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Article

The GC/MS Identification of Volatiles Formed During the Roasting of High Molecular Mass Coffee Aroma Precursors

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Os compostos voláteis formados nas frações torradas obtidas do café verde foram analisados e identificados por CG/EM. As frações precursoras de aroma estudadas foram: fração 1, insolúvel em água contendo predominantemente polissacarídeos e proteínas; e fração 2, correspondente aos lipídeos totais do grão verde. Após a torração da fração 1, houve uma perda de 46% de arabinose da arabinogalactana indicando grande susceptibilidade desse polissacarídeo à torração. Os resultados mostram que a arabinogalactana é o principal polissacarídeo envolvido na formação de derivados do furano presentes no café torrado e que aminoácidos ligados a proteína são precursores importantes de pirazinas. Uma parte dessas pirazinas parece originar-se da pirólise da treonina. Os lipídios desenpenhariam um papel secundário na formação do aroma no café torrado.

Volatile compounds formed during the roasting of green coffee fractions were analyzed by GC/MS. The green coffee aroma precursor fractions studied were: fraction 1, water-insoluble and rich in polysaccharides and proteins, and fraction 2, representing the total lipids from the green bean. A loss of 46% of arabinose was observed in fraction 1, indicating that arabinogalactan, present in this fraction, is more sensitive to roasting than other polysaccharides such as mannan and glucan. The results indicate that arabinogalactan is the main polysaccharide involved in the formation of furans in roasted coffee. Amino acids, particularly threonine, appear to be the source of pyrazine during coffee roasting. Lipids appear to contribute very little to aroma formation in roasted coffee.

Keywords: *capillary gas chromatography, mass spectrometry, coffee, aroma compounds*

Introduction

The attribute of aroma is of great relevance for the assessment of the quality of coffee products. Aroma formation in roasted coffee occurs via Maillard and Strecker reactions and pyrolysis[1] which are extremely complex and difficult to monitor. Simpler models containing free amino acid and sucrose have been submitted to coffee roasting conditions[2,3]. Such models provide invaluable insights into the underlying aroma formation but they do not take into account high molecular mass aroma precursors present in the green coffee bean.

We have previously reported a method for isolating fractions from green coffee based on its water solubility and molecular mass[4]. Specific groups of compounds isolated from green coffee were then used to study aroma formation under coffee roasting conditions. To obtain volatile profiles produced by the roasted fractions, we used an off-line headspace method which allows the collection of the aroma fraction immediately after roasting. The purpose of the present study was to identify the volatile compounds formed by roasting high molecular mass water-insoluble fractions using high resolution gas chromatography/mass

spectrometry (HRGC/MS). The flavor precursors of these fractions were monitored before and after roasting.

Materials and Methods

Materials

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

Isolation of fractions

Finely ground green coffee was submitted to Soxhlet extraction with petroleum ether[5]. The defatted material (100 g) was then extracted with 4 L hot bi-distilled water (80 °C) in a shaker-water bath for 15 min. The insoluble material obtained by centrifugation at 100 x g for 10 min and freeze-drying was called fraction 1. The ether soluble material (total lipids) obtained by Soxhlet extraction was called fraction 2.

Flavor precursors

The amino acids were analyzed after hydrolysis in fraction 1 as described by Coelho *et al.* [9], and the crude protein by the Kjeldahl method[5]. The total CGA and the trigonelline method were in accordance with Trugo *et al.*[7,8] Sugar determination was based on the method described by Albersheim *et al.*[6]

Headspace method

Individual 0.5 g samples of fractions 1 and 2 were roasted in an oven at 200 ± 2 °C for 14 min in test tubes having screw caps (Duran, Germany) with a hole (1.5 mm dia) and with septum (10043 - Chrompack, Germany), according to De Maria *et al.*[4] The sealed tube was then submitted to vacuum (1 min) using a microsyringe connected to a vacuum pump (E2M8, EDWARDS, Brazil). Immediately after roasting, a 4 mL headspace sample was collected and injected by means of a 10 mL gas-tight syringe (Hamilton, USA) into the chromatographic column.

Chromatography and MS

HRGC was determined according to De Maria *et al.*[4]. A 30 m x 0.25 mm (id) Supelcowax 10 bonded-phase fused-silica capillary column (Supelco, USA) was installed into a Carlo Erba model 4300 gas chromatograph (Carlo Erba, Italy). Injection was in the split mode with a 1:20 ratio. The detector (FID) and injector temperatures were 280 °C and 100 °C, respectively. The oven temperature was held at 40 °C for 6 min and then programmed to reach 190 °C at 3 °C/min. The linear flow rate of hydrogen gas was 40 cm/s.

HRGC/MS analysis was carried out using a mass spectrometer (HP5987- A) in the EI mode with a RTE - 6 / E data system with a cyclic scan of 1 s and a mass range of m/z 50 -500. Modified Kóvats indices were calculated according to van den Dool and Kratz[10].

Results and Discussion

Table 1 shows the amount of aroma precursors in the high molecular mass water-insoluble fraction 1. After roasting, there was a 46% loss of arabinose residue from the arabinogalactan, which is an indication of its greater susceptibility to roasting when compared to mannan and glucan.

The distribution of amino acids produced in protein hydrolysis is also presented in Table 1. There was a reduc-

Table 1. Distribution of carbohydrates, tigonelline, total CGA, crude protein and amino acids before and after roasting the water-insoluble fraction obtained from green coffee.

Compounds	fraction 1 (g%)	
	non-roasted	roasted
galactose	24.8	23.9
arabinose	11.0	6.0
mannose	38.0	41.7
glucose	20.0	22.0
xylose	nd	nd
sucrose	trace	nd
trigonelline	nd	nd
total CGA	0.5	0.6
protein (N x 6.25)	7.0	6.4
fraction 1 (g% of each amino acid / 100g of total protein)		
lysine	2.9	2.5
histidine	2.5	2.2
arginine	2.8	2.3
aspartic acid	9.7	10.2
threonine	1.9	1.3
serine	0.4	0.3
glutamic acid	25.2	28.4
proline	6.4	5.9
glycine	9.7	9.4
alanine	7.1	6.4
valine	7.3	6.9
isoleucine	5.0	4.6
leucine	13.3	13.6
tyrosine	0.8	0.7
phenylalanine	5.0	5.5

Results are the average of duplicate determinations on defatted and dry basis; Polysaccharides are expressed as the monosaccharides produced on hydrolysis; nd - not detected.

tion in the amounts of individual amino acids present after roasting (alanine, valine, proline, threonine, arginine, lysine), as expected. Conversely, glutamic acid showed an apparent increase with roasting. Differences in the stability of the amino acids under roasting may be attributed to both their individual chemical properties and their position within the protein structure.

Aroma precursors from fraction 2 (lipid fraction) were not determined because after roasting only dimethyl benzene isomers which are not characteristic of coffee aroma, were identified (Fig. 1 and Table 2).

Aroma compounds identified in fraction 1 by MS are presented in Fig. 1 and Table 2. Methyl pyrazine was the most important pyrazine found in fraction 1, but other pyrazines (ethylpyrazine and pyrazine) were also detected in this fraction. In previous work, pyrazines were also encountered in roasted high molecular mass water-soluble fractions, but not in low molecular mass fractions[11]. So, amino acids that are bound in proteins are important pyrazine precursors in roasted coffee. It has been previously reported that the pyrolysis of hydroxyamino acids form alkylpyrazines[2,12], but that other amino acids do not[13]. In Table 1, there was a decrease (31.6%) in threonine after roasting fraction 1. Thus, pyrazines could be in part directly derived from pyrolitic degradation of protein-bound threonine residues. Indeed, we observed previously that threonine is markedly degraded in the high molecular mass water-soluble fractions submitted to roasting[11].

Table 2 also lists the furan derivatives found in fraction 1. The 2 - furfural was the major furan found in roasted fraction 1. As furans are well-known as carbohydrate breakdown products[1], the furan derivatives found in the roasted fraction originate from polysaccharide degradation. So, we suggest that arabinogalactan, the major poly-

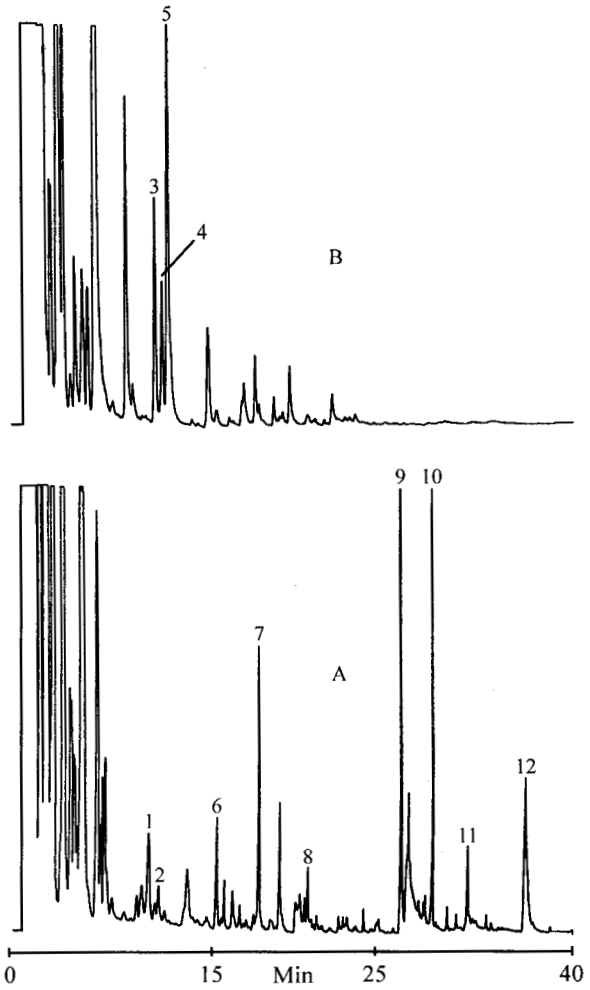

Figure 1. Profiles of headspace volatiles from roasted fractions 1 and 2, isolated from green coffee. A) Fraction 1: water-insoluble residue. B) Fraction 2: total lipids. Chromatographic conditions[4]: Carlo Erba model 4300 GC, splitless injector, FID, Supelcowax 10 column (30 x 0.25 mm), H_2 (40 cm/s). See Table 2 for a key to peak numbering.

Table 2. Some aroma compounds identified by GC/MS after the roasting of water-insoluble fractions obtained from green coffee.

Compounds	IK	fraction 1	fraction 2	Ref.**
1 1-methylpyrrole	530	+	nd	14
2 5-methyl - 2 - vinylfuran	541	+	nd	3
3 1,4-dimethylbenzene*	541	nd	+++	15
4 1,3-dimethylbenzene*	547	nd	+	15
5 1,2-dimethylbenzene*	552	nd	++++	15
6 Pyrazine*	599	+	nd	16
7 Methylpyrazine*	639	+++	nd	16
8 Ethylpyrazine*	689	+	nd	16
9 2-furfural*	786	++++	nd	3
10 Pyrrole	820	++++	nd	14
11 5-methyl-2-furfural	860	+	nd	3
12 2-furfuryl-alcohol	934	+	nd	3

Results are the average of duplicate determinations; * Standard compounds available; ** References to the published spectrum; IK modified retention index[10]; nd - not detected; + Very low concentration; ++ Low concentration; +++ High concentration; ++++ Extremely high concentration.

saccharide affected after roasting fraction 1 (Table 1), is a precursor of furans in roasted coffee.

Pyrrole and 1-methyl pyrrole were also identified in large amounts in fraction 1 (Fig. 1 and Table 2). This is unexpected since pyrroles are very reactive, and may be an indication that the formation rate is higher than the rate of further reactions.

In Fig. 1, peaks in the range of 0 - 5 min were detected in fractions 1 and 2, but it was not possible to identify the volatiles due to poor resolution between the peaks.

Conclusions

- Protein-bound amino acids are important pyrazine precursors in roasted coffee. Pyrazines may in part be directly derived from threonine pyrolysis.
- Arabinogalactan appears to be the main polysaccharide involved in the formation of furans in roasted coffee.
- Lipids seem to make little contribution to the aroma formation of roasted coffee.

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References

1. M.N. Clifford and K.C. Willson, Ed.; Chemical and physical aspects of green coffee and products. In: *Coffee: Botany, Biochemistry and production of beans and beverage,* Croom Helm.; 1985 p. 305.
2. Baltes, W.; Bochmann, G. *J. Agric. Food Chem.* **1987**, *35*, 340.
3. Baltes, W.; Bochmann, G. *Z. Lebensm. Unters Forsch* **1987**, *184*, 179.
4. De Maria, C.A.B.; Trugo, L.C.; Moreira, R.F.A.; Werneck, C.C. *Food Chem.* **1994**, *50*, 141 (1994).
5. Coelho, R.R.R.; Linhares, L.F.; Martin, J.P. *Plant and Soil* **1985**, *87*, 337.
6. Pearson, D. *The Chemical Analysis of Foods*; Churchill Livingstone, 1976; 7th ed., p. 11.
7. Trugo, L.C., De Maria, C.A.B.; Werneck, C.C. *Food Chem.* **1991**, *42*, 81.
8. Trugo, L.C.; Macrae, R.; Dick, J. *J. Sci. Food Agric.* **1983**, *34*, 300.
9. Albersheim, P.; Nevins, D.J.; English, P.D.; Karr, A. *Carbohyd. Res.* **1967**, *5*, 340.
10. Van den Doll, H.; Kratz, P.D. *J. Chromatogr.* **1963**, *11*, 463.
11. De Maria, C.A.B.; Trugo, L.C.; Aquino Neto, F.R.; Moreira R.F.A.; Alviano, C.S. *Food Chem.* **1996**, *55* (3), 203.
12. Kato, S.; Kurata, T.; Ishitsuka, R.; Fujimaki, M. *Agr. Biol. Chem.* **1970**, *34*, 1826.
13. Wang P.S.; Odell, V. *J. Agric. Food Chem.* **1973**, *21*, 868.
14. Baltes W.; Bochmann, G. *Z. Lebesm. Unters Forsch.* **1987**, *184*, 478.
15. Stenhagen, E.; Abrahamsson, S.; McLafferty, F.W. *Atlas of Mass Spectral Data*, Wiley, 1969; Vol. 1.
16. Baltes W.; Bochmann, G. *Z. Lebesm. Unters Forch.* **1987**, *184*, 485.