An Innovative Approach to Coffee Characterization, Using Size Exclusion Chromatography and High Resolution Gas Chromatography

A combination of high performance SEC and GC were applied to the analysis of six coffee samples. Data obtained from the two techniques were statistically evaluated by principal components analysis (PCA). The results confirmed the potential of these techniques for coffee analysis.

Key Words:
- coffee quality
- size exclusion chromatography
- principal components analysis
- headspace extraction

Coffee beans are one of the largest traded commodities at international levels — important to producers and consumers. Coffee bean quality traditionally has been judged by specially trained individuals who taste, smell, and visually examine the beans (sensory analysis).

Today's international business trade requirements are stricter. However, methods for detecting trading fraud and chemical standards for characterizing coffee beans have yet to be developed. Efficient methods for characterizing coffee beans are becoming a necessity.

New techniques for characterizing coffee beans combine GC or reversed phase LC with sensory analysis. High resolution GC, using headspace extraction, is the preferred method for analyzing components related to aroma. This method provides a better profile of volatile chemical compounds when compared to distillation techniques, which tend to form inhibitor compounds that interfere with some analytical protocols. HPLC techniques define flavor components rather than aroma components.

Investigators at the Universidade Federal do Rio de Janeiro, Instituto de Química, Brazil, characterized coffee beans by using size exclusion chromatography (SEC), headspace-GC, and principal component analysis (PCA). Six samples of green coffee beans from various countries of origin were first identified as high or low quality by sensory analysis — beans A, B, and C were high quality; and beans D, E, and F were low quality. These beans were then analyzed by the following methods.

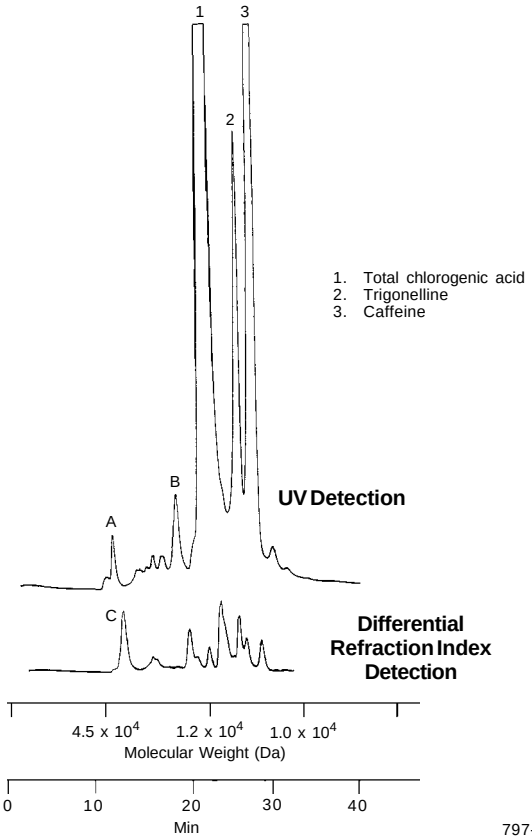
Size Exclusion Chromatography

To extract a sample for SEC analysis, a peeled bean was granulated and passed through a 0.75mm mesh filter, then mixed with distilled water at 80°C for 15 minutes. A 0.5g sample was withdrawn, cooled to ambient temperature, filtered into a flask, and taken to 50mL volume with distilled water. The samples were separated on a TSKgel G3000SW column, with UV detection and differential refraction index detection (Figure A).

SEC excludes, or fractions, compounds according to their molecular weight — molecules with larger molecular weights are eluted from the column before those with smaller molecular weights. The TSKgel G3000SW column was calibrated using protein standards, which split the chromatogram into several regions. The chromatograms in Figure A present a distinct profile showing low and high molecular weight regions.

Figure A. Low-Quality Coffee Bean by SEC

Column: **TSKgel G3000SW, 30cm x 7.5mm ID, 10µm particles**
Cat. No.: **805789**
Mobile Phase: 0.05% N_3Na in twice-distilled water
Flow Rate: 0.5mL/min
Det.: UV, 272nm, 0.16 AUFS
differential refraction index, 64 scale factor of 40
Inj.: 10µL of prepared sample from coffee bean D



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The regions up to 21 minutes consist of larger molecules with molecular weights greater than 1.2×10^4. The regions from 21 to 40 minutes consist of molecules with low molecular weights, less than 1.2×10^4. The latter regions include very important substances — total chlorogenic acid, trigonelline and caffeine — shown respectively in Figure A as peaks 1, 2, and 3 (1).

The compounds shown as peaks A, B, and C contribute 90% of the varieties' quality attributes among the samples — the peak areas are the principal parameters representing the variability of the analyses. Therefore, these small peaks represent the final quality characteristics of coffee preparations.

In addition to the conventional size exclusion mechanism, the presence of some hydrophobic interactions may explain the better resolution of the low molecular weight regions, relative to the higher molecular weight regions.

Headspace-Gas Chromatography

Headspace-GC analysis was performed according to the method described by De Maria et al. (2). A coffee bean sample of 0.5g was roasted at 280°C for 14 minutes in a vacuum-sealed test tube. 4mL of the resulting gaseous phase was injected into the GC. These samples were separated on a bonded phase SUPELCOWAX™ 10 column. Figure B shows the large quantity of peaks resulting from this procedure, which demonstrates the complexity of the samples. However, the number of extraneous peaks decreases the ease in identifying those that are important quality characteristics.

Principal Components Analysis

Results from the chromatographic analyses were processed by the statistical method PCA. In PCA the following methods are used to assess discrimination stability: PCA, modified PCA, and discriminate function analysis with cross validation. System integrity and reproducibility are maintained as long as the original sample conditions are kept constant.

Highly correlated variables are analyzed using nonlinear multiple regression analysis, partial regression analysis, and least square techniques. Sensory test data were correlated to the analytical techniques used, for each green coffee sample. Variation of a complex system of variables can be expressed by a simple, graphical representation using PCA.

Results were analyzed statistically using the peak area but, due to the complexity of the headspace-GC chromatograms, a relationship between the techniques and the chromatograms was needed to correlate the data. Variation coefficients were calculated from characteristic peaks on ten repetitive runs per sample. Peaks having a variation coefficient of less than 15% were selected and subjected to PCA, individually and as a group.

To examine sample dispersion, a scatterplot graph was made with the two principal values corresponding to the main variation peaks (Figure C). From these graphs we can tell that the dispersion was high. These results were seen during previous attempts, and dispersion was higher when each peak from each chromatographic technique was analyzed individually. We suggest that further grouping can remain according to sample characteristics, as long as testing continues and sample size increases.

Figure B. Coffee Bean by Headspace-GC

Column: **SUPELCOWAX 10, 30cm x 0.25mm ID, 15µm film**
Cat. No.: **24287**
Oven: 40°C (10 min) to 190°C (15 min) at 3°C/min
Carrier: hydrogen, 40cm/sec
Det.: FID, 280°C
Inj.: 4mL headspace of coffee bean

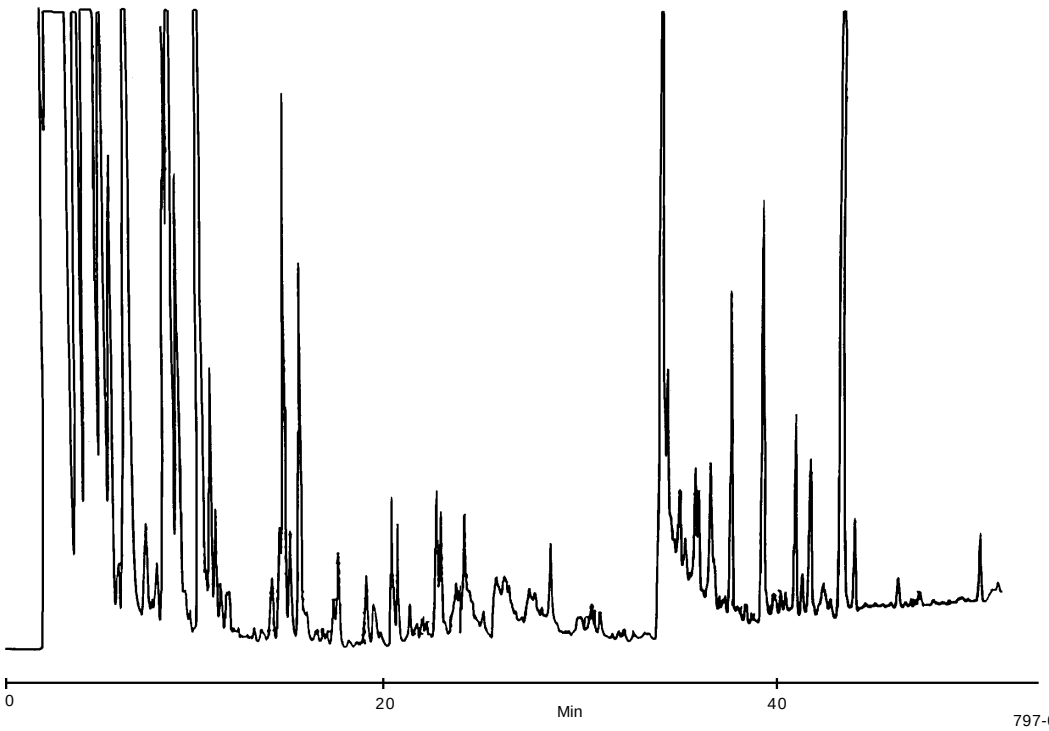

Figure C. Scatterplot Graph Showing Sample Dispersion for Each Coffee Bean

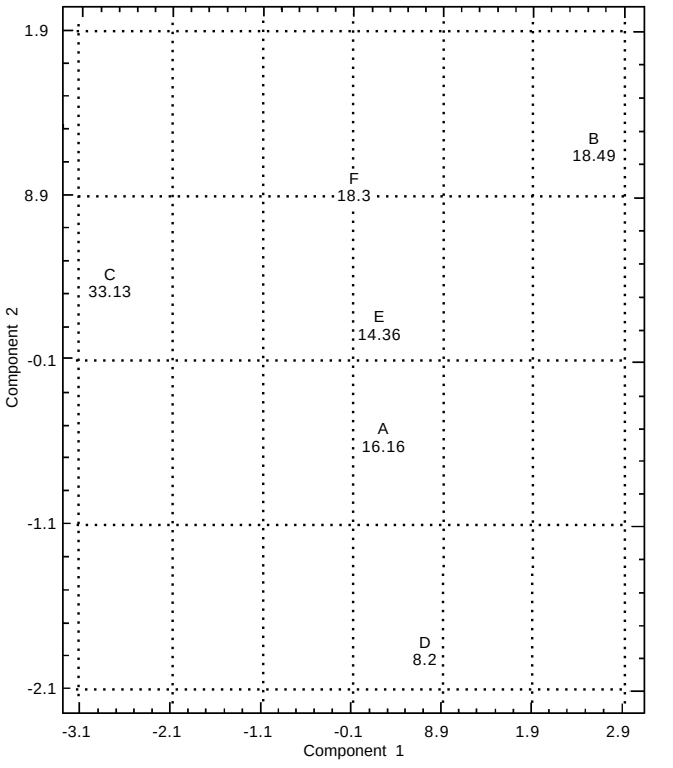

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Table 1 offers a relationship of the percent variance obtained through PCA for each peak area — SEC compared to traditional reversed phase chromatography. SEC performed as well as reversed phase chromatography. In fact, SEC presents certain advantages — SEC is capable of simultaneous analysis of the important peaks, whereas reversed phase HPLC requires different chromatographic conditions for each compound (1). Additionally, SEC uses water as the mobile phase, which is attractive from a cost containment perspective.

Further studies are necessary to provide detailed identification of components. Additional complementary analytical techniques, such as mass spectrometry and GC or LC, may help obtain a detailed characterization profile of most aroma and bouquet compounds for coffee.

Table 1. Average Results for CGA, Trigonelline, and Caffeine

Samples	Methods					
	CGA		Trigonelline		Caffeine	
	SEC	RP	SEC	RP	SEC	RP
Mean	8.0	9.0	1.1	1.1	1.1	1.2
Std. Dev.	0.32	0.31	0.05	0.04	0.03	0.04
Coef. Var. (%)	4.0	3.4	4.5	3.6	2.7	3.3
B	6.7	7.7	1.2	1.2	1.1	1.3
C	7.4	8.1	1.0	1.1	1.1	1.1
D	7.2	8.0	1.6	1.6	0.7	0.7
E	6.8	7.1	1.5	1.4	1.1	1.3
F	6.8	7.0	1.2	1.2	1.2	1.3

SEC — size exclusion chromatography; RP — reversed phase liquid chromatography, a different technique was used for each component

A — results are means for 12 replicates originating the respective standard deviation and coefficient of variation; B-F — results are means for duplicate determinations.

Ordering Information:

Description	Cat. No.
TSKgel G3000SW Gel Filtration Column 30cm x 7.5mm ID, 10µm particles	**805789**
SUPELCOWAX 10 Capillary Column 30m x 0.25mm ID, 0.15µm film	**24287**

References

1. De Maria, C. A. B., L. C. Trugo, and R. F. A. Morreira, *Food Chemistry,* **52:** 447 (1995).
2. De Maria, C. A. B., L. C. Trugo, R. F. A. Morreira, and C. C. Werneck, *Food Chemistry,* **49:** 141 (1994).

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Information in this bulletin used with permission from Química Nova, **20:** 1:5-8, (1997) Professor Luiz C. Trugo, Universidade Federal do Rio de Janeiro, Instituto de Química, Brazil, and Sociedade Brasileira de Química, Professors Ricardo F.A. Moreira and Carlos A.B. De Maria.

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For more information on these analyses and PCA, please contact Professor Luiz C. Trugo directly — email: cabm@acd.ufrj.br.

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