

controller. The samples were loaded into the sample cup and allowed to equilibrate for 20 min at the desired temperature. The samples were subjected to shear rate ranging from 0.5 to 100 rpm in 3 min, followed by a steady shear at 100 rpm for 5 min and finally a linearly decreasing shear rate from 100 rpm to 0.5 rpm in 3 min.

2.5 Pasting behavior analysis

The pasting behavior of a 3% (dry basis) TKP suspension was determined by the Brabender viscoamylograph Model D-47055 (Brabender Instrument Inc., Germany) operated with a bowl speed of 160 rpm. The weighed samples were mixed with distilled water and made up to 500 g. The suspension was heated from 25 °C at the rate of 1.5 °C/min to 95 °C, held at this temperature for 30 min. After which, TKP paste was cooled down to 50 °C at the same rate and this temperature was held for 30 min.

2.6 Shear thinning properties

The shear thinning properties of TKPs were determined in a Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Australia) as the method of Chen et al. (2003). Two grams of TKPs was mixed with distilled water to give a total weight of 28 g in the RVA sample cell. Three programmed heating profiles were used. The cells containing samples were held at 50 °C for 1.5 min, heated to the desired temperature (75, 85 or 95 °C) at a rate of 10 °C/min, and then held isothermally for a further 30 min. The shear rate (160 rpm) was kept constant over the RVA procedure. The degree of shear thinning (DST%) was calculated as $100 \times (\eta_p - \eta_{30}) / \eta_{30}$, where η_p = the peak viscosity and η_{30} = the final viscosity after 30 min of isothermal stirring.

2.7 Purification of tamarind xyloglucan

Xyloglucan was extracted and purified by a modification of the method of York et al.(1990). TKP (30 g) was defatted using hexane extraction and was dissolved in 3 liter of distilled water. The solution was incubated with constant stirring for 30 min at 80 °C, centrifuged at 10,000 rpm for 30 min, and the pellet was discarded. Soluble polysaccharides were precipitated overnight in 70% ethanol at 4 °C and centrifuged at 10,000 rpm for 30 min, then the pellet was lyophilized.

2.8 Gel permeation chromatography

Gel permeation chromatography was performed on a Sepharose CL-2B (Pharmacia Fine Chemicals, Sweden) column (1.6 × 100 cm) operating with the peristaltic pump at the flow rate of 15 ml h⁻¹, using distilled water containing 0.02% sodium azide as eluent. Purified xyloglucan was dissolved in distilled water with constant stirring and heating until the temperature was raised to 80 °C. The heating was stopped as soon as 80 °C was reached and the solutions were left covered overnight, with stirring at room temperature to allow further hydration to occur (Picout et al., 2003). An aliquot (3 ml) of the solution containing 12 mg of the sample was loaded on the column. Fraction (3 ml) was collected and analyzed for carbohydrate content by phenol-sulphuric acid reagent method (Dubois et al., 1956).

2.9 Molecular weight determination

Weight average molecular weight was determined using a multi-angle laser light scattering (MALLS). Measurement was carried out simultaneously at 30 °C in the

angular range 30 to 180 ° using a Mulvern 4700 light scattering system (Mulvern Instruments Ltd., UK) with an Ar⁺ ion laser operating at 480 nm. The scattering of benzene was used as the primary standard and the refractive index increment, dn/dc was chosen as 0.146 ml g⁻¹ as recommended by Picout et al. (2003). Xyloglucan solutions were prepared using the same method as prepared solution for gel permeation chromatography except that distilled water was filtered three times through 0.45 µm filter before being used as a solvent. Zimm plots were constructed from measurements of purified xyloglucan at seven concentrations (0.02 to 0.2% w/v). The solutions were filtered directly into the cylindrical light scattering cuvette using a 0.45 µm syringe filter. All solution preparation stages were carried out in a laminar airflow carbinet to minimize contamination with dust.

2.10 Statistical analysis

All experiments were performed in triplicate. One-way analysis of variance (ANOVA) and Duncan's post hoc test to detect significant differences were performed using a statistical software package (SPSS Inc., USA).

3. Results and Discussion

3.1 Characteristics of TKPs from different dehulling process

As shown in Table 1, the % yield of TKPs from different dehulling process was about 50 % and the main composition of these TKPs (Table 2) was not significantly different except for the lowest ash content of TKP from non-heating process. This may be due to the lowest remaining testa of the seed from the washing step of the process.

The starch contents of TKPs have not been reported in the literature. However, in the present study it was found that TKPs contained about 1% starch (dry basis). The low starch content of 0.4% (dry basis) was also analyzed in detarium seed flour which is also as rich in xyloglucan as tamarind seed (Wang et al., 1996). The obvious effect of the dehulling process on color of TKPs is seen in Table 3. The color lightness (L^*) and whiteness of TKP1 was higher than that of TKP2 and TKP3 and there were significant differences between TKP1 and TKP3.

3.2 Rheological and pasting behavior

Typical rheograms of TKPs from different dehulling processes evaluated at 25 °C are shown in Fig.1 for comparison. A clearly non-Newtonian, shear-thinning (pseudoplastic) and time-independent behavior of all treatments was observed. The experimental results of the variation of shear stress (τ) with shear rate ($\dot{\gamma}$) were fitted to the power law model by linear regressions which were significant at the 95% probability level. The non-linear regression of τ and $\dot{\gamma}$ were examined and showed the negative intercept value. The plots of square root values of shear rate and shear stress values using the Casson model were also plotted but not fitted (data not presented) indicating that none of the solutions showed a yield value for TKP at 3% concentration. Prabhanjan and Zakiuddin Ali (1995) also found that solutions of TKP at concentrations up to 5% exhibited pseudoplastic flow behavior and did not show a yield stress. On the other hand, Bhattacharya et al. (1991) indicated that the TKP suspension at 2-10% concentration showed pseudoplastic and time-dependent; thixotropic behavior with yield stress. The different results may be due to the different sample preparation

methods for rheological measurement of the earlier study based on the non-homogeneity of the TKP suspension prepared without heating.

According to data analysis and calculation, since an increase in shear rate resulted in increasing shear stress, the TKP suspensions were evaluated as a non-Newtonian pseudoplastic type. The suspensions did not exhibit a yield stress at the concentration studied. The experimental results of the variation of shear stress (τ) with shear rate ($\dot{\gamma}$) were fitted to the power-law model. Therefore, the rheological parameters (flow behavior index; n and consistency coefficient; k) were evaluated from logarithmic plots of shear stress versus shear rate. Table 4 lists the rheological parameters calculated from the power law model and the regression coefficient. The flow behavior index (n) of TKP1 was the lowest whereas the consistency coefficient (k) was significantly higher than that of TKP2 and TKP3, which corresponded to the higher viscosity. Rao (1999) indicated that the degree of pseudoplasticity could be measured by the n value, which decreases when pseudoplasticity increases and the increase in k value was due to higher viscosity of the fluid. Therefore, it could be concluded that the dehulling process by heating decreased the non-Newtonian behavior of TKP. The Brabender viscogram of TKP suspensions is shown in Fig. 2 and the data (Table 5) indicated that the maximum and final viscosity of TKP1 was significantly higher than that of TKP2 and TKP3. These results corresponded to the rheological properties measured by the Brookfield viscometer.

3.3 Shear thinning properties

It was found that after 30 min of isothermal stirring at 75 and 85 °C, the viscosity of TKP paste from all treatments increased simultaneously (Fig. 3). Whereas, isothermal stirring at 95 °C caused viscosity reduction in TKP paste of TKP2 and TKP3 and the DST (%) were calculated as shown in Table 6. The data could indicated the more consistency of the TKP from the non-heating dehulling process than the two heating dehulling processes.

3.4 Gel permeation chromatography

The gel permeation pattern of xyloglucan from gel permeation chromatography was shown in Fig. 4. All xyloglucan samples were eluted as a broad peak, suggesting a wide range of polymer size. Obviously, the peak of xyloglucan of TKP1 was eluted at a lesser elution volume, indicating a higher Mw polymer than that of xyloglucan of TKP2 and TKP3. It could be concluded that the decrease in Mw of the two xyloglucan is due to the molecular degradation of the polymer during the heating dehulling process. Franco et al. (1996) also found that galactomannan extracted from Brazilian legume seeds by hot water extraction had lower Mw than that extracted by a mechanical process.

3.5 Molecular weight determination

In this study, light scattering technique was used in order to estimate the Mw (weight-average molecular weight) of purified xyloglucan. The data summarized in Table 7 shows little difference in Mw range from $3.304\text{--}3.831 \times 10^6$ g/mol,

nevertheless, the highest Mw of xyloglucan was from TKP1. This result is in agreement with the gel permeation pattern in Fig. 4. It is observed that radius of gyration (Rg) value of xyloglucan from TKP3 was higher than that of TKP1 and TKP2 but the Mw was the lowest. For most polymers, the Rg depends on the molar mass of the particles (Burchard, 1994). However, water of hydration (non-free-draining water) can create long distances between non-free-draining coils of the polymer, giving a long Rg (Walter, 1998). The Mw and Rg of tamarind seed xyloglucan determined by MALLS was reported as 8.8×10^5 Da (Rg = 110 nm), 8.0×10^5 Da (Rg = 115 nm) and 1.16×10^6 Da (Rg = 122 nm) for xyloglucan from Dinippon Pharmaceutical Co., Japan (Gidley et al., 1991; Picout et al., 2003; Hiroshi et al., 2002) and as 8.33×10^5 Da (Rg = 136 nm) for xyloglucan from Megazyme, Ireland (Sims et al., 1998). These values are lower than that reported in this research. This variation may be generated by the difference in sample source, dehulling process and xyloglucan extraction methodology.

4. Conclusion

This study revealed that TKP suspension obtained from different dehulling processes showed pseudoplastic flow behavior but did not exhibit yield stress. The dehulling process of tamarind seed by heating affected the color and rheological properties of TKP. The obvious effects were found in TKP from the dehulling process by roasting at 200 °C, 15 min. It resulted in the lowest L* value, whiteness and viscosity but the highest degree of shear thinning. The difference in rheological behavior of TKP from different dehulling processes could be explained in terms of the difference in molecular weight of xyloglucan, the main polysaccharide component of the kernel

powder. The highest whiteness and viscosity and the tolerance to shearing during thermal treatment of TKP from non-heating process (TKP1) should be useful in the manufacturing process with high temperature.

Acknowledgements

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Table 1
% Yield (dry basis) of tamarind kernel powders from different dehulling process

Dehulling process	Yield (g/100g)
Non-heating	57.15 ± 2.64 ^a
Heating at 150 °C, 15 min	58.04 ± 1.26 ^a
Roasting at 200 °C, 2 min	60.46 ± 2.38 ^a

Means within a column with different letters are significant different (p< 0.05)

Table 2
Composition of tamarind kernel powders from different dehulling processes

Samples	Composition (%dry weight)				
	Protein	Fat	Fiber	Ash	Starch
Non-heating	18.05 ± 0.46 ^a	7.39 ± 0.53 ^a	0.37 ± 0.02 ^a	2.26 ± 0.11 ^a	0.94 ± 0.30 ^a
Heating at 150 °C, 15 min	18.88 ± 0.08 ^b	7.64 ± 0.60 ^a	0.43 ± 0.08 ^a	2.67 ± 0.11 ^b	1.00 ± 0.22 ^a
Roasting at 200 °C, 2 min	18.39 ± 0.28 ^{ab}	7.33 ± 0.54 ^a	0.41 ± 0.04 ^a	2.63 ± 0.05 ^b	1.10 ± 0.21 ^a

Means within a column with different letters are significant different (p< 0.05)

Table 3

L*, a*, b* value and whiteness of tamarind kernel powders from different dehulling processes

Samples	L* value	a* value	b* value	Whiteness
Non-heating	91.55 ± 0.22 ^a	0.37 ± 0.09 ^a	10.45 ± 0.09 ^a	68.93 ± 0.81 ^a
Heating at 150 °C, 15 min	90.82 ± 0.01 ^{ab}	0.30 ± 0.09 ^a	10.82 ± 0.14 ^a	66.77 ± 0.40 ^{ab}
Roasting at 200 °C, 2 min	90.34 ± 0.65 ^b	0.24 ± 0.15 ^a	11.36 ± 0.98 ^a	65.8 ± 1.87 ^b

Means within a column with different letters are significant different (p< 0.05)

Table 4

Rheological data of tamarind kernel powders from different dehulling processes at 25 °C

Samples	Flow behavior index (n)	Consistency index (Pa.s) (k)	R ²
Non-heating	0.66 ± 0.004 ^a	8.19 ± 0.19 ^a	0.99
Heating at 150 °C, 15 min	0.69 ± 0.02 ^{ab}	6.66 ± 0.86 ^b	0.99
Roasting at 200 °C, 2 min	0.71 ± 0.02 ^b	5.61 ± 0.38 ^b	0.99

Means within a column with different letters are significant different (p< 0.05)

Table 5
Brabender viscosity characteristics of TKPs from different dehulling processes

Samples	Maximum viscosity (BU)	Final viscosity (BU)	Setback (BU)
Non-heating	301.0 ± 8.72^a	661.33 ± 12.90^a	360.33 ± 8.02^a
Heating at 150 °C, 15 min	254.0 ± 12.12^b	548.0 ± 35.02^b	294.33 ± 25.89^b
Roasting at 200 °C, 2 min	219.67 ± 12.10^c	506.67 ± 22.19^b	287.0 ± 10.15^b

Means within a column with different letters are significant different ($p < 0.05$)

Table 6
Degree of shear thinning of tamarind kernel powders from different dehulling processes

Sample	Viscosity (cp)		Degree of shear thinning (%)
	At peak viscosity	After holding at 30 min at 95 °C	
Non-heating	572.36 ± 14.22^a	579.83 ± 9.68^a	—
Heating at 150 °C, 15 min	516.83 ± 14.67^b	492.42 ± 17.20^b	4.97 ± 0.70^a
Roasting at 200 °C, 2 min	479.50 ± 46.76^b	450.94 ± 9.78^b	6.03 ± 1.34^a

Means within a column with different letters are significant different ($p < 0.05$)

Table 7

Molecular weight (Mw) and radius of gyration (Rg) of purified xyloglucan from tamarind kernel powders prepared by different dehulling processes

Xyloglucan From different dehulling processes	Mw (x 10 ⁻⁶ g/mol)	Radius of gyration (nm)
Non-heating	3.831 ± 0.83 ^a	75.60 ± 2.23 ^a
Heating at 150 °C, 15 min	3.440 ± 0.30 ^a	72.67 ± 1.29 ^a
Roasting at 200 °C, 2 min	3.304 ± 0.39 ^a	130.83 ± 6.96 ^b

Means within a column with different letters are significant different (p< 0.05)

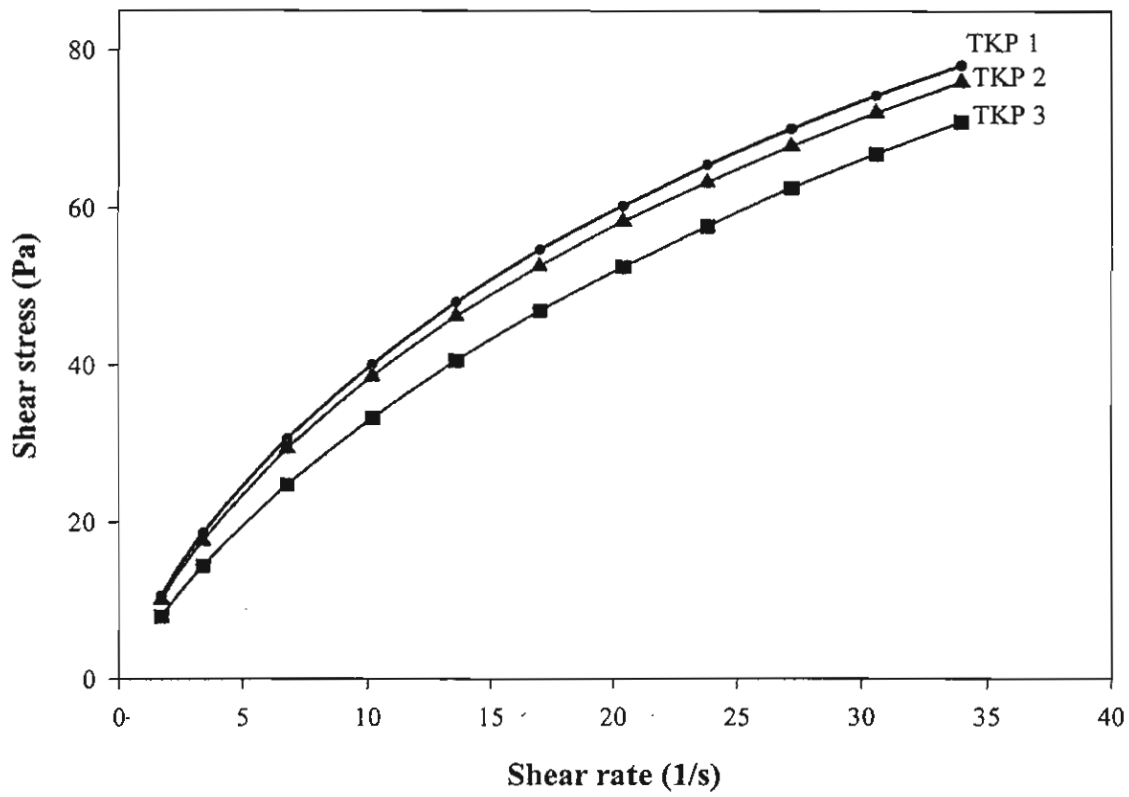


Fig. 1. Rheogram of 3% (w/w) tamarind kernel powders prepared from different dehulling processes measured at 25 °C.

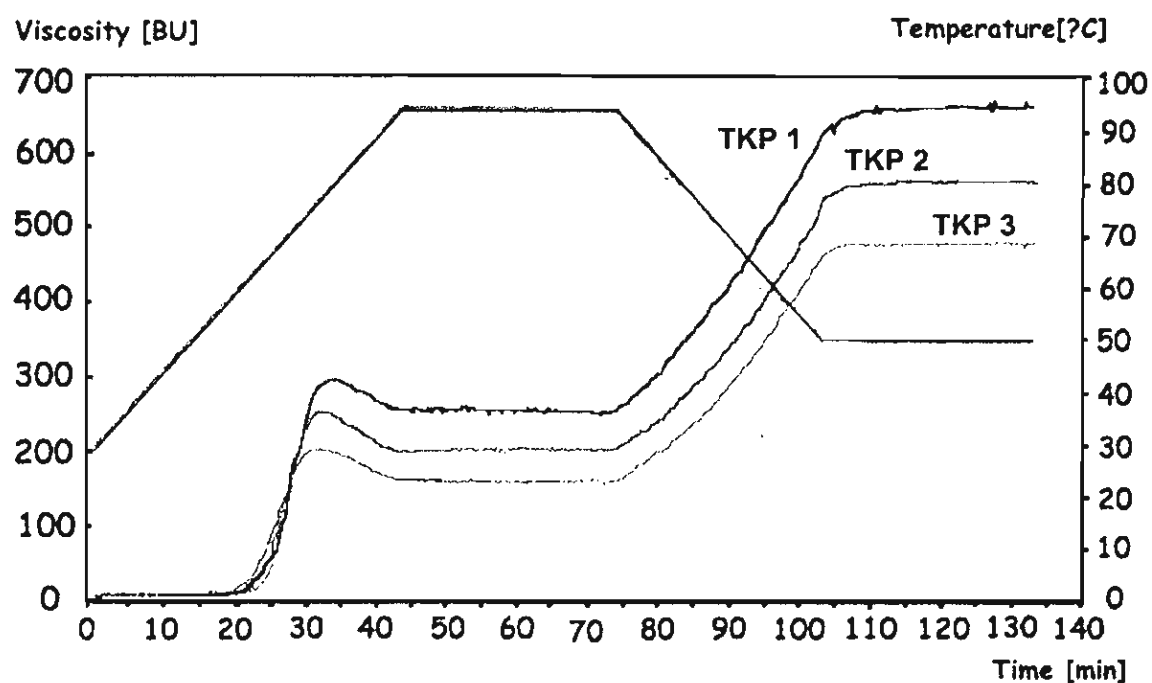


Fig. 2. Paste viscosity profiles of tamarind kernel powders prepared from different dehulling processes when determined by a Brabender Visco Amylograph using 15.0 g (dry basis) of tamarind kernel powder in 470 ml distilled water.

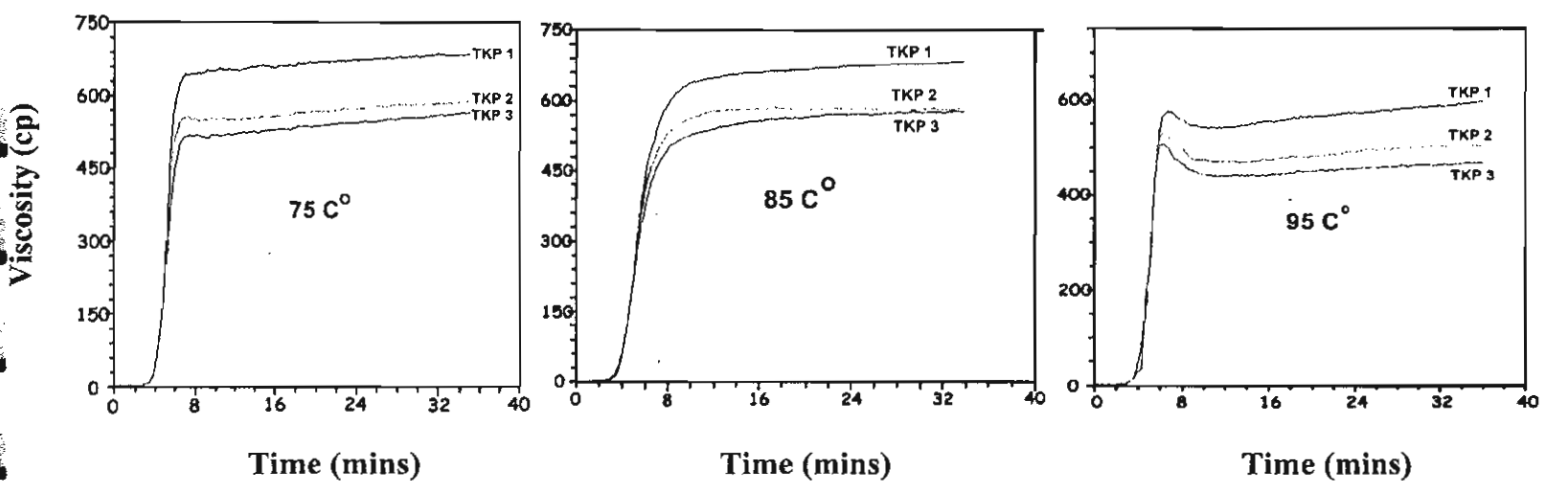


Fig. 3. Viscosity developments of tamarind kernel powders prepared from different dehulling processes during isothermal pasting at 75, 85 and 95 °C in term of pasting time when determined by a Rapid Visco Analyser.

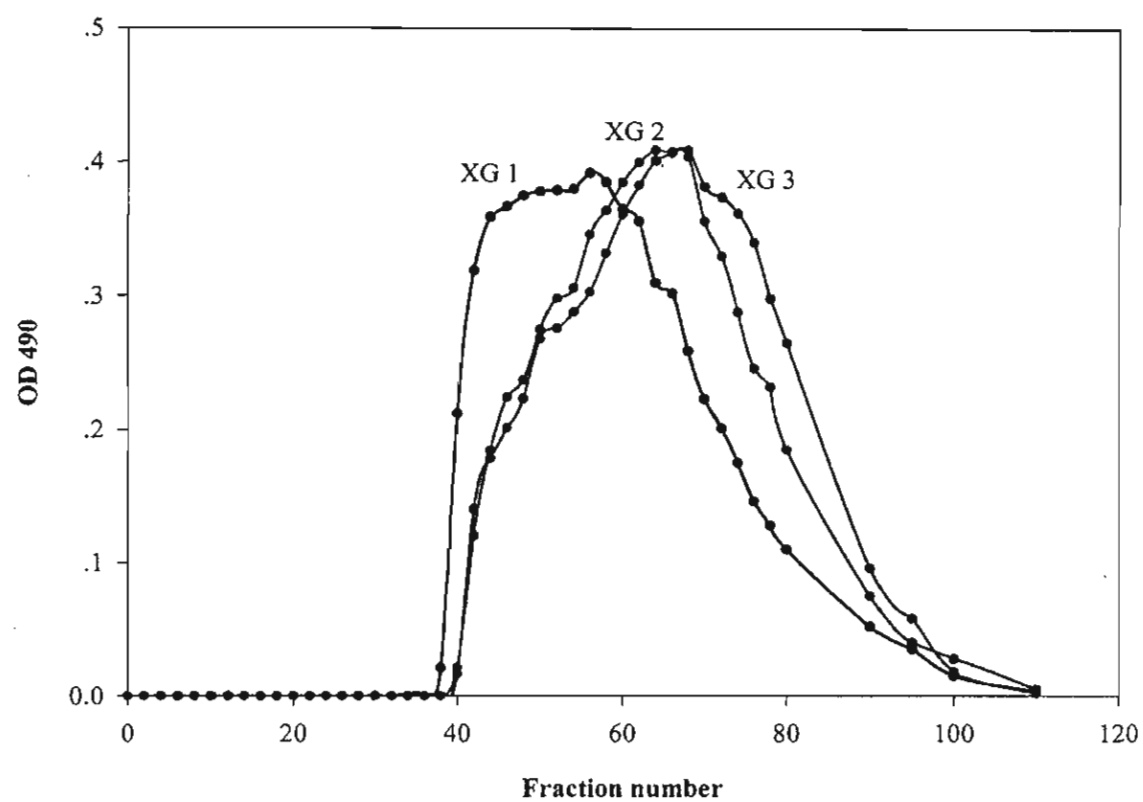


Fig. 4. Gel permeation chromatography on Sepharose CL-2B of purified xyloglucan from tamarind kernel powders (TKPs). XG1, XG2 and XG3 are xyloglucan from TKP1, TKP2 and TKP3 respectively.

**22nd International
Carbohydrate Symposium
Scottish Exhibition & Conference Centre (SECC)
Glasgow, UK
23-27 July 2004**

Introduction

The Organic Division and Carbohydrate Group of Royal Society of Chemistry (RSC) and the International Carbohydrate Organisation (ICO) are pleased to announce that the 22nd International Carbohydrate Symposium will be held at the SECC, Glasgow from Friday 23 to Tuesday 27 July 2004. The Symposium is sponsored by IUPAC.

The Symposium will cover new and emerging trends, with particular emphasis on synthetic methodology and applications in the design and synthesis of bioactive organic compounds. Relevant aspects of biologically active natural products and materials chemistry will also contribute to a programme catering for a wide range of interests in contemporary carbohydrate chemistry, biochemistry and medicine and offering a visionary perspective on future challenges and opportunities.

The programme will consist of a mix of plenary, keynote, shorter contributed lectures, two formal poster sessions in the themes

- Carbohydrate Chemistry and Enzymology
- Carbohydrates in Medicine and Biology
- Carbohydrate Materials and Biopolymers

Previous meetings in this series were held in: Cairns, Australia (2002), Hamburg, Germany (2000) and San Diego, USA (1998).

About the International Carbohydrate Organization

International Carbohydrate Symposium (ICS) is a biennial event organised in the selected host country on behalf of the International Carbohydrate Organization (ICO). The ICS is the premier meeting occasion for glycoscientists. The International Carbohydrate Organization was established in 1980. Prior to that date Carbohydrate Symposia were coordinated by an International Steering Committee first formed in 1965. The ICO Council comprises representatives of about 30 countries accredited by the ICO. The aims of the ICO, as stated in its constitution, are to encourage research, communication and education in glycoscience.

The Glasgow 2004 Symposium will be the twenty second in the series that began in Gif-sur-Yvette in 1960. The purpose of the Symposium is to gather together the world's carbohydrate chemists, biochemists and glycobiologists to facilitate exchange of ideas and information. The meeting presents and tracks recent developments in the field.

International Carbohydrate Organization

President:	Professor Robert Stick (Australia)
Past President:	Professor Dr Joachim Thiem (Germany)

Secretary:	Professor Berit Smestad Paulsen (Norway)
Symposium Chair:	Professor Elizabeth Hounsell (United Kingdom)

Roy L Whistler International Award in Carbohydrate Chemistry

In 1984, the International Carbohydrate Organisation established a US \$10,000 award in honour of Professor Roy L Whistler, to recognize scientists; who have made contributions of excellence in carbohydrate chemistry and biochemistry and with promise of continuing significant contributions'.

The awards will be jointly presented to Anne Imberty and Thomas Peters.

Past winners include:

2002	Stephen G Withers	1992	Andrea Vasella
2000	Anne Dell	1990	Johannis Kamerling
1998	Ole Hindsgaul	1988	Davis Bundle
1996	Constance van Boeckle and Maurice Petitou	1986	Klaus Bock
1994	Chi-Huey Wong	1984	Tomoya Ogawa

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Professor Elizabeth F Hounsell (Chair), Professor David H G Crout, Professor Rob A Field and Mrs Nicola J Cuthbert.

**Fractionation of xyloglucan oligosaccharides from tamarind seed
and its prebiotic effect**

Chitsuda Chaisakdanugull^{1*} and Klanarong Sriroth²

Abstract : Oligosaccharide fragments were prepared by hydrolysis of xyloglucan extracted from tamarind seed by cellulase enzyme (Sigma Chemical Co.). Four oligosaccharide fractions were obtained when fractionated by gel permeation chromatography (Bio-Gel P-2, Bio-Rad Co.) The molecular weight of these fractions as determined by matrix assisted laser adsorption ionization-time of flight (MALDI-TOF) mass spectrometry was ranging from 90 to 170 daltons. The effects of these xyloglucan oligosaccharides on the growth of bifidobacteria (*Bifidobacterium bifidum*) and lactic acid bacteria (*Lactobacillus casei* and *Lactobacillus acidophilus*) were evaluated and compared with inulin, a commercially used prebiotic in many food products. The maximum cell growth of microbial improved by 10% when cultured in basal medium containing 0.1% xyloglucan oligosaccharides. The maximum cell growth of microbial cultured with xyloglucan oligosaccharides was about 40% when compared to the maximum cell growth of microbial cultured with inulin. The results suggest that xyloglucan oligisaccharides are able to be applied as a prebiotic health food.

Keywords: tamarind seed xyloglucan, oligosaccharide, MALDI-TOF mass spectrometry, prebiotic effect

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