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Composition of green coffee fractions and their contribution to the volatile profile formed during roasting

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A sequential fractionation method to isolate water-soluble fractions of green Arabica coffee is described. Low- and high-molecular-weight fractions were obtained by treating the dried water extract with aqueous ethanol (80%, v/v). High-performance gel filtration chromatography was used to show some differences in molecular weight distribution between fractions. Flavour precursors were detected in both fractions. Arabinogalactan was the main polysaccharide found in the water-soluble high-molecular-weight fraction, together with large amounts of protein. Important flavour precursors such as sucrose, trigonelline and chlorogenic acid were detected in the low-molecular-weight fraction. A simple and rapid headspace method was developed to obtain the volatile profiles produced by the roasted fractions. Volatiles were immediately collected after roasting in a sealed tube and submitted to gas chromatography. The chromatographic profiles obtained showed considerable differences between fractions and revealed an important contribution to aroma formation.

INTRODUCTION

Many complex and poorly-defined reactions take place during roasting to produce the characteristic flavour of coffee. The importance of Maillard and Strecker reactions, sugar caramelisation and degradation of trigonelline, chlorogenic acids (CGA), proteins and polysaccharides in the formation of coffee attributes has been well described (Clifford, 1985; Dart & Nursten, 1985). The amounts and composition of flavour precursors in green coffee can have a dramatic effect on the quality of the final roasted product. Polysaccharides, lipids and proteins appear to be the major constituents of the green bean (Underwood & Deatherage, 1952; Clifford 1985). However, minor components such as free amino acids, trigonelline, CGA, free sugars (mainly sucrose) and others. are also relevant (MacDonald & Macrae, 1985; Trugo & Macrae, 1989). The minor compounds are of particular interest because they are an important source of coffee aroma.

A number of papers has recently been published concerning the model reactions of roast aroma formation by treating one or two selected amino acids with sugars under coffee roasting conditions (Baltes & Bochmann. 1987*a–e*; Baltes & Mevissen, 1988; Kunert-Kirchhoff & Baltes, 1991*a,b*). Although these studies are useful, they may represent the formation of artefacts because some coffee components are not available to inter-react. The situation is further complicated by the fact that some of the aroma precursors can be encountered as macromolecular constituents.

Our aim was to develop a method for the isolation of low- and high-molecular-weight fractions from green coffee and to obtain the volatile profiles produced by the water-soluble roasted fractions by the use of a simple headspace method. High-performance gel filtration chromatography was used to show relevant differences between the water-soluble fractions obtained. Reverse-phase HPLC and capillary gas chromatography (GC) were applied to the analyses of some flavour precursors and aroma compounds.

MATERIAL AND METHODS

Materials

Green Arabica coffee was obtained from a local industry. Finely ground green coffee was extracted with petroleum ether to remove lipids. All analyses were carried out with freeze-dried samples. All reagents were of analytical-reagent grade. Standard sugars were obtained from Merck (Germany), chlorogenic acid (5-caffeoylquinic acid) from Carl Roth (Germany) and trigonelline from Sigma (USA).

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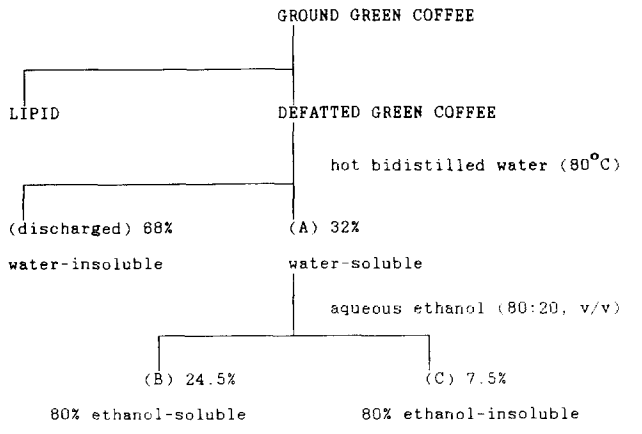

Fig. 1. Fractions isolated from green Arabica coffee.

Methods

Isolation of fractions

A sequential fractionation procedure used to isolate some fractions from green coffee is described (Fig. 1). Finely ground defatted green coffee (100 g) was extracted with 4 litres hot bidistilled water (80°C) in a water bath with shaking for 15 min. The insoluble fraction was removed by filtration under gravity and the resulting soluble fraction (A) was then freeze-dried. A 20 g sample of fraction (A) was dissolved in 25 ml of bidistilled water (50°C) and (100 ml) ethanol was then added. The mixture was maintained under shaking for 1 h and centrifuged at 100 × *g* for 10 min. The residue was extracted again four times for 30 min each time. The combined supernatants were filtered and transferred to a round-bottom flask. Both filtrate (B) and residue (C) were freeze-dried separately.

Crude protein, sugars and CGA

Crude protein was determined by the standard Kjeldahl procedure (Pearson, 1976). Sugars were determined by GC after sample hydrolysis. Fractions (0·15 g) were hydrolysed in 72% (v/v) sulphuric acid (135 ml) (agitation, 15 min, 50°C), followed by addition of fucose (internal standard), dilution to 9% acid and heating (refluxing, 4 h 105°C). Sodium carbonate was added (pH 6) and the resultant mixture filtered. The filtrate was concentrated and the resultant monosaccharides were converted to the alditol acetate derivatives and their analyses were performed by GC using an OV 225 column (Intralab-Brazil) (20 m × 0·25 mm i.d.) (Albersheim *et al.*, 1967).

Total CGA determination was carried out using the method described by Trugo *et al.* (1991). Trigonelline was determined as described by Trugo *et al.* (1983). These methods were applied to the analysis of fractions (B) and (C).

High performance gel filtration chromatography

A 0·5 g sample of finely ground defatted green coffee was extracted with 30 ml of hot bidistilled water (80°C) and maintained under shaking for 15 min. The extract (fraction A) was filtered into a 50-ml volumetric flask and made up to volume. An aliquot was refiltered using a Millipore filter (0·45 μm). Fractions (B) and (C) (0·5 g) were just dissolved in bidistilled water (50 ml) and filtered on a Millipore membrane. All Millipore filtrates were examined by chromatography using a TSK-G 3000 SW (300 × 8 mm id) gel-filtration column (LKB-Sweden) as described by Trugo *et al.* (1991).

Headspace analysis

Individually 0·5 g of ground defatted green coffee and fractions (B) and (C) were roasted at 220 ± 2°C in a oven (Heraeus, Germany) for 14 min in test tubes with screw caps (Duran, Germany) and septa (No. 10043-Chrompack, Germany). A hole (1·5 mm dia.) was made in the screw caps in order to connect a microsyringe through the septum. Before roasting the sample, the sealed tube was 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, Switzerland) into the chromatographic column. A 30 m × 0·25 mm (id) Supelcowax 10 bonded-phase fused-silica capillary column (Supelco USA) was installed on a Carlo Erba model 4300 gas chromatograph (Carlo Erba Germany). 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 programmed to 190°C at 3°C/min (Shimoda & Shibamoto, 1990). The linear flow rate of hydrogen gas was 40 cm/s.

RESULTS AND DISCUSSION

The sequential fractionation procedure, described in this work appears to be adequate to study isolated groups of components from green coffee and their participation in the roasting reactions and aroma formation. Furthermore, the composition of the aroma formed is more genuine than in model reactions containing only selected standard components. The fractions studied were the water-soluble fractions (B) and (C) derived from (A) by treatment with aqueous ethanol 80% (Fig. 1). Fraction (A) contains the water-soluble material important for flavour formation. Fractions (B) and (C) contain separately, the most relevant water-soluble flavour precursors of low and high molecular weight.

Green coffee and fractions (B) and (C) were submitted to high-performance gel filtration chromatography using a TSK-G3000 SW column. Before chromatography, the dissolved fraction (C) was filtered to remove a small amount of residue formed probably due to some protein denaturation during ethanol precipitation. Detection at 280 nm showed relevant differences between these fractions due to the solvent

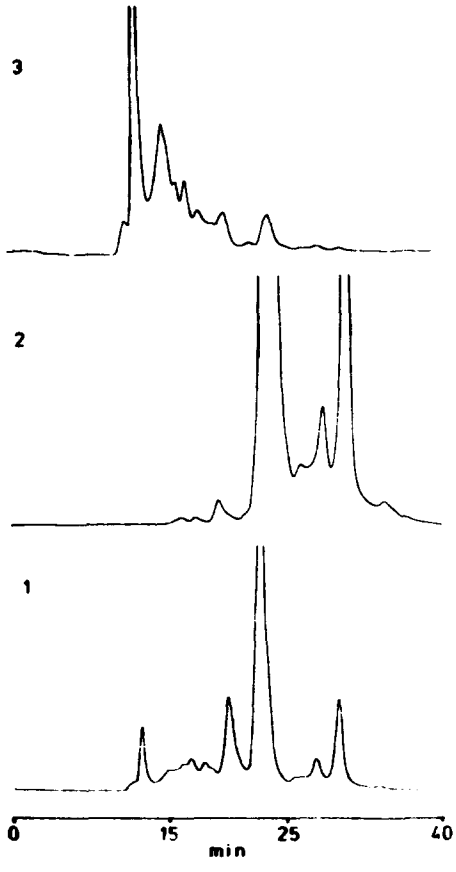

Fig. 2. Gel filtration chromatography of green coffee (1) and fractions B (2) and C (3), using the TSK-G 3000 SW column. Mobile phase was bidistilled water containing 0·05% (w/v) of sodium azide at flow rate of 0·5 ml/min. Detection at 280 nm.

fractionation. Fraction (B) showed concentrated amounts of low-molecular-weight material as compared to fraction (A) and the opposite was observed in fraction (C) (Fig. 2). Fractions (B) and (C) presented relevant water-soluble flavour precursors of low and high molecular weight including polysaccharide, protein, trigonelline, sucrose and total CGA (Table 1). Polysaccharides were expressed as the monosaccharides produced on hydrolysis. Fraction (C) was mainly

Table 1. Distribution of carbohydrates, trigonelline, total CGA and protein in fractions obtained from green coffee

	Fractions[a] (g%)	
	80% ethanol-soluble (B)	80% ethanol-insoluble (C)
Galactose	tr[b]	21·2
Arabinose	tr	18·1
Mannose	tr	6·9
Glucose	tr	1·9
Xylose	tr	1·9
Sucrose	17·4	—
Trigonelline	5·3	—
Total CGA	33·2	—
Protein (N×6·25)	7·5	40·0

[a] Results are averages of duplicate determinations; g% dry basis.
[b] tr, trace.

protein and polysaccharide with galactose, arabinose and mannose, as well as less xylose and glucose. components. A water-soluble arabinogalactan which has been shown to occur in green coffee has a linked galactan main chain, with galactose- and mainly arabinose-containing side chains with an arabinose–galactose ratio very close to 2 : 5. (Wolfrom & Patin, 1965). In this study the arabinose–galactose ratio found 2 : 2·4) was higher and this difference may be due to the more drastic treatment with chlorine dioxide used by Wolfrom and Patin (1965). It has been reported that chlorine dioxide may degrade polysaccharides (Masschelein, 1979). A mannan was also isolated by Wolfrom *et al.* (1961) but it was less soluble in water than arabinogalactan. The mannan present in fraction (C) comes from a water-soluble material and it may then constitute a very small part of the original mannan from green coffee.

In a pyrolytic reaction, the relative stability of the glycans decreases in the sequence glucan, mannan, galactan and araban (Clifford, 1985). So arabinogalactan is the most affected polysaccharide present in coffee. In fact, Wolfrom and Anderson (1967) isolated an arabinogalactan residue (2 : 25) ratio from instant coffee that had a much lower arabinose content than the arabinogalactan (2 : 5 ratio) from green coffee (Wolfrom & Patin, 1965). Considering that the arabinogalactan is the major polysaccharide in fraction (C), it appears that it will contribute significantly to flavour formation in coffee. Fraction (B) contains sucrose as the most important free sugar. On roasting, sucrose is extensively degraded and largely involved in the formation of flavour including aroma material (Trugo & Macrae, 1989). Carbocyclic compounds are known to be present in volatiles from sucrose pyrolysis (e.g. cycloten) (Nishimura & Mihara, 1990). Sucrose is also involved in Maillard reactions forming, for example, furanones, monocyclic furans, acetylpyridines (Baltes & Bochmann, 1987*b,e*). Sucrose is therefore an important participant in aroma formation of fraction (B).

Protein was another important constituent found in fraction (C) (Table 1) which is consistent with data of Underwood and Deatherage (1952) who reported 20–30% of water-soluble protein of the total protein in green coffee. Other nitrogen components were only found in trace amounts in this fraction. Roasting causes significant chemical modifications, including loss of protein nitrogen, i.e. as heterocyclic volatiles (Clifford, 1985). Therefore, protein of fraction (C) can be another important source of volatiles in this fraction. Protein in fraction (B) comprises amino acids and peptides. These free amino acids will be readily available for roasting reactions when compared to protein from fraction (C). Trigonelline is an important flavour precursor and its degradation products include a range of volatiles such as pyridines and pyrroles (Viani & Horman, 1974). Since 50% of non-protein nitrogen, in fraction (C), is represented by trigonelline, this will certainly have a relevant role during aroma formation.

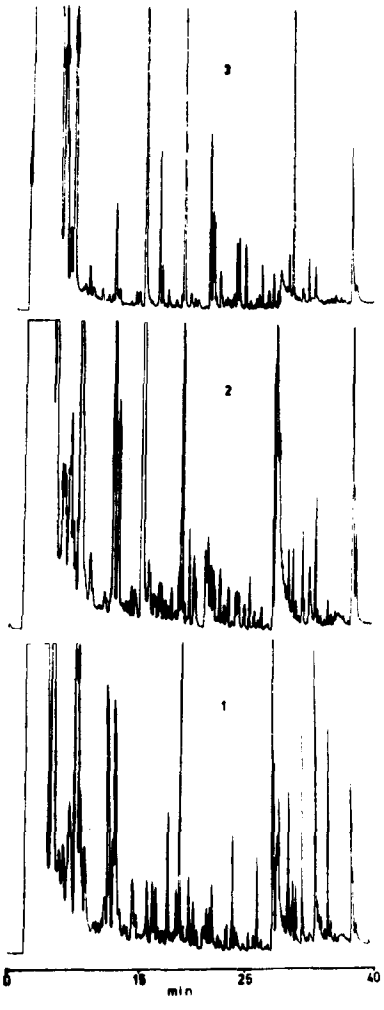

Fig. 3. Profiles of headspace volatiles from roasted defatted green coffee (1) and fractions B (2) and C (3), by gas chromatography, using the Supelcowax 10 column and FID detector. Conditions as in headspace analysis section.

The esters of quinic acid known as CGA are major components of green coffee and roasting causes progressive loss of extractable CGA with production of the characteristic flavour components (Clifford, 1985). Thirty phenols have been isolated from coffee products (e.g. guaiacol, 4-vinylguaiacol and phenol) (Tressl *et al.*, 1978). A part of aroma components formed from fraction (B) may be derived from degradation of phenolic acids, since CGA is present in large amounts (Table 1).

Headspace methods have been used by different workers to study aroma components of coffee. These methods are generally quicker, simpler and yielding of true aroma profile, as there is less possibility of artefact formation than in classical distillation techniques (Dart & Nursten, 1985; Shimoda & Shibamoto, 1990). The headspace method proposed in the present work allows study of the aroma arising immediately after roasting of the fractions. The headspace is collected and injected immediately after roasting the samples at a controlled temperature. Since the method is not intended to be quantitative, the chromatographic profiles of replicate samples were just compared and showed no qualitative differences (data not shown). Volatile profiles of roasted green coffee and fractions (B) and (C) are illustrated in Fig. 3. A complex pattern appeared in the roasted samples. Some peaks obtained in the fractions were not found in the original roasted green coffee. This is an indication that there was some concentration of coffee volatiles in the roasted fractions. However, some peaks detected in the roasted green coffee were not encountered in the fractions, showing that some volatiles might be formed by reactions between low- and high-molecular-weight precursors. It is apparent that fraction (B) produces a much richer profile than fraction (C) and this is due to the presence of low-molecular-weight flavour precursors present (CGA, trigonelline, sucrose and amino acids) which are readily available to react. Fraction (C) has less diversity and availability of flavour precursors to react than fraction (B) and their formed volatiles may then have a minor impact in the aroma of coffee.

Identification of specific components revealed in the profiles are now in progress using the same headspace technique applied to GC–MS. The distribution of non-volatile components in the fractions after roasting will be also investigated.

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