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An Investigation of Coffee Roasting Using High Performance Gel Filtration Chromatography

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ABSTRACT

Changes in the molecular weight distribution of components in green coffee during roasting were investigated using high performance gel filtration chromatography (HPGFC) with TSK-PW 4000 columns. The chromatographic separation of sample extracts obtained from green and roasted coffees, after different degrees of roasting, was monitored by refractive index (RI) detection and UV detection at 280, 325 and 420 nm. The chromatograms obtained with RI detection showed significant differences in molecular weight profiles. UV detection showed similar patterns at 280 and 325 nm, suggesting that phenolic compounds were probably bound to proteins. The formation of pigments was clearly demonstrated when chromatography was carried out with detection at 420 nm and the coloured material was found to be distributed throughout the molecular weight range studied. The use of HPGFC provided a more rapid analysis compared with traditional gel filtration and proved to be useful in monitoring changes in molecular weight which occur during coffee roasting.

INTRODUCTION

Many complex reactions take place during coffee roasting, including condensation reactions between amino groups and carbonyl compounds

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(Maillard reaction), caramelisation of sucrose and degradation of trigonelline, chlorogenic acids, proteins and polysaccharides. The characteristic flavour of coffee is formed during roasting and involves these complex and poorly understood processes. These reactions also significantly change the molecular weight profile of the components of green coffee and, therefore, a more detailed study of the nature of the high molecular weight material formed will assist in a better understanding of the mechanisms of flavour formation, aroma retention and the changes which may occur during extraction in instant coffee production.

Investigations of the molecular weight profiles of coffee extracts have mainly been carried out using gel filtration chromatography with Sephadex (Maier *et al.*, 1968; Nakabayashi & Watanabe, 1977) or Bio-gel (Barbetti & Chiappini, 1978). However, these techniques frequently provide poor resolution and are time consuming. More recently, new column packing materials have been developed for gel filtration and many of these are mechanically stable and can therefore be used as small particles under high pressures. Some examples of these new chromatographic phases are TSK-gel PW type (Hashimoto *et al.*, 1978) which is made of cross-linked hydrophilic polymers and TSK SW type (Rokushika *et al.*, 1979) which consists of silica gel chemically bonded with hydrophilic compounds. Analyses using these materials can often be carried out with a significant reduction in time and, in most cases, with a considerable improvement in resolution. In this work TSK-PW 4000 columns were used to study the molecular weight distribution of green and roasted coffee as a function of the degree of roasting. The results presented are an extension of the preliminary data reported at the 10th ASIC Colloquium (Trugo & Macrae, 1983).

MATERIAL AND METHODS

Sample preparation

Ground green or roasted Robusta (Uganda) coffee (*ca.* 2 g) was extracted with boiling distilled water (20 ml) by shaking for 3 min in a boiling water bath. The contents were immediately filtered under reduced pressure and, after cooling to room temperature, refiltered using a Millipore filter (0·45 μm). This filtrate was then used for chromatography.

Chromatography

Chromatographic separation was achieved using two TSK 4000-PW columns (300 × 8 mm inside diameter each) (Varian Associates Ltd, Great Britain). Deionised water was used as mobile phase at a flow rate of 0·8 ml min^{-1}. The temperature of the column was kept at 20 °C by means of a column water jacket. Detection was achieved either using a refractive index (RI) detector (Model 750/14, Applied Chromatography Systems Ltd, Great Britain) at attenuation × 4, in series with a variable wavelength detector (Model CE 212, Cecil Instruments Ltd, Great Britain) set at 420 nm and 0·5 AUFS, or two UV detectors, one at 280 nm (Model 750/11, ACS Ltd, Great Britain) and the other at 325 nm (Model CE 212), both at 1 AUFS. Injection was by means of a 50 μl fixed loop injection valve (Model 7120, Rheodyne Incorporated, USA).

RESULTS AND DISCUSSION

The chromatograms obtained using RI detection showed major differences between the green and the roasted coffees in both the high molecular weight region ($> 2{\cdot}5 \times 10^5$) and the low molecular weight region (*ca.* 10^3). Very high molecular weight material is also formed during the roasting process but the corresponding peaks decreased when severe roasting conditions were used, indicating that some of this material was degraded (Fig. 1). The peak tentatively assigned as sucrose indicated a very high content in the green coffee but this decreased considerably during roasting. In the intermediate molecular weight region a more complex pattern appeared in the roasted samples, in comparison to the green coffee, suggesting that more complex compounds have been formed. Other peaks were further retained on the column (below 10^3) indicating that, for these components, adsorption, as well as gel filtration, is important.

The formation of pigments is clearly demonstrated when chromatography was performed with detection at 420 nm. Very little coloured material appeared in the green coffee, as would be expected, but many peaks were detected in the roasted samples, showing the formation of considerable amounts of pigments. The coloured material seems to be distributed throughout the molecular weight range studied with a considerable amount being formed with a molecular weight above

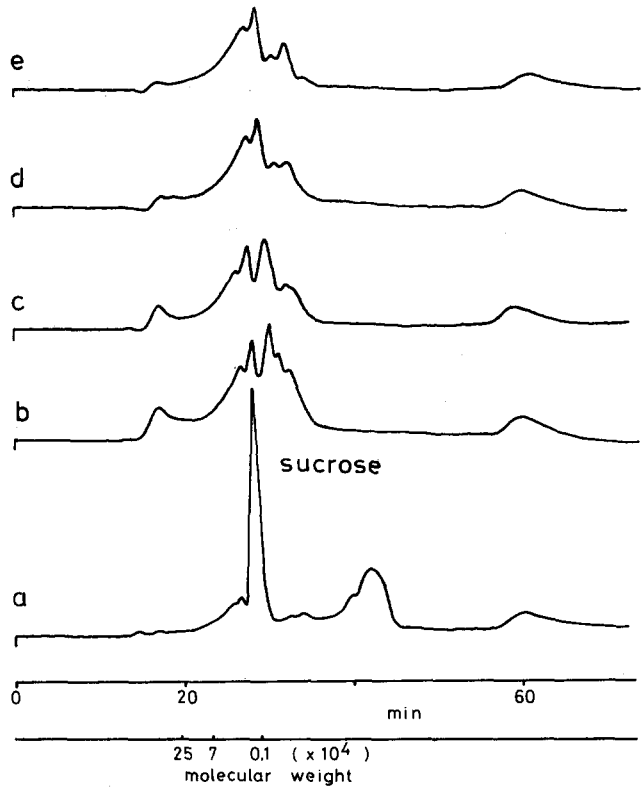

Fig. 1. Gel filtration chromatography of green and roasted Robusta (Uganda) coffee using refractive index detector. Chromatography with two TSK-PW 4000 columns in series (300 × 8 mm inside diameter each) and deionised water at 0·8 ml min^{-1}. Chromatograms of (a) green, (b) light roasted, (c) medium roasted, (d) dark roasted and (e) very dark roasted coffees.

$2{\cdot}5 \times 10^5$ (Fig. 2). A relatively simple pattern was obtained for the green coffee when detection was carried out at 325 nm (Fig. 3). Three major peaks predominated in the chromatogram and, in contrast to the detection at 420 nm and with RI detection, no significant peaks were found above $2{\cdot}5 \times 10^5$. However, high molecular weight material was detected in the roasted samples and this may be an indication that phenolic compounds, which absorb strongly at 325 nm, participate in condensation reactions. This fraction increased with degree of roasting except under the two most severe conditions where it remained approximately constant.

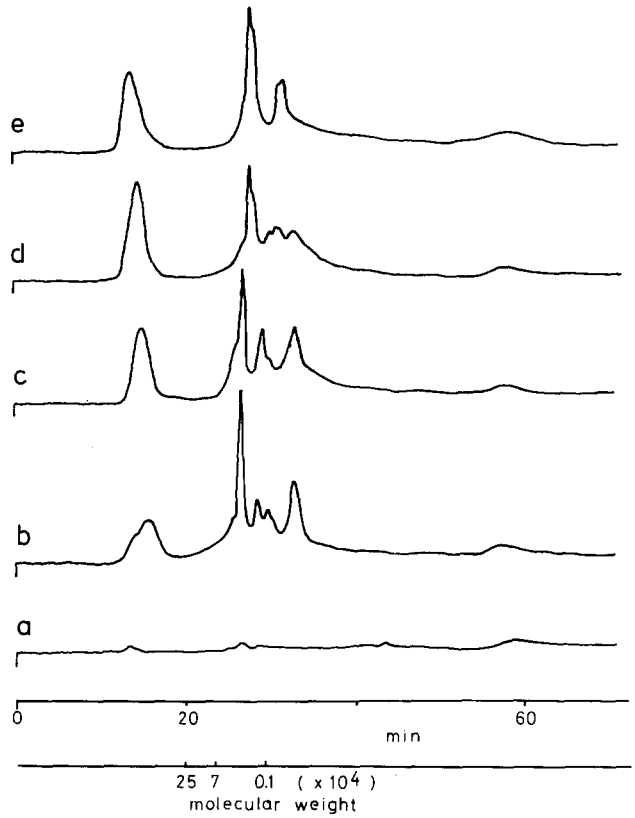

Fig. 2. Gel filtration chromatography of green and roasted coffee with detection at 420 nm. Conditions as in Fig. 1.

UV detection at 280 nm in gel filtration chromatography is commonly used to assess the molecular weight distribution of proteins. However, when this wavelength was used for the detection in the coffee samples, similar patterns to those at 325 nm were obtained for both green and roasted coffees, particularly above molecular weights of 7×10^4 (Figs 3 and 4). Most of the peaks detected at 325 nm in the green coffee also appeared in the traces at 280 nm, with similar shapes and distribution, but were generally somewhat smaller. High molecular weight proteins (above $2{\cdot}5 \times 10^4$) appear not to be present in significant amounts in the green coffee. Although total protein in green coffee is often reported at levels of 10–13% (Pearson, 1976), it has been demonstrated that water-soluble proteins are only present at levels of 3% and that other nitrogen

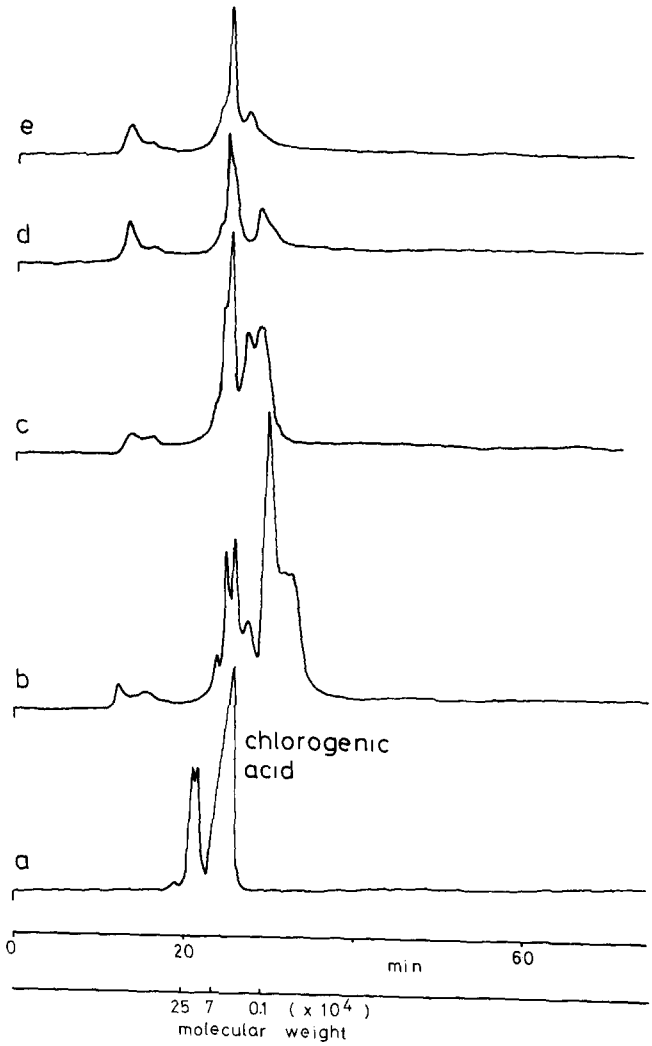

Fig. 3. Gel filtration chromatography of green and roasted coffee with detection at 325 nm. Conditions as in Fig. 1.

compounds (such as trigonelline, caffeine, proteoses, peptones, amines and possibly components related to Schiff bases) contribute to the total nitrogen values frequently used to calculate the crude protein value (Underwood & Deatherage, 1952). Thus, the chromatograms obtained using detection at 280 nm indicate that most of the water-soluble proteins of green coffee are distributed in the intermediate molecular weight

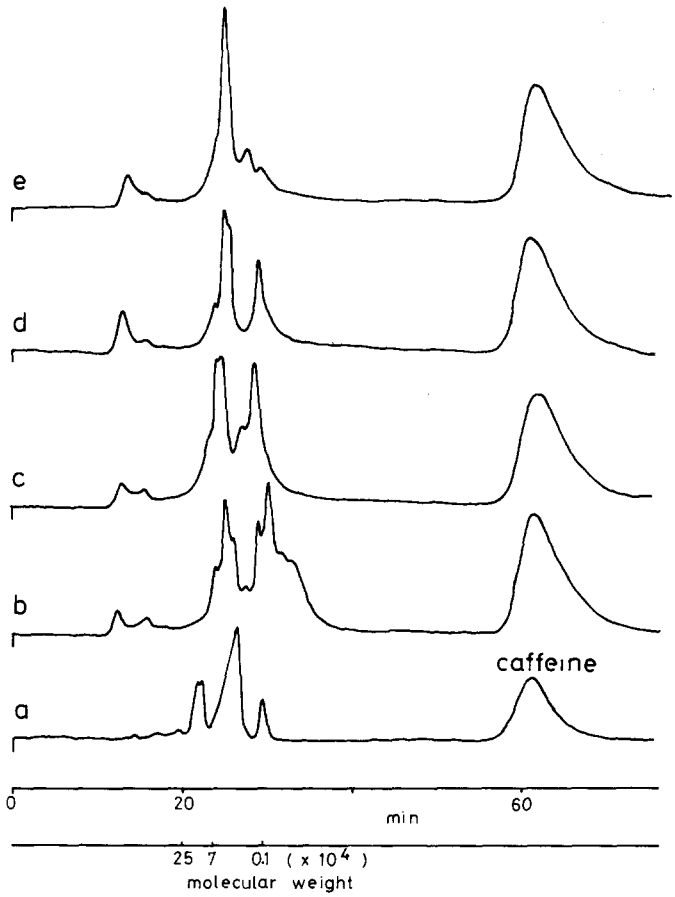

Fig. 4. Gel filtration chromatography of green and roasted coffee with detection at 280 nm. Conditions as in Fig. 1.

region. The similarity between the chromatograms with detection at 280 nm and 325 nm suggests that phenolic compounds (chlorogenic acids) are probably bound to some extent to the proteins, as has been also suggested by other workers (Amorim & Josephson, 1975). This is also consistent with the results obtained by other workers who have been able to isolate polypeptide–phenol and polypeptide–carbohydrate complexes. These have been characterised after hydrolysis, showing the presence of some twelve phenols in the former (Clifford, 1972; Klöcking *et al.*, 1971) and galactose, mannose and arabinose in the latter (Maier *et al.*, 1968; Maier & Buttle, 1973).

There are indications that most of the proteins present in green coffee are denatured and rendered insoluble in the roasting process. Therefore, the appearance of high molecular weight material which absorbs at 280 nm in the water-soluble extract of roasted coffee may not, in fact, be due entirely to protein but to other components which absorb at this wavelength (e.g. chlorogenic acid) condensed with water-soluble macromolecules (e.g. polysaccharides). This high molecular weight material increased with degree of roasting and stayed constant under the most severe conditions (Fig. 4).

With detection at 280 nm, a strongly retained large peak, tentatively identified as caffeine, was detected in both green and roasted coffees, being eluted only after 60 min (42 ml) as shown in Fig. 4.

The use of high performance gel filtration chromatography provides a more rapid technique for monitoring changes in molecular weight profiles which occur during coffee roasting and can provide useful information about chemical changes during further processing. However, improvement in resolution would certainly be necessary for a more detailed study of individual components to achieve a better assessment of the mechanisms involved in the complex reactions of flavour formation of roasted coffee.

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