Effect of pectic-oligosaccharides from Japanese orange peels as prebiotic in animal feed

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ABSTRACT: The study focuses on the treatments for Japanese orange peels by enzymatic degradation (Sclase A®) and the use as prebiotics in animal feed. This study was carried out using a completely randomized design (CRD) and consists of two experiments. The first experiment consisted of 6 treatments using 2 types of Japanese orange peels (Taguchi wase and Nichinan ichigo) and 3 levels of the enzyme (0, 1 and 5% (w/w)). Each treatment consisted of 5 replicates. Parameters measured in this study were chemical compositions, reducing sugar contents and oligosaccharides. The chemical compositions were significantly different among treatments (P<0.05) except crude protein. Taguchi wase peel treated with 5% enzyme had lower crude fiber content as compared to other treatments (P<0.05). (They were 22.71 (T1), 24.74 (T2), 25.84 (T3), 21.14 (T4), 12.20 (T5) and 18.34 % (T6), respectively. Enzyme treatments reported higher gross energy than other treatments (P<0.05). Moreover, reducing sugar contents in the peels were significantly higher with enzyme than without enzyme (P<0.05). Japanese orange peels treated with enzyme had higher reducing sugar content than other treatment (P<0.05). They were 582.69 (T1), 616.00 (T2), 882.67 (T3), 849.33 (T4), 1,049.33 (T5) and 1,016.00 mg/g (T6), respectively. Oligosaccharides analysis by thin-layer chromatography method found that all treatments released oligosaccharides. The second experiment was carried out to examine prebiotic properties (concentration of sugar 1,500 µg/ml), and the findings lead to the insights that products from enzyme treatments could increase the growth of Lactobacillus plantarum but could not decrease the growth of Escherichia coli. In conclusion, it was suggested that Japanese orange peels treated with an enzyme has the potential to be used as prebiotics due to it could increase the growth of probiotics.

Keywords: Japanese orange peels; prebiotics; oligosaccharides

Introduction

Prebiotics are oligosaccharides, a class of non-digestible carbohydrates by the digestive enzymes and are able to pass to the small intestine. Function of prebiotics are to stimulate the growth of probiotics such as Lactobacilli and Bifidobacteria (Al-Sheraji et al., 2013). The most common prebiotics are fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), manno-oligosaccharides (MOS) and pectic-oligosaccharides (POS). Pectin are one of the most complex carbohydrates and mostly found in the plant cell wall. The pectin is broken down by enzyme and are able to release pectic-oligosaccharides (POS). The peel of citrus, apple and sugar beet pulp are source of POS which could be obtained from agricultural by-products (Baldassarre et al., 2018). Taguchi wase and Nichinan ichigo orange are mandarin oranges which belong to various citrus species in the family of Rutaceae. Orange peel consisted of 9.74% crude protein and 14.19% crude fiber, 48.12% glucose and 31.19% arabinose (Manderson et al., 2005;
Feumba et al., 2016). Therefore, the aim of this study aims to improve quality of Japanese orange peels with an enzymatic degradation (Sclase A®) and to be used as prebiotics in animal feed.

Materials and Methods

Preparation of enzyme treated fruit peel: In first experiment, Japanese orange peels (Taguchi wase and Nichinan ichigo) were obtained from Experimental farm of Meijo University, Kasugai, Japan. This experiment consists of 6 treatments and 5 replicates per treatment. The peels were blended and treated with 3 levels (0, 1 and 5 %w/w) of a commercial enzyme (Sclase A®) dissolved in 0.05M phosphate buffer (pH4). The peels treated with enzyme were incubated at 40°C for 19 hours. After that, the samples were centrifuged at 10,000 rpm, 25°C, for 10 min. The supernatant was collected for reducing sugar and oligosaccharides analyses. While, the solid sample was dried in hot air oven at 60°C for 48 hours and stored in a refrigerator for proximate analysis.

Determination of reducing sugar: Approximately 0.5 ml of the supernatant was transferred into a sample tube that contained 0.5 ml of dinitrosalicylic acid (DNS) reagent. The mixture was boiled for 10 min and then immediately cooled down by immersing the sample tube into the cold water. The absorbance of the sample was read at 540 nm using distilled water as blank (Miller, 1959). Different concentrations of glucose (0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml) were prepared to develop a glucose standard curve using a similar procedure as described above.

Determination of oligosaccharides: Oligosaccharide analysis was performed using thin-layer chromatography (TLC) described by Cabrera and Van Cutsem (2005). The supernatant sample was spotted near the bottom of silica gel plate (Merck & Co., Inc. art. No.1.05554 size 20×20 cm). The TLC plate was then placed in a shallow pool of a solvent (2-propanol: ammonium hydroxide: distilled water, 7:1:2 ratio (in a developing chamber so that only the very bottom of the plate was in the liquid. This liquid acted as mobile phase, and it slowly rose up the TLC plate by capillary action. After thoroughly drying the TLC plate with 10% v/v sulfuric acid in an ethanol solution in an operating hood, and heated at 100°C until dry. The spot on TLC plate was compared with glucose and cellobiose.

Determination of chemical compositions: Dried-centrifuged solid samples were analyzed for dry matter (DM), crude protein (CP), ash, crude fiber (CF), ether extract (EE) (AOAC, 1990). Gross energy was also determined using bomb calorimeter (CAL 2K, South Africa).

Prebiotic and antibacterial activities: In second experiment, the products from untreated and enzyme treated Japanese orange peels were used to evaluate its ability to support the growth of beneficial bacteria (Lactobacillus plantarum) and pathogenic bacteria (Escherichia coli). Overnight culture broths from stock culture were diluted to 0.5 optical density (OD) with nutrient broth (NB) (0.05% cysteine) for L. plantarum and NB for E. coli. The culture broths with bacteria were incubated into medium supplemented with oligosaccharides at 1,500 µg/ml and incubated at 37°C. The OD at 660 nm of L. plantarum and E. coli growth were measured every 3 hours by using spectrophotometer (SPECTROstar Nano, Germany).

Statistical analysis: The experimental data were subjected to analysis of variance and Tukey’s test mean using R-studio software. Differences were considered significant at 5% probability.
Results and Discussion

Chemical compositions: Chemical compositions of untreated and enzyme treated Japanese orange peels are shown Table 1. Moisture of Japanese orange peels were approximately 70.41 to 71.23%. The results showed that chemical compositions were significantly different among treatments (P<0.05) except CP. Taguchi wase peel treated with 5% enzyme had lower CF content as compared to other treatments (P<0.05). They were 22.71 (T1), 24.74 (T2), 25.84 (T3), 21.14 (T4), 12.20 (T5) and 18.34 % (T6), respectively. The peels treated with enzyme had higher gross energy than other treatments (P<0.05). Sclease A® is a crude enzyme which contributed to the breakdown of fiber in orange peels. Feumba et al. (2016) reported that orange peels contain 9.74% CP and 14.19% CF which were higher than Japanese orange peel. The results obtained in this study remains in agreement with the previous report by Saenphoom et al. (2020) which highlighted that lime peel treated with enzyme had lower cellulose than untreated samples. In a similar study, Ahmadi et al. (2015) found that orange peel fermented with Trichoderma spp. could decrease neutral detergent soluble content as compared to non-fermented orange peel.

Reducing sugar: Reducing sugar contents of untreated and enzyme treated Japanese orange peels are shown in Table 1. The results showed that Japanese orange peels treated with enzyme had higher reducing sugar content than other treatments (P<0.05). They were 582.69 (T1), 616.00 (T2), 882.67 (T3), 849.33 (T4), 1,049.33 (T5) and 1,016.00 mg/g (T6), respectively. Orange peel consist of 48.12% glucose, 31.19% arabinose, 9.59% galactose, 6.21% galacturonic, 2.44% xylose, 2.13% rhamnose and 0.24% fucose (Manderson et al., 2005). Enzymes can break down plant cell wall and release more reducing sugar than raw orange peel. In a similar result, Saenphoom et al. (2020) also reported that mango peel treated with enzyme had higher reducing sugar than samples without enzyme treatment as a similar result.

Oligosaccharides: Oligosaccharides products of untreated and enzyme treated Japanese orange peels are shown in Figure 1. The oligosaccharides products were found in both untreated and enzyme treated fruit peels (column 2 to 7) and lighter than standard glucose and cellulbiose. The oligosaccharides products from the untreated sample originated from preparation of sample such as grinding and heating. The glucose products could be due to galacturonic acid and POS. In similar study, Chimtong et al. (2016) reported that enzyme (Pentozyme®) could degrade spent tea leaves to oligosaccharides as a similar result.

Prebiotic and antibacterial activities: The NB containing 1,500 µg/ml of products from enzyme treatments could increase the growth of L. plantarum and could not decrease the growth of E. coli when cultured at 37 ºC. (Figure 2 and 3). Taguchi wase peel treated with 1% enzyme had higher the growth of L. plantarum as compared to other treatments at 48 hours. Martinez et al. (2010) reported that POS had more prebiotic properties than pectin because the POS could increase the growth of Bifidobacteria and lactic acid bacteria. In similarly study, Mandalari et al. (2007) reported that bergamot peel treated with enzyme was a good source of prebiotic because it could increase the population of Bifidobacteria, Lactobacilli and Eubacteria and decrease the population of Clostridia.
### Table 1  Chemical compositions and reducing sugar of untreated and enzyme treated Japanese orange peels (% on a dry matter basis)

<table>
<thead>
<tr>
<th>Items</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>SEM</th>
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<tbody>
<tr>
<td>Ash (%)</td>
<td>5.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>9.88</td>
<td>8.18</td>
<td>7.93</td>
<td>7.88</td>
<td>7.88</td>
<td>7.87</td>
<td>0.26</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>1.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.18</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>22.71&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>24.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11</td>
</tr>
<tr>
<td>Gross energy (kcal/kg)</td>
<td>6,400.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6,490.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7,052.76&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6,841.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6,780.50&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6,598.69&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>52.25</td>
</tr>
<tr>
<td>Reducing sugar (mg/g)</td>
<td>582.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>616.00&lt;sup&gt;de&lt;/sup&gt;</td>
<td>882.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>849.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,049.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,016.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.38</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>, Means with different superscripts in row are significantly different (P<0.05), T1 = Untreated Taguchi wase peel, T2 = Untreated Nichinan ichigo peel, T3 = Taguchi wase peel treated with 1% enzyme, T4 = Nichinan ichigo peel treated with 1% enzyme, T5 = Taguchi wase peel treated with 5% enzyme, T6 = Nichinan ichigo peel treated with 5% enzyme, SEM = Standard error of mean.

### Figure 1  Oligosaccharides products of untreated and enzyme treated Japanese orange peels

T1 = Untreated Taguchi wase peel, T2 = Untreated Nichinan ichigo peel, T3 = Taguchi wase peel treated with 1% enzyme, T4 = Nichinan ichigo peel treated with 1% enzyme, T5 = Taguchi wase peel treated with 5% enzyme, T6 = Nichinan ichigo peel treated with 5% enzyme.
Figure 2 Effect of oligosaccharides products (1,500 µg/ml) on the growth of L. plantarum

T1= Untreated Taguchi wase peel, T2= Untreated Nichinan ichigo peel, T3 = Taguchi wase peel treated with 1% enzyme, T4= Nichinan ichigo peel treated with 1% enzyme, T5= Taguchi wase peel treated with 5% enzyme, T6= Nichinan ichigo peel treated with 5% enzyme

Figure 3 Effect of oligosaccharides products (1,500 µg/ml) on the growth of E. coli

T1= Untreated Taguchi wase peel, T2= Untreated Nichinan ichigo peel, T3 = Taguchi wase peel treated with 1% enzyme, T4= Nichinan ichigo peel treated with 1% enzyme, T5= Taguchi wase peel treated with 5% enzyme, T6= Nichinan ichigo peel treated with 5% enzyme

Conclusion

Enzyme hydrolysis effectively breaks down the cell wall of Japanese orange peels and released reducing sugar and oligosaccharides. Moreover, the oligosaccharides products from enzyme treatments could stimulate the growth of L. plantarum but do not inhibit the growth of E. coli. Taguchi wase peel treated with 1% enzyme had higher growth of L. plantarum than other treatments at 48 hours. In
conclusion, it was suggested that Japanese orange peels treated with enzyme could be used as prebiotics due to the capability to increase the growth of probiotics.

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References