Coffee carbohydrates

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This review summarises recent advances in the chemistry, physiology and molecular properties of coffee carbohydrates with a particular focus on the cell wall polysaccharides. The results of detailed chemical studies have demonstrated novel structural features of both the galactomannans and the arabinogalactan polysaccharides of the green and roasted coffee bean. For the first time immunological probes based on monoclonal antibodies for specific polysaccharide epitopes were used to reveal the patterns of distribution of the galactomannans, arabinogalactans and pectic polysaccharides in the coffee bean cell wall. Finally, the results of physiological and molecular studies are presented which emphasise the growing awareness of the potential role the metabolic status of the green bean may play in final coffee beverage quality.
Key words: *Coffea*, cell wall, beverage quality, seed.

Carboidratos do café: Este artigo sumariza os mais recentes desenvolvimentos nas áreas de química, fisiologia e propriedades moleculares dos carboidratos do café, com um particular interesse nos polisacarídeos presentes nas paredes celulares. Os resultados dos estudos químicos detalhados demonstraram novas características estruturais tanto nos galactomananos como nos polissacarídeos dos arabinogalactanos do grão de café verde ou torrado. Pela primeira vez, estudos imunológicos baseados em anticorpos monoclonais foram usados para revelar a distribuição dos galactomananos, dos arabinogalactanos e dos polissacarídeos pectídicos na parede celular do grão de café. Finalmente, os resultados dos estudos fisiológicos e moleculares são apresentados de maneira a sublinhar a influência do status metabólico do grão verde do café na qualidade final da bebida.
Palavras-chave: *Coffea*, parede celular, qualidade da bebida, semente.

INTRODUCTION

The importance of carbohydrates in coffee can be attributed to not only their high concentration in the bean but also to the complex changes they undergo during the roasting process which contribute to the organoleptic appeal of the coffee beverage. In this review we will focus on the coffee carbohydrate literature of the last 5 years. Before this time, an excellent summary of coffee carbohydrates was reported in the volume, "Coffee, Recent developments" (Bradbury, 2001). It included a detailed report of the low molecular weight sugars in green and roasted coffee beans of many varieties of Arabica and Robusta, which will not be repeated in the present review. In the last few years interest has grown in the physiology and biochemistry of green coffee bean development and the role this could play in coffee quality. This includes work to understand sugar metabolism in coffee in relation to sink-source relationships during bean development. However, to date there is very little published literature relating to the carbohydrate physiology of coffee. What there is will be covered in the present review but of necessity the major focus will be the polysaccharides of the coffee bean cell wall, where significant advances in understanding their chemistry, biochemistry and distribution within the endosperm have been made in the last 5 years.

Carbohydrate status of developing grains

Free sugars: The driver for coffee research has been to consider the various components of the coffee grains as potential precursors of coffee beverage flavour and aroma. Whether focusing on the low molecular weight sugars or the polysaccharides, the great majority of such studies have been done on the mature grains of Arabica and Robusta coffee. There is a lack of information on the evolution of

the carbohydrate profile of the grain during its growth and development and the postharvest stages of bean processing. The metabolic status of the green bean at these stages will affect the final chemical composition of the mature green bean and the influences which modulate this metabolic status are factors which impinge on coffee bean quality.

Rogers et al., (1999) conducted a study of the changes to the content of sugars, and sugar alcohols in developing grains from varieties of Robusta and Arabica. The grains were harvested between 12–30 weeks after flowering (WAF) for Arabica and 18-40 WAF for Robusta. In the early stages of development, up to the halfway stage of maturation, glucose and fructose were the major free sugars with glucose consistently twice the concentration of fructose. Glucose levels were higher in the Arabica varieties (between 8-12 % dry weight) than in Robusta (2-4 % dry weight). At the end of grain development concentrations of glucose and fructose had decreased for both species to 0.03 and 0.04 % dry weight respectively, while sucrose at 5-12 % of the dry weight was essentially 100 % of the total free sugars in mature grains.

A more detailed analysis in one variety of Arabica was done by separating the perisperm tissues from the endosperm and analysing the free sugar concentration in each. The higher concentrations of glucose and fructose compared to sucrose in the early stages of development were always associated with perisperm tissue. In the endosperm even at the earliest stages of maturation, sucrose was the dominant sugar.

The authors speculated that the catabolism of sucrose in the perisperm would be consistent with the requirement by the tissue for an increase in osmotic pressure to enable both the initial expansion within the locular space and a sink function.

Results of the analysis of sugar alcohols (mannitol) and oligosaccharides (raffinose, stachyose) did not show any discernible trend that would indicate fundamental changes to the metabolism of these compounds during grain development.

Another recent study (Geromel et al., 2004) investigated the biochemical and molecular characterisation of sucrose synthase (Susy EC2.4.1.13) and invertase during coffee bean development. The objective of this work was to understand sugar metabolism in coffee in relation to sink-source relationships during bean development. To this end they measured the sugar concentrations and activities of the two enzymes in the pulp, perisperm and endosperm from *Coffea arabica* at stages of bean development. Susy was more active than invertase functioning more towards sucrose degradation than synthesis. This was supported by the fact that no expression of invertase-encoding genes was observed in any of the tissues tested. The Susy activity peaked in the final stages of perisperm development suggesting that the tissue played a role in controlling bean size and in the build up of sugars in the green bean. At the molecular level 2 cDNAs were cloned encoding Susy isoforms which showed differences in their spatial and temporal expression in coffee fruits.

The importance of seed metabolism during postharvest processing methods as it relates to coffee quality has been emphasised by Mazzafera and Purcino (2004). They drew attention to the postharvest physiology of the coffee bean and described changes to several groups of metabolites, including the carbohydrates, which are likely to be influenced by the nature of the postharvest processing methods. Just to what extent the final beverage quality is influenced by the metabolic status of the bean at harvest has yet to be determined but it remains an area which needs more attention from coffee researchers.

Polysaccharides: The polysaccharides which make up ~50 % of the green bean's dry weight, consist of three major types: mannans or galactomannans, arabinogalactan-proteins (AGPs) and cellulose. In addition, there are small amounts of pectic polysaccharides (Redgwell et al., 2002a) and recently xyloglucan was also shown to be present (Oosterveld et al., 2003).

Galactomannans: If there is little information on the biosynthesis of free sugars during coffee bean development there is even less on the biosynthesis of the cell wall polysaccharides. Nevertheless, with the advent of molecular techniques there is now heightened interest in the mode of biosynthesis of coffee cell wall polymers. This is particularly pertinent in relation to the galactomannans, the solubilisation of which is a critical factor in determining the yield of soluble coffee powder during commercial extraction (Clifford, 1985). Cellulose apart, the most resistant polymers to solubilisation are the galactomannans. One of the principal determinants of galactomannan solubility is the frequency of substitution of the mannan backbone with galactose residues. In theory an increase in the degree of galactosylation of the mannans may increase the degree of solubilisation of the galactomannans.

In order to manipulate the final structure of the galactomannans it is necessary to understand the metabolic steps involved in their synthesis. In particular, what are the

crucial steps which dictate the final degree of galactosylation? It is known that in some plants, the final degree of galactosylation is determined by the action of an α-galactosidase which cleaves galactosyl residues from the primary synthetic product. If such a mechanism operated in the coffee bean, then down regulation of the α-galactosidase gene could result in coffee beans with a higher Gal/Man ratio. Fischer et al. (1999) determined the monosaccharide composition of coffee bean cell walls 12, 17 and 29 WAF and reported that early in development the galactomannans were more highly substituted than at maturity. In a more detailed study Redgwell et al. (2003), isolated and characterised galactomannans from the endosperm of coffee beans 11, 15, 21, 26, 31 and 37 WAF. At the earliest stage of development the galactomannans accounted for ~10 % of the polysaccharides but were highly substituted, with Gal/Man ratios between 1:2 and 1:7. At maturity the galactomannan became the predominant polysaccharide accounting for ~50 % of the total endosperm polysaccharides but their degree of substitution decreased with Gal/Man ratios between 1:7 and 1:40. The decrease in the Gal/Man ratio of the galactomannans commenced between 21 and 26 WAF and was in synchrony with a rise in free galactose. It was concluded that the final Gal/Man ratio was to an extent the result of galactose removal from the primary synthetic product by an α-galactosidase.

Other polysaccharides: Of the other polysaccharides in the coffee bean cell wall little has been reported on the subject of developmental changes to their structural features. In the same study which reported developmental changes to the galactomannans (Redgwell et al., 2003) data was published for the monosaccharide composition of the cell wall material at different stages of development (table 1). Making the reasonable assumption that the arabinose and galactose are derived mostly from the arabinogalactans and the rhamnose and galacturonic acid are structural components of the pectic polysaccharides, some discernible trends can be deduced for the structural features of these polymers during grain development. The Gal/Ara ratio of the arabinogalactans in the earliest stage of growth was 1.3:1 but this gradually increased during grain growth and reached 2.6:1 at maturity. In addition, at the earliest growth stage the arabinogalactan accounted for ~50 % of the total polysaccharides but this decreased to 34 % in the mature grain.

In the case of the pectic polysaccharides the endosperm of the youngest growth stage contained ~20 % of its weight as pectic polymers, which dropped dramatically in the mature grain (~4 %). This is to be expected, as the earliest formed layer during the period of rapid growth and cell division is the cell plate, which is rich in pectic polymers.

In summary during growth and development of the coffee bean cell wall there is a progressive change in both the relative content of the different polysaccharide types and their structural features. At the earliest stages of growth, cellulose and arabinogalactan appear to be the primary products of cell wall synthesis with the former the predominant polysaccharide (Fischer et al., 1999). During the middle stages of growth, cellulose synthesis appears to cease and there is a progressive increase in mannan synthesis relative to the other wall polysaccharides as the grain approaches maturity. The close stereochemistry of cellulose and mannan prompts the speculation that perhaps the same catalytic membranes, which lead to cellulose synthesis, are also involved in mannan synthesis later in the growth of the endosperm with the additional intervention of an enzyme capable of interconverting GDP-mannose and GDP-glucose such as GDP-mannose-2-epimerase.

Table 1. Monosaccharide composition of alcohol-insoluble residue from coffee bean endosperm at several stages of development

WAF	Monosaccharide composition (mole %)							
	Rha	Ara	Xyl	Man	Gal	Glc	GlcA	GalA
11	3.7	18.9	1.9	9.3	23.9	21.7	6.6	14.0
15	3.7	17.5	3.0	13.4	25.7	20.8	4.7	11.0
21	1.7	14.7	0.5	19.6	33.1	21.7	3.3	5.3
26	0.5	10.1	0.4	37.0	27.7	18.2	2.4	3.6
31	0.5	8.9	1.1	45.4	23.0	15.9	2.1	3.2
37	0.6	9.1	1.0	45.6	23.2	15.7	1.9	3.1

(Redgwell et al., 2003)

Molecular and biochemical characterisation of polysaccharide modifying enzymes

Once the function and expression of the enzymes responsible for the biosynthesis and breakdown of coffee polysaccharides is understood, targeted manipulation of the morphological properties of the bean becomes a realistic proposition. To date there has been little published data on the biochemical or molecular characterisation of the endogenous enzymes governing polysaccharide metabolism in the coffee bean. Those that have been targeted include endo-β-mannanase and α-galactosidase, two enzymes which mediate changes to the galactomannans. Biochemical studies involving endogenous enzymes of coffee, which specifically promote metabolic changes to the arabinogalactans, have not been reported.

The galactomannans play a dominant role in the physicochemical properties of the coffee grain, a major factor influencing industrial extractability. The two major enzymes concerned with modification of the galactomannans are α-galactosidase and β–(1→4) endo-mannanase. Both have been fairly well characterised in relation to their biochemical and molecular properties. Zhu and Goldstein (1994) reported the cloning and functional expression of a cDNA encoding coffee bean α-galactosidase and demonstrated that the enzyme had a preference for α-1,3- and 1,4-glycosidic linkages. If, as the results of Redgwell et al. (2003) suggested, α-galactosidase is involved in the determination of the final galactose content of coffee endosperm galactomannans, then potentially, coffee plants transformed by down regulation of the α-galactosidase gene could contain galactomannans with a higher degree of substitution than the wild type. The opposite effect has already been demonstrated by Joersbo et al. (2001), who cloned and transformed the α-galactosidase gene expressed in immature senna seeds into a species of guar (*Cyamopsis tetragonoloba*). Approximately 30 % of the guar transformants produced endosperm with galactomannans where the galactose content was significantly reduced.

Until recently reports on endo-β-mannanase activity in coffee grains were limited to two studies. Dirk et al., (1995) reported multiple isozymes of the enzyme in dry and imbibed seeds while Giorgini and Comoli (1996) measured the effect of growth regulators on the activity of the enzyme during germination. The first molecular characterisation was reported by Marraccini et al. (2001) who cloned and sequenced two endo-β-mannanase cDNAs (man A and man B) from germinating coffee grains (*Coffea arabica L.).* Northern hybridizations with man A- and man B-specific probes showed that mRNA transcripts for both cDNAs were present during the same periods of bean germination, with expression peaking 20 days after imbibition of water. Activity and mRNA levels appeared to be tightly coordinated and unlike the reports of Dirk et al. (1995) they stated that enzyme activity did not exist in grains prior to germination. Thus, transcripts of the enzyme were not detected during grain maturation or in other tissues of the plant (roots, leaves, stems, flowers). The enzyme showed no activity with mannotriose or mannobiose and required oligomers with at least 5 or more units for maximum efficiency.

Despite the obvious potential to manipulate the physicochemical properties of coffee beans by using transformation technology, which can change the polysaccharide structure of the grain cell wall, it is unlikely that the approach will yield any significant benefits to the coffee industry in the short to medium term. Primarily this is because the relationship between genetically induced changes and the biochemistry, physiology and quality traits of the coffee bean is far from being elucidated. In addition, since the FLAV SAVR tomato became the first genetically engineered whole fruit to become commercially available, the application of biotechnology in agriculture has been intensively discussed, and consumer polls, particularly in Europe, have shown a general ambivalence and some hostility in attitudes (Schibeci et al., 1997).

Chemistry of the polysaccharides

Advances in our knowledge of the chemistry of coffee bean polysaccharides during the last 5 years has focused primarily on elaborating the detailed structural features of the arabinogalactans and the (galacto)-mannans. Some additional information has also been revealed on the structural features of the pectic and hemicellulosic polysaccharides. The availability of a range of immunological probes, which are specific for certain epitopes of the polysaccharides has permitted localisation studies to reveal additional information on the architecture of the coffee bean cell wall. To date there has been no published work on the characteristics of coffee cellulose.

Arabinogalactans: Several structural studies have revealed that the type II arabinogalactans in the coffee bean consist for the most part of a backbone of β –(1→3) - linked galactosyl residues, substituted at intervals in the 0-6 position with various combinations of arabinosyl and galactosyl residues (Bradbury, 2001). Redgwell et al., (2002a) reported two important additional pieces of structural information. Firstly,

that the polysaccharides carried a negative charge due to the presence of up to 10 % of their structure as glucuronosyl residues which occurred as non-reducing terminal units on a 1→6 linked galactosyl side chain. Secondly, all or some of the arabinogalactans are in fact arabinogalactan-proteins (AGPs). The existence of a covalent link between the arabinogalactan moiety and protein was premised on the continued association of carbohydrate and protein during purification, the positive reaction to the β-glucosyl-Yariv reagent and the amino acid composition of the protein moiety which was hydroxy-proline rich, a characteristic of many reported AGPs (Clarke et al., 1979). The protein content of three different AGP fractions isolated from Arabica Yellow Caturra was 0.4, 1.1 and 1.9 %. The AGPs were shown to exist in several varieties of both Arabica and Robusta coffee beans.

Previous studies characterised coffee arabinogalactans, which represented only fractions of the total cell wall polymer. This can be attributed to the fact that the arabinogalactans are not readily extractable from green beans despite the fact that they and AGPs in general are extremely water-soluble. Bradbury and Halliday (1990) used 20 % NaOH at 100°C to extract arabinogalactan from green beans and reported that 45 % remained in the insoluble fraction. Fischer et al. (2001a,b) used a sequence of extractants, which included 8M KOH and $NaClO_2$ with similar results. Oosterveld et al., (2003) used water, EDTA and 4 M NaOH and released less than 10 % of the polysaccharides. Bradbury (2001) speculated that the insolubility of coffee arabinogalactan was evidence that it was covalently linked to a less soluble component of the cell wall (e.g. cellulose or mannan). However, a second possibility is that the compact structure of the coffee cell wall, which is made up mostly of the insoluble polymers cellulose and mannan, entraps much of the AGP within its structure, rendering it effectively insoluble. Evidence to support this idea was provided by Redgwell et al. (2002a) who were able to solubilise almost all the AGPs in green beans by treating the insoluble residue remaining after 8 M KOH treatment with a mixture of cellulase and mannanase enzymes. The 8 M KOH treatment was necessary to render the mannan/cellulose polymers more susceptible to the enzymes and probably did this by causing the cellulose/mannan fibrils to swell, making them more accessible substrates.

Redgwell et al. (2002a) reported that the AGPs existed as an extremely heterogeneous mixture containing between 6-10 % glucuronic acid and possessing a Mw average of ~650 kDa. The heterogeneity related particularly to their degree of branching and monosaccharide composition of their side chains. Five different AGP fractions were isolated with Gal/Ara ratios which varied markedly. For Arabica Caturra, Catimor and Sarchimor the Gal/Ara ratios ranged from 0.9 to 3.1, 1.5 to 3.2 and 1.2 to 3.0, respectively. For Robusta Indes, Conillon and Ivoire the values were 0.9 to 3.1, 1.1 to 3.0 and 0.9 to 3.1, respectively. The putative structural features of the major arabinogalactan fraction which was liberated only following enzyme treatment of the insoluble residue of CWM isolated from Arabica Yellow Caturra, is given in figure 1.

The wide heterogeneity of coffee arabinogalactans supported the earlier findings of Fischer et al. (2001a,b) which reported that Robusta contained a highly soluble arabinogalactan which possessed more branch points and more

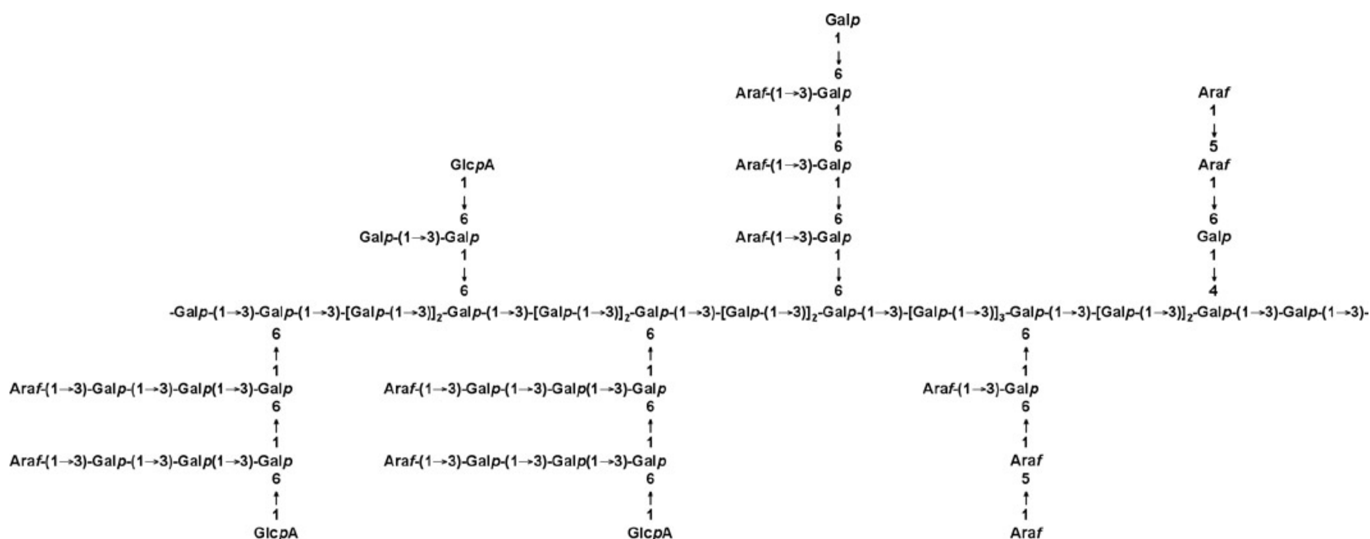

Figure 1. Possible structure of arabinogalactan moiety of coffee AGP (Redgwell et al., 2002a).

extended side chains than those found in Arabica and argued that this may be the reason why the arabinogalactans from Robusta are more easily solubilised than those of Arabica.

Galactomannans: The galactomannans are the predominant components in the coffee bean cell wall accounting for 50 % of the polysaccharides. Recent research has provided information on the degree of galactosylation of the mannans, the presence and distribution of other substituents (e.g. acetyl groups), the possibility that other sugar residues exist in the primary structure of the molecule (e.g. arabinose and glucose) and the location of the mannans in the endosperm of the cell wall.

Coffee bean mannan consists for the most part of linear chains of β1→4-mannosyl residues with single galactose units α-linked at C-6 of a mannosyl residue. The literature reports wide-ranging degrees of substitution from 47:1 (Wolfrom and Patin, 1961), 130:1 (Bradbury and Halliday, 1990), 30:1 (Fischer et al., 2001a), 1:7 and 1:40 (Redgwell et al., 2003) and 3:1 and 9:1 (Oosterveld et al., 2004). It is likely that the mannan molecules in coffee consist of a heterogeneous mixture of substituted and unsubstituted polymers but definitive data on the exact nature of this mixture is not available. One of the reasons for this is that most of the linkage analyses for coffee mannans have been done only on fractions of the total wall mannan, usually those which can be solubilised by various forms of solvent extraction. Invariably they consist of the more galactosylated galactomannan fractions which are more readily soluble than the less substituted molecules which remain in the insoluble residue. This is confirmed by the study of Redgwell et al., (2003) where almost all the mannan was solubilised from mature grains by a combination of chemical and enzymatic treatments. The chemically solubilised polymers (8 M KOH) possessed Man/Gal ratios of 7:1. The 8 M KOH-insoluble residue was solubilised by enzymic hydrolysis with a mixture of cellulase and mannanase. The arabinogalactan which was solubilised at the same time was easily removed by dialysis allowing the Man/Gal ratio (40:1) of the galactomannan fragments derived from the insoluble residue to be determined.

A pure mannan is able to form a hard insoluble structure much like cellulose because of interchain hydrogen bonding. One of the principal effects of galactose substitution is to disrupt the interchain hydrogen bonding and this can lead to increased solubility. However, galactose is not the only substituent, which could induce such an effect. The presence of other moieties (e.g. acetyl groups) could also disrupt interchain hydrogen bonding and may be an explanation for why some galactomannans with an apparently low degree of galactose substitution are relatively soluble. Oosterveld et al. (2004) reported that galactomannans extracted from green Arabica coffee beans were acetylated. In this study the galactomannans were extracted in water at 90°C for 1 h. The galactomannans were separated into two neutral fractions by anion-exchange chromatography which suggested that other mechanisms such as molecular weight must also have played a part in the separation. One neutral fraction of average molecular weight of 2000 kDa possessed both a high degree of galactosylation (30 %) and acetylation (9 % of the mannose groups were acetylated). The second neutral fraction had a molecular weight average of 20 kDa and the degree of galactosylation and acetylation was much lower (11 % and 4 % respectively). The results of Oosterveld et al. have been reinforced in a study by Nunes et al. (2005) who reported that the galactomannans in hot water infusions of green coffee beans are acetylated at a level of 11 mole %. They provided evidence that the acetyl groups were attached to the O-2 and O-3 positions (sometimes both) on the mannose residue. Contiguously acetylated mannosyl residues were also found.

Another possibility for disrupting interchain hydrogen bonding would be the interruption of the mannan backbone with glucose residues and/or the substitution of the mannan backbone with sugar residues other than galactose. Navarini et al., (1999) reported the possibility that the galactomannans were substituted with arabinose. These structural features were looked for, but not found, in the Oosterveld et al. study (2004). However, the Nunes et al. report (2005) provided evidence that terminally linked arabinosyl residues (2 mole %) were attached at O-6 of the mannose residues. In addition, they stated that β-(1→4)-linked glucosyl residues (6 mole %) were present in the mannan backbone and concluded that green coffee mannans extracted with hot water contained acetylated arabinogalactoglucomannans.

Cytochemical and immunolabelling of cell wall polysaccharides

Chemical analysis has revealed an increasingly sophisticated picture of the coffee cell wall polymers. Just how these different types of polysaccharide and different structural forms of the same type of polysaccharide, contribute to the architecture of the wall has until recently been largely unknown. Sutherland et al. (2004) used a range of cytochemical and immunological probes to reveal the spatial arrangement

of the arabinogalactan-proteins, galactomannans and pectic polysaccharides in the cell wall of the endosperm of green coffee beans (*Coffea arabica* L.Yellow Caturra).

Arabinogalactan-proteins: AGPs were localised by labelling with the AGP-specific β-glucosyl Yariv reagent and the monoclonal antibody LM2 (Sutherland et al., 2004) which recognises a carbohydrate epitope containing glucuronic acid. Glucuronic acid has been shown to occupy terminal positions on some of the side chains of coffee AGPs (Redgwell et al., 2002a). Both forms of labelling showed a widespread distribution of the AGP across the cell wall. However, there was more intense staining with the Yariv reagent in the region adjacent to the cell lumen. The labelling pattern for LM6, a monoclonal specific for several contiguous arabinosyl residues, was quite different to that of LM2. Whereas LM2 labelled across the whole wall, LM6 was found in two specific locations (figure 2). LM6 gave intense labelling of the epidermal cells across the whole width of the cell wall indicating that these cells were enriched in 1,5-α-arabinan compared to the endosperm cells. The second location was in a compact band adjacent to the cell wall lumen of the endosperm cells. No label was found in the main body of the cell wall. This indicated the existence of a different structural form of arabinan polymer in the region adjacent to the cell wall lumen, which was absent in the rest of the cell wall. Since the Yariv reagent also showed increased staining in this location one explanation could be that the AGPs in this region have more 1,5-α-arabinosyl residues incorporated into their side chains. However, all antibody data must be interpreted with caution, as there is a possibility that the antibody LM6 is reacting with similar epitopes on completely unrelated molecules (e.g. rhamnogalacturonans and AGPs). An alternative explanation is that LM6 was labelling a rhamnogalacturonan type molecule which carried side chains of 1,5-α-arabinosyl residues. The presence of rhamnogalacturonans in coffee bean cell walls which contain moderate amounts of 1,5-α-arabinosyl residues has been demonstrated (Redgwell et al., 2002a). The argument for the arabinosyl residues being structural features of some pectic polysaccharides is supported by the fact that LM6 does not cross react with the AGP in gum acacia. On the other hand chemical analysis of the coffee bean does indicate the presence of a mixture of AGPs which are polydisperse with regard to their 1,5-arabinosyl residue content. In addition, the inhibition of LM6 labelling by similar concentrations of pure 1,5 arabinan and a pectin-depleted AGP fraction from coffee, suggested that coffee AGPs do cross-react with LM6.

Mannans: When mannans were labelled with the β-1,4-mannan-specific monoclonal antibody BGM C6, the antibody labelled across the entire wall. However, there was a variation in intensity of the labelling across the wall with more intense staining adjacent to the lumen of the cell and the middle lamella. These two zones were separated by a region of only moderately intense staining (Sutherland et al., 2004). Evidence that galactomannans with different degrees of galactosylation were located at different sites in the wall was provided by the use of BS-1 lectin, which is specific for

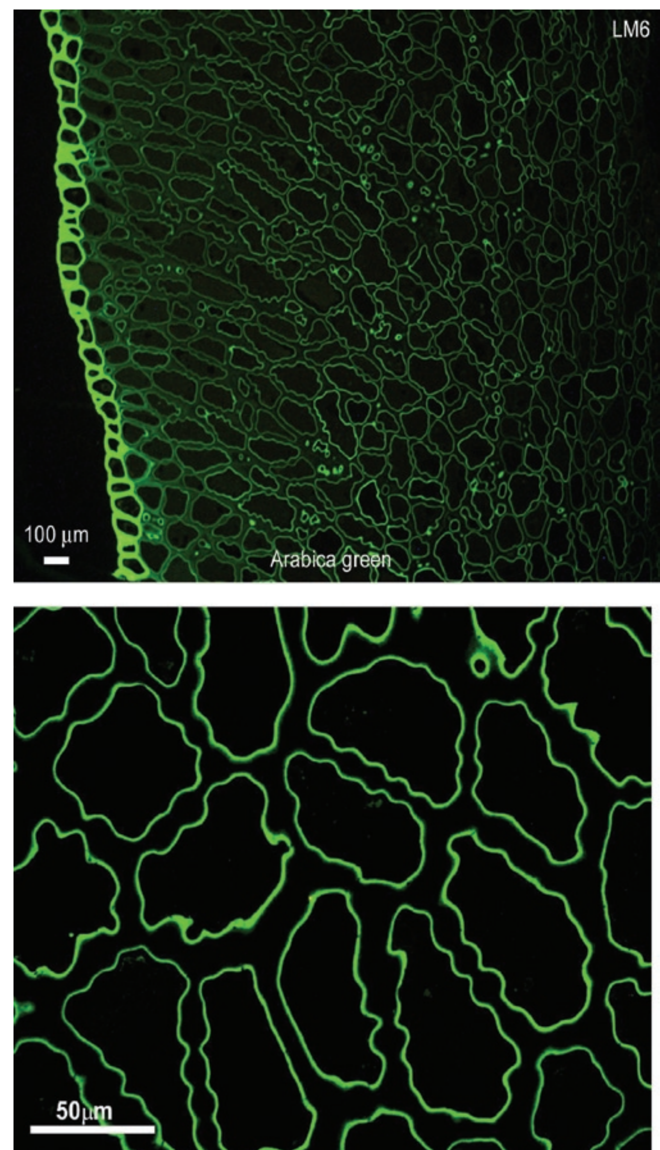

Figure 2. Localisation of 5-linked arabinan with monoclonal antibody LM6 (Sutherland et al., 2004. Upper: Low magnification showing intense labelling of epidermal layer. Lower: High magnification showing labelling of layer adjacent to the cell lumen.

terminal α-galactose (Hayes and Goldstein, 1974). In contrast to the BGM C6 monoclonal antibody, the lectin did not uniformly label the cell walls. The lectin appeared to be concentrated in a compact band adjacent to the cell wall lumen in the single layer of epidermal cells and in the inner zone of the endosperm cells. The lectin did not label across the whole wall as the BGM C6 did. Assuming that all the α-galactose is associated with galactomannans and that the intensity of staining is related to the frequency of galactose substitution of the mannan backbone, the result suggested that structural forms of galactomannan with different degrees of substitution are differentially located in the wall.

Pectic polysaccharides: Location of the pectic polysaccharides was determined by the use of the monoclonal antibodies JIM 5 and JIM 7 which are specific for homogalacturonan type pectic polysaccharides. The monoclonal antibody JIM 7 only labelled the middle lamella area of the endosperm. JIM 5 did not bind to any sites within the wall. Differences in the degree of esterification do not significantly effect JIM 7 binding over the range from 15 to 80 %, whereas JIM 5 binding increases markedly by esterification levels up to 40 % (Willats et al., 2000).

Roast coffee

The roasting process is of central importance, both for flavour development and for the subsequent extractability of the coffee bean for the production of soluble coffee. The relevance of these two areas to the coffee in industry has encouraged continued research into understanding the relationship between roasting and changes to the sugars and cell wall polysaccharides in the coffee bean which accompany the roasting process (Clarke and Vitzthum, 2001).

It has been well documented that low molecular weight sugars are almost totally degraded and minimal amounts remain in the beans after roasting. By contrast the structural features of the cell wall polysaccharides, although modified in some cases, remain largely intact in others, although a significant amount of degradation does take place (Clarke and Vitzthum, 2001). Studies during the last 5 years have focused on providing more details on the nature of roasting induced changes to the polysaccharides and the extent of their degradation at different roasting times. These studies can be separated into two categories. Those which looked at the polysaccharide profile in water soluble extracts of roasted beans and those which attempt to characterise the effect of roasting on the total polysaccharide content of the coffee cell wall. In addition, the roasting induced changes to the structure of the cell wall and the distribution of the polysaccharides within it, have been studied at the cellular level by microscopic methods and immunolabelling.

Nunes and Coimbra (2001, 2002a,b) made a series of reports which described the chemical characterisation of the high molecular weight material extracted with hot water infusions from green and roasted coffee. They reported the degree of polymerisation and the degree of branching of the galactomannans decreased with the increase of degree of roasting. The amounts of terminally linked arabinosyl residues of the arabinogalactans decreased during roasting as did the average molecular weight of the polymers.

Redgwell et al. (2002b) and Oosterveld et al. (2003) reported studies on the changes to the total cell wall polysaccharide content of Arabica beans during roasting. Redgwell et al. (2002b) reported that up to 40 % of the polysaccharides were degraded after a long roast and these results were confirmed by Oosterveld et al. (2003). Based on linkage data the latter reported a decrease in molecular weight of the galactomannans during roasting. At the same time they stated that extracts from roasted coffee contained galactomannans with a greater molecular size than in green bean extracts. This was attributed to the possibility that the roasting process allowed the solubilisation of galactomannans with a higher molecular weight. This idea would support the findings of Redgwell et al., (2002b) which reported no change in the molecular weight of galactomannans extracted by 8M KOH from green and roasted beans.

Coffee arabinogalactans are known to be the most susceptible of the polysaccharides to degradation during roasting, particularly the more heat labile arabinofuranosyl residues. The polymers are depolymerised showing a sharp decrease in molecular weight after a light roast which represented at least a 10-fold reduction in molecular weight (Redgwell et al., 2002b). Although longer roasting continued to degrade the polymer, the extent of further structural modification was only moderate compared to that which followed the initial roast (Redgwell et al. 2002b). The magnitude of the molecular weight decrease was more than could be predicted from the loss of arabinosyl residues from the side chains and must therefore have resulted from fission of the galactan backbone. Ooosterveld et al. (2003) reported that the debranching of the arabinose sidechains occurred more rapidly than hydrolysis of the galactan backbone

The carbohydrate-protein link of the AGP appeared to remain intact to some degree. Even after a dark roast AGP purified from the roasted beans still gave a clear positive result for the Yariv test (Redgwell et al., 2002b).

The fate of the AGPs during roasting was also followed by immunolabelling using the monoclonal antibody LM6 which is specific for contiguous 5-linked arabinosyl residues. Both Arabica and Robusta beans showed a pattern of label reduction for LM6 even after the lightest roasting. At a medium roast only the epidermal layer labelled. At the lighter roasts it was apparent that the labelling for 1,5-arabinan first decreased in the inner regions of the cell wall and near the mucilage cells (Redgwell et al., 2004). Why the 1,5- arabinan should be more rapidly degraded in the inner cells of the wall during roasting when obviously the surface of the bean suffers a much more rapid rise in temperature is not clear. One possibility is that there is more trapped free water in the internal structure which as it heats becomes a more effective agent for hydrolysing the arabinan side chains.

Undoubtedly the degradation of the cell wall polysaccharides plays a key role in the increased solubilisation of coffee polysaccharides. This degradation takes two forms. Hydrolysis of the polysaccharide backbones resulting in a significant decrease in their molecular weight and removal of sidechain sugars which results in a linearisation of the polymers. As a consequence the degree of entanglement between the AGPs, galactomannans and the mannan/cellulose matrix is not as marked allowing their easier solubilisation by aqueous extraction. However, roasting conditions also have a major impact on the structural properties of the bean at the multicellular level. Schenker et al. (2000) used volumetry, mercury porosimetry and electron microscopy to study structural product properties. Following roasting, the beans showed greater bean volume, and larger micropores in the cell walls compared to green or low temperature roasted coffees. Thus, there is an increased accessability of the cell wall polymers to any extractant which favours greater solubilisation of polysaccharides.

Conclusion

The last five years have seen steady progress in understanding the complex chemistry of the coffee bean cell wall. The discoveries that the arabinogalactans are AGPs and that the galactomannans may in some instances contain sugar residues other than galactose and mannose are major advances in the continuing effort to define the structural features of coffee carbohydrates. However, most of this work has focused solely on the polysaccharides in isolation. More work needs to be done to reveal the complex array of interactions between the carbohydrate fraction of the coffee bean and other molecules (proteins, melanoidins) which take place during the roasting process and just what role these interactions play in both flavour development and extractability of the bean. On the physiology side there is a dramatic lack of knowledge on the metabolism of coffee carbohydrates both in the immediate pre-harvest and post harvest stages. The carbohydrate status of the bean at these phases of development may play an important role in the final coffee beverage quality. Before the roasting stage the coffee bean is a living, dynamic structure. If the carbohydrate status of the green bean is a contributing factor to coffee quality then the degree to which postharvest treatments mediate this status becomes an important area for further study.

REFERENCES

Bradbury AGW, Halliday DJ (1990) Chemical structures of green coffee bean polysaccharides. J. Agric. Food Chem. 38:389-92.

Bradbury AGW (2001) Carbohydrates. In: Clarke RJ, Vitzthum OG (eds), Coffee Recent Developments, pp.1-17. Blackwell Science, Oxford.

Clarke AE, Anderson RL, Stone BA (1979) Form and function of arabinogalactans and arabinogalactan-proteins. Phytochemistry 18:521-540.

Clarke RJ, Vitzthum OG (2001) Coffee, Recent Developments. Blackwell Science, Oxford.

Clifford MN (1985) Chemical and Physical Aspects of Green Coffee and Coffee Products. In: Clifford MN, Willson KC (eds), Coffee: Botany, Biochemistry and Production of Beans and Beverage, pp305-374. The AVI Publishing Company Inc., London, Sydney.

Dirk LMA, Griffen AM, Downie B, Bewley JD (1995) Multiple isozymes of endo-β-mannanase in dry and imbibed seeds. Phytochemistry 40:1045-1056.

Fischer M, Reimann S, Trovato V, Redgwell RJ (1999) Structural aspects of polysaccharides from Arabica Coffee. In: Proceed. 18th ASIC Colloquium, Helsinki, pp.91-94.

Fischer M, Reimann S, Trovato V, Redgwell RJ (2001a) Polysaccharides of green Arabica and Robusta coffee beans. Carbohyd. Res. 330:93-101.

Fischer M, Redgwell RJ, Reimann S, Trovato V (2001b) Polysaccharides Composition in Arabica and Robusta green coffee beans : Similar but different? In: Proceed. 19th ASIC Colloquium, Trieste.

Geromel C, Ferreira LP, Cavalari AA, Pereira LFP, Vieira LGE, Leroy T, Mazzafera P, Marraccini P (2004) Sugar metabolism during coffee fruit development. In: Proceed. 20th ASIC Colloquium, Bangalore.

Giorgini JF, Comoli E (1996) Effect of embryo and exogenous GA_3 on endospermic endo-β-mannanase activity of *Coffea arabica* L. during germination and early seedling growth. Rev. Bras. Fisiol. Veg. 8:43-49.

Hayes CE, Goldstein IJ (1974) An α-D-galactosyl-binding lectin from *Bandeiraea simplicifolia* seeds. J. Biol. Chem. 249:1904-1914

Joersbo M, Marcussen J, Brunstedt J (2001) In vivo modification of the cell wall polysaccharide galactomannan of guar transformed with a α-galactosidase gene cloned from senna. Mol. Breed. 7:211-19.

Marracini P, Rogers WJ; Allard C; Andre ML; Caillet V; Lacoste N; Lausanne F; Michaux F (2001) Molecular and biochemical characterization of endo-β-mannanases from germinating coffee (*Coffea arabica*) grains. Planta 213: 296-308.

Mazzafera P, Purcino RP (2004) Post harvest processing methods and physiological alterations in the Coffee fruit. In: Proceed. 20th ASIC Colloquium, Bangalore.

Navarini L, Gilli R, Gombac V, Abatangelo A, Bosco M, Toffanin R (1999) Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: isolation and characterization. Carbohyd. Polym. 40:71-81.

Nunes FM, Coimbra MA (2001) Chemical characterization of the high molecular weight material extracted with hot water from green and roasted Arabica coffee. J. Agric. Food Chem. 49:1773-1782.

Nunes FM, Coimbra MA (2002a) Chemical characterization of the high-molecular-weight material extracted with hot water from green and roasted robusta coffees as affected by the degree of roast. J. Agric. Food Chem. 50:7046-7052.

Nunes FM, Coimbra MA (2002b) Cheminal characterization of galactomannans and arabinogalactans from two coffee infusions as affected by the degree of roast. J. Agric. Food Chem. 50:1429-1434.

Nunes FM, Domingues MR, Coimbra MA (2005) Arabinosyl and glucosyl residues as structural features of acetylated galactomannans from green and roasted coffee infusions. Carbohyd. Res. 340:1689-1698.

Oosterveld A, Coenen GJ, Vermeulen NCB, Voragen AGJ, Schols HA (2004) Structural features of acetylated galactomannans from green *Coffea arabica* beans. Carbohyd. Polym. 58:427-434.

Oosterveld A, Harmsen JS, Voragen AGJ, Schols HA (2003) Extraction and characterisation of polysaccharides from green and roasted *Coffea arabica* beans. Carbohyd. Polym. 52:285-296.

Redgwell RJ, Curti D, Fischer M, Nicolas P, Fay LB (2002a) Coffee bean arabinogalactans: Acidic polymers covalently linked to protein. Carbohyd. Res. 337:239-253.

Redgwell RJ, Trovato V, Curti D, Fischer M (2002b) Effect of roasting on degradation and structural features of polysaccharides in Arabica coffee beans. Carbohyd. Res. 337:421-431.

Redgwell RJ, Curti D, Rogers J, Nicolas P, Fischer M (2003) Changes to the galactose/mannose ratio in galactomannans during coffee bean (*Coffea arabica* L.) development: implications for in vivo modification of galactomannan synthesis. Planta 217:316-326.

Redgwell RJ, Fischer M, Curti D, Sutherland P, Hallett I, Macrae E (2004) Galactomannans and arabinogalactan-proteins in the Coffee bean cell-wall: Heterogeneity and localisation. In: Proceed. 20th ASIC Colloquium, Bangalore

Rogers WJ, Michaux S, Bastin M, Bucheli P (1999) Changes to the content of sugars, sugar alcohols, myo-inositol, carboxylic acids and inorganic anions in developing grains from different varieties of Robusta (*Coffea Canephora*) and Arabica *(C. arabica)* coffees. Plant Sci. 149:115-123.

Schenker S, Handschin S, Frey B, Perren R, Escher F (2000) Pore structure of coffee beans affected by roasting conditions. Journal of Food Science 65:452-457.

Schibeci R, Barns I, Kennealy S, Davison A (1997) Public attitudes to gene technology: the case of the MacGregor's tomato. Public Understanding of Science 6:167-183.

Sutherland PW, Hallett IC, MacRae E, Fischer M, Redgwell RJ (2004) Cytochemical and immunolocalisation of cell wall structures and polysaccharides in the endosperm of green Arabica coffee beans. Protoplasma 223:203-211.

Willats WGT, Knox JP, Limberg G, Christensen TMIE, Mikkelsen JD, Buchholt HC, Van Alebeek GJ, Voragen A, Benen J, Visser J (2000) Analysis of pectic epitopes recognised by hybridoma and phage display monoclonal antibodies using defined oligosaccharides, polysaccharides, and enzymatic degradation. Carbohyd. Res. 327:309-320.

Wolfrom ML, Laver ML, Patin DL (1961) Carbohydrates of the coffee bean. II. Isolation and characterization of a mannan. J. Org. Chem. 26:4533-4535.

Zhu, Goldstein IJ (1994) Cloning and functional expression of a cDNA encoding coffee bean alpha-galactosidase. Gene 140:227-231.