Rapid Analysis of Cholesterol-Elevating Compounds in Coffee Brews by Off-Line High-Performance Liquid Chromatography/ High-Resolution Gas Chromatography

María Luisa Ruiz del Castillo, Marta Herraiz,* and Gracia P. Blanch

Instituto de Fermentaciones Industriales, c/Juan de la Cierva 3, 28006 Madrid, Spain

A new method is proposed to analyze the cholesterol-elevating cafestol and kahweol which allows their rapid and reliable determination in different coffee brews. The method involves the preseparation of the sample by high-performance liquid chromatography, the collection of the selected fraction, and its subsequent analysis by high-resolution gas chromatography using a programmed temperature vaporizer operated in the split mode as sampling system. Under the experimental conditions investigated, recoveries as high as 87% (cafestol) and 94% (kahweol) were achieved while detection limits equal to 0.06 and 0.04 ppm for cafestol and kahweol, respectively, were obtained. Examples are given comparing levels of cafestol and kahweol resulting from the same ground roasted coffee by different brewing methods, which show the lowest values for brews prepared from coffee bags.

Keywords: *Coffee; cafestol; kahweol; off-line RPLC/GC; brewing method*

INTRODUCTION

Over the past decades coffee lipid composition has been widely studied mainly with regard to the effect of prolonged coffee intake on human health. Some results supported the concept that coffee lipids may be hypercholesterolaemic, and they also appear to modify the activity of specific enzymes (Zock et al., 1990; Superko et al., 1991). In this respect, it has been recently suggested that some diterpenic alcohols could be the lipid components responsible for such an effect so that their determination in different coffee brews may be useful to predict their capacity to affect lipoprotein metabolism and liver function (Urgert et al., 1995a,b; van Rooij et al., 1995). Specifically, it has been reported that high intakes of coffees rich in cafestol and kahweol may be responsible for increases of both low-density lipoprotein cholesterol (and, consequently, the risk of coronary heart disease) and the rise of serum alanine aminotransferase concentrations (Heckers et al., 1994; Weusten van der Wouw et al., 1994; Ratnayake et al., 1995; Urgert et al., 1996). Moreover, it has been recently established that cafestol is universally present in *Coffea* species, although Arabica coffee contains more than Robusta, whereas kahweol occurs in Arabica but in only trace amounts in Robusta (de Roos et al., 1997).

Further evidence has been published concerning the influence of different brewing methods on diterpene content, and it is known that cafestol and kahweol are retained by a paper filter so that paper-filtered coffee has no or little influence on serum cholesterol increase (Ahola et al., 1991; van Dusseldorp et al., 1991; Fried et al., 1992; Ratnayake et al., 1993), whereas high diterpene levels have been detected in other brews, namely, Scandinavian boiled coffee and Turkish/Greek coffee, which do not require the use of filter paper (Urgert et al., 1995b).

* Corresponding author [fax (341) 564 48 53; e-mail mherraiz@fresno.csic.es].

It has also been pointed out that coffee diterpenes might also exhibit anticarcinogenic properties as its intake reduced the frequency of colon adenocarcinoma (Gershbein, 1994), although there is certainly more evidence on the cholesterol-raising effect of cafestol and kahweol.

All in all, it is clear that further research is required concerning the effect on human health of coffee lipids and, in this respect, it is also evident that reliable, simple, and rapid analytical methods to analyze diterpenic alcohols in coffee brews are needed. However, methods currently used often involve a laborious sample preparation step (including saponification, fractionation, and lipid extraction and formation of silyl derivatives), which demands the use of different organic solvents (Frega et al., 1994; Lercker et al., 1995; Urgert et al., 1995b).

The purpose of this work was to develop a rapid and reliable method to analyze cafestol and kahweol in coffee brews by off-line high-performance liquid chromatography (LC) and high-resolution gas chromatography (GC). Special attention was given to the replacement of time-consuming conventional procedures of sample preparation, which are often a source of error and uncertainty that render unreliable the analysis. For that reason, the LC step was used to fractionate the coffee compounds into different classes and the GC analysis provided the efficiency required for the analysis of the previously selected fractions. To increase the sensitivity achievable with the GC analysis, a programmed temperature vaporizer (PTV) operated in the split mode was used to make possible large volume sampling (Herraiz et al., 1989; Villén et al., 1989, 1992).

A further aim of our work was to evaluate the effect of using coffee bags on cafestol and kahweol contents.

EXPERIMENTAL PROCEDURES

Samples and Materials. Cafestol and kahweol used for identification purposes were purchased from LKT Laborato-

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ries, Inc. (MN); coffee samples were obtained in the commercial market. Coffee bags were provided by Cofesa (Almería, Spain). Methanol (HPLC grade) was obtained from Lab Scan (Dublin, Ireland), and the water used was collected from a Milli-Q water purification system (Millipore, Milford, MA).

Preparation of Brews in the Laboratory. We analyzed samples of the same ground roasted coffee (100% Arabica) prepared in the laboratory in different ways.

The *Scandinavian coffee* was brewed by boiling the coffee with water for 5 min followed by 5 min of settling time and subsequent decantation of the liquid. *French press coffee* was prepared by pouring boiling water onto the ground coffee in a plunger pot. After stirring, a settling time of 2−3 min was established, and finally the plunger was pushed down and the coffee filtered through a metallic filter.

Some brews were simply prepared by pouring boiling water onto a *coffee bag* in a cup and stirring, followed by 2−3 min of settling time. In this case, we examined two different papers (named A and B) consisting of cellulose and thermoplastic fibers, which exhibited a suitable heat-seal strength in hot water. When using an *electric drip filter coffee maker,* the coffee was in a paper filter while the water contained in the pot was heated. We examined three different papers, including that obtained from Melitta (Gorinchem, The Netherlands) (named paper C) and the same two papers that were used for coffee bags (i.e., papers A and B).

All brews were prepared from a 7.5-g sample weight of coffee per a 100-mL volume of water. Before its analysis by off-line LC/GC, each brew prepared in the laboratory was simply filtered through a 0.22-μm Pro-X filter (Teknokroma, Madrid, Spain), no further pretreatment being required.

LC Preseparation. The sample preparation step was carried out using exclusively a HPLC system that consisted of a Hewlett-Packard model 1050 (Wilmington, DE) chromatograph equipped with a manual injection valve (model 7125, Rheodyne, Cotati, CA) having a 20-μL sample loop and an ultraviolet (UV) detector. Throughout the experimentation a slurry-packed 10-μm column (50 × 4.6 mm i.d., Vydac 214 TPB) was used. The column was maintained at 45 °C while the UV detection was operated at 205 nm, 2000 μL/min being the flow rate during LC preseparation. Acquisition of data from the UV detector was performed with a HP Chemstation (Hewlett-Packard).

Different compositions of the eluent (methanol/water, v/v) were tested to optimize the preseparation of the fraction of interest, namely, 60:40, 55:45, and 50:50. Collection of the selected fractions was performed by using a multiport valve, placed immediately after the UV detector of the LC system, and a fused silica tube (80 cm × 0.33 mm i.d.), which allowed the sample to flow from the LC system to a vial. After each HPLC run, the column was washed for at least 30 min with methanol (100%).

GC Analysis. A Perkin-Elmer (Norwalk, CT) model 8500 gas chromatograph fitted with a PTV injector and a flame ionization detector (FID) was used. GC separations were performed on a poly(5% diphenyl−95% dimethylsiloxane) fused silica column (25 m × 0.25 mm i.d., 0.25-μm film thickness) with helium as the carrier gas. The equipment was also coupled to a HP Chemstation (Hewlett-Packard), which was used for data acquisition from the FID.

In all cases, a 30-μL volume of the fraction resulting from the LC preseparation was sampled onto GC via PTV. To prevent injector overflow, the injections were made by discharging the syringe slowly while maintaining the split valve open and the carrier gas stream switched off. In this way, solvent elimination was obtained through the split exit and an internal GC concentration was achieved by trapping in the adsorbent packed in the glass liner. Subsequently, the carrier gas was turned on and thermal desorption and transfer of the retained material were performed by raising the PTV temperature (at 14 °C/s) to different values, as described below. In each case, the final temperature was maintained for 11 min.

To retain the investigated compounds, a plug length of ∼7 cm (15 mg) of silanized glass wool (Phase Separation, Deeside, U.K.) was placed in the glass liner (80 mm × 1 mm i.d. × 2 mm o.d.) of the PTV injector. The glass wool had been conditioned under a helium stream prior to its use.

Table 1. Recoveries of Cafestol and Kahweol under Different Experimental Conditions

	recoveries (%)	
	cafestol	kahweol
end PTV temp (°C)[a]		
150	nd[d]	nd
200	nd	nd
225	nd	nd
250	87	94
275	d[e]	d
end oven temp (°C)[b]		
250	87	94
270	76	77
280	d	d
290	d	d
FID temp (°C)[c]		
250	87	94
280	d	d
320	d	d

[a] End oven temperature and FID temperature: 250 °C in all cases. [b] End PTV-temperature and FID-temperature: 250 °C in all cases. [c] End PTV-temperature and end oven-temperature: 250 °C in all cases. [d] nd, nondesorbed. [e] d, total decomposition.

As cafestol and kahweol are very prone to thermal decomposition, variables affecting GC operation during both sampling of the LC fraction and analysis itself were carefully evaluated to optimize desorption, elution, and detection of cafestol and kahweol, avoiding, or at least minimizing, eventual decomposition. The investigated variables and their respective values were as follows: end PTV temperature, 150, 200, 225, 250, and 275 °C; end oven temperature, 250, 270, 280, and 290 °C; FID temperature, 250, 280, and 320 °C.

RESULTS AND DISCUSSION

Data initially obtained by performing the experimentation under the different GC conditions given under Experimental Procedures were evaluated in terms of the percentage of decomposition of cafestol and kahweol peaks calculated from the sum of peak areas corresponding to new peaks observed in each chromatogram and those areas obtained for cafestol and kahweol. Table 1 shows the actual recovery data of cafestol and kahweol for each of the GC conditions tested. As can be seen, some experimental conditions do not allow the desorption (or the elution or detection) of the investigated compounds. From the data in Table 1, the highest temperatures admissible to avoid decomposition while allowing desorption, elution, and detection were finally selected. The conditions giving the most satisfactory analysis were as follows: PTV temperature during desorption, 250 °C; highest temperature in the GC oven, 250 °C; FID temperature, 250 °C. Actually, under the experimental conditions proposed, we also detected small amounts of decomposition products of cafestol (ranging from 7 to 14%) and kahweol (6−16%), probably formed by loss of the 16-OH function due to dehydration produced during the analysis (Urgert et al., 1995b). In any case, it is interesting to emphasize that performing the experimentation at different temperatures did not yield satisfactory results as either desorption of cafestol and kahweol from the adsorbent material was not possible or unacceptable percentages of decomposition products were observed. Consequently, the selection of the temperature (i.e., the end PTV temperature, the oven temperature, and the detector temperature) must be carefully considered when the analysis is performed.

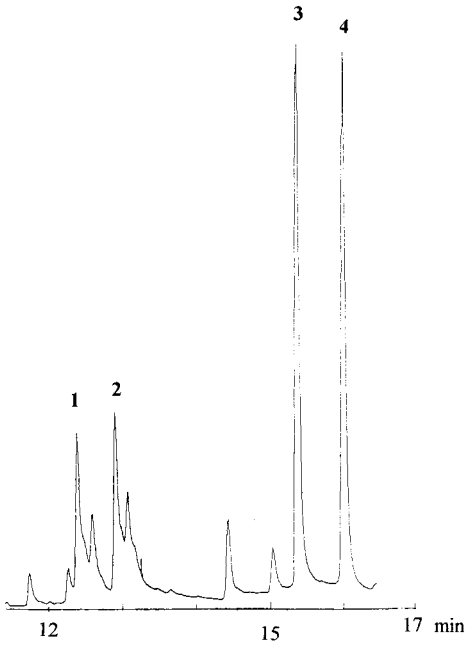

Figure 1. Gas chromatogram of a standard solution of cafestol and kahweol. Fused silica capillary column: 25 m × 0.25 mm i.d., coated with a 0.25-μm layer of poly(5% diphenyl−95% dimethylsiloxane). Oven temperature was started at 130 °C and programmed at 10 °C/min to 230 °C and then at 2 °C/min to 250 °C. Split ratio: 1:1. Identification peak numbers: 1, kahweol decomposition product; 2, cafestol decomposition product; 3, kahweol; 4, cafestol.

To illustrate this aspect, Figure 1 shows the relative positions of cafestol, kahweol, and their decomposition products.

Evaluation of the different eluent compositions mentioned under Experimental Procedures was performed by considering both the resolution and the elution time observed in the LC preseparation of the fraction to be collected. As a result, methanol/water eluent (55:45, v/v) was finally selected as the best option because other compositions either provided worse resolution (i.e., 60:40) or required more time to be carried out (i.e., 50:50).

Figures 2−4 show the gas chromatograms obtained from injection of a 30-μL fraction resulting from the LC preseparation of three different brews (Scandinavian boiled coffee, electric drip filter coffee maker, and coffee bag, respectively). The resolution achieved in Figure 2 allows an acceptable integration of the obtained area for cafestol using the software detailed under Experimental Procedures.

Throughout the experimentation, the presence or absence of both cafestol and kahweol was established by matching their retention times with those obtained for the authentic reference compounds, and it was confirmed from the subsequent analysis carried out under identical experimental conditions of the original sample spiked with a known amount of each analyte. Quantification was performed from peak areas obtained from the original and the spiked samples for the compounds of interest.

Table 2 shows the average values and the relative standard deviations obtained (from three replicates) when the off-line LC/GC analysis of cafestol and kahweol in different brews was performed. As expected, the Scandinavian boiled coffee gave the highest values, whereas the use of filter paper allowed retention of the diterpenic alcohols, thus resulting in clearly lower

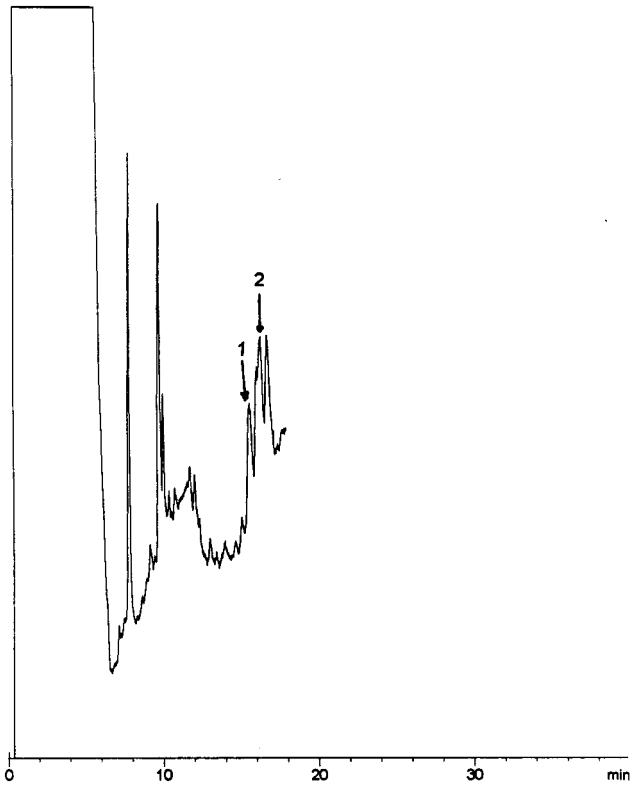

Figure 2. Gas chromatogram obtained from injection of a 30-μL fraction of methanol/water eluent (55:45), resulting from the LC preseparation of a Scandinavian boiled coffee. Capillary column and experimental conditions are as in Figure 1. Identification peak numbers: 1, kahweol; 2, cafestol.

values in the corresponding coffee brews. An interesting observation concerns the cafestol and kahweol content detected when using coffee bags as brewing method as it resulted in the lowest levels. Depending on the type of paper used for the coffee bag, clear differences regarding its retention power for cholesterol-elevating compounds are observed, and keeping in mind that the same roasted ground coffee was used throughout the experimentation, it is evident that paper characteristics were major determinants of cafestol and kahweol contents. Evidently, one factor that may influence lipid levels is the mesh width of the filter grid, but considering values given in Table 2, it is also clear that the effect of the paper on the diterpene content depends on the brewing method. When we apply paper in an electric drip filter coffee maker, resulting diterpenes levels can be higher than those for coffee bags, probably due to the fact that the contact surface between coffee and paper for coffee bags is approximately double that for the electric drip filter coffee maker.

On the other hand, it should be emphasized that under the experimental conditions proposed, mean average recoveries as high as 87% (cafestol) and 94% (kahweol) were estimated from peak areas obtained for both compounds (peak areas corresponding to decomposition products were not considered). This estimation was performed using as a reference peak areas resulting from splitless injection into GC of a standard solution of cafestol and kahweol. Moreover, a spike method was also used to check these data. In this case, similar amounts of both compounds (~100 ng of each) were added to the mixture and recovery values close to 90% were then achieved for cafestol and kahweol from the peak areas obtained in the GC analysis (split ratio =

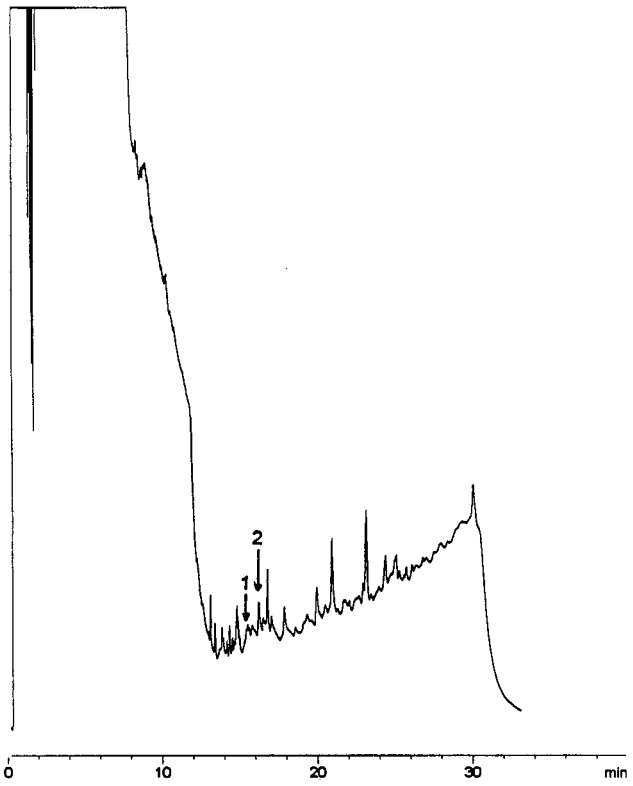

Figure 3. Gas chromatogram obtained from injection of a 30-μL fraction of methanol/water eluent (55:45), resulting from the LC preseparation of a brew prepared with an electric drip filter coffee maker (paper B). Capillary column, experimental conditions, and identification peak numbers are as in Figure 2.

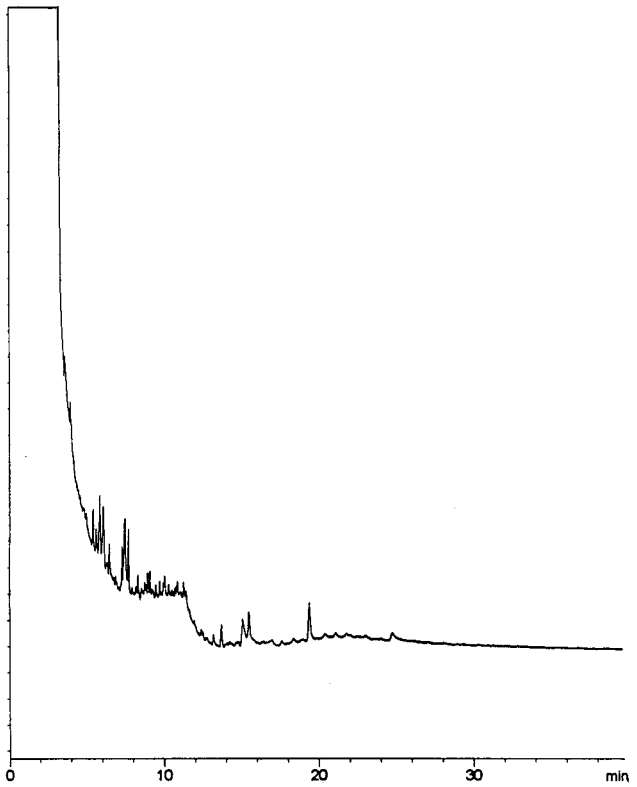

Figure 4. Gas chromatogram obtained from injection of a 30-μL fraction of methanol/water eluent (55:45), resulting from the LC preseparation of the brew prepared from a coffee bag (paper B). Capillary column and experimental conditions are as in Figure 2.

1:1). The detection limits (0.06 and 0.04 ppm for cafestol and kahweol, respectively) were obtained from the amount of product giving a signal 5 times as high as the background noise (defined from the standard deviation of the baseline signal over a certain period of time). As far as precision of the analysis is concerned, relative standard deviation values lower, in general, than 10% were obtained (Table 2). The overall analysis (i.e., including LC preseparation, fraction collection, and GC analysis) takes only 30−35 min. Under the experimental conditions proposed, the performance of the HPLC column was maintained over >200 coffee brews.

The fact that the proposed method does not require a sample preparation step clearly demonstrates its advantages with respect to the saponification procedures, which may be a source of unreliability. However, as previously mentioned, the current method demands the careful control of the end PTV temperature (and the oven and detector temperatures as well) to minimize the percentage of decomposition products observed during analysis. The method proposed by Gross et al. (1997) is an interesting approach that involves reverse-phase HPLC analysis, although in this case a sample preparation step, namely, a solid-phase extraction, is also required.

It is interesting to emphasize that the values found for cafestol and kahweol in the present work for some coffee brews seem to be much higher than the hitherto reported values (Urgert et al., 1995b; Gross et al., 1997). However, it is evident that relative amounts of coffee weight and volume of water used to prepare each brew should be known to comparatively study data reported by different authors. Moreover, the mentioned differences may be related not only to the geographical distributions of the *Coffea* species investigated but also to the use of either *Coffea arabica* (commonly called Arabica) or *Coffea canephora* (commonly called Robusta).

Table 2. Average Values and Relative Standard Deviation Values of Cafestol and Kahweol in Different Coffee Brews

	cafestol		kahweol	
brew	mean[a]	RSD[b]	mean[a]	RSD[b]
Scandinavian	615	4.7	666	6.8
French press	279	2.9	266	10.9
coffee bag (paper A)	11	6.0	25	9.2
coffee bag (paper B)	t[c]		t[c]	
electric maker (paper A)	t[c]		16	9.2
electric maker (paper B)	139	2.5	100	13.2
electric maker (paper C)	173	1.0	153	6.7

[a] Average value (mg/L) obtained from three replicates. [b] Relative standard deviation values (%) from absolute peak areas obtained from three replicates. [c] Trace amount.

In summary, our data suggest that the effects of consumption of coffee prepared from coffee bags on serum lipids through its cafestol and kahweol content can be negligible. Consequently, the use of coffee bags might have a favorable effect on coronary heart disease risk.

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