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Review:

Depolymerized konjac glucomannan: preparation and application in health care

Min JIANG¹, Heng LI¹, Jin-song SHI^{†‡1,2}, Zheng-hong XU^{3,4}

1 School of Pharmaceutical, Jiangnan University, Wuxi 214122, China

² Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Pharmaceutical Science, *Jiangnan University, Wuxi 214122, China*

3 National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi 214122, China

4 School of Biotechnology, Jiangnan University, Wuxi 214122, China

† E-mail: shijs@163.com

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Abstract: Konjac glucomannan (KGM) is a water-soluble polysaccharide obtained from the roots and tubers of konjac plants. Recently, a degraded product of KGM, depolymerized KGM (DKGM), has attracted attention because of its low viscosity, improved hydrophily, and favorable physiological functions. In this review, we describe the preparation of DKGM and its prebiotic effects. Other health benefits of DKGM, covering antioxidant and immune activity, are also discussed, as well as its safety. DKGM could be a candidate for use as a tool for the treatment of various diseases, including intestinal flora imbalance, and oxidative- and immune-related disorders.

Key words: Konjac glucomannan; Depolymerized konjac glucomannan; Prebiotics; Immune response; Antioxidant https://doi.org/10.1631/jzus.B1700310 **CLC number:** N8

1 Introduction

Konjac glucomannan (KGM) is a water-soluble polysaccharide obtained from the roots and tubers of the konjac plant (*Amorphophallus konjac*). It consists of D-mannose and D-glucose units at a molar ratio of about 1.6:1.0. These units are joined by β-(1,4) glycosidic bonds to form the backbone, and a few branches are formed by β-(1,6)-glycosidic units. Part of the short side branches can be formed via linkage between the C-3 positions of the mannoses and the acetyl groups randomly found at the C-6 position of a sugar unit. These sugar units with acetyl groups comprise about 1/9th to 1/20th of the total sugar units. Owing to its high molecular weight (mostly ranging from 1×10^4 to 2×10^6 Da) (Al-Ghazzewi et al., 2007) and non-caloric nature, KGM plays a beneficial role in blood cholesterol and sugar reduction, weight loss, promotion of intestinal activity and immune function. Therefore, KGM has been consumed for centuries in East Asia, but in low-added value food forms, such as KGM tofu, noodles, and jellies.

Recently, many researchers have studied the preparation of depolymerized KGM (DKGM) (Behera and Ray, 2016; Tester and Al-Ghazzewi, 2017). Compared with native KGM, DKGM showed significantly reduced viscosity, which is more favorable for food processing. Besides, DKGM displays many unique biological characteristics, especially its prebiotic function. Because of these advantages, DKGM

[‡] Corresponding author

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has attracted increasing attention. In this review, we discuss its preparation strategies, safety concerns, and biological function assessments, including its prebiotic, antioxidant, and immune-regulator effects. Factors affecting the extent of its physiological benefits are also discussed.

2 Preparation techniques

Recently, a number of studies have been devoted to exploring efficient methods for degrading KGM. In general, these methods can be divided into two classes: physicochemical degradation and biodegradation.

2.1 Physicochemical degradation

Various physicochemical degradation methods, such as acid-treatment (Suzuki et al., 2010), thermal treatment (Jin et al., 2014b), oxidative degradation (Zheng et al., 2015; Zhang et al., 2016), ultrasound treatment (Huang et al., 2006; Li et al., 2017), and irradiation treatment (Xu et al., 2007; Jian et al., 2013, 2017; Pan et al., 2013; Li et al., 2018), have been used to degrade polysaccharides. Although strong acid is powerful in breaking down the glycosidic chains of KGM, the process is not very controllable and carries a high risk of environment pollution. The use of γ-rays is a simple and manageable way to degrade polysaccharides in any physical form (e.g. solid, suspension, paste, or solution) without heating. The ionizing energy can rapidly penetrate the granule and randomly rupture the backbone of KGM. However, the molecular mass distribution of the final product is broad. Degradation of KGM by irradiation is believed to be dosedependent (Xu et al., 2007; Jian et al., 2013, 2017). While there is agreement among international experts that irradiation dosages up to 10 kGy are safe (Jian et al., 2013, 2017; Jin et al., 2014a), carbonyl groups and double bonds might be formed at relatively high irradiation doses, leading to browning of the final product (Xu et al., 2007). Therefore, irradiation dosage is the priority consideration during the process.

Besides irradiation dosage, the solvent used to dissolve KGM is important. A higher efficiency of KGM degradation by irradiation is observed in some solvents than that found by irradiation alone. For example, the hydrogen peroxide solution alone cannot degrade KGM efficiently, but produces hydroxyl radicals, and the concentration of hydroxyl radicals increases after irradiation. The hydroxyl radicals are powerful oxidative agents, and could directly break the glucosidic linkages of KGM by absorbing carbonbound hydrogen atoms. Therefore, the molecular weights of products with irradiation alone $(6.58 \times 10^4 \text{ Da})$ and synergetic degradation $(6.48 \times 10^4 \text{ Da})$ are much lower than that of the untreated sample $(4.80 \times 10^5 \text{ Da})$ (Pan et al., 2013). Nevertheless, many factors are involved in the process: (1) free radicals, generated by the solvent, probably play either a positive or negative role in radiation; (2) molecular conformation varies with the solvent type and concentration. For instance, KGM molecular conformation and hydrogen atoms played a restrained effect during KGM irradiation in ethanol. At low irradiation doses, stability predominated, leading to the viscosity-average molecular weights of pre-immersed samples being higher than those of irradiation controls. However, at high doses, the inhibition effect was relatively less significant, and the irradiation effect played a leading role in the hydrolysis process. For this reason there was no significant difference between ethanol-treated and untreated groups (Jin et al., 2014a).

So far, there have been only a few studies of the use of ultrasound degradation of KGM. It was thought that ultrasound treatment could weaken the intermolecular interactions between KGM molecules in an aqueous solution as well as the intramolecular interactions between the KGM polymer chains, leading to their degradation. The changes in the structure and degradation of KGM depended on the ultrasound power and other treatment parameters (Huang et al., 2006; Chen and Qian, 2008). Moreover, the degradation process of KGM by ultrasound was deemed to fit firstorder polymer degradation kinetics (Li et al., 2017).

2.2 Enzyme hydrolysis

Compared with physicochemical degradation, enzymes have been widely used in polysaccharide hydrolysis because of their advantages such as high extraction yield, reproducibility, environmental-friendliness, energy efficiency, and simple protocols (van Zyl et al., 2010; Bhotmange et al., 2017; Jiao et al., 2017).

The molecular structure of KGM can provide multiple cutting sites. Thus, it can be degraded by different enzymes (Chen J et al., 2013; Jian et al., 2013; Mikkelson et al., 2013; Liu HX et al., 2015; Chen CY et al., 2016; Yang et al., 2017). Cellulase and hemicellulase derived from different strains have been most well studied so far (Table 1). The commercial cellulase product (a mixture of endoglucanase, exoglucanase, and glucosidase) has been the enzyme most commonly used for KGM hydrolysis. In the

mixed product, the glucanase can break the β -(1,4)glycosidic bond between glucose and mannose, the exoglucanase can remove the 1,4-glucopyranose units at the non-reducing end, and the β-glucosidase can cleave glucobiose into glucose units (Zhang et al., 2003). As for the hemicellulase, β-mannanase has been the

Category	Origin	Reaction condition	Product information	Reference
Hemicellulase		pH 6.5, 20% konjac, 45 °C,	KGM oligosaccharide yield 35%, mainly	He et al., 2013
		E/S 250 U/g, 0.83 h	consists of triose and tetrose units	
	Aspergillus niger	pH 4.2, 2% konjac, 65 °C,	KGM oligosaccharide yield 32.3%	Xu et al., 2005
		E/S 108 U/g, 4 h		
	A. niger	pH 7.0, 15% konjac, 50 °C,	KGM oligosaccharide yield 100% after	Li et al., 2007
		E/S 50 U/g, 6 h	yeast fermentation	
	A. niger	pH 7.0, 24% konjac, 50 °C, E/S 120 U/g, 8 h	The average DP of DKGM was 1.8–1.9	Xu et al., 2008
	A. niger	pH 5.0, 1% konjac, 30 °C, E/S 46.2 U/g, 24 h	Oligosaccharide covering the whole range of DP $3-9$	Albrecht et al., 2009
		pH 5.5, 1% konjac, 45 °C, E/S 100 U/g, 1.5 h	KGM oligosaccharide yield 100% after yeast fermentation	Wu et al., 2010
	A. niger	pH 3.5–8.5, 0.05%–3.05%	The molecular weight and molecular weight Yao et al.,	
		konjac, 14–62 °C; 20% konjac, 0.04–1.00 h	distribution depended on the enzymatic parameters	2011
	A. niger	pH 3.5, 18% konjac, 65 °C, E/S 30 U/g, 4 h	KGM oligosaccharide yield 35.73% after yeast fermentation	Xu SC et al., 2011
	Bacillus subtilis	pH 6.0, 12.5% konjac, 50 °C, E/S 200 U/g, 3 h		Xu LP et al., 2011
	B. subtilis	pH 7.5, 5% konjac, 45 °C,	Oligosaccharide covering the whole range	Kang et al.,
		E/S 50 U/g, 24 h	of DP 2-9	2012
		pH 6.0, 61.3% konjac, 55 °C, E/S 1500 U/g, 4.2 h	DKGM yield 76.34% after ethanol precipitate, Qin et al., galactopyranose type DKGM covering the whole range of DP $2-10$	2013
		pH 6.0, 61.3% konjac, 55 °C, E/S 1500 U/g, 4.2 h	DKGM yield 52.67%	Deng et al., 2013
	Trichoderma reesei	pH 5.0, 1% konjac, 45 °C, 48 h	17% DKGM (DP 2-6)	Mikkelson et al., 2013
		pH 7.1, 20% konjac, 41 °C, E/S 0.49, 3.4 h		Chen et al., 2013
	Thermobifida fusca	50 °C, 24 h	The weight-average molecular weight 3089 Da	Cheng et al., 2016
		E/S 360 kU/g, 24 h	pH 4.0, 0.2% konjac, 50 °C, The molecular mass lower than 2200 Da after combination of γ -irradiation and enzymatic hydrolysis	Jian et al., 2013
	B. subtilis	pH 7.0, 0.33% konjac, 60 °C, E/S 6 U/g, 1 h	DKGM yield 35.96%, most DKGM with DP 2–6	Cheng et al., 2016
Cellulase	Trichoderma viride	pH 5.0, 10% konjac, 40 °C, E/S 50 U/g, 2 h	Weight-average molecular weight 7160 Da, number-average molecular weight 5100 Da	Zhang et al., 2003
		pH 4.5, 10% konjac, 60 °C, E/S 450 U/g, 1 h		Elamir et al., 2008
	T. viride	pH 5.0, 1% konjac, 30 °C, 24 h		Albrecht et al., 2009
		E/S 58 U/g, 24 h	pH 3.5, 0.5% konjac, 30 °C, Oligosaccharide covering the whole range of DP 3-14	Albrecht et al., 2009
		10% konjac, 50 °C, 1.3 h		Dang et al., 2015
	T. reesei	pH 5.0, 1% konjac, 45 °C, 48 h	18.0%, 4.7% DKGM (DP 2–6) for Cel7A and Cel7B, respectively	Mikkelson et al., 2013

Table 1 KGM-degrading enzymes

E/S: the ratio of enzyme to substance; DP: degree of polymerization

most commonly used enzyme. It is an endo-acting hydrolase which acts by randomly catalyzing the $β-(1,4)$ -mannosidic linkages, resulting in the production of DKGM (Dhawan and Kaur, 2007; van Zyl et al., 2010; Liu HX et al., 2015). Therefore, the choice of enzyme determines the structural characteristics of the final product. For example, M(mannan)G(glucose), MM, MMG, and MMM are abundant when KGM is hydrolyzed by endoglucanase, whereas plenty of GM, MM, GGM, and GMM are obtained by using β-mannanase (Cescutti et al., 2002). Consistent with this, 99% of DKGM obtained from catalysis by β-mannanase had M as the reducing end pyranosyl unit, while KGM hydrolyzed by endoglucanase had both M and G as the reducing end pyranosyl unit (Mikkelson et al., 2013).

Compared with enzymatic hydrolysis alone, chemical and physical pretreatments enhance the KGM enzymatic degradation process. Jian et al. (2013) found that the molecular weight of KGM (1.68×10⁶ Da) following γ -irradiation pre-treatment could be further reduced to 2200 Da with enzymes. Similarly, the molecular weight distribution of KGM enzymatic hydrolysis with or without mechanical shear pre-treatment was investigated. Compared with non-treated controls, the average molecular weights of the supernatant and pellet of DKGM following mechanical shear pre-treatment were further reduced by nearly 5% and 35%, respectively (Yang et al., 2017).

Although enzymatic degradation has been widely studied, most of these studies aimed mainly to reduce the molecular weight of KGM, and there is lack of information about its structure and purity. We believe that the health benefits of DKGM are closely associated with its molecular characteristics (e.g. its molecular structure and degree of polymerization (DP)). Therefore, controllable and limited enzymatic degradation as well as product purification is still the most challenging problems in its application.

3 Safety

It is widely regarded that overconsumption of oligosaccharides might induce flatulence and even cause an imbalance of essential nutrients, which would be counterproductive (Liu et al., 2016). Although the intrinsic safety of DKGM is supported by

its natural occurrence in konjac, the safety of DKGM is still worthy of investigation. So far, only a few studies of DKGM safety have been reported. A previous study by our research group demonstrated that DKGM (DP=2–7) of up to 7.5 g/kg daily was safe for rats for 90 d. The results of genotoxicity studies in vitro (using the Ames assay and erythrocyte micronucleus assay) and in vivo (using a sperm malformation test in mice) indicated that administration of DKGM up to 10.0 g/kg had no mutagenic potential. In addition, DKGM up to 2.5 g/kg showed neither maternal toxicity nor teratogenicity in pregnant rats (Jiang et al., 2016).

4 Biological activity

More recently, DKGM, especially low molecular weight DKGM, has been widely studied because of its health benefits. In this part, its prebiotic effects and relevant factors are summarized. Potential applications of DKGM in the fields of anti-oxidation and immune function are also discussed.

4.1 Prebiotic activity

The prebiotic activity of DKGM has been mentioned briefly in some reviews which focused mainly on native KGM (Bateni et al., 2013; Behera and Ray, 2016; Tester and Al-Ghazzewi, 2017). In general, recent studies (Table 2) revealed that DKGM is valuable as a prebiotic via a number of mechanisms:

(1) It can selectively stimulate *Lactobacillus* and *Bifidobacterium* in the colon (Feng et al., 2015; Liu et al., 2016), cecum (Wang et al., 2016a), vagina and skin surface (Tester et al., 2012).

(2) By blocking their adhesion on mucosal surfaces of animals, DKGM can restrain the growth of pathogenic bacteria such as *Coliforms*, *Enterococci*, *Staphylococcus aureus* (Al-Ghazzewi et al., 2012; Liu et al., 2016), *Propionibacterium acnes* (Al-Ghazzewi and Tester, 2010), *Clostridium* (Chen et al., 2005), and *Salmonella typhi* (Al-Ghazzewi et al., 2012).

(3) During the fermentation of DKGM, short chain fatty acids (SCFAs) (mainly acetic, propionic, and butyric acid) are generated, leading to a lowering of the gut pH. These SCFAs are a source of nutrition for gut microbes, and show positive effects for the treatment of inflammation and carcinogenesis in the

DP: degree of polymerization; IBD: inflammatory bowel disease; SCFA: short chain fatty acid

gut and other organs (Tester et al., 2012; Bateni et al., 2013; Wan et al., 2015; Sivaprakasam et al., 2016; Tao et al., 2016; Unger et al., 2016; Primec et al., 2017).

Generally, the fermentation process of DKGM is a time- and dose-dependent procedure (Tester et al., 2012; Behera and Ray, 2016). The DP, structural characteristics, and constituents of DKGM change with the preparation methods, often leading to different fermentation behaviors (Wang et al., 2008; Albrecht et al., 2009). Four substrates (a monomerfree DKGM with a cellulose digest, a monomer-free DKGM with an endo-β-(1,4)-glucanase digest, a monomer-containing DKGM with a cellulose digest, and native KGM) were fermented in a fecal slurry consisting of a mix of feces from three human volunteers for 72 h. Optical density and pH showed that all DKGM were fermented well except native KGM. The monomer-free endo-β-(1,4)-glucanase digest behaved similarly to the cellulose digests. However, the fermentation of the monomer-free cellulose digest showed a different pattern compared to the monomercontaining digest. For the monomer-free digest, the monomers were fermented during the first 24 h, and then most of the dimers and trimers were fermented

between 24 and 72 h. No significant change was observed for the larger oligomers. In contrast, for the monomer-containing cellulose digest, no change was observed in the first 24 h. Moreover, DP 6 was completely degraded and DP 5 mostly degraded between 24 and 72 h, whereas DP 4 was more resistant (Albrecht et al., 2009). Similarly, DKGM with a different structure and constituent was obtained following treatments with endo-β-(1,4)-glucanase, endo-β-(1,4)-mannanase, and an equivalent mixture (1:1) of cellulose-mannanase. Their utilization by lactic acid bacteria was then evaluated. The *Lactobacillus* growth profiles showed that DKGM produced with cellulose enzymes was the most effective growth promoter among the three groups (Al-Ghazzewi and Tester, 2012).

Although lots of research has demonstrated the prebiotic effect of DKGM, its selectivity towards specific microorganisms and the fermentation profile are still unclear. According to Wang et al. (2016b) and Yang et al. (2017), the fermentation of DKGM varies among species. DKGM with different molecular weights was prepared by mechanical-pretreated enzymatic hydrolysis, and fermentation by five *Lactobacillus* and three *Bifidobacterium* was investigated in vitro. Results showed that all the test bacteria (except *Lactobacillus delbrueckii* subsp. *bulgaricus*) were DKGM fermenters, but *Bifidobacterium* strains were not as strong as *Lactobacillus.* Moreover, there was variation among the *Lactobacillus* strains (Yang et al., 2017). Similarly, Wang et al. (2016b) investigated the regular fermentation patterns of DKGM by four acid bacteria (*Lactobacillus animals*, *Lactobacillus plantarum*, *Enterococcus faecalists*, and *Lactobacillus reuteri*) in vitro. Results showed that *L. plantarum* and *E. faecalists* could quickly and completely consume DKGM with DP 2–3, whereas *L. animals* and *L. reuteri* utilized only the DKGM with DP2.

In vivo studies in healthy animals (Elamir et al., 2008; Qin et al., 2014; Wan et al., 2015; Wang et al., 2016a), ulcerative colitis animals (Feng et al., 2015; Liu et al., 2016), and humans suffering from IBD (Suwannaporn et al., 2013) (Table 2) verified the prebiotic effects of DKGM. Mechanism studies revealed that oral administration of DKGM not only promotes the proliferation of probiotics (mainly *Lactobacillus* and *Bifidobacterium*), which is consistent with the in vitro results, but also influences the intestinal environment (increasing intestinal villi height and SCFAs) and reduces the levels of inflammatory factors (malondialdehyde, inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-α (TNF- α), and interleukin-1 β (IL-1 β)). Furthermore, the physiological effects of DKGM in the gut were verified to be effective in the skin and vagina of humans (Tester et al., 2012; Bateni et al., 2013), implying that the use of DKGM can be expanded to other ecological systems beyond the gut.

4.2 Anti-oxidative activity

In recent years, there has been increasing interest in natural antioxidants because synthetic antioxidants might induce teratogenicity, carcinogenicity or mutagenicity. A few studies have demonstrated that DKGM could have great potential as a natural antioxidant (Wang et al., 2008; Liu J et al., 2015; Jian et al., 2017). In general, its anti-oxidative effects may result from two pathways:

(1) It could act directly as an antioxidant which not only scavenges oxidants, but also activates intracellular antioxidant enzymes. Antioxidant experiments in vitro showed that DKGM (DP 5.2), obtained by β-mannanase degradation, not only could eliminate hydroxyl radicals (OH) and 1,1-diphenyl-2 picrylhydrazyl radicals (DPPH), but also had great reducing power (Liu J et al., 2015). Moreover, cytological tests in human hepatic cells revealed that DKGM (53 kDa) degraded by 100 kGy irradiation not only significantly increased cellular survival and activity of glutathione peroxidase and intracellular activity of catalase, but also reduced the levels of lactate dehydrogenase, malondialdehyde, and intracellular accumulation of reactive oxygen species (ROS), providing a protective effect against oxidative damage by H_2O_2 (Jian et al., 2017).

(2) Fermentation products of DKGM produced by bacteria also exert anti-oxidative effects. However, the mechanisms vary depending on its molecular weight and the bacterial strains (Wang et al., 2008). Native KGM and DKGM with different DPs within five colonic bacteria were compared. The results suggested that native KGM produces anti-oxidative activity mainly through initiation of ferrous ioninduced peroxidation. Nevertheless, fermentation of DKGM (DP=5) produced anti-oxidative effects by reducing ferrous ion-induced peroxidation and the formation of lipid peroxide products and increasing radical-eliminating ability, whereas fermentation of DKGM (DP=10) produced anti-oxidative effects by increasing radical-scavenging ability and eliminating lipid peroxide formation.

4.3 Immune activity

Administration of DKGM has positive immunomodulatory effects on various animal species, including mammals (Suzuki et al., 2010) and fish (Zheng et al., 2015, 2016). Mechanism studies revealed that DKGM at an optimal size (between 10 and 500 kDa) could inhibit the production of immunoglobulin E (IgE) and enhance interferon-γ (IFN-γ) in vitro, thereby easing atopic disease in mice (Suzuki et al., 2010). DKGM $(4.7\times10^5 \text{ Da})$ and low molecular-DKGM (L-KGM; 9.29×10^3 Da), obtained by H_2O_2 and HCl degradation, were used to evaluate effects on immune response in *Schizothorax prenanti*. Data suggested that the ideal dosage of L-DKGM for enhancement of the immune system was 8.0 g/kg and that the immunomodulatory mechanism involved a significant enhancement of lysozyme, superoxide dismutase (SOD) activity, and immune factors (1L-1 β and TNF- α) and a significant reduction in the malondialdehyde level, leading to better resistance to *Aeromonas hydrophila* (Zheng et al., 2015, 2016).

5 Conclusions

The health benefits of DKGM have been confirmed and accepted. However, most studies have focused mainly on producing DKGM with low molecular weight. In addition, the relation between its physicochemical properties and its health benefits is still unclear. Therefore, further studies focused on DKGM are needed to develop manageable and physiological benefit-oriented technologies to produce DKGM with a specific structure, providing guidelines for industrial product design.

Compliance with ethics guidelines

Min JIANG, Heng LI, Jin-song SHI, and Zheng-hong XU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

- 题 目:解聚型魔芋葡甘露聚糖的制备以及生理活性研究 的进展
- 概 要:魔芋葡甘露聚糖是从魔芋块茎中提取的一种高分 子水溶性多糖。近些年研究表明,其解聚产物, 除了具有高溶解性和低粘度等良好的理化性质 外,还具有调节微生物菌群结构、抗氧化、免疫 调节等多种生理活性。本文重点综述了解聚型葡 甘露聚糖的制备方法以及菌群调节功能。除此之 外,对其抗氧化、免疫调节功能以及安全性评价 也进行了全面的总结,为解聚型葡甘露聚糖的研 究与应用提供一定的依据与思路。
- 关键词:魔芋葡甘露聚糖;益生 功能;免疫活性;抗氧化