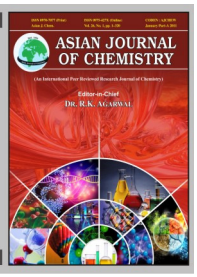
Asian Journal of Chemistry; Vol. 26, No. 1 (2014), 122-126

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15346



Extraction and Characterization of Gum from *Cordia myxa*

MUHAMMAD ABDUL HAQ[1,*], ABID HASNAIN[1], KHALID JAMIL[2] and MUHAMMAD SAMEE HAIDER[2]

[1]Department of Food Science & Technology, University of Karachi, Karachi-75270, Pakistan
[2]Food & Marine Resources Research Centre, PCSIR Laboratories Complex, Off University Road, Karachi-75280, Pakistan

*Corresponding author: Tel: +92 21 992613006; Ext. 2452, 3292; E-mail: abdul.haq@uok.edu.pk

Received: 18 February 2013; *Accepted*: 30 April 2013; *Published online*: 26 December 2013; AJC-14486

This study aimed to develop an extraction method of gum cordia and characterize the resulting gum from the fruits of *Cordia myxa*. Heating the fruits at 90 °C for 0.5 h with water (fruit: water, 1:4) containing 1000 mg kg^{-1} sodium metabisulfite was found to be sufficient to produce low colour gum. The yield of gum was approximately 1.5 % based on fresh fruit weight. The gum was found to be different in composition than previously reported gum from *C. abyssinica*. The gum was high in protein (12 %) and low in degree of methylation (10 %). Gel permeation chromatography resolved two fractions in the gum having average molar mass of 1.9×10^6 and 4×10^3 g mol^{-1} respectively.

Keywords: *Cordia myxa*, Gum, Polymer, Mucilage, Molecular mass.

INTRODUCTION

Cordia is a genus of flowering plants in Family Boraginaceae. There are about 250 species in this genus[1] which are found mostly in warm climate. Four species namely, *C. dichotoma*, *C. latifolia*, *C. Abyssinia* and *C. myxa*, are explored up to a certain extent for various applications in scientific literature[2-6]. However, in general this potentially beneficial plant resource has remained unexplored. Our research group has recently reported the successful application of gum cordia to retard the oxidation in nuts[7-9].

Method of extraction, specie and geographical location may affect the properties of the gums. The first report of the extraction of gum cordia dated back to 1976 when Ifzal and Qureshi[5] reported the monosacharides in *Cordia myxa*. They used cold and hot water along with ammonium oxalate and sodium hydroxide to extract the gum but did not perform any purification step (precipitation). In recent years two different extraction methods have been reported. In the first method, un-extracted or water extracted gum was precipitated by hydrochloric acid[3,10-12] while in the second method, ethanol along with sodium chloride was used as precipitant[3]. In all these studies, the mucilage was first separated from the skin and stone manually. This limits the utilization of the process at large scale. However, the method of extraction reported by our research group[7] has the potential of commercialization. The objective of this study was to characterize the gum cordia extracted by using newly developed method from *C. myxa*.

EXPERIMENTAL

The fruits of *C. myxa* were collected from Karachi region of Pakistan as described previously[7]. In entire study analytical reagent grade chemicals were used, purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Physical properties of fruit of *C. myxa*: The fruits were separated in 3 parts manually into skin, pulp and stone. One hundred fruits were taken in triplicate for this purpose. The size and shape of the fruit was determined from 100 randomly selected fruits. For each fruit, two principal dimensions, namely length and diameter were measured using a vernier caliper with a sensitivity of 0.01 mm. Geometric mean diameter (D_g) and sphericity (Φ) were calculated by using the following equations:

$$D_g = (L.D^2)^{1/3}$$

$$\phi = \frac{D_g}{L}$$

Thousand fruit mass (M1000) was measured by an electronic balance with a sensitivity of 0.0001 g. The fruit volume and density were determined using the liquid displacement method[13]. Toluene was used as a displacement liquid because it is not absorbed by the fruit and its low surface tension allows shallow dips in the fruits[14]. The true density (D_t) was evaluated by finding the ratio of the true mass (M_t) to that of the volume (V_t) as shown in equations:

$$D_t = \frac{M_t}{V_t}$$

The bulk density was determined by filling the fruits in a 1 L container. The mass of the fruit that filled the container was determined. This mass of the fruit is referred as bulk mass (M_b) and the volume it occupied is bulk volume (V_b). The bulk density was calculated by:

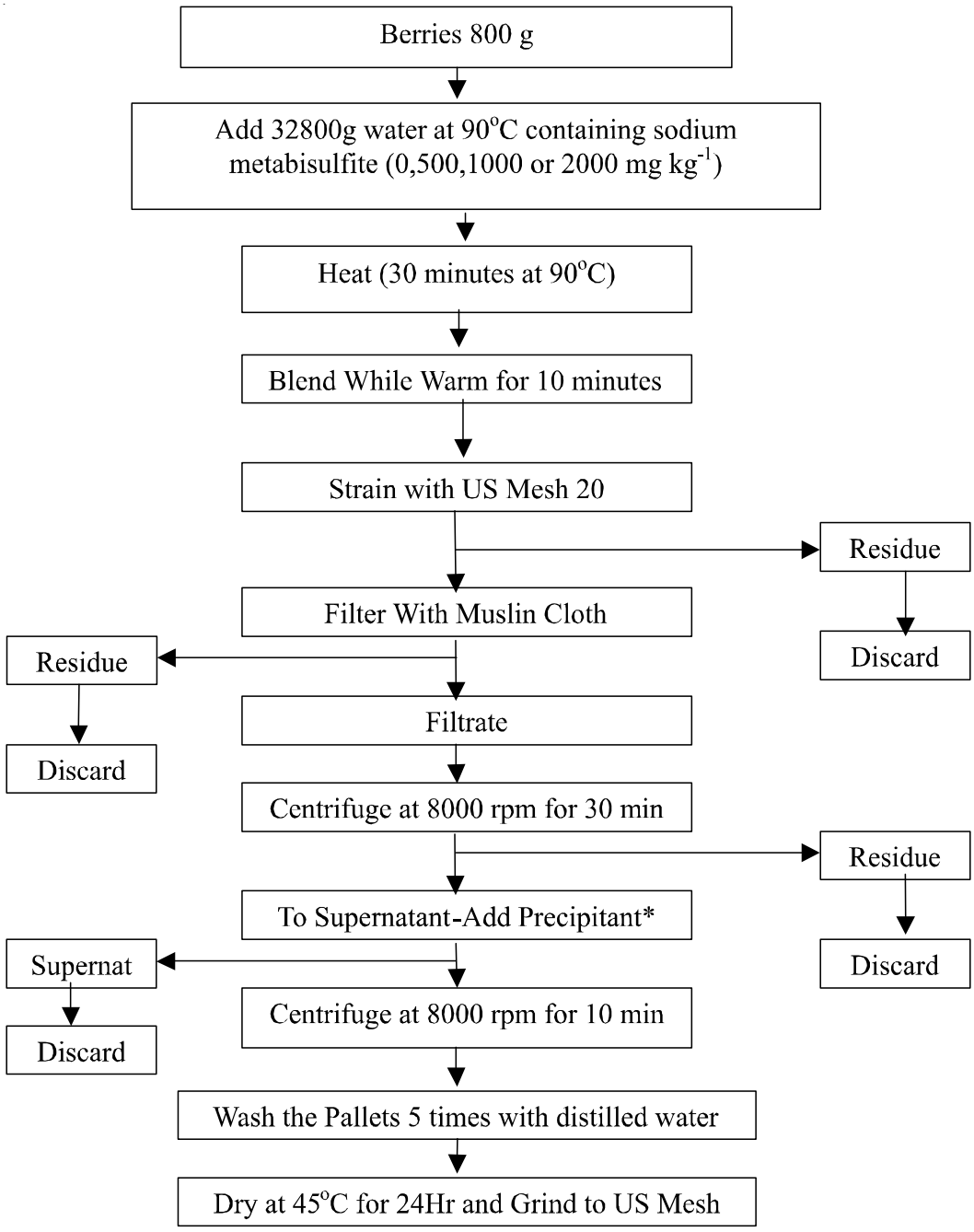
$$D_b = \frac{M_b}{V_b}$$

The porosity of the fruit was calculated using the below equation:

$$\varepsilon = \left(1 - {}^{D_b}/_{D_t}\right) \times 100$$

Extraction of the gum: Extraction was done by described method[7]. This scheme of extraction is given in Fig. 1 and Table-1. For comparison the method of Benhura and Chidewe[3] was used.

Physico-chemical methods: Moisture was determined by loss on drying method in oven at 70 °C till constant weight. Protein was determined by Kjeldahl method AOAC method



***Precipitant 1**: 1% Hydrochloric acid, **Precipitant 2**: 4 volume ethanol after adding the sodium chloride so that its concentration is 0.25M based on supernatant

Fig. 1. Extraction scheme for gum cordia

TABLE-1
CONDITIONS FOR EXTRACTION OF GUM CORDIA

Code	Heating Conditions		Sodium metabisulfite (mg kg^{-1})	Precipitant
	Time (min)	Temperature (°C)		
T1*	None	None	None	1 % Hydrochloric acid**
T2	30	90	None	1 % Hydrochloric Acid
T3	30	90	500	1 % Hydrochloric Acid
T4	30	90	1000	1 % Hydrochloric Acid
T5	30	90	2000	1 % Hydrochloric Acid
T6	30	90	1000	4 Volume ethanol***

*Manual separation of pulp as described by Benhura and Chidewe[3] was used.
**Based on volume of mother liquor.
***Added to 0.25M sodium chloride containing gum solution.

955.04[15]. The conversion factor for N to protein was taken as 6.25. Ash was determined by furnace method AOAC method 942.05[15]. The optical rotation of the gum solution at 0.25 % (w/v) was measured by the method of Benhura and Chidewe[10]. Uronic acid content was determined by the method of Filisetti-Cozzi and Carpita[16]. The degree of methylation was determined by the method of Voragen *et al.*[17]. CIE colour parameters of powdered gum were measured using a spectrophotometer (JASCO V-670), equip with powder sample holder accessory in reflectance mode with 2° Standard observer and D65 light source. The colour was expressed as CIE parameters L^* a^* and b^*. Total colour difference between untreated and treated samples were calculated using following equation

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differentials between the colour parameter of the treated samples and the colour parameter of untreated sample[18]. Molecular mass of the gum was determined using gel permeation chromatography (GPC) on Agilent series 1200 HPLC system. One percent gum in 0.01N NaOH was injected into GPC column (PL-aquagel-OH 30 8um 300 × 7.5 mm, Agilent). Mobile phase was distilled water at a rate of 1 mL min^{-1}. The eluted fractions were detected using refractive index detector. Polystyrene of various molecular mass was used to calibrate the system. Data was collected and analyzed on software ChemStation ver. 10.0. Following parameters were determined: number average molar mass (M_n), weight average molar mass (M_w), average molar mass (M_z) and polydispersity index (M_w/M_n).

The component monosaccharides were determined by hydrolyzes with triflouroacetic acid and subsequent determination of released monosaccharides by HPLC as described by Nikolov *et al.*[19] and optimized by Benhura and Chidewe[10] for gum cordia.

RESULTS AND DISCUSSION

Physical properties of the fruit of *C. myxa*: The knowledge of the physical properties of the fruit is not only important for processing such as sorting, cleaning, separation and extraction but also help to classify the various varieties[20]. These properties have been determined for many grains, fruits and vegetables[20]. However, such data was not available for *C. myxa* before this study (Table-2).

Extraction and characterization of the gum: Preliminary studies showed that either blending or squeezing of whole fruit without heat results in extensive browning and thus coloured gum. This is expected to be due to the enzyme polyphenol oxidase (PPO) which has been reported to cause browning in many fruits[21]. Heating the fruit at 90 °C for 0.5 h was found to stop the activity of PPO; judged by the colour of the gum. However the colour was still not satisfactory. Therefore, sodium metabisulfite as source of sulfur dioxide was added into heating medium (water). This treatment greatly reduced the colour of the resulting gum (Table-3). This effect was observed till 1000 ppm sodium metabisulphite but further increase in concentration did not improve the colour.

The method of extraction may affect the properties of biopolmer. Therefore, gum cordia extracted from this newly developed method was characterized. Table-4 shows that there was no major difference in properties of manually squeezed and heat and/or sulfur dioxide treated gum cordia. Although, the uses of gum cordia from different species have been documented in literature[11,12,22] but the detailed physicochemical characteristics are only reported for *Cordia abyssinica* by Benhura and Chidewe[3]. The yield of the acid precipitated gum was found to be about 1.5 % based on fresh fruit weight (Table-4) which is comparable to the previously reported yield of 1.2 % from *Cordia abyssinica*. However, the yield from ethanol precipitation was four times lower than the yield reported for

TABLE-2
PHYSICAL PROPERTIES OF THE FRUITS OF *C.myxa*

Physical properly	Unit of measurement	Mean value	Minimum value	Maximum value	Range
Length	mm	14.55	12.07	16.46	4.39
Diameter	mm	16.36	13.12	18.23	5.11
Geometric mean diameter	mm	15.72	13.76	16.89	3.13
Volume	mm^3	1747.57	887.27	2341.15	1453.88
Sphericity	%	108.39	90.895	125.234	34.339
Thousand fruit mass	g	1956.85	1892.51	2005.42	112.91
Bulk density	kg/m^3	554	550	560	10
True density	kg/m^3	691	687	697	10
Porosity	%	19.91	19.70	20.21	0.51
Skin	%	34.12	33.24	35.67	2.43
Pulp	%	47.43	46.13	48.56	2.43
Seed	%	19.34	18.09	20.48	2.39

TABLE-3
CIE COLOUR PARAMETERS OF GUM CORDIA EXTRACTED UNDER DIFFERENT CONDITIONS

Treatment	L*	a*	b*	ΔE
T1	17.61 ± 0.22[a]	-0.84 ± 0.11[a]	0.68 ± 0.17[a]	N/A**
T2	67.58 ± 1.22[b]	6.75 ± 1.81[b]	14.23 ± 2.65[b]	52.46 ± 3.24[a]
T3	76.61 ± 0.69[c]	4.23 ± 1.01[c]	11.41 ± 1.57[c]	60.24 ± 1.22[b]
T4	90.76 ± 1.65[d]	0.86 ± 0.08[d]	4.29 ± 1.41[d]	73.27 ± 2.58[c]
T5	91.85 ± 0.77[d]	0.71 ± 0.15[d]	4.55 ± 1.36[d]	74.37 ± 1.07[c]
T6	89.50 ± 1.81[d]	0.77 ± 0.14[d]	4.54 ± 1.25[d]	72.03 ± 2.87[c]

Different superscript alphabet in a column indicate statistically significant difference at $p \leq 0.05$; ** Not applicable.

TABLE-4
EFFECT OF HEATING, SULFUR DIOXIDE AND TYPE OF PRECIPITANT ON SOME PROPERTIES OF THE GUM EXTRACTED FROM *Cordia myxa*

Treatment Code/Parameter	T1	T2	T3	T4	T5	T6
Yield (%)	1.56 ± 0.11[a]	1.55 ± 0.19[a]	1.53 ± 0.12[a]	1.55 ± 0.17[a]	1.53 ± 0.11[a]	0.52 ± 0.09[b]
Moisture (%)	8.34 ± 0.41[a]	8.27 ± 0.32[a]	8.32 ± 0.43[a]	8.25 ± 0.12[a]	8.11 ± 0.29[a]	8.45 ± 0.21[a]
Protein (%)	12.75 ± 0.34[a]	12.42 ± 0.41[a]	12.21 ± 0.21[a]	12.1 ± 0.32[a]	12.23 ± 0.87[a]	15.45 ± 0.12[b]
Specific optical rotation	-46 ± 0.61[a]	-48 ± 0.32[a]	-48 ± 0.26[a]	-47 ± 0.51[a]	-48 ± 0.42[a]	-48 ± 0.57[a]
Uronic Acid (%)	10.22 ± 0.51[a]	10.17 ± 0.34[a]	10.32 ± 0.56[a]	10.47 ± 0.18[a]	10.21 ± 0.21[a]	7.31 ± 0.10[b]
DM (%)	11.21 ± 0.77[a]	10.89 ± 0.64[a]	11.44 ± 0.49[a]	10.96 ± 0.11[a]	10.53 ± 0.72[a]	8.81 ± 0.29[b]
Ash (%)	0.98± 0.11[a]	0.95 ± 0.23[a]	0.95 ± 0.64[a]	0.96 ± 0.53[a]	0.99 ± 0.16[a]	1.78 ± 0.21[b]

Different superscript alphabet in a row indicate statistically significant difference at $p \leq 0.05$.

C. abyssinica. In fact, it was very difficult to precipitate the gum by ethanol even after the addition of sodium chloride (very fragile precipitate was formed).

Chemical characteristics of the gum extracted in this study were found to be different than of the gum of *C. abyssinica*. Protein content was found to be about 12 % compared to 4 %. Uronic acid was almost same but the degree of methylation was only about 10 % compared to 38 %, while the ash was comparable but not found to be very high on ethanol precipitated gum as reported from *C. abyssinica*. This shows that the gum extracted from different species of the genus *Cordia* are different in characteristics, which is not unlikely due to variation in geographical location and species characteristics.

The molecular mass of the gum cordia is shown in Table-5. Two fractions were eluted from acid precipitated gum during GPC (Fig. 2), having average molar mass of 1.9×10^6 and 4.0×10^3g mol^{-1} respectively, which was not affected by heat or sulfur dioxide. It has been previously reported that gum from *C. abyssinica* and *C. dichotoma* has two different molecular fractions[2,3]. The average molar mass of first fraction is relatively higher than other plant based gums which are generally in the order[23] of 10^5g mol^{-1}. Similarly, the polydispersity index (PI) for both fractions are close to unity which is not common in plant based gums, *e.g.*, PI values of 2.27 and 3 have been reported for gum acacia and κ-carrageenan respectively[24,25]. Polydispersity index is related to the heterogeneity within the polymer chains, its value close to one indicates that the polymer chains in gum cordia have very narrow molecular mass distribution. This is advantageous for applications in edible film because homogenous chains may align better to give higher tensile strength[26].

The elution profile of ethanol precipitated gum was found to be different than of acid precipitated gum (Figs. 2 and 3). Three major peaks were identified, two having the same

TABLE-5
MOLECULAR MASS CHARACTERISTICS OF GUM CORDIA

Treatment/Parameter	M_n	M_w	M_z	M_w/M_n
		Fraction 1		
T1	1.68×10^6	1.81×10^6	1.96×10^6	1.08
T2	1.71×10^6	1.80×10^6	1.95×10^6	1.05
T3	1.66×10^6	1.83×10^6	1.94×10^6	1.10
T4	1.75×10^6	1.78×10^6	1.99×10^6	1.02
T5	1.63×10^6	1.89×10^6	1.81×10^6	1.16
T6	1.28×10^6	1.54×10^6	1.80×10^6	1.20
		Fraction 2		
T1	3.79×10^3	3.89×10^3	4.00×10^3	1.03
T2	3.83×10^3	3.85×10^3	3.95×10^3	1.01
T3	3.69×10^3	3.84×10^3	4.11×10^3	1.04
T4	3.81×10^3	3.90×10^3	3.99×10^3	1.02
T5	3.82×10^3	3.84×10^3	3.95×10^3	1.01
T6	4.13×10^3	4.69×10^3	5.41×10^3	1.14
		Fraction 3		
T6	3.94×10^4	4.04×10^4	4.13×10^4	1.02

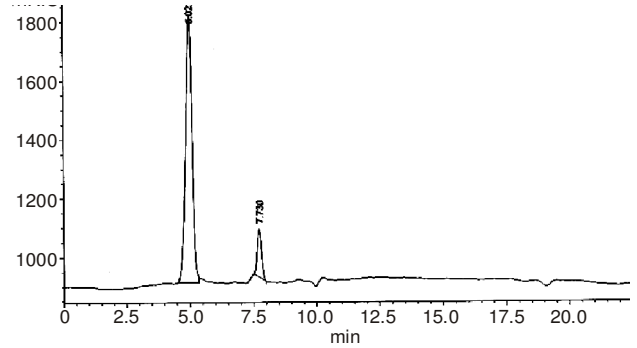


Fig. 2. Representative gel permeation chromatogram of acid precipitated gum cordia

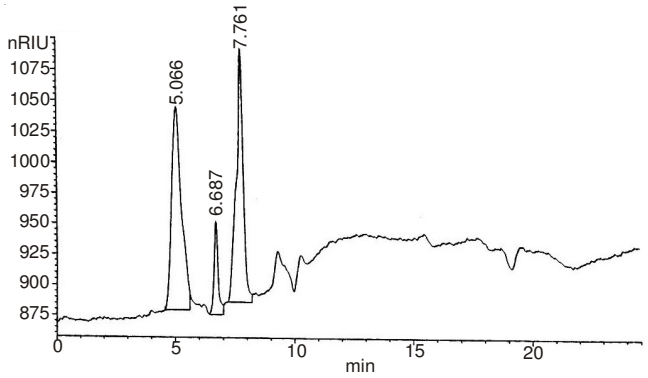


Fig. 3. Gel permeation chromatogram of ethanol precipitated gum cordia

retention time than of acid precipitated gum and the third one was between these two. Furthermore, the base line of ethanol precipitated gum was very noisy; indicating that ethanol precipitated material may contain other unrelated polymers present in the mother liquor. This hypothesis is also supported by the high PI of fraction 1 and 2 of ethanol precipitated gum. The sugar released by the hydrolysis of gum cordia is shown in Table-6. An attempt to quantify the sugars on absolute mass basis was failed due to incomplete digestion, therefore, ratios are mentioned. These ratios were found to be different than of previously reported values for *C. abyssinica*, however no conclusion can be drawn due to incomplete digestion in both studies[10].

TABLE-6
RATIO OF SUGARS RELEASED ON HYDROLYSIS OF GUM CORDIA

Sugar	*Cordia myxa*		*Cordia abyssinica**
	Acid precipitated gum	Ethanol precipitated gum	
Galactose	31	33	27
Rhamnose	17	16	21
Mannose	11	12	18
Xylose	6	5	11
Glucose	18	21	10
Arabinose	11	8	9
Uronic acid	6	5	4

*Acid precipitated reference: Benhura and Chidewe[10].

REFERENCES

1. Y.J. Nasir, in eds.: S. I. Nasir and E. Ali, "Araceae," in Flora of West Pakistan, vol. 120 (1978).
2. N.G. Basu, P.K. Ghosal and S. Thakur, *Carbohydr. Res*., **131**, 149 (1984).
3. M. Benhura and C. Chidewe, *Afr. J. Biochem. Res*., **5**, 95 (2011).
4. B.S. Siddiqui, S. Perwaiz and S. Begum, *Nat. Prod. Res*., **20**, 131 (2006).
5. M.S. Ifzal and A. Qureshi, *Pak. J. Sci. Ind. Res*., **19**, 64 (1976).
6. M. Afzal, C. Obuekweb, A. Khanc and H. Barakata, *Electron. J. Environ. Agric. Food Chem*., **6**, 2236 (2007).
7. M.A. Haq, M.J. Alam and A. Hasnain, *LWT-Food Sci. Technol*., **50**, 306 (2013).
8. M.A. Haq and A. Hasnain, *J. Food Process. Preserv*., (2013) (In Press).
9. M.A. Haq and A. Hasnain, *Asian J. Chem*., **25**, 343 (2013).
10. M.A.N. Benhura and C. Chidewe, *Food Chem*., **76**, 343 (2002).
11. M. Ahuja, M. Yadav and S. Kumar, *Carbohydr. Polym*., **80**, 161 (2010).
12. S. Dinda and B. Mukharjee, *Acta Pharm. Sci*., **51**, 189 (2009).
13. S.D. Deshpande, S. Bal and T.P. Ojha, *J. Agric. Eng. Res.*, **56**, 89 (1993).
14. H. Ö g, *J. Agric. Eng. Res*., **69**, 273 (1998).
15. AOAC, Official Methods of Analysis of AOAC International, AOAC International, Gaithersburg, 2000.
16. T.M.C.C. Filisetti-Cozzi and N.C. Carpita, *Anal. Biochem*., **197**, 157 (1991).
17. A.G.J. Voragen, H.A. Schols and W. Pilnik, *Food Hydrocoll.*, **1**, 65 (1986).
18. T.M. Paschoalick, F.T. Garcia, P.J.A. Sobral and A.M.Q.B. Habitante, *Food Hydrocoll.*, **17**, 419 (2003).
19. Z.L. Nikolov, M.M. Meagher and P.J. Reilly, *J. Chromatogr. A*, **319**, 51 (1985).
20. I. Akinci, F. Ozdemir, A. Topuz, O. Kabas and M. Canakci, *J. Food Eng*., **65**, 325 (2004).
21. M.V. Martinez and J.R. Whitaker, *Trends Food Sci. Technol*., **6**, 195 (1995).
22. M. Yadav and M. Ahuja, *Carbohydr. Polym*., **81**, 871 (2010).
23. R.J. Redgwell, C. Schmitt, M. Beaulieu and D. Curti, *Food Hydrocoll.*, **19**, 1005 (2005).
24. C. Sanchez, D. Renard, P. Robert, C. Schmitt and J. Lefebvre, *Food Hydrocolloids*, **16**, 257 (2002).
25. I.S. Chronakis, J.L. Doublier and L. Piculell, *Int. J. Biol. Macromol*., **28**, 1 (2000).
26. Y. Jiang, R. Huang and H. Liang, *J. Chem. Phys*., **123**, 124906 (2005).