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MINI REVIEW

A review on konjac glucomannan and hydrolysed konjac glucomannan

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Abstract

Konjac glucomannan (KGM) and hydrolysed konjac glucomannan (HKGM) are natural polysaccharides that have many applications in the food and non-food fields (chemical and health industries) due to their non-hazardous, non-toxic, biocompatible properties and good solubility. KGM was produced from pulverising chips, followed by air classification of solid particles to separate them from impurities or ethanol precipitation. Production of KGM from the genus *Amorphophallus* by the extraction and purification methods was critically reviewed. Characteristics of the physicochemical properties of KGM were outlined, and the preparation of HKGM was also discussed. The potential of KGM and HKGM to provide health benefits, such as being prebiotics or anti-inflammatory and anti-tumour agents, were considered. A brief discussion on the safe usage of KGM and HKGM is included. Meanwhile, HKGM was prepared by physical-acid treatments or enzymic degradation. Clinical studies have demonstrated that supplementing the diet with KGM or HKGM significantly lowers plasma glucose and reduces inflammation in rat models. Tumour was reduced when KGM was added to the test meals.

1. Introduction

The genus *Amorphophallus* belongs to the Araceae family (Hetterscheid, 1994). About 170 species are spread worldwide, whether in natural conditions, mainly in the forest, or cultivated and consumed. Out of the 170 species of *Amorphophallus,* those that are commonly consumed are: *A. konjac or A. riveri*, *A. mulleri*, *A. yuloensis*, *A. yunnanensis*, and *A. krausei* and *A. paeoniifolius.* However, *A. paeoniifolius* contains only e few amounts of KGM and is cultivated because of its high starch content (Behera and Ray, 2017). *Amorphophallus* as a food source and traditional medicine has been carried out in China, Japan and Southeast Asia since a few centuries ago (Chua *et al*., 2010; Zhao *et al*., 2010). In China, the curative effect of *Ammarphophallus* was recorded as early as the late Han Dynasty (Zhao *et al.*, 2010). The parts of the plants of *Amorphophallus* species utilised are corms or tubers, which are the primary sources of konjac glucomannan (Yanuriati *et al*., 2017). Konjac Glucomannan (KGM) is a water-soluble polysaccharide extracted from *Amorphophallus konjac* K.Koch tubers. It is also known as a non-calorie food and a source of indigestible dietary fibre, which is resistant to hydrolysis by digestive enzymes in the human gut (Huang *et al*., 2002; Anderson *et al*., 2009). The health benefits of KGM include reducing weight, modification of carbohydrate metabolism in people with diabetes, reducing cholesterol (Huang *et al.*, 2002), improving the vitamin B-6 metabolism (Hayakawa *et al*., 1999), modification of intestinal microbial metabolism, improving intestinal activity (Fujiwara *et al*., 1991), or reducing plasma cholesterol in rats (Levrat-Verny *et al.*, 2000) and improving immune function in humans (Onitake *et al*., 2015).

The two researcher teams have studied hydrolysed konjac glucomannan (HKGM) (Behera and Ray, 2016; Tester and Al-Ghazzewi, 2016). The biological activities of HKGM were demonstrated by dissolving KGM in an aqueous sulfuric acid solution which showed high anti-HIV activity and blood anticoagulant activities (Bo *et al*., 2013). Acid hydrolysis of KGM to prepare a

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microcrystal of HKGM and facilitate more functional applications was reported by Wang *et al*. (2015). The present review paper discussed the production technologies of HKGM, its safety, biological functions and mechanism.

2. Physicochemical properties of konjac glucomannan

Konjac glucomannan (KGM) includes dietary fibre, which is easily soluble in water, fermentable, and has high viscosity. It is found in nature, especially in softwoods (hemicellulose), tubers, roots, and bulbils of some plants (Keithley and Swanson, 2005; Alonso-Sande *et al*., 2009). KGM is a heteropolysaccharide composed of D-glucose and D-mannose units linked by β-1,4 glycosidic bonds with few acetyl groups (Takigami, 2009; Wang *et al*., 2012). Glucomannan sources influence the ratio of glucose and mannose monomers (Maeda *et al*., 1980; Gao and Nishihari, 2004). Generally, KGM has a mannose and glucose molar ratio of 1: 1.6 (Alonso-Sande *et al*., 2009; Wang *et al*., 2012) or 2: 3 (Takigami, 2009; Wang *et al*., 2012) with degrees of polymerisation of 6,000 (Tester and Al-Ghazzewi, 2016; Behera and Ray, 2017). The acetyl group is at 9-19 units of sugar glucomannan and is connected to the main chain via C3 or through C3 on D-glucose and D-mannose residues and or as branch points in C2 and C4 (Kato and Matsuda, 1969) or C3 or C4 (Maeda *et al*., 1980). This polysaccharide has an acetylation degree of between 5-10%, mostly about 8%. The composition of glucose and mannose in glucomannan consists of several patterns. It has been reported that the repeating unit of the glucomannan is pattern A; G-G-M-M-M-M-G-M and pattern B; G-G-M-G-M-M-M-M (Kato and Matsuda, 1969), where M and G represent D-mannopyranosyl and D-glycopyranosyl residues, respectively. The chemical structure of glucomannan can be seen in Figure 1.

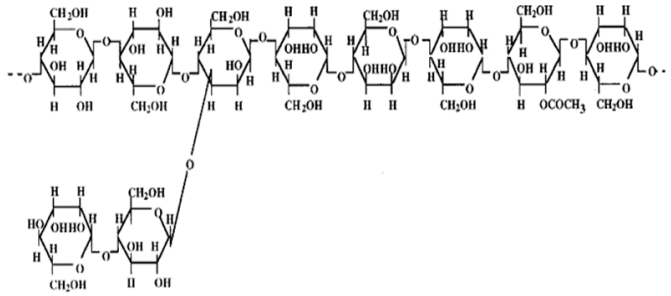

Figure 1. Chemical Structure of glucomannan (Okimasu and Kishida, 1982)

Konjac Glucomannan (KGM) has a molecular weight ranging from 200 - 2000 kD and ranges from 1000 kD during storage and processing (Parry, 2010; Keithley and Swanson, 2005). Xu *et al.* (2013) explained that konjac glucomannan has a molecular weight of 2.476×10^5 g/mol using a combination method of laser light scattering and refractive index (GPC-LLS-RI) online method. The first method resulted in a ~~B~~ molecular weight of 2. 508×10^5 g/mol. The molecular weight of KGM is influenced by cultivars, origin, processing methods, and storage time. KGM is one of the most important chemical components in the *Amorphophallus konjac K. Koch* tubers (Takigami *et al*., 1997). The *Amorphophallus konjac* tubers were served using a Hitachi S-2460N chilled scanning electron microscope (SEM) equipped with an energy-dispersive X-ray spectrometer (EDX), direct magnification ×200, to uncover the konjac mannan cell of the tissue structure of a 2-year-old Konjac tuber. Most cell morphology that could be observed was composed of glucomannan granules with a diameter in the range of 0.25 – 2 mm. Glucomannan granules are 10-20 times larger than starch cells. In *Amorphophallus muelleri* (porang) or other konjac species tubers, glucomannan granules are located in an egg-shaped idioblast cell in the parenchymal tissue as a single cell and are protected by a kind of cell wall. Glucomannan granules are evenly distributed in the konjac tubers. Microscopically, glucomannan granules have cell wall surfaces that resemble scales and are compact (Chua *et al*., 2012).

Glucomannan is a hydrophilic molecule and is quickly soluble in water. Water solubility is affected by hydrogen bonds and acetyl groups contained in glucomannan. KGM lost its solubility in water by strong hydrogen bonds once it had been purified or dried (Kohyama *et al*., 1993). At the same time, the presence of acetyl groups can inhibit the formation of intramolecular and intermolecular hydrogen bonds between glucomannan molecules, therefore, it can cause a decrease in the solubility of glucomannan in water with increasing degrees of acetylation (Chen *et al*., 2011). The highly hydrophilic KGM macromolecules show difficulty in water solubility relatively quickly without heating (Zhang *et al*., 2014). The viscosity of glucomannan is influenced by the concentration and pH of the solution but is not affected by salt concentration (Cui *et al*., 2013).

Moreover, glucomannan gel was stable in hot conditions with alkali and low pH (Dave *et al.*, 1998). Glucomannan is a molecule capable of interacting synergistically with other polysaccharides such as xanthan, carrageenan, agar, and locust bean gum and forms a reversible gel when exposed to heat. Adding sugar can increase the synergy of interactions, and adding salt can reduce the synergy of glucomannan interactions with other polysaccharides (Saha and Bhattacharya, 2010).

Another property of KGM is to quickly absorb water so that the molecules are susceptible to swelling. Adding a little water to glucomannan through hydrogen bonding, molecular dipole, and induced dipole, the instantaneous dipole can cause glucomannan macromolecules to change shape and contribute to decreasing molecular solubility. Glucomannan has a water absorption value close to 105.4 grams of water/gram glucomannan, while glucomannan acetylation reduces this value to 1 g of water/gram glucomannan (Zhang *et al*., 2014). Glucomannan powder expands in water by 138-200% compared to ordinary starch, which can only expand 25% in water (Parry, 2010). Glucomannan solution in water has the properties of glue, but when adding acetic acid or acid in general, the properties of the glue will disappear altogether. The glucomannan solution can be precipitated by recrystallisation by ethanol, and the crystals that will be formed can be dissolved with dilute hydrochloric acid. Glucomannan can be converted into mannose and glucose by methylation or acetylation (Ohtsuki, 1967). Glucomannan will decompose at a temperature between 250°C and 350°C. Glucomannan is also sensitive to the activity of enzymes such as β-D-glucanase and β-D-mannase. In addition, glucomannan is sensitive to fermentation bacteria in the digestive tract (Parry, 2010).

3. Production technologies

3.1 Production of konjac glucomannan

Crude konjac flour (CKF) is d as a powder with light colour obtained from *Amorphophallus konjac* (Behera and Ray, 2016). The characteristics of CKF are the low glucomannan content (37.78-45.89%) and low viscosity, high calcium oxalate content and dark colour (Faridah and Widjanarko, 2013) and a typical fish-like smell (Suzuki, 1980). Widjanarko *et al*. (2014) proposed the production of CKF from *A. muelleri* by washing, slicing, drying, milling chips to 60-40 mesh powder, then air classification to separate starch and other impurities from konjac flour using a cyclone. Usually, a cycle will be carried out three times to reduce tachiko or tobico (dust) from konjac flour (Zhao *et al*., 2010). Purification of CKF using multiple ethanol leaching with 40% concentration, followed by 60% and 80% respectively for 4 hrs each time and assisted by ultrasound wave has successfully attained to produce purified yellow konjac flour (Widjanarko *et al*., 2014). Yanuriati *et al*. (2017) achieved the isolation of glucomannan using two different methods, water dissolving using $Al_2(SO_4)_3$ as a flocculant and purification using ethanol (Methods 1) and repeated milling using ethanol (5 – 7 times) and glucomannan granules filtration to separate them from impurities without further purification (Methods 2). Farmers prepared crude konjac flour (CKF) by slicing konjac corms, drying and pounding the chips. Current commercial production of konjac flour: Before processing dried chips, corms were washed to remove dirt and soil. The outer skin of corms was peeled and sliced into chips followed by drying in hot air, which contains sulphur dioxide to prevent the darkening of konjac chips. The dried chips are pulverised to produce CKF, a fish-like smell and an acrid taste. Common konjac flour (food grade) is produced by removing impurities such as starch, protein, cellulose, fats and low molecular weight sugars from CKF. This can be achieved by using two-four cyclones of a grinder or ethanol precipitation (Chua *et al*., 2010).

3.2 Production of hydrolysed konjac glucomannan

Several attempts have been devoted to preparing HKGM. In most cases, the preparation of HKGM consists of physical, chemical and enzymatic degradations.

3.2.1 Physico-chemical degradations

Physical degradation of KGM by using γ-rays that penetrate the granule and randomly rupture the backbone of KGM in any physical form (Xu *et al*., 2007). The safety aspect of the irradiation technique is still questionable, but the dosage of up to 10 kGy is safe (Jian *et al*., 2013; Jin *et al*., 2014). Increasing the efficiency of KGM degradation by irradiation is obtained by adding a solvent such as a hydrogen peroxide solution. The degradation process of KGM with γ-rays and hydrogen peroxide solution will also produce the hydroxyl radical. It is well known to be an oxidative agent. The hydroxyl radicals could break down the glycosidic linkages of KGM, and the molecular weight of synergic degradation is lower than the untreated sample (Pan *et al*., 2013). The physicochemical properties and cellular protection of HKGM prepared by γ-irradiation were revealed by Jian *et al*. (2017). They reported that the dosage of 100 kGy irradiation produced a good degradation product and contributed to the antioxidant activity. These findings provide new products of health food and medicinal products.

Ultrasound-assisted extraction has been used to extract bioactive compounds from cash crops (Bubalo *et al*., 2016; Wen *et al*., 2018). It was reported that ultrasound-HCl treated KGM had a degree of hydrolysis, significantly different at $p>0.05$ than KGM-sonification or untreated samples (Cheng *et al*., 2010). Ultrasound-acid hydrolysis of KGM with trifluoroacetic acid (TFA) exhibited much lower molecular weight fractions than untreated and enhanced the growth of bifidobacteria *in vitro* experiments (Song *et al*., 2018).

3.2.2 Enzymatic hydrolysis

Enzymatic hydrolysis to degrade polysaccharides has been widely used to lower molecular weight (Koutinas *et al*., 2001). The molecular structure of KGM has multiple cutting-edge sides; thus, it facilities the degradation by enzymic hydrolysis. Structural characteristics of the oligosaccharide fragments of KGM that had been hydrolysed by endo-glucanase (EG) and endo-mannanase (EM) had been studied by Albrecht *et al.* (2011). The results showed that KGM polysaccharides were composed of short mannose and glucose sequences at the backbone of KGM. Hydrolysis of 1% KGM by hemicellulase extracted from *Trichoderma reesei* at pH 5.0, 45°C for 48 hrs resulted in 17% HKGM (DP 2-6) (Mikkelson *et al*., 2013). Furthermore, cellulase from *Trichoderma viride* was used to hydrolyse 0.5% KGM at reaction conditions such as pH 3.5, 30°C, enzyme: substrate ration 58 U/g for 24 hrs exhibited molecule oligosaccharide covering of DP 3-14 (Albrecht *et al*., 2011). Hydrolysis of 12 mg KGM in 0.02 M NaOAc solution, with the presence of β-Mannase from *Aspergillus niger* at the processing condition of pH 4.5 at 40°C for 24 hrs or cellulase from *Penicilium funiculosum*, where 11 mg polysaccharide dissolved in 0.05 M NaOAc buffer at pH 4.8, 37°C for 24 hrs produced dimer MM dyads 26%, GG dyads 16% (Cescutti *et al*., 2002).

4. Safety

The beneficial effects of consuming konjac glucomannan have been reviewed by Keithley and Swanson (2005), and only limited information on HKGM safety has been reported. The effects of ingested depolymerised glucomannan on wound healing were observed (Al-Ghazzewi *et al.*, 2015). The results showed faster healing for the group of mice drinking water containing depolymerised KGM (DKGM). The administration of DKGM up to 10.0 g/kg had no mutagenic effects. In addition to that, DKGM up to 2.5 g/kg exhibited neither maternal nor foetal teratogenicity in pregnant rats (Jiang *et al*., 2018).

5. Health benefits of konjac glucomannan and its derivatives

5.1 Konjac glucomannan and hydrolysed konjac glucomannan as prebiotics

Generally, prebiotics is defined as non-digestible polysaccharides and oligosaccharides (NDO), which promote the growth of beneficial lactic acid bacteria (LAB) in the colon and exert antagonism against *Salmonella* sp. or *Escherichia coli,* limiting their proliferation (Patel and Goyal, 2012). Many researchers reported about effects of prebiotics using *in vivo* and human studies. Chen *et al*. (2008) investigated time-course and dose-dependent unhydrolysed KGM and acid -hydrolysed glucomannan (HKGM) on the cecal and faecal microflora in balb/c mice. This study showed that the KGM and HKGM significantly increased cecal anaerobes bifidobacteria counts at weeks 2 and 4, respectively. KGM and HKGM significantly decreased cecal *Clostridium perfringens* counts only at week 4. In addition, acetate and propionate concentration in cecal contents were increased by KGM and HKGM diets at weeks 2 and 4. HKGM increased cecal bifidobacteria counts only at the 2.5% level but increased faecal bifidobacteria count and suppressed *Clostridium perfringens* counts at each dose level compared with KGM. Al-Ghazzewi *et al.* (2007) investigated the hydrolysed konjac glucomannan as a prebiotic by studying the growth profile of 2 bacteria strains (lactobacilli and bifidobacteria) on de Man, Rogosa and Sharpe (MRS) media supplemented with hydrolysate or in UHT milk supplemented with oligosaccharides (hydrolysate KGM or inulin). The HKGM stimulated the growth of all the bacteria strains, and colony size was significantly bigger than those grown on pectin or xylan hydrolysate. In addition, the number of colony-forming units (CFU) in milk supplemented with HKGM was higher than in those supplemented with inulin. A similar result was also reported by Elamir *et al.* (2008). Elamir and co-workers investigated the effects of depolymerised mannans and specifically HKGM on the colonic microflora of mice. The results reported that HKGM promotes the growth of anaerobes and lactobacilli and significantly reduces *Clostridium perfringens* and *Escherichia coil* counts in faeces. Harmayani *et al.* (2014) also studied the effect of prebiotics from *porang* konjac flour (PKF) on male Wistar rats. The diet supplemented with PKF was able to inhibit the growth of *Escherichia coli*, enhance the production of total SCFA, and reduce the pH value of cecal content.

Several researchers reported the effect of prebiotic KGM on a human. Chen and co-workers studied the effect of prebiotic KGM supplements in healthy adults (Chen *et al.*, 2006) and constipated adults (Chen *et al*., 2008) during a twenty-one day placebo period and a seven-day adaptation period. The stools were fully collected on days 15 to 21 of placebo for further analysis. These results showed that the KGM supplement significantly promotes the faecal concentration of lactobacilli, bifidobacteria, and total bacteria evaluated by the fluorescence in situ hybridisation methods. In addition, the KGM supplement also promotes colonic fermentation and growth of lactic acid in decreased faecal pH and increased faecal short-chain fatty acid concentration. Glucomannan hydrolysate (HKGM) at a

concentration of 0.1, 0.5, 1.0 and 2.0% w/v inhibit the growth of *Candida albicans* in the presence of *Lactobacillus* and *Lactococcus* species when compared with inulin and glucose. GMH promoted the growth of LAB (even at concentrations as low as 0.1%) and consequently increased inhibition of *Candida albicans* under anaerobic conditions, 5% CO_2 or aerobic conditions. Inhibition of C. *albicans* growth was generally higher than that with glucose or inulin, and, of the LAB strains, *Lactobacillus jensenii* exhibited the most pathogen inhibition.

5.2 Anti-inflammatory effects

Inflammation occurs when tissues and cells are exposed to biological or physical (virus, bacteria) infection. Human organs' infection site indicates local swelling and pains because of the accumulation of plasma and white blood cells. In the process of inflammation, cells release inflammatory mediators, such as interleukins, chemokines, tumour necrosis factors, interferons and eicosanoids (Medzhitov, 2008). Some studies suggest that natural polysaccharides may be used as folk medicine to treat inflammation. Zheng *et al.* (2019) claimed that the ultrasound-degraded oxidised konjac glucomannan (U-OKGM) decreased significantly ($p>0.05$) the secretion levels of TNF-α and IL-1β in LPS -stimulated RAW264.7 cells. It should be noted that inflammatory cytokines, which are TNF-α and IL-1β are known to be pro-inflammatory factors (Wang *et al*., 2020). The anti-inflammatory effects of KGM and its derivatives were supported by the findings of Liu *et al*. (2016). They found that when Konjac oligosaccharides (KOS) were administrated in rats, the expression of the pro-inflammatory factors (TNF-α and IL-1β) was lower in rats with TNBS-induced colitis than in TNBS-treated rats.

5.3 Anti-tumour activity

Medical treatment of tumours has been complex, so scientists could not overcome it for a long time. The present treatment for tumour patients includes resection, irradiation and chemotherapy, but these treatments have adverse side effects on the human body such as nausea, loss of hair, loss of appetite and others. As a result, people seek immunological treatments that create a more comfortable feeling (Li *et al*., 2019). A high-fat corn oil diet on Sprague-Dowley rats significantly elevated faecal β-glucoronidase and mucinase activities and total bile acid concentration, known to be the risk factors for colon cancer carcinogenesis. The administration of KGM and inulin into the high-fat fibre-free diet beneficially reduced the faecal β-glucoronidase and mucinase activities and secondary bile acid concentration (Wu and Chen, 2011). They claimed that KGM could be used as a supplementary diet for high-fat diet-induced colon cancer risk patients. KGM was tested using an MTT assay on multidrug-resistant (MDR) cancer cells, including 5-fluorouracil (5-FU) resistance, to investigate the reversal effect of KGM on 5-FU. PCR and western blotting were also performed to determine the effect of 5 -FU and KGM on the expression of MDR-associated genes. The results showed that KGM significantly inhibited the growth of HepG2/5-FU in nude mice. KGM may be a promising agent against the resistance of HepG2/5-FU cells (Chen *et al.*, 2020).

6. Conclusion

KGM and HKGM are natural polysaccharides widely applied in various industries for food processing and bring health benefits by having biological activities including prebiotics, anti-inflammatory, anti-tumour, and other health-related applications. The preparation of crude konjac flour has been discussed, and the purification of crude konjac flour includes air classification or ethanol precipitation. Hydrolysis of KGM includes physicochemical and enzymatic methods. KGM and HKGM appeared to have health benefits, including prebiotic, anti-inflammatory and anti-tumour. The future of KGM and its derivatives is becoming crucial for maintaining the health of human beings.

Conflict of interest

The authors declare no conflict of interest.

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