Conversion of konjac powder into glucomannan-oligosaccharides, mannose, and glucose by hydrolysis facilitated by microwave heating and HCl catalyst

Cuong Viet Bui, Wanwipa Siriwatwechakul, Wareepat Tiyabhorn,

Thammanont Wattanasiritham, Natthaphat Limpraditthanont

and Siwarutt Boonyarattanakalin[*]

Abstract

The reaction conditions to hydrolyze konjac powder (KP) were investigated by using a combination of microwave radiation heating and HCl as a catalyst to explore the possibility of glucomannan-oligosaccharides, mannose, and glucose extractions from the polysaccharides of KP. Selective reaction conditions to produce glucomannan-oligosaccharides, mannose, and glucose were determined. The highest yield for glucomannan-oligosaccharides production, at 19.2%, was achieved under the reaction conditions of 110°C, 15 min, 2 M HCl, and 10:1 (mL/g) ratio of reaction volume to KP mass. The highest yield for both mannose and glucose production, at 35.8% and 30.2%, respectively, was achieved under the reaction conditions of 110°C, 15 min, 1.2 M HCl, and 10:1 (mL/g) ratio of reaction volume to KP mass. Only a small amount of 5-HMF at 0.3% was formed and a low level of UV absorbance (284 nm) of hydrolyzed products (HP) at 1.7 was found under these conditions. The combination of microwave radiation (MCR) heating and HCl effectively hydrolyzed konjac powder selectively into glucomannan-oligosaccharides and/or monosaccharides in a relatively short reaction time.

School of Bio-Chemical Engineering and Technology, Sirindhorn International Institute of Technology, Thammasat University,

Pathum Thani, 12121, Thailand

[*] Corresponding author; E-mail: siwarutt@siit.ac.th Received 16 March 2016 , Accepted 7 June 2016

1. Introduction

Konjac (*Amorphophallus konjac*) is a herbaceous plant grown in some Asian countries (China, Japan, South East Asia), and it has been consumed by humans for more than 2000 years [1]. Konjac powder (KP) is consumed for benefits as medicine (detoxification, tumor-suppression, asthma, etc.), food additive, and dietary supplement (noodles, rubbery jelly, etc.) [1-2]. The major polysaccharide found in konjac is glucomannan, in which glucose and mannose are linked via β–1,4-glycosidic linkages with a ratio of 1:1.6 (w/w) [1, 3-6]. In addition, the structure of glucomannan polysaccharide is 8% branched. The monosaccharide ratio of the terminal units is approximately 2:1 (w/w) of glucose to mannose [6].

Prebiotics are functional foods [7] which have attracted a lot of interests recently because of their health benefits [7-9]. Glucomannans are a promising prebiotic obtained by hydrolysis of konjac. Salivary and pancreatic amylase of an animal digestion system cannot break the β-1,4-glycosidic linkages in glucomannans to release monomers. However, they can be fermented by useful bacteria in the colon [1]. This has satisfied one of the important requirements for prebiotic activities. In both *in vitro* and *in vivo* tests, glucomannan-oligosaccharides extracted from konjac have been demonstrated to have better prebiotic activities than glucomannan polysaccharides [2, 4, 10-11]. In addition, both mannose and glucose obtained

from konjac are also useful products. Food additives, including humectants, sweeteners, stabilizers, texturizers [12], and pharmaceutical products for therapeutic purposes [13], are major applications of mannose. Glucose has plenty of applications in many sectors, including food industries as energy drinks, and in the health sector as a portion of medicine [14].

Extractions of glucomannans from konjac have been investigated by several methods, including acid degradation, enzymatic hydrolysis, oxidative degradation, and physical methods [3-15]. However, the processing time for these methods is very long (3.4 h) [4, 16-17]. In addition, the enzymatic method has its limitations, including high cost, complicated processes, substrate solubility, a narrow range of temperature activity for the enzyme, and longer reaction time [18-19].

In recent years, the heat generated by microwave radiation (MCR) has been explored for its applications in various industries [20-21], such as food processing [22-23], food drying [24-25], polymers [26-27] and organic synthesis [28-29]. MCR was also used to hydrolyze KP to obtain monosaccharides in mild reaction conditions [30-31]. In comparison to conventional heating, for the purpose of a complete digestion of KP to monosaccharides to analyze the KP contents, the time required to completely hydrolyze KP was shorter [31]. In addition, the amount of acid catalyst required was reduced by twenty-fold.

Furthermore, the structure and quality of products were not affected by MCR heating [31]. To the best of our knowledge, the hydrolysis of KP by a combination of microwave radiation and HCl as a catalyst, to selectively extract glucomannan-oligosaccharides has not been reported.

Therefore, we aim to employ the tremendous advantages of the combination of MCR heating and HCl as a catalyst in the KP hydrolysis process, in order to circumvent the shortcomings mentioned in the previous methods and to determine conditions to produce both glucomannan-oligosaccharides and monosaccharides. The obtained glucomannan-oligosaccharides may be used as a more effective prebiotic agent than the intact glucomannan-polysaccharides.

2. Material and methods

2.1 Material and reagents

2.1.1 Konjac and konjac powder

Fresh konjac was purchased from Rayong province, Thailand. The raw material was washed with tap water and cut into small pieces, and it was dried in a hot air oven at 60°C for 2 days. The dried konjac flakes were ground by a household blender and sieved to obtain KP with particle sizes of less than 250 μm. The KP was kept in an airtight container for further usage.

2.1.2 Reagents

Phenol, sulfuric acid (95 - 97%), dihydroxyacetone (DHA), Coomassie Brilliant Blue G-250, and albumin were purchased from Merck (Germany). 3,5-Dinitrosalicylic acid, sodium hydroxide, 5-hydroxymethyl-2-furfuraldehyde (5-HMF), methanol, and HCl were purchased from Sigma-Aldrich (USA). Potassium sodium tartrate was purchased from Ajax FineChem Pty Ltd (Australia). Mannose and xylose were purchased from Senn Chemicals (Switzerland). Galactose and glucose were purchased from Fluka (USA). The standard mannans were synthesized according to a published report [32]. Other reagents were of analytical grade.

2.2 Physicochemical hydrolysis of KP

KP was dried at 60°C overnight in a hot air oven to remove moisture before being hydrolyzed by a combination of MCR (CEM, Discover SP 909155, USA) and HCl as a catalyst. KP (0.1 g) was mixed with diluted HCl (aq., 1 mL) at a specified concentration in a batch-type closed-vessel of 10 mL at certain time intervals. Maximal pressure of 290 psi, maximal power of 150 watts, and ramping time of 2 min were used as the set conditions of MCR for all of the hydrolysis reactions.

Afterward, the vessel was allowed to cool to room temperature. Reverse osmosis (RO) water (8 mL) was added and stirred for 1 h to separate the soluble and insoluble portions.

The residual solid was collected by filtering through a Whatman No. 1 paper. Centrifugation (SCILOGEX, D3024R, USA) was used to obtain the supernatant, at 14,000 rpm, at 4°C, for 20 min. The combined residual solids, collected from both filtration and centrifugation, were dried at 60°C overnight in a hot air oven to obtain a constant weight.

The hydrolyzed products (HP) were kept as an aqueous solution in a refrigerator at 4°C for further analyses. All reactions were repeated three times.

2.3 Experiment conditions

2.3.1 The effect of temperature on the hydrolysis of KP

The reaction temperatures investigated in this study were set at a range of 110°C - 200°C (10°C increments), with 15 min of reaction time, 0.05 M HCl as a catalyst, and 10:1 (v/w) of ratio of reaction volume to KP mass.

2.3.2 The effect of HCl concentration on hydrolysis of KP

A temperature that is higher than 120°C accelerates Maillard browning and caramelization reactions (MBCR) over reaction time [33]. Thus, the reaction temperature was fixed at 110°C. The HCl concentration was 0.05 M and 0.2 M – 2.0 M (0.2 M HCl increments in the range of 0.2 – 2 M HCl). Reaction time and ratio of reaction volume to KP mass were 15 min and 10:1 (v/w), respectively.

2.4 Methods

All data are expressed as mean ± standard deviation

2.4.1 Proximate composition analysis of KP

The standard methods set by the Association of Analytical Communities (AOAC) [34] were used for proximate composition analysis of KP. Briefly, a hot air oven drying at 100°C for 16 – 18 h, drying the ash at 550°C for 4 – 6 h, Kjeldahl method with 6.25 as conversion coefficient, and Soxhlet extraction method were used to determine moisture, total ash, crude protein, and crude fat in KP, respectively.

2.4.2 Solid loss (SL) determination

The SL was calculated by Eq. (1),

$$SL = ((IS - RS)/IS) \times 100\% \qquad (1)$$

where,

SL: Solid loss (%, based on the dried weight of KP),

IS: Initial dried solid (g),

RS: Residual dried solid (g).

2.4.3 Total carbohydrate (TC) determination

Phenol-sulfuric acid assay was used to quantify TC in HP [35]. The HP (0.2 mL) was diluted to a total of 10 mL by RO water in a volumetric flask. The diluted HP (1 mL) was mixed with 1 mL of aqueous phenol solution (5%) in a closed test tube, and then 5 mL of sulfuric acid (95 - 97%) was added to the mixture. The mixture was thoroughly mixed and kept in a water bath

at 25°C for 20 min. A UV-VIS spectrophotometer (Thermo Fisher Scientific, G10S UV-VIS, USA) was used to record the absorbance of the mixture at 490 nm. A mixture of RO water, 5% aqueous phenol solution, and sulfuric acid (95 - 97%) with a ratio of 1:1:5 (v/v/v), was used as blank. Aqueous solutions of mannose and glucose, with a ratio of 1.6:1 (w/w) at different concentrations, were used to construct a standard curve. TC was reported in % (g of TC in 100 g of the dried weight of KP).

2.4.4 Reducing sugar (RS) determination

The RS in the HP was measured by dinitrosalicylic acid assay [36]. Dinitrosalicylic acid (1 g) and potassium sodium tartrate (300 g) were mixed with 200 mL of 2 M NaOH as dinitrosalicylic acid reagent. The mixture was then adjusted to 800 mL by RO water. The HP (0.2 mL) and the dinitrosalicylic acid reagent (2 mL) were mixed thoroughly in a closed test tube, and then the mixture was immersed in boiling water for 10 min. Afterward, it was rapidly cooled to room temperature by ice water. The absorbance of the mixture was recorded at 570 nm by using a UV-VIS spectrophotometer (Thermo Fisher Scientific, G10S UV-VIS, USA). A mixture of RO water and dinitrosalicylic acid reagent (0.2:2, v/v) was used as blank. Aqueous solutions of mannose and glucose with a ratio of 1.6:1 (w/w) at different concentrations were used to construct a standard curve. RS was reported in % (g of RS in 100 g of the dried weight of KP).

2.4.5 Protein determination

The Bradford method was used to quantify protein in HP [37-38]. Bradford reagent was prepared by dissolving 0.2 g of Coomassie Brilliant Blue G-250 in 100 mL of ethanol (95%), and then 200 mL of phosphoric acid (85%) was added. The mixture was thoroughly mixed and diluted to 2 L in a volumetric flask. Bradford reagent was filtered through Whatman No. 1 paper before using. HP (0.1 mL) was diluted to 1 mL by RO water, and then 5 mL of Bradford reagents was added. The mixture was incubated at room temperature for 5 min. The absorbance of a mixture was recorded at 595 nm by a UV-VIS spectrophotometer (Thermo Fisher Scientific, G10S UV-VIS, USA). A mixture of RO water and Bradford reagent with a ratio of 1:5 (v/v) was used as blank. Albumin aqueous solutions with different concentrations were used to construct a standard curve. The value of protein determination was reported in % (g of protein in 100 g of the dried weight of KP).

2.4.6 UV absorbance level for determination of intermediate degradation products of MBCR

A UV-VIS spectrophotometer (Thermo Fisher Scientific, G10S UV-VIS, USA) was used to record the UV absorbance level of the HP with 50-fold dilutions at 284 nm [39-40]. RO water was used as blank.

2.4.7 5-hydroxymethyl-2-furfuraldehyde (5-HMF) determination

5-HMF in HP was quantified by HPLC (Agilent 1260 Infinity, G1329B, Germany), equipped with a C18 column (Agilent, ZORBAX Eclipse Plus C18, 959961-902, USA) [19]. The diluted HP was neutralized and filtered through a 0.2 µm membrane, and 20 µL of the sample was injected into the HPLC. A mixture of deionized water (DI) and methanol (90:10, v/v), filtered through 0.2 µm membrane, was used as a mobile phase with a flow rate of 1 mL/min [19]. Absorbance of 5-HMF in HP was recorded at 284 nm by a UV detector [39-40]. 5-HMF aqueous solutions with different concentrations were used to construct a standard curve. Dihydroxyacetone (DHA) was used as an internal standard for all HP and the standard curve. 5-HMF was reported in % (g of 5-HMF in 100 g of dried KP).

2.4.8 Saccharide composition analysis of HP

An HPLC (Agilent 1260 Infinity, G1329B, Germany), equipped with a guard column (Transgenomic CARBOSEP CHO682, Guar Kit, CHO-99-2354, USA) and a carbohydrate column (Transgenomic CARBOSEP CHO682, LEAD column, CHO-99-9854, USA), was used to analyze saccharide compositions of HP. The diluted HP was neutralized and filtered through a 0.2 µm membrane. The sample (20 µL) was then injected into the HPLC. DI water, filtered through 0.2 µm membrane, was used as a mobile phase, with a flow rate of 0.4 mL/min. The column's temperature was maintained at 80°C. Monosaccharides and oligosaccharides were monitored by a refractive index detector. Mannose, glucose, galactose, xylose, and mannan oligosaccharides aqueous solutions with different concentrations were used to construct the standard curves. DHA, as an internal standard, was applied to all HP and the standard curve. Saccharide compositions were reported in % (g of each saccharide in 100 g of dried KP)

3. Results and discussion

3.1 Proximate composition analysis of KP

KP is rich in carbohydrates, especially mannose and glucose [6-31]. The TC content is 81% (Table 1) of the dried weight of KP, which is considered very high.

Table 1 Proximate compositions of KP (%)

Moisture	3.39 ± 0.12
Total ash	6.44 ± 0.192
Crude protein	8.91 ± 0.18
Crude fat	0.42 ± 0.01
Total carbohydrate	80.96 ± 0.34

In this study, we aim to extract most of the TC in KP and preserve the quality of extracted glucomannan-oligosaccharides and monosaccharides in HP, with a set of optimized reaction conditions.

3.2 HPLC analyses for oligosaccharides and types of monosaccharides

The type and percentage of monosaccharides and oligosaccharides in HP were directly determined by HPLC without the need of chemical derivatizations. The retention times of the common monosaccharides, including glucose, xylose, galactose, arabinose, and mannose on HPLC chromatogram were 20.6, 22.4, 24.5, 27.0, and 28.9 min, respectively. The retention times of the standard mannan oligosaccharides and the glucomannan-oligosaccharides in HP were distinctively shorter, at around 9 to 16 min. A carbohydrate column (Transgenomic CARBOSEP CHO682, LEAD column, CHO-99-9854, USA) allows the HPLC assay to directly profile the saccharide contents in HP while avoiding possible errors from the incomplete chemical derivatizations that often occur in typical analysis methods [19-41].

3.3 The effect of reaction temperature on the hydrolysis of KP

The reaction temperature has significantly affected the hydrolysis of KP. In general, SL (Eq. (1)), TC, RS, and protein increased with an increase in the reaction temperature (Fig. 1a).

The SL increased dramatically with the temperature from 110°C to 130°C, to reach 73.4%. Afterward, it increased slightly and reached a maximal value of 86.8% of the dried weight at 200°C. When higher temperatures were applied, some carbohydrates and protein were burned and a black residual solid was generated. This observation corresponds with a decrease in TC and protein at a temperature higher than 180°C. The formation of 5-HMF and intermediate degradation of MBCR required the consumption of proton (H^+) [33-42], and thus, the final pH of HP fluctuated between 2.33 and 2.52 (Fig. 1a).

TC in HP increased dramatically and reached the highest value of 76.5% at 170°C, which accounted for most of TC in KP (81%, Table 1). At higher reaction temperatures, TC decreased because the carbohydrates were degraded to form MBCR products [33] and/or burned to produce a black residual solid, which also corresponds to a dramatic increase of 5-HMF at the high reaction temperature (Fig. 1b).

The RS level increased and reached a maximal value of 61.7% at 190°C because the polysaccharides in KP were hydrolyzed to produce oligosaccharides, which continued degrading to form monosaccharides at high temperature. The released proteins increased with increasing temperature and reached the highest value of 3.9% at 160°C. Then, it decreased and disappeared at 200°C because proteins were degraded at high temperature.

The combination of the heat generated by MCR and 0.05 M HCl as a catalyst accelerates the hydrolysis of polysaccharides in KP to produce glucomannan-oligosaccharides, which were continually degraded to

form monosaccharides. Generally, the obtained monosaccharides and total monosaccharides (TM) were increased while the oligosaccharide level decreased with increasing temperature (Table 2).

The oligosaccharide yield reached a maximal value of 7.6% at 140°C, and then it decreased with increasing temperature because the oligosaccharides were degraded to form monosaccharides (glucose, xylose, galactose, mannose), and to form other intermediate degradation products of MBCR (methylglyoxal, α-dicarbonyl compounds) at high temperature [43-46]. The major monosaccharides found in HP were glucose and mannose, which dramatically increased and reached maximal values of 12.2% and 11.9% at 200°C, respectively. When the higher temperatures were applied, more glucose and mannose were obtained because the extracted glucomannan-oligosaccharides were hydrolyzed to form monosaccharides, which also led to an increase of the TM in HP from 1.8% at 110°C to 28.8% at 200°C.

The degree of MBCR depends on reaction temperature, time, pH, and water activity, and 5-HMF is a key intermediate of the degradation products of MBCR [33]. 5-HMF is produced from monosaccharides, which were hydrolyzed from KP. The UV absorbance level of the HP at 284 nm refers to intermediate degradation products of MBCR [39-40]. During hydrolysis process of KP, the undesirable products of MBCR were generated. Thus, the amount

of 5-HMF in HP was quantified, and the UV absorbance level of the HP was recorded to determine the effect of reaction temperature, HCl concentration on the formation of the intermediate degradation products of MBCR. This information will assist the optimization conditions for the hydrolysis reaction of KP, to selectively produce glucomannan-oligosaccharides and monosaccharides.

In general, 5-HMF and the UV absorbance level of HP increased with increasing reaction temperature (Fig. 1b). 5-HMF and UV absorbance levels of HP increased slightly with the temperature from 110°C to 160°C and then they suddenly increased and reached the maximal values of 8.5% and 2.8 at 200°C, respectively.

The extents of MBCR are proportional to the reaction temperature [33]. Thus, the 5-HMF and UV absorbance levels of HP increase at the high temperatures. This also corresponds to a decrease of oligosaccharides, TM, and protein. In addition, the low reaction temperatures tend to generate more oligosaccharides and significantly limit MBCR.

In summary, the reaction temperature has dramatically affected the hydrolysis of KP. SL, TC, RS, glucose, mannose, and TM increased with increasing temperature. However, the extracted oligosaccharides were still low, and the intermediate products of MBCR were high, which was indicated by 5-HMF proportion and the UV absorbance level of HP at high temperature.
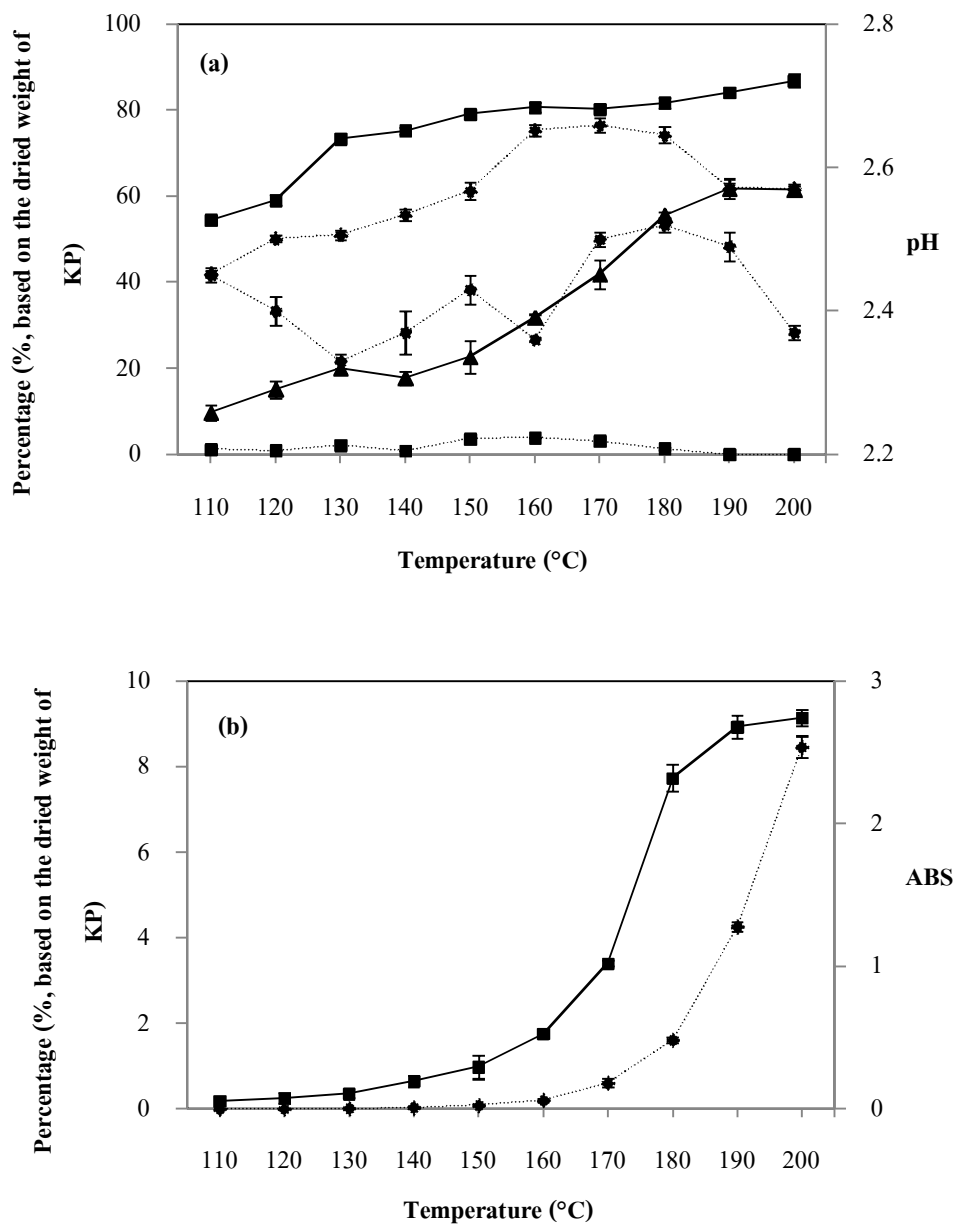
Fig. 1. The effect of temperature on the hydrolysis of konjac powder: (a) solid loss (■, solid line), total carbohydrates (♦, dotted line), reducing sugars (▲, solid line), final pH (●, dotted line), and protein (■, dotted line), and (b) 5-HMF (♦, dotted line) and UV absorbance level (■, solid line) of the hydrolyzed products (15 min, 0.05 M HCl, 10:1 of ratio of reaction volume to konjac powder mass)

Table 2 The effect of reaction temperature on the saccharide compositions of HP

Temperature	Saccharide composition (%, based on the dried weight of KP)					TM
(°C)	Oligosaccharides	Glucose	Xylose	Galactose	Mannose	
110	2.96±0.06	0.94±0.27	0.86±0.14	ND	ND	1.80±0.41
120	6.37±0.13	0.72±0.10	0.75±0.10	ND	ND	1.47±0.20
130	7.10±0.07	0.74±0.27	0.96±0.25	ND	ND	1.70±0.53
140	7.63±0.18	2.63±0.27	2.72±0.41	ND	ND	5.35±0.66
150	6.04±0.26	1.90±0.44	1.52±0.25	ND	ND	3.42±0.52
160	2.12±0.25	3.16±0.39	3.21±0.26	1.01±0.10	1.20±0.26	8.58±0.90
170	1.47±0.08	1.65±0.58	3.16±0.35	1.10±0.00	2.33±0.27	8.24±0.77
180	1.45±0.10	7.10±0.40	5.96±0.28	1.51±0.04	8.00±0.67	22.57±1.20
190	1.44±0.06	9.24±0.18	6.20±0.23	1.20±0.02	10.77±0.16	27.41±0.44
200	1.37±0.04	12.23±0.16	3.59±0.38	1.06±0.03	11.91±0.17	28.80±0.34

ND: Not detectable

3.4 The effect of HCl concentration on the hydrolysis of KP

In general, SL (Eq. (1)), TC, RS and the protein levels dramatically increased with increasing HCl concentration, from 0.05 to 0.4 M (Fig. 2a). The SL and TC increased significantly from 54.5% and 42%, to 81.7% and 76.1% with the increase of HCl concentration from 0.05 M to 0.4 M, respectively. The TC in HP at 0.4 M (76.1%) was accounted for almost all TC in KP (81%, Table 1). However, the increase in HCl concentrations in the range of 0.4 M to 1.4 M did not affect the TC and SL levels. At HCl concentrations higher than 1.4 M, TC and SL decreased because the extracted carbohydrates were degraded to form MBCR intermediate products [33]. RS, which represents the monosaccharides and low molecular weight oligosaccharides levels, dramatically increased and reached the highest value of 70.8% at 0.8 M HCl. It then decreased because some portions of the extracted monosaccharides were degraded to form MBCR intermediate degradation products at high HCl concentration [33]. A decrease of RS at high HCl concentration also corresponded to a decrease of monosaccharides (Table 3), and an increase of 5-HMF at the high HCl concentrations (Fig. 2b). The released protein reached a maximal value of 5.5% at 0.4 M HCl, and it then decreased with increasing HCl concentration.

HCl concentration is a major reaction parameter that positively affects the hydrolysis of KP to generate glucomannan-oligosaccharides, glucose, and mannose at a lower reaction temperature of 110°C. It has been well established that without HCl, in order for the effective hydrolysis of mannans in coconut meal to take place, a set of extreme reaction conditions (250°C) under the subcritical water conditions is required [19]. The amount of extracted oligosaccharides dramatically increased when higher concentrations of HCl were applied. TM increased when the applied HCl concentrations were from 0.05 M to 1.2 M, and TM decreased with the HCl concentrations higher than 1.2 M (Table 3). The yields of glucose, mannose, and TM were vastly improved and reached the highest values of 30.2%, 35.8%, and 69.4% at 1.2 M HCl, respectively. At 1.2 M HCl concentration, the combined yield of glucomannan-oligosaccharides (11.6%) and TM (69.4%) was accounted for all TC in KP (81%, Table 1). When the HCl concentrations were increased to 2 M, the yields of glucose, mannose, and TM decreased gradually to 22%, 22.7%, and 46.3% at 2 M HCl, respectively. However, the yield of oligosaccharides increased and reached a maximal value of 19.2% at 2 M HCl.

The results support the critical strategies developed in this study by combining MCR heating and the HCl catalyst for the hydrolysis of KP. The medium concentration of HCl (1.2 M) accelerates the KP hydrolysis for glucose and mannose for monosaccharide production, while the higher concentration of HCl (2 M) is selective for glucomannan-oligosaccharide production.

A low temperature of 110°C limited the formation of intermediate degradation of MBCR (Fig. 2b). 5-HMF was generated in a small amount, only 0.7%, at 1.4 M HCl concentration, and then it decreased when the higher HCl concentrations were applied. The presence of 5-HMF is undesirable. At high HCl concentrations, the conversions of 5-HMF to other compounds such as, levulinic, formic acids, etc., are accelerated [47-49]. Therefore, it would also be advantageous to use higher HCl concentrations in order to degrade 5-HMF. The UV absorbance level of HP reached a maximal value at 2.3 at 1.6 M HCl, and at higher HCl concentrations, the UV absorbance decreased to 1.7 because more MBCR intermediates were degraded at higher HCl concentrations.

In summary, HCl concentration was the major factor in the hydrolysis of KP process at a low temperature (110°C). The SL, TC, RS, oligosaccharides, monosaccharides, and TM were increased with increasing HCl concentration. Furthermore, the formations of intermediate degradation products from MBCR were significantly minimized by the use of a low reaction temperature at 110°C.
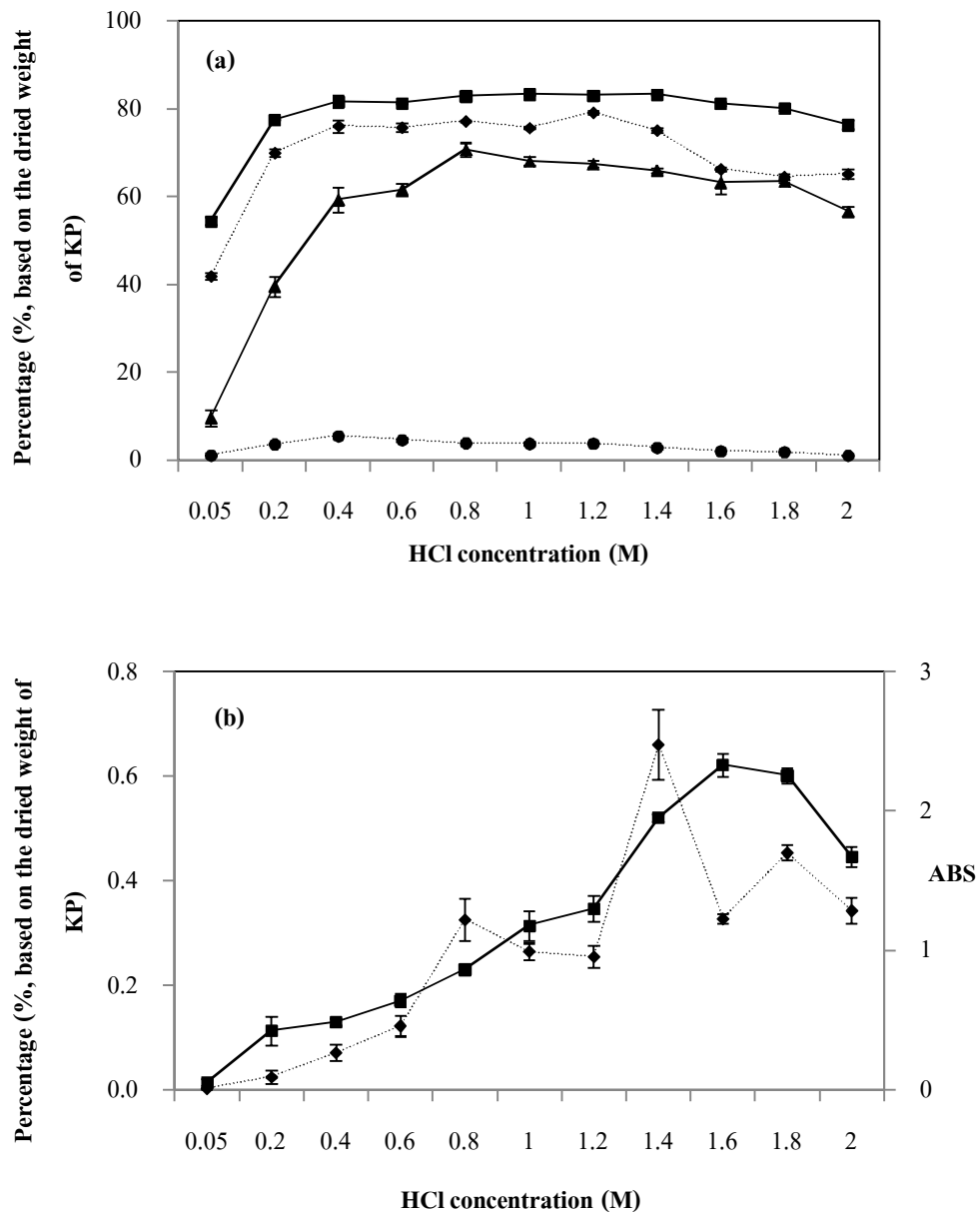
Fig. 2. The effect of HCl concentration on the hydrolysis of konjac powder: (a) solid loss (■, solid line), total carbohydrates (♦, dotted line), reducing sugars (▲, solid line), and protein (●, dotted line), and (b) 5-HMF (♦, dotted line), and UV absorbance level of HP (■, solid line) (110 °C, 15 min, 10:1 of ratio of reaction volume to konjac powder mass).

Table 3 The effect of HCl concentration on the saccharide compositions of HP

HCl concentration (M)	Saccharide composition (%, based on dried weight of KP)					TM
	Oligosaccharides	Glucose	Xylose	Galactose	Mannose	
0.05	2.96±0.06	0.94±0.27	0.86±0.14	ND	ND	1.80±0.41
0.20	2.77±0.32	4.05±0.25	4.81±0.03	0.59±0.10	4.65±0.33	14.09±0.21
0.40	4.76±0.57	13.59±0.74	9.07±0.35	1.18±0.44	20.75±0.81	44.58±1.55
0.60	7.29±0.23	16.77±0.73	3.74±0.46	1.14±0.17	34.43±0.80	56.08±1.60
0.80	8.37±0.49	28.56±0.47	3.45±0.60	1.23±029	34.78±0.50	68.03±1.80
1.00	9.17±0.26	27.37±0.82	3.09±0.22	1.25±0.16	34.74±0.70	66.46±1.42
1.20	11.64±0.84	30.18±1.01	2.11±0.65	1.33±0.20	35.81±0.96	69.44±1.07
1.40	13.23±0.39	27.87±0.37	2.11±0.51	1.21±0.18	30.81±0.30	62.01±0.30
1.60	14.52±0.70	22.12±0.86	1.49±0.61	1.18±0.27	27.54±0.60	52.33±1.97
1.80	15.44±0.64	20.63±0.90	1.42±0.67	1.14±0.13	24.75±0.36	47.95±1.75
2.00	19.23±0.71	21.95±0.64	0.64±0.14	1.02±0.27	22.68±0.78	46.29±1.33

ND: Not detectable

4. Conclusion

In this study, we successfully developed a rapid method to selectively produce glucomannan-oligosaccharides, mannose, and glucose, as well as exactly profile the saccharides obtained from the hydrolysis of KP by using an HPLC assay. The temperature and HCl concentration have considerably affected the hydrolysis of KP. The reaction conditions with high HCl concentration, at 2 M, and low temperature, at 110°C, selectively hydrolyze konjac powder into glucommanan-oligosaccharides. A set of conditions with low temperature, at 110°C, with a medium HCl concentration, at 1.2 M, generates the highest amounts of glucose and mannose. The combination of microwave radiation heating and HCl allows the selective hydrolysis of konjac powder into glucommanan-oligosaccharides. The developed method is also able to extract most of the carbohydrate contents in konjac powder under relatively mild reaction conditions.

5. Acknowledgements

6. References

[1] M. Chua, T.C. Baldwin, T.J. Hocking and C. Kelvin, "Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex NE Br", Journal of Ethnopharmacology 128, 2010, pp. 268-278.

[2] H.L. Chen, H.C. Cheng, Y.J. Liu, S.Y. Liu and W.T. Wu, "Konjac acts as a natural laxative by increasing stool bulk and improving colonic ecology in healthy adults", Nutrition 22, 2006, pp. 1112-1119.

[3] W. Jian, Y. Sun, H. Huang, Y. Yang, S. Peng, B. Xiong, T. Pan, Z. Xu, M. He and P. Jie, "Study on preparation and separation of Konjac oligosaccharides", Carbohydrate polymers 92, 2013, pp. 1218-1224.

[4] J. Chen, D. Liu, B. Shi, H. Wang, Y. Cheng and W. Zhang, "Optimization of hydrolysis conditions for the production of glucomanno-oligosaccharides from konjac using β-mannanase by response surface methodology", Carbohydrate polymers 93, 2013, pp. 81-88.

[5] W. Fang and P. Wu, "Variations of konjac glucomannan (KGM) from *Amorphophallus konjac* and its refined powder in China", Food Hydrocolloids 18, 2004, pp. 167-170.

[6] K. Katsuraya, K. Okuyama, K. Hatanaka, R. Oshima, T. Sato and K. Matsuzaki, "Constitution of konjac glucomannan-chemical analysis and 13 C NMR spectroscopy", Carbohydrate Polymers 53, 2003, pp. 183-189.

[7] S.H. Al-Sheraji, A. Ismail, M.Y. Manap, S. Mustafa, R.M. Yusof and F.A. Hassan, "Prebiotics as functional foods - A review", Journal of Functional Foods 5, 2013, pp. 1542-1553.

[8] M. Roberfroid, "Prebiotics - the concept revisited", The Journal of nutrition 137, 2007, pp. 830S-837S.

[9] P.M. Rolim, "Development of prebiotic food products and health benefits", Food Science and Technology 35, 2015, pp. 3-10.

[10] C.H.L. Chen, Y.H. Fan, M.E. Chen and Y. Chan, "Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice", Nutrition 21, 2005, pp. 1059-1064.

[11] F.H. Al-Ghazzewi, S. Khanna, R.F. Tester and J. Piggott, "The potential use of hydrolysed konjac glucomannan as a prebiotic", Journal of the Science of Food and Agriculture 87, 2007, pp. 1758-1766.

[12] A.R. Oroskar, N.S. Sudharsan, P.A. Oroskar and O.M. Kulkarni, "Mannose production from palm kernel meal using simulated moving bed separation", USA Patent, US20140039180 A1.

[13] A.R. Oroskar, N.S. Sudharsan, P.A. Oroskar and O.M. Kulkarni, "Mannose production from palm kernel meal using simulated moving bed separation", USA Patent, US9163050 B2.

[14] B. Kranjčec, D. Papeš and S. Altarac, "D-mannose powder for prophylaxis of recurrent urinary tract infections in women - a randomized clinical trial", World journal of urology 32, 2014, pp. 79-84.

[15] S. Wang, B. Zhou and B. Li, "Preparation and characterization of konjac glucomannan microcrystals through acid hydrolysis", Food Research International 67, 2015, pp. 111-116.

[16] M. Chua, K. Chan, T.J. Hocking, P.A. Williams, C.J. Perry and T.C. Baldwin, "Methodologies for the extraction and analysis of konjac glucomannan from corms of *Amorphophallus konjac* K. Koch", Carbohydrate Polymers 87, 2012, pp. 2202-2210.

[17] K. Kato and K. Matsuda, "Studies on the Chemical Structure of Konjac Mannan - Part III. Theoretical Aspect of Controlled Degradation of the Main Chain of the Mannan", Agricultural and Biological Chemistry 36, 1972, pp. 639-644.

[18] S. Sen and J.E. Puskas, "Green Polymer Chemistry: Enzyme Catalysis for Polymer Functionalization", Molecules 20, 2015, pp. 9358-9379.

[19] P. Khuwijitjaru, A. Pokpong, K. Klinchongkon and A. Shuji, "Production of oligosaccharides from coconut meal by subcritical water treatment", International Journal of Food Science and Technology 49, 2014, pp. 1946-1952.

[20] C.O. Kappe and D. Dallinger, "Controlled microwave heating in modern organic synthesis-highlights from the 2004 - 2008 literature", Molecular Diversity 13, 2009, pp. 71-193.

[21] C.O. Kappe, "Controlled microwave heating in modern organic synthesis", Angewandte Chemie International Edition 43, 2004, pp. 6250-6284.

[22] M.S. Shaheen, A.H. El-Ghorab, F.M. Anjum and K.F. El-Massry, "Microwave applications in thermal food processing", In: W. Cao (Ed.) "The Development and Application of Microwave Heating", INTECH Open Access Publisher. 2012.

[23] J. Ahmed and H.S. Ramaswamy, "Microwave pasteurization and sterilization of foods", Food Scicen and Technology 167, 2004, pp. 691.

[24] M. Zhang, J. Tang, A. Mujumdar and S. Wang, "Trends in microwave related drying of fruits and vegetables", Trends in Food Science and Technology 17, 2006, pp. 524-534.

[25] M. Berteli, E. Rodier and A.M. Jr, "Study of the microwave vacuum drying process for a granulated product", Brazilian Journal of Chemical Engineering 26, 2009, pp. 317-329.

[26] F. Wiesbrock, R. Hoogenboom and U.S. Schubert, "Microwave assisted polymer synthesis - state of the art and future perspectives", Macromolecular Rapid Communications 25, 2004, pp. 1739-1764.

[27] A. Sosnik, G. Gotelli and G.A. Abraham, "Microwave assisted polymer synthesis (MAPS) as a tool in biomaterials science-how new and how powerful", Progress in Polymer Science 36, 2011, pp. 1050-1078.

[28] A. Hoz, A.D. Ortiz and A. Moreno, "Microwaves in organic synthesis. Thermal and non-thermal microwave effects", Chemical Society Reviews 34, 2005, pp. 164-178.

[29] B. Wathey, J. Tierney, P. Lidström and J. Westman, "The impact of microwave assisted organic chemistry on drug discovery", Drug Discovery Today 7, 2002, pp. 373-380.

[30] A. Richel and M. Paquot, "Conversion of carbohydrates under microwave heating", In: C.F. Chang (Ed.) "Carbohydrates-Comprehensive Studies on Glycobiology and Glycotechnology", INTECH Open Access Publisher. 2012.

[31] Y. Tanaka, K. Okamoto, A. Matsushima, T. Ota, Y. Matsumoto and T. Akasaki, "Microwave assisted Acid Hydrolysis of Konjac Products for Determining the Konjac Powder Content", Analytical Sciences 29, 2013, pp. 1049-1053.

[32] C. Yongyat, S. Ruchirawat and S. Boonyarattanakalin, "Polymerization of mannosyl tricyclic orthoesters for the synthesis of α(1-6) mannopyranan - the backbone of lipomannan", Bioorganic and medicinal chemistry 18, 2010, pp. 3726-3734.

[33] M.C. Mart´ınez, N. Corzo, M. Villamiel and M.D. Castillo, "Browning Reactions", In: B.K. Simpson (Ed.) "Food biochemistry and food processing", John Wiley and Sons, Inc Publishers, Iova (USA). 2006.

[34] A.O.A.C, "*Official Methods of Analysis of AOAC International* (17th ed.)", AOAC International, 2000.

[35] M. Dubois, K.A. Gilles, J.K. Hamilton, P. Rebers and F. Smith, "Colorimetric method for determination of sugars and related substances", Analytical chemistry 28, 1956, pp. 350-356.

[36] M.F. Chaplin and J.F. Kennedy, "*Carbohydrate analysis: a practical approach* (2nd ed.)", IRL Press Ltd., 1994.

[37] M.M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding", Analytical biochemistry 72, 1976, pp. 248-254.

[38] J.E. Noble and M.J. Bailey, "Quantitation of protein", Methods in enzymology 463, 2009, pp. 73-95.

[39] B. Jiang, Y. Liu, B. Bhandari and W. Zhou, "Impact of caramelization on the glass transition temperature of several caramelized sugars. Part I - Chemical analyses", Journal of agricultural and food chemistry 56, 2008, pp. 5138-5147.

[40] S. Haghparast, B. Shabanpour, H. Kashiri, G. Alipour and M. Sudagar, "A comparative study on antioxidative properties of carameled reducing sugars; inhibitory effect on lipid oxidative and sensory improvement of glucose carameled products in shrimp flesh", Journal of Agricultural Science and Technology 15, 2012, pp. 87-99.

[41] D.B. Gomis, D.M. Tamayo and J.M. Alonso, "Determination of monosaccharides in cider by reversed phase liquid chromatography", Analytica Chimica Acta 436, 2001, pp. 173-180.

[42] L.W. Kroh, W. Jalyschko and J. Häseler, "Non-volatile Reaction Products by Heat-induced Degradation of α-Glucans. Part I-Analysis of Oligomeric Maltodextrins and Anhydrosugars", Starch-Stärke 48, 1996, pp. 426-433.

[43] P.H. Farkas, F. Örsi and L.W. Kroh, "Methylglyoxal determination from different carbohydrates during heat processing", Food chemistry 59, 1997, pp. 157-163.

[44] A. Hollnagel and L.W. Kroh, "Degradation of oligosaccharides in nonenzymatic browning by formation of α-dicarbonyl compounds via a "peeling off" mechanism", Journal of agricultural and food chemistry 48, 2000, pp. 6219-6226.

[45] A. Hollnagel and L.W. Kroh, "3-Deoxypentosulose-an α-dicarbonyl compound predominating in nonenzymatic browning of oligosaccharides in aqueous solution", Journal of agricultural and food chemistry 50, 2002, pp. 1659-1664.

[46] S. Benjakul and M. Tanaka, "Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince", Food Chemistry 90, 2005, pp. 231-239.

[47] B.F. Kuster and H.M.G. Temmink, "The influence of pH and weak acid anions on the dehydration of D-fructose", Carbohydrate research 54, 1977, pp. 185-191.

[48] F.S. Asghari and H. Yoshida, "Acid catalyzed production of 5-hydroxymethyl furfural from D-fructose in subcritical water", Industrial and Engineering Chemistry Research 45, 2006, pp. 2163-2173.

[49] F.N. Gomes, L.R. Pereira, N.F. Ribeiro and M.M. Souza, "Production of 5-Hydroxylmethylfurfural (HMF) via fructose dehydration - Effect of solvent and salting out", Brazilian Journal of Chemical Engineering 32, 2015, pp. 119-126.