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Use of Ethanol Solution for Extruding Konjac Glucomannan to Modify Its Water Absorption and Water Solubility

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ABSTRACT

Konjac glucomannan (KGM) has promising health benefits; however, high water absorption can limit its use in nutraceuticals. In this study, extrusion with alcohol addition successfully modified its water absorption. KGM, with different solids content, was extruded through a twin screw extruder at different barrel temperatures and screw speeds, and the corresponding effects on the water absorption index (WAI) and water solubility index (WSI) were determined using response surface methodology (RSM). Increasing solids content resulted in an increased WAI while an inversely proportional effect was observed with temperature. The WSI decreased with increased temperature but increased with higher solids content. The WAI values ranged from 154.6 (control) to 47.5 for samples with 20% solids extruded at 110 ^{o}C. The WSI values ranged from 0.71 (control) to 0.36 for samples under similar extruding conditions. In both cases, the RSM models showed that greater modification was attained with a decreased solids level and a higher extrusion temperature. The screw speed had little influence on the WAI or WSI.

Keywords: extrusion, response surface methodology, konjac glucomannan, water absorption, water solubility

INTRODUCTION

Extrusion is a process in which a pliable material is forced through a die orifice to produce a long, continuous product with a uniform cross-section. Extrusion has long been used to manufacture uniform synthetic polymers and metal pieces as well as foodstuffs such as pet foods and starch-based snacks (Moscicki and van Zuilichem, 1983; Laarhoven *et al*., 1991). The process has also been used to modify starch structures and offers several advantages over chemical modification, including lower costs and higher throughput (Lopez-Rubio *et al*., 2007). The granular structure of starch may be disrupted by extrusion, as native crystalline regions are melted and macromolecules are partially disrupted. Portions of the starch may be transformed to a crystalline state, an amorphous state or an unstable V-type crystal state, depending on the polymer type and processing conditions such as the screw speed, the solids flow rate, the water flow rate and the barrel temperature (Biliaderis, 1998; Lopez-Rubio *et al*., 2007). Previous studies have shown that several properties of starch may be improved or at least modified by extrusion, for example

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the average molecular weight, viscosity, water absorption index (WAI) and water solubility index (WSI) (Gomez and Aguilera, 1983; Davidson *et al*., 1984; Sriburi *et al*., 1999). For instance, the WAI of extruded corn starch increased in comparison with non-extruded corn starch (Gomez and Aguilera, 1983), and a change of the WAI of extruded starch also depended on the degree of starch conversion (Sriburi *et al*., 1999). While extrusion shows much promise for the physical modification of the polymer structure, only a few studies on non-starch polysaccharides have been performed (Cote and Willet, 1999; Sereno *et al*., 2007).

Konjac glucomannan (KGM) is a water-soluble polysaccharide found abundantly in the konjac tuber (*Amorphophallus Konjac*), and extraction of KGM from konjac tubers typically uses an ethanol solution (Ohashi *et al*., 2000). This tuber is grown in several Asian countries including China, Japan, and Thailand (Techapattaraporn, 1992; Takigami, 2000; Li and Xie, 2003). KGM is a neutral polysaccharide which contains mannose and glucose at a ratio of approximate 1.6 to 1.0 (Takigami, 2000). The glucomannan backbone has randomly placed β-1,4 linked *D*-mannose and *D*-glucose, with an approximately 8% degree of branching through β-1,6 glucosyl linkages; branches are typically 2 to 3 units long (Li and Xie, 2003; Takigami, 2000). Furthermore, the chain has a 5–10% acetyl group substitution (Takigami, 2000). KGM is an amorphous polymer and has a molecular weight in the range of 1×10^5 to 1×10^6 Da (Li and Xie, 2003). The viscosity of KGM is very high being the highest viscosity among various gum types (Yaseen *et al*., 2005). The gelation behavior of KGM is dependent on the presence of acetyl groups in the chain, which inhibit interchain interactions (Kishida *et al*., 1978). After heating in the presence of alkali agents such as calcium hydroxide, KGM solutions transform into a strong, elastic and irreversible gel (Takigami, 2000). An alkali solution is required for the gelation of KGM at a low solid content (Gao and Nishinari, 2004); however, KGM can also form a gel in the absence of alkali when extruded with a high solids content (Dave *et al*., 1998). KGM has also been shown to form gels in the absence of alkali, when co-gelled with other hydrocolloids (Williams *et al*., 1993).

KGM has many valuable properties useful for applications in drug delivery systems, coating materials, encapsulation materials, emulsifiers, and cosmetics (Zhang *et al*., 2005). Several studies have shown the health benefits of KGM (Chen *et al*., 2005; Martino *et al*., 2005; Wood *et al*., 2007), and many conventional and health foods contain KGM as an ingredient. It is not hydrolyzed by digestive enzymes in human beings and is considered an indigestible dietary fiber and has been used to treat constipation, as it reduces the fecal transit time (Chen *et al*., 2005). KGM is also known to reduce cholesterol levels (Martino *et al*., 2005), so it has a potential role to play in the prevention of coronary heart disease (Vuksan *et al*., 1999). KGM significantly increases fecal anaerobes and bifidobacteria in mice, thus it has been investigated for its prebiotic effects (Chen *et al*., 2005); moreover, it has been introduced in the USA as a GRAS (generally recognized as safe) substance (Takigami, 2000). However, the use of KGM in health foods and neutracutical products is limited by its relatively high water absorption index, which can be as high as 100 g of water per gram of sample (Koroskenyi and McCarthy, 2001). To improve these properties, the use of chemical modifications such as acetylation, methylation and oxidation has been reported (Kishida *et al*., 1978; Gao and Nishinari, 2004), but the procedures are both inconvenient as well as time consuming. Physical methods such as sonication, irradiation and pressure-temperature treatments have also been suggested as alternatives (Samil Kök *et al*., 2009), but these processes are not practical on an industrial scale. The extrusion process can subject the extruding materials to substantial pressures, temperatures and shear conditions. These circumstances could cause degradation and affect the structure of the material resulting in changes to the physical properties (Sriburi *et al*., 1999). In the case of extruded konjac glucomnan, a melting temperature of 1.0% (weight per weight; w/w) KGM gel was reported as being approximately 40 °C (He *et al.,* 2012); therefore, the current study hypothesized that the use of cooking extrusion might break the konjac glucomannan chains and might change their properties. Few studies have been performed on the use of extrusion to modify polysaccharides other than starch. Thus, this research aimed to access the potential of the extrusion process in altering the properties of KGM, particularly those related to water absorption and solubility. As ethanol solutions are commonly used to extract KGM from the tuber and flour (Ohashi *et al*., 2000), its use for extrusion was investigated compared with water. In addition, response surface methodology was used to study the effects of the extrusion variables (solid content, barrel temperature and screw speed) on both the water absorption and water solubility.

MATERIALS AND METHODS

KGM was purchased from DKSH (Thailand) Limited and ethanol (95% purity) was acquired from The Liquor Distillery Organization, Thailand. The carbohydrate and moisture contents of KGM were 89.04% and 9.13% respectively. The ethanol was used and mixed with distilled water as required for extrusion.

Extrusion was performed in a co-rotating twin screw extruder (ZE25x 33D; Hermann Berstorff Laboratory; Hannover, Germany). A length-to-diameter ratio of 33:1 and a screw diameter of 25 mm were used in the present study. The hole diameter of the die plate was set at 30 mm. The extruder barrel contained seven independent heating zones. Temperature profiles in the first to the fifth sections were kept constant at 30, 40, 50, 70 and 90 °C, respectively. The temperature of the sixth section was set at 90, 100 or 110 °C, and the temperature of the seventh section was kept at 10 °C below that of the sixth heating section. KGM was metered into a feed hopper and mixed with the ethanol solution (20%) to attain the desired solid contents. The screw speed was varied between 250 and 350 rpm. The extruded KGM was dried in a hot air drier at 45 °C for 15 hr, then ground and sieved through a 100 mesh (approximately 150 µm) screen. The resultant KGM powder was stored with silica gel in sealed polyethylene/aluminum pouches until use.

A response surface methodology (RSM) was used in the experimental design to establish the optimal extrusion conditions for the WAI and WSI values. A 3-factor 3-level Box-Behnken design consisting of 15 experimental runs complete with 3 replicates at the center point was used to map the dependent variables. The independent variables were solid content (Sc), barrel temperature (Bt) and screw speed (Sp), coded as X_1, X_2 and X_3, respectively. The relationships between the variables are shown in Tables 1 and 2.

Table 1 Independent variables, symbol and code level used in the response surface methodology, and probability levels for water absorption index (WAI) and water solubility index (WSI) for extrusion of konjac glucomannan.

Independent variable	Symbol	Code level			Probability (*P* level)	
		-1	0	1	WAI	WSI
Solid content (%)	X_1	15	20	25	0.000	0.000
Barrel temperature (°C)	X_2	80	90	100	0.000	0.001
Screw speed (revolutions per minute)	X_3	250	300	350	0.006	0.078

Table 2 Code variables and variable levels used in the response surface methodology experimental design, and experimental data for water absorption index (WAI) and water solubility index (WSI) for konjac glucomannan samples in each treatment.

Treatments	Code variables			Variable levels			Experimental data	
	X_1	X_2	X_3	Sc (%)	Bt (°C)	Sp (rpm)	WAI	WSI
1	1	-1	1	40	90	300	156.86 ± 2.06[k]	0.73 ± 0.02[i]
2	0	-1	-1	30	90	250	94.68 ± 0.54[ef]	0.54 ± 0.03[ef]
3	0	-1	1	30	90	350	113.61 ± 1.12[g]	0.62 ± 0.02[h]
4	-1	-1	0	20	90	300	76.12 ± 2.19[c]	0.46 ± 0.01[bc]
5	1	0	1	40	100	350	146.04 ± 0.76[j]	0.63 ± 0.04[h]
6	0	0	0	30	100	300	92.76 ± 3.58[e]	0.51 ± 0.03[de]
7	1	0	-1	40	100	250	133.42 ± 2.90[i]	0.60 ± 0.02[gh]
8	0	0	0	30	100	300	95.98 ± 2.70[ef]	0.55 ± 0.01[ef]
9	-1	0	1	20	100	350	81.96 ± 0.43[d]	0.48 ± 0.02[cd]
10	0	0	0	30	100	300	96.67 ± 0.31[f]	0.54 ± 0.02[ef]
11	-1	0	0	20	100	300	69.43 ± 0.65[b]	0.42 ± 0.01[b]
12	-1	0	-1	20	100	250	118.90 ± 0.88[h]	0.57 ± 0.03[fg]
13	0	1	-1	30	110	250	74.69 ± 1.93[c]	0.45 ± 0.02[bc]
14	0	1	1	30	110	350	66.89 ± 2.54[b]	0.45 ± 0.01[bc]
15	-1	1	0	20	110	300	47.51 ± 0.98[a]	0.36 ± 0.00[a]
16	-	-	-	-	-	-	154.59 ± 2.63[k]	0.71 ± 0.03[i]

Sc = Solids content, Bt = Barrel temperature of the sixth head, Sp = Screw speed, rpm = Revolutions per minute.
Experimental data are shown as mean ± SD.
Treatment 16 was non-extruded konjac glucomannan.
[a-k] = Different superscript letters after values in the same column indicate a significant difference between samples ($P \leq 0.05$).

A multiple regression analysis was used to fit the second-order polynomial equation (Equation 1):

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j \quad (1)$$

where β_o, β_i, β_{ii} and β_{ij} are coefficients for the intercept, linear, quadratic and interactive effects, respectively. The Minitab 16 statistical software (State College, PA, USA) was used to fit the data and develop the plots. The optimal model with no significant lack-of-fit ($P > 0.05$) and the highest correlation coefficient (R^2) was selected. Contour and surface plots were also generated as a function of two variables, with the third variable kept at the optimum level.

All samples were determined for water sorption, water solubility and crystallinity. The water absorption index (WAI) and water solubility index (WSI) were determined based on the methods of Anderson *et al*. (1969). A 0.150 g sample was weighed and suspended in 30 mL of distilled water. The sample was then decanted into a 50 mL centrifuge tube, vortexed and inverted to disperse any clumps. The mixture was stirred on a magnetic stirrer for 30 min. The mixture was again centrifuged at 1500×*g* for 15 min. The supernatant was then poured into a tarred metal canister and dried at 80 °C for 24 hr to determine the dried weight of the dissolved solids. The tube of the residual gel was weighed after removal of the supernatant fraction to determine the wet weight of the sediment. All measurements were performed in triplicate. The WAI and WSI were calculated using the Equations 2 and 3, respectively;

$$\text{WAI} = \frac{wt_{\text{ sediment}}}{(wt_{\text{sample}})(\text{1-soluble solids fraction})} \quad (2)$$

$$\text{WSI} = \frac{wt_{\text{ solids in supernatant}}}{(wt_{\text{dry solids}})} \quad (3)$$

where $wt_{\text{ sediment}}$ is the weight of the sediment, $wt_{\text{ sample}}$ is the weight of the sample, $wt_{\text{solids in supernatant}}$ is the weight of the solids in the supernatant and $wt_{\text{ dry solids}}$ is the weight of the dry solids. The crystallinity indicates the degree of tight organization of the molecular chain in the polymer. The crystallinity values of the extruded KGM and non-extruded KGM samples (average particle sizes of samples were approximately 100–140 µm, determined with a mastersizer) were determined with X-ray diffraction (XRD; Phillips X'Pert; Amsterdam, the Netherlands). The tube voltage and tube current applied were 40 kV and 30 mA where a continuous measurement was scanned from 5 to 60° at a rate of 5.00°.min^{-1}.

To validate the generated models, three new extrusion conditions were tested in triplicate to compare the experimental and predicted values. The average results along with the standard deviations were reported for the verification test using an analysis of variance (ANOVA) and Duncan's multiple range test generated using the SPSS application version 10.0.0 (IBM SPSS, Chicago, IL, USA). Results were considered significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

Use of ethanol solution for extruding KGM

The aim of the present study was to decrease the water absorption of KGM by means of the extrusion process. Several studies have shown extrusion was able to modify the water absorption properties of starch (Gomez and Aguilera, 1983; Davidson *et al*., 1984; Sriburi *et al*., 1999; Hagenimana *et al*., 2006), but only a few studies have been performed on non-starch polysaccharides (Cote and Willet, 1999, Sereno *et al*., 2007). Preliminary studies were conducted in which KGM was extruded with distilled water only. At a solid contents level of 20% (w/w), a multitude of lumps were observed in the mixture (Figure 1). In addition, the mixture was so thick that it was difficult to operate the screw in a smooth and continuous fashion. As noted previously, aqueous KGM dispersions have the highest viscosity among a number of tested gums (Yaseen *et al*., 2005). The KGM mixed well with 20% (v/v) ethanol at a solids content of 20% (w/w) (Figure 1). The mixture was found to yield a coarse texture that was suitable for extrusion without slippage of material during extrusion. The extrusion of the KGM samples was also operated without the die insert in some instances to allow for a more homogeneously continuous product. Previous study indicated that extrusion without the application of a die was potentially useful to obtain desirable properties in rice flour extrudates (Guha *et al*., 1997).

Model fitting and verification

The RSM was used to investigate the influence of the extrusion process variables on the water absorption and solubility of the KGM extrudate. The data were fitted to a polynomial equation comprised of linear, quadratic, cubic and interaction terms for the three independent variables (solids content, barrel temperature and screw speed) in accordance with ANOVA procedures. The selection of the best models was based on lack-of-fit tests and an R^2 analysis. Good fit was achieved with a second order polynomial model, with R^2 values of 0.994 and 0.971 for the WAI and WSI, respectively. The models were also examined for lack-of-fit and found that there was no significant lack-of-fit for both of the WAI and WSI predicted equations (data not shown). Thus, the best-fitting equations for WAI and WSI were found to be Equations 4 and 5, respectively:

$$WAI = -779.80 - 1.66(Sc) + 16.24(Bt) + 0.66(Sp) + 0.12(Sc\times Sc) - 0.08(Bt\times Bt) + 3.92E-05(Sp\times Sp) - 0.02(Sc\times Bt) + 4.50E-05(Sc\times Sp) - 0.01(Bt\times Sp) \quad (4)$$

$$WSI = -2.23 + 0.03(Sc) + 0.03(Bt) + 0.01(Sp) + 7.08\times 10\text{-}5(Sc\times Sc) - 1.04\times 10\text{-}4(Bt\times Bt) - 3.17E-06(Sp\times Sp) - 1.50E-04(Sc\times Bt) - 1.50E-05(Sc\times Sp) - 4.0E-05(Bt\times Sp) \quad (5)$$

where Sc is the solids content, Bt is the barrel temperature and Sp is the screw speed. To further verify the fitted models, KGM was extruded under three new extrusion conditions (Table 3). The predicted values for the new WAI and WSI conditions were then compared to the experimental data. Reasonably good predictions were attained with differences between 1.81 and 8.18% for WAI, and 0 and 7.78% for WSI.

Influences of extrusion variables on water absorption and water solubility

The mean values of the WAI and WSI for all extruded trials in comparison with the non-extruded KGM are shown in Table 2. Most of the extruded KGM samples had lower WAI values and were significantly different from the non-extruded control (WAI = 154.59). The WAI values after extrusion ranged from 47.51 to 156.86. Similarly, most values of the WSI were lower than and significantly different from the control (0.71). After extrusion, the WSI values ranged from 0.36 to 0.73. The variables for the WAI and WSI values (Table 1) were determined to investigate the significance of the tested effects. Both the solids content and temperature were significant factors for the WAI and WSI. However, the screw speed

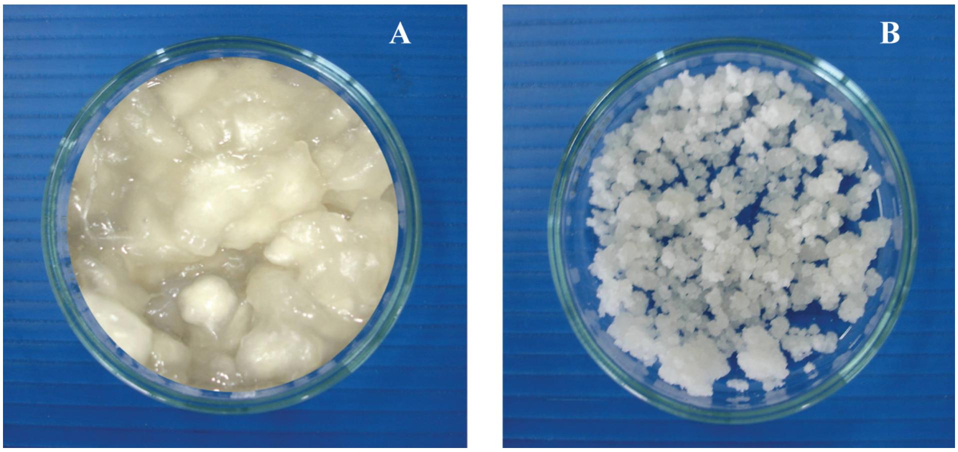

Figure 1 Appearance of konjac glucomannan mixtures 20% weight per weight with: (A) distilled water; (B) 20% ethanol solution at 35 °C.

Table 3 Experimental conditions and differences between experimental and predicted data for water absorption index (WAI) and water solubility index (WSI) for extruded konjac glucomannan without a die.

Conditions			WAI		WSI		WAI - WSI (%)	
Sc (%)	Bt (°C)	Sp (rpm)	Experiment	Prediction	Experiment	Prediction	WAI	WSI
34	90	330	133.38	126.57	0.66	0.66	5.10	0.00
31	100	270	87.73	94.91	0.52	0.53	-8.18	-1.61
21	110	310	53.10	52.14	0.42	0.39	1.81	7.78

Sc = Solids content, Bt = Barrel temperature of the sixth head, Sp = Screw speed, rpm = Revolutions per minute.

was only significant for the WAI. The contour plots showed the effects of the solids content and barrel temperature on the WAI and WSI of KGM held at a constant screw speed of 300 rpm (Figures 2 and 3).

The effects of screw speed and barrel temperature on the WAI are clearly illustrated by the contour and surface plots (Figure 2). These plots show that an increased solids content in the feed resulted in a higher WAI, while

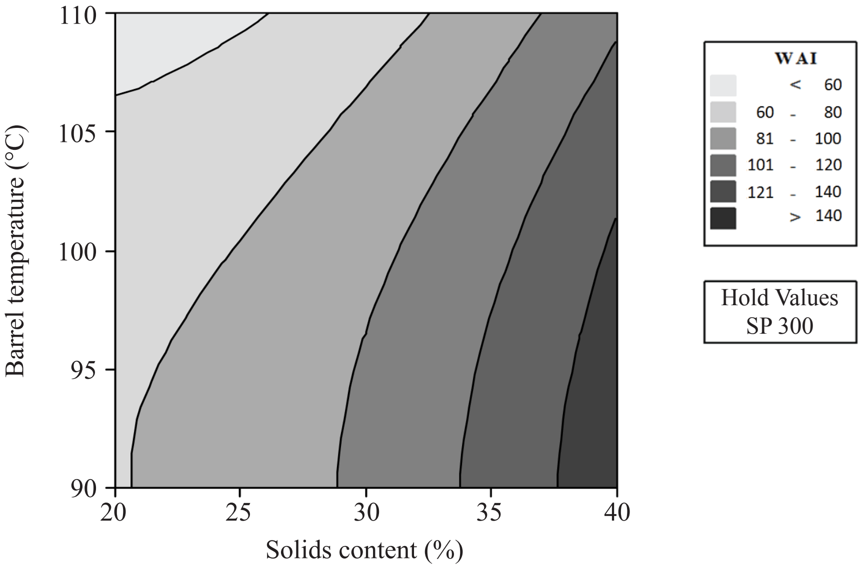

Figure 2 Contour plot showing the effect of solids content and temperature on the water absorption index (WAI) of the extruded konjac glucomannan at a constant screw speed. On legend, "Hold values SP 300" is constant screw speed at 300 rpm.

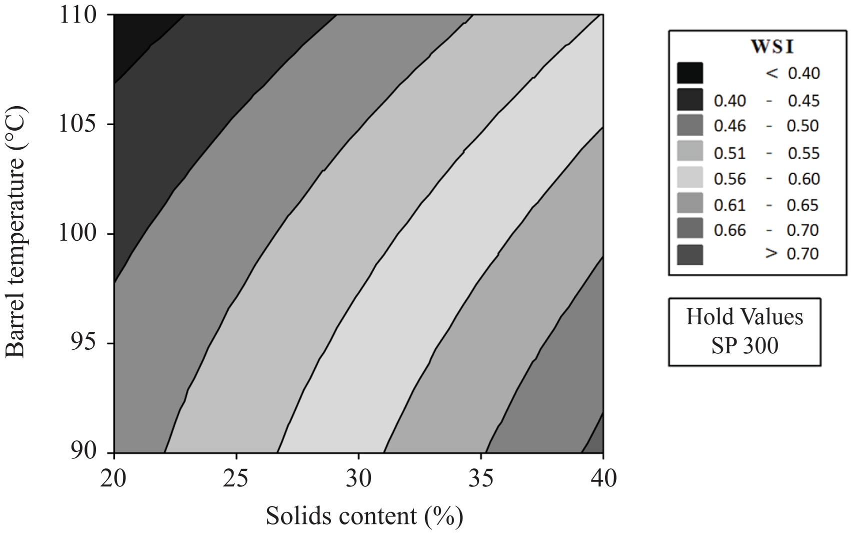

Figure 3 A contour plot showing the effect of solids content and temperature on the water solubility index (WSI) of the extruded konjac glucomannan at a constant screw speed. On legend, "Hold values SP 300 is constant screw speed at 300 rpm.

increasing temperatures resulted in a lower WAI. In addition, since no local minimum for the WAI was observed and the WAI decreased with a lower solids content and higher temperatures, the study found that extensive extrusion could reduce the weighted average molecular weight of glucomannan (data not shown). The reduction in molecular size potentially decreased the entanglements which encouraged extensive gel formation and consequently resulted in a structure which entrapped water to a lesser degree. Previous research on starch and flour found the WAI values to vary widely and to depend on the initial moisture content, temperature, screw speed and amylose content (Gomez and Aguilera, 1983; Sriburi *et al*., 1999; Hagenimana *et al*., 2006). Hagenimana *et al*. (2006) studied extruded rice flour which resulted in increased WAI values for 19–22% moisture levels. They suggested that water acted as a plasticizer during extrusion, and thus was able to reduce the starch granule degradation causing enhanced water absorption. In contrast, at a lower moisture content (16%), increasing temperature levels decreased the WAI values. It should be noted that starch is compacted in granules with various semi-crystalline and amorphous regions whereas KGM is an amorphous polysaccharide with limited crystallinity. Thus, the mechanisms of the physical modification may not be identical in the two polymers.

To clarify the effects of extrusion on the structural features that might reduce the WAI for extruded KGM, powdered XRD was used to investigate the crystallinity of non-extruded extruded KGM samples (Figure 4). Non-extruded KGM was found to have a major peak at $2\theta = 20°$ and a minor one at 35–40°. For the extruded samples, particularly those prepared at higher temperatures and shear, the maximum peak intensity was greater and the peaks were narrower. This indicated that the extruded samples had a higher degree of crystallinity, which also potentially led to a lower WAI, as the ability of the KGM chains to interact at junction zones decreased, and the ability of the structure to entrap water was reduced.

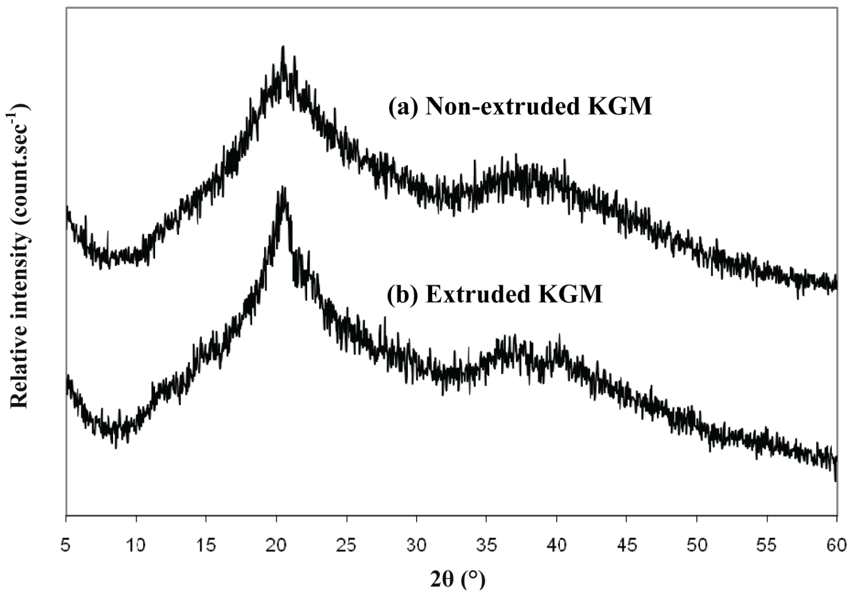

Figure 4 X-ray diffraction pattern of samples (scale = 1.5x:y) of konjac glucomannan (KGM): (a) Non-extruded KGM; (b) Extruded KGM.

The WSI decreased with raised barrel temperatures while the WSI values increased as a function of the solids content (Figure 3). Based on the coefficients for the predicted WSI (Equation 5), temperature had the strongest effect. This was followed by the solids content, while the screw speed had little influence on the WSI. Preliminary studies indicated that a 20% (v/v) ethanol solution was required to effectively extrude the KGM. These conditions provided a mass that could be extruded, yet still allowed enough shear to be imparted to the dough to degrade the KGM molecular chains. The lower WSI values indicated that fewer soluble solids were present in the supernatant fraction of the centrifuged water-KGM mix.

Use of the ethanol solution led to an increased crystallinity in the KGM polymer leading to a reduction in the water solubility of the extruded KGM with a lower solids content (increasing ethanol levels) or raised barrel temperatures (the reaction was accelerated). The addition of ethanol is likely to help release of some sugars from the matrix, particularly at lower solids levels, which however may become trapped in the sediment matrix. Although the ethanol solution evaporated easily at these operating temperatures and the extruded KGM was safe enough for many applications, its suitability for an application should take into account that there may be some remnant compound under some extrusion conditions. For example, extruding KGM at a low solids content (high ethanol solution) at a high temperature led to a condensation film on its wall so the solution was trapped and undesirable compounds might be generated by the interaction of the remaining solution and the KGM. Moreover, chains of polysaccharide might be decomposed during extrusion, particularly at high temperatures. According to Thommes *et al.* (2007), extruded pellets based on κ-carrageenan were produced with a drying temperature above 70 °C. In the case of extruded konjac glucomnan, the melting temperature of 1.0 % (w/w) KGM gel was reported to be approximately 40 °C (He *et al.,* 2012). Thus, the use of cooking extrusion in the present study might break the konjac glucomannan chain and also form undesirable compounds. Nonetheless, the exact mechanism responsible for such a reaction remains unclear. However, this extrusion application is useful to modify the desirable water absorption and water solubility properties of KGM.

CONCLUSION

Extrusion processing was effective in modifying the water absorption and water solubility properties of KGM with high throughput. The water absorption and water solubility of the extruded KGM increased as a function of the solids content while these values decreased with increasing temperatures. The ability to modify these properties could facilitate gum-producing manufacturers to control viscosity and gelling behaviors. In addition, the ability of extrusion to reduce excessive moisture absorption was likely to alleviate concerns associated with KGM ingestion.

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