Low Molecular Weight Carbohydrates, Prebiotic Content, and Prebiotic Activity of Selected Food Plants in Thailand

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Abstract: The study aimed to determine the content of Low Molecular Weight Carbohydrate (LMWC), prebiotic, and prebiotic activity of thirteen food plants commonly consumed in Thailand. The prebiotics (inulin and fructooligosaccharide (FOS)) and LMWC content (glucose, maltose, sucrose, isomaltose, maltotriose, and maltotetraose) were determined using High Performance Liquid Chromatography (HPLC). Prebiotic activity of plant extracts (including LMWC and prebiotics) obtained from potential food plants was evaluated through cell density of Lactobacillus acidophilus grown on the extracts, commercial inulin, FOS, and glucose relative to that of Escherichia coli grown on similar media cultures. The results indicated that garlic contained significantly highest of inulin with the amount of 41.72% dry weight followed by shallot (33.22 %), and onion (27.17 %) whereas only small amount of inulin was detected in sweet potato, white radish, cassava, and yam bean (0.42-2.14%). There were no inulin and FOS found in rice. In the study on LMWC content, shallot contained the highest concentration of sucrose (11.42%) and glucose (2.91%) followed by onion. There were no maltose, isomaltose, maltotriose, and maltotetraose found in the bulbs and root/tuber crops studied, whilst the significant concentrations were observed in the germinated rice. For the study on prebiotic activity, Lactobacillus acidophilus grown on the extracts from onion, shallot, and garlic indicated the highest prebiotic activity scores comparable to that of commercial inulin while the prebiotic activity score of germinated rice were lowest and close to zero (0.17 and 0.23 in germinated non waxy and waxy rice, respectively).

Key words: Prebiotic, low molecular weight carbohydrates, inulin, fructooligosaccharide, maltooligosaccharides, prebiotic activity

INTRODUCTION

Recently, the growing awareness of consumers on the relationship between foods and health has led to an increasing demand for functional foods; foods that provide health benefits further than the basic function of contributing nutrients. Prebiotic is one of the most promising functional foods as a component presented in foods. Prebiotics contribute health benefits by (1) promoting the growth of beneficial bacteria (probiotics) in the intestinal tract (Gibson et al., 1995), (2) being as a soluble dietary fiber with low caloric value and health related benefits (Roberfroid, 1999), (3) supporting the inhibition of the growth of lesions, such as adenomas and carcinomas in the gut, and thus reduce the risk factors involved in colorectal diseases (Reddy et al., 1997), and (4) enhancing the absorption of certain minerals, such as calcium and magnesium (Scholz-Ahrens et al., 2001; Scholz-Ahrens et al., 2007).

According to Gibson and Roberfroid (1995), “prebiotics defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon”. Bifidobacteria and Lactobacilli are examples of bacteria in the colon which have the potential to improve host health. Foods classified as a prebiotic must demonstrate that it is not broken down in the stomach or absorbed in the gastrointestinal tract. It is fermented by the gastrointestinal microflora and selectively stimulates the growth and activity of beneficial bacteria (probiotics) or a limited number of bacteria in the colon and thus improves host health Gibson and Roberfroid (1995).

Prebiotics are found naturally in a variety of foods and can also be synthesized enzymatically. The most common prebiotics are inulin and FOS (also sometimes termed oligofructose or oligofructans) (Messina, 1995; Velazquez et al., 1996). Inulin is presented in significant quantities in vegetables such as artichokes, asparagus, leeks, onions, and garlic. FOS is also found in substantial amounts in vegetables such as Jerusalem artichokes, onions, chicory root, garlic, asparagus, and some cereals such as barley and wheat (Espinosa-Martos et al., 2006; Judprasong et al., 2011). However, some oligosaccharides...
(molecules containing 2 to 10 monosaccharide units) are non-digestible oligosaccharides and currently considered as prebiotics, such as glucooligosaccharides, maltooligosaccharides, lactulose, xylooligosaccharides, stachyose, and raffinose (Orban et al., 1997; Patterson and Burkholer, 2003). Oligosaccharides are subgroup of Low Molecular Weight Carbohydrates (LMWC) in foods, which consisted of digestible mono- and disaccharides and non-digestible oligosaccharides such as glucose, fructose, sucrose, raffinose, stachyose, and FOS. According to Matsuhiro et al. (2009), LMWC are carbohydrates with molecular weight lower than 3500 Daltons, herein called LMWC, which mainly consisted of mono- and disaccharides and oligosaccharides. Most of the few naturally occurring prebiotics and oligosaccharides are found in vegetables; however, some oligosaccharides can also be found in degraded starch from cereals such as maltooligosaccharides and some may be found in other food plants such as in root and tuber crops, which have been during investigation for their prebiotic activity, recently.

In this study, thirteen plants (as shown in material section) were selected because they are able to grow well and well recognized as common foods consumed in Thailand. Moreover, the germinated rice was selected to study its LMWC content because, at the present time, germinated rice products have been gained a lot of popularity consumed as functional food. During germination of rice seeds, starch, as the major component, was partially degraded by enzymes to lower molecular weight carbohydrates. Saman et al. (2008) reported that several types of sugars and oligosaccharides such as maltotriose, isomaltooltriose, maltotetraose, and maltotriose were detected during germination of rice seed. These oligosaccharides may perform as a prebiotic but no investigation has been done on LMWC extract on the growth of prebiotic. Therefore, the study was carried out to determine the content of LMWC and prebiotic in selected food plants and then LMWC extracts obtained from plants were evaluated for prebiotic activity. The evaluation of prebiotic activity was based on the growth of selected probiotic strain in specific substrates and LMWC extract. These results may be helpful in identifying potential food plants for use as prebiotic sources. Moreover, this study may find a more cost-effective way to produce prebiotic oligosaccharides directly extracted from food plants as LMWC.

MATERIALS AND METHODS

Materials: Samples and sample preparation: Thirteen food plants were selected including 3 species of bulb crops (onion (Allium cepa var. cepa L.), shallot (Allium cepa var. aggregatum), and garlic (Allium sativum L. var. sativum)), 7 types of root/tuber crops (sweet potato (red; Ipomoea batatas), white radish (Raphanus sativus), yam bean (Pachyrhizus erosus), taro (Colocasia esculenta), cassava (Manihot esculenta), yam (round tuber; Dioscorea esculata), and yam (purple; D. alanta), and 4 types of rice; Oryza sativa including waxy rice (RD6), non waxy rice (KDML105), germinated RD6, and germinated KDML105.

Fresh plant samples, except rice, approximately 2-3 kg of each plant were purchased from local retailers, Mahasarakham, Thailand, crop year 2009. The samples were washed and cleaned with tap water, blotted, and peeled to remove inedible portion prior to cutting into small pieces and then freeze dried. The freeze-dried samples were finely ground using a coffee grinder and kept in refrigerator until used. All samples were prepared in triplicates and the compositions determined were calculated as dry matter basis.

Rice samples: Rough rice of Oryza sativa L., cultivar RD6 and Khao Dok Mali 105 (KDML 105) was purchased from a local rice-milling factory in Mahasarakham province, Thailand. Brown rice (ungerminated rice) was prepared by removing a husk of the ungerminated rough rice using a laboratory de-husker.

Germinated rice preparation: The preparation of germinated rice was done by following the method reported by Moongngarm and Saetung (2010). Briefly, rough rice seed (5 kg) was soaked in tap water at room temperature for 48h. Then the soaked rice seed was placed in plastic baskets covered by cheese cloth prior to germination in a germinating cabinet for 48h at 28-30°C and 90-95% relative humidity. After germination, the germinated seeds were dried at 50°C to approximately 10% of moisture content. The hull, root, and shoot were separated using laboratory de-husker before finely ground (40 mesh) to obtain germinated rice flour.

Both rice flour and germinated rice flour were further extracted with distilled water to obtain the extracted powder of rice flour by following the method reported by Moongngarm and Saetung (2010).

Commercial prebiotics: FOS and inulin, the most extensively studied prebiotics and the current market leader, were chosen as a positive control for in vitro fermentation studies, while glucose, a non-prebiotic carbohydrate, was used as a nonselective control. The commercial fructooligosaccharide (FOS) product was purchased from Sigma-Aldrich (USA) and inulin (from chicory) were obtained from Fluka (Singapore).

Methods:
Determination of sugar compositions: Total available carbohydrates (Anthrone method) were determined according to AOAC (1990). The individual sugars were
analyzed by HPLC-RI detector by following the method reported by Ruperez and Toledano (2003) and Espinosa-Martos et al. (2006).

Preparation of plant extracts for LMWC determination: The dried food plant and rice flour samples from sample preparation section (400 mg) was extracted by following the method studied by Ruperez and Toledano (2003). Samples were extracted with ethanol (85% v/v; 40 mL) in screw-capped tubes, which incubated at 50°C in a water bath with constant shaking for 1 h. After that samples were cooled down to room temperature and were centrifuged at 4000 rpm for 15 min. The supernatants (10 mL) containing LMWC were evaporated to dryness by vacuum rotary evaporator (Buchi, Switzerland). The LMWC powder was separated in to two portions, one was kept for prebiotic activity study and the other was re-dissolved in deionized water (1.5 mL) before performing the HPLC analyses and the samples were passed through 0.45 μm nylon filter.

Determination of low-molecular-weight carbohydrate by HPLC: LMWC were analyzed by HPLC-Reflective Index Detector (RID) by following the method reported by Espinosa-Martos et al. (2006) and Espinosa-Martos et al. (2006). The column was a REZEX RSO Oligosaccharide (200 x 10.00 mm I.D) Phenomenex, USA). The column was eluted isocratically with deionized water at 60°C, a flow rate of 0.2 mL/min, and an injection volume of 10 μL. LMWC were identified by their retention times and quantified by comparison with known carbohydrate standards (inulin, FOS, isomaltotriose, maltotriose, maltotetraose, maltose, sucrose, glucose, fructose). The auto sampler and Waters differential refractometer R-401 were used. The run time was 60 min. Peaks were eluted by the decreasing order of molecular weight. Thus, inulin was first, then FOS, disaccharide (maltose and then sucrose), and finally the monosaccharide (glucose and fructose).

Bacterial strains and prebiotic activity assay: L. acidophilus and E. coli (freeze dried cells) were purchased from the TISTR Culture Collection at Bangkok MIRCEN, Thailand Institute of Scientific and Technology Research and used for the study of prebiotic activity of potential prebiotic plants. Both bacteria were activated before use in the experiment. The freeze dried cells of L. acidophilus were incubated in Lactobacillus MRS Broth (Difco Laboratories, Sparks, MD, USA) containing 15% (wt/vol) glycerol for 24 h, then streaked on to MRS agar plate and incubated for another 24 h prior to being transferred into MRS broth and incubated for 24 h at 37°C. For E. coli culture, cells were incubated in Tryptic Soy Broth (TSB; Difco Laboratories, USA) containing 15% (wt/vol) glycerol for 24 h and were streaked on to Tryptic Soy Agar (TSA) plate followed by incubation at 37°C for 24 h. Then, one colony from each plate of each strain was transferred into 10 mL of MRS broth incubated at 42°C for L. acidophilus and TSB for the E. coli strain, an additional transfer of 1% (vol/vol) from a TSB over night culture (E. coli) into 10 mL of M9 minimal medium broth and incubated overnight at 37°C prior to the prebiotic activity assay was performed. The assay was carried out by adding 1% (vol/vol) of an overnight culture of each probiotic strain to separate tubes containing MRS Broth with 1% (wt/vol) glucose or 1% (wt/vol) prebiotic or plant extract (LMWC) which only five types of studied plants were chosen, based on the concentration of prebiotics, for prebiotic activity assays. The cultures were incubated at 37°C under anaerobic conditions (85% N, 10% CO, and 5% H) in an anaerobic chamber for L. acidophilus strains. After 0 and 24h of incubation, samples were enumerated on MRS agar using spread plating technique. For E. coli, the overnight culture (1% (vol/vol) was added to separate tubes containing M9 broth with 1% (wt/vol) glucose or 1% (wt/vol) prebiotic or plant extract (LMWC). The cultures were incubated at 37°C at ambient atmosphere, and enumerated on TSA after 0 and 24 h of incubation. Three replications were conducted for each strain.

Prebiotic activity score: According to Huebner et al. (2007), prebiotic activity indicated the ability of a given substrate to promote the growth of probiotic (L. acidophilus) compared with that of other organisms, i.e. E. coli in this study. Therefore, substrates have a positive prebiotic activity score if they are metabolized as well as glucose by probiotic strains but not by other intestinal bacteria. The prebiotic activity score was calculated using the following equation.

\[
\text{Prebiotic activity score} = \frac{(\text{probiotic log cfu/mL on the prebiotic at 24 h} - \text{probiotic log cfu/mL on the prebiotic at 0 h})}{(\text{probiotic log cfu/mL on glucose at 24 h} - \text{probiotic log cfu/mL on the glucose at 0 h})} - \frac{(\text{enteric log cfu/mL on the prebiotic at 24 h} - \text{enteric log cfu/mL on the prebiotic at 0 h})}{(\text{enteric log cfu/mL on glucose 24 h} - \text{enteric log cfu/mL on the glucose at 0 h})}
\]

RESULTS AND DISCUSSION

The composition of LMWC extracted from plants in this study consisted of fructose, glucose, maltose, sucrose, maltotriose, isomaltotriose, maltotetraose, FOS and inulin. LMWC of roots/tubers and bulbs contained fructose, glucose, sucrose, FOS, and inulin (Table 1) whereas the rice samples comprised fructose, glucose, maltose, sucrose, maltotriose, isomaltotriose, maltotetraose (Table 2). The FOS and inulin are prebiotic whilst maltotriose, isomaltotriose, maltotetraose were grouped as
non-digestible maltooligosaccharide and considered as prebiotic in this study. Therefore, in the study on prebiotic activity, the LMWC extracts obtained from selected food plants were used as prebiotic carbohydrates of probiotic bacteria to evaluate prebiotic activity.

Based on the contents of total carbohydrates, inulin, FOS, and sugars of root/tuber and bulb crops presented in table1, the results could be classified into two groups; (1) high sugar and inulin (bulbs) and (2) low sugar and inulin (roots/tuber crops). For the sugar contents in bulbs, glucose and sucrose were significant highest in shallot (2.91 and 11.42%, respectively) followed by onion (9.71 and 2.03%, respectively) whereas the content of fructose from all three bulbs were comparable. These results were somewhat varied from those studied by Espinosa-Martos et al. (2006) and Judprasong et al. (2011). The FOS in all samples varied in a relatively wide interval, ranged between 1.35-6.46% in bulbs and 0 to 0.39% in roots/tubers. The FOS concentration reported in the literature (Espinosa-Martos et al., 2006; Galdon et al., 2009; Jaime et al., 2001; Judprasong et al., 2011) were also reported in a wide range. The inulin content was highest in garlic (41.72%), followed by shallot (33.22%) and onion (27.17%) whilst only small amounts were detected in the root/tuber crops (0 to 0.33%). The inulin content in garlic was about twenty times higher than those of the root/tuber crops, in this work, which these results were in agreement with those reported by Judprasong et al. (2011).

The root/tuber crops contained higher and broader range (74.63-86.42%) of total carbohydrates than that of onion, shallot, and garlic (62.33-69.97%). These results were similar to those studied by Espinosa-Martos et al. (2006), which could be due to several factors such as cultivar variation, location, and pre and post harvest management. The sugar contents (fructose, glucose, and sucrose) in root/tuber crops were ranged between 0.07 to 8.89% for fructose, 0.09 to 14.84% for glucose, and 1.52 to 8.07% for sucrose. When the sugar content was compared between the roots/tubers, they were observed that all sugars were highest in yam bean.

Table 2 shows the content of total carbohydrates, LMWC, and inulin of different rice types. All rice types contained comparable amount of total carbohydrates (81.79-84.64%) which closed to the reference values of carbohydrate content in rice reported by Moongngarm and Saetung (2010). The germinated rice had higher level of LMWC, consisting of glucose, fructose, maltose, sucrose, maltotriose, isomaltotriose, and maltotetraose than those of ungerminated rice (brown rice). The LMWC detected by HPLC showed retention times that could be assigned to oligosaccharide molecules that DP was higher than DP4 (data not shown). They could be seen by the presence of peaks that of maltotetraose and inulin, but this study was unable to identify those peaks because of lacking of oligosaccharide standards. These results were similar to that studied by Saman et al. (2008) who found that the oligosaccharides detected in malted rice including maltotriose, maltotetraose, maltpentaose, maltohexose, and maltoheptose. The inulin and FOS did not present in any type of rice, these results were in agreement with that reported by Espinosa-Martos et al.
Table 3: Cell density between time 0 h and time 24 h, reported as log cfu/mL, for bacterial cultures grown with various LMWC extracts

<table>
<thead>
<tr>
<th>Source</th>
<th>Escherichia coli (log cfu/mL)</th>
<th>Lactobacillus acidophilus (log cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Glucose</td>
<td>8.47 ± 0.10^d</td>
<td>9.01 ± 0.04^d</td>
</tr>
<tr>
<td>Inulin</td>
<td>8.35 ± 0.12^a</td>
<td>9.16 ± 0.05^a</td>
</tr>
<tr>
<td>FOS</td>
<td>8.16 ± 0.08^c</td>
<td>9.25 ± 0.08^c</td>
</tr>
<tr>
<td>Onion</td>
<td>8.31 ± 0.04^d</td>
<td>9.09 ± 0.06^d</td>
</tr>
<tr>
<td>Shallot</td>
<td>8.26 ±0.15^c</td>
<td>8.75 ± 0.03^b</td>
</tr>
<tr>
<td>Garlic</td>
<td>8.39± 0.03^c</td>
<td>9.45± 0.03^c</td>
</tr>
<tr>
<td>GRRD6</td>
<td>8.21± 0.24^d</td>
<td>8.61± 0.05^a</td>
</tr>
<tr>
<td>GRK105</td>
<td>8.22± 0.09^c</td>
<td>8.67± 0.08^c</td>
</tr>
</tbody>
</table>

GRRD6 and GRK105 refer to germinated rice cultivar RD6 and KDML105, respectively. Results in the same column with the same superscript were not significantly (p<0.05) different.

Growth of L. acidophilus and E. coli on prebiotics and LMWC extracts: The increases in cell densities of Lactobacillus following 24 h growth on 1% (wt/vol) glucose or 1% (wt/vol) LMWC extract are presented in Table 3. For a given substrate to have prebiotic activity, that substrate should be metabolized by a test strain similar or close to that of glucose. The growth of both strains, used in this study, on the inulin, FOS, onion, shallot, and garlic was higher than on glucose (p<0.05) except the growth of L. acidophilus on LMWC extracted from germinated rice.

Prebiotic activity: Prebiotic activity was evaluated through the prebiotic activity score, as shown in Fig. 1. The results were obtained from the cell density values from Table 3. The highest prebiotic activity scores were L. acidophilus grown on inulin (2.22), garlic (2.15), shallot (2.09), and onion (1.94) whereas L. acidophilus had prebiotic activity scores close to zero (0.17 and 0.23) when grown on LMWC extracted from germinated rice.

A low or negative prebiotic activity score was obtained if the test strain grew less well (based on cell densities) on the prebiotic compared with that on glucose and/or had less growth on the prebiotic than the growth of the E. coli on the prebiotic carbohydrate. These results may due to the types of prebiotic carbohydrates presented in germinated rice which mainly contained maltooligosaccharides whereas those presented in bulbs were inulin and FOS. Different prebiotics are metabolized differently by probiotic members in the gastrointestinal tract. The similar results were obtained in the study of Huebner et al. (2007). These carbohydrates have the capacity to influence the population of probiotics in the gastrointestinal tract due to their selective utilization. Therefore, the effectiveness of a prebiotic depends on its ability to be selectively fermented by and to support growth of specific targeted organisms.
CONCLUSION

The major prebiotic contents, found in selected food plants in Thailand, were inulin and FOS and could be detected in substantial amount in garlic, shallot, and onion whereas tubers/roots crop and rice were not potential sources of these compounds. Germinated rice contained maltooligosaccharides and also simple sugars in significant amounts; therefore, when LMWC extract applied as prebiotic it may need to remove simple sugars successfully before use. The highest prebiotic score was obtained for L. acidophilus grown on inulin, garlic, shallot, and onion while the lowest score was found when grown on germinated rice. However, in this study, only one probiotic strain was applied to evaluate the prebiotic activity whereas there are varieties of probiotic strains presented in the gastrointestinal tract, therefore, more probiotic strains are needed to be tested to evaluate the prebiotic activity. LMWC extracted from selected food plants (garlic, shallot, onion) could be a useful source for use as prebiotic whilst LMWC extracted from germinated rice may need some further processes to improve the concentration of maltooligosaccharides.

ACKNOWLEDGMENT

This study was supported by a grant from Mahasarakham University and National Research Council of Thailand (NRCT)

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