Carbohydrate Polymers 92 (2013) 1218–1224

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Study on preparation and separation of Konjac oligosaccharides

Wenjie Jian[a,b], Yuanming Sun[a,*], Huan Huang[a], Youhui Yang[a], Shuhui Peng[c], Bo Xiong[d], Tingtiao Pan[d], Zhenlin Xu[a], Mingxiang He[e], Jie Pang[d,*]

[a] College of Food Science, South China Agricultural University, Guangzhou 510642, People's Republic of China
[b] Department of Biological Technology, Xiamen Ocean Vocational College, Xiamen 361012, People's Republic of China
[c] Department of Food Science, Guangzhou City Polytechnic, Guangzhou 510405, People's Republic of China
[d] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, People's Republic of China
[e] Fuqing Quality Metrology Inspection Institute, Fuzhou 350300, People's Republic of China

ARTICLE INFO

Article history:
Received 9 August 2012
Received in revised form 3 September 2012
Accepted 24 September 2012
Available online 23 October 2012

Keywords:
Konjac Glucomannan
Irradiation
Enzymatic hydrolysis
Oligosaccharides
Ultrafiltration

ABSTRACT

To study the preparation and separation of Konjac oligosaccharides, Konjac Glucomannan was degraded by the combination of γ-irradiation and β-mannanase, and then the degradation product was separated by ultrafiltration. To our interest, for most of Konjac oligosaccharides obtained by this method, the molecular mass was lower than 2200 Da. In addition, the 1000 Da molecular weight cut off membrane could effectively separate the Konjac oligosaccharides. In conclusion, the combination of γ-irradiation and β-mannanase was an efficient method to obtain Konjac oligosaccharides, and the oligosaccharides of molecular mass lower than 1000 Da could be effectively separated by ultrafiltration.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Konjac Glucomannan (KGM) is an essential polysaccharide composed of glucose and mannose in the ratio of 1:1.6 by the linkage of β-1, 4 glycoside (Jian, Zeng, Xiong, & Pang, 2011). It has been widely applied on food, biological materials and pharmaceutical industry because of the excellent properties, such as thickening, gelatin, and biological compatibility (Pang, Lin, Zhang, Tian, & Sun, 2003).

Recent researches found that its degradation products with different molecular mass have particular biological functions, such as anti-tumor (Vuksan et al., 2000), immunoregulation (Onishi et al., 2005), and cytothesis (Yeh, Lin, & Chen, 2010). These findings promote researchers to pay more attention to the research and development of Konjac degradation product (Suzuki et al., 2010). Presently, several strategies have been developed to obtain oligosaccharides by the depolymerization of Konjac Glucomannan (Courtois, 2009), such as acid degradation, enzymatic hydrolysis (Albrecht et al., 2011), oxydative degradation, and physical methods (Pang et al., 2012). In the previous researches, we found that γ-irradiation could effectively degrade Konjac Glucomannan. But the molecular mass distribution of the products was wide. Besides, the molecular weight is always higher than 400,000 Da in the safe irradiation dose (Xu, Sun, Yang, Ding, & Pang, 2007). Enzymatic hydrolysis has been widely applied in the degradation of polysaccharide because of the characteristics, safety and in room temperature (Qi, Li, & Zong, 2003). However, it is difficult to obtain plenty of oligosaccharides only by enzymatic hydrolysis.

Besides degradation method, the mainly problem limiting the wide use and research of Konjac degradation production is the method to obtain pure and narrowly distributed molecular mass oligosaccharides. So, it is significant to find a suitable method to effectively and precisely separate the degradation products. So far, column chromatography has been extensively used in the separation and purification of polysaccharides (Wu, Li, Che, Zhu, & Kang, 2012). But Column chromatography exhausts much time and elution, and the concentration of product is low.

Ultrafiltration has been widely applied on the separation of proteins, because its device is simple, and it has several advantages, such as good selectivity and low energy requirement (Guan, Shi, Yu, & Xu, 2011; Zhang et al., 2009). In earlier stage, with the technological limit, it has not been applied in the separation of polysaccharides. However, with the development of membrane filtration technology, several researches about the application of ultrafiltration on polysaccharides have been reported (Prabhakar, Ghosh, Bindal, & Tewari, 2011; Xing & Li, 2009).

In this experiment, we decomposed Konjac Glucomannan by the combination of γ-irradiation and β-mannanase; furthermore, the oligosaccharides were separated by ultrafiltration technology.

* Corresponding authors. Tel.: +86 20 33867896; fax: +86 20 85288279.
E-mail addresses: ymsun@scau.edu.cn (Y. Sun), pang3721941@163.com (J. Pang).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.09.065

This work will provide a new method and theoretical guide for the preparation and separation of oligosaccharides.

2. Materials and methods

2.1. Materials and enzymes

The KGM polysaccharide was obtained from San Ai Konjac Food Co., (Yibin City, China). The KGM had a molecular mass of approx. 500 kDa, with the purity up to 85%.

Endo-β-(1, 4)-mannanase (EC 3.2.1.78) was purchased from Huzhou Biolily Technology Co., Ltd. (Zhejiang, China). The enzymatic activity was 3600 kU/g, and its optimal condition was in pH 3.5–5.5, 50 °C. All chemicals used were of analytical grade.

2.2. Method

2.2.1. Purification of KGM

10 g KGM was washed with 50 mL 50% (V/V) ethanol containing 0.1% sodium azide for three times so as to remove water-soluble impurity. After drying in air, the powder was degreased in 50 mL mixed solvent of absolute aether/absolute ethanol [2:1(V/V)] at 40 °C under stirring condition for 8 h. Afterwards the degreased sample was dissolved in distilled water to be a 0.6% (W/V) hydrosol. The fibrin and other insoluble impurity were removed with overspeed freezing centrifuge at a rate of 16,000 r/min for 20 min. Then the clear solution was gotten rid of, appropriate amount of amylase was added to remove amylum at room temperature till enzymatic hydrolysis was complete. Deproteinization was carried out on the enzymatic hydrolysis liquid with sevag method for five times. The centrifugation process was carried out again and its water phase was obtained. The same volume 95% ethanol (V/V) was added to deposit sample and then washed with absolute ethanol and aether. Finally, the sample was treated by vacuum freeze drying to obtain white flocculent KGM.

2.2.2. Degradation by γ-irradiation

The purified KGM powder (100 g) was sealed in polyethylene bags and irradiated at Radiation Center of Guangdong Province with a ^{60}Co source at room temperature. The target dose was 10 kGy and 20 kGy respectively. Samples were stored in refrigerator (at 4 °C) for further use.

2.2.3. Enzymatic hydrolysis

Digestions with endo-β-(1, 4)-mannanase were performed in 0.01 M sodium citrate buffer at pH 4.0. After γ-irradiation, 2 g KGM powder was slowly added to 1000 mL vigorously stirred sodium citrate solution and swelled for about 30 min. Then, 0.2 g endo-β-(1, 4)-mannanase was added to the solution. The incubation was carried out in 50 °C for 24 h at shaking conditions. After hydrolysis, the enzymes were inactivated (10 min, 100 °C), and the hydrolysate was centrifuged (20 min, 7000 rpm, room temperature).

2.2.4. Desalination of the hydrolysate

After centrifugation, the hydrolysate was desalted by cationic exchange resin (732) and anion-exchange resins (717). The column for the two resins was 45 cm × 3.5 cm. About 1000 mL hydrolysate solution flowed through anion-exchange resins (732) and anion-exchange resins (717) in the speed of 300 mL/h.

2.2.5. Separation of the hydrolysate

Following desalination, the degradation products of Konjac Glucomannan was separated by molecular weight cut off of the membrane through ultrafiltration. The operation was performed in membrane separating devices (Shanghai Shiyuan biological engineering equipment Co., Ltd., China), with the Centramate T-series cassette membrane (Pall Corporation, USA). The molecular weight cut off for the membrane used was 3×10^5 Da, 1×10^5 Da, 5×10^4 Da, 5×10^3 Da, and 1×10^3 Da respectively.

In order to separate effectively, the separation was operated successively from maximum molecular weight cut off (3×10^5 Da) to the least molecular weight cut off (1×10^3 Da). Meanwhile, the dialysate of the previous operation was used as the feed solution of the next operation. So, the degradation products were fractioned into 6 groups of different molecular mass range.

2.2.6. Analytical methods

For the fractions whose molecular weight up to 1000 Da, molecular weight distribution and composition of the degradation products was determined by gel permeation chromatography (GPC) using Waters 600 controller HPLC system (Waters), equipped with Waters 2414 refractive index detector (Milford, MA, USA). 20 μL sample solutions (2 mg/mL) were injected and separation was performed by a Tosoh TSK gel superAW column (TSK-GEL G4000PWXL, Tosoh Bioscience, Tokyo, Japan). For elution, degassed water was used at a flow rate of 0.5 mL/min at 30 °C.

Before determination of samples, the system was calibrated by a series of standard dextran of different weight-average molecular mass (360 kDa, 210 kDa, 113 kDa, 48,800 Da, 21,700 Da, 10 kDa, 6000 Da, 5300 Da, and 1200 Da). The chromatography of standard dextran was displayed in Fig. 1. The relationship between molecular mass and retention time was established by the following equation.

$$l_g M_W = -0.2796t + 9.3041, \quad R^2 = 0.9956$$

M_W stands for weight-average molecular mass, Da; t stands for retention time, min.

For the oligosaccharides of the degradation products ($M_W < 1000$), the separation analysis was performed in Waters 600 controller HPLC system (Waters), equipped with Sugar Park 1 Column (ion exchange column), and Waters 2414 refractive index detector. 20 μL sample solutions (2 mg/mL) were injected. For elution, EDTA-Ca solution (0.05 mg/mL) was used at a flow rate of 0.5 mL/min at 90 °C.

3. Results and discussion

3.1. The results of γ-irradiation

Fig. 2 showed the gel permeation chromatography of KGM irradiated at 0 kGy, 10 kGy and 20 kGy. It was found that the weight-average molecular weight (Mw) for KGM was 1.68×10^6 Da, and it decreased to 1.27×10^6 Da and 1.45×10^6 Da respectively after irradiated at 10 kGy and 20 kGy. However, the safe dose for γ-irradiation applied in food was within10 kGy. So, it could be speculated that single use of γ-irradiation could not effectively degrade KGM into oligosaccharides.

3.2. Analysis by gel permeation chromatography

In order to compare the effect of γ-irradiation on the composition of degradation product of Konjac Glucomannan, the native KGM, KGM irradiated by 10 kGy, and KGM irradiated by 20 kGy was used as enzymatic raw materials respectively. The degradation product of Konjac Glucomannan was separated into 6 groups through ultrafiltration (Group 1, >300K; Group 2, 100–300K; Group 3, 50–100K; Group 4, 5–50K; Group 5, 1–5K; Group 6, <1K). Although, it was separated into 6 groups by the molecular weight cut off of the membrane, its molecular weight distribution should

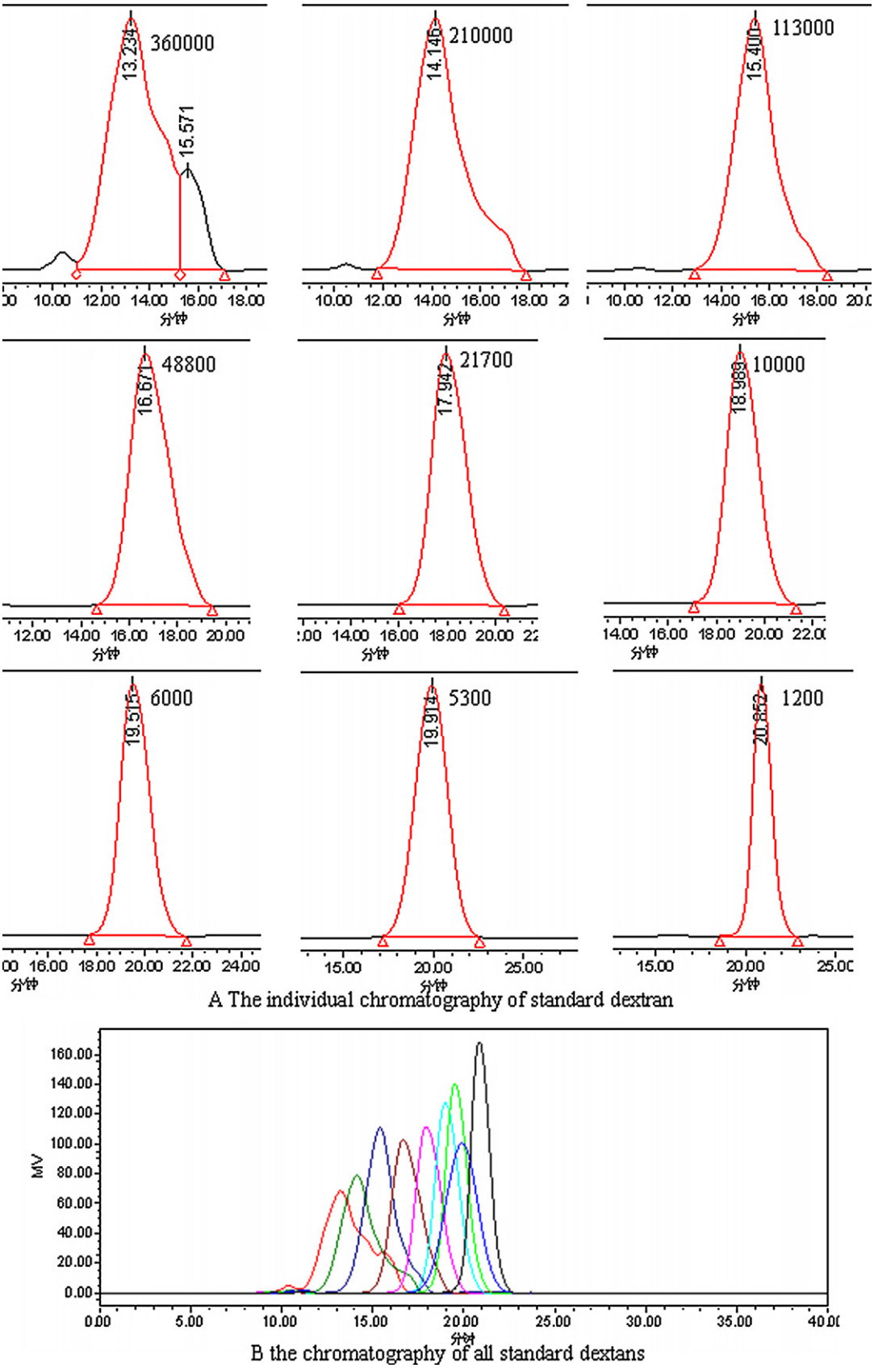


Fig. 1. The chromatography of standard dextrans.

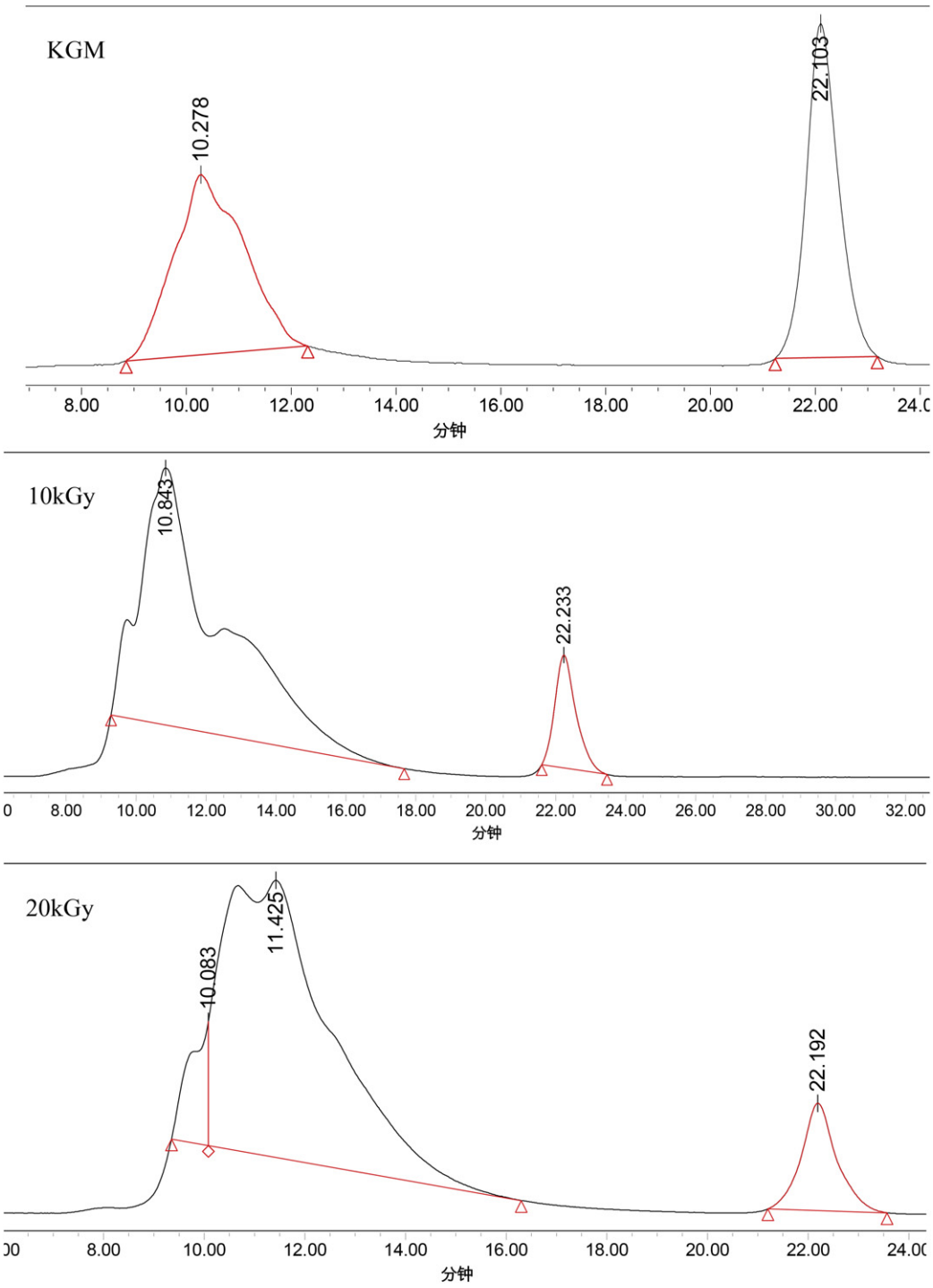
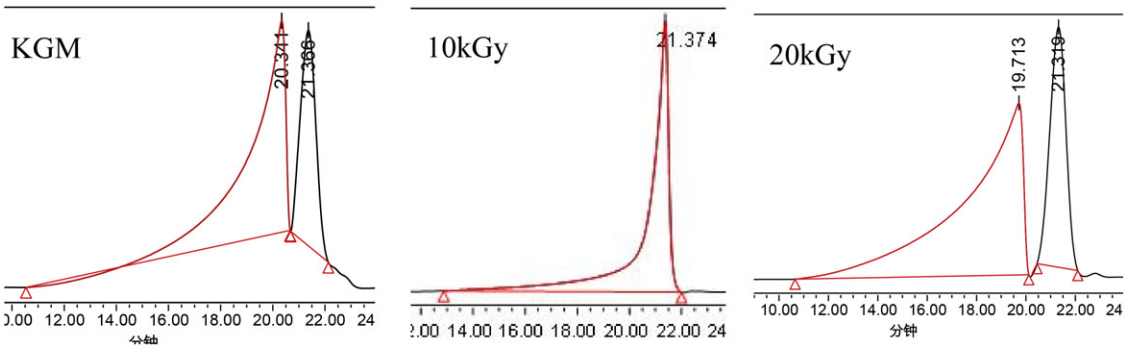


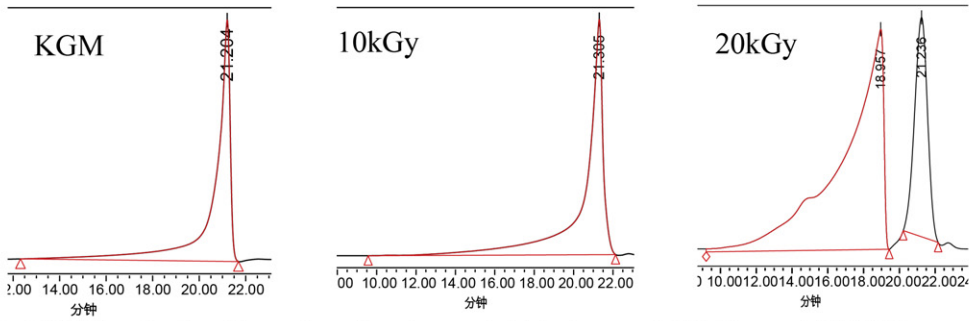
Fig. 2. GPC for KGM and KGM irradiated by γ-irradiation.

be verified. The GPC for the degraded products was displayed in Fig. 3.

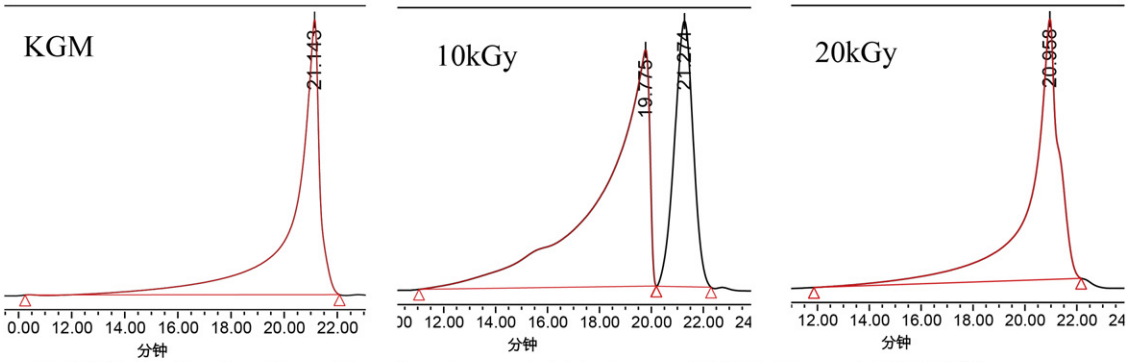
Because the exclusion limit of the GPC column used was from 1 K to 700 K, the degradation product with molecular weight up to 1000 Da for 3 samples was analyzed by gel permeation chromatography respectively. To our interest, despite the degradation product was separated into 6 group fractions by molecular weight cut off for the membranes, most of the degradation product with molecular weight up to 1000 Da were distributed from 2000 Da to 3000 Da, i.e. the average polymerization degree of the degradation product is between 10 and 18. The highest molecular mass was 10,086 Da for KGM irradiated by 20 kGy. The detailed compositions of the 3 samples were listed in Table 1. It was found that for the native KGM, the product was mainly distributed from 2100 Da to 2400 Da. However, the compositions of degradation products for γ-irradiated KGM were more complex compared to the native KGM. The product of KGM irradiated by10 kGy could be classified into two grades, which were 6200 Da and 2000 Da. Meanwhile, the product of KGM irradiated by 20 kGy could be classified into three grades, which were 10,086 Da, 4200 Da, and 2100 Da.



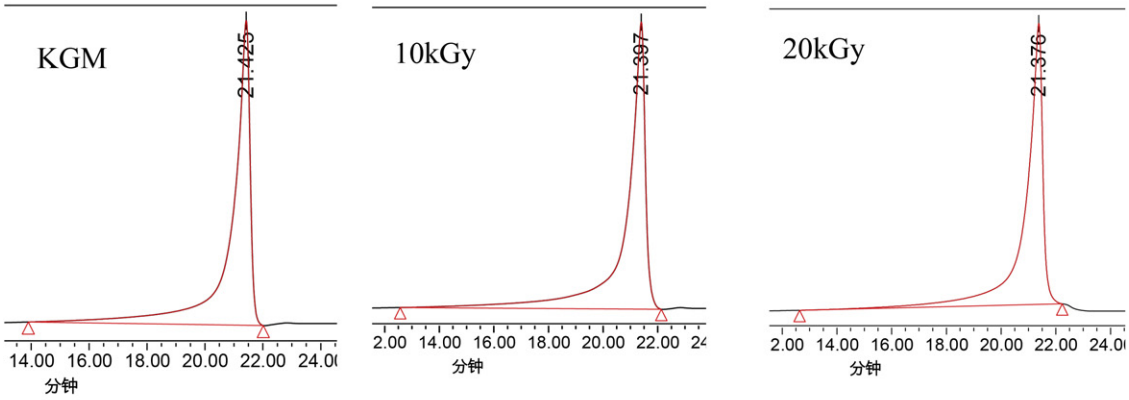
A. GPC for the fraction of molecular weight between 1000Da and 5000 Da



B. GPC for the fraction of molecular weight between 5000 Da and 50000Da



C. GPC for the fraction of molecular weight between 50000 Da and 100000Da



D. GPC for the fraction of molecular weight between 100000Da and 300000Da

Fig. 3. GPC for the fractions separated by ultrafiltration.

The mechanism for the difference in composition for the 3 degradation products should be that difference in molecular weight distribution before enzymatic hydrolysis. As we know, γ-irradiation cuts the molecular chain randomly, and it produces a lot of products of different molecular weight. So, the enzymatic raw materials from γ-irradiation were more complex than the native KGM. In conclusion, the method used in this paper could effectively degrade KGM to the oligosaccharides.

Table 1
The composition of the degradation products.

KGM		KGM irradiated by 10 kGy		KGM irradiated by 20 kGy	
Retention time (min)	Weight-average molecular mass (M_W)	Retention time (min)	Weight-average molecular mass (M_W)	Retention time (min)	Weight-average molecular mass (M_W)
21.204	2373.896	19.713	6199.336	18.957	10086.09
21.143	2468.978	19.775	5956.759	20.341	4137.675
21.374	2127.794	21.274	2269.288	21.23	2334.49
21.425	2059.065	21.305	2224.447	21.36	2147.059
–	–	21.319	2204.487	21.376	2125.056
–	–	21.397	2096.519	–	–

3.3. Analysis by ion exchange column in HPLC

The oligosaccharides with molecular mass less than 1000 Da from the degradation product for 3 samples were analyzed by ion exchange column in HPLC respectively. It was shown from Fig. 4 that there were 5 peaks in every sample, i.e. there were 5 oligosaccharides in every sample. Except for the first peak, the retention time of all other peak was nearly the same. So, it could be speculated that the five peaks maybe stand for pentasaccharides, tetrasaccharides, pentasaccharides, disaccharide, and monosaccharide respectively. After enzymatic hydrolysis, 3 kinds of KGM powder all produced the oligosaccharide with polymerization degree lower than 5. It could be concluded that the oligosaccharide of molecular weight lower than 1000 could be produced from combination of γ-irradiation and enzymatic hydrolysis. Besides, the oligosaccharides with polymerization degree lower than 5 could be separated effectively from the degradation product using 1000 Da molecular weight cut off ultrafiltration membrane.

3.4. Conclusion and discussion

So far, no detailed research on how to effectively obtain Konjac oligosaccharides has been reported. Most of the researches only used the single degradation method, and the composition of the degradation products was not investigated (He, Zhang, & Huang, 2001; Prawitwong, Takigami, & Phillips, 2007). In this work, we effectively obtained the oligosaccharides of molar mass lower than 2200 Da by the combination of γ-irradiation and enzymatic

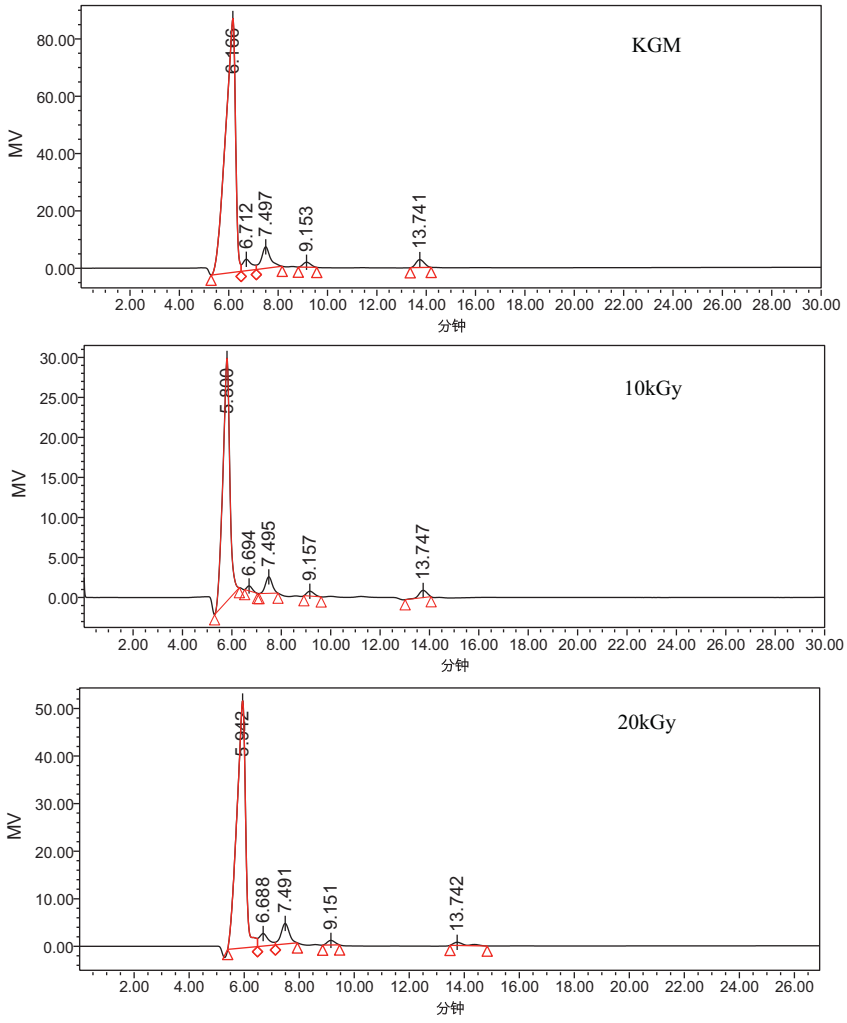


Fig. 4. Ion exchange column analysis in HPLC for the fraction molecular mass lower than 1000 Da.

hydrolysis. The average polymerization degree of most of the products is 14. Furthermore, we found the effective method to separate the oligosaccharides, i.e. the 1000 Da molecular weight cut off membrane could effectively separate and purify Konjac oligosaccharides.

Compared to the literatures (Xu et al., 2007; Zhang et al., 2009), our work fully investigated the degradation products by the combination of gel permeation chromatography and ion exchange column in HPLC, so it was innovative in analysis method.

In conclusion, our work will be theoretically beneficial for the study of preparation and separation of Konjac oligosaccharides. At the mean time, it will also be beneficial for the study of degradation of other polysaccharides.

Acknowledgments

This work was supported by China Postdoctoral Science Foundation (2012M510199), Natural Science Foundation of Fujian Province (2012J05046), Fujian Education Department Science Program (JA11327), National Natural Science Foundation of China (31271837, 30901004 and 31071518), Key Research Project of Guangdong Province (2010B080701079), the doctoral program of higher education of the specialized research fund for the project funded by the United (20113515110010), Yangcheng Scholars Technology Project of Guangzhou (10B005D) and Fuzhou Science and Technology Plan Project (2011-N-44).

References

Albrecht, S., van Muiswinkel, G. C. J., Xu, J., Schols, H. A., Voragen, A. G. J., & Gruppen, H. (2011). Enzymatic production and characterization of Konjac glucomannan oligosaccharides. *Journal of Agricultural and Food Chemistry*, *59*(23), 12658–12666.

Courtois, J. (2009). Oligosaccharides from land plants and algae: Production and applications in therapeutics and biotechnology. *Current Opinion in Microbiology*, *12*(3), 261–273.

Guan, G. Y., Shi, W. H., Yu, S. J., & Xu, X. B. (2011). Ultrafiltration of caramel color solutions reduces 5-hydroxymethyl-2-furaldehyde. *Journal of Membrane Science*, *380*, 9–12.

He, Z., Zhang, J., & Huang, D. (2001). A kinetic correlation for konjac powder hydrolysis by beta-mannanase from *Bacillus licheniformis*. *Biotechnology Letters*, *23*(5), 389–393.

Jian, W., Zeng, Y., Xiong, H., & Pang, J. (2011). Molecular simulation of the complex of konjac glucomannan-borate in water. *Carbohydrate Polymers*, *85*(2), 452–456.

Onishi, N., Kawamoto, S., Nishimura, M., Nakano, T., Aki, T., Shigeta, S., et al. (2005). A new immunomodulatory function of low-viscous konjac glucomannan with a small particle size: Its oral intake suppresses spontaneously occurring dermatitis in NC/Nga mice. *International Archives of Allergy and Immunology*, *136*(3).

Pang, J., Jian, W., Wang, L., Wu, C., Liu, Y., He, J., et al. (2012). X-ray photoelectron spectroscopy analysis on surface modification of Konjac glucomannan membrane by nitrogen plasma treatment. *Carbohydrate Polymers*, *88*(1), 369–372.

Pang, J., Lin, Q., Zhang, F. S., Tian, S. P., & Sun, Y. M. (2003). Progress in the application and studies on functional material of konjac glucomannan. *Chinese Journal of Structural Chemistry*, *22*(6), 633–642.

Prabhakar, S., Ghosh, A. K., Bindal, R. C., & Tewari, P. K. (2011). Outside skin tubular ultrafiltration membranes for separation of proteins and polysaccharides and removal of turbidity from seawater. *Desalination and Water Treatment*, *27*(1-3), 231–236.

Prawitwong, P., Takigami, S., & Phillips, G. O. (2007). Effects of gamma-irradiation on molar mass and properties of Konjac mannan. *Food Hydrocolloids*, *21*(8), 1362–1367.

Qi, L., Li, G. J., & Zong, M. H. (2003). Enzyme-catalyzed controllable degradation of konjac glucomannan. *Acta Polymerica Sinica*, (5), 650–654.

Suzuki, H., Oomizu, S., Yanase, Y., Onishi, N., Uchida, K., Mihara, S., et al. (2010). Hydrolyzed Konjac glucomannan suppresses IgE production in Mice B cells. *International Archives of Allergy and Immunology*, *152*(2), 122–130.

Vuksan, V., Sievenpiper, J. L., Owen, R., Swilley, J. A., Spadafora, P., Jenkins, D. J., et al. (2000). Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with the insulin resistance syndrome: Results of a controlled metabolic trial. *Diabetes Care*, *23*(1).

Wu, Y., Li, X., Che, T., Zhu, Z., & Kang, J. (2012). Separation and quantitative analysis of the composition of low molecular weight sulfated polysaccharides by size exclusion chromatography. *Se pu = Chinese journal of chromatography/Zhongguo hua xue hui*, *30*(2), 178–183.

Xing, J.-m., & Li, F.-f. (2009). Separation and purification of aloe polysaccharides by a combination of membrane ultrafiltration and aqueous two-phase extraction. *Applied Biochemistry and Biotechnology*, *158*(1), 11–19.

Xu, Z., Sun, Y., Yang, Y., Ding, J., & Pang, J. (2007). Effect of gamma-irradiation on some physiochemical properties of konjac glucomannan. *Carbohydrate Polymers*, *70*(4), 444–450.

Yeh, S.-L., Lin, M.-S., & Chen, H.-L. (2010). Partial hydrolysis enhances the inhibitory effects of konjac glucomannan from Amorphophallus konjac C. Koch on DNA damage induced by fecal water in Caco-2 cells. *Food Chemistry*, *119*(2).

Zhang, M., Chen, X. L., Zhang, Z. H., Sun, C. Y., Chen, L. L., He, H. L., et al. (2009). Purification and functional characterization of endo-beta-mannanase MAN5 and its application in oligosaccharide production from konjac flour. *Applied Microbiology and Biotechnology*, *83*(5), 865–873.