inflammatory properties, butyrate in the intestine not only affects key functions of the colonic epithelium but also plays an important role in colon cancer suppression.

Considering the benefits of SCFA, it would be beneficial for intestinal health to increase the SCFA concentrations in the gut. Research suggests that prebiotic dietary fibers intervention can improve gut environment and change the intestinal microbial composition. The plant-derived non-digestible polysaccharides such as inulin, which is one of the most extensively studied prebiotics. It can be fermented by Lactobacilli and Bifidobacteria in the colon and produce SCFA, which may exert direct effects on human health. As a fructan-type polysaccharide, inulin consists of (2→1) linked by β-D-fructosyl residues which each fructose chain is usually terminated with an (1→2) α-D-glucose end moiety. The degree of polymerization of inulin varies from 2 to 60 and the average molecular weight is about 5500. Given the β-(2→1) chemical structure in inulin fructose monomers, inulin cannot be hydrolyzed by digestive enzymes which are specific for α-glycosidic bonds in the human gut. Therefore, inulin can escape digestion in the upper gastrointestinal tract and be fermented in colon which can produce prebiotics effects. Indeed, it is no longer surprising that the colon plays an important role in our digestive system. Many in vivo studies have indicated that a rise in the amount of inulin in food can obviously enhance the number of bifidobacterium in the intestine.

Consequently, in order to further improve the performance of inulin and meet the needs of the diversity of the modern food industrial applications, an effective way is the chemical modification via introduction of the individual functional moieties to inulin molecules. Many efforts have been devoted to the chemical modification of inulin, such as octenyl...
succinic anhydride modified inulin and amino modified inulin.\textsuperscript{[19–21]} However, although the inulin SCFA ester derivatives have been reported and described, to our knowledge there are few studies focused on chemical modification of inulin with propionic anhydride.\textsuperscript{[11,22,23]} Furthermore, there are no reports on the synthetic technology conditions optimal of inulin propionate ester.

Inulin can be selected as desirable carrier to deliver high concentration SCFA to the colon and regulate the composition of the intestinal microflora.\textsuperscript{[24–27]} This study aimed to develop a method for the synthesis of inulin propionate which can be used to increase the amount of SCFA. Thus, in the current study, we presented the synthesis, characterization of inulin propionate ester (INP). Further, the synthetic technology conditions were optimized with the single-factor experiments and Box-Behnken design (BBD) along with Response Surface Methodology (RSM) to fabrication of INP with a high DS. The chemical structures of the inulin derivatives were characterized by FTIR, \(^1\)H NMR, and \(^{13}\)C NMR.

2. Experimental Section

2.1. Materials Analytical Methods

Inulin (from chicory) was procured from Sensus (Roosendaal, the Netherlands). The properties of inulin were as follows: the average degree of polymerization was around 10 fructosyl fructose, the purity was about 91\%, and the molecular weight was about 1600. Deuterium oxide (D\(_2\)O), deuterochloroform (CDCl\(_3\)), and propionic anhydride were purchased from the Sigma–Aldrich Chemical Corp (Shanghai, China). All other reagents were all analytical grade and used as received.

2.2. Synthesis of INP

The INP was synthesized by using the improved synthesis method,\textsuperscript{[22–25]} inulin was dissolved in a round bottom flask in oil bath pot and stirred to dissolve, using pyridine as solvent. Then, using drip funnel, dropwise addition of propionic anhydride followed. Meanwhile, a stirring device was used to mix the inulin solution and anhydride uniformly. Once the addition of anhydride was finished, the reaction mixture was stirred for 24 h at the set temperature. Furthermore, the product was poured into about 100 mL water and then transferred into a separating funnel, using 200 mL dichloromethane extracted for two times. The organic phase was collected and repeatedly washed by saturated sodium chloride solution and saturated sodium bicarbonate solution to remove excess pyridine and the remnants of anhydride. Finally, the neutral solution was condensed by rotary evaporator and dried through vacuum pump until product was obtained as white powder (Scheme 1).

2.3. Characterization of Inulin and INP

The synthesized INP was characterized by Fourier transform infrared spectroscopy (FTIR), \(^1\)H Nuclear magnetic resonance (\(^1\)H NMR), and \(^{13}\)C nuclear magnetic resonance (\(^{13}\)C NMR). Fourier transform infrared (FTIR) spectra were recorded range from 4000 to 400 cm\(^{-1}\) with resolution of 4.0 cm\(^{-1}\) using a Jasco-4100 FT-IR spectrometer (Japan, provided by JASCO Co., Ltd. Shanghai, China). A 1 mg of sample was mixed with 100 mg of KBr, and the mixture was pressed into pills with a compression and prepared pellets were used for studies. \(^1\)H Nuclear magnetic resonance (\(^1\)H NMR) and \(^{13}\)C nuclear magnetic resonance (\(^{13}\)C NMR) spectra were recorded using Bruker AVIII-500 Spectrometer (n500 MHz, Switzerland, provided by Bruker Tech. and Serv. Co., Ltd. Beijing, China). For spectral recording inulin was dissolved in D\(_2\)O, INP was dissolved in CDCl\(_3\). The tetramethylsilane (TMS) was used for internal standard and the chemical shifts were expressed as \(\delta\) (ppm).

The degree of substitution (DS) of inulin propionate ester was calculated via its \(^1\)H NMR spectrum by using the ratio of integrals of resonance peaks at 1–2 ppm and 3.5–5.5 ppm which correspond to ethyl protons of propionylation and protons of

Scheme 1. Synthetic routes of INP.
inulin fructose skeleton using Equation (1) for INP.\textsuperscript{[31,28,29]}

The Degree of Substitution (DS) of Propionylation

\[
\text{DS} = \frac{A_{335}/S_{575}}{A_{435}}
\]

(1)

2.4. Response Surface Methodology Analysis

RSM was carried out to optimize the conditions of the synthesis method for improving the DS.\textsuperscript{[30]} Single factor experiments were used to determine the effect of the ratio of anhydride to inulin \(X_1\), concentration \(X_2\), and reaction temperature \(X_3\) on DS. All the results are means of triplicate tests. Based on the single factor experiments, a three-level, three-factor BBD was employed to optimize the synthesis conditions. \(X_1\), \(X_2\), and \(X_3\) were the independent variables while the DS \(Y\) was taken as the response of the design experiments. As Table 1 presented, the reaction experiment was carried out with three factors and three levels. The independent variables and their levels was as follows: ratio of anhydride to inulin (3.5, 4, 4.5), the concentration (20, 25, 30 g mL\(^{-1}\)), the temperature (20, 30, 40 °C). The range of each factor level was based on the preliminary experiments. And the general form of the second-order polynomial equation is as follows:

\[
Y = \beta_0 + \Sigma_{i=1}^{3} \beta_i X_i + \Sigma_{i=1}^{3} \beta_{ii} X_i^2 + \Sigma_{i=1}^{3} \beta_{ij} X_i X_j
\]

(2)

where \(Y\) is the response variable, \(\beta_0\) is constant, \(\beta_i\), \(\beta_{ii}\), and \(\beta_{ij}\) are the regression coefficients for intercept, linearity, square, and interaction, respectively; \(X_i\) and \(X_j\) are the independent variables \((i \neq j)\).

2.5. In vitro Fermentation of Inulin and Inulin Propionate

The in vitro fermentation was performed according to the method of Wronkowska and Stark with moderate modifications.\textsuperscript{[31,32]} Briefly, the compounds (inulin and inulin propionate ester) were used as carbon sources and dissolved in the fermentation medium at a concentration of 1.0 g L\(^{-1}\). The supernatant was poured into a separate tube and frozen at −80 °C for SCFA analyses.\textsuperscript{[33]}

2.6. Short Chain Fatty Acid Analysis

The total SCFA concentrations of acetate, propionate, and butyrate was measured by the method of Kotani with slight modifications using high-performance liquid chromatography. Supernatant samples from each replicate at each time point were taken from the −80 °C freezer and thawed at room temperature. The determination was carried out using the reverse C18 column (4.6 × 150 mm\(^2\), 5 μm), the mobile phase of the 0.02 mol mL\(^{-1}\) KH\(_2\)PO\(_4\) and methanol (98:2, v/v).\textsuperscript{[34]}

2.7. Statistical Analysis

The data were expressed as the mean ± SD. Each experiment was performed three times to verify the validity of the statistical experimental strategies. The single-factor experiments were analyzed using one-way analysis of variance (ANOVA) followed by LSD test. The total SCFA was analyzed with the Mann–Whitney test. Design Expert software (Trial Version 8.0.5) was used to estimate the response of independent variables. Differences were considered to be significant when \(p < 0.05\). The results were processed by the computer programs: Design Expert, Origin, and SPSS.

3. Results and Discussion

3.1. Chemical Syntheses and Characterization

The synthesized inulin propionate ester was characterized by FT-IR (Figure 2), \(^1\)H NMR (Figure 3), and \(^13\)C NMR (Figure 4). As shown in Figure 1, in the FTIR spectrum of inulin contained the following characteristic bands: 3394, 2927, 1423, and 1033 cm\(^{-1}\). The wide peak appeared at approximately 3394 and 1423 cm\(^{-1}\) indicating the presence of the hydroxyl groups of inulin which produced by −O−H stretching vibration and shear vibration, respectively. The band at 2927 cm\(^{-1}\) could be ascribed to the methylene C−H stretching vibration. The peak appeared at 1033 cm\(^{-1}\) was attributed to the stretching vibrations of the tetrahydrofuran ring of fructose C−O stretch. In the fingerprint region, two peaks were observed at 933 and 817 cm\(^{-1}\), which were attributed to the stretching vibrations of the tetrahydrofuran ring C−O and C−H stretch. Compared to inulin, the new characteristic absorption peaks can be observed in INP spectrum. The new peak appeared at 1743 cm\(^{-1}\) was the ester carbonyl absorption peak which formed by anhydride and inulin in INP. Similarly, we can discover the −C−O binding peaks at 1164 cm\(^{-1}\) which consistent with the propionyl group (−COCH\(_2\)CH\(_3\)) in INP. The wide peak appeared at approximately 2981 and 2946 cm\(^{-1}\) could be attributed to the stretching vibration of C−H of ethyl group (−CH\(_2\)CH\(_3\)) in INP. All these characteristic peaks demonstrated that the propionyl group was connected with inulin successfully.
Figure 2 showed the $^1$H NMR spectra of inulin and inulin propionate ester. The peaks at 3.5–5.5 ppm were the skeleton protons of fructose ring of inulin and INP. Compared with inulin spectra, the new distinct and sharp signals for $-\text{CH}_3$ and $-\text{CH}_2$ appear at $\approx 1.2$ and $\approx 2.0$ ppm of $-\text{CH}_2\text{CH}_3$ only in the spectrum of INP. Using the ratio of integrals of the skeleton protons of fructose ring and the protons of alkyl group, the reaction the DS was calculated. In addition, it can be observed...
that the shift of skeleton protons of fructose ring shifting to a lower field which would be put down to the presence of the electron-withdrawing group carbonyl. Further, Figure 3, showed the $^{13}\text{C}$ NMR of inulin and the products. Apparently, the chemical shifts at ≈62.55 ppm were the $^{13}\text{C}$ NMR of skeleton carbons of fructose ring.[30] In the $^{13}\text{C}$ NMR of INP, the new signals for $-\text{CH}_3$ and $-\text{CH}_2$ appeared at 10–40 ppm while the characteristic chemical shift of carbonyl appears at ≈173.92 ppm. By comparing the $^{13}\text{C}$ NMR of inulin and the propionate ester, it can be extrapolated that the peak intensity of C-6 at 60.1 ppm was weakened which may due to the diminish of terminal glucose units.[11] These results demonstrated that the inulin propionate ester have been synthesized successfully.

3.2. Effects of Various Factors on the Degree of Substitution

3.2.1. Effect of the Ratio of Anhydride to Inulin on DS

The effect of different ratio of anhydride to inulin on DS was presented in Figure 4a. The ratio of anhydride to inulin on DS was set at 1.5, 2, 2.5, 3, 3.5, 4, and 4.5, while other reaction conditions were presented as follows: the reaction temperature 60°C, the concentration 25%, the reaction time 24 h. According to one-way analysis of variance, the DS increased significantly with the increasing ratio of anhydride to inulin ($p < 0.01$). This was because of the enhanced concentration of the reactants would increase the reaction chances of hydroxyl groups of inulin with anhydride.[35] Although the DS continued to increase while the ratio of anhydride to inulin exceeded 4.5, increasing the amount of anhydride will increase not only the cost of the reaction but the by-products. Meanwhile, the difficulty for the separation and purification of reaction product was also increased. As shown in Figure 4a, significantly different from the optimal ratio of 4.5 compared with the ratio from 1 to 4 with the analysis of LSD’s post hoc test ($p < 0.01$). Thus, the ratio of anhydride to inulin should be fixed at less than 4.5:1.

3.2.2. Effect of Inulin Concentration on DS

The concentration of inulin was a factor that would influence the DS of INP. The low concentrations of inulin reduced the chance of reaction of hydroxyl and anhydrides which also reduced DS. Conversely, the viscosity increased with the increasing of the inulin concentrations which reduced reaction activity and the DS.[36] The effect of inulin concentration on the DS was shown in Figure 4b. The inulin concentration was set at 15%, 20%, 25%, 30%, 35%, and 40% while other reaction conditions were presented as follows: the ratio of anhydride to inulin 4:1, the reaction temperature 60°C, the reaction time 24 h. The results of one-way analysis of variance indicated that the DS of INP increased significantly with the increase of the inulin concentration ($p < 0.01$) at first. When the inulin concentration was higher than 25%, the DS of INP stayed down significantly ($p < 0.01$) which can be explained by the fact that increase the inulin concentration may increase the viscosity of the reaction system and hinder the reaction of inulin and anhydride.[37] As shown

Figure 3. $^{13}\text{C}$ NMR spectra of inulin and INP.
3.3. Statistical Analysis and the Model Fitting

Response surface optimization was more advantageous than the traditional single parameter optimization in that it saves time, space, and raw material. The current BBD with three factors and three levels were carried out to optimize the mutual effect of three independent variables (ratio, concentration, and temperature) on the DS of inulin. Table 2 showed the experimental conditions and the results of DS. The low, medium, and high levels were selected based on the results from preliminary experimentation. Maximum DS (2.86) was recorded under the optimal reaction conditions of the ratio of anhydride to inulin of 4.5:1, the concentration of 30%, the temperature of 40°C. The by applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

\[
Y = 0.37 + 0.34X_1 + 0.084X_2 + 0.016X_3 + 0.028X_1X_2 \\
+ 0.087X_1X_3 + 0.13X_2X_3 - 0.035X_1^2 - 0.017X_2^2 \\
+ 0.052X_3^2
\]  

(3)

The fit statistics of DS (Y) for the selected quadratic predictive model was shown in Table 3. The quadratic regression model showed the value of the determination coefficient \(R^2 = 0.9733\), which implied that 97.33% of the variations could be explained by the fitted model. The model F-value was 28.31 and the model p-value was smaller than 0.0001, implied that the model was highly significant. The value of the adjusted determination coefficient \(R^2_{adj} = 0.9389\) also testified the minute difference between the experimental values in Figure 4b, there was a significant difference from the optimal concentration of 25% compared with the concentration 20%, 30%, and 35% with the analysis of LSD’s post hoc test \(p < 0.01\). Therefore, the inulin concentration about 25% was the best option in this experiment.

### Table 2. Box–Behnken experimental design and results for DS of inulin.

<table>
<thead>
<tr>
<th>No.</th>
<th>X1/ratio [mol mol⁻¹]</th>
<th>X2/concentration [g mL⁻¹]</th>
<th>X3/temperature [°C]</th>
<th>DS</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.37</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.37</td>
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<td>1</td>
<td>0</td>
<td>2.69</td>
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<td>4</td>
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<td>-1</td>
<td>0</td>
<td>2.55</td>
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<td>5</td>
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<td>0</td>
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<td>1.91</td>
</tr>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>2.86</td>
</tr>
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<td>7</td>
<td>-1</td>
<td>0</td>
<td>-1</td>
<td>2.09</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>2.37</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>-1</td>
<td>1</td>
<td>2.19</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
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<td>-1</td>
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</tr>
<tr>
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<td>0</td>
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</tr>
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<td>0</td>
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</tr>
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<td>1</td>
<td>2.69</td>
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<tr>
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<td>-1</td>
<td>2.37</td>
</tr>
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<td>-1</td>
<td>1</td>
<td>0</td>
<td>2.03</td>
</tr>
<tr>
<td>17</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>2.00</td>
</tr>
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</table>

#### 3.2.3. Effect of Reaction Temperature on DS

The effect of reaction temperature on DS was shown on Figure 4c. The reaction temperature was set at 20, 30, 40, 50, 60, and 70°C, while other reaction conditions were presented as follows: the ratio of anhydride to inulin 4:1, the concentration 25%, the reaction time 24 h. As the results of one-way analysis of variance revealed that the DS increased significantly from 2.3 to 2.37 as the temperature increased from 20° to 30° \(p < 0.05\). However, when temperature exceeded 30°, the DS significantly decreased \(p < 0.01\). It indicated that with the increase of temperature, the reactivity of inulin and anhydride was accelerated which lead to the increase of DS.\(^{38}\) Nevertheless, when the temperature exceeded 30°, the anhydride would be hydrolyzed which attribute to the decrease of DS. As shown in Figure 4c, there was a significant difference of the optimal temperature of 30° compared with the data points 20° \(p < 0.05\) and 40 to 60° \(p < 0.01\) with the analysis of LSD’s post hoc test. Thus, the reaction temperature fixed at 30° was the appropriate option.
Table 3. Analysis of variance for the fitted second-order polynomial model of DS of Inulin.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F-value</th>
<th>Prob &gt; F</th>
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</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.12</td>
<td>9</td>
<td>0.12</td>
<td>28.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$X_1$</td>
<td>0.95</td>
<td>1</td>
<td>0.95</td>
<td>215.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.056</td>
<td>1</td>
<td>0.056</td>
<td>12.72</td>
<td>0.0091</td>
</tr>
<tr>
<td>$X_3$</td>
<td>2.112E-0.03</td>
<td>1</td>
<td>2.112E-0.03</td>
<td>0.48</td>
<td>0.5112</td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>3.025E-0.03</td>
<td>1</td>
<td>3.025E-0.03</td>
<td>0.69</td>
<td>0.4349</td>
</tr>
<tr>
<td>$X_1X_3$</td>
<td>0.031</td>
<td>1</td>
<td>0.031</td>
<td>6.94</td>
<td>0.0337</td>
</tr>
<tr>
<td>$X_2X_3$</td>
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<td>1</td>
<td>0.063</td>
<td>14.17</td>
<td>0.0070</td>
</tr>
<tr>
<td>$X_1^2$</td>
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<td>5.158E-0.03</td>
<td>1.17</td>
<td>0.3154</td>
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<tr>
<td>$X_2^2$</td>
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<td>1.289E-0.03</td>
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<td>0.6055</td>
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<td>$X_3^2$</td>
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<td>1</td>
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<td>2.63</td>
<td>0.1488</td>
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<tr>
<td>Residual</td>
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<tr>
<td>Lack of fit</td>
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<tr>
<td>Cor total</td>
<td>1.15</td>
<td>16</td>
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</table>

$R^2 = 0.9733$  $R^2_{adj} = 0.9389$  CV = 2.80

and predicted values. A relatively low value of coefficient of the variation (CV = 2.80) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. Table 3 also demonstrated that the linear coefficients ($X_1$, $X_2$), cross product coefficients ($X_1X_2$, $X_2X_3$) were significant ($p < 0.05$) while the other terms’ coefficients were not significant ($p > 0.05$).

3.4. Option of the Reaction Parameters

In the response surface plot and contour plot, the DS of inulin was obtained along with two continuous variables, while the other two variables were fixed constant at their respective zero. Figure 5 described the regression Equation (3) by means of graphical approach, which provided a method to visualize the mutual effect of test variables at different levels on DS and the reciprocal interactions between variables. The maximum predicted value indicated by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours are obtained when there is a perfect interaction between the independent variables. Figure 5a and b confirmed that the ratio of anhydride to inulin, concentration were significant for DS ($X_1X_3$). As shown in Figure 5b and c, it can be seen that the temperature had positive synergistic effects when coupled with concentration ($X_2X_3$). The singular effect of temperature on DS was insignificant. In combination as a pair, however, all intercept terms were statistically significant in terms of their $p$ value, except the combination of the ratio of anhydride to inulin with concentration ($X_1X_3$).

According to Figure 4, and above single parameter analysis, it can be concluded that optimal reaction condition is the concentration of 30%, the temperature of 40°C, the ratio of anhydride to inulin of 4.5:1. Under the modified conditions, the experimental DS of inulin was 2.86, which was close to the predicted value. Among the three extraction parameters studied, the ratio of anhydride to inulin was the most significant factor to affect the DS of inulin, followed by the concentration, temperature according to gradient of slope in the 3-D response surface plot (Figure 5).

3.5. Verification of Predictive Model

According to Figure 5, it could be inferred that the optimal reaction conditions are as follows: the ratio of anhydride to inulin ($X_1$) 4.5:1, the concentration ($X_2$) 30%, the temperature ($X_3$) 40°C. This set of conditions was determined to be optimum by the RSM optimization approach and was also used to validate experimentally and predict the values of the responses using the model equation. The average DS of inulin was 2.80 ± 0.07% ($n = 3$), obtained from real experiments, confirmed that the validation of the RSM model, indicating that the model was adequate for reflecting the reaction process.

3.6. Short Chain Fatty Acid Analysis of In Vitro Fermentation

The total amount of SCFA (acetate, propionate, and butyrate) and the molar ratios after 48 h of in vitro fermentation of inulin and inulin propionate were shown in Figures 6–7, respectively. In Figure 6, the total SCFA was analyzed with the Mann–Whitney test. The production of total SCFA by *Bifidobacterium* fermentation of inulin and propionylated inulin were increased significantly with the fermentation time from 6 to 30 h ($p < 0.05$) where the total amount mainly depends on the increase of acetate and propionate. As Figure 6 presented, there was a significant difference from the control inulin at the same time point with the analysis of the Mann–Whitney test. ($p < 0.05$). The SCFA were produced by microbial fermentation of inulin and propionylated inulin under anaerobic conditions. During in vitro fermentation, the proliferation of *Bifidobacterium* caused the increase of acetic acid and propionate which was consistent with the study of Maria et al. and Sarbini et al. The propionylated inulin resulted in 117.2 mmol mL⁻¹ of total SCFA during the 48 h of fermentation, compared with 90.2 mmol mL⁻¹ of total SCFA produced by inulin of an equal amount. Figure 7 showed the molar ratios of acetate, propionate, and butyrate after 48 h of in vitro fermentation of inulin and inulin propionate. For the control inulin, the ratio of acetate and butyrate were slightly higher than propionylated inulin. However, the ratio of propionate in propionylated inulin was higher than the control inulin which was consistent with our expectation. Additionally, the increased propionate ratio was compensated for the decrease of butyrate ratio.

As the samples were fermented over the course of 48 h, additional acetate, propionate, and butyrate were produced by *Bifidobacterium*. This increase of SCFA would bring down the pH and affect the growth of certain bacteria and types of metabolites produced during the fermentation. Dietary fibers like inulin
and propionylated inulin which escape digestion by host enzymes in the upper gut, are metabolized by the microbiota in the cecum and colon. The major products from the microbial fermentative activity in the gut are SCFA.\footnote{1,47} SCFA have been reported to affect various physiological processes and may contribute to health and disease. Thus, the inulin-propionate ester can be used as a novel carrier molecule whereby propionate is chemically bound by an ester bond to inulin.\footnote{17,48,49} Meanwhile, the majority of propionate chemically bound to inulin would be released when the inulin polymer is fermented by the colonic microbiota, and increasing the amount of total SCFA.

Figure 5. Response surface plots and contour plots (a, b and c) showing the interactive effects of the ratio of anhydride to inulin (A), inulin concentration (B) and reaction temperature (C) on DS.
Finally, the in vitro fermentation indicated that the chemical most suitable conditions, we enhanced the DS which up to 3.05.

Factor experiments and BBD along with RSM and under the (ratio, concentration, and temperature) through the single-method of inulin propionate whereby propionate is chemically derived SCFA in ameliorating the colon health. Increasing the amount of SCFA in colon is therefore an attractive method for this paper, we proposed a synthetic functions of inulin SCFA esters in vivo.

4. Conclusion

Intestinal health is increasingly viewed as an important problem which has attracted more and more attention in nutrition and health science. Moreover, previous studies have demonstrated the significance of microbiota-generated inulin-derived SCFA in ameliorating the colon health. Increasing the amount of SCFA in colon is therefore an attractive method for health regulation. In this paper, we proposed a synthetic method of inulin propionate whereby propionate is chemically bound by an ester bond to inulin and it can be used to increase the SCFA concentrations in colon, especially the concentration of propionate. At first, we optimized the reaction parameters (ratio, concentration, and temperature) through the single-factor experiments and BBD along with RSM and under the most suitable conditions, we enhanced the DS which up to 3.05. Finally, the in vitro fermentation indicated that the chemical modification of inulin would be beneficial to increase not only the amount of total SCFAs but the ratio of propionate. According to our study, we speculate that acylation of inulin may be an effective way to deliver high amounts of SCFA in colon and improve body health. Consequently, comprehensive studies need to be carried out to investigate the biological functions of inulin SCFA esters in vivo.

Abbreviations

ANOVA, analysis of variance; \(^{13}\)C NMR, \(^{13}\)C nuclear magnetic resonance spectrometer; DS, degree of substitution; FTIR, fourier transform infrared spectroscopy; \(^{1}\)H NMR, \(^{1}\)H nuclear magnetic resonance spectrometer; INP, inulin propionate ester; RSM, response surface methodology; SCFA, short-chain fatty acids.

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Conflict of Interest

The authors have declared no conflicts of interest.

Keywords

DS, inulin, inulin propionate ester, RSM, SCFA

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Figure 6. Total short chain fatty acids (acetate, propionate, and butyrate) produced during in vitro fermentation of inulin and inulin propionate ester. Values are means ± S.D. (n = 3).

Figure 7. Molar ratios of acetate, propionate, and butyrate after 48 h of in vitro fermentation of inulin and inulin propionate ester. Values are means ± S.D. (n = 3).