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Composition of green coffee water-soluble fractions and identification of volatiles formed during roasting

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Flavour precursors from water-soluble green coffee low molecular weight (B) and high molecular weight (C) fractions were investigated and the volatiles formed during roasting were identified by high resolution gas chromatography/mass spectrometry (HRGC/MS). Roasting promoted extensive degradation of trigonelline, sucrose and amino acids in fraction (B) and of arabinogalactan in fraction (C). The analyses of the roasted isolated fractions showed that furans are not only formed by sucrose degradation but also by arabinogalactan pyrolysis. Also, pyrazines appear to be mainly formed by pyrolysis of hydroxy amino acids from fraction (C). The results also showed that pyridine found in roasted coffee is not exclusively formed by trigonelline degradation but also by protein pyrolysis.

INTRODUCTION

Aroma is an important attribute to define consumer acceptance of coffee products. The reactions involved in coffee aroma formation are very complex with Maillard and Strecker reactions, and the degradation of sugar, trigonelline, chlorogenic acids (C and A), proteins and polysaccharides are some examples (Clifford, 1985; Dart & Nursten, 1985). One approach to study the mechanisms of aroma formation is the use of sugar or sugar degradation products together with one or two selected amino acids under coffee roasting conditions (Baltes & Bochmann, 1987*d,e*, 1992; Baltes & Knoch, 1993). Although these models are very useful, they do not take into consideration other aroma precursors, particularly macromolecular constituents (e.g. polysaccharides).

In a previous work (De Maria *et al.*, 1994), we reported a procedure to study coffee aroma, based on the isolation and roasting of low and high molecular weight water-soluble fractions of green coffee. We used this procedure and high resolution gas chromatography/mass spectrometry (HRGC/MS) to achieve the identification of volatiles formed in the roasted water-soluble fractions. The distribution of flavour precursors in these fractions before and after roasting were also investigated.

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MATERIALS AND METHODS

Materials

Green arabica coffee was obtained from a local industry. All reagents were of analytical-reagent grade. All analyses were carried out with freeze-dried samples.

Methods

Isolation of fractions

Ground defatted green coffee (100 g) was extracted with 4 litres of hot bidistilled water (80°C) for 15 min. Twenty grammes of freeze-dried water-soluble fraction (A) was then dissolved in ethanol water (80:20 v/v, 50°C) with shaking for 1 h and centrifuged at 100 g for 10 min (De Maria *et al.*, 1994). Both filtrate (B) and residue (C) were freeze-dried for further analyses.

Amino acids, crude protein, sugars, CGA and trigonelline

Amino acid analysis was based on the procedure described by Coelho *et al.* (1985). Crude protein (N × 6.25) was determined by the Kjeldahl method (Pearson, 1976). The analysis of sugars was carried out using the method described by Albersheim *et al.* (1967). Total CGA and trigonelline were determined by methods described elsewhere (Trugo *et al.*, 1983, 1991).

Headspace analysis

The headspace analysis was based on a previously described technique (De Maria *et al.*, 1994): 0.5 g of ground defatted green coffee and fractions (B) and (C) were put in test tubes with screw caps and septa and submitted to vacuum. Each sample was roasted at 220°C ±2°C (14 min). Immediately after roasting, a 4 ml headspace sample was injected into the chromatographic column.

HRGC/MS

HRGC was carried out using a Carlo Erba 4300 with FID and Supelcowax fused silica capillary column (30 m × 0.25 mm i.d.). The oven temperature was held at 40°C for 6 min and programmed to 180°C at 3°C/min. The linear flow rate of hydrogen carrier gas was 40 cm/s. HRGC/MS was carried out using an HP 5987-A mass spectrometrer with a RTE-6/E data system with a cyclic scan of 1 s and mass range of m/z 50–500. Calculation of modified Kóvatz indices was based on the method of Van den Dool & Kratz (1963).

RESULTS AND DISCUSSION

Fractions were isolated from green defatted coffee based on water solubility and molecular weight. Specific groups of compounds were then considered in the fractions for the studies of aroma formation under coffee roasting conditions.

Table 1 shows the distribution of carbohydrates, trigonelline, total CGA and protein in the low and high molecular weight fractions (B) and (C) before and after roasting. In fraction (B), sucrose was completely degraded on roasting. There was a great loss of trigonelline (70%) and protein (67%). The major compounds in fraction (C) were protein and polysaccharide which was expressed as the monosaccharides produced on hydrolysis (Table 1). Arabinogalactan was the major polysaccharide found in fraction (C) and it was affected by roasting with the consequent loss of arabinose (82.3%). According to literature data (Wolfrom & Patin, 1965) the arabinogalactan has a linked galactan main chain with galactose and mainly arabinose-containing side chains. Therefore, the greater susceptibility of arabinose to roasting is due to its arrangement in the arabinogalactan. It is supposed that this polysaccharide is the major carbohydrate aroma precursor in fraction (C). There was an increase of mannan which may be due to its lesser susceptibility to roasting (Clifford, 1985). Some compounds found in the roasted fractions were not considered because they are not as relevant as aroma precursors (i.e. caffeine, ash, organic acids). Consequently, these compounds together with humic acids should make up the balance of the fractions in Table 1.

The distribution of amino acids in fractions (B) and (C) before and after roasting is shown in Table 2. Since roasted fraction (B) showed only trace amounts of free amino acids, any protein nitrogen detected appears to come from ethanol-soluble peptides. Serine and threonine were the most affected amino acids in fraction (C) indicating their importance as flavour precursors (Table 2). On the assumption that the glutamic acid content does not change during roasting, amino acid results may also be expressed after normalizing for glutamic acid.

Loss of weight during roasting is higher in fraction (B) (33.6%) than in fraction (C) (13.8%). This was expected since fraction (B) contains more labile flavour precursors (sucrose, amino acids, trigonelline, CGA) than fraction (C) which is mainly formed by polysaccharides and proteins.

The volatile compounds of roasted coffee and of the roasted fractions (B) and (C) are listed in Table 3. In the course of this work, about 30 volatiles were identified by HRGC/MS. More than 90% of the volatile compounds

Table 1. Distribution of carbohydrates, trigonelline, total CGA and protein after roasting of water-soluble fraction[a] obtained from green coffee

Compounds	Fractions (g%)					
	80% ethanol-soluble[b] (B)			80% ethanol-insoluble[c] (C)		
	non-roast	roast	variation (%)	non-roast	roast	variation (%)
Galactose	nd	nd	—	21.2	14.0	−34.3
Arabinose	nd	nd	—	18.1	3.2	−02.3
Mannose	nd	nd	—	6.9	20.0	+190
Glucose	nd	nd	—	1.2	1.3	+8.3
Xylose	nd	nd	—	1.1	0.9	−18
Sucrose	17.4	nd	−100	nd	nd	—
Trigonelline	5.3	1.6	−70	nd	nd	—
Total CGA	33.2	29.9	−10	nd	nd	—
Protein (N × 6.25)	7.5	2.5	−67	50	46.2	−7.6

Results are averages of duplicate determinations on defatted and dry basis; nd, not detected.

[a]Low and high molecular weight water-soluble fractions were obtained by ethanol extraction as described in Methods.

[b]Low molecular weight water-soluble fraction.

[c]High molecular weight water-soluble fraction.

Table 2. Distribution of amino acids before and after roasting of water-soluble fractions[a] obtained from green coffee

Compounds	Fractions (g%)				
	80% ethanol-soluble[b] (B)	80% ethanol-insoluble[c] (C)			
	non-roast[d]	non-roast	roast	variation (%)[e,f]	
Lysine	1.9	8.6	8.4	−2.3	−29.3
Histidine	2.9	3.5	2.8	−20	−40.0
Arginine	5.1	5.4	4.2	−22	−44.4
Aspartic acid	8.9	7.2	7.0	−2.9	−30.0
Threonine	3.5	2.7	0.3	−89	−92.0
Serine	17.5	4.6	1.5	−67	−76.5
Glutamic acid	28.4	20.0	27.7	+38.5	—
Proline	3.8	3.8	4.6	+21.1	−12.0
Glycine	2.5	5.1	4.5	−11.8	−36.4
Alanine	9.5	4.9	5.3	+8.2	−22.0
Valine	2.8	5.3	4.9	−7.5	−33.6
Isolerncine	3.1	4.1	3.6	−12.2	−38.6
Leucine	3.9	6.5	7.4	+13.0	−17.0
Tyrosine	5.3	3.8	3.5	−7.9	−33.7
Phenylalanine	1.1	14.5	14.3	−1.4	−28.8

[a,b,c] as in Table 1.
[d]Roasted fraction (B) showed only trace amounts of amino acids.
[e]In relation to non-roast.
[f]after normalizing for glutamic acid.
Results are averages of duplicate determinations on defatted and dry basis; data of amino acids are expressed in g% of each amino acid × 100 g of total protein.

encountered in roasted coffee were also found in the roasted fractions (B) and (C) which shows a good recovery of original coffee volatiles after fractionation. The higher amount of volatiles found in the roasted fractions as compared to the whole coffee is due to the concentration of aroma precursors after fractionation.

Seven identified furans are listed in Table 3. It has been well documented that the presence of furans in roasted coffee is due to sucrose degradation (Clifford, 1985; Dart & Nursten, 1985). In fact, sucrose is the principal carbohydrate present in fraction (B) which contains furans. However, furans were also identified in fraction (C) which did not contain sucrose (Table 1). Furans of the fraction (C) should be formed via arabinogalactan pyrolysis. On the other hand, as already mentioned by Baltes & Mevissen (1988), sugar degradation is more pronounced via Maillard reactions rather than pyrolysis. This may explain why furans were found in lesser quantity in roasted fraction (C) than in fraction (B) (Table 3). Consequently, the furans present in roasted coffee appear to be formed mainly by sucrose degradation but also by arabinogalactan pyrolysis.

Pyrazines are another group of powerful aroma compounds present in roasted coffee (Dart & Nursten, 1985; Goldman *et al.*, 1967). In roasted fraction (C), 11 alkylpyrazines were detected; however, none of them were found in the roasted fraction (B) (Table 3). The absence of pyrazines in fraction (B) may be owing to the low content of amino acids, in comparison to sucrose, which were not present in sufficient amounts to form detectable pyrazines. Based on these observations it is reasonable to conclude that high molecular weight water-soluble compounds of fraction (C) are the most relevant precursors of alkylpyrazines in roasted coffee. It is also suggested that aroma compounds from fraction (C) are mainly formed via pyrolysis due to the predominance of high molecular weight material. In fact, typical Maillard reaction compounds (i.e. acylpyrroles and furanones) were not found in this fraction. It is well documented that pyrolysis of hydroxy amino acids produce alkylpyrazines (Baltes & Bochmann, 1987*a*,*d*; Kato *et al.*, 1970; Wang & Odell, 1973). In this study there is strong evidence that threonine and also serine are the most important alkylpyrazine precursors since they were the most intensely degraded amino acids in fraction (C). Furthermore, only hydroxy amino acids produce pyrazines by pyrolysis (Wang & Odell, 1973). Consequently, pyrazines of roasted coffee are mainly formed by hydroxy amino acid pyrolysis.

Pyridines are the most important group of volatiles obtained by heating of trigonelline (Viani & Horman, 1974). Therefore, high loss of trigonelline during roasting (Table 2) was related to the high amounts of pyridine found in the roasted fraction (B) (Table 3). On the other hand, trigonelline was not found in roasted fraction (C) (Table 2) but pyridine was identified after roasting (Table 3). However, pyridine may also be formed by hydroxyamino acid pyrolysis (Baltes & Bochmann, 1987*a*) which are present in this fraction. These results indicate that pyridine of roasted coffee is formed not only by trigonelline degradation, but also by protein pyrolysis.

Cyclotene (2-hydroxy-3-methyl-2-cyclopentene-1-one) was readily detected in fraction (B) (Table 3) but only a

Table 3. Some compounds identified after roasting of coffee and of the water-soluble fractions[a]

Compounds	IK	Fractions			Ref.
		Roasted coffee	80% ethanol-soluble (B)	80% ethanol-insoluble (C)	
1-Methyl-pyrrole[d,e]	530	+ + +	+ + + +	+ +	1
5-Methyl-2-vinylfuran[e]	541	+ + +	+ + +	(+)	2
1-Ethyl-pyrrole[e]	571			(+)	1
Pyridine[d,e]	585	+ +	+ + + +	+ +	3,4
Pyrazine[d,e]	599	+ + +		+ + +	4,5,6
2-Methyl-tetrahydro-3-furanone[e]	633	+ +	+ + + +		2
Methylpyrazine[d,e]	639	+ + + +		+ + + +	4,5,6
2,5-Dimethyl-pyrazine[d,e]	678	+		+ + +	4,5,6
2,6-Dimethyl-pyrazine[d,e]	883	+		+ +	4,5
Ethylpyrazine[d,e]	809	+ +		+ +	4,6
2,3-Dimethyl-pyrazine[d,e]	897	(+)		+	5
2-Ethyl-6-methylpyrazine[d,e]	729	(+)		+ +	4,5,6
2-Ethyl-5-methylpyrazine[d,e]	732	(+)		+ +	4,5
Trimethyl-pyrazine[d,e]	737	+ + +*		+ +	4,5,6
2-Methyl-3-[2H]-furanone[e]	737	+ + +*	+ + +		2
2,6-Diethyl-pyrazine[e]	766	(+)		+ +	4,5,6
2,3-Diethyl-pyrazine[d,e]	774			+	5
Acetic acid[d,e]	783		+ + +		7
2-Furfural[d,e]	786	+ + +	+ + + +	(+)	2,8
Furfuryl-methanoate[e]	804	+ +			2,9
Pyrrole[e]	820		+ + + +		1
2-Methyl-pyrrole[e]	845		+ +		1
5-Methyl-2-furfural[e]	860	+ + +	+ + +	+ +	2,8
2-Formyl-1-methylpyrrole[e]	883	+ + +	+ + +		8
2-Acetyl-1-methylpyrrole[e]	886	+ +	+ +		1,8
2-Furfuryl-alcohol[e]	934	+ + +	+ + + +	+ + +	2,8
Cyclotene[e]	1014	+	+ +		3
2-Methoxy-phenol[d,e]	1049	+	+ +	(+)	8
Phenol[d,e]	1141	+	+ +	(+)	8

Results are averages of duplicate determinations; IK, modified retention index (Van den Dool & Kratz, 1963);[a,b,c] as in Table 1; [d]reference compound available; [e]identification via a published spectrum: 1, Baltes and Bochmann (1987*c*); 2, Baltes and Bochmann (1987*b*); 3, Baltes and Bochmann (1987*e*); 4, Goldman *et al.* (1967); 5, Baltes and Bochmann (1987*d*); 6, Kato *et al.* (1970); 7, Stenhagen *et al.* (1969); 8, Stoll *et al.* (1967). (+) Trace compound; + very low concentration; + + low concentration; + + + high concentration; + + + + extremely high concentration. * Estimate of abundance in the co-eluted compounds.

trace amount was found in fraction (C). Sucrose degradation is the main source of carbocyclic compounds (Nishimura & Mihara, 1990) and this appears to be the route of cyclotene formation in roasted coffee.

In the present work, six pyrroles were identified. It has been reported that alkylpyrroles can be formed via hydroxyamino acid pyrolysis (Baltes & Bochmann, 1987*c*). Thus, the formation of alkylpyrroles in fraction (C) appears to follow this route. Although pyrrole is extremely reactive, large amounts still remain in roasted fraction (C) (Table 3). This may in fact be expected because fraction (C) contains high molecular weight aroma precursors which are more difficult to interreact.

The results found in this work showed that fractions (B) and (C) are relevant and representative of coffee aroma precursors. The approach used to collect the headspace fraction formed after roasting proved to be rapid and appropriate for the analysis of the aroma compounds produced.

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REFERENCES

Albersheim, P., Nevins, D. J., English, P. D. & Barr, A (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. *Carbohydr. Res.*, **5**, 340–5.

Baltes, W. & Bochmann, G. (1987*a*). Model reactions on roast aroma formation. I: Reaction of serine and threonine with sucrose under the conditions of coffee roasting and identification of new coffee aroma compounds. *J. Agric. Food Chem.*, **35**, 340–6.

Baltes, W. & Bochmann, G. (1987*b*). Model reactions on roast aroma formation. II: Mass spectrometric identification of furans and furanones from the reaction of serine and threonine under the conditions of coffee roasting. *Z. Lebensm. Unters Forsch.*, **184,** 179–88.

Baltes, W. & Bochmann, G. (1987*c*). Model reactions on roast aroma formation. III: Mass spectrometric identification of pyrroles from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Z. Lebensm. Unters Forsch.*, **184,** 478–84.

Baltes, W. & Bochmann, G. (1987*d*). Model reactions on roast aroma formation. IV: Mass spectrometric identification of pyrazines from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Z. Lebensm. Unters Forsch.*, **184**, 485–93.

Baltes, W. & Bochmann, G. (1987*e*). Model reactions of roast aroma formation. V: Mass spectrometric identification of pyridines, oxazoles, and carboxyclic compounds from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Z. Lebensm. Unters Forsch.*, **185,** 5–9.

Baltes, W. & Knoch, E. (1993). Model reactions on roast aroma formation. II: The formation of some uncommon N-heterocyclic compounds and furans after roasting of tryptophan with reducing sugars and sugar degradation products. *Food. Chem.*, **48,** 343–9.

Baltes, W. & Mevissen, L. (1988). Model reactions of roast aroma formation. VI: Volatile reaction products from the reaction of phenylalanine with glucose during cooking and roasting. *Z. Lebensm. Unters Forsch.*, **187,** 209–14.

Bohnenstengel, C. & Baltes, W. (1992). Model reactions on roast aroma formation. XII: Reaction of glucose with aspartic acid or asparagine at three different temperatures. *Z. Lebensm. Unters Forsch.*, **194**, 366–71.

Clifford, M. N. (1985). Chemical and physical aspects of green coffee and coffee products. In *Coffee: Botany, Biochemistry and Production of Beans and Beverage*, eds M. N. Clifford & K. C. Willson. Croom Helm, Ltd, London, pp. 305–74.

Coelho, R. R. R., Linhares, L. F. & Martin, J. P. (1985). Amino acid distribution in some fungal melanins and of soil humic acids from Brazil. *Plant & Soil*, **87,** 337–46.

Dart, S. R. & Nursten, H. E. (1985). Volatile components. In *Coffee: Chemistry* (Vol. 1), 3rd edn. eds R. J. Clarke & R. Macrae. Elsevier Applied Science, London, pp. 223–65.

De Maria, C. A. B., Trugo, L. C., Moreira, R. F. A. & Werneck, C. C. (1994). Composition of green coffee fractions and their contribution to the volatile profile formed during roasting. *Food Chem.*, **49,** 141–5.

Goldman, I. M., Seibl, J., Flament, I., Gautschi, F., Winter, M., Willhalm, B. & Stoll, M. (1967). Sur l'aróme de café, II: Pyrazines et pyridines. *Helv. Chim. Acta*, **50**, 694–705.

Kato, S., Kurata, T., Ishitsuka, R. & Fujimaki, M. (1970). Pyrolysis of ß-hydroxy-amino acids, especially L-serine. *Agric. Biol. Chem.*, **34,** (12), 1826–32.

Nishimura, O. & Mihara, S. (1990). Investigation of 2-hydroxy-2-cyclopenten-1-ones in roasted coffee. *J. Agric. Food Chem.*, **38,** 1038–41.

Pearson, D. (1976). *The Chemical Analysis of Foods*, 7th edn. Churchill Livingstone, London, pp. 11–12.

Stenhagen, E., Abrahamsson, S. & McLafferty, E. M. (1969). *Atlas of Mass Spectral Data* (Vol. I). Wiley, New York, p. 264.

Stoll, M., Winter, M., Gautschi, F., Flament, I. & Willhalm, B. (1967). Sur l'aróme de café. I. *Helv. Chim. Acta*, **50,** 628–93.

Trugo, L. C., De Maria, C. A. B. & Wernerck, C. C. (1991). Simultaneous determination of total chlorogenic acid and caffeine in coffee by high-performance gel filtration chromatography. *Food Chem.*, **42,** 81–7.

Trugo, L. C., Macrae, R. & Dick, J. (1983). Determination of purine alkaloids and trigonelline in instant coffee and other beverages using high-performance liquid chromatography. *J. Sci. Food Agric.*, **34,** 300–6.

Van den Dool, H. & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *J. Chromatogr.*, **11**, 463–71.

Viani, R. & Horman, I. (1974). Thermal behavior of trigonelline. *J. Food Sci.*, **39**, 1216–17.

Wang, P. S. & Odell, G. V. (1973). Formation of pyrazines from thermal treatment of some amino-hydroxy compounds. *J. Agric. Food Chem.*, **21,** 868–70.

Wolfrom, M. L. & Patin, D. L. (1965). Carbohydrates of the coffee bean. IV. An arabinogalactan. *J. Organic Chem.*, **30,** 4060–83.