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Chemical Investigation on the Polysaccharides Present in the Mesocarp of Chalkumra (*Benincasa hispida*) Fruit

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The mesocarp tissues of kumra (*Benincasa hispida*) fruit were homogenized and an alcohol-insoluble residue (BAIR) was isolated. BAIR was rich in carbohydrate (745.4 mg g^{-1}), and contains large amount of pectic substances. The extractable juice (EJ) amounts to 65.9% of the fresh mesocarp tissue and contains, *inter alia*, pectic polymers, proteins and arabinose. The polysaccharide present in the juice was isolated and characterized by its sugar composition, molecular weight distribution and infrared spectra. It contained 30.6% of galacturonic acid and 69.4% of neutral sugars, mainly galactose, arabinose and glucose. On size exclusion chromatography this pectic substacnce shows almost an asymmetrical peak.

INTRODUCTION

Benincasa hispida (Thunb) Cogn., also called chalkumra, grows commericially in almost every part of India[1, 2]. The juice of this fruit is used as traditional medicine for the treatment of hemorrhagic diseases, epilepsy and is applied directly on the affected part of the body to prevent dandruff and other skin diseases. The decoction of the fruit is a laxative, a popular anti-mercurial and also used to cure internal hemorrhage and diseases of the respiratory track[1, 2]. The reason for these effects remains unknown. Several polysaccharides from plants have effects on the human immune system[3–5]. So some of these medicinal properties might be due to biological active polysaccharides. Different parts of this plant have been investigated to some extent, and found to contain various low molecular weight compounds[1–14]. For example, it has been known that the fruit of this plant contains amino acids[1, 6], vitamins (B_1 and C)[7], steroids, phenolic acid[8], free sugars like glucose, rhamnose, mannitol etc.[1, 2] and even some trace metals[9], whereas its leaves contain free amino acids, carotene, sucrose, fructose and a large proportion of citric acid together with trace amount of oxalic acid and malic acid[1, 2]. The seeds of this fruit contain sterols[10, 11], fatty acids[12, 13], amino acids and proteins[14]. The ethanolic extract of *Benincasa hispida*, when administered orally to rats failed to lower blood sugar or to depress the peak value, after glucose load[15]. The sugar composition of the insoluble non-cellulose

polysaccharides (INCP) and soluble dietary fibre (SDF) has been partly determined[16]. Despite the importance of chalkumra (*Benincasa hispida*) as an edible vegetable, in the production of petha (a kind of sweet dish) and as folk medicine no information about the polysaccharides present in chalkumra fruit is yet available. The full use of these polysaccharides requires an in-depth knowledge of the relationship between their structure and properties. This report presents the preparation of alcohol-insoluble residues from the mesocarp of *Benincasa hispida* fruit and to characterize it in order to perform further rheological and structural investigations of the important fractions. Extraction of the fruit juice and chemical investigation of the purified polysaccharides present therein is also presented.

EXPERIMENTAL

Plant material: Chalkumra (*Benincasa hispida* (Thunb) Cogn.) syn *B. cerifera* Savi (Fam. Cucurbitaceae) fruits were obtained from plants grown in Tentulia village of Birbhum district, West Bengal. From a number of mature kumra harvested in October 1999, those between 3.5 and 4.5 kg were selected and stored for 10 days at room temperature before analysis.

The skin and the central core of the fruit were removed with a knife and the remaining mesocarp, which was cut into pieces ($\sim 0.5 \times 0.3 \times 3$) cm^3, was homogenized using a household blender. The homogenized inner tissue zone (820 g) of the fruit was filtered to separate the extractable juice (EJ) from the solid residue. The latter was sequentially extracted with 2.5 L of EtOH for 30 min (under reflux) and then with 70% EtOH (2×1 L) for 1.5 h at room temperature to yield the alcohol-insoluble residue (BAIR, 9.1 g). The alcoholic extracts were combined (Alcohol soluble material, ASM) and a part of it was subjected to chemical analysis. A part of the fruit juice (EJ) was dialyzed and the retentate diluted with ethanol to make the final ethanol concentration 70% and the precipitate formed was then collected by centrifugation and dried after solvent exchange over anhydrous P_2O_5 under vacuum to yield juice alcohol precipitable material (JAPM).

Gel permeation chromatography: The polysaccharide (JAPM, 3 mg) was dissolved in 400 mM sodium acetate buffer, pH 5.0, and passed through a Sephadex G-200 column (2.3×50 cm) equilibrated with the same buffer at 20 mL/h. Fractions (5 mL) were collected and assayed for the neutral sugar and uronic acid contents. Elution of polysaccharide from GPC is expressed as K_{av} [$K_{av} = (V_e - V_0)/(V_t - V_0)$ with V_t and V_0 being the total and void volumes of the column (determined as the elution volume of glucose and blue dextran, respectively) and V_e is the elution volume of the sample]. The column was calibrated with standard dextrans with a molecular-weight range of 10,000 to 500,000.

Analytical methods: Neutral sugars were determined by the phenol-sulfuric acid assay[17]. Total uronic acids were assayed colorimetrically as anhydrogalacturonic acid using *m*-phenylphenol color reagent[18]. All fractions were hydrolyzed

in 1 M sulfuric acid (3 h, 100°C) for measurement of individual neutral sugar, with an additional pre-treatment of 72% sulfuric acid (1 h, 20°C) for insoluble residues. The individual sugars were reduced, acetylated and analyzed as their alditol acetate by GLC on columns of 3% SP-2340 on Supelcoport 100–120 mesh, and DB-225 (JW) as described and by GLC/MS[19, 20]. Myo-inositol was used as internal standard[21]. TLC as described[22] also analyzed the sugars in the acid hydrolysates.

Protein was measured in the insoluble residues by estimating the total nitrogen and multiplying the value with 6.25 and in the soluble material by the method of Lowry *et al.*[23] using bovine serum albumin as standard. Amino acids were released by hydrolysis with 6 M HCl at 110°C for 22 h in a sealed tube. Protections were done for cysteine, methionine and tyrosine using proper protecting reagents. Pharmacia LKB ALPHA PLUS amino acid analyzer analyzed the liberated amino acids.

IR spectra were obtained on an FT-IR spectrophotometer (Jasco FT/IR-420) using a KBr discs containing finely ground samples. UV-VIS spectra were recorded with a Shimadzu UV-160 A spectrophotometer.

RESULTS AND DISCUSSION

In order to have an idea about the chemical composition of the *Benincasa hispida* fruit the mesocarp tissue was homogenized. The procedure used for the isolation of various fractions is shown in Fig. 1. In order to inactivate endogenous enzymes that could alter the structure of polymers during their isolation, the fruit was rapidly cut into pieces, homogenised and blended for 30 min in boiling ethanol. The mesocarp tissue of this fruit consists of 95.5–96.5% water. The

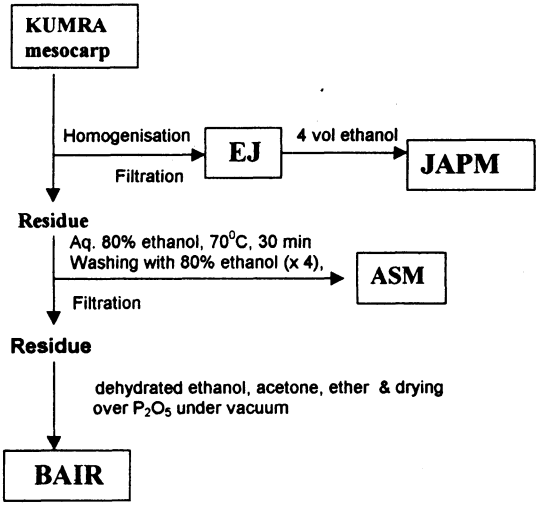

Fig. 1. Scheme for the isolation of various fractions from the mesocarp of *Benincasa hispida* fruit.

extractable juice (EJ) which amounted to 65.9% of the fresh tissue contains protein 1.8 mg/mL. In contrast to earlier observations[1, 2], which showed the presence of glucose, rhamnose, fructose and mannitol the fruit juice (EJ) isolated in our laboratory contained only arabinose as free sugar (as indicated by TLC, GLC and GLC/MS analysis).

To facilitate storage, an alcohol-insoluble residue (BAIR) was prepared from the mesocarp of *Benincasa hispida* fruit. The yield and sugar content of BAIR, EJ, ASM and JAPM fractions obtained from chalkumra pulp was determined and is shown in Table-1. The yields of alcohol-insoluble residue obtianed were 11 mg per gram fresh tissue. The yield may be compared with values of 6.7 mg/g fresh weight for kiwi fruits[24], 1.8–2.4 mg/g for grape berries cell walls[25] and 5–6 mg/g for tomato fruit walls[26]. The compositions of BAIR are shown in Table-2. The total nitrogen value of the BAIR was 1.14% and this corresponds to protein content 7.1% (w/w). The amino acid compositions of proteins associated with BAIR show that the most abundant amino acid was glycine. Other abundant amino acids included alanine, aspartic acid, glutamic acid, leucine etc. The high contents of galacturonic acid, which are higher than those from carrot, onion, apple, suggested the presence of an important amount of pectic polysaccharides. Galactose, arabinose and xylose were the main non-cellulosic sugars and the first two sugars are generally associated with pectin. The content of galactose was high (BAIR, 31.8%), the bulk originated from pectin but a fraction (24.1%) was not released by mild acid hydrolysis.

TABLE-1
YIELD AND SUGAR CONTENT OF FRACTIONS OBTAINED FROM THE FRUIT OF *Benincasa hispida* (SEE 'EXPERIEMNTAL' FOR IDENTIFICATION OF REACTIONS)

Sample	Yield[a]	TS[a]	UA[a]	RS[a]	Protein[a]
EJ	658.70	9.13	0.78	0.573	8.26
JAPM	1.01	–	0.09	–	–
BAIR	11.10	6.51	2.75	–	–
ASM	–	33.14	1.66	–	5.48

RS = Reducing sugar, TS = Total sugar, UA = Uronic acid.
[a] Weight in g per kg of fruit mesocarp.
– not determined.

Sugar analysis revealed that galacturonic acid, galactose and arabinose were the main sugars found in JAPM fraction together with smaller quantities of xylose, mannose, glucose and traces of rhamnose and fucose. The presence of high amount of galacturonic acid (confirmed by TLC analysis) in this fraction suggests the presence of pectic polysaccharides. GLC analysis and colorimetric estimations also showed that the ratio of acidic to neutral sugars in JAPM was around 31 galacturonic acid molecules for 69 neutral sugar residues (rhamnose inside the chain and arabinose, galactose and other sugars in lateral branches). The presence of phenolic acids was indicated from UV absorption spectrum of JAPM fraction.

TABLE-2
SUGAR COMPOSITION OF THE FRACTIONS ISOLATED FROM *Benincasa hispida* FRUIT

	BAIR	JAPM
NS[a]	49.5 (39.6)[c]	25.0
UA[a]	25.0 (25.0)	8.3
Rha[b]	1.3 (1.5)	0.2
Fuc[b]	1.5 (1.0)	0.4
Ara[b]	3.9 (3.1)	17.2
Xyl[b]	7.9 (5.0)	2.4
Man[b]	1.3 (1.3)	1.4
Gal[b]	22.2 (49.8)	42.1
Glc[c]	3.2 (1.9)	5.6
UA[b]	58.5 (36.3)	30.6

[a]Percentage weight of fraction dry weight.
[b]Mol per cent.
[c]Values in parenthesis are results obtained from experiments with prehydrolysis whereas other values are results from hydrolysis with 1 M sulfuric acid.

The JAPM fraction was further characterized by its molecular weight distribution as determined by gel permeation chromatography. Fig. 2, contains the chromatogram obtained using Sephadex G-200 column. The fractionation range of this column was 10,000–200,000. As indicated by the K_{av} values at the front and tail ends of the chromatograms, the JAPM fraction elutes well within the fractionation range of the column. *ca.* 34.9% of the polymers had molecular weights lower than 40,000 whereas the rest part have molecular weight between 2,00,000 to 40,000 based on calibration with dextrans.

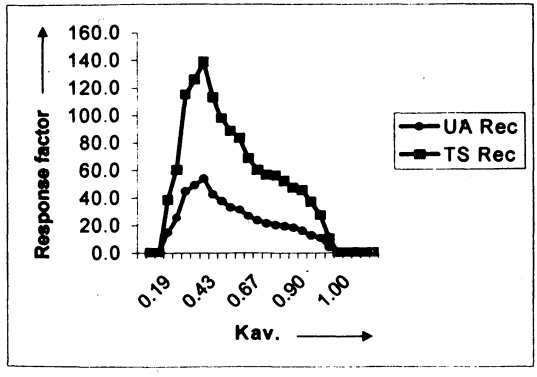

Fig. 2. Gel permeation chromatography of JAPM fraction obtained from the mesocarp of *Benincasa hispida* fruit on Sephadex G-200.

The FT-IR spectrum of the JAPM and BAIR fraction is given in Fig. 3. The broad band between 3600 and 3000 cm^{-1}, corresponding to vibrations of the hydroxylic groups appeared to be similar in all the spectra. Methyl and methylene group vibrations appeared around 2927 cm^{-1} and were present in the spectra of all fractions. The pectic polymers present in the JAPM (Fig. 3a) and the BAIR itself (Fig. 3b) show a band in the region 1739 cm^{-1} related to the >C=O stretching of the acetyl group[27]. After de-esterification a reduction of the absorbance band at 1739 cm^{-1} was observed (Fig. 3c). Structural features arising from particular conformations around the glycosidic bond of the pectins are observable in the 1200–850 cm^{-1} region[28]. For example, the bands in 1101–1014 cm^{-1} region characteristics of the uronic acid residues of the pectic polysaccharides[29] are clearly visible in most of the spectra (Fig. 3a).

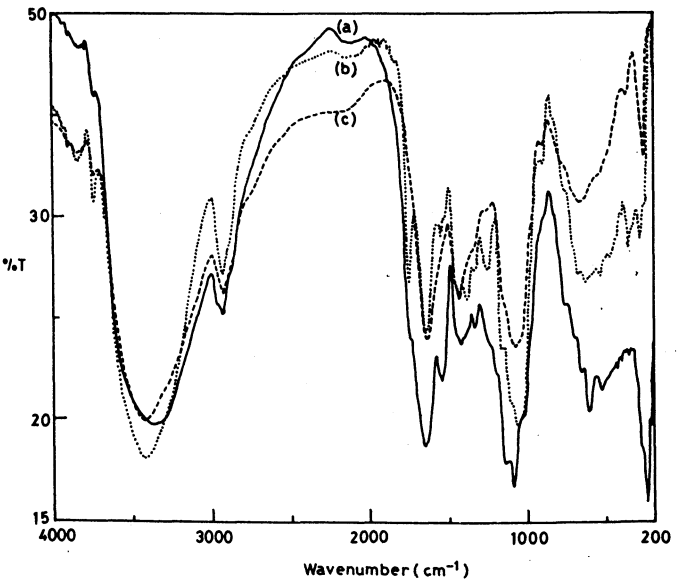

Fig. 3. FT-IR spectra of polysaccharide fractions: (a) JAPM, (b) BAIR and (c) de-acetylated BAIR obtained from *Benincasa hispida* fruit pulp. See experimental section for identification of the fractions.

In conclusion, an alcohol-insoluble residue (BAIR) was isolated from the mesocarp of Chalkumra (*Benincasa hispida*) fruit. The BAIR was rich in pectin and contains very high amount of carbohydrate. The extractable juice (EJ) amounts to 65.9% of the fresh mesocarp tissue and contains, amongst other pectic substances, protein and arabinose. The pectic polysaccharide contains 30.6% of galacturonic acid and 69.4% of neutral sugars, mainly galactose, arabinose and glucose. This polymer gave viscous solutions. Further research will be directed towards a detailed characterization of the polysaccharides present in the BAIR.

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