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Structure Elucidation of Soluble Dietary Fiber from Cauliflower

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Abstract

Arabinogalactan and rhamnogalactglucuronan were isolated from soluble dietary fiber (SDF) of cauliflower. The isolated SDF was fractioned into neutral and acidic parts by ion-exchange column chromatography using DEAE-Sepharose Cl-6B gel. Structure elucidation of the isolated SDF polysaccharides were determined sugar analysis, ^{1}H-NMR, H-H COSY and TOCSY spectroscopic studies. Neutral fraction of cauliflower was found to be composed of galactose, arabinose and rhamnose whereas the acidic part was found to contain rhamnose, galactose, galacturonic and glucuronic acids. The main chain of the neutral fraction was found to be a 1,4-β-galactan where terminal rhamnose and arabinose residues were attached to the main chain at the 3-position of by α-linkage.The main chain of the acidic fraction was composed of 1,4-β-linked galacturonic acid and 1,2-β-linked rhamnopyranose residues. α-Glucuronic acid and α-galactose were attached to the main chain of galacturonic acid at its 3-position.

Key Words:Soluble Dietary Fiber; Cauliflower; Arabinogalactan; Rhamnogalactglucuronan

1. Introduction

Dietary fibers (DF) play an important role in human health management; it reduces the risk of colon cancer, heart disease and risk of gallstone formation, helps to reduce blood glucose & blood cholesterol level and other complications of diabetes like hypertension, atherosclerosis and hyperlipidaemia[1]. The fibers are also helpful to control irritable bowel syndrome[2]. The soluble components of DF increase viscosity of the stomach contents, thereby retarding gastric emptying. This then affects the rate of digestion and the uptake of nutrients and creates a feeling of satiety. This protective effect is associated with a selective decrease in biliary cholesterol [3-4]. Epidemiological data show that high fiber rich diet generally reflects a healthier life style and fiber intake can be viewed as a marker of a healthy diet[5-8]. Increasing awareness of the importance of dietary fiber (DF) to a healthy diet has highlighted the need to provide meaning and reproducible data on the DF content of the foods that we consume where the vital sources are fruits and vegetables.

Free sugar (FS) and dietary fiber (DF) are the two major nutritional constituent of all fruits and vegetables. Pectins are one of the important dietary fibers and have the ability to form gels which are common acidic polysaccharide found in the cell walls of fruits[9]. Content and composition of soluble and insoluble DF of a large number of local fruits and vegetables have been reported[10-13]. Isolation and structure elucidation of a number of soluble dietary fibers from fruits have also been reported[14-20]. But there is no report on structural studies of dietary local vegetables. In continuation of our work on dietary fibers, in this paper we are reporting structure elucidation of soluble dietary fiber of cauliflower, which has been reported earlier.

II. Materials and Methods

Isolation of dietary fiber

Good quality of cauliflower was purchased from local vegetable market of Dhaka city. The vegetable was chopped into small pieces, made into pulp by a kitchen blender and extracted with 80% aqueous ethanol (volume of alcohol was adjusted considering water content of the fresh vegetable). Aquaeous alcohol was separated by decantation followed by centrifugation. The extractive free vegetable was further extracted with water (2 hrs) in boiling water bath. The water extract was collected by squeezing the pulp with pre-cleaned cloth filter, concentrated, and was poured into 4 times of its volume of absolute alcohol. The polymeric precipitate was collected by centrifugation, re-dissolved in water, centrifuged again; the clear supernatant was concentrated and freeze-dried to get pure water soluble polysaccharide (500 mg).

Enzymes

Protease from *Streptomyces caespitosus* (purified Type IV, Sigma Chemical Company, USA), and Termamyl 120L (NOVO A/S Copenhagen, Danmark) & amyloglucosidase-from *Aspergilliusniger* (Boehringer-Mannheim, Germany) were used for enzymatic degradation of protein and starch, respectively.

1D and 2D NMR Total Correlation Spectroscopic (TOCSY) studies

NMR spectrum was recorded by dissolving the SDF sample in D_2O using TSP (deuteriatedtrimethylsilyl sodium propionate) on 600 MHz Bruker NMR machine. 2D-NMR spectra were recorded with standard TOCSY, relayed COSY, double-relayed COSY, COSY experiments were performed with (90^o-t_1-90^o-t_2) for higher sensitivity.

Removal of Starch and Protein by enzymatic degradation[15-20]

The isolated water soluble polysaccharide (200 mg) was suspended in phosphate buffer (0.1 M, 300 mL), treated with protease (0.5 mg), heated in an thermostatic water bath (60^0C, 3h) and protein[12] free mucilaginous water extract was collected by dialysis followed by freeze-drying (180 mg). The protein free material (170 mg) was again suspended in acetate buffer (0.1 M; pH 5.0, 200 mL), treated with termamyl (2 µL), heated in an thermostatic shaker (1 h, 60^0C), cooled, amyloglucosidase (2 µL) was added to the mixture and shook again (16 h), the enzyme-treated mixture was dialyzed (48 h), concentrated,

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centrifuged and the starch free material was collected by dialysis (molecular weight cut off 14000) and freeze drying (165 mg) and termed as soluble dietary fiber.

De-esterification of starch and protein free material[15-20]

SDF fraction of cauliflower (160 mg) was suspended in water, NaOH (1M) was added drop wise to reach its pH 12 and kept at 0^0C for 2 h, neutralized with 10% acetic acid, dialyzed and freeze-dried (158 mg)

Fractionation of SDF

Fractionation of SDF of cauliflower (150 mg) was done by ion-exchange column chromatography using DEAE-Sephadex CL-6B gel column (Pharmacia gel column) and acetate buffer (0.1 M, pH 6.5) was used as mobile phase. The neutral fraction was eluted with mobile phase and the acidic fraction with 1 M NaCl in the mobile phase. The eluted fractions were collected in test tubes by an automatic fraction collector (Pharmacia Frac-100; flow rate 1 mL/min was maintained by a Peristaltic pump), monitored by phenol sulphuric acid and Carbazoletests[21](13%). The neutral and acidic fractions were collected by dialysis followed by freeze-drying (26 and 32 mg, respectively).

Reduction of carboxylic functional group of acidic SDF[22]

Acidic SDF fraction (10 mg) was dissolved in water (1 mL) in a small beaker and an electrode of pH meter was immersed in the solution (pH meter was calibrated with buffers of pH 5 and 7), diethyl sodium carbadimide (EDC, 5 mg in 2 mL water) solution was added to the SDF solution drop wise maintaining pH 4.5 by adding NaOH (0.1M) simultaneously drop wise. The solution was kept at pH 4.5 for 1 hour and $NaBH_4$ (10 mg; 2mL) in solution was added to the EDC complex by maintaining pH 7.0 by adding HCl (1M) simultaneously drop wise. Evolution of gas vapor was controlled by addition 1-2 drops of 1-octanol. The solution was kept in that condition for 2h, dialyzed and freeze dried to get uronic acid reduced acidic SDF (8 mg).

Sugar analysis[23]

The parent SDF, its neutral, acidic and carboxyl-reduced fractions (5 mg in each case) were hydrolyzed with 2 M TFA (1mL; 2h, 120^0C), evaporated with added rectified spirit, re-dissolved in water (3 mL), reduced with $NaBH_4$ (2 mg; 2 h), acidified with Dowex H^+, filtered and the reduced material was evaporated dryness. All the neutral sugar were converted into their corresponding alditol acetates[15] with acetic anhydride in dry pyridine (1mL; 1:1, 20 min, 96^0C) and were analyzed by GC-FID. Rhamnose (Rha*p;*15%), arabinose (Ara*f;*34%), galactose (Gal*p*;25%) & glucose (Glc*p*;26%); galactose (80%), rhamnose (12%) & arabinose (8%); rhamnose (48%), galactose (44%) & arabinose (14%) and rhamnose (42%), galactose (45%) arabinose (7%) & glucose (6%) were identified in parent SDF, neutral SDF and acidic SDF and carboxyl reduced acidic SDF, respectively.

1D and 2D NMR Total Correlation spectroscopic studies

^{1}H NMR spectroscopic signals neutral SDF fraction of cauliflower were found at 5.20, 5.10, 4.55, 4.15 , 4.00-3.30 and 1.25 ppm and acidic SDF fraction showed the signals at 5.2-5.1, 4.70, 4.60, 4.10, 4.00-3.40 and 1.22 ppm.^{1}H NMR and TOCSY spectra of neutral SDF fraction are given in Fig 1 & 2 and spectral data in Table 1. Similarly ^{1}H NMR and TOCSY spectra of acidic SDF fraction are given in Fig 3 & 4 and spectral data are presented in Table 2.

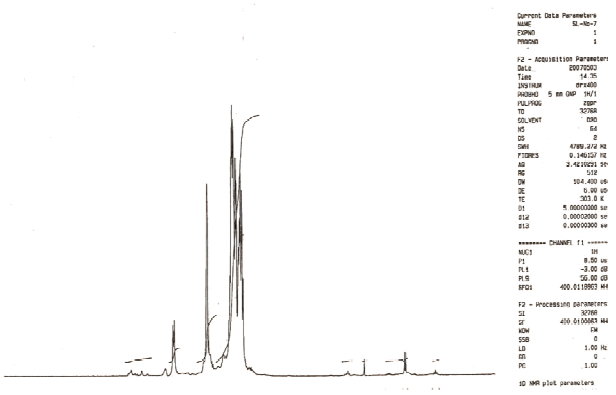


Fig. 1. ^{1}H-NMR spectrum of neutral SDF of cauliflower

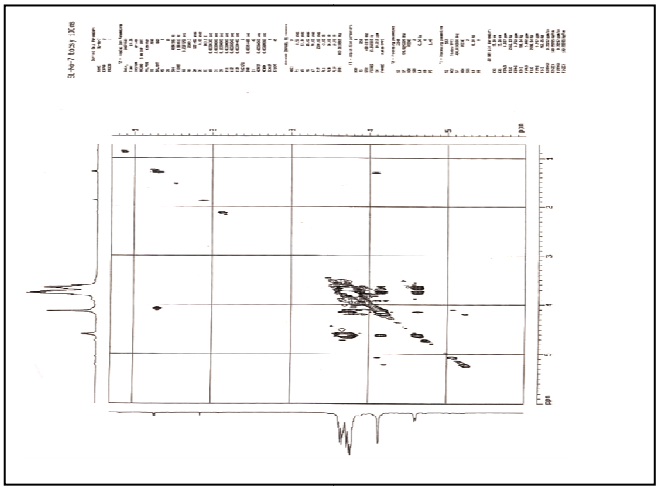


Fig. 2. H-H TOCSY spectrum of neutral SDF of cauliflower

III. Results and Discussion

^{1}H-NMR spectroscopic studies of neutral SDF fraction

In the ^{1}H-NMR spectrum (Fig-1) of neutral SDF gave three anomaric signals at 5.22, 5.06 and 4.60 ppm (Table-2). Signal at 5.22 ppm was assigned to the anomeric proton of arabinose and the signal at 5.06 ppm assigned to the anomeric proton of rhamnose. Both the signals were unresolved and were appeared at downfield indicated that these sugar residues had α-anomeric configuration. Anomeric signal (doublet) at 4.60 ppm assigned to the β-anomeric protons of galactopyranose residues. The intense singal of β-galactopyranose indicated that the main chain

was composed by β-galactose. The signals between 4.20 to 3.30 ppm assigned to the oxymethyne protons present in the ring at different sugar residues. The signal at very up field chemical shift at 1.35 ppm assigned to the methyl group protons (H-6) of the deoxy sugar α-rhamnose residue in polysaccharide of neutral SDF of cauliflower.

Total correlation spectral (TOCSY) studies of neutral SDF fraction

In H-H total correlation spectrum (TOCSY) of neutral SDF gave three anomeric signals (Fig-2; Table-1) at 5.22, 5.06 and 4.60 ppm which were accounted for α-arabinose, α-rhamnose and β-galactose residues, respectively. Chemical shifts of all the three sugar residues were assigned from the total correlation spectrum. The H-1 proton signal of galactose residue at 4.60 ppm coupled with H-2 at 3.75 ppm, H-2 coupled with H-3 at 3.65 ppm, H-3 coupled with H-4 at 4.15 ppm. H-3 of galactose had two other cross peaks with H-1 proton of rhamnose at 5.06 and H-1 of arabinose at 5.22 ppm, respectively which showed that rhamnose and arabinose were linked to the 3-position of galactose residue. From integration of anomeric protons it was evident that galactose was the major sugar residue. Therefore, the main chain must be 1,4-β-linked galactan. The protons of arabonose and rhamnose were also assigned from the H-H TOCSY spectrum. Anomeric proton H-1 of α-arabinofuranose at 5.22 ppm coupled with H-2 at proton at 4.15 ppm, H-2 coupled with H-3 at 3.75 ppm and H-3 coupled with H-4 at 3.65 ppm. H-1 had also cross peak H-3 of galactose which further proved linkage of arabinose at the 3-position of β-galactose. In case of α-rhamnose, anomeric proton at 5.06 ppm coupled with H-2 at 4.15 ppm. Besides H-1 of rhamnose had also cross peak with H-3 of galactose at 3.65 ppm indicated that α-rhamnose was attached to 3-position of galactose residue in the main chain.

^{1}H-NMR spectroscopic studies of acidic SDF fraction

In ^{1}H-NMR spectrum (Fig-2) of acidic SDF of cauliflower was complex, there were five signals at 5.75, 5.22, 5.08, 4.60 and 4.40 ppm (Table-2). The major signal at 5.08 ppm was assigned for the anomeric proton of galacturonic acid residue having coupling constant ($J_{1,2}$ ~6 Hz) which indicated galacturonic acid had β-anomeric configuration. The signal at 5.22 ppm assigned to the anomeric proton of α-arabinofuranose and the doublet signal at 4.60 ppm assigned for the anomeric proton of β-galactose sugar residues. The very downfield signal at 5.75 ppm having coupling constant ($J_{1,2}$ ~1.2 Hz) was assigned for α-rhamnose residue. The very small doublet at 1.32 ppm was assigned[24] for H-6 protons of the deoxy sugar residue, α-rhamnopyranose. The signals between 4.25 to 3.25 ppm were assigned to the oxymethyne protons of different sugar residues.

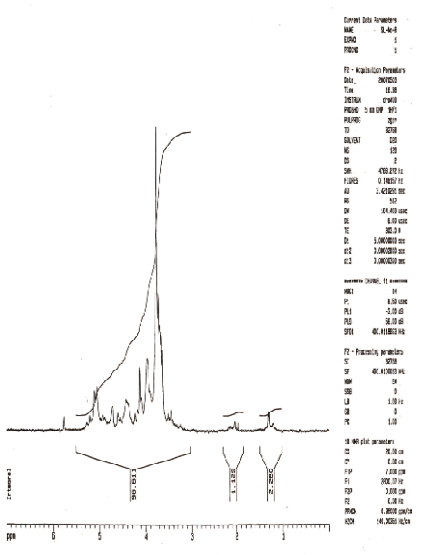


Fig. 3. ^{1}H-NMR spectrum of acidic SDF of cauliflower

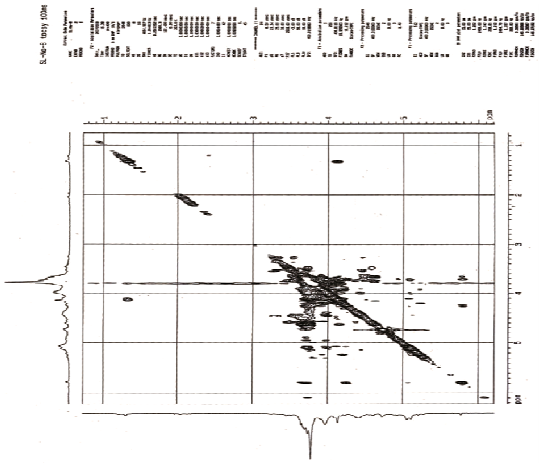


Fig. 4. H-H TOCSY spectrum of acidic SDF of cauliflower

H-H Total correlation spectral (TOCSY) studies of acidic SDF fraction

In the total correlation spectrum (Fig.2, Table.2) of acidic SDF, a downfield signal at 5.08 ppm was assigned for D-galacturonic acid (Glc*p*A residue). In the spectrum, two different types of uronic acid residues were present, which were assigned for galacturonic and glucuronic acid residues. In case of gal*p*A, anomeric proton H-1 at 5.08 ppm coupled with H-2 at 4.12 ppm. H-2 coupled with H-3 at 3.95 ppm and H-3 coupled with H-4 at 3.75 ppm. In addition H-4 of galacturonic acid had cross peak with anomeric proton H-1 of rhamnose at 5.75 ppm which indicated that the rhamnose residues were 1,4-linked with galacturonic acid. In ^{1}H-NMR spectrum, coupling constant ~6Hz indicated that rhamnose residue were β-linked *i.e.*rhamnose was 1,4-β-linked with galacturonic acid in main chain. In glucuronic acid, anomeric proton H-1 at 5.08 ppm coupled with H-2 at 3.75 ppm. H-2 at 3.75 ppm coupled with H-3 at 3.95 ppm.

Table. 1. TOCSY spectral data of neutral SDF fraction

β-Gal*p* residue (ppm)	α-Ara*f*residue (ppm)
H-1 (4.60) ⟷ H-2 (3.75) H-2 (3.75) ⟷ H-3 (3.65) H-3 (3.65) ⟷ H-4 (4.15) H-4 (4.15) ⟷ H-1 gal (4.60) H-3 (3.65) ⟷ H-1 ara (5.22)	H-1 (5.22) ⟷ H-2 (4.15) H-2 (4.15) ⟷ H-3 (3.75) H-3 (3.75) ⟷ H-4 (3.65) H-1 (5.22) ⟷ H-3 gal (3.65)
α-Rha residue (ppm)	
H-1 (5.06) ⟷ H-2 (4.15) H-1 (5.06) ⟷ H-3 gal (3.65)	

In addition H-1 of β-galacturonic acid at 5.08 ppm had cross peak with H-2 of rhamnose at 3.75 ppm which indicated that galacturonic acid residues were 1,2-β-linked with β-rhamnose. Besides the cross peak of H-3 of galacturonic acid at 3.95 ppm with anomeric proton H-1 of β-glucuronic acid at 4.45 ppm indicated that the β-glucuronic acid residues were attached to the β-galacturonic acid residues of main chain at the position 3 as a side chain.

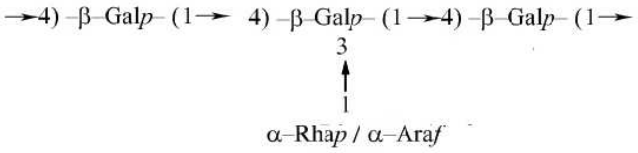
Table. 2. TOCSY spectral data of acidic SDF fraction

β-Gal*p*A residue (ppm)	β-Glc*p*A residue (ppm)	β-Gal*p* residue (ppm)
H-1 (5.08) ⟷ H-2 (4.12) H-2 (4.12) ⟷ H-3 (3.95) H-3 (3.95) ⟷ H-4 (3.75) H-4 (3.75) ⟷ H-1 rha*p* (5.75)	H-1 (5.08) ⟷ H-2 (3.75) H-2 (3.75) ⟷ H-3 (3.95) H-1 (5.08) ⟷ H-2 rha*p* (3.75) H-3 (3.95) ⟷ H-1 glc*p*A (4.45)	H-1 (4.60) ⟷ H-2 (3.45) H-2 (3.45) ⟷ H-3 (3.75) H-3 (3.75) ⟷ H-4 (3.95) H-1(4.60) ⟷ H-3 gal*p*A(3.95)
β-Ara*f* residue (ppm)	β-Rha*p* residue (ppm)	
H-1 (5.22) ⟷ H-2 (4.20) H-2 (4.20) ⟷ H-3 (4.12) H-1 (5.22) ⟷ H-2 gal*p*A (3.75)	H-1 (5.75) ⟷ H-2 (3.75) H-2 (3.75) ⟷ H-3 (3.95) H-3 (3.95) ⟷ H-4 (4.12) H-1 (5.75) ⟷ H-4 gal*p*A (3.75) H-2 (3.75) ⟷ H-1 gal*p*A (5.08)	

In β-rhamnose, anomeric proton H-1 at 5.75 ppm coupled with H-2 proton at 3.75 ppm of same residue. H-2 proton of rhamnose coupled with H-3 proton at 3.95 ppm. H-3 at 3.95 ppm coupled with H-4 at 4.12 ppm. In β-rhamnoseresidue, it had two cross peaks, H-1 of rhamnose at 5.75 ppm cross peak with H-4 of galacturonic acid at 3.75 ppm and H-2 of rhamnose at 3.75 ppm cross preak with anomeric proton H-1 of glucuronic acid at 5.08 ppm indicate that galacturonic acid and rhamnose was 1,2-β-linked. In case of galactose, anomeric proton H-1 at 4.60 ppm coupled H-2 proton at 3.45 ppm. H-2 at 3.45 ppm coupled with H-3 at 3.75 ppm. H-3 coupled with H-4 at 3.95 ppm. In addition, cross peak of anomeric proton H-1 of β-galactose at 4.60 ppm with H-3 of β-galacturonic acid at 3.95 ppm showed that the β-galactose residue was attached to the galacturonic acid residue of main chain at position 3. Again in arabinofuranose, the anomeric proton H-1 at 5.22 ppm coupled with, H-2 at 4.20 ppm. H-2 at 4.20 ppm also coupled with another proton H-3 at 4.12 ppm of same residue. Cross peak of anomeric proton H-1 of β-arabinose at 5.22 ppm with H-2 of galacturonic acid at 3.75 ppm indicated that β-arabinose residue also attached to the galacturonic acid residue of main chain at position 2. From ^{1}H and H-H TOCSY NMR spectroscopic studies and comparing the NMR data with SDF from other sources[16-20;24-25], it was concluded that the main chain of acidic SDF of cauliflower composed of 1,4-linked β-galacturonic acid and 1,2-linked β-rhamnose sugar residues, 1,4-β-galactruronic acid had side chain at it 3 position by α-rhamnopyranose and α-glucuronic acid as terminal residues. In macro molecule of polysaccharide a few number of α-arabinose might be attached to the galacturonic acid at position 3 in main chain. Partial structures of neutral and aciic SDF are given below.

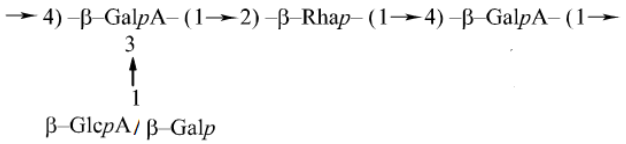
The neutral SDF of cauliflower was found to be a 1,4-β-galactan having side chain at position 3 by

rhamnose and arabinose residues (Partial structure 1).



Partial structure 1

The acidic SDF was found to be a rhamnogalacturonan having branching of galacturonic acid by galactose and glucuronic acid residues at position 3 galactose (Partial structure 2).



Partial structure 2

IV. Conclusion

Structural studies of isolated soluble dietary fiber of cauliflower showed that it is complex mixture neutral and acidic polymeric carbohydrate materials and these were present in the parecymatous tissues[26-27] of cauliflower.The SDF polymeric carbohydrates will be beneficial for human health.

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