A comparative study of arabinogalactan-protein isolates from instant coffee powder of *Coffea arabica* beans

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Summary

Parallel extraction of instant coffee powder prepared from roasted beans of *Coffea arabica* by industrial process according to the procedure of Wolfrom and Anderson afforded two arabinogalactan-protein isolates, i.e. AG1 and AG2 in yields of 14.6% and 14.4% of a crude polysaccharide complex A (w/w), respectively. However, their yields were about twice lower in comparison with AG isolation described by Wolfrom and Anderson forty years ago. Stronger roasting and extraction conditions during the industrial process used at that time for producing instant coffee powder used for AG isolation are supposed to be the main reason. This conclusion is supported by the fact that molecular masses of newly isolated arabinogalactans AG1 and AG2 were higher (5 200–5 400) then that of arabinogalactan isolated in the past (1 800 only). The presence of proteins in AG1 and AG2 suggests that these polysaccharides are present in the form of arabinogalactan-protein. Saccharide analysis of both polysaccharides showed similar composition: over 85% of galactose and 8% of arabinose residues, accompanied by smaller amounts of sugars as contaminants, which were not determined in previous isolation. Identical structure of both arabinogalactan-proteins was confirmed on the basis of ^{1}H and ^{13}C NMR spectra.

Keywords

Coffea arabica; roasted beans; instant coffee powder; arabinogalactan-protein; nuclear magnetic resonance; Fourier transform infrared spectroscopy

More than 40 years ago, Wolfrom and Patin [1–3] put in evidence that "green *Coffea arabica* beans have a high content (50–60%) of polysaccharides, the constituent sugars of which have been determined to be D-mannose (preponderant), L-arabinose, D-galactose and D-glucose". The same group performed, at that time, the first attempt to characterize the primary structure of the polysaccharides in view of the obtained monosaccharide content. In particular (galacto)-mannan, arabinogalactan and cellulose have been suggested as saccharide polymers of raw *C. arabica* beans. As far as arabinogalactan is concerned, a backbone of β-(1,3)-galactosyl residues with frequent arabinosyl and galactosyl side chains linked at the O-6 position of the galactosyl units in the main chain was proposed. At that time, Wolfrom and Anderson [4] isolated from instant coffee powder (described as commercial soluble coffee without any information both on raw material and processing) and preliminary characterized both mannan and arabinogalactan constituents. For the latter, the authors evidenced the much lower L-arabinose content in comparison with the arabinogalactan isolated from green coffee beans. They also evidenced a difference in the specific optical rotations of the two materials which was attributed, in addition to the lower arabinose content, to possible linkage changes induced by processing.

Up to now, several investigations have been aimed at studying the polysaccharides from green as well as from roasted coffee. Many of them have been focussed to confirm and to further elucidate the chemical structure, as well as to explore the solution properties and the biological activity of arabinogalactans [5–17].

It is now well known that arabinogalactans from green coffee beans exist as an extremely heterogeneous mixture of arabinogalactan-proteins (AGP) containing between 6% and 10% glucuronic acid and 0.4–1.9% protein [11]. This heterogeneity mostly resides in both degree of branching (Gal/Ara ratio) and monosaccharide composition of side chains. The result of this complexity is that different structural elements can be found depending on the adopted isolation and fractionation (if any) procedure [11, 17].

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This, in turn, is related to the widespread distribution of the polymers across the cell walls and to possible different structural forms characterizing the arabinogalactans present in the different cell walls regions [17–19].

Roasting strongly affects coffee polysaccharides, inducing degradation, depolymerization and structural modification [12]. Arabinogalactans are particularly susceptible to thermal degradation in comparison with the other *C. arabica* polysaccharides being depolymerized after a light roast both by fission of the galactan backbone and loss of arabinose from the side chains. OOSTERVELD et al. [19] reported that debranching of the arabinose side chains occurred more rapidly than hydrolysis of the galactan backbone. The sensitivity to thermal degradation of arabinogalactan from *C. arabica* has been shown to depend on coffee variety [12] in particular for three different varieties (namely Catimor CIFC, Yellow Caturra and Sarchimor). On the other hand, independently on variety about 50% of arabinose side chains were destroyed after light roasting and about 80% after dark roasting, The galactan part after light roasting was degraded as little as 6% for Catimor CIFC and more than 30% for Yellow Caturra. After dark roasting degree, these differences between varieties were much less apparent, since approximately 50% of the galactan part of the arabinogalactan were degraded in all three varieties. The protein moiety survives the roasting [12].

Very recently, BEKEDAM et al. [20] and BEKEDAM [21] investigated melanoidins (brown-coloured compounds) from high molecular weight roasted coffee brew fractions and they found that arabinogalactan-proteins were incorporated in negatively charged coffee brew melanoidins. In addition to the negative charge, the AGP-melanoidin complex has been found to be molecularly decorated with galactomannan fragments and chlorogenic acids. It has been suggested that both uronic acids in arabinogalactans and chlorogenic acids contribute to the negative charge on coffee melanoidins [21].

In spite of the relevant steps ahead performed up to now in the knowledge of both green and roasted coffee beans arabinogalactans, little effort has been dedicated to investigate arabinogalactans from instant coffee powder (soluble coffee) [22]. Industrial soluble coffee processing, in addition to roasting, includes a further thermal treatment, the extraction, which is normally performed at high temperature and is accompanied by hydrolysis [23]. This process produces both oligosaccharides and saccharide polymers, but the former only attracted the attention due to the need of classifying the huge range of coffee-based soluble products and of monitoring the presence of non-coffee bean components resorting to relatively simple and well-established analytical procedures [9]. It has been reported that the saccharide polymers are more abundant in extracts obtained from dark roasts whereas mono- and oligosaccharides are in greater amount in light and medium roast extracts [23]. Moreover, the final step of the soluble coffee processing, i.e. drying, can be performed by two different processes: spray drying and freeze drying. Of course, for the former, an additional thermal treatment is necessary and this may induce further degradation and alteration of the polysaccharides present in the instant coffee powder.

As far as we know, apart from the pioneering work by WOLFROM and ANDERSON [4] and some observations regarding the presence of arabinogalactan-derived polymer material in soluble coffee [9], no detailed structural characterization of arabinogalactan-protein from soluble coffee has been reported in the literature and the experimental data by WOLFROM and ANDERSON [4] have not yet been re-discussed.

In the present work, arabinogalactan-proteins have been isolated from freeze-dried instant coffee powder produced from dark roasted *C. arabica* according to WOLFROM and ANDERSON [4] and characterized by chemical and spectroscopic methods. In order to ascertain the reproducibility of the isolation procedure, two different samples were prepared in a sort of isolation ring test. The two samples were compared in terms of chemical composition and structural elements, and their chemical modifications in respect to the chemical structure of the native polymer proposed in the literature were determined.

MATERIAL AND METHODS

Plant material

Coffea arabica blend for espresso coffee (100%) was dark roasted (average roasting weight loss of 19% w/w) and industrially processed (grinding to particle size of 2.3–2.4 mm with 10% particles less than 1 mm; extraction at 150–180 °C; concentration under vacuum at 50 °C and freeze drying) to give a low yield of freeze-dried instant coffee powder.

General methods

Solutions of saccharides were concentrated under diminished pressure at a bath temperature of 40 °C. Polysaccharide samples were hydrolysed with 2 M trifluoroacetic acid at 120 °C during 1 h. The quantitative determination of neutral monosaccharides was carried out in the form of their trifluoroacetates by gas chromatography on a Hewlett-Packard 5890 Series II chromatograph (Hewlett-Packard, Palo Alto, California, USA) using a PAS-1701 column (0.32 mm × 25 m; Hewlett-Packard) using a temperature programme of 110–125 °C (2 °C·min^{-1}) –

165 °C (20 °C·min^{-1}) and a flow rate of hydrogen of 20 ml·min^{-1} [24]. The uronic acid content was determined with the 3-hydroxybiphenyl reagent [25]. Elemental analysis was performed with EA 1108 apparatus (Fisons Instruments, East Grinstead, United Kingdom). Contents of proteins were calculated from nitrogen contents (%N × 6.25). Optical rotations were measured with an automatic polarimeter Model 241 (Perkin-Elmer, Waltham, Massachusetts, USA) in 0.5% aqueous solutions at 20 °C.

Molecular size determination of polysaccharides was performed with Shimadzu apparatus (Shimadzu, Kyoto, Japan) using a tandem of two columns HEMA-BIO 100 followed by HEMA-BIO 40 column (Tessek, Prague, Czech Republic) of dimensions of 8 mm × 250 mm. As a mobile phase, 0.02 M phosphate buffer (pH 7.2) containing 0.1 M NaCl was used at a flow rate of 0.8 ml·min^{-1}. A set of pullulan standards was used for calibration of the column (Gearing Scientific, Baldock, United Kingdom). Fourier transform infrared spectra of samples were recorded with a Magna 750 spectrometer (Nicolet, Madison, Wisconsin, USA) with a DTGS (deuterated triglycine sulfate) detector (Nicolet) and OMNIC 3.2 software (Nicolet). The polysaccharides were measured in the form of KBr pellets with a sample/KBr ratio of 1 mg : 200 mg.

The ^{13}C NMR spectra of polysaccharides were recorded at 25 °C in D_2O on a Bruker Avance DPX 300 spectrometer (Bruker, Rheinstetten, Germany) operating at 300 MHz for ^{1}H, and 75.46 MHz for ^{13}C. The ^{1}H NMR spectra were measured on Varian 600 MHz Unity Inova 600 NB spectrometer (Varian, Darmstadt, Germany) in 5 mm 1H{13C, 15N}PFG Triple Res IDTG600-5 probe. Acetone was used as the internal standard (δ 2.225 for ^{1}H and 31.07 for ^{13}C).

Isolation of arabinogalactan-proteins from freeze-dried instant coffee powder

Step 1: The polysaccharide was isolated by the procedure of Wolfrom and Anderson [4] with minor modifications. Briefly, the coffee powder (75.0 g) was dissolved in Milli-Q (Millipore, Billerica, Massachusetts, USA) water (750 ml), cooled to 4 °C and formic acid (40 ml of an 85% solution; Carlo Erba, Milano, Italy) was added. This mixture was centrifuged at 4 230 *g*, during 15 min at room temperature. The supernatant was treated with 5 volumes of methanol (Fluka, Seelze, Germany). The light brown precipitate was removed by centrifugation (4 230 *g*, 5 min) and the supernatant was discarded. The sediment was washed with methanol and ethyl ether (Fluka) to yield a crude polysaccharide fraction A.

Step 2: The crude polysaccharide fraction A (16.5 g) was dissolved in 0.05 M sodium hydroxide solution (Aldrich, Steinheim, Germany; 170 ml) and precipitated by saturated aqueous barium hydroxide solution (Aldrich; 220 ml). The brown precipitate formed was removed by centrifugation (4 230 *g*, 5 min, room temperature) and discarded. The supernatant was treated dropwise with 1.0 N sulfuric acid until pH of 4 was achieved. The precipitate was removed by centrifugation (4 230 *g*, 5 min, room temperature) and discarded. The supernatant was poured into five volumes of absolute ethanol (Fluka) to give a crude arabinogalactan. The material was dissolved in Milli-Q water (500 ml) and passed through a column (25 mm × 300 mm) containing 41 g of Amberlite MB 150 (H^+, OH^-; Supelco, Bellafonte, Pennsylvania, USA). The effluent was then freeze-dried to give the arabinogalactan (AG1).

In order to verify the reproducibility of step 2, the crude polysaccharide fraction A (25 g) was solubilized in 0.05 M sodium hydroxide solution (250 ml) and precipitated by saturated aqueous barium hydroxide solution (325 ml). A mixture was incubated in the refrigerator overnight and then centrifuged (2 351 *g*, 10 min) to give sediment (a crude galactomannan) and supernatant. The supernatant was further slightly acidified to pH 4 and the precipitate formed was centrifuged and discarded. The supernantant was poured into 96% ethanol (1 : 5), the precipitate was removed by centrifugation, dissolved in water and applied to the column of Dowex resins (H^+ and OH^-; Serva, Heidelberg, Germany), and eluted with water. The eluted solution containing saccharides was concentrated and freeze-dried to give the arabinogalactan (AG2) as a grey-white material. Both arabinogalactans, i.e. AG1 (Trieste, Italy) and AG2 (Bratislava, Slovakia), isolated in different laboratories, were subjected to structural characterization by chemical and spectroscopic methods.

RESULTS AND DISCUSSION

Coffea arabica blend was industrially processed, i.e. roasted, ground, extracted, concentrated and freeze-dried to give an instant coffee powder. Water extraction of coffee powder followed by methanol precipitation and freeze-drying yielded a crude polysaccharide complex A in 22.0 % yield (16.5 g) of starting material (w/w). In both procedures, the polysaccharide complex A was solubilized in sodium hydroxide solution and its dominant polysaccharide, a galactomannan, was removed by barium hydroxide precipitation. The supernatant was slightly acidified and the formed precipitate of non-saccharide

origin was removed by centrifugation. A crude arabinogalactan was recovered by ethanol precipitation, followed by freeze-drying. First isolation afforded a brown-coloured product in a yield of 5.2% yield (3.9 g) of starting coffee powder (w/w). It was futher dissolved in water and purified on a column of Amberlite or Dowex resins (H^+/ OH^-) to give the arabinogalactan (AG1) as a nearly colourless fluffy amorphous solid in 3.2% (2.4 g) yield of coffee powder or 14.6% of crude polysaccharide complex A.

The repeated procedure gave a grey-white arabinogalactan (AG2) in 14.4% yield of crude polysaccharide complex A (w/w). In both polymers, a low content of nitrogen was estimated, thus suggesting the presence of proteins (Tab. 1) and indicating that the polymer was present in coffee beans in the form of arabinogalactan-protein complex (AGP). The above mentioned facts shows that both independent extractions gave very similar yields and indicated thus a high degree of reproducibility of this arabinogalactan isolation procedure. On the other hand, WOLFROM and ANDERSON [4] isolated AG from instant coffee powder in 27% yield of crude polysaccharide complex A (w/w). This yield was about 50% higher in comparison with the presented results. There could be several reasons for this discrepancy: different coffee bean species (it was not specified by WOLFROM and ANDERSON if pure *Coffea arabica*, pure *Coffea canephora*, known as robusta, or their blend were used to produce the instant coffee powder) or geographic region of cultivated coffee plants. As a matter of fact, it was shown by FISHER et al. [8] that green robusta beans contained amounts of a highly soluble arabinogalactan which possessed more branch points and more extended side chains than arabinogalactan found in *Coffea arabica* var. caturra and this has been indicated as one of the reasons to explain that the arabinogalactans of robusta were more easily solubilized than those of *Coffea arabica*. Moreover, NUNES and COIMBRA [13] reported on differences in the arabinogalactan content of polysaccharide fractions isolated from coffee infusions from *Coffea arabica* of two geographical origins.

Tab. 1. Characterization of arabinogalactan-proteins AG1 and AG2 from *C. arabica.*

	AG1	AG2
Monosaccharide composition [% w]		
Arabinose	8.2	8.3
Xylose	traces	traces
Mannose	2.7	2.9
Galactose	85.0	85.2
Glucose	1.4	1.2
Heptose	2.7	2.4
N [% w]	0.25	0.27
Inorganic salts [% w]	1.7	2.2
Molecular mass (M_w)	5,400	5,200
$[\alpha]_D$ (c 0.5, water)	+ 14°	+ 10°

Industrial processes. i.e. roasting, grinding, extraction and drying, which were used for preparation of instant coffee powder forty years ago, highly probably respresented drastic conditions used to achieve very high commercial yields and in the same time to maximize the polysaccharide depolymerization [26] for a better performance of spray-drying. Freeze-drying, the process used to produce the instant coffee powder in the present work, is a recently introduced technology in the soluble coffee industry, which offers the advantage of avoiding the additional thermal treatment necessary for spray-drying reflected by an increase in the product quality. It has also to be stressed that, according to OOSTERVELD et al. [14], the water extraction of roasted *Coffea arabica* at 170 °C yielded a larger quantity of arabinogalactan than compared to extraction carried out at 90 °C, in spite of the fact that the total yield and the polysaccharide contents of the extracts at 170 °C were comparable to those obtained at 90 °C.

This hypothesis may be supported by the fact that HPLC analyses of AG1 and AG2 showed similar molecular mass distribution patterns for both polymers. Their average M_w was found to be in the range of 5200–5400, whereas the osmometrically determined molecular size of AG isolated by WOLFROM and ANDERSON [4] was ~1800. This fact further suggests that processing used in the industrial production of instant coffee powder in the case of AG isolation was stronger than for AG1 and AG2.

Saccharide compositional analysis of investigated AG1 and AG2 did not reveal any significant difference in the monosaccharide composition of AG1 and AG2, since in both samples galactose (> 85.0%) and arabinose (> 8.0%) dominated, accompanied with smaller amounts of mannose and glucose, and traces of xylose (Tab. 1). The galactose/arabinose ratio was approx. 9 : 1. In AG, presence of two saccharides was reported only, i.e. galactose (94%) and arabinose (6%), with the galactose/arabinose ratio of approx. 16 : 1. The lower content of arabinose residues in AG in comparison with AG1 and AG2 may reflect the different processing conditions adopted for soluble coffee production. In particular, the preparation of instant coffee powder under stronger processing conditions is favourable to cleave the arabinofuranosyl residues more efficiently. This finding is in full agreement with yield and molecular weight data discussed above when comparing the polymers of the present investigation with those of a previous one [4].

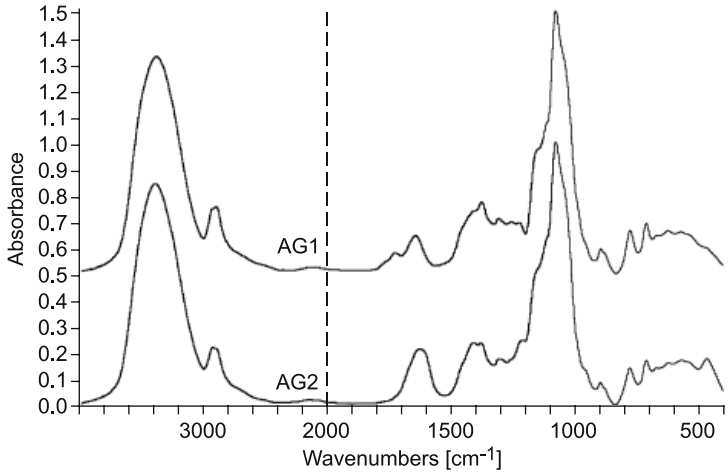

Fig. 1. FT-IR spectra of arabinogalactan-proteins AG1 (Trieste) and AG2 (Bratislava) from *C. arabica*.

Both glycoconjugates, i.e. AG1 and AG2, showed low values of positive specific optical rotations of +14° and +10°, respectively. These values are comparable with the optical rotation of AG (+9.5°) determined by WOLFROM and ANDERSON [4].

The Fourier transform infrared spectroscopy (FT-IR) spectra of AG1 and AG2 (Fig. 1) showed the presence of strong bands at 1 076 cm^{-1}, which are characteristic for a galactopyranose backbone in arabinogalactan Type II. Besides that, in the anomeric

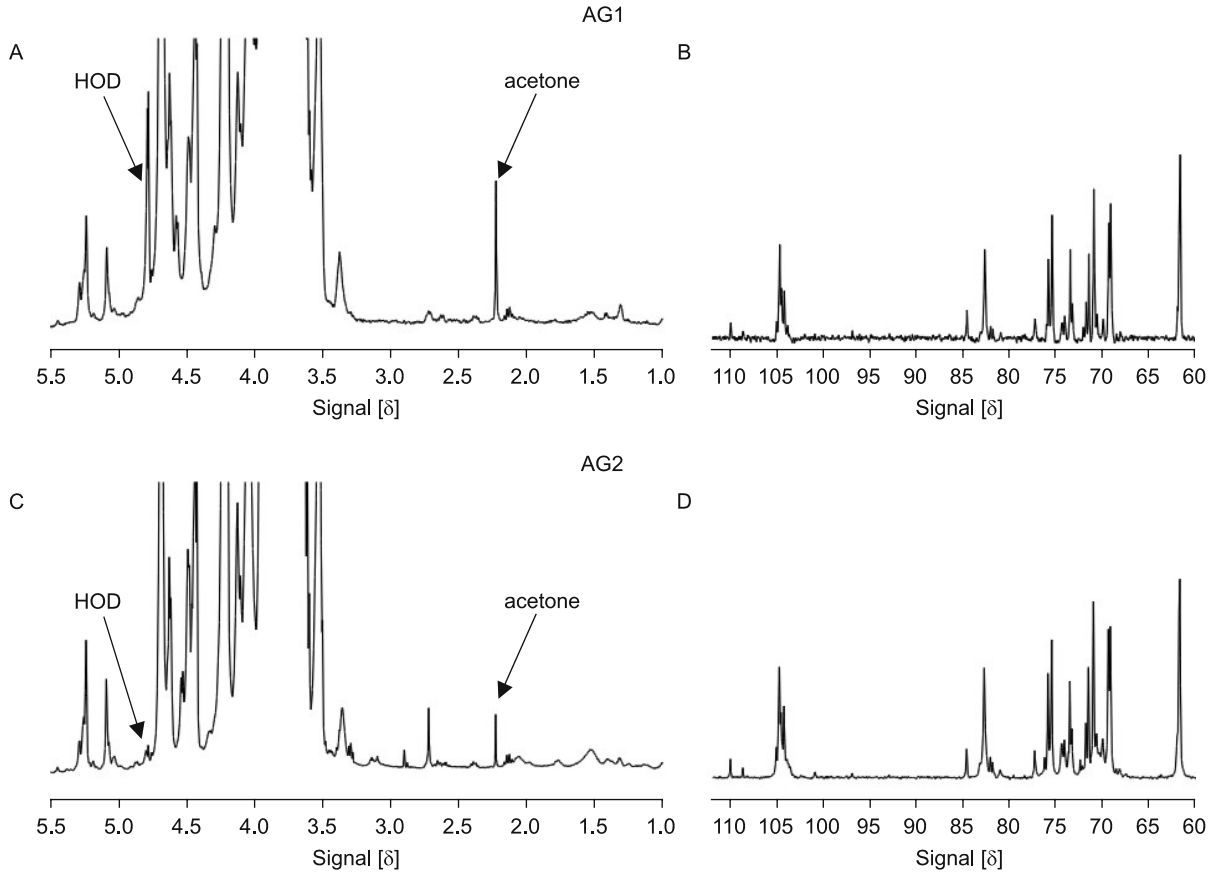

Fig. 2. NMR spectra of arabinogalactan-proteins AG1 and AG2 from *C. arabica.*
A – ^{1}H NMR spectrum of AG1, B – ^{13}C NMR spectrum of AG1; C – ^{1}H NMR spectrum of AG2, D – ^{13}C NMR spectrum of AG2. AG1 – arabinogalactan-protein isolated in Trieste, AG2 – arabinogalactan-protein isolated in Bratislava; HOD – residual signal of water; acetone was used as an internal standard.

region, absorption bands at 894 cm^{-1} (AG1) and 896 cm^{-1} (AG2) were observed , which are characteristic for β-linked galactose residues. Bands at 1 045 cm^{-1} that are characteristic for arabinose were not very well distinguished, which was probably due to low contents of these saccharide residues in both polysaccharides [27]. As can be seen from the Fig. 1, the FT-IR spectra of both polysaccharides showed similar profiles of the saccharide parts, some differences were observed due to a different content of inorganic material only.

For both arabinogalactan-proteins, AG1 and AG2, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured (Fig. 2). Anomeric H1 signals present in AG1 and AG2 ^{1}H NMR spectra were attributed, according literature data, to the following: signal at δ 5.24 to terminal arabinofuranose [17], β-(1,3,6)-linked galactose at δ 4.50, β-(1,6)-linked galactose at δ 4.43 and β-(1,3)-linked galactose at δ 4.69. Comparison of ^{1}H NMR spectra of both polysaccharides showed a high degree of similarity with only a small difference in the H1 signal intensity at δ 4.69 due to non-substituted internal β-(1,3)-linked galactose units indicating their lower content in AG2. Different types of H^{+} and OH^{-} columns used for separation might have been a reason for this observation.

Signals characteristic for proteins were present in the ^{1}H NMR spectra of both AG1 and AG2 samples (Fig. 2). These were observed in regions δ 3.0–0.5 (important intensity) and some signals could also be identified in the region characteristic of aromatic signals (8–6 δ). In addition, signals due to quinic part of the molecule of chlorogenic acid were not detected, which indicated absence of this compound in the samples. Signal at δ 4.57 in AG1 might suggest the presence of GlcA, however, we were not able to prove its presence by an HMBC experiment due to a very low intensity of this signal. In addition, this signal was absent in AG2. Thus the presence of GlcA was not proved by NMR.

In ^{13}C NMR spectra, chemical shifts of signals due to galactose and arabinose were also in accordance with literature data [17]. High degree of similarity was confirmed also by ^{13}C NMR spectra in which C1 signals due to arabinose units were present at δ 110.0 and 108.7, while those of galactose units appeared between δ 105.2–103.9, C3 involved in a 1,3-glycosidic linkage at δ 83.3. Both ^{1}H and ^{13}C NMR spectra of AG1 and AG2 confirmed the identical structure of both polymers.

CONCLUSIONS

The preliminary results on arabinogalactan-proteins isolated by Wolfrom and Anderson procedure from instant coffee powder prepared from *Coffea arabica* showed that this procedure affords reproducible results from the point of view of yield, distribution of molecular sizes and monosaccharide composition. Only slight differences were detected by ^{1}H NMR in the contents of internal non-substituted 1,3-linked β-Gal residues and in FT-IR spectra. However, in comparison with AG isolation described forty years ago, both new arabinogalactan-protein isolates, i.e. AG1 and AG2, were obtained in lower yields, higher molecular weight and with a higher content of arabinose. Different roasting, extraction and overall processing conditions used to industrially produce the instant coffee powder (from *C. arabica* beans in the present work but from unknown raw material in the work of Wolfrom and Anderson) might be the principal reason for such discrepancies. The present investigation is a stimulus for further studies aimed at elucidating primary structure and functional properties of this class of coffee components.

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