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Cocoa bean carbohydrates: roasting-induced changes and polymer interactions

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Abstract

Roasting induced change to carbohydrates and cell wall polysaccharides was investigated in three varieties of cocoa beans. The concentrations of glucose and fructose decreased after roasting but levels of the non-reducing sugars, sucrose, raffinose, stachyose and verbascose, were not markedly affected. Approximately 10% of the arabinose content of the polysaccharides was degraded but, overall, the pectic and hemicellulosic polymers remained intact after roasting. The degree of esterification and acetylation of the pectic polysaccharides were unaffected by roasting. Roasting did promote an interaction between polysaccharides, proteins, polyphenolics and Maillard products. This led to the formation of insoluble complexes which co-purified with, and augmented, the levels of cell wall material isolated from roasted compared to unroasted beans. The implications of the results are discussed in relation to the role that ''Klason lignin'' plays in the formation of these chemical amalgams during roasting.

Keywords: Cocoa beans; Polysaccharides; Roasting; Lignin; Interactions; Protein

1. Introduction

A knowledge of the state of cocoa (*Theobroma cacao* L.) bean carbohydrates after roasting is important for a better understanding of their possible roles as flavour precursors and in the case of polysaccharides, as both dietary fibre components, and interactive texturising components in cocoa products. When the chemistry of a minor polymeric component allows it to promote a diverse range of interpolymeric interactions, it can potentially exert a major influence on the final physicochemical properties of complex mixtures. The pectic polysaccharides are just such molecules and they have recently been characterised as major components of cocoa bean polysaccharides (Redgwell & Hansen, 2000). However, the effect of the subsequent roasting step on their structural features has been the subject of limited study. Valiente, Esteban, Molla, and Lopez-Andreu (1994), reported the effect of roasting on the dietary fibre composition of cocoa beans for a single variety and concluded that roasting caused no change in total dietary fibre content. However, they did report a sharp increase in Klason lignin after roasting which suggested that a redistribution of polymer types (e.g. polysaccharide, protein, polyphenols) had occurred during roasting.

There have been separate studies on both the free sugar content of cocoa beans (Reineccius, Anderson, Kavanagh, & Keeney, 1972) and the fate of reducing sugars during roasting (Rohan & Stewart, 1966) but no publications which report roasting induced changes of both mono-, oligo- and polysaccharides in the same beans.

The present investigation reports the nature of roasting induced modification of low molecular weight carbohydrates and cell wall polysaccharides in three varieties of cocoa bean and the extent to which polysaccharide–protein–polyphenol interactions were effected by the roasting process.

2. Materials and methods

2.1. Plant material

Three varieties of cocoa (*Theobroma cacao* L.) bean were obtained from the Broc factory. Ecuador superior

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Arriba-flavour beans, Ghana good fermented and Ivory Coast good fermented. Each variety was obtained as unroasted and roasted beans. Roasting conditions for the three varieties were: dried at 150 °C, 14 min, roasted 148 °C, 13 min and cooled to 25 °C, 22 min. Total time was 49 min.

2.2. General

Polysaccharide fractions and CWM were hydrolysed with either 2 M TFA for 1h at 110 °C or by solubilisation in 72% H_2SO_4 and then hydrolysis in 2 M sulphuric for 2 h at 100 °C (Selvendran, March, & Ring, 1979). Sugar mixtures were converted into alditol acetates for GLC analysis. The column, SP-2330 fused silica (30 m × 0.32 mm), was maintained at 120 °C for 2 min and then raised to 220° C at 25° C/min.The degree of esterification and acetylation of CWM was detemined by the HPLC method of Voragen, Schols, and Pilnik (1986). Protein content was obtained by multipying the nitrogen content, estimated by GLC, by a factor of 6.25. Amino acids were released by hydrolysis with 6 M HCl containing 1% phenol and analysed as their isothiocyanate derivatives by HPLC. Uronic acid was determined by the method of Blumenkrantz and Asboe-Hansen (1973).

2.3. Isolation of cell wall materials

The skins were removed from the beans, and the latter frozen in liquid nitrogen and cryo-milled in liquid nitrogen. Duplicate samples were assigned for the unroasted and roasted beans. Frozen powder (20 g) was suspended in 150 ml of methanol/chloroform (1:1) at −20° C, homogenised with a polytron and stirred at 4° C for 2 h. The suspension was centrifuged (10 min at 6000 *g*) and the supernatant decanted. The process was repeated twice and the combined supernatants dried.

The residue was re-extracted with 150 ml of water for 1 h at room temperature. The water extraction was repeated. The aqueous supernatants were combined, concentrated and freeze-dried.

The insoluble residue was suspended in 150 ml of phenol:acetic acid:water (2/1/1 w/v/v, PAW) and stirred overnight at ambient temperature. After centrifuging, the residue was extracted a second time in 150 ml of PAW for 3 h at ambient temperature. The PAW-soluble fractions were combined dialysed and freeze-dried.

The PAW-insoluble residue was suspended in 150 ml of 100% DMSO and stirred at room temperature for 5 h. The supernatant was decanted following centrifuging and another 150 ml of 90% DMSO were added to the residue which was stirred overnight. All supernatants were recovered, an aliquot dialysed and the polymers recovered after freeze drying.

The DMSO-insoluble residue (CWM) was suspended in water, dialysed at 4 °C and freeze-dried.

2.4. Fractionation of CWM

CWM (1 g) was stirred overnight at 4 °C in 100 ml of 0.05 M CDTA/ 0.05 M Na_2CO_3, containing 20 mM $NaBH_4$ and then for 3 h at ambient temperature. The supernatant was recovered after centrifuging (7000 *g*) and the residue washed in 50 ml of water and the combined supernatants neutralised (pH 5.0) and dialysed.

The residue was resuspended in 100 ml 4 M KOH (20 mM $NaBH_4$) and stirred for 2 h at ambient temperature. The supernatant was neutralised with acetic acid and dialysed. The residue was suspended in water, neutralised, dialysed and freeze-dried.

2.5. Analysis of oligosaccharides and monosaccharides in water-soluble extract

The freeze-dried water-soluble fractions (2 g) were dissolved in 20 ml water and dialysed overnight in a 3.5 kDa MWCO membrane against 1000 ml water. This separated the low molecular weight sugars and oligosaccharides from trace amounts of polysaccharide which were also solubilised by the water extraction. The dialysis was repeated with a fresh 1000 ml of water and the low molecular weight fraction recovered, following concentration and freeze-drying of the dialysis water.

The sugars (200 mg) in 20 ml of water were passed through two Sephadex ion-exchange columns (1.5 × 6 cm bed volume) of SP-C 25 (cation) and QAE-A 25 (anion), in series (Redgwell, 1980). Amino acids were removed by the SP-Sephadex while acidic components were retained on the QAE-Sephadex. The neutral carbohydrates (glucose, sucrose, fructose and oligosaccharides) were not held on the exchangers and were collected from the outlet of the QAE column, dried down and stored at −20° pending analysis. Each fraction was analysed by HPLC on the Dionex.

3. Results and discussion

3.1. Monosaccharide and oligosaccharide composition

The major sugars in cocoa beans were fructose and sucrose (Table 1). The higher amounts of fructose compared to glucose have been reported previously (Reinecius et al., 1972) and ascribed to preferential metabolism of glucose during fermentation. In the Ghana and Ivory Coast beans, fructose was present at a higher concentration than sucrose but, in Ecuador beans, the reverse was true. It is unlikely that this difference is a varietal one. The variable concentrations of these sugars are probably caused by differing conditions of fermentation which markedly affect the amount of sucrose in cocoa beans. The Ecuador Arriba beans are normally fermented for shorter periods than other bean varieties

Table 1
Changes to monosaccharides and oligosaccharides during roasting of cocoa beans

	Mono-and oligosaccharides mg/20 g					
	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Verbascose
Ghana						
Non-roasted	12.4	83.6	31.6	4.0	15.1	1.9
Roasted	0.9	11.9	28.2	5.4	10.6	1.3
Ivory coast						
Non-roasted	15.9	56.0	31.0	3.0	12.2	0.9
Roasted	1.0	8.7	40.5	5.5	14.4	0.9
Ecuador						
Non-roasted	16.8	34.4	96.6	12.2	20.1	0.8
Roasted	2.1	12.1	124.8	14.9	22.2	0.9

and this would explain their higher sucrose levels. Glucose and stachyose shared the third highest concentration of any sugar component. Raffinose and verbascose were also present.

During roasting, most of the fructose and nearly all the glucose was destroyed. There were no decreases in the concentration of sucrose, raffinose, stachyose or verbascose during roasting. This is a predictable result since glucose and fructose are reducing sugars and can interact with amino acids during roasting to form other compounds. The remaining sugars are non-reducing and are unable to undergo similar interactions to any extent.

3.2. Isolation and composition of CWM

The water-insoluble residue was extracted with PAW and DMSO to remove proteins and starch from the CWM. Among the bean varieties, there was little variation in yield of individual fractions during CWM preparation (Tables 2 and 3). However, in roasted beans,

Table 2
Yield of CWM and fractions isolated during CWM preparation from three varieties of non-roasted cocoa beans

Fraction	Cocoa bean type					
	Ivory Coast		Ghana		Ecuador	
	Yield (g)	%	Yield (g)	%	Yield (g)	%
Fat	10.5	57.8	10.0	55.5	10.8	60.8
Water-sol	1.9	10.3	2.1	11.5	1.5	8.5
PAW-sol	1.8	10.0	2.0	11.1	2.0	11.0
DMSO-sol	1.6	9.0	1.5	8.2	1.1	5.9
CWM	2.3	12.8	2.4	13.5	2.4	13.3
Total	18.2	–	18.0	–	17.7	–

Table 3
Yield of CWM and fractions isolated during CWM preparation from three varieties of roasted cocoa beans

Fraction	Cocoa bean type					
	Ivory Coast		Ghana		Ecuador	
	Yield (g)	%	Yield (g)	%	Yield (g)	%
Fat	10.9	57.8	10.2	54.9	9.3	49.4
Water-sol	1.9	9.6	1.9	10.5	1.7	9.0
PAW-sol	1.1	5.5	1.4	11.1	2.3	12.3
DMSO-sol	1.1	5.8	1.1	5.8	1.1	5.8
CWM	4.1	21.0	3.9	21.1	4.3	23.0
Total	19.4	–	18.5	–	18.9	–

the amount of CWM was approximately twice that obtained from the non-roasted beans.

Compositional analysis of the CWM showed that the increase could not be attributed to polysaccharides, as almost all the monosaccharide components of the polysaccharides were present at half the levels found in the CWM from the non-roasted beans (Table 4). The exception was the glucose content of the roast CWM, which was disproportionately higher, than the other monosaccharides. Treatment with amylase lowered the glucose levels of the CWM (data not shown), showing that the additional glucose content in the CWM of the roasted beans was caused by residual starch. The material which accounted for the increased weight in the CWM from roasted beans was derived from protein and "Klason lignin" and these aspects will be discussed in Section 3.5.

3.3. Degradation of polysaccharides during roasting

Table 5 shows the monosaccharide content of the total polysaccharide component of cocoa beans before and after roasting. It shows a small (~10%) but consistent loss of arabinose from all three of the cocoa varieties during roasting. No other monosaccharide constituent showed a significant change. This result is

Table 4
Monosaccharide composition of cell wall material from three varieties of non-roasted and roasted coffee beans

Bean variety	Monosaccharide composition (μg/mg)								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	Total
Non-roasted									
Ecuador	8.7	2.5	57.2	18.8	10.1	54.3	166	132	449
Ghana	9.5	2.3	57.2	18.8	9.5	47.3	174	153	471
Ivory coast	6.4	2.6	61.1	21.9	9.6	50.1	172	145	468
Roasted									
Ecuador	3.7	1.3	26.3	9.0	5.0	28.3	142	73.5	289
Ghana	4.8	1.3	28.8	9.6	5.1	27.4	155	94.0	326
Ivory coast	3.1	1.5	31.0	11.4	5.8	29.6	16.0	90.1	332

Table 5
Amounts of monosaccharide constituents in polysaccharides determined before and after roasting of beans

Bean variety	Amount (mg monosaccharide per 20 g beans)							
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic
Ecuador								
Non-roasted	20.5	5.9	141	46.6	32.3	157	1301	415
Roasted	16.9	5.7	128	41.4	27.1	159	1257	413
Ghana								
Non-roasted	21.8	5.5	147	44.6	33.2	138	1416	462
Roasted	18.9	5.1	137	42.1	32.9	130	1392	467
Ivory coast								
Non-roasted	15.4	5.7	158.9	54.4	33.1	138	1613	455
Roasted	14.8	5.6	143.3	54.1	32.3	137	1548	461

consistent with the known thermal stability of arabinose, which is markedly degraded from the arabinogalactans of coffee during roasting (Redgwell, Trovato, Curti, & Fischer, 2002). In cocoa beans, most of the arabinose is present as sidechains on the pectic polysaccharides, although some is associated with hemicellulosic polymers (Redgwell & Hansen, 2000).

Individual pectic and hemicellulosic polysaccharide fractions were isolated from the CWM by sequential extraction with solutions of CDTA/Na_2CO_3 and 4 M KOH, respectively. There were increased amounts of the 4 M KOH-soluble fraction recovered from the CWM of the roasted beans compared to that recovered from the unroasted samples (Table 6). Concomitantly, there was a decrease in the levels of CWM-res recovered from the roasted samples. Compositional analysis (Table 7) showed increased amounts of glucose in both 4 M KOH fractions and the CWM-sol fraction (material solubilised during dialysis of the CWM) of the roasted beans and the results are consistent with the solubilisation, by the 4 M KOH, of some of the additional starch and protein present in the CWM.

Table 6
Yield of fractions isolated from CWMs after sequential extraction with CDTA/Na_2CO_3, and 4 M KOH

Bean variety	Yield (mg /1 g)				
	CDTA/Na	4 M KOH-sol	CWM-sol	CWM-res	Total
Non-roasted					
Ecuador	74.4	219	68.1	598	959
Ghana	89.9	217	80.1	561	948
Ivory coast	94.6	222	103	500	920
Roasted					
Ecuador	90.2	394	72.5	393	949
Ghana	111.2	347	61.7	401	921
Ivory coast	104.5	354	73.0	412	943

Table 7
Monosaccharide composition of CWM fractions isolated from non-roasted and roasted Ecuador cocoa beans

Fraction	Composition (mole%)								
	Rha	Fuc	Ara	Xyl	Man	gal	Glc	UA	Total μg/mg
CDTA/Na-sol									
Non-roasted	2.2	–	5.9	2.5	1.3	6.1	41.2	40.9	361
Roasted	1.7	–	9.6	3.3	–	4.8	26.3	54.3	217
4 M KOH-sol									
Non-roasted	1.2	2.3	7.0	13.0	7.0	14.6	37.0	17.9	579
Roasted	0.8	1.5	6.7	9.1	6.0	11.6	47.1	17.7	412
CWM-res-sol[a]									
Non-roasted	2.7	0.3	13.4	5.6	0.6	16.1	8.5	52.9	552
Roasted	1.7	0.3	10.0	4.0	0.2	14.6	33.8	35.7	465
CWM-res									
Non-roasted	1.7	0.4	16.6	3.8	1.4	11.5	37.1	27.6	512
Roasted	1.5	0.3	14.2	2.9	1.4	9.7	66.2	20.7	457

[a] Polymers solubilsed during dialysis of the 4 M KOH-insoluble residue.

The loss in arabinose from the total polysaccharide of the bean was not apparent in the individual pectic and hemicellulosic fractions of the CWM. The magnitude of the arabinose loss was probably masked by variations between the fractionation pattern of the polysaccharide fractions solubilised from the non-roasted and roasted beans. Another possibility is that the degraded arabinose originated, not from the more insoluble polymers of the CWM, but from the polysaccharide contained in the PAW-soluble fractions.

3.4. Degree of esterification and acetylation of pectic polysaccharides

Important structural characteristics of pectic polysaccharides are the esterification of galacturonic acid residues with methanol and, for certain pectins, the acetylation of hydroxyl groups. The degree of esterification (DE) is defined as the percentage of carboxyl groups esterified with methanol. The degree of acetylation (DAc) is defined as the percentage of galacturonosyl groups esterified with one acetyl group. The degree of esterification and/or acetylation of the pectic polysaccharides in cocoa bean cell walls was determined for the three varieties of non-roasted and roasted beans (Table 8).

The DE of the pectin was between 43 and 55% for the three varieties. Pectin with a DE above 50% is normally classified as highly esterified while, below 50%, it is regarded as low-esterified pectin. Cocoa is therefore in the middle range. The DE was unaffected by roasting.

Table 8
Degrees of esterification and acetylation of pectic polysaccharides of cocoa bean before and after roasting

Bean variety	DE (%)	DAc (%)
Ecuador		
Non-roasted	53	71
Roasted	55	66
Ghana		
Non-roasted	49	54
Roasted	46	61
Ivory Coast		
Non-roasted	47	67
Roasted	43	60

The DAc for the cocoa bean pectin was moderately high but was not altered by roasting. The effect of acetyl groups on the pectin is to make the molecule more hydrophobic and also to decrease its susceptibility to hydrolysis by pectin-degrading enzymes, such as endo-polygalacturonase. These results suggest that, to maximise the enzymatic breakdown of cocoa pectin, it would be necessary to include an acetyl esterase in any mixture of pectin degrading enzymes.

3.5. Increased CWM in roasted beans—"Klason lignin"

Roasting was responsible for an increase in the amount of CWM isolated from the roasted beans. The increase was caused by a redistribution of several components in the bean—polysaccharide, protein, "Klason lignin" and starch. The major contributors to the increase during roasting appeared to involve protein and "Klason lignin". During roasting, the proportion of these two components in the CWM increased relative to the polysaccharide content (Table 9).

Table 9
Amount of polysaccharide, protein and "Klason lignin" in CWM from cocoa beans. Values expressed as% of whole beans

Bean variety	% dry wt.		
	Polysaccharide	Protein	"Klason lignin"
Non-roasted			
Ecuador	5.4	1.7	4.3
Ghana	5.7	1.7	4.1
Ivory coast	5.4	1.6	4.0
Roasted			
Ecuador	6.2	7.3	9.2
Ghana	6.3	5.5	8.6
Ivory coast	6.4	6.0	9.0

Analysis of the CWM proteins, before and after roasting, showed similar amino acid compositions (Table 10). This suggested that the additional protein fraction in the roasted CWM was not a consequence of an interaction between the cell wall polysaccharides and a specific protein (i.e. basic protein). It seemed to result from a non-specific interaction between the whole protein content of the bean and other components in the CWM.

"Klason lignin"has been, and still is, used to describe a fraction of plant material which remains insoluble after treatment with 12 M H_2SO_4 for 3 h at room temperature and for another 2 h in 1M H_2SO_4 at 100 °C. In many plants, the insoluble material resulting from this treatment represents lignin. However, tannin/protein complexes and Maillard products from roasting are also largely insoluble after this treatment. Therefore, in material which contains significant amounts of these complexes, the term "Klason lignin" is meaningless as a measurement of actual lignin. This has significance for the estimation of dietary fibre in such materials, because lignin is included in the definition of dietary fibre whereas tannin/protein complexes and Maillard products are not.

Despite the fact that non-roasted, fermented cocoa beans contained moderate amounts of "Klason lignin" there is indirect evidence that it is not lignin. CWM prepared from unfermented cocoa beans (before oxidative polymerisation of polyphenolics has occurred) contained no insoluble material after sulphuric acid treatment and therefore, by definition, no "Klason lignin". If there was no lignin present in the cocoa beans before fermentation then it is unlikely that lignin synthesis would take place during fermentation. What is more probable, is that, during fermentation, oxidative polymerisation of polyphenols leads to the formation of the insoluble protein/tannin complexes, which account for the approximately 30% of the CWM which was insoluble in 72% sulphuric acid. During roasting, these complexes are augmented by amounts of insoluble material generated by the formation of Maillard products, leading to a further increase in "Klason lignin" in the CWM.

The findings of the present study confirm the results of Valiente et al. (1994) who reported a marked increase in "Klason Lignin" during roasting of cocoa beans. They attributed the formation of extra "Klason lignin" to the involvement of fibre sugars (neutral and uronic acid). Our results found no decrease in any polysaccharide component, which would indicate their involvement in Maillard reactions. It is more likely that the increase in the "Klason lignin" fraction is in part due to the Maillard reactions between proteins and the reducing sugars, fructose and glucose. In addition, the extent of the increase in the insoluble component of the CWM, indicates that other chemical changes, which

Table 10
Protein amino acid composition of CWM from cocoa beans

Amino acid	Ecuador non-roasted		Ecuador roasted		GhanaNon-roasted		Ghana roasted		Ivory Coast non-roasted		Ivory Coast roasted	
	μmol/g	%	μmol/g	%	μmol/g	%	μmol/g	%	μmol/g	%	μmol/g	%
Asp	82	10.2	219	10.5	64	9.5	169	10.6	69	9.3	180	10.3
Glu	107	23.2	327	15.7	104	15.5	259	16.3	109	14.7	271	15.5
Ser	66	8.1	166	7.9	56	8.3	125	7.9	60	8.1	139	7.9
Gly	66	8.2	169	8.1	56	8.3	133	8.3	60	8.1	145	8.3
His	18	2.2	31	1.5	14	2.0	24	1.5	16	2.2	27	1.6
Arg	38	4.7	116	5.6	36	5.4	89	5.6	38	5.1	102	5.8
Thr	40	4.9	106	5.1	32	4.7	83	5.2	34	4.6	94	5.4
Ala	60	7.4	145	6.9	47	7	106	6.7	51	6.8	116	6.6
Pro	68.4	8.5	137	6.6	57	8.5	105	6.6	67	9.1	123	7.0
Tyr	27	3.4	70	3.3	23	3.4	50	3.1	26	3.6	57	3.2
Val	56	6.9	142	6.8	43	6.4	104	6.5	49	6.6	113	6.5
Met	2.6	0.3	19	0.9	0	0	11	0.7	0	0	13	0.7
Cys	0	0	13	0.6	0	0	11	0.7	0	0	12	0.7
Ile	33.4	4.1	84	4.0	26	3.8	64	4.0	29.9	3.9	70	4.0
Leu	60	7.4	156	7.5	46	6.8	120	7.6	50	6.7	130	7.4
Phe	33	4.1	97	4.7	24	3.5	64	4.0	28	3.7	74	4.2
Lys	53	6.5	90	4.3	46	6.9	72	4.5	55	7.4	86	4.9
Total	810	100	2088	100	672	100	1589	100	739	100	1751	100

promote an association between macromolecular species, have occurred during roasting. The mechanisms of such processes are not known but if they occur between polymeric species then they are unlikely to require the presence of a reducing end group to facilitate their reaction.

4. Conclusions

Roasting of cocoa beans effected a small loss in the arabinose content of the polysaccharides but, in general, the pectic and hemicellulosic polymers were resistant to thermal degradation under the conditions of cocoa bean roasting. This contrasts with the magnitude of the loss of the monosaccharide constituents of coffee beans, where up to 40% of the polysaccharide can be degraded under some roasting conditions (Redgwell et al., 2002). It seems that roasting at high temperature for a short time (as in coffee roasting) is more conducive to thermal breakdown of cell wall polymers than an extended roast at a lower temperature. Nevertheless, the conditions during cocoa bean roasting are sufficient to promote changes in the interactions between polysaccharides, protein and Maillard products resulting in the formation of increased amounts of insoluble complexes. The formation of such complex chemical amalgams has implications, not only for the physicochemical properties of the resultant cocoa products, but from a human nutritional standpoint, for the *in-vivo* status of the material.

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References

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acid. *Analytical Biochemistry*, *17*, 484–489.

Redgwell, R. J. (1980). Fractionation of plant extracts using ion-exchange Sephadex. *Analytical Biochemistry*, *107*, 44–50.

Redgwell, R. J., & Hansen, C. (2000). Isolation and characterisation of cell wall polysaccharides from cocoa (*Theobroma cacao* L.) beans. *Planta*, *5*, 820–823.

Redgwell, R.J., Trovato, V., Curti, D., & Fischer, M. (2002). Effect of roasting on degradation and structural features of polysaccharides in arabica coffee beans. *Carbohydrate Research*, *337*, 421–431.

Reineccius, G. A., Anderson, D. A., Kavanagh, T. E., & Keeney, P. G. (1972). Identification and quantification of free sugars in cocoa beans. *Journal of Agriculture and Food Chemistry*, *20*, 199–202.

Rohan, T. A., & Stewart, T. (1966). The precursors of chocolate aroma: changes in the sugars during the roasting of cocoa beans. *Journal of Food Science*, *31*, 206–209.

Selvendran, R. R., March, J. F., & Ring, S. G. (1979). Determination of aldoses and uronic acid content of vegetable fibre. *Analytical Biochemistry*, *96*, 282–292.

Valiente, C., Esteban, R. M., Molla, E., & Lopez-Andreu, F. J. (1994). Roasting effects on dietary fiber composition of cocoa beans. *Journal of Food science*, *59*, 123–124.

Voragen, A. G. J., Schols, H. A., & Pilnik, W. (1986). Determination of the degree of methylation and acetylation of pectins by HPLC. *Food Hydrocolloids*, *1*, 65–70.