Production of Resistant Starch Type III from Native Sago Starch as a Potential Prebiotic

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Abstract: Resistant starch (RS) is the sum of starch that escape upper gastrointestinal digestion and can be fermented by colonic microorganisms to produce beneficial metabolites such as short chain fatty acids (SCFA). Resistant starch can be a potential prebiotic, a non-digestible food ingredient that selectively stimulate the growth and/or activity of gut microorganisms, thus promoting the host health. The main focus of this study is to produce resistant starch type III from native sago starch. The resistant starch was produced via retrogradation process and with the aid of pullulanase enzyme. The quantification of total resistant starch content was determined based on the method approved by AOAC 2002.02 and AACC 38-40.01. From this study, it showed that there are no significant different between the resistant starch content of native and modified sago starches (RS type III).

Keywords: Resistant starch, Prebiotic, Retrogradation, Sago starch.

1. INTRODUCTION

Malaysia is one of the largest sago starch producers in the world with annual production of 102,600 tonnes [1]. The most important sago starch-producing crop in Malaysia is Metroxylon sagu. The starch content derived from the trunk of the sago palm is very high compare to other typical starch sources. Each palm can yield 216 to 219 kg of starch, 3 to 4 times higher than rice or corn and 17 times higher than cassava. Amylose content of sago is in the range of 21.7 to 31% while its amylpectin content is in the range of 69 to 78.3%. To date, sago is still considered an underutilized crop with limited research carried out on the properties and functionality of the starch [2]. When starch is consume, only their small percentage are able to escape digestion into the bowel. This portion of starch that are able to resist the upper gastrointestinal starch is termed as resistant starch [3]. It is transported to the lower gut for microbial anaerobic fermentation and may act as prebiotic. Resistant starch can be classified into five types. Type I are referred as physically inaccessible starches, such as partially milled grains and seeds. Type II are native starches with highly packed starch granules structure, such as uncooked potato starch. Type III are physically modified starches while type IV are chemically modified starches and are industrially produced through cross-linking and/or substitution. Type V are newly proposed RS, consisting of amylose-lipid complexes. Their resistance is the result of interaction between amylose and amylpectin with fatty acids and fatty alcohols [4].

Resistant starches are substrates for microbial fermentation that produces metabolites which includes short chain fatty acids (SCFAs), such as acetate, lactate and butyrate which benefits the host [5]. The SCFAs are believed to have an important roles in gastrointestinal health. Moreover, RS has the ability to regulate gut microbiota favouring RS degradation process and SCFA production thus it can be considered as a prebiotic candidate [6]. In this study, retrograded sago starch (RS III) are produced through a cycle of heating and cooling process, with debranching enzyme pullulanase.

2. METHODOLOGY

2.1. Preparation of Retrograded Starch

An amount of 10 g sago starch was dispersed in 40 ml of water, and then pressure-cooked in an autoclave at 121°C for 20 minutes. The autoclaved starch paste was allowed to cool to room temperature and then stored at 4°C for 24 h. The prepared starch paste was re-exposed to the cooking-cooling cycle twice and oven dried at 50°C for 24 h. The resulting retrograded sago starch (RSS) was then milled and sieved through 0.3 mm sieve. RS content in all prepared samples was analysed. Each preparation was carried out in triplicate. Fibersym®, provided by MGP Ingredients, Inc. (Atchison, Kansas, US), was used for comparative study. Fibersym® is RS type 4 and was produced by modification of wheat starch via cross-linking and substitution agent of sodium trimetaphosphate and sodium tripolyphosphate. It is a food grade cook-up resistant starch.
2.2. Preparation of Debranched-Retrograded Starch with Pullulanase Enzyme

Debranching of gelatinized and retrograded sago starch was carried out using method described by Ozturk et al. [7]. Promozyme (Novo Nordisk, Bagsvaerd, Denmark), a heat-stable pullulanase type I was used. The enzyme was of technical grade with a specified standard activity of 200 PUN/g (one Pullulanase Unit Novo) and a density of approximately 1.25 g/ml. It acts by hydrolysing primarily $\alpha$-1-6 linkages. An amount of 10 g sago starch was dispersed in 40 ml water. The mixture was pressure-cooked in an autoclave at 121°C for 20 minutes, and then cooled to 60°C. An amount of 1 ml pullulanase solution (enzyme activity 30 PUN per ml) was added to the gelatinized starch paste. During debranching, the gelatinized sago starch was incubated at 60°C for 4, 8 and 12 hours respectively under continuous agitation. The resulting pullulanase-treated starch (DSS) was then heated to 100°C for 10 minutes to inactivate the enzyme, and then cooled to room temperature, before being stored at 4°C for 24 hours. The starch was then retreated with two cycles of cooking at 121°C for 20 minutes and cooling to 60°C. All the pullulanase treated starch, DSS4, DSS8 and DSS12 was then dried and milled (numbers following DSS refer to time of incubation with pullulanase enzyme). Each preparation was carried in triplicate.

2.3. Isolation of Resistant Starch

In a 2 ml microcentrifuge tube, 20 mg of starch sample was washed with 1.8 ml of 90% ethanol to eliminate soluble sugar and monosaccharides. The mixture was then warmed to 60°C for 5 minutes with occasional vortexing before being centrifuged at 1500 g for 10 minutes. The supernatant was decanted and the washing was repeated twice. Then, 1.8ml of distilled water was added to solubilise the starch before being vortexed. The solution was then heated on a boiling water bath for 5 minutes and centrifuged at 1500 g for 10 minutes to obtain the isolated resistant starch in the pellet. RS portion was analysed using method adopted from total starch kit (Abcam, Cambridge, UK).

2.4. Total Resistant Starch Content

In a universal bottle, suspended pellet was mixed with 2 ml of 10 N potassium hydroxide (KOH) to break-down the resistant starch into soluble starch and heated on boiling water bath for 5 minutes. To neutralize the solution, 2 ml of 10 M of $\text{H}_2\text{PO}_4$ was added slowly. Next, 5 µl of aqueous extract of the flour was added to 500 µl (4%) phenol and 2.5ml (96%) $\text{H}_2\text{SO}_4$. The RS content was measured colorimetrically by ELISA microplate reader at 490 nm. The concentration of the sample was extrapolated from a standard curve obtained by serially diluting 1 mg/ml glucose standard to concentrations ranging from 0.01 µg/ml to 0.5 µg/ml. The result was expressed in percentage. RS portion was analysed using method adopted from total starch kit (Abcam, Cambridge, UK).

2.5. Statistical Analysis

Total resistant starch content in the samples were analyzed statistically using analysis of variance to detect treatment effect. Means of treatments were compared using Tukey’s Test ($P < 0.05$). The statistical software used was Statistical Analysis System (SAS) version 9.3 (SAS 2009).

3. RESULTS

All of the treatments showed no significant different among each other, with exception of Fibersym®. The percentage of total resistant starch are shown in Table 1. All substrate have total resistant starch content lower than 2.0%. Fibersym® showed a significantly higher RS content than native and all modified sago starch at 1.7% ($P < 0.05$). Statistically there are no significant difference among the native (NSS) and all modified sago starches (RSS, DSS4, DSS8 and DSS12).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatment</th>
<th>RS concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibersym™70 (F)</td>
<td>-</td>
<td>1.72 (0.100)*</td>
</tr>
<tr>
<td>Native Sago Starch (NSS)</td>
<td>-</td>
<td>1.22 (0.000)*</td>
</tr>
<tr>
<td>Retrograded Sago Starch (RSS)</td>
<td>-</td>
<td>1.05 (0.104)*</td>
</tr>
<tr>
<td>Pullulanase-treated Sago Starch</td>
<td>4 hours debranching</td>
<td>1.01 (0.173)*</td>
</tr>
<tr>
<td></td>
<td>8 hours debranching</td>
<td>1.14 (0.153)*</td>
</tr>
<tr>
<td></td>
<td>12 hours debranching</td>
<td>1.07 (0.132)*</td>
</tr>
</tbody>
</table>

Significant differences among substrates ($P < 0.05$) were indicated with different superscript alphabets. Standard deviation is shown in parentheses ($n = 3$).
However, DSS8 showed a slightly higher percentage of RS than DSS4 and DSS12. The RS content increased by 0.07% from 4 hours to 8 hours, before decreasing again by 0.07% at 12 hours. Statistically, there are also no significant difference between retrograded starch (RSS) and debranched retrograded starch (DSS). However, there are still slight differences of RS content percentages that can be seen when comparing the samples.

4. DISCUSSION

Fibersym® is a commercialized RS type IV. Their highly resistant properties is due to its chemically cross-linked structure [8], which is reflected in the present data. It was initially predicted that the pullulanase-treated samples, DSS will have higher RS content than NSS and RSS. However, all have shown to have a non-significant difference of RS content ($P < 0.05$). This may be due to the extra heating treatment in preparation of DSS samples. As in the methodology above, the DSS starches undergone heating and cooling cycles after the first gelatinization process. In which, they are heated up to 60°C to initiate the debranching process and further heated up to 100°C to stop the pullulanase activity. Under these conditions, the starch was gelatinized again as the gelatinization temperature of sago starch is around 72°C [9]. Gelatinization greatly increases the digestibility of starch, presumably due to both granular disorganization and increased porosity of the substrate [10]. The amount of RS depends on the degree of gelatinization and amylose retrogradation during cooling of cooked food [11]. Hence, instead of making the starch more resistant, the extra processing would increase its digestibility. The additional heating treatment in DSS samples caused the starch to be more digestible and RS to be easily lost throughout the process.

The best incubation time for debranching of α-1,6 glucosidic linkages in starch by pullulanase is at 8 hours [9]. However, it was shown that there is no significant difference among the DSS samples at all debranching times ($P < 0.05$). The slight rise of RS content from 4 hours to 8 hours indicate that the debranching process has released medium to small chains that were able to form RS [9]. The branches produced during the period of 4 to 8 hours are long enough to form RS. The yield of RS varied with average chain length of the amylose and yield of RS formed in amylose with short chain can be lower [12]. Therefore, the lower RS acquired in 12 hours was most likely due to the accumulation of short chains that were unable to form RS structure.

Among the debranched samples, although not significant, DSS8 does showed the highest RS percentage compared to RSS. This difference is most probably due to the debranching by pullulanase, which might promote the association of free linear chain segments and their close packing. Subsequently, the number of perfect starch crystals would increase. Without disentanglement from the amylopectin molecule, the association of the linear segments of starch units and subsequent crystallite formation may be tough due to lesser flexibility of the starch chain segments owing to their closer proximity to the branching points [13].

5. CONCLUSION

Resistant starch type III can be produce through the process of retrogradation and/or enzymatic hydrolysis. Gelatinised sago starch, when subjected to enzymatic hydrolysis by pullulanase could trigger the formation of RS. From data acquired, although not statistically significant, the debranched at 8 hour sago starch sample (DSS8) showed the highest RS starch. With the advanced of enzyme technology, it is possible that a complete debranching process of sago starch can be conducted at sub gelatinization temperature and a more economical process can be developed to produce product with high yield of resistant starch. Different methods of RS determination could also be conducted in future study to obtain and compare for the best result. Further investigation on the optimisation of RS from sago can be a value add for sago.

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