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Molecular properties and gut health benefits of enzyme-hydrolyzed konjac glucomannans

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Abstract: Konjac glucomannan (KGM) with a molecular weight (MW) of 823.4 kDa was partially degraded by endo-1,4-β-mannanase. Two hydrolyzed KGM fractions (KGM-M-1: 147.2 kDa and KGM-M-2: 21.5 kDa) were characterized and applied to the animal tests in comparison with the native KGM. After oral feeding to the mice, KGM-M-1 and KGM-M-2 significantly increased the levels of short chain fatty acids (SCFAs) in the colonic contents and the native KGM increased SCFAs in the cecum. The more significant effect of the native KGM in the cecum may be attributable to its high viscosity, slowing down the movement of intestinal microflora through the cecum, while the lower MW KGM-M-1 and KGM-M-2 could move more easily through the colon to be fermented by colonic bacteria. This new finding may be useful for future research and development of low-MW KGM polysaccharides through enzyme hydrolysis for the desired gut health benefits.

KEYWORDS: Konjac glucomannan; Enzyme hydrolysis; Molecular weight; Viscosity; Intestinal microflora; Short chain fatty acids.

1. Introduction

Konjac glucomannan (KGM) from the tuber and roots of *Amorphophallus konjac* C. Koch is a water soluble polysaccharide containing both (1→4)-β-D-glucopyranose and (1→4)-β-D-mannopyranose (Felix da Silva et al., 2019). It is a linear and random copolymer, with approximately 1 in 19 sugar units being acetylated at the side C-6 position. It is slightly branched with side chains consisting of mannose and glucose

(Behera & Ray, 2017). KGM is widely applied in food, pharmaceutical and cosmetic products because of its thickening and gelling effects, and biocompatibility (Yang et al., 2017; Cui, Chen, & Yang, 2014; Devaraj, Reddy, & Xu, 2019). It has been widely studied for its various biological activities, including anti-tumor, immunomodulation, wound healing, anti-obesity, anti-hyperglycemic, and prebiotic activities (Behera & Ray, 2017). However, the native KGM is highly viscous and forms gels in water due to its high molecular weight (MW) (over 1000 kDa) and long chain. It is envisaged that partial degradation of KGM to a lower MW may improve the water solubility and favor the application as health products.

Recently, many studies have evaluated various bioactivities of KGM and its partial hydrolyzed derivatives, such as hypoglycemic (Chen, Sheu, Tai, Liaw, &Chen, 2003), relieving constipation (Chen, Cheng, Liu, Liu, & Wu, 2006), and regulating cholesterol metabolism (Tsuji, Tsuji, & Suzuki, 1975). In addition to the native KGM, the partially degraded products of KGM with lower MW have exhibited improved biological functions, such as immunomoldulation (Onishi et al., 2005), antioxidation (Liu et al., 2015; Jian et al., 2017) and growth promotion of probiotics (Al-Ghazzewi & Tester, 2012; Al-Ghazzewi, Khanna, Tester, &Piggott, 2010; Yang et al., 2017). These findings have stimulated the research interest in the partial degradation of KGM for new and improved bioactivities and health benefits. A previous study (Yeh, Lin, & Chen, 2010) has shown that the partial hydrolysis of KGM resulted in greater protective effects against water-induced DNA damage. In another study (Mao et al., 2018), the partially degraded KGM with power ultrasound has been applied to bifidobacterial cultures for protection against selected antibiotics.

Among various methods for depolymerization, enzymatic hydrolysis is one of the most widely used in the degradation of high MW polysaccharides including KGM

(Albrecht et al., 2011; Jian et al., 2013) because of its biocompatibility, selectivity and effectiveness. This study was to evaluate the process of enzymatic hydrolysis of KGM with β-mannanase and the beneficial effects of native and degraded KGM on the intestinal health.

2. Materials and methods

2.1 Materials

Konjac glucomannan (KGM) (Catalogue number KJ30) was kindly provided by Hubei Konson Konjac Gum Co., Ltd (Wuhan, China). Endo-1,4-β-mannanase (*Aspergillus niger*) was purchased from Megazyme (Ireland), which was supplied as an ammonium sulphate suspension in 0.02% sodium azide with an enzyme activity of 600 U/mL. Monosaccharide standards including fucose (Fuc), rhamnose (Rha), arabinose (Ara), glucose (Glc), galactose (Gal), xylose (Xyl), mannose (Man), fructose (Fru), galacturonic acid (GalA) and glucuronic acid (GlcA) were from Merck Corp. (Darmstadt, Germany) or Sigma Chemical Corp. (St. Louis, USA). Xylose (Xyl) was from Shanghai Aladdin Biochemical Technology Co. (Shanghai, China). Short chain fatty acids (SCFAs) including acetic, propionic, butyric and i-valeric acid over 99% purity from Shanghai Aladdin Biochemical Technology Co. (Shanghai, China) were used to as standards. All other reagents were of analytical grade.

2.2 Enzymatic degradation of KGM

2.2.1 Degradation of KGM with different amounts of enzyme

Mannanase was used to degrade konjac glucomannan according toa previous study (Chen et al., 2012). Firstly, 4 g of the native KGM was dissolved in 200 mL distilled water (at concentration of 2% w/v) with stirring magnetically until full swelling. Various amounts (1, 10, 20, 100 μL) of endo-1,4-β-mannanase were then added to the KGM solution and incubated at 37 ℃ for 40 min for evaluation of the enzyme amount on the KGM hydrolysis. The hydrolysis reaction was stopped by heating the solution at 95 ℃ in a water bath for 20 min to inactivate the enzyme. After cooling to room temperature, the solution was mixed with pre-cooled ethanol (4 ℃) at a final concentration of 20%, and kept at 4 ℃ for 6 hours. The supernatant was removed by high-speed refrigerated centrifugation (10000 × *g*, 4 ℃, 15 min) and the precipitate was lyophilized, yielding the degraded KGM fraction.

2.2.2 Degradation of KGM for different time periods

As in the last section, the native KGM was completely dissolved in distilled water at 2% (w/v). Then, 20 μL of endo-1,4-β-mannanase was added into 200 mL of the KGM solution and incubated at 37 ℃ for 1, 5, 20 or 40 min. After the given reaction period, the enzyme was inactivated and the degraded KGM product was recovered with the same methods and conditions as mentioned in the lasted section.

2.3 Preparation of partial degraded polysaccharides for animal tests

Two degraded KGM fractions were prepared, KGM-M-1 and KGM-M-2, by

hydrolysis of the native KGM for 20 min with 2 μL and 20 μL of endo-1,4-β-mannanase, respectively. The procedures and conditions for the enzyme hydrolysis of native KGM and the subsequent recover of the degraded KGM products were the same as described in section 2.2.1.

2.3.1 Analysis of composition and molecular weight

Total sugar content was determined by phenol-sulfuric acid with mannose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was determined by Coomassie brilliant blue method with bovine serum albumin as a standard (Bradford, 1976). Uronic acid content was measured by carbazole-sulfuric acid method with galacturonic acid as a standard.

The polysaccharide sample (5 mg) was firstly hydrolyzed by 12 M H_2SO_4 (0.5 mL) in an ice bath for 0.5 h, then diluted with ultra-pure water for another 2 h of hydrolysis at 100℃ according to literature (Liu et al., 2017). The hydrolysate was diluted and applied to monosaccharide analysis using a Dionex ICS-5000 System (Therom Scientific, CA, USA), which was equipped with a CarboPac^{TM} PA20 guard column (3 mm×30 mm) and a CarboPac^{TM} PA20 analytical column (3 mm×150 mm) in series connection

The molecular weight (MW) of polysaccharides was determined by high performance size exclusion chromatography (HPSEC) with an instrument system consisting of a multi-angle laser light scattering (MALLS) detector (wavelength of 663.6 nm, eighteen angles) (Dawn Heleos II, Wyatt), a refractive index (RI) detector

(OPTILAB T-rEX, Wyatt) and a viscometer (Visco Star II, Wyatt). Three SEC columns in series were used, including an Ohpak SB-G guard column (8×50 mm), an Ohpak SB-806 HQ column (8×300 mm) and an Ohpak SB-804 HQ column (8×300 mm) from Showa Denko K.K. (Tokyo, Japan). The solution (0.1 mol/L $NaNO_3$ containing0.02% NaN_3) was used as mobile phase (0.6 mL/min), and sample injection volume was 100 μL. The column temperature was kept at 35.0 ℃. Data were analyzed using the ASTRA software (Version 6.1.1.84).

2.3.2 FT-IR and SEM

An appropriate amount of polysaccharide sample was ground with KBr powder in the mortar. It was scanned with a Fourier transform infrared spectrometer (Nicolet 5700, Thermo Fisher Scientific, Madison, USA) from 4000 to 500 cm^{-1}, with a resolution of 4 cm^{-1}.

The polysaccharide samples were completely dissolved in ultra-pure water at 1.0 mg/mL, and then placed in liquid nitrogen for 10 minutes. After being freeze-dried, gold film was evaporated by gold spray treatment. Scanning electron microscope (SEM) observation was performed on a JMS-6701 F Field Emission SEM (Japan Electronics Co. Ltd., Tokyo, Japan).

2.4 Animal tests on KGM and hydrolyzed samples for gut health

2.4.1 Animal experiment design

Kunming mice, male, weighing 15.0-18.0 g [Grade II, Certificate Number SCXK (Xiang) 2016-0002], were purchased from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China). All animals used in this study were raised according to the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institute of Health (NIH, Publication No. 85-23, 1996). All experimental procedures were approved by the Animal Care Review Committee of Nanchang University. Mice were raised in cages in a room with controlled temperature (25 ± 1 ℃), relative humidity (50 ± 5%), and 12/12 h of light-dark periods. They were treated with *ad libitum* food and water before starting the experiments.

Mice were randomly divided into four groups (12 in each). Mice in the treatment groups received KGM, KGM-M-1 and KGM-M-2 at a dosage of 200 mg/kg bw and the control group received an equal dose of physiological saline by oral gavage at 8:30 am every day. The body weight (bw) of mice was recorded every day. On day 14, they were fasted for 24 h with free access to water after the last oral administration. All mice were sacrificed on day 15, from which the ceca and colons were aseptically removed and placed on an ice-cold plate. The cecal and colonic contents were collected for the determination of SCFAs level, pH, and moisture and for the measurement of the colon. The colon index was equal to the net weight of colon divided by the body weight of animal (Liu, Wang, Ma, Nie, & Yin, 2016).

2.4.2 Determination of fecal moisture content

The feces were collected on the day 0, day 7 and day 14 of treatment from each two mice combined, and dried to constant weight in an oven at 105 ± 5 ℃.

2.4.3 Analysis of SCFAs and pH

Sample preparation followed the method as described previously (Hu, Nie, & Xie, 2013). Briefly, colonic and cecum contents were diluted with deionized water (1:14 w/v), mixed intensively on a vortex mixer for 5 min and sonicated for another 5 min. The mixture was then cooled in an ice water bath for 20 min, and centrifuged at 4 ℃ for 20 min (4800 ×*g*) to retain the supernatant for analysis. The pH was measured directly with pH meter.

The SCFAs in the fecal samples were analyzed by gas chromatography (GC) as reported previously (Hu et al., 2013). The analysis was performed on an Agilent 6890N GC system with a GC column (30 m × 0.32 mm × 0.25 μm, HP-FFAP) (Agilent Technologies Inc., California, USA). The fecal supernatant was filtered through 0.22 μm membrane before injection. Nitrogen gas was used as the carrier gas at a flow rate of 12 mL/min with a split ratio of 1:10.

2.4.4 Statistical processing of experimental data

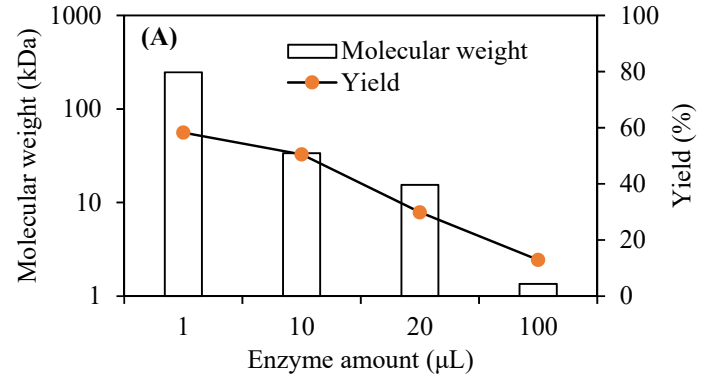
The results of animal experiments were represented as mean ± standard deviation (SD). Statistical analysis of experimental data was performed using SPSS 22.0 statistical software (SPSS Inc., Chicago, United States). One-way analysis of variance

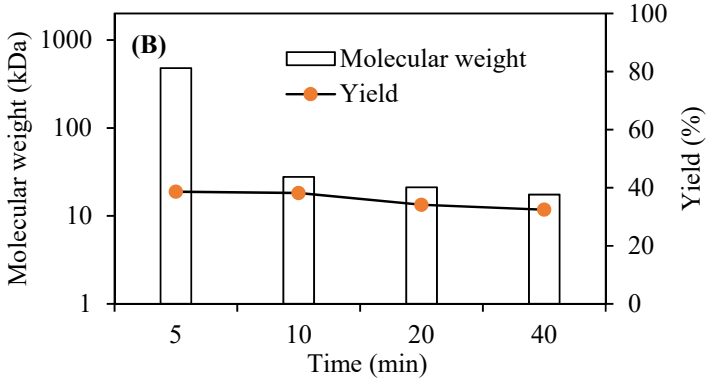
(ANOVA) was adopted to compare the significant differences among all of groups using Tukey's analysis. Differences were considered to be significant at $P< 0.05$.

3. Results and Discussion

3.1 Effects of enzymatic treatment on molecular properties of KGM

Fig.1 shows effects of endo-1,4-β-mannanase treatment on the MW and yield of degraded KGM products. The MW showed a steady decrease with the amount of enzyme (Fig. 1A). The molecular weight of KGM decreased sharply in the initial period of 10 minutes and only slightly in the later period. The results indicated that KGM could be effectively degraded by this enzyme in a short period of time. The yield of degraded products also decreased steadily with the reduction of molecular weight.





208 **Fig.1.** Molecular weight change of KGM and yield of degraded KGM product with (A)
209 different amount of endo-1,4-β-mannanase and (B)various time.
210

211 Table 1 shows physicochemical properties of the native KGM and its degraded
212 products, KGM-M-1 and KGM-M-2. The sugar content of KGM was up to 85.3% (Yin,
213 Ma, Siu, & Wu, 2019), but was decreased to 68.5% for KGM-M-1 and to 65.3% for
214 KGM-M-2. The protein content of KGM was negligible but was detectable in KGM-
215 M-1 and KGM-M-2, which were mainly from the added enzyme. The molecular weight
216 dramatically decreased as the enzyme concentration increased, 21.5 kDa for KGM-M-
217 2 compared to 147.2 kDa for KGM-M-2. Similarly, both intrinsic viscosity and R_h
218 decreased appreciably after the enzymatic hydrolysis.

219 The two degraded KGM fractions were composed of Man and Glc in different ratios.
220 The percentage of Glc and Man in KGM was 44.0% and 33.5%, as the ratio was 1.3:1.0
221 (Yin et al., 2019). In comparison with the literature data, there was a small difference
222 in molar ratio of Man/Glc in KGM (Nishinari, Williams, & Phillips, 1992) due probably
223 to different sources. After the enzymatic treatment, the molar ratio of Man/Glc was
224 1.7:1.0 for KGM-M-1 and 1.3:1.0 for KGM-M-2.
225

Table 1 Physicochemical properties of KGM and its enzyme-degraded products (KGM-M-1 and KGM-M-2 were derived from enzymatic degradation of KGM with 2 μL and 20 μL of endo-1,4-β-mannanase, respectively).

	KGM[d]	KGM-M-1	KGM-M-2
Yields (%) [a]	1	84.0	66.2
Neutral sugar content (%) [b]	85.3±0.15	68.5±2.07	65.3±2.36
Uronic acid content (%) [c]	n.d.	n.d.	n.d.
Protein content (%)[b]	n.d[c]	0.17±0.30	0.30±0.20
M_w (kDa)	823.4	147.2	21.5
M_n (kDa)	540.7	90.8	15.6
M_w/M_n	1.5	1.6	1.4
$[\eta]$	1803.5	229.4	48.6
Rh (nm)	60.0	15.8	5.1

[a] mass ratio to native KGM.
[b] mean ± standard deviation (SD) at $n = 3$.
[c] n.d.: not detectable.
[d] Properties of KGM from our previous report (Yin et al., 2019).

Fig.2 shows the FT-IR spectra of the three KGM polysaccharides. There was no significant difference in the spectra among them. The absorption peaks near 3354 cm^{-1} and 2890 cm^{-1} were the stretching vibration of the hydroxyl and methyl groups, respectively. There was a characteristic peak of acetyl group at 1732 cm^{-1} (Yu, Huang, Ying, & Xiao, 2007). The peaks of 885 cm^{-1} and 806 cm^{-1} were characteristic absorption of mannopyranose (He, Pan, & Wang, 2009).

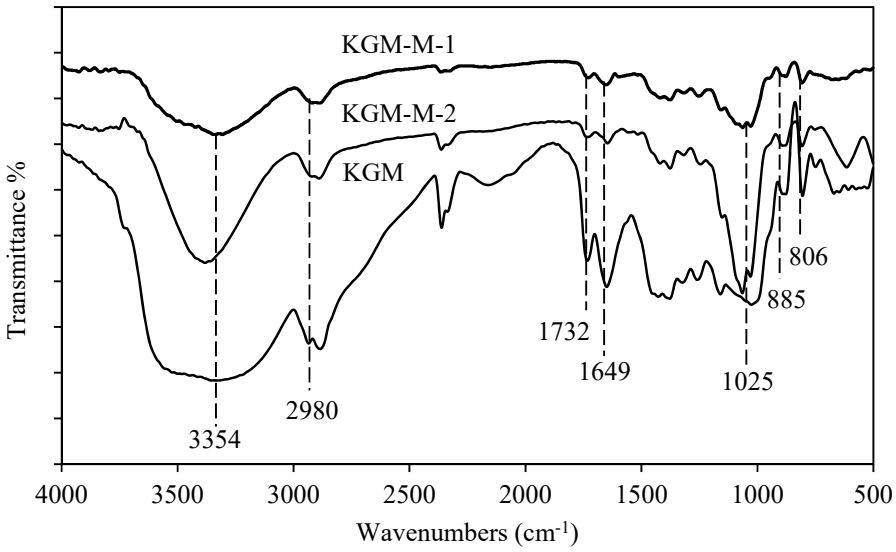


Fig.2. FT-IR spectra of KGM, KGM-M-1 and KGM-M-2. Data of KGM was from literature (Yin, Ma, Siu& Wu, 2018).

Fig. 3 shows the SEM images of KGM and hydrolyzed products. The native KGM appeared like a bundle of rice straws with the stems well oriented in the same direction and the branches randomly cross-linked. However, the enzyme degraded products of KGM, KGM-M-1 and KGM-M-2, appeared as irregular and amorphous fragments of various shapes including planner, fibrous and particulate. These changes in the aggregate morphology of KGM were similar to those for KGM after degradation by power ultrasound in our previous study (Yin et al., 2019).

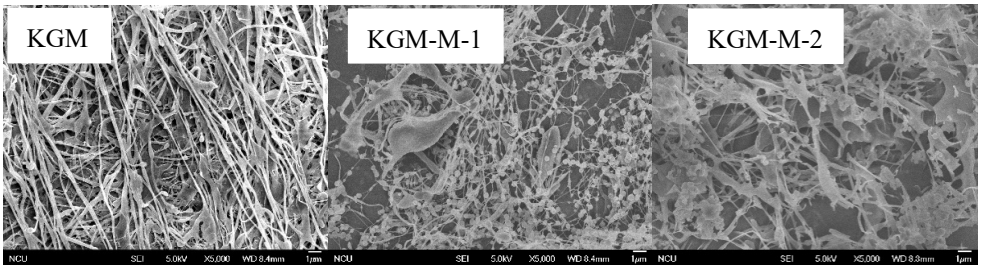


Fig.3. SEM images of KGM-M-1 and KGM-M-2 (×5000). Data of native KGM was from the literature (Yin, Ma, Siu & Wu, 2018).

3.2 Effects of KGM on intestinal functions

Table 2 summarizes effects of the three konjac polysaccharides on body weight, colonic weight, colonic length and colonic index of mice. After 7 days of oral administration, average body weight of the polysaccharide-treated groups was higher than that of the control group, but no significant difference was observed except the KGM group. After day 14, there was no difference in body weight among the groups. The group treated with KGM-M-1 had significantly larger colon length and colonic index than those of the control group and the groups treated with KGM or KGM-M-2. Colonic length and colonic index are two important indices to describe the health of the colon and bowel disease leads to shorter colon length (Jädert, Phillipson, Holm, Lundberg, & Borniquel, 2014), while the increase of colon length and colonic index indicates improved intestinal function (Min, Wan, Nie, & Xie, 2014). The results suggest that moderate but not extensive degradation of KGM could enhance the beneficial effects on the intestinal functions.

Table 2 Effects of three KGM polysaccharides on body weight, colonic weight, colonic length and index of colon in mice.

Group	Body weight (g)		Colonic	Colonic	Colonic
	Day 7	Day 14	weight (g)	length (cm)	index (%)
Control	32.59±1.37^{a}	38.81±1.94^{a}	0.20±0.04^{a}	5.9±0.79^{a}	0.54±0.07^{a}
KGM	34.23±1.03^{b}	38.57±1.71^{a}	0.21±0.06^{ab}	6.58±1.78^{a}	0.54±0.17^{a}
KGM-M-1	33.39±1.31^{ab}	37.77±1.26^{a}	0.27±0.04^{c}	7.68±1.42^{b}	0.72±0.08^{b}
KGM-M-2	33.19±1.40^{ab}	38.19±1.68^{a}	0.24±0.03^{bc}	6.42±0.60^{a}	0.65±0.08^{b}

Note: each value was the mean ± SD (n = 6); values in the same column with different

letters were significantly different ($P < 0.05$)

3.3 Effects of KGM on fecal moisture content

Fig. 4 shows the moisture contents of animal feces on day 0, day 7 and day 14 of KGM polysaccharide feeding. At the beginning, the water contents of animal feces in control and the polysaccharide-treated groups were in the range of 57.8%-60.4% without any significant difference. After oral feeding of polysaccharides for 7 days, the fecal water contents of polysaccharide-treated group increased significantly ($P< 0.05$). However, there was only a small increase in fecal water content by day 14. Intake of the native and degraded KGM polysaccharides can increase the fecal water content, especially KGM-M-2 ($P < 0.05$). The results showed that the intake of KGM polysaccharides could accelerate the peristalsis of the intestine and promote the defecation of the mice.

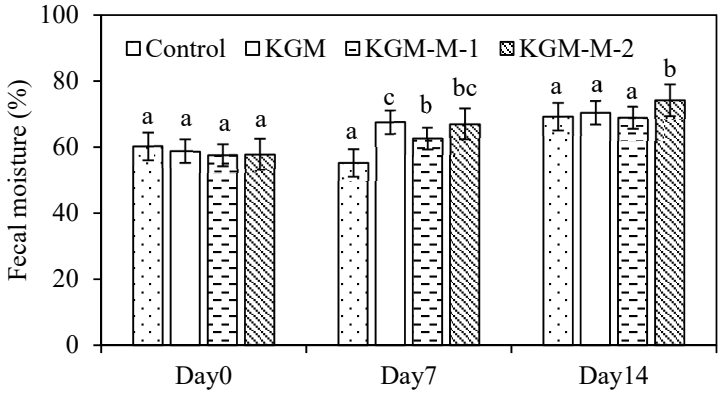
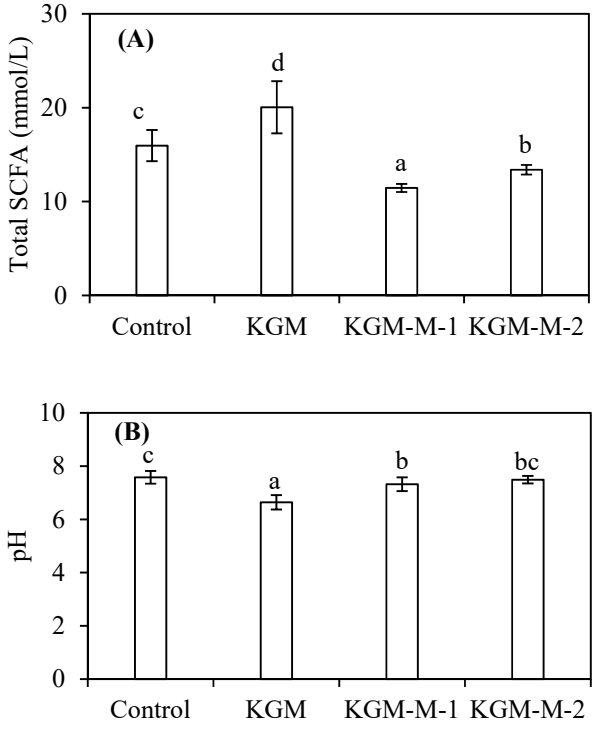


Fig.4. Effects of three konjac polysaccharides on fecal moisture content of mice. Data was expressed as the mean ± SD (n =12). Values in each test period with different letters were significantly different ($P < 0.05$).

3.4 Effects of KGM polysaccharides on SCFAs and pH in cecum

290 Fig. 5 shows the total concentration of short-chain fatty acids (SCFAs) including
291 acetic acid, propionic acid and butyric acid in the cecum of the KGM polysaccharide-
292 treated groups and the control group. Compared with the control (15.96 mmol/L), there
293 was a significant decrease in the total SCFAs in KGM-M-1 (11.45 mmol/L) and KGM-
294 M-2 (13.39 mmol/L), while that of the KGM group increased significantly (20.04
295 mmol/L) ($P< 0.05$). The pH value from the lowest to highest was KGM group (6.64),
296 KGM-M-1 group (7.32), KGM-M-2 group (7.49) and control group (7.58),
297 successively. Comparing with the control group, the pH of the polysaccharide-treated
298 group decreased in varying degrees. A previous study (Xu, Qin, Wu, & Zhong, 2015)
299 has shown that the carboxymethyl KGM derived from chemical modification of KGM
300 had a lower effect on the SCFAs of caecum contents in mice.



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302

303 **Fig.5.** Effects of three konjac polysaccharides on (A) total SCFA concentration and (B)
304 pH in cecum contents of mice. Data was expressed as the mean ± SD (n =12). Different

305 letters in each figure mean significantly different from each other ($P< 0.05$).

306

307 Fig. 6 shows the concentrations of four individual SCFAs in the cecum contents
308 of polysaccharide-treated and control group. In comparison with the control group, the
309 concentrations of acetic acid, propionic acid and butyric acid in the KGM group were
310 significantly higher than those of control group ($P< 0.05$), while concentrations of these
311 three acids in the other polysaccharide-treated group were much lower. In the KGM-
312 treated group, acetic acid and n-butyric acid increased more significantly ($P< 0.05$).
313 Butyric acid is an important substance in intestinal fermentation products, and the most
314 important source of energy for human colon and cecum epithelia cells. The results
315 suggest that microorganisms in the cecum of mice can use KGM for production of more
316 butyric acid.

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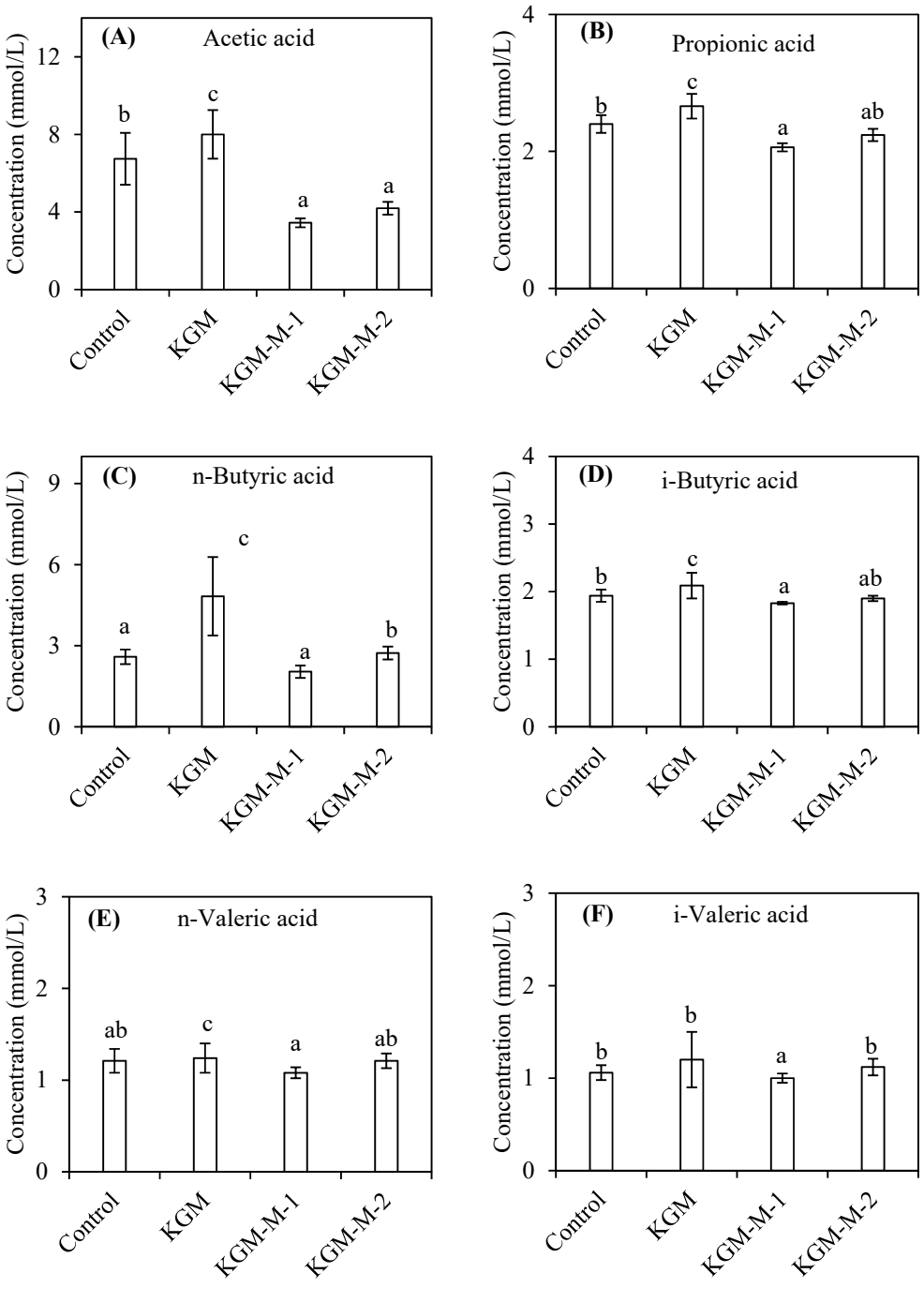
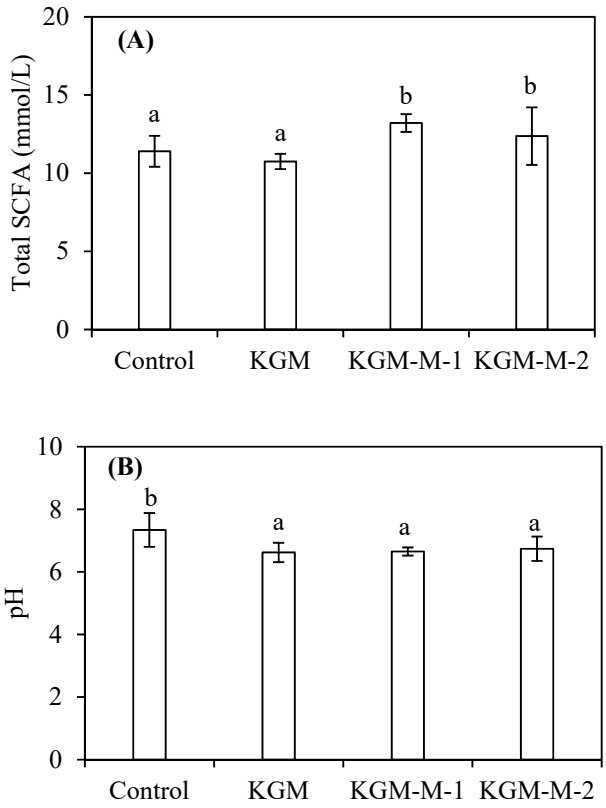


Fig.6. Effects of three konjac polysaccharides on the concentration of (A) acetic acid, (B) propionic acid, (C) n-butyric acid, (D) i-butyric acid, (E) n-valeric acid and (F) i-valeric acid in cecum contents of mice. Data was expressed as the mean ± SD (n =12). Different letters in each figure mean significantly different from each other ($P< 0.05$).

3.5 Effects of polysaccharides on SCFAs and pH in colon

Fig.7 shows the total SCFA concentration in the colonic contents of polysaccharide-treated groups and control group. The total SCFA concentrations in the polysaccharide-treated groups were significantly higher than those in the control group except for the KGM group ($P< 0.05$). The biological effects of polysaccharides in the animals are dependent on the structural characteristics and physical properties such as monosaccharide composition, molecular weight or degree of polymerization, and solubility. In general, polysaccharides with a lower molecular weight (or degree of polymerization) and a higher water solubility can produce a higher amount of SCFAs during colonic fermentation (Nilsson & Nyman, 2005). Concomitantly, the pH of the polysaccharide-treated groups was significantly lowered ($P< 0.05$). SCFAs are mainly produced from indigestible carbohydrates in food by anaerobic bacteriolysis in the colon lumen (Chen, Cao, & Liu, 2006). SCFAs have many physiological functions, such as providing energy for intestinal mucosal cells, promoting cell growth and metabolism, reducing the pH value in the colon and inhibiting the growth of harmful bacteria (Bae, Park, Ji, & Park, 2013). Decrease of the colonic pH can create an acidic environment, inhibiting the growth of harmful bacteria and lowering the risk of intestinal dysfunction (Hernandez, Sanz, Kolida, Rastall, & Moreno, 2011).



345

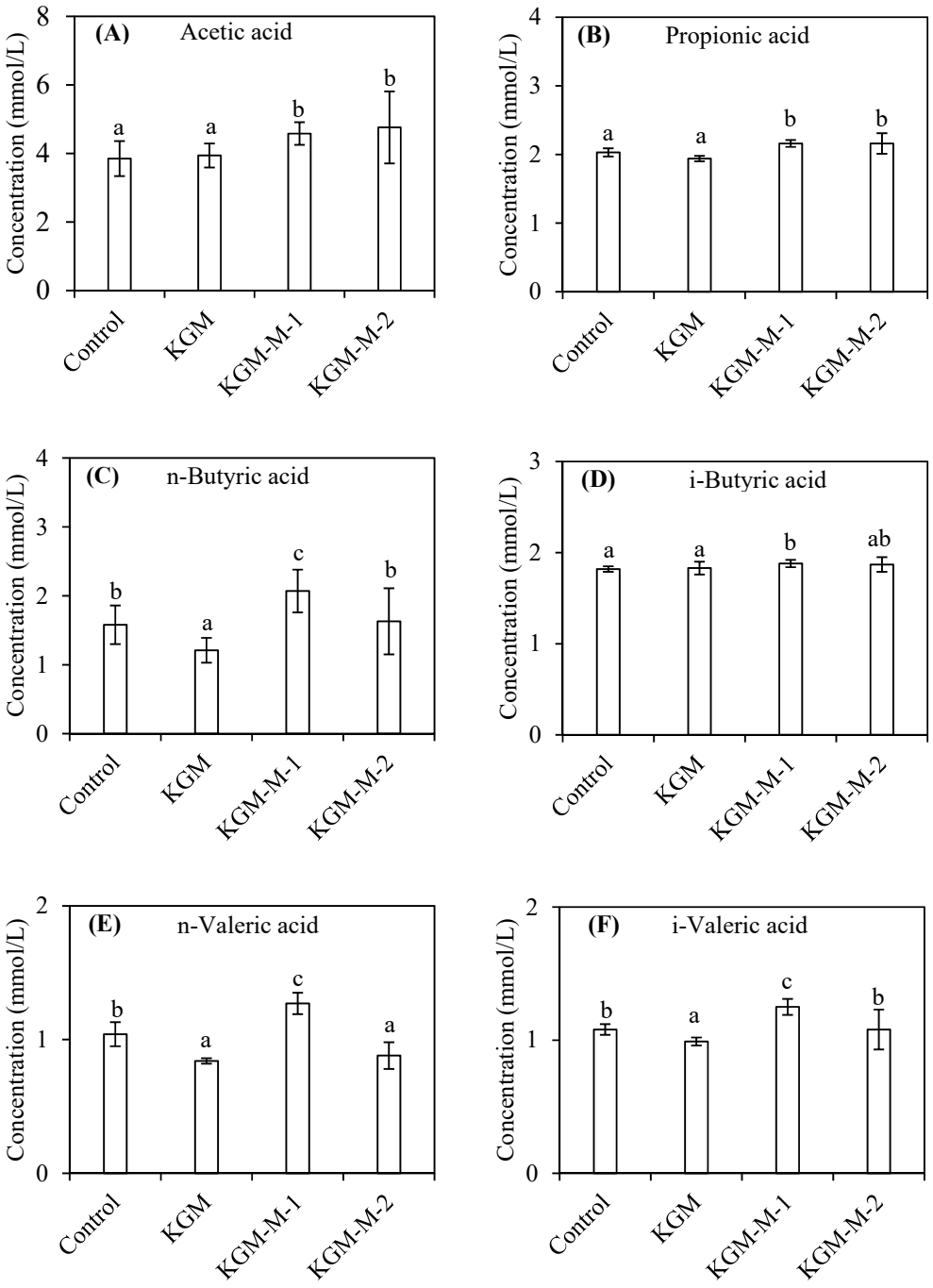
346

347 **Fig.7.** Effects of three kinds of konjac glucomannan on (A) total SCFA concentration
348 and (B) pH in colon contents of mice. Data were expressed as the mean ± SD (n =12).
349 Different letters in each figure mean significantly different ($P < 0.05$) from each other.

350

351 As shown in Fig.8A, the concentration of acetic acid increased notably in the
352 groups treated with KGM-M-1 (4.58 mmol/L) and KGM-M-2 (4.76 mmol/L), which
353 were both significantly higher than that in the KGM group (3.94 mmol/L) ($P< 0.05$). In
354 Fig.8B, the propionic acid concentrations in the KGM-M-1 and KGM-M-2 group
355 increased significantly ($P< 0.05$). The concentration of valeric acid in the groups treated
356 with KGM and KGM-M-1 were lower than the control group. The concentration of n-
357 butyric acid in the KGM-M-1 treated group was significantly higher than in the control
358 group. Acetic acid is the main product of protein degradation and amino acid
359 fermentation in colon (Bianchi et al., 2011). It is involved in the metabolism of muscles,
360 spleen, heart and brain. Butyric acid can be absorbed by colonic epithelial cells and is
361 preferred as energy source of cecum (Pryde, Ducan, Hold, Stewart, & Flint, 2002).

362 Liver and cholesterol metabolism are affected by propionic acid.



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365

366 **Fig.8.** Effects of KGM polysaccharides on the concentration of (A) acetic acid, (B)
367 propionic acid, (C) n-butyric acid, (D) i-butyric acid, (E) n-valeric acid, and (F) i-valeric
368 acid in colonic contents of mice. Data were expressed as the mean ± SD (n =12).
369 Different letters in each figure mean significantly different from each other ($P< 0.05$).

370

371 The fermentation of glucomannans by the anaerobic bacteria in the large intestine
372 results in the production of SCFAs. The above results showed that the native KGM was
373 good for the gut health of mice. Previous studies have shown that hydrolyzed konjac
374 glucomannan was an effective prebiotic in mice (Elamir et al., 2008; Pan, Chen, Wu,
375 Tang, & Zhao, 2009), even better than the native one (Chen, Fan, Chen, & Chan, 2005).
376 In the present study, enzymatic degradation of native KGM at the given conditions
377 mainly caused reduction of the MW but not major structural changes. The intake of
378 KGM-M-1 and KGM-M-2 significantly increased the total concentration of SCFAs in
379 the colonic contents, while the native KGM increased the total concentration of SCFAs
380 in the cecum. The native KGM with a much higher MW may have a more significant
381 impact in the cecum because of its high viscosity and slow movement. KGM-M-1 and
382 KGM-M-2 with a lower MW and a lower viscosity can pass through the cecum quickly
383 to reach the colon, to be fermented in the colon.

384

385 **4. Conclusions**

386 Enzymatic hydrolysis of native KGM was effective to produce low-MW products
387 with similar structure and slightly different chemical composition. The enzyme
388 degradation also caused disruption of the microstructure of KGM from orderly and
389 compact linear structure to loose and amorphous irregular structures. In association
390 with the physiochemical changes, the enzyme-degraded KGM polysaccharides showed
391 functional differences from the native KGM as a dietary fiber and functional

polysaccharides in the animal experiments. The high MW native KGM appeared to be active mainly in the cecum, while the low-MW KGM hydrolysates were more active in the colon. This difference was probably attributed to the difference in the MW and the resulting liquid viscosity between the KGM polysaccharides, which determined or affected their movement through the large intestine. These findings may be useful for understanding the relationship between the MW of polysaccharides and its gut health promotion activities. It is of interest to examine the MW-dependent effect of KGM polysaccharides on the microbial composition of the gut microbiota in future studies.

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