International Food Research Journal 17: 1043-1050 (2010)

Ultrasound mediated acid hydrolysis of konjac glucomannan

*Cheng, L.H., Nur Halawiah, H, Lai, B. N., Yong, H. M. and Ang, S. L.

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

Abstract: Konjac glucomannan (KGM) was treated with or without sonication and/or hydrochloric acid (HCl). Hydrolysis effects on KGM were studied for properties such as degree of hydrolysis, fluidity, molecular mass, and creep properties. The degree of hydrolysis for sonicated KGM and acid treated KGM were not significantly different. However, the combination treatment of acid hydrolysis and sonication was found effective in yielding a smaller molecular weight fraction of KGM and solution with higher fluidity. From the creep analysis, KGM treated with combination treatment exhibited the highest compliance among samples tested. In general, ultrasound mediated acid hydrolysis was found to be a promising technique in degrading high molecular weight biopolymer. This could be attributed to a localized high temperature and high shear forces generated during cavitation that facilitated the endothermic acid hydrolysis.

Keywords: acid hydrolysis, sonication, konjac glucomannan, molecular mass

Introduction

High molecular weight biopolymers, such as konjac glucomannan (KGM), are found not suitable for certain applications due to the fact that such biopolymer tends to incur high viscosity at low solid content. Depolymerization is one of the strategies to produce smaller molecular weight fractions to acquire desired properties (Szu *et al*., 1986; Lii *et al*., 1999; Liu, *et al.,* 2006; Desai *et al.*, 2008; Iida *et al*., 2008). The common treatments for biopolymer degradation are enzymatic hydrolysis and acid hydrolysis. Both methods have received extensive attention from researchers and substantial amount of work has been focusing on starch (Gorinstein *et al*., 1993; VanSoest *et al*., 1995; Wang *et al*., 1995; Mélo *et al*., 1996; Cote and Willet, 1999).

Sonication is another mean of depolymerisation of macromolecules. According to Desai *et al*. (2008), ultrasonication has been proven to be an effective means to depolymerise macromolecules, because it reduces the molecular weight of a polymer by simply splitting the most susceptible chemical bond without causing any changes in the chemical nature of the polymer during cavitation. It was reported that prolonged exposure of macromolecules solutions to high energy ultrasonic waves produced a permanent reduction in viscosity resulted from degradation of molecules (Desai *et al*., 2008; Grönross *et al*., 2003). On the other hand, Iada *et al*. (2008) studied the changes in the viscosity of starches and polysaccharides by sonication and found that glucomannan with 1% concentration showed a drastic depression in viscosity by the sonication. Cote and Willet (1999) revealed that sonication was much more effective in producing lower molecular weight fragments of macromolecules than extrusion or jet cooking.

To the best of our knowledge, the research work on KGM hydrolysis is relatively scarce. Enzymatic degradation on KGM was first attempted by Mayeda (1922), using the growing culture of a sporulating bacterium isolated from konjac flour, as reported in Kato *et al.* (1970). Using crude and purified cellulases, Kato *et al.* (1970) managed to isolate oligosaccharides from the KGM hydrolysates. In 2005, Chen *et al*. studied the effect of unhydrolyzed KGM and acid hydrolyzed glucomannan on cecal and fecal microflora and found that the hydrolyzed glucomannan exerts a greater prebiotic effect than the non-hydrolyzed KGM in Balb/c mice. This is inline with work reported by Al-Ghazzewi *et al.* (2007), Alonso-Sande *et al*. (2009) and Al-Ghazzewi and Tester (2010).

This research was conducted with the aim of producing KGM with modified physical properties by degradation. The specific aim was to study the effects of ultrasound mediated acid hydrolysis on KGM physical properties.

Materials and Methods

Materials

A purified KGM powder (PROPOL A) was purchased from Shimizu Chemical Corporation, Japan. All chemicals used are analytical reagents.

Sample preparation

Native KGM (35 g) was suspended in 250 ml absolute ethanol in order to ensure a low viscosity suspension system was created. The suspension was poured into a 1 L reaction flask and was purged with nitrogen gas to create an inert environment within the reaction flask. The suspension was stirred for 30 min at ambient temperature using an overhead stirrer. Where necessary, approximately 20 ml concentrated hydrocholoric acid (HCl, 36.7%) was

*Corresponding author.
Email: *lhcheng@usm.my*
Tel: +60-4-6532118, Fax: +60-4-6573678;

added and/or the suspension was subjected to sonication for 30 min at 35 kHz with a ELMA T 700 H Sonicator (Lazer Scientific, Inc.). After treatment, the suspension was filtered with Whatman No. 1 filter paper and rinsed with 70% ethanol to neutral. The sample was then left in a fumehood to evaporate off the ethanol before being dried in a vacuum oven at 50°C. The samples produced were coded as KGM (control sample), KGM-HCl (acid treated sample), KGM-SONI (sonicated sample) and KGM-HCl-SONI (ultrasonic acid treated sample).

Sample characterization

Moisture content determination

Sample (2g) was dried in a hot air oven at 105°C and the determination was triplicated.

Total reducing end determination

Total reducing end of samples was determined with Somogyi-Nelson method (Nielsen, 1998). Samples with a concentration of 0.2% (w/v) were prepared. To eliminate insoluble substances, the solutions were centrifuged at 3000 rpm for 30 min. Somogyi copper solution (2 ml) was added to a 2 ml sample solution. The mixture was then heated in a water bath and at 95°C for 10 min. After cooling, 1 ml of arsenomolybdate was added into the mixture. The mixture was made up to 10 ml with distilled water and absorbance was read at 620 nm with Shimadzu UV-160A Spectrophotometer. Maltose was used as a standard. The percentage of total reducing materials reported as maltose equivalent was defined as the degree of hydrolysis (Haska and Ohta, 1992).

Weight-average molecular weight ($\overline{M}_w$) and polydispersity (I) determination

The weight-average molecular weight ($\overline{M}_w$) and polydispersity (*I*) of the samples were determined using a one-column (PL-aquagel-OH 8µm, 300 x 7.5 mm, Polymer Laboratories Ltd., UK) HPSEC system (Waters 1525, Binary HPLC Pump and PL-ELS 1000 detector, Polymer Laboratories Ltd., UK). Each sample solution (0.05% w/v) was prepared in distilled water by stirring at ambient temperature for 4 hrs. The solution was then centrifuged for 40 min at 3000 rpm to remove insoluble substances. A sample of 100 µl was injected into the system with distilled deionised water used as eluent at a flow rate of 0.6 ml/min. The system was calibrated using Shodex molecular weight 5.9E3 to 7.88E5 pullulan standard (Separation & HPLC Group, Japan).

Specific viscosity (η_{sp}) determination

Sample solutions of 0.08 g/100 ml were prepared with distilled water by stirring for 4 hrs at ambient temperature. The solution was centrifuged for 30 min at 3000 rpm to remove insoluble substances. Viscosity of the supernatant was measured with a Ubbelohde-type viscometer (Cannon Instrument, State College, PA) at various concentration (0.05g/100 ml to 0.08 g/100 ml) by adding 1 ml of distilled water at a time at 30°C. The reciprocal of η_{sp} (η_{sp}^{-1}) was plotted against concentration of samples. η_{sp}^{-1} was defined as fluidity of the sample solution (Sugiyama *et al*., 1973).

Viscoelastic properties determination

Sample solutions of 1.5% (w/v) were prepared following the preparation steps aforementioned. Creep analysis was performed using CLS^2 100 Carri-Med Rheometer (TA Instruments, New Castle, DE, USA) with a 60 mm diameter and 2.0 mm gap parallel plate geometry. The Peltier-controlled was pre-set at 25°C. The linear viscoelastic region (LVR) of the samples was pre-determined. Creep compliance of the samples was determined within 0.8 to 1.5 N for 300 s, the recovery compliance was also recorded for 300 s at zero stress (0 N). The compliance against time graphs were analyzed using the Burger Model with TA Instrument Rheology Solutions Software, DATA V1.1.6.

Result and Disscussion

Moisture content

Table 1 shows the moisture content of the samples prepared in this study. The moisture content of the samples ranged from 4.24% to 4.66% on wet basis.

Degree of hydrolysis

Sugar with reducing property, viz. with the presence of potential aldehyde or keto group, are called the reducing sugars. When heated with alkaline copper tartrate, reducing sugar tend to reduce the copper from the cupric to cuprous, which in turn will reduce molybdic acid to molybdenum (Sadasivam and Manickam, 2005). The percentage of reducing materials formed was defined as degree of hydrolysis (Haska and Ohta, 1992). Figure 1 shows the degree of hydrolysis of KGM, KGM-HCl, KGM-SONI and KGM-HCl-SONI. The amount of reducing materials detected in samples treated with acid or sonication only was found not significantly different from the control. Whereas, for those treated with both acid and sonication, it was found that a significantly higher amount of reducing materials was detected. This indicates that ultrasonic acid hydrolysis is more efficient in degrading KGM when compared to the single treatment of acid hydrolysis or sonication.

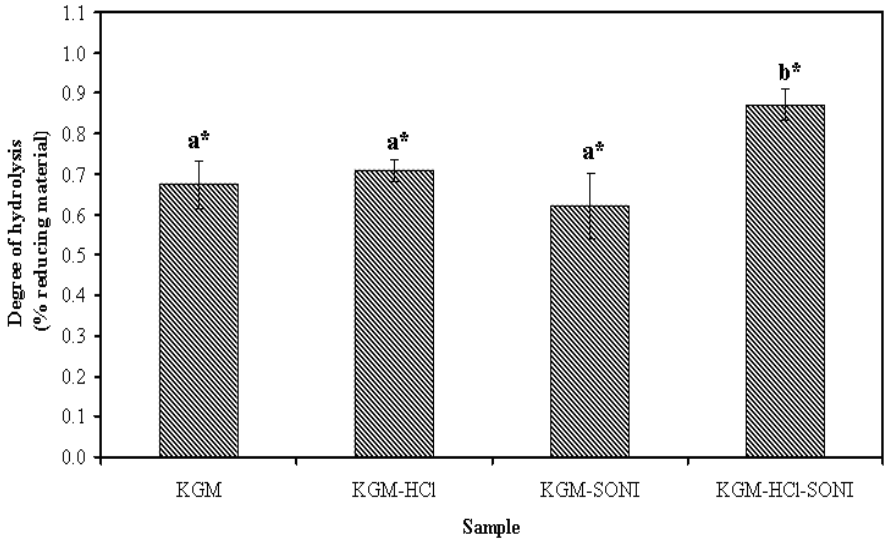
Weight-average molecular weight ($\overline{M}_w$) and polydispersity index (I)

Figure 2 shows the molecular profile of the samples prepared. As evident, the samples were started to elute at 8th minute and were fully eluted at 10th minutes and 40 seconds. Overall, the elution curves for all sample types were symmetric in shape except for KGM-HCL-SONI, which showed a sign of more obvious tailing indicating the presence of relatively smaller molecular weight fractions in the sample. The statistical analysis (Table 2) showthat the $\overline{M}_w$ and *I* for all the samples were not significantly different ($p>0.05$). Polydispersity index, *I*, provides a simple definition of molecular weight distribution. The

Table 1. Moisture content of control sample (KGM), acid treated sample (KGM-HCl), sonicited sample (KGM-SONI) and ultrasound mediated acid treated sample (KGM-HCl-SONI)

Sample Coding	Moisture content on wet basis (%)
KGM	4.66 ± 0.04
KGM-HCl	4.24 ± 0.02
KGM-SONI	4.53 ± 0.03
KGM-HCl-SONI	4.48 ± 0.02

Mean ± standard deviation (n=3)



* Bars with the same letter are not significantly different from each other (p>0.05).
Vertical bar represents plus and minus one standard deviation from the mean.

Figure 1. Degree of hydrolysis of control sample (KGM), acid treated sample (KGM-HCl), sonicated sample (KGM-SONI) and Ultrasound mediated acid treated sample (KGM-HCl-SONI)

higher the value of *I*, the greater spread of the molecular weight distribution (Chanda and Roy, 1998).

The $\overline{M}_w$ and *I* for KGM-HCl-SONI were found to be the lowest and the highest, respectively among the samples tested. There means to say that KGM-HCl-SONI sample had a relatively smaller molecular weight fractions as compared to the other samples. This is in agreement with the qualitative observation as noted in Figure 2 , in which KGM-HCl-SONI was found showing tailing in the later stage of the elution. Therefore, it is proven that the combination of acid hydrolysis and sonication showed greater effect on KGM's weight-average molecular weight and polydispersity.

According to Kim *et al*. (2001), acid hydroysis is an endothermic reaction which requires energy higher than its activation energy to trigger the reaction. In other words, increasing the system temperature will enhance the rate of hydrolysis. Therefore, during sonication a localized high temperature and pressure was generated following cavitation. This higher temperature subsequently triggered the acid hydrolysis of KGM and contributed substantially to reducing materials. Apart from this, the high shear forces generated during implosion of cavities was another factor that accelerated the hydrolysis of KGM. These forces have been shown to be sufficient to fragment polymer chains (Mason, 1991). As a result, KGM with smaller molecular weight fractions and higher polydispersity was produced with the combination treatment of acid hydrolysis and sonication.

Specific viscosity (η_{sp}) and fluidity

Figure 3 shows the fluidity of the samples prepared at different concentration. The higher the value of η_{sp}^{-1}, the higher the fluidity of the samples, i.e. the easier for the sample to flow (Sugiyama *et al*., 1973). It is commonly known that polymer with higher molecular weight tends to produce a viscous solution than the lower molecular weight counterparts (Grassino, 2000). This happens because when a polymer with high molecular weight is dissolved, it shows a bigger hydrodynamic volume as a result of higher interaction between solvent and solutes. Consequently, the solution resists to flow and hence show lower fluidity.

At a given concentration, KGM-HCl-SONI showed the highest fluidity followed by KGM-HCl, KGM-SONI and KGM. This shows that KGM-HCl-SONI sample possesses relatively lower $\overline{M}_w$ when compared to KGM, KGM-HCl and KGM-SONI. This is in agreement with observations made in previous analyses.

Creep analysis

Creep analysis is one of the methods used widely to study the rheological properties of a sample. This analysis allows one to differentiate between the viscous and elastic responses of a sample. Figure 4 shows the creep-recovery response of all samples tested. The creep recovery response of samples treated with acid or sonication only was not significantly different from the control. At any time, KGM-HCl-SONI shows the highest compliance value followed by KGM-HCl, KGM and KGM-SONI. This indicates that KGM-HCl-SONI sample experienced greater deformation under the same applied stress than the others. On the other hand, the creep profile shows more structure is retained for KGM, KGM-HCl and KGM-SONI samples.

This characteristic was also reflected by the instantaneous compliance (J_o) values of the samples (Table 3). The J_o values for KGM, KGM-HCl and KGM-SONI samples showed no significant different, whereas the ultrasonic acid hydrolysed ones is significantly ($P<0.05$) higher. This indicates that KGM molecules have lost

Table 2. Molecular mass properties of control sample (KGM), acid treated sample (KGM-HCl), sonicated sample (KGM-SONI) and ultrasound mediated acid treated sample (KGM-HCl-SONI)

Sample	$\overline{M}_w$ **x** 10^{-5}	***I***
KGM	6.77±0.02[b]	1.02±0.01[a]
KGM-HCl	6.11±0.03[a,b]	1.05±0.01[a,b]
KGM-SONI	6.26±0.46[a,b]	1.03±0.02[a]
KGM-HCl-SONI	5.60±0.23[a]	1.09±0.02[b]

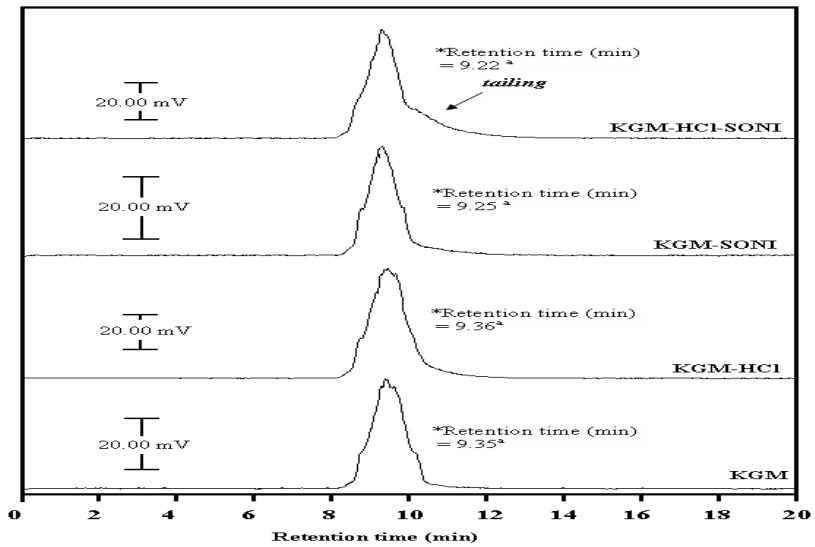


Figure 2. Elution profile of control sample (KGM), acid treated sample (KGM-HCl), sonicated sample (KGM-SONI) and ultrasound mediated acid treated sample (KGM-HCl-SONI)

its rigidity (Lynch and Mulvihill, 1994) drastically upon ultrasonic acid hydrolysis, giving a higher J_o. Besides, the smaller molecular weight fraction present within the samples could serve as an antagonist to sample rigidity as well. According to Rahalkar (1992), small molecular weight fractions will pack into the void spaces and disrupt the interaction between molecules and subsequently weaken the structure built up. As a result, there is less structural resistance to flow and therefore the rigidity of KGM-HCl-SONI was lower as compared to the others.

As for Newtonian viscosity (η_o), KGM-HCl-SONI shows the lowest values among the samples tested. This indicates that KGM-HCl-SONI samples had the lowest resistance to flow due to the interference of interaction between molecular chains by the smaller molecular weight fractions present.

Conclusions

Acid treatment or sonication alone was not effective in degrading konjac glucomannan (KGM). A combination of both treatments would help to produce KGM with lower weight-average molecular weight and broader molecular weight distribution. With the presence of higher amount of lower molecular weight fraction, KGM solution rheological properties can be modified.

Acknowledgments

This work was supported by the Fundamental Research Grant Scheme (Grant No.:203/PTEKIND/6711100) funded by the Ministry of Higher Education, Malaysia.

Table 3. Instantaneous compliance (J0) and Newtonian viscosity (η0) for solution of control sample (KGM), acid treated sample (KGM-HCl), sonicated sample (KGM-SONI) and ultrasound mediated acid treated sample (KGM-HCl-SONI)

Sample	J_0 x 10^3 (Pa^{-1})	η_0 (Pa·s)
KGM	0.77 ± 0.34[a]	943.13 ± 23.07[c]
KGM-HCl	0.89 ± 0.71[a]	732.55 ± 30.78[b]
KGM-SONI	1.09 ± 0.83[a]	1323.5 ± 72.69[d]
KGM-HCl-SONI	12.65 ± 4.64[b]	37.61 ± 0.52[a]

Mean ± standard deviation (n = 4). Means within a column with the same letter are not significantly different (p>0.05).

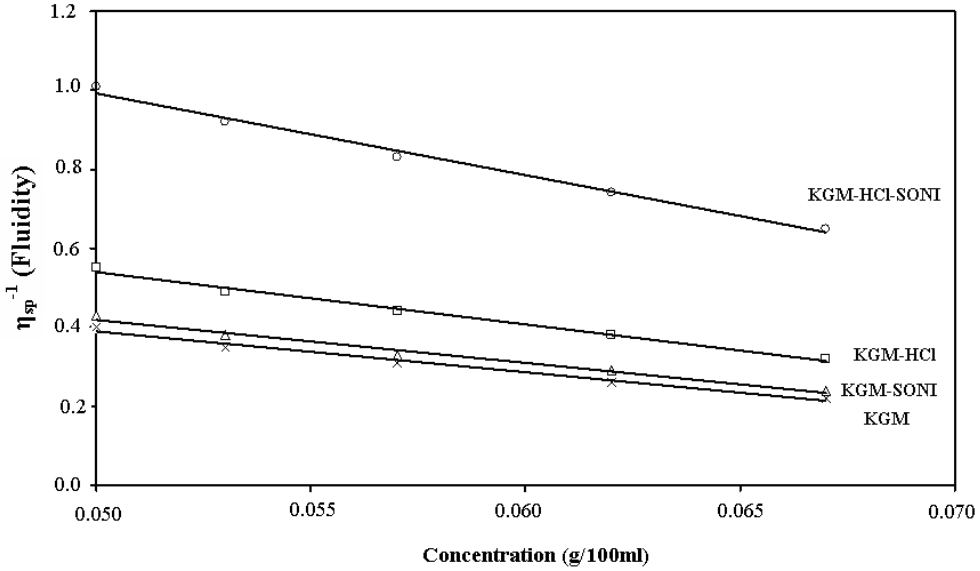


Figure 3. Fluidity against concentration for control sample (KGM), acid treated sample (KGM-HCl), sonicated sample (KGM-SONI) and ultrasound mediated acid treated sample (KGM-HCl-SONI)

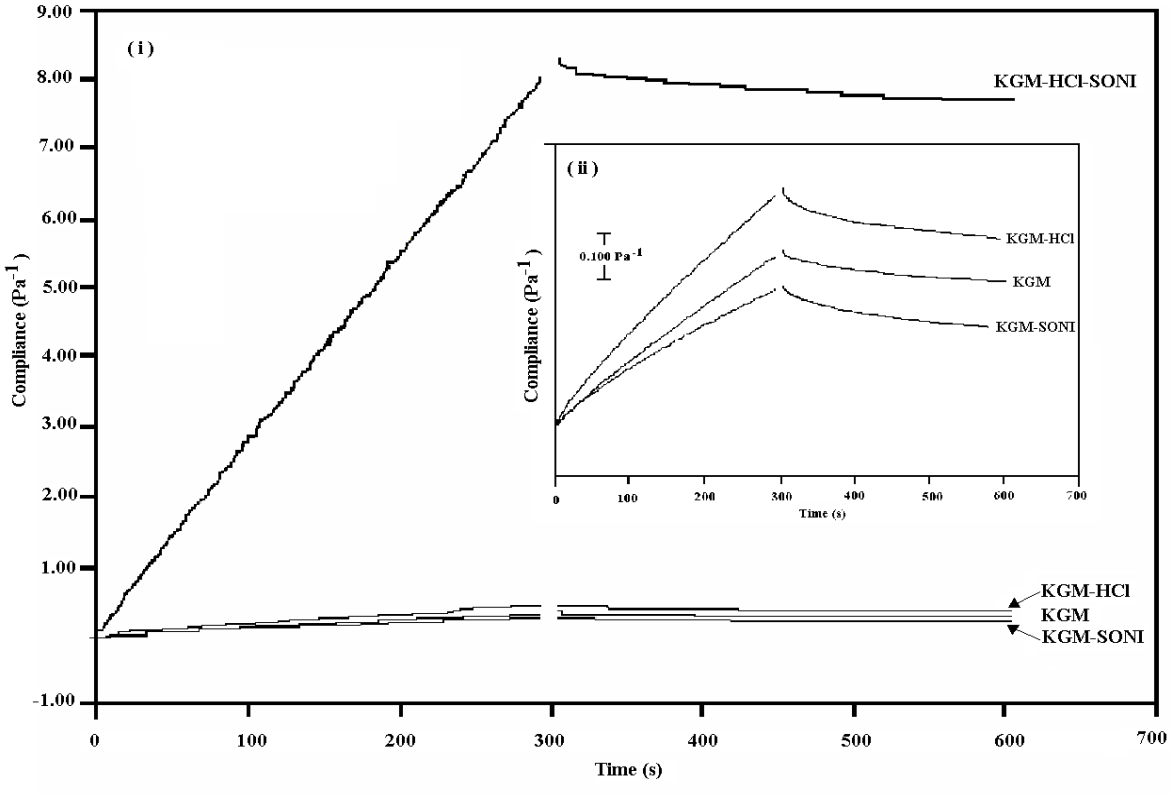


Figure 4. Creep-recovery response of (i) of control sample (KGM), acid treated sample (KGM-HCl), sonicated sample (KGM-SONI) and ultrasound mediated acid treated sample (KGM-HCl-SONI); (ii) Inclusion: acid treated sample (KGM-HCl), control sample (KGM) and sonicated sample (KGM-SONI)

References

Al-Ghazzewi, F.H., Khanna, S., Tester, R.F. and Piggott, J. 2007. The potential use of hydrolysed konjac glucomannan as a prebiotic. Journal of The Science of Food and Agriculture 87(9):1758-1766.

Al-Ghazzewi, F.H. and Tester, R. F. 2010. Effect of konjac glucomannan hydrolysates and probiotics on the growth of the skin bacterium *Propionibacterium acnes in vitro.* International Journal of Cosmetic Science 32: 139-142.

Alonso-Sande, M., Teijeiro-Osorio, D., Remunan-Lopez, C. and Alonso, M. J. 2009. Glucomannan, a promising polysaccharide for biopharmaceutical purposes. European Journal of Pharmaceutics and Biopharmaceutics 72: 453-462.

Chanda, M. and Roy, S. K. 1998. Plastic Technology Handbook, 3rd Edition. New York: Marcel Dekker.

Chen, H. –L., Fan, Y. –H., Chen, M. –E. and Chan, Y. 2005. Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice. Nutrition 21: 1059-1064.

Cote, G. L. and Willet, J. L. 1999. Thermomechanical depolymerization of dextran. Carbohydrate Polymers 39: 119-126.

Desai, V., Shenoi, M. A. and Gogate, P. R. 2008. Ultrasonic degradation of low-density polyethylene. Chemical Engineering and Processing 47: 1461-1466.

Gorinstein, S., Oates, C. G., Chang, S. M. and Lii, C. Y. 1993. Enzymatic hydrolysis of sago starch. Food Chemistry 49: 411-417.

Grönross, A., Pirkonen, P. and Ruppert, O. 2003. Ultrasonic depolymerization of aqueous carboxymethylcellulose. Ultrasonics Sonochemistry 11: 9-12.

Haska, N. and Ohta, Y. 1992. Mechanism of hydrolysis of the treated sago starch granules by raw starch digesting amylase from *Penicillium Brunneum.* Starch/Stärke 44: 25-28.

Iida, Y., Tuziuti, T., Yasui, K., Towata, A. and Kozuka, T. 2008. Control of viscosity in starch and polysaccharide solutions with ultrasound after gelatinization. Innovative Food Science and Emerging Technologies 9: 140-146.

Kato, K., Watanabe, T. and Matsuda, K. 1970. Studies on the chemical structure of konjac mannan Part II. Isolation and characterization of oligosaccharides from the enzymatic hydrolyzate of the mannan. Agricultural Biological Chemistry 34: 532-539.

Kim, J. S., Lee, Y. Y. and Torget, R. W. 2001. Cellulose hydrolysis under extremely low sulphuric acid and high temperature conditions. Applied Biochemistry and Biotechnology 91-93: 331-340.

Lii, C. Y., Chen, C. H., Yeh, A. I. and Lai, V. M. F. 1999. Preliminary study on the degradation kinetics of agarose and carrageenans by ultrasound. Food Hydrocolloids 13: 477-481.

Liu, H., Bao, J., Du, Y., Zhou, X. and Kennedy, J. F. 2006. Effect of ultrasonic treatment on the biochemphysical properties of chitosan. Carbohydrate Polymers 64: 553-559.

Lynch, M. G. and Mulvihill, D. M. 1994. The influence of caseins on the rheology of iota-carrageenan gels. Food Hydrocolloid 8: 312-329.

Mason, T. J. 1991. Practical Sonochemistry: User's guide to application in chemistry and chemical engineering. UK: Ellis Horwood.

Mayeda, M., 1922. Preliminary communication on mannanase and laevidulinase. Journal of Biochemistry (Tokyo), **1**: 131–137. Cited in Kato *et al*. (1970).

Mélo, E.de A., Vieira, F., Krieger, N., Guerra, N. B., Silva, M. P. C. and Kennedy, J. F. 1996. Enzymatic hydrolysis of starch from Jacatupé (*Pachyrhizus erosus* L. Urban) by thermostable amylolytic enzymes. Starch/Stärke 48: 101-104

Nielsen, S. S. 1998. Food Analysis, 2nd Edition. USA: Aspen.

Rahalkar, R. R. 1992. Viscoelastic properties of oil-water emulsions. In: Viscoelastic Properties of Foods.(Rao, M.A. & Steffe, J.F., Eds.), London: Elsevier Applied Science.

Sadasivam, S. and Manickam, A. 2005. Biochemical Methods, 2nd Edition. New Delhi: New Age International.

Sugiyama, N., Shimahara, H., Andoh, T. and Takemoto, M. 1973. Konjac-Mannanase from Tubers of Amorphophallus konjac C. Koch. Agricultural Bioliological Chemistry 37(1): 9-17.

Szu, S. C., Zon, G., Schneerson, R. and Robbins, J. B. 1986. Ultrasonic irradiation of bacterial polysaccharides. Characterization of the depolymerized products and some applications of the process. Carbohydrate Research 152: 7-20.

VanSoest, J. J. G., Benes, K. and DeWit, D. 1995. The influence of acid hydrolysis of potato starch on the stress-strain properties of thermoplastic starch. Starch/Stärke 47: 429-434.

Wang, W. J., Powell, A. D. and Oates, C. G. 1995. Sago starch as a biomass source: Raw sago starch hydrolysis by commercial enzymes. Bioresource Technology 55: 55-61.