Optimization of enzymatic hydrolysis of copra meal: compositions and properties of the hydrolysate

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Abstract This study demonstrated that oligosaccharides from copra meal could be prepared by using a commercial enzyme preparation containing mannanase for use as a prebiotic. The conditions giving the highest hydrolysis rate of the copra meal by the enzyme were pH 4 and temperature of 40 °C with an enzyme to substrate ratio (E/S) of 1092.69 β -mannanase activity unit/g of the dried copra meal. Monosaccharide was the predominant product of the hydrolysis reaction followed by di- and tri-saccharide. Activated carbon treatment of the copra meal hydrolysate reduced the monosaccharide content resulting in 36.13% of monosaccharide, 54.26% of disaccharide and 9.61% of trisaccharide. All monosaccharides were eliminated by incubating the copra meal hydrolysate with *Saccharomyces cerevisiae* for 48 h, which promoted the growth of *B. breve*, *L. plantrorum*, *B. bifidum*, *L. bugarius*, *L. acidophilus*, *L. brevis*, *L. casei*, *B. longum*, and *S. thermophiles* while retarding the growth of the pathogenic bacteria, *S. aureus* and *E. coli*.

Keywords Copra meal · Enzymatic hydrolysis · Mannanase · Mannooligosaccharides · Prebiotic

Introduction

Coconut residue, or copra meal, is a by-product remaining after pressing the cream and oil out of the coconut meat, which is produced in quantity in coconut product factories. The utilization of copra meal is also considered a valuable livestock feed (Guarte et al. 1996). It contains 43–45% of carbohydrates, which are mainly in the form of mannose polysaccharides (61%) furthermore, the sugar composition of copra meal polysaccharide contains 63–79% of mannose, 13–24% of glucose, 6% of galactose and 1–4% of arabinose (Khuwijitjaru et al. 2012; Kusakabe et al. 1987). Copra meal also has a linear β -1, 4-mannan backbone with only a few β -galactosyl substitutes (Pangsri et al. 2015). β -mannanases attack the internal glycosidic bonds of the mannan backbone chain releasing short β -1,4-mannooligosaccharides (MOS). Kusakabe et al. (1983) prepared MOS from copra mannan by using mannanase from *Streptomyces* sp. No. 493. The hydrolysis reaction resulted in mannose, mannobiose (M2), mannotriose (M3), mannotetraose (M4) and mannopentaose (M5). Similarly, Meryandini (2015) hydrolyzed copra meal by using mannanase from *Streptomyces* sp. BF3.1 resulting in glucose, mannose and a mannoooligosaccharide, M2–M6.

Probiotics are food supplements that contain live microorganisms; such as, *Bifidobacterium breve*, *Lactobacillus plantrorum*, *Bifidobacterium bifidum*, *Lactobacillus bugarius*, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium longum*. These supplements produce bacteriocin or inhibitory compounds to combat pathogenic bacteria (*Escherichia coli*), compete for adhesion sites, and stimulate immune functions, which help to maintain the balance of intestinal microbiota. Therefore, MOS serves as a functional food component that can be used as a prebiotic. The copra meal hydrolysate obtained

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by mannanase S1 from the *Klebsiella oxytoca* KUB-CW2-3 treatment enhances the growth of *Lactobacillus reuteri* KUB-AC5, which is a beneficial bacterium in the intestine but inhibits pathogenic bacterium (*Salmonella* serovar Enteritidis S003), indicating potential prebiotic properties (Chantorn et al. 2013b). In addition, the hydrolysis of defatted copra meal with a purified mannanase preparation from *Bacillus circulans* NT 6.7 resulted in MOS, which promoted beneficial bacteria, especially from the *Lactobacillus* group, and inhibited pathogenic bacteria: *Shigella dysenteriae* DMST 1511, *Staphylococcus aureus* TISTR 029, and *Salmonella enterica* serovar Enteritidis DMST 17368 (Pangsri et al. 2015).

Commercial enzyme preparations have been successfully used for the production of oligosaccharide mixtures to act as a prebiotic (Im et al. 2016; Montilla et al. 2011). Commercial cellulase preparation from *Trichoderma longibrachiatum* was used for large-scale production of chitooligosaccharides (Tegl et al. 2016). Since the commercial enzyme preparations are relatively inexpensive, they are more practical for the large-scale production of oligosaccharides. Various studies (Chantorn et al. 2013b; Pangsri et al. 2015) have also investigated MOS production by hydrolyzing copra meal using purified or partially purified fungal or bacteria producing mannanase. However, the production of oligosaccharides from copra meal using commercial enzyme preparation containing β -mannanase has not been previously reported.

The objective of this research was to optimize the hydrolysis of copra meal using a food grade commercial enzyme preparation containing β -mannanase. The copra meal hydrolysate was treated with activated charcoal and *S. cerevisiae* to eliminate bitterness and monosaccharides. The oligosaccharide compositions of the copra meal hydrolysate were analyzed by high-performance liquid chromatography (HPLC), and the properties of the oligosaccharides were investigated in artificial gastric digestive juice and in the culture media to ascertain the usefulness of oligosaccharides as a potential prebiotic enhancing probiotic growth.

Materials and methods

Preparation of the raw material

Copra meal was obtained from a local market in Phitsanulok, Thailand. The coconut milk from local markets in Thailand is commonly made by grating the white inner flesh of a brown coconut and mixing the shredded coconut meat with a small amount of water. The grating process is carried out by a grater. The process is repeated three times before obtaining the copra meal. In the laboratory, the

copra meal was dried in a hot air oven at 60 °C overnight. To reduce the fat content, 100 g of the dried copra meal was mixed with hexane (1 L) for 18 h at room temperature. The hexane was then evaporated by keeping the copra meal in a hot air oven (Shel Lab 1375FX, Cornelis, Oregon) at 45 °C for 3 h (Raghavendra et al. 2004). Protein in the defatted copra meal was further removed by using an alkaline treatment in accordance with the method used by Chambal et al. (2012). In brief, 20 g of defatted dried copra meal was suspended in 400 ml of distilled water and the pH was adjusted to 11.0 by using a Na_3PO_4 (≈ 0.7 M) aqueous solution. The suspensions were heated in a water bath at 50 °C at a constant pH for 2 h. After removing the liquid portion of the treated copra meal, the dry copra meal was washed with distilled water until achieving a neutral pH and then dried at 60 °C. The fat and protein content of the treated copra meal was analyzed using standard methods. The fat content was 8.65% and the protein content was 1.96%.

The Actipro SF-RTM enzyme

Actipro SF-RTM or SF-R is an enzyme preparation derived from a selected strain of *Aspergillus niger* containing hemicellulase, which is used in commercial food preparation. The sample of the Actipro SF-RTM used for this research was provided by the Thailand agent of DSM Food Specialties (the Netherlands). The enzyme was approved for food use by the Food and Drug Administration of Thailand (FDA Thailand). The high hemicellulase activity at a reasonable price made it possible for use in a large scale operation. Therefore, this enzyme was selected for this study. The enzyme contains β -mannanase activity (3.60 units), β -mannosidase activity (79.45 units), endoglucanase (3053.33 units), and β -glucosidase (781.70 units). As the gel diffusion assay was positive, this indicated that the enzyme had endo- β -mannanase activity. The following assays were used to determine the hemicellulase activities of the SF-R.

The presence of the endo- β -mannanase was determined by a gel diffusion assay in accordance with the method used by Still et al. (1997). The endo- β -mannanase enzyme activity was detected by the gel diffusion assay from the appearance of a clear zone.

The β -mannanase activity was determined with an optimal pH of 5.0 and temperature of 60 °C. One unit of β -mannanase activity was defined as the amount of enzyme that released 1 μmol of reducing sugar content, which was equivalent to D-mannose per minute per ml of enzyme under experimental conditions [using 0.5% of locust bean gum as a substrate as used by Ratto and Poutanen (1988)].

One unit of β -mannosidase was defined as the amount of enzyme that liberated 1 nmol of p-nitrophenol per minute

per ml of enzyme using p-nitrophenyl- β -D-mannopyranoside as a substrate at 50 °C with a pH of 7.0.

One unit of endoglucanase was defined as the amount of enzyme that liberated the μ mol/ml of glucose equivalent in 1 min per 0.1 ml of enzyme, according to Ghose (1987), using 1.0% of hydroxyethyl cellulose as a substrate at 50 °C with a pH of 4.8.

The β -glucosidase was determined using p-nitrophenyl- β -D-glucopyranoside (PNPG) as a substrate in accordance with the AOAC Official Method 994.09 (AOAC 1997). One unit of activity was the amount of β -glucosidase that freed 1 nmole of p-nitrophenol per minute per ml of enzyme from the PNPG substrate under the conditions of the assay (pH of 5.0 and temperature of 50 °C).

Optimization of the enzymatic hydrolysis of the copra meal

The Response Surface Methodology (RSM) was applied to predict the optimal hydrolysis conditions of the treated dried copra meal by the SF-R with the highest hydrolysis rate. The Optimal Exact Three Factor design (Borkowski 2003) was employed in this study. The selected variables were: enzyme content, temperature (40–80 °C) and pH (4–8). The ranges of the enzyme content used were 38–760 units. One unit of the β -mannanase activity was defined as changes in the absorbance at 540 nm due to the reduced groups formed per minute (0.001 Δ A540/min) under the assay conditions. The response measured was the rate of the reducing sugar produced. The determination of the reducing sugar content was conducted using the 3,5-dinitrosalicylic acid method.

For each hydrolysis experiment, four grams of the treated dried copra meal was mixed with distilled water at a ratio of 1:33 (w/v). The pH of the mixture was adjusted to the required pH. After adding the enzyme, the hydrolysis was performed in a circulating water bath (Poly Science 8205, Pleasant Prairie, Wisconsin) for 6 h at the temperature and pH studied. The reactions were terminated by heating the mixture at 85 °C for 10 min. The separation of the insoluble portion was performed using centrifuge and filtration (Whatman paper #4).

The experimental data obtained were fitted by the following quadratic (second-degree) polynomial equation as shown below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where Y is the predicted response, b_0 the constant, b_1 , b_2 , and b_3 the linear coefficients, b_{12} , b_{13} , and b_{23} the cross-

product coefficients, and b_{11} , b_{22} , and b_{33} are the quadratic coefficients.

Treatment of the copra meal hydrolysate

The objective of this step was to remove the salt and concentrate the copra meal hydrolysate. The treatment consisted of desalting, partial purifying and concentrating the copra meal hydrolysate using activated carbon (AC). For the desalting step, a volume of 70 ml of the hydrolyzed solution was added to 50 g of a food-grade cation ion exchange resin (Mazuma, Thailand) contained in a shaken flask. Adsorption was held for overnight ($\sim$ 15 h) at 25 °C, and agitation of 130 rpm was conducted by a shaker. The desalted solution was collected and the resin was washed with 140 ml of distilled water. The filtrated water was then combined with the desalted solution. Adsorption of the sugars onto the AC was conducted by adding 35 ml of the desalted sample solution into 18 g of the food-grade activated carbon (Mazuma, Thailand) then shaken for 2 h at 25 °C at 150 rpm of agitation. After the absorbed activated carbon was washed well with water, the absorbed sugars were eluted with ethanol by shaking the mixture for 2 h at 150 rpm of agitation. The desorbing solution was collected and evaporated at 65 °C to remove the ethanol and concentrate sugars using a rotary evaporator. The concentrated copra meal hydrolysate was collected after centrifugation to remove the carbon particles. The reducing sugar contents of the AC treated copra meal were adjusted to obtain 100 mg/ml and stored at -20 °C until used.

In some cases, the monosaccharide was completely removed from the copra meal hydrolysate by subjecting it to incubation with *Saccharomyces cerevisiae* for 48 h. The pH of the copra meal hydrolysate was adjusted to 4.5 and *S. cerevisiae* was added into the solution. Then, the solution was incubated with shaking at room temperature for 48 h. The absence of the monosaccharide was confirmed by high-performance liquid chromatography (HPLC).

Properties of the treated copra meal hydrolysate

Sugar compositions of the copra meal hydrolysate

The oligosaccharide compositions in the hydrolyzed solution were analyzed by using a HPLC system (Shimadzu, Kyoto, Japan). The neutralized hydrolysis solution (2 ml) was filtered through a 0.45 μ m filter and a portion (10 μ l) of the filtrate was injected into the HPLC column (Rezex RSO oligosaccharide Ag + column, 200 mm $\times$ 10 mm, Phenomenex, Torrance, CA). HPLC-grade water was used as a mobile phase and pumped at a flow rate of 0.3 ml/min at 75 °C. The HPLC was performed using a refractive index detector with a detection limit of 0.01 mmol/L.

The mono- and di-saccharide compositions in the hydrolyzed solution were analyzed by using a HPLC system described previously using a HPLC column (Inertsil® NH2, GL Sciences). Ethanol/ethyl acetate/acetonitrile/water at a ratio of 30:30:23:17 was used as the mobile phase at 1 ml/min flow rate at 26 °C.

D-mannose (Merck, Germany), 1,4-beta-D-mannobiose, 1,4-beta-D-mannotriose and 1,4-beta-D-mannotetraose (Megazyme, Ireland) were used as the sugar standards.

In vitro digestion of copra meal oligosaccharides

Hydrolysis with an artificial gastric juice was performed with a test solution consisting of 7.0 mL of HCl, 2.0 g of NaCl and 3.2 g of Pepsin (porcine gastric mucosa, Merck, Germany) (in 1L). The test solution was added to the concentrated copra meal hydrolysate (1%, w/v) at a ratio of 2:1 and incubated at 37 °C for 4 h. Hydrolysis with an artificial pancreatic juice was performed with α -amylase from the porcine pancreas (Sigma, USA). Porcine pancreatin was dissolved in 50 mM of a potassium phosphate buffer (pH of 8.0) at an amylase activity of 70 units/ml. One unit would liberate 1.0 mg of glucose from the starch in 1 min at a pH of 5.0 at 37 °C. The artificial pancreatic juice was added to the concentrated copra meal hydrolysate at a ratio of 2:1, incubated at 37 °C for 4 h, and heated in a boiling water bath for 5 min to stop the reaction (Asano et al. 2003).

The oligosaccharides remaining after digestion were measured by HPLC, and the results were shown as the average of three experiments.

Probiotic growth on the copra meal hydrolysate

Preparation of bacterial inoculums

The health benefits of bacterial probiotics: *B. breve*, *L. plantrarum*, *B. bifidum*, *L. bugaricus*, *L. acidophilus*, *L. casei*, and *B. longum* were purchased from DSMZ, Germany. A lyophilized culture was reactivated at 37 °C for 24 h on MRS agar medium (Himedi, India) and the growth media (MRS, EMB, and peptone nutrient). Pathogenic bacteria, *S. aureus* and *E. coli* were among the most prevalent species of gram-positive and gram-negative bacteria, respectively, that induced the clinical pathogens. They were purchased and incubated on EMB agar medium from TISTR, Thailand. The purified colony was inoculated into a nutrient broth and incubated at 37 °C at 80 rpm for 16–18 h to use as an inoculum.

Microbial cultivation

Inoculums (10%) were added into the culture media (The yeast treated copra meal hydrolysate with a reducing sugar content of 10 g/L, which was filter sterilized (0.2 μ m), the growth media or distilled water) and incubated at a temperature of 37 °C with shaking at 80 rpm, followed up for 24–48 h. The optical density of the cells' cultures were measured at 600 nm by a spectrophotometer (Specord40, Analytik Jena, Germany).

Data analysis

The specific growth rates in the culture were estimated from the abundance of data by linear regression as the slope of the line representing the natural polynomial of the bacterial abundance against the time during the exponential growth phase. The maximum specific growth rates were calculated from the mean $\pm$ standard deviation of the triplicated culture. All the data were analyzed by the one-way analysis of variance (ANOVA) at $p < 0.01$ using SPSS 14.0 (SPSS Inc. Chicago, IL, USA), and the test for significant differences in the groups (p value < 0.01) was done using Duncan's new multiple range test.

Results and discussion

Optimization of the enzymatic hydrolysis of the copra meal

The effect of the enzyme content (X_1), temperature (X_2) and pH (X_3) on the hydrolysis rate was measured for the reducing sugar production rate, which indicated the progress of the enzymatic hydrolysis of the copra meal using SF-R that was investigated by using the three-factor design for the response surface method (RSM). The experimental design and results are shown in Table 1 and the quadratic models generated from the raw data are shown in Eq. 1.

To check the adequacy of the model, a residual analysis was performed. The normal probability plot of the residuals was linear indicating that the normal assumption was satisfied. The plot of the residuals versus the predicted responses showed that the residuals were randomly scattered, which indicated that the model was adequate. Both the normal probability plot of the residuals and the plot of the residuals versus the predicted responses resulted in patterns that indicated the predicted models were reliable. This suggests that the generated models could explain the data variations and represent the actual relationships between the parameters.

The plots showing the effects of temperature and pH on the hydrolysis rate at a constant level of the enzyme (data

Table 1 Experimental design and results obtained by the hydrolysis of copra meal by SF-R

Runs	Variables			Response
	X ₁	X ₂	X ₃	
1	0.2912	− 1	− 1	57.13
2	− 1	0.2912	− 1	9.06
3	− 1	− 1	0.2912	5.53
4	− 0.1925	1	− 0.1925	17.38
5	− 0.1925	− 0.1925	1	4.68
6	1	− 0.1925	− 0.1925	56.27
7	− 1	1	1	10.66
8	1	1	− 1	0.62
9	1	− 1	1	17.52
10	1	1	1	11.28

The equation beneath the table indicates a relationship between hydrolysis rate (Y) and the variables. Y is the average value of two sets of the experiment

*Y is the hydrolysis rate or reducing sugar production rate (mg mannose eq./g dried copra meal min)

The quadratic models generated for SF-R hydrolysis $Y = 36.8441 + 14.1557x_1 - 10.1116x_2 - 10.3125x_3 + 1.0948x_1^2 - 11.1846x_1x_2 - 7.7104x_2^2 - 2.1187x_1x_3 + 17.5812x_2x_3 - 17.3479x_3^2$

not shown) indicated that as temperature increased so the hydrolysis rate also increased. The temperature and optimum pH to obtain the highest hydrolysis rate were 40–46 °C with a pH of 4. At a constant temperature, the hydrolysis rate increased as the enzyme content increased.

Using the data from the RSM experiment, the relationship between the hydrolysis rate and monosaccharides, disaccharides and trisaccharides liberated in the supernatant of the reaction mixture is shown in Fig. 1. It was observed that the monosaccharides and disaccharides content increased as the hydrolysis rate increased. A higher content of monosaccharides was produced than the disaccharides regardless of the hydrolysis rate.

Table 2 shows that glucose and mannose were the major sugars detected in the hydrolysis solution after the copra meal hydrolysis at an optimum temperature and pH at various treatment times. As the hydrolysis time increased, the glucose, mannose and disaccharide content increased, but the tetrasaccharide content decreased as the hydrolysis time increased. The decrease in the tetrasaccharide was due to the action of the enzyme from the prolonged hydrolysis time. Furthermore, disaccharide was the major oligosaccharide detected in the hydrolysis solution.

The copra meal had a linear β-1, 4-mannan backbone with only a few β-galactosyl substitutes (Pangsri et al. 2015). The predominant products of the enzymatic hydrolysis of the copra meal were mannose, glucose and mannooligosaccharides. Analysis of the monosaccharide

Fig. 1 Relationships between hydrolysis rate and monosaccharide and disaccharides (using mannose and mannobiose as standards) liberated during hydrolysis of copra meal using SF-R. The values were averages of the two replicates

and oligosaccharide content in the enzymatic hydrolysate of the copra meal by HPLC showed that the products of the hydrolysis reaction appeared at the peak retention times of the standard glucose, mannose, 1,4-beta-D-mannobiose, 1,4-beta-D-mannotriose and 1,4-beta-D-mannotetraose. The SF-R enzymatic hydrolysis process used with the copra meal liberated the monosaccharide (glucose and mannose), disaccharide (using mannobiose as the standard) and trisaccharide (using mannotriose as the standard) whereas other oligosaccharides with DP > 4 were below the detectable level. Disaccharide was the major oligosaccharide released from the copra meal by the enzymatic hydrolysis process. It is possible that the disaccharide and trisaccharide in the hydrolysate were mannobiose and mannotriose, respectively; however, further studies on the structure of the oligosaccharides would have to be conducted to confirm this statement.

The production of either short- or long-chain oligomannosides could vary depending on the source of the mannanase, the presence of other enzymes, and the hydrolysis conditions (Puls 1997). β-1,4-MOS produced from the copra meal was reported in some articles which indicated differences in degree of polymerization (DP), depending on the hydrolysis method. Chantorn et al. (2013a) investigated crude mannanase from *Penicillium oxalicum* KUB-SN2-1 and found that the enzyme hydrolyzed the copra mannan to become mainly mannotriose; moreover, mannobiose and monosaccharides were observed on thin layer chromatography (TLC). Rungrasamee et al. (2014) also produced MOS (M1 to M6 of 6.08, 8.81, 10.41, 11.87, 1.21 and 0.21 g/100 g MOS, respectively) through the hydrolysis of copra meal using crude

Table 2 Sugar (mg/ml) released into the hydrolysis solutions of the hydrolysis of copra meal by SF-R. The hydrolysis condition was at optimum condition selected from RSM [S = 3.2% (w/v), E/S = 1092.69 unit/g (β -mannanase activity) at 40 °C and pH 4.0] for various times

Hydrolysis time (h)	Glucose	Mannose	Disaccharide	Trisaccharide	Tetrasaccharide
2	3.044 $\pm$ 0.438	2.392 $\pm$ 0.719	3.564 $\pm$ 0.419	1.858 $\pm$ 0.817	0.243 $\pm$ 0.097
4	3.592 $\pm$ 0.466	3.780 $\pm$ 0.892	5.034 $\pm$ 0.384	2.230 $\pm$ 0.629	0.104 $\pm$ 0.048
6	4.274 $\pm$ 0.701	5.919 $\pm$ 0.694	6.303 $\pm$ 0.989	1.963 $\pm$ 0.133	0.064 $\pm$ 0.009

The sugar content determination was conducted by HPLC using HPLC column (Rezex RSO oligosaccharide Ag + column, 200 mm $\times$ 10 mm, Phenomenex, Torrance, CA)

mannanase from *Bacillus subtilis* CAe24. Additionally, Pangsri et al. (2015) reported that mannanase from *Bacillus circulans* NT 6.7 hydrolyzed defatted copra meal into mannooligosaccharides; these were mainly mannotriose, mannotetraose and mannopentaose. These results correlated with those of Titapoka et al. (2008), who reported that the products hydrolyzed from copra meal by purified mannanase S1 from *K. oxytoca* KUB-CW2-3 were mannotriose and mannotetraose. Furthermore, Kusakabe et al. (1983) prepared beta-1,4-mannooligosaccharides from copra mannan using *Streptomyces* sp. No.493 and found that the predominate product was mannobiose with some amounts of M1 to M5. Similarly, Meryandini (2015) found that the major product of copra meal hydrolyzed by using crude mannanase from *Streptomyces* sp. BF3.1 was mannobiose. Monosaccharide and M3-M6 were also produced but in much lesser content. In another study, when mannanase from *Penicillium purpurogenum* was used to hydrolyze the copra meal, mannose and mannobiose were the two products observed (Kusakabe et al. 1987).

The monosaccharides obtained from the hydrolyzed products by crude mannanase, reported in those studies, were in very low amounts, which was unlike the results from the present study. The crude mannanase used in those studies either had very low activity of β -glucosidases and β -mannosidase or none for the purified one. On the contrary, the commercial enzyme preparation contained β -glucosidases and β -mannosidase in order to enhance the hydrolytic properties of the commercial enzyme. The production of the monosaccharide was due to the β -mannosidase and β -glucosidase activity of the SF-R. The β -glucosidases removed the 1,4-glucopyranose units at the non-reducing end of the oligomers galactoglucomannan of the copra meal (Moreira and Filho 2008). β -mannosidase, an exo-type enzyme, also cleaved β -1,4-linked mannoses releasing mannose from the non-reducing end of the mannans and mannooligosaccharides (Dhawan and Kaur 2007). Therefore, the authors of the present study predicted the presence of the monosaccharides in the prepared copra meal hydrolysate by using a commercial enzyme preparation.

Treatment of the copra meal hydrolysate

Hydrolysis of copra meal using a food grade commercial enzyme preparation containing mannanase produced significant amounts of monosaccharides. To obtain a higher portion of the oligosaccharides and reduce the impurities, activated carbon (AC) treatment was used. Previously, Wang and Lu (2013) used an AC treatment to remove the impurities from xylooligosaccharides prepared from wheat bran. They found that the removed macromolecules included starch, pectin, tannin, and protein. To produce fructooligosaccharides (FOS) from the fermentation of sucrose by *Aureobasidium* sp., Nobre et al. (2012) also used AC to improve the purity from 56 to 92.9% with recovery of 74.5%. AC is cheap and has a large surface area and pore volume with the potential to separate monosaccharides from oligosaccharides.

Since copra meal hydrolysate contains a majority of monosaccharides and disaccharides, the authors investigated the influence of the AC treatment on the recovery of the monosaccharides and disaccharides using mannose and mannobiose as the standards, respectively. Figure 2 illustrates that both the monosaccharides and disaccharides were absorbed by the AC and that the ethanol concentration level influenced the release of the absorbed sugars in the AC. Treatment with 5–15% (v/v) of ethanol allowed a higher recovery of the monosaccharides than the disaccharides. The recovery of the mono- and disaccharides was similar for the treatment with 20–25% (v/v) of ethanol. The recovery of both kinds of sugar was highest when treated with 25% (v/v) of ethanol. The yield of the disaccharide was 75.88% of the original content after desorption by 25% of ethanol of 7.23 g per 100 g of dried copra meal. At 30 and 40% of ethanol, the recovery of the disaccharide was higher than that of the monosaccharide. A major portion of the carbon surface of AC is non-polar or hydrophobic, and the hydrophobic character of the sugars relates to the number of the CH groups. Therefore, as the oligosaccharides have an increased chain of CH groups, it is probable that they are more adsorbed by AC than small saccharides (Nobre et al. 2012). However in the case of the present study, it could be seen that both the monosaccharides and

Fig. 2 Mono- and disaccharide content after AC treatment shown as percentage recovered after desorption from AC using ethanol in water solution of 5–40% (v/v). The monosaccharide measured as mannose in the copra meal hydrolysate before AC treatment was 439.84 ± 27.19 and the disaccharide measured as mannobiose was 381.29 ± 97.75 mg/4 g dried copra meal. The sugar content determination was conducted by HPLC using an HPLC column (Inertsil® NH2, GL Sciences). Error bars represent standard deviation ($n = 2$)

disaccharides were absorbed into the AC as their molecular weights were not that different. For the desorption process, several authors used water or a very small percentage of ethanol to recover the monosaccharides whereas between 5 and 10% of ethanol in water was used to recover the disaccharides. For the oligosaccharides, 15–50% of ethanol was used by Nobre et al. (2012). In the case of the present study, to reduce some of the monosaccharides present in the copra meal hydrolysate, desorption of the absorbed sugar using 5% of ethanol prior to 25% of ethanol was selected. When 100 mg/ml of the reducing sugar of the concentrated copra meal hydrolysate after the AC treatment was reached, the sugar compositions were 54.88 mg/ml of monosaccharide, 82.41 mg/ml of disaccharide and 14.59 mg/ml of trisaccharide. Although the AC treatment could not remove all of the monosaccharides, the monosaccharide:disaccharide:trisaccharide ratio changed from 0.55:0.34:0.11–0.36:0.54:0.10 indicating the increase in the disaccharide portion in the copra meal hydrolysate. After the AC treatment, the copra meal hydrolysate was shown as a clear solution with no bitter taste or off-flavor; as such, the solutions were only slightly sweet.

The AC treatment was unable to completely remove the monosaccharides. The copra meal hydrolysate was treated with *S. cerevisiae* under optimum conditions for yeast growth for 48 h. Figure 3a, b show the depletion of the monosaccharides in the copra meal hydrolysate by the yeast treatment from the conversion of the monosaccharides into ethanol and CO_2 . The yeast treatment showed no influence on the content of the disaccharides of the copra

Fig. 3 HPLC chromatogram using an HPLC column (Inertsil® NH2, GL Sciences) showing mono- and disaccharide compositions of the copra meal hydrolysate before (a) and after yeast treatment (b). HPLC chromatogram using an HPLC column (Rezex RSO oligosaccharide Ag + column) showing compositions of the copra meal hydrolysate after AC treatment (c), after In vitro digestion using artificial gastric juice (d) and after In vitro digestion using porcine pancreatin (e)

meal hydrolysate, and this sample was used to conduct a probiotic growth study. Yoon et al. (2003) reported similar results using carbohydrate solutions of glucose and galactose standards. Moreover, Hernández et al. (2009) showed that yeast treatment removed the monosaccharides and allowed a high recovery of the galactooligosaccharides and disaccharides. Kusakabe et al. (1987) also used the *Candida perapsilosis* var. *komabaensis* yeast to eliminate the mannose in the enzymatic hydrolysate of white copra meal with mannanase.

Although, the authors' AC treatment was not able to completely eliminate the monosaccharides in the copra meal hydrolysate, it was noticed that this treatment removed the bitterness, which was probably due to the presence of aromatic peptides or protein. AC also had the potential to desalt the solutions. The FOS fractions were free of salt after the AC treatment (Nobre et al. 2012). Therefore, AC treatment was considered necessary for the preparation of the oligosaccharides by the enzymatic hydrolysis of the copra meal using a commercial enzyme preparation. In addition, it helped to concentrate the oligosaccharides after the yeast treatment.

Probiotic growth on the copra meal hydrolysate

The authors conducted a study of the in vitro digestion of the copra meal hydrolysate in which it was found that the oligosaccharides prepared from the enzymatic hydrolysis of copra meal using the SF-R were not digested at all by artificial gastric juice and artificial pancreatic juice (Fig. 3c–e).

Figure 4 shows the specific growth rate of the microorganisms. Of the nine bacterial growth experiments conducted using the yeast treated copra meal hydrolysate, the highest specific growth rate observed was of *L. plantarum* of 0.037 h^{-1} after 48 h of incubation ($p < 0.01$) (Fig. 4a). The growth of both the pathogenic bacteria of *S. aureus* and *E. coli* were completely inhibited in this medium although they grew normally in the growth media. However, most bacteria had limited growth in the growth media after 24 h of incubation after which the growth rate declined except for *L. bulgaricus* where the highest specific growth rate of 0.026 h^{-1} occurred after 48 h of incubation ($p < 0.01$) (Fig. 4b). On the contrary, the seven beneficial bacteria continued to grow in the yeast treated copra meal hydrolysate after 48 h of incubation.

The copra meal hydrolysate prepared from mannanase from different sources showed the promotion of the beneficial bacteria, especially the *Lactobacillus* group; such as, *L. reuteri* KUB-AC5 and *B. circulans* NT 6.7 (Chantorn et al. 2013a). Meanwhile, the copra meal hydrolysate inhibited the pathogenic bacteria: *Shigella dysenteriae* DMST 1511, *Staphylococcus aureus* TISTR 029, and

Fig. 4 Microbial growth rate from measuring turbidity of a culture after 24 and 48 h incubation at 37 °C; **a** the yeast treated copra meal hydrolysate, **b** the growth media and **c** distilled water (A = *B. breve*, B = *L. plantarum*, C = *B. bifidum*, D = *L. bulgaricus*, E = *L. acidophilus*, F = *L. brevis*, G = *L. casei*, H = *B. longum*, I = *S. thermophilus*, J = *S. aureus*, K = *E. coli*)

Salmonella enterica serovar Enteritidis DMST 17368, indicating the potential prebiotic properties (Chantorn et al. 2013b; Pangri et al. 2015).

In this study, seven strains of beneficial bacteria were cultivated in the copra meal hydrolysate and also in commercial media. Several researchers have demonstrated that glycosidic linkages and degrees of polymerization of the oligosaccharides contribute toward the selectivity of the fermentation by the beneficial bacteria (Rowland and Tanaka 1993; Sanz et al. 2005, 2006). Higher molecular

oligosaccharides may be slowly fermented, exhibiting a higher colonic persistence than low molecular weight carbohydrates reaching the most distal regions where most intestinal disorders are encountered (Montilla et al. 2011). Furthermore, Asano et al. (2003) studied the digestibility and fermentation of a β -1,4-mannobiose and mannoooligosaccharides mixture prepared from spent coffee grounds. By utilizing an in vitro fecal incubation method, it was found that the mannoooligosaccharides were fermented by human fecal bacteria to produce short chain fatty acids. These acids were thought to improve the large intestinal environment, as they were absorbed and utilized by the host as an energy source. The products are supposed to inhibit harmful bacteria that can affect the digestive system.

On the basis of results, the production of copra meal hydrolysate prepared by commercial enzymatic hydrolysis of copra meal composed mostly of disaccharide with some trisaccharide was indigestible oligosaccharides and would reach the large intestine without digestion and be used by intestinal bacteria. The result suggested that the copra meal hydrolysate could represent an opportunity in the future development of prebiotics. It is further expected that the copra meal hydrolysate has the potential to promote healthful human intestinal microflora as a prebiotic and to prevent the growth of pathogenic bacteria.

Conclusion

A commercial enzymatic preparation containing mannanase from *Aspergillus niger* (Actipro SF-R) was used to produce MOS from copra meal. The conditions that led to the highest hydrolysis rate were pH of 4 and temperature of 40 °C at an E/S of 1092.69 β -mannanase activity unit/g of dried copra meal. Monosaccharide was the predominant product of the hydrolysis reaction followed by disaccharide and trisaccharide. AC treatment not only reduced the monosaccharide portion in the copra meal hydrolysate, but also removed the bitterness. The monosaccharide:disaccharide:trisaccharide ratio changed from 0.55:0.34:0.11 to 0.36:0.54:0.10 after the AC treatment indicating an increase in the disaccharide ratio. The yeast treatment using *S. cerevisiae* eliminated all monosaccharides in the copra meal hydrolysate without any significant influence on the oligosaccharide contents. The yeast treated copra meal hydrolysate promoted the growth of *B. breve*, *L. plantarum*, *B. bifidum*, *L. bugarius*, *L. acidophilus*, *L. brevis*, *L. casei*, *B. longum*, and *S. thermophiles*, and more importantly, retarded the growth of pathogenic bacteria (*S. aureus* and *E. coli*). This research study showed that it was possible to use a commercial enzymatic preparation containing mannanase to prepare oligosaccharides from copra

meal to be used as a prebiotic. Although monosaccharide could be eliminated by AC and yeast treatment, further studies should be conducted to test other commercial enzyme preparations containing mannanase to investigate the commercial enzyme that produces less or no monosaccharides.

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