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Structure of arabinogalactan oligosaccharides derived from arabinogalactan-protein of *Coffea arabica* instant coffee powder

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ABSTRACT

Arabinogalactan-protein, previously isolated from instant coffee powder of *Coffea arabica*, has been subjected to partial mild acidic and enzymatic hydrolyses. Separation of obtained mixtures by size exclusion and HPLC chromatographies afforded series of oligosaccharides, structure of which were studied by NMR spectroscopy. Mild acidic hydrolysis afforded oligosaccharides without any αAra*f* substituent while after enzymatic hydrolysis αAra*f* was found in di-, tri-, and tetrasaccharides. In all cases αAra*f* was a terminal substituent linked separately to O3, O6, and to both, O3 and O6, of βGal residues. Identification of di-, tri-, and tetrasaccharides containing α-Ara*f* enabled to distinguish in the ^{1}H NMR spectra αAra*f* signals linked to O6 and O3 of neighboring βGal unit. Composition of polymeric residues after enzymatic and mild acidic hydrolyses was also analyzed.

1. Introduction

Coffee belongs to the most widely used beverage in the world. Usually it is prepared by using beans of two commercially exploited species, *Coffea arabica* and *Coffea canephora* known as *robusta*, or their blends. *C. arabica*, originating from east Africa, thanks to its appreciated quality, is the most cultivated coffee species, representing over 70% of coffee cultivated worldwide. Wolfrom and Patin[1–4] put in evidence that green *C. arabica* beans are constituted by a high content (50%) of polysaccharides and their suggested primary structure was mannan, galactomannan, arabinogalactan, and cellulose. As far as arabinogalactan type II in coffee beans is concerned, its backbone is composed of β-1,3-linked galactosyl (Gal) residues frequently substituted at O6 by side chains formed by 1,6- and 1,3-linked βGal units and α-arabinosyl residues.[5] Up to now, several investigations have been aimed at polysaccharides from green as well as from roasted coffee, and many of them have been focused to confirm and further elucidate arabinogalactans (AG) chemical structure and to explore their solution properties and biological activity.[5–17] It is now well known that AGs from green coffee beans exist as an extremely heterogeneous mixture of arabinogalactan-proteins (AGPs) containing between 6% and 10% glucuronic acid and 0.4–1.9% protein.[11] This heterogeneity mostly resides in both, the degree of branching (Gal/Ara ratio) and a monosaccharide composition of side chains. Depending on the adopted isolation and fractionation (if any) different structural elements can be found[11,17] due to a widespread distribution of these polymers across the cell walls as well as supposed different structural forms of arabinogalactans in different cell wall regions.[17–19]

Preliminary characterization of AG constituents isolated from instant coffee powder by Wolfrom and Anderson[4] showed much lower Ara content in comparison with the AG isolated from green coffee beans and a difference in specific rotations of two materials. This difference was attributed not only to the lower content of Ara, but also to possible changes caused by industrial processing. It was found that roasting strongly affects coffee polysaccharides by inducing a degradation, depolymerization, and structural modifications. AGs are particularly susceptible to thermal degradation including a loss of arabinofuranose (Ara*f*) from side chains already after a light roast or galactan backbone depolymerisation.[12] The de-branching of the Ara*f* occurred more rapidly than hydrolysis of the galactan backbone.[19] The sensitivity to thermal degradation of AG from *C. arabica* has been shown to depend on coffee variety.[12] Industrial soluble coffee production includes many steps with high temperature processing (roasting, extraction) which promote polymers hydrolysis.

Little effort has been dedicated to investigate AGs structure from instant coffee powder (soluble coffee).[20] The pioneering work on AGP isolation procedure from instant coffee powder performed

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by Wolfrom and Anderson[4] has been re-discussed in one of our previous works.[21] We have found that general structural features of AGP already described by them were similar to those found for low molecular weights (M_w ~5400 and 5600) AGPs, isolated from commercial *arabica* instant coffee powder by two independent researcher groups. In our recent work, dealing with chemical and spectroscopic studies on these isolates, we showed a complex structure of these AGPs.[22] Obtained data confirmed a β-1,3-linked galactopyranosyl backbone branched at C-6 by side chains composed of single βGal and 1,6- and 1,3,6-linked βGal residues. Based on the proposed structure of the high molecular weight AG from green coffee[20] and obtained data, we could make following conclusions: (i) from the total amount of 3,6-linked βGal (23%) about 17% could have the origin in the backbone and over 6% in side chains, (ii) from 22% of terminal βGal units ~17% have their origin in side chains as single stubs and about 5% was located in short side chains formed by two or three 6- or 1,3,6-linked βGal, (iii) side chains always terminated by non substituted Gal residues, (iv) the presence of H1/C1 signals due to reducing α and βGal in NMR spectra confirmed a low molecular weight of the AGP polymer, (v) 11% of side chain Gal residues was without any substituent due to severe roasting conditions which caused the de-branching of Ara*f* substituents, (vi) no glucuronic acid or rhamnose residues were found, (vii) obtained data indicated that during drastic conditions of instant coffee industrial production not only Ara*f* linkages were broken, but also the backbone was split producing thus a mixture of AGP fragments, (viii) in general, in any AGP NMR structural analysis sets of H1/H2/C1 chemical shifts of βGal residues could be used as structural reporter groups.[22]

Instant coffee beverage, representing a complex mixture of molecules, is consumed by people as an everyday drink. In the view of beneficial effect of arabinogalactans on human health recognized recently and lack of structural information on AGP from soluble instant coffee, we found relevant to know its chemical structure. For this purpose partial mild acidic and enzymatic hydrolyses of instant coffee AGP isolated according the Wolfrom and Anderson procedure[4] was performed. The present study reports on results obtained during a detailed structural analysis of afforded arabinogalactan oligosaccharides in order to receive additional information about fine structure features of side AGP chains.

2. Material and methods

2.1. Isolation of coffee arabinogalactan-protein

The Wolfrom procedure,[4] with minor modifications,[22] has been used for isolation of arabinogalactan-protein (AGP) from freeze-dried instant coffee powder of *C. arabica* blend.

Shortly, instant coffee powder was dissolved in distilled water, cooled to 4 °C, and treated with formic acid overnight in a cool room. The insoluble portion was removed by centrifugation and the supernatant was precipitated into 96% ethanol (1:5). The precipitate was removed by centrifugation, dissolved in distilled water, and freeze-dried to yield a crude polysaccharide fraction A. It was further dissolved in 0.05 M sodium hydroxide solution and precipitated by saturated aqueous barium hydroxide solution. The brown precipitate was removed by centrifugation and the supernatant was treated dropwise with sulfuric acid (1 N) until a pH of 4 was obtained. The precipitate was removed by centrifugation (discarded) and the supernatant was poured into 96% ethanol (1:5). The precipitate was centrifuged, dissolved in distilled water, and applied on the column of Amberlite MB 150 (H+/OH) and eluted with distilled water. The water eluent was concentrated and freeze-dried to give the arabinogalactan-protein (AGP).

2.2. Partial acid hydrolysis (PH) of AGP

Samples of AGP from instant *Arabica* coffee (~1–2 mg) were partly hydrolyzed with 1 M TFA for 30, 40, 50, 60, 70, and 80 min at 100 °C and their degraded profiles were monitored by HPLC on an analytical HPLC column (300 × 7.8 mm) of MetaCarbo Oligo (a stationary phase: sulfonated polystyrene resin, Metachem, Torrance, USA) using de-ionized water as an elution system and differential refractometer as a detector. For preparative purposes, the AGP (500 mg) was partially hydrolyzed with 1 M TFA (50 mL) for 60 min at 100 °C. TFA was evaporated under reduced pressure and a hydrolyzed mixture was separated by size exclusion chromatography on a column of Bio-Gel P2. Individual fractions were further purified by combination of HPLC and preparative paper chromatographies (PPCH).

2.3. Partial enzymatic hydrolysis (EH) of AGP

AGP from instant *Arabica* coffee was dissolved in 50 mM acetate buffer (pH 5.0), exo-β-1,3-galactanase produced by fungi *Phanerochaete chrisosporium*[23] was added and the mixture was maintained at 35 °C for 24 h. Enzymes were inactivated by heating for 10 min and the hydrolyzed mixture was separated by size exclusion chromatography on a column of Bio-Gel P2. Individual fractions were further purified by combination of HPLC and preparative paper chromatographies (PPCH).

2.4. Separation of oligosaccharides

(i) Size exclusion chromatography—Mixtures of oligosaccharides after partial acid (PH) and enzymatic hydrolysis (EH) were separated on a column (200 × 2.5 cm) of Bio-Gel P2. Fractions of 5 mL were collected, analyzed for the carbohydrate content by phenol–sulfuric acid assay,[24] concentrated, and freeze dried. The PH mixture (Fig. 1) afforded nine fractions: a polymeric residue (1F), oligosaccharides (2–8F), and a fraction of monosaccharides (9F). The EH mixture (Fig. 2) afforded five fractions: a polymeric residue (1F), oligosaccharides (2–5F), and monosaccharides (6F).

(ii) HPLC chromatography—Low molecular EH mass fractions of dp 2–4 obtained after separation on Bio-Gel P2 were further purified on a TSK-GEL Amide-80 column (300 mm × 0.25 nm) using elution system acetonitrile–water (60–40%). In the TSK-GEL Amide-80 column (Tosoh Bioscience, Tokyo, Japan) the stationary phase consists of nonionic carbamoyl groups that are chemically bonded to the silica gel particles. Differential refractometer was used for detection.

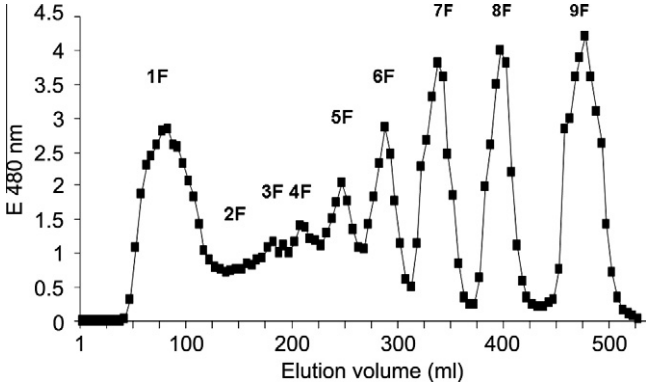

Figure 1. Bio-Gel P2 elution profile of AGP-derived oligomers formed during partial acidic hydrolysis of AGP. 9F—dp 1, 8F—dp 2, 7F—dp 3, 6F—dp 4, 5F—dp 5, 4F–2F—higher molecular mass oligosaccharides, 1F—polymeric residue.