Chemometric Evaluation of Adulteration Profile in Coffee Due to Corn and Husk by Determining Carbohydrates Using HPAEC–PAD

Livia Maria Zambrozi Garcia[1], Elis Daiane Pauli[1], Valderi Cristiano[1], Carlos Alberto Paulinetti da Camara[3], Ieda Spacino Scarminio[2], and **Suzana Lucy Nixdorf[3,*]**

[1]Natural Resources ChemistryPost-Graduate Program, Universidade Estadual de Londrina, Londrina, PR, Brazil, [2]Natural Science Chemiotherapy Laboratory, Universidade Estadual de Londrina, Londrina, Parana, Brazil, and [3]Instrumentation, Automation and analytical Methodology Development Laboratory (DIA), Universidade Estadual de Londrina (UEL), Londrina, Parana, Brazil

Abstract

The detection of impurities in coffee samples already roasted and ground is a constant concern mainly in order to verify the incidence of frauds. Carbohydrates contents are important as variations on original constitutes of different matrixes may be able to reveal the final composition of the product. In this sense, a study is performed in this paper in order to evaluate the quality through concentration of total carbohydrates in Arabic roasted and ground coffee. Chemometric methods were applied in order to verify an adulteration pattern by coffee husk and corn, by the mixture of different amounts of these contaminants to coffee, following a statistical design of Simplex-Centroid. It could be concluded that this method was efficient in distinguishing different matrixes. In pure coffee, higher concentrations were found for galactose and mannose with levels of 8.25% and 9.65% (w/w), respectively. However, in the pure husks, the main carbohydrates were mannitol (with a level of 0.64%), arabinose (with 4.24%), and xylose (with 3.40%). For the corn sample, glucose was detected in greater quantity, with 52.53% (w/w). Models presenting the influence of adulterants incorporated to coffee in the carbohydrate level were obtained.

Introduction

Coffee is a symbol of hospitality and a daily stimulant in thousands of households and work places. As it is one of the most consumed beverages in the world. Due to its great commercial importance, its polysaccharides have been investigated for quite some time (1).

Carbohydrate analysis is important in this issue because variation in original constituents from different raw materials may be able to reveal the final composition of the product (2). In other words, if it is pure or if it has been adulterated, it could provide information on the proportion of the adulteration, as a function of the proportion of each mono-, oligo-, and polysaccharides (3).

The quality of the coffee beverage, characterized by its flavor and aroma, is influenced by several factors both before and after harvesting, ensuring the final expression of the product's quality. Among the pre-harvesting factors, the species and variety of coffee, the area it has been cultivated, the maturing of grains, the incidence of microorganisms, and the effect of fertilization (4) can be highlighted. As for the post-harvesting factors, enzyme and microbial fermentation, storage of the grains (5), the blending and the roasting of grains can be brought into attention. The polysaccharide content is reduced during roasting due to degradation of arabinogalactan and galactomannans, predominating carbohydrates in coffee beans and extracts, to low molecular weight carbohydrates (mono and oligosaccharide (6,7). This stage is essential for the formation of various types of composed flavors (8).

Detection of impurities in samples of roasted and ground coffee is a constant concern, mainly to verify the occurrence of frauds (by fraud understand the mixture, intentional or not, of materials foreign to the product, normally of a low cost, which alter its quality and harm consumers, especially economically) (2). In Brazil, impurities or mixtures found with greater frequency in roasted and ground coffee are husks, sticks, roasted corn, cocoa seed husks, barley, wheat middling, brown sugar, soybeans, and more (9). Some of the main problems found in identifying these substances are related to the analytical methods applied because these are time-consuming, subjective, and many times provide conflicting results (3). Techniques that do not rely on the determination of specific chemical tracers, but rather on strictly physical measurements, were proposed for the detection of adulterants in coffee [e.g., the use of photo-acoustic spectroscopy (10), of infra-red spectroscopy (11), of scanning electron microscopy (12), and of thermal lens spectrometry (13)]; however, most of the analytical techniques for soluble coffee were based on the determination of the carbohydrate

*Author to whom correspondence should be addressed: email snixdorf@uel.br.

chromatographic profiles coupled to multivariate statistical analysis of chromatographic data (3,14–17).

The chromatographic technique known as high-performance anionic exchange (HPAE) chromatography was developed to separate carbohydrates. Associated to pulse amperometric detection (PAD), it enables the direct quantification of carbohydrates without derivatization, with low levels of concentration and minimal preparation and clean-up of the sample. HPAE used in the analysis of carbohydrates uses the mildly acid trace of carbohydrates to grant high selectivity in an elevated pH, using a stationary phase of strong anionic-ionic exchange. In elevated pH, carbohydrates suffer electro-catalyzed oxidations on the surface of the gold electrode by applying a positive potential (18). The current generated in the detector is proportional to the concentration of carbohydrates, which, after being separated by the ionic exchange mechanism, can be detected and quantified (19). The HPAE-PAD technique was adopted to determine carbohydrates in coffee for being simple, precise, and sensitive, and it allows a complete separation of all major carbohydrates in one single run. It has been collaboratively tested within the Association of Soluble Coffee Manufacturers of the European Community (AFCASOLE). The method was published as International Standard ISO 11292: 1995 (20) and has been adopted as a First Action by AOAC International.

Carbohydrate profiles of a wide variety of soluble commercial products, all sold as pure soluble coffee, were determined (3,14,16). Through these studies, it was possible to characterize the profiles and tendencies of content for free and total carbohydrates, depending on the use of husk and other cereals, enabling the determination of a maximum total xylose limit of 0.40%, above which a soluble coffee should be considered as adulterated. Maximum limits of 0.30% and 2.10% were also proposed for free mannitol and total glucose contents, respectively (21).

While there have been numerous publications concerning the carbohydrate content, especially in soluble coffee, in green and roasted coffee, only a few publications provide quantitative data, with somewhat conflicting ranges for individual carbohydrates (3). Blanc et al. (3) present data for roasted arabic coffee, used for the production of soluble coffee, by UV–vis post-column method. Contents varied in % (w/w): from 0.08–0.14 xylose; 1.03–6.29 arabinose; 5.91–19.50 mannose; 1.29–2.00 glucose; and 3.19–20.93 for galactose, depending on the extraction and time conditions adopted: 30 to 240 min and temperatures of 150°C to 190°C. Redgwell et al. (22), have found the composition of monosaccharides following hydrolysis in 72% H_2SO_4 for 3 h at room temperature and then for 2 h at 110°C in 1 M H_2SO_4. The hydrolysates were separated by HPAEC–PAD on Dionex DX-500 using a CarboPac PA1, equilibrated in 150 mM NaOH. Contents obtained in % (w/w) varied according to the degree of roasting: light, medium, or dark, as well as the type of coffee used: arabinose 0.74–4.29; xylose 1.1–3.65; mannose 15.97–24.89; galactose 5.50–12.10, and glucose 6.1–8.01.

Considering the necessity of controlling the quality of the products with greater efficiency, as a consequence of a market more and more globalized and competitive, the present study evaluated the quality of arabic coffee sample by means of its carbohydrate concentrations. Data were treated using chemometric methods in order to verify an adulteration standard by coffee husks and corn. The mixtures of different proportions of these contaminants to the collected coffee were prepared following a statistic design of a simplex-centroid type of the mixtures of three components (23). The coffee was analyzed in relation to the influence of roasting degree in the variation of carbohydrates in green coffee, roasted, and ground coffee in medium and dark roast.

Materials and Methods

Samples

Coffee samples were collected *in natura* from raw coffee, berries, husks, and roasted and ground coffee, in the Farm School from the State University of Londrina (UEL).

The corn sample was obtained in a commercial store in Londrina, PR. The sample was roasted in a roaster until reaching a color similar to coffee, ground, and sieved in the same manner as the other samples.

For the study of adulterants, the preparation of mixtures was made by means of a statistic design of the simplex-centroid type of mixtures with Axel points, samples from 1 to 10 (Table I). All samples were analyzed in duplicate. The coffee used for this study was the previously described one in medium roast. Preparation was made by weighing different proportions of matrixes in order to reach 0.3000 g.

For the preliminary evaluation of the influence of the roasting degree in the samples of coffee, a raw coffee sample was used (sample 11), ground, and sieved; one sample of roasted and ground coffee in medium roasting degree (sample 1), ground and sieved, and one sample of roasted and ground coffee in a dark roasted degree supplied by the Farm School (sample 12) (Table I).

Preparation of samples

Samples were prepared following the procedure described in the norm ISO 11292 (20). The quantity of 0.3000 g was weighed

Table I. Composition of the Sampling for Evaluation of Adulterants and Degree of Roasting used in the Coffee (Simplex-Centroid Design)

Samples	Coffee (%)	Husk (%)	Corn (%)
1–1a*	100	0	0
2–2a	0	100	0
3–3a	0	0	100
4–4a	50	50	0
5–5a	50	0	50
6–6a	0	50	50
7–7a	66	17	17
8–8a	17	66	17
9–9a	17	17	66
10–10a	34	33	33
11–11a (raw)	100	0	0
12–12a (dark)	100	0	0

* duplicate.

for each sample in dry base and transferred to a 500 mL erlenmeyer with screw lid, and a solution of 50 mL cloridric acid 1.00 mol/L was added. The recipient was placed in a thermostatized water-bath with lid at 85ºC for 150 min. After every 30 min, the mixture was manually agitated. It was cooled down to room temperature by placing it under running tap water. The solution was then filtered in white stripe paper filter, and collected in a 100.00-mL volumetric balloon, completing up to the mark with ultrapure water. An aliquot of 10 mL from this solution was filtered in Sep-Pak C_{18} (Waters, Milford, MA), preconditioned with methanol and water, and in 0.22 µm Polivinylidenefluoride disposable membrane (GVWP 02500–Millipore, Billerica, MA) discarding the first milliliters. The filtered was collected in 4-mL vial and sent to the chromatographic system for analysis.

Standards

In order to quantify the carbohydrates in the samples, a standard stock solution was prepared with concentrations similar to those found in the roasted and ground coffee samples. Dilutions were made for the construction of analytical curves, which enable the determination of the linearity applied in the chromatographic analysis. The standard was refrigerated. Only certified analytical degree reagents were used for the preparation of all solutions, provided by Merck (Whitehouse Station, NJ).

Due to the high hygroscopicity of carbohydrates, standards were stored in a glass drier, in vacuum and phosphorous pentoxide (Merck), they and were used only after one week of drying for the preparation of the standard.

For the preparation of the carbohydrate storage standard mixture solutions, 0.0160 g D(–)-mannitol; 0.0200 g D(–)-arabinose; 0.1200 g D(+)-galactose; 0.0400 g D(+)-glucose; 0.0100 g D(+)-xylose; 0.0800 g D(+)-mannose; 0.0500 g D(–)-fructose, were weighed, added to a volumetric balloon of 100.00 mL and using ultrapure water to complete it. The solution was kept for 10 min in ultra-sonic bath.

Mobile phases

The preparation of the mobile phase is a critical stage in analyzing carbohydrates by HPAEC. In order to minimize contamination with carbonate, in pH ≥ 12, avoiding the drastic decrease in selectivity, resolution, efficiency, and retention time, the solutions were prepared on the day of the evaluation. Mobile phases of sodium hidroxide 1.4 mmol/L (eluent) and 300 mmol/L (regenerator) were prepared from the aliquots extracted from the center of the certified solution of NaOH 50% (m/m) (Fisher: 19.23 mol/L), and completed with ultrapure water (Milli-Q), filtered through 0.45-µm filtering membrane (HATF 04700 – Millipore), attached to the filtering system (Filterware Labglass), and degasser by aspersion of 99.997% purity nitrogen, Linde for 30 min. After preparation, the solutions were stored in 2-L HDPE Nalgene containers sealed with lids, to avoid the entrance of CO_2 from the exterior, with exit of phase through sinterized filter and Teflon tubes.

Analytical conditions applied in chromatographic analysis

The instrumental system applied consisted in an inert liquid chromatography (PEEK) composed of: two containers to store the mobile eluent phase (Nalgene); an inert high-pressure LC-10Ai (Shimadzu pump, Kyoto, Japan; a solenoid low pressure valve of 3 ways NResearch, 1367-72; a "lab-made" external activation circuit to the solenoid valve; a high pressure valve of 10 ways with electronic activation VICI, C2-2340 EP (20-µL loop); a pre-column (CarboPac PA1, Dionex), and an anionic exchange column CarboPac PA1 (Dionex, 250 mm × 4 mm, 10 µm polyestirene–divinilbenzene, backbone of the pellicular resine cross linking, covered with quaternary amine); a thermostatized oven for the column (Waters), controled by temperature controler CHX 650 (Pickering Laboratories, Mountain View, CA); an electrochemical cell ED-50 (Dionex), composed by gold working electrode, Ag/AgCl gel reference electrode, and counter-electrode; a potentiostat Autolab PGStat 30 (Eco-Chemie); one Autolab (Eco Chemie, Utrecht, the Netherlands) interface; a data acquisition and treatment system composed by one Pentium IV micro-computer, with the program GPES (General Purpose Electrochemical System, Eco Chemie).

The analytical conditions adopted were an injection volume of 20 µL, with the system operating in isocratic mode with temperature at 28°C. Flow rate of the mobile phase was of 1.1 mL/min, with solution of NaOH 1.4 mmol/L being used as eluent with a chromatographic run of 0 to 45 min. The NaOH 300.0 mmol/L phase was applied for the regeneration column from 45.1 to 57.5 min, in order to remove impurities retained in the active sites of the column, preventing retention or promoting the appearance of peaks in subsequent runs. After this regeneration, the solution of 1.4 mmol/L, was once more applied in 57.6–72.6 min to re-balance the column before every new injection. Autolab acted as a detector, applying different oxidation potentials and reduction in the ED-50 electrochemical cell, conducting the reading of the generated currents, which are proportional to the concentration of carbohydrates. The waveform of the amperometric pulse applied was of + 0.25 V for 200 ms in order to determine carbohydrates; + 0.75 V for 400 ms for the oxidation of gold; and –0.15 V for 200 ms for reduction.

Standard solution was injected after every two injections of the samples in order to quantify any alterations in retention time or electrode surface, which might cause changes in the peak integrations.

Every first injection in each day was disposed of because the

Table II. Regression Equations, Correlation Coefficients, and Dynamic range for all Seven Carbohydrates

Carbohydrate	Regression Equation	Correlation Coefficient	Dynamic range (µg/mL)
Mannitol	$y = 1.87 \times 10^{-09}x - 8.48 \times 10^{-10}$	$r = 0.9994$	28.0–80.0
Arabinose	$y = 2.13 \times 10^{-09}x - 1.87 \times 10^{-08}$	$r = 0.9999$	35.0–100.0
Galactose	$y = 1.58 \times 10^{-05}x + 6.90 \times 10^{-08}$	$r = 0.9997$	210.0–600.0
Glucose	$y = 2.36 \times 10^{-09}x - 4.22 \times 10^{-08}$	$r = 0.9996$	70.0–200.0
Xylose	$y = 3.05 \times 10^{-09}x - 4.29 \times 10^{-08}$	$r = 0.9903$	17.5–50.0
Mannose	$y = 1.93 \times 10^{-09}x - 3.96 \times 10^{-08}$	$r = 0.9993$	140.0–400.0
Fructose	$y = 1.22 \times 10^{-09}x - 7.44 \times 10^{-08}$	$r = 0.9996$	87.5–250.0

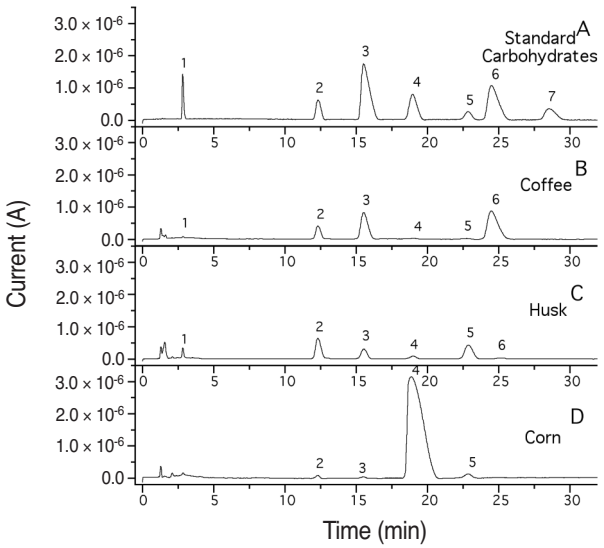

Figure 1. Chromatograms of carbohydrates: (A) Standard, (B) Roasted Coffee arabica, (C) Roasted Husks, (D)Roasted Corn. Peaks: (1) Mannitol, (2) Arabinose, (3) Galactose, (4) Glucose, (5) Xylose, (6) Mannose, (7) Fructose using HPAE-PAD. Mobile phase- isocratic: NaOH 1.4 mmol/L (eluent:0–45 min and re-equilibrate: 57.6–72.6 min.) and NaOH 300 mmol/L (regeneration: 45.1- 57.5 min.). Flow: 1.1 mL/min; injection vol: 20 µL; pre-column and column: CarboPac PA-1 and T: 28 °C; amperometric pulse waveform ED-50-Au: +0.25 V (200 ms); +0.75 V (400 ms) e -0.15 V(200 ms).

balance of the system is only obtained after one cycle, therefore, in the second chromatographic run.

Results and Discussion

Quantitative analysis of carbohydrates

The calibration curve showed to be linear in the tested range. Table II shows the regression equations and correlation coefficients for mannitol, arabinose, galactose, glucose, xylose, mannose, and fructose.

The repeatability calculated from the areas of 4 successive repetitions of the standards presented variation coefficiency of 3.95%, 2.13%, 1.67%, 1.98%, 4.79%, 1.55%, and 3.24% for mannitol, arabinose, galactose, glucose, xylose, mannose, and fructose, respectively, being lower than 5.0% for all the carbohydrates analyzed.

Figure 1 presents the chromatographic profile (HPAEC–PAD) of monosacharides present in the pure matrixes of coffee, coffee husks, and corn, and mixture for standards, used for quantifying carbohydrates, through external standardization method. The analyzed carbohydrates were well resolved in a single run.

Table III synthesizes the results in percentages (w/w) of the carbohydrates obtained in the analysis of the roasted, ground, and sieved samples of arabica coffee, pure corn, and husks, and

Table III. Average Levels for Carbohydrates Found in Determinations by HPAEC-PAD, Run in Duplicates, in Pure Samples of Coffee, Corn, and Coffee Husks, and in Different Proportions of Mixtures Adopted According to the Simplex-Centroid Chemometric Design

Sample codes* Composition	Carbohydrates (%) (w/w) (n = 2)									
	1 coffee 100% ± s.d.	2 husk 100% ± s.d.	3 corn 100% ± s.d.	4 coffee 50% husk 50% ± s.d.	5 coffee 50% corn 50% ± s.d.	6 husk 50% corn 50% ± s.d.	7 coffee 66% husk 17% corn 17% ± s.d.	8 coffee 17% husk 66% corn 17% ± s.d.	9 coffee17% husk 17% corn 66% ± s.d.	10 coffee 34% husk 33% corn 33% ± s.d.
Mannitol	0.04	0.64	n.d.	0.39	n.d.	0.52	0.03	0.50	0.36	0.41
Mannitol†	0.04	0.65	n.d	0.31	n.d.	0.41	0.05	0.69	0.02	0.18
Média ± SD	0.04 ± 0.00	0.64 ± 0.01	n.d	0.35 ± 0.06	n.d.	0.46 ± 0.08	0.04 ± 0.01	0.59 ± 0.13	0.19 ± 0.23	0.29 ± 0.16
Arabinose	2.37	4.37	0.50	2.99	1.28	2.07	2.27	3.01	1.28	2.33
Arabinose†	2.24	4.11	0.35	2.83	1.32	2.15	1.72	2.75	1.17	1.37
Média ± SD	2.31 ± 0.10	4.24 ± 0.18	0.42 ± 0.11	2.91 ± 0.12	1.30 ± 0.03	2.11 ± 0.05	2.00 ± 0.39	2.88 ± 0.18	1.23 ± 0.08	1.85 ± 0.68
Galactose	8.45	2.92	n.d	5.38	4.33	0.89	6.42	3.41	1.86	4.17
Galactose†	8.04	2.79	n.d	5.15	4.27	1.18	4.52	2.84	1,68	2.24
Média ± SD	8.25 ± 0.30	2.85 ± 0.09	n.d.	5.27 ± 0.16	4.30 ± 0.04	1.03 ± 0.21	5.47 ± 1.34	3.12 ± 0.40	1.77 ± 0.13	3.21 ± 1.37
Glucose	0.22	0.74	57.81	0.34	30.63	24.94	11.98	12.74	35.97	21.63
Glucose†	0.14	0.67	47.25	0.26	30.31	30.92	8.47	10.59	36.78	13.12
Média ± SD	0.18 ± 0.06	0.70 ± 0.05	52.53 ± 7.47	0.30 ± 0.05	30.47 ± 0.23	27.93 ± 4.23	10.22 ± 2.48	11.67 ± 1.52	36.37 ± 0.58	17.38 ± 6.02
Xylose	0.14	3.49	1.14	1.58	0.28	1.87	0.74	2.80	0.96	1.45
Xylose†	0.14	3.31	0.75	1.49	0.41	2.35	0.33	2.28	0.92	0.50
Média ± SD	0.14 ± 0.00	3.40 ± 0.12	0.94 ± 0.27	1.54 ± 0.06	0.35 ± 0.09	2.11 ± 0.33	0.54 ± 0.29	2.54 ± 0.37	1.13 ± 0.03	0.98 ± 0.67
Mannose	10.79	0.46	n.d	5.58	5.62	n.d	7.69	2.05	1.30	4.13
Mannose†	8.56	0.17	n.d	5.49	5.44	n.d	5.48	1.56	1.21	1.93
Média ± SD	9.68 ± 1.57	0.31 ± 0.20	n.d	5.53 ± 0.06	5.53 ± 0.12	n.d	6.59 ± 1.57	1.81 ± 0.35	1.25 ± 0.07	3.03 ± 1.56
Fructose	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fructose†	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Média ± SD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* See Table I.
† duplicate, s.d. = standard deviation; n.d. = below LOD.

the blends of these adulterants in different proportions.

Observing chromatograms from Figure 1 and analyzing the carbohydrate contents for each of the three pure matrixes in Table III, it is possible to note that they present distinct characteristics. In the pure coffee sample (Figure 1B), galactose (8.25%) (w/w) and mannose (9.65%) (w/w) are at the highest concentrations. For the pure husk sample (Figure 1C), the highest levels are for mannitol (0.64%) (m/m), arabinose (4.24%) (w/w), and xylose (3.40%) (w/w). Moreover, for the pure corn sample (Figure 1D) elevated concentration of glucose (52.53%) (w/w) can be noticed. It can also be observed that there is greater correspondence of carbohydrates from the coffee husks in relation to coffee, with higher levels of mannose, galactose, and arabinose, which is justified by the predominant polysaccharides in the grain because both come from the same fruit, when compared with corn, which has a very different matrix, with a high value of glucose (52.53%) (w/w), characteristic of cereals, according to what has been described in literature (6–8,21).

Chromatogram overlapping in Figure 2 allows for the observation of the increase or decrease of a specific carbohydrate, according to its proportion in the blend coffee-husk-corn.

Comparing data from this study to those related by Redgwell (2002) and Blanc (3) for roasted arabica coffee, it can be observed that all levels obtained by Redgwell were higher, possibly due to the drastic hydrolysis condition used by him. Data obtained for arabinose and lactose presented levels within the rate described by both authors. However, xylose value of 0.14% (w/w) is in agreement with those described by Blanc, but much lower than those described by Redgwell (1.1 to 3.6%). The level of glucose (0.18%) found was much lower than the value stated by both authors, of 1.29–2.00 and 6.1–8.01%, respectively, possibly due to the milder condition of extraction at 85°C. Fructose was not detected in any of the hydrolized samples. Mannitol level could not be compared because the authors did not conduct its quantification. However, according to Prodolliet (21) maxim limits of 0.30% were proposed for free mannitol, being used for the establishment of the Code of Practice for the Soluble Coffee Industry in the United Kingdom, which, in addition, sets a maximum acceptable content for free fructose of 0.60%, for soluble coffee. This shows that its increase is also associated to the adding of adulterants, which may be demonstrated by the value of 0.64% (w/w), found in the pure husk sample, against 0.04% (w/w) for the pure coffee (Table III).

Although it may be difficult to compare levels for ground and roasted coffee, due to differences between the type of coffee, roasting, extraction conditions, and analysis methods, it is possible to notice a similar behavior to what has been described by Blanc (3), Davis (14), and Prodolliet (21) for soluble coffee. Pure soluble coffee is characterized by high amounts of total galactose and total mannose. Products adulterated with coffee husks or parchments present high levels of free mannitol, total glucose, and total xylose. Products adulterated with starch-containing substitutes (e.g., cereals or malt), maltodextrins or caramelized sugar present huge levels of total glucose.

Table IV synthesizes the results of carbohydrates determined in the analysis of raw coffee, coffee (medium roast), and coffee (dark roast) samples.

Observing the variation in carbohydrate levels according to roast in Table IV, it is possible to note that there is a sensible lowering of these levels, from raw to roasted coffee. From medium to darker roast, some carbohydrates could not be detected any longer, such as mannitol, xylose, glucose, and fructose. However, the total value considering the sum of all individuals remains practically constant, ~ 15% (Table IV). This decrease in values is in accordance with papers analyzing the influence of the degree of roasting in the carbohydrate levels (8,22).

Chemometric analysis of carbohydrates

Figures 3 and 4 refer to the results of the analysis of factors from the chromatogram matrixes. Data matrix is represented by 2800 variables and 16 samples (chromatograms). Each line of the matrix corresponds to one mixture from Table I. Initially, the data matrix was composed containing 10 samples, analyzed in duplicate (Table 1). However, four of these samples (5a, 6a, 8, and 10) were disregarded in the statistic study for presenting variations above acceptable in relation to the other samples. Samples 11, 11a, 12, and 12a did not enter the statistic treatment in that

Table IV. Average Carbohydrates Levels found in the Determinations by HPAEC-PAD, Run in duplicate, in Pure Samples of Coffee at Different Degrees of Roasting

	Carbohydrates (%) (w/w) (n = 2)		
Sample codes (Table II)	11	1	12
Type of coffee sample	Raw	Medium roast	Dark roast
Mannitol	0.07 ± 0.00	0.04 ± 0.00	n.d.
Arabinose	3.32 ± 0.01	2.31 ± 0.10	1.35 ± 0.11
Galactose	5.90 ± 0.11	8.25 ± 0.30	5.86 ± 0.46
Glucose	2.77 ± 0.21	0.18 ± 0.06	n.d.
Xylose	0.87 ± 0.04	0.14 ± 0.00	n.d.
Mannose	3.55 ± 0.02	9.65 ± 1.67	7.83 ± 0.73
Fructose	0.90 ± 0.01	n.d.	n.d.
Total carbohydrates	23.48	14.47	15.04

*n.d. = below LOD.

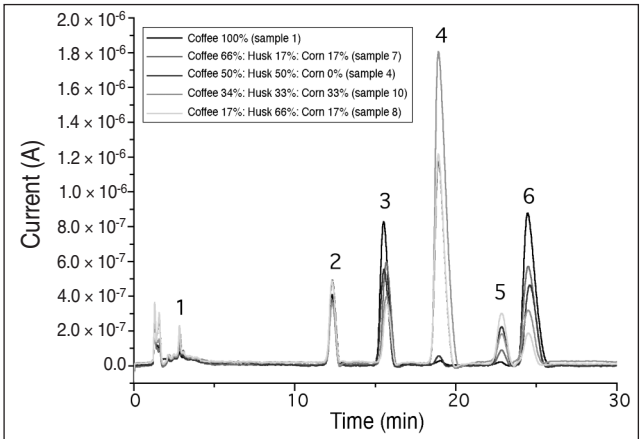

Figure 2. Carbohydrates Chromatographic profiles of samples showing changes occurred with the addition of husk and corn to pure coffee. Peaks: 1, Mannitol; 2, Arabinose; 3, Galactose; 4, Glucose; 5, Xylose; 6, Mannose.

these were only to verify the influence of the roasting degree in the variation of the carbohydrate levels.

Figure 3 shows the score graph for factor 1 and factor 2, which together could explain 63% of total variance of data. In this graph, the formation of 4 groups can be noticed. Group I is composed by samples 1 and 1a (pure coffee), group II by samples 2, 2a, 4, and 4a (50% coffee and husk), group III by samples 7, 7a, 8a, and 10 (mixtures with more coffee and husk) and group IV by samples 3, 3a, 6, 5, 9, and 9a (mixtures with more corn).

Hierarchical analysis was applied to be used as a classification base. The dendrogram obtained for the collection of 16 samples and 2800 variables is presented in Figure 4. In the distance 54 value, the formation of 4 groups can be observed. These groups are identical to those groups verified in Figure 3, in the projection of factor 1 with factor 2, excluding sample 6.

Factor loadings for the chromatographic retention times are illustrated in Figure 5 for the first two factors.

The first factor separates the groups I and II from groups III and IV, by differences in the concentration of all carbohydrates because all peaks are negative. The most negative peak corresponds to glucose. This means that samples with more negative score values in factor 1 (Figure 3), contain more glucose in its composition. Factor 2 shows that group IV is located in the most positive region in this factor. Observing Figure 5, the only positive peak corresponds to glucose. This means that the level of glucose discriminates the samples containing corn from those of coffee.

Figure 6 shows the graph for scores to factors 1 and 4. It can

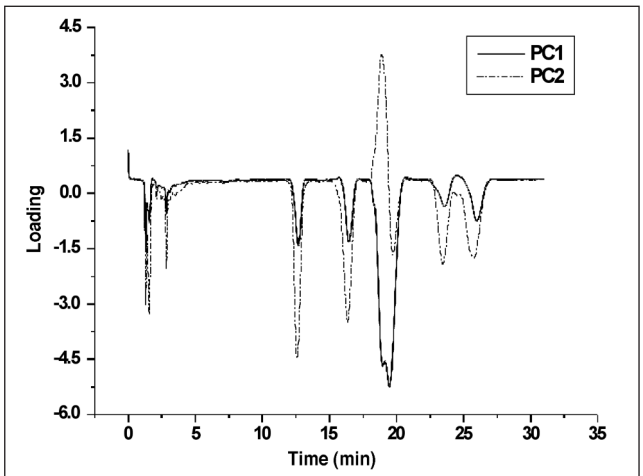

Figure 5. Graph of Factor 1 with Factor 2 loadings from the chromatographic data from the 16 samples.

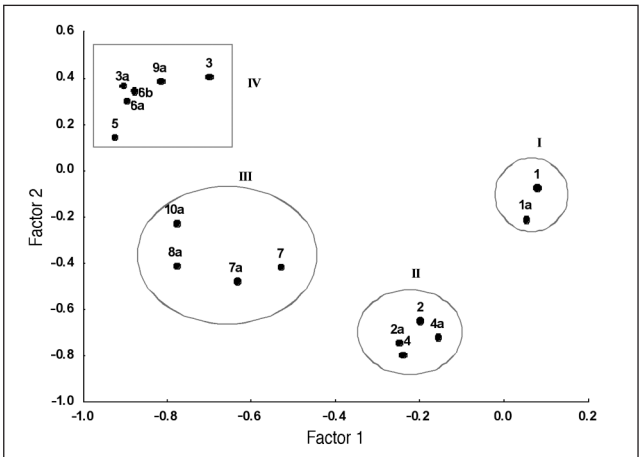

Figure 3. Graph for scores of Factor 1 with Factor 2 from the chromatographic data from the 16 samples.

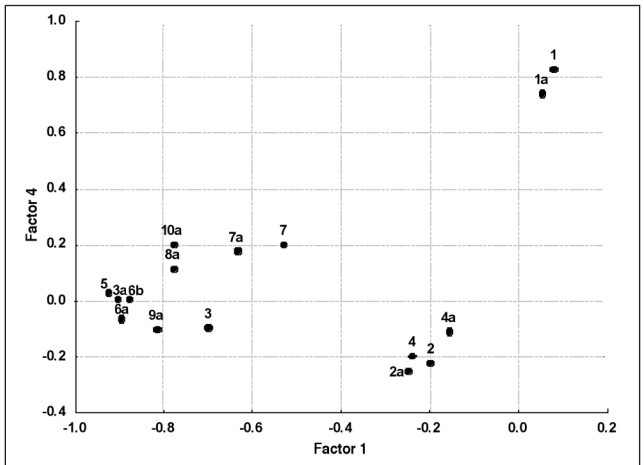

Figure 6. Graph of Factor 1 scores with Factor 4 for chromatographic data of the 16 samples.

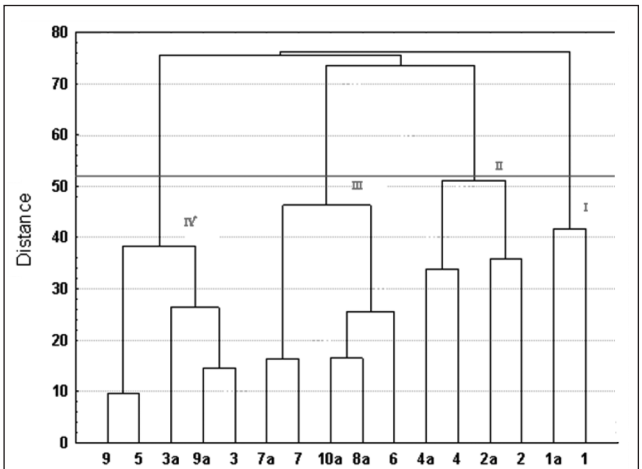

Figure 4. Dendrogram obtained from the analysis of hierarchical groupings based on chromatographic data from the 16 samples.

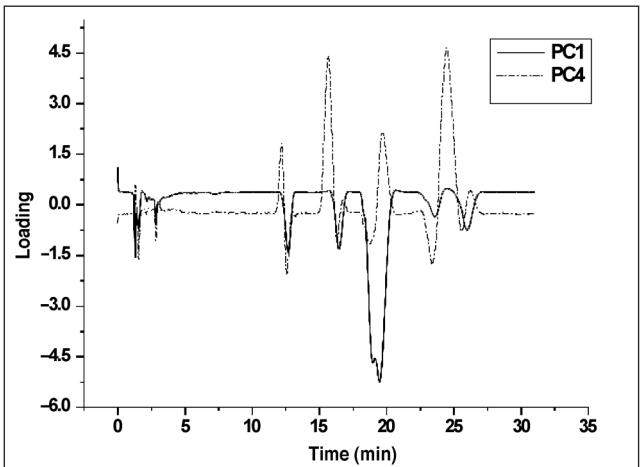

Figure 7. Graph of Factor 1 with Factor 4 loadings of chromatographic data for the 16 samples.

be noticed in this graph that the samples that do not contain corn are located more to the right in factor 1, while all samples containing corn in its composition are located to the left of this factor, that is, glucose, for presenting higher values of negative loadings in this component (Figure 5), is the main factor responsible for this discrimination. Factor 4 shows that group I is located in the most positive region of this factor.

Observing Figure 7, it can be noticed that the greatest values of positive loadings correspond to the carbohydrates mannose and galactose. This means that the level of these carbohydrates discriminate the samples of pure coffee.

In order to evaluate the effect of mixtures in the concentration of carbohydrates, linear and square models were used. All linear models were adjusted to a 95% confidence level. ANOVA of the regression results is presented in Table V. The linear models are given by:

Table V. ANOVA Tables for Linear Models of Six Carbohydrates

Variation source	Sum of squares	Degrees of freedom	Mean square	F-value	Probability
Mannitol					
Regression	1.012523	2	0.506261	46.16130	0.000000
Residuals	0.186443	17	0.010967		
Lack of fit	0.076913	7	0.010988	1.00317	0.481646
Pure Error	0.109529	10	0.010953		
Total	1.198966	19	0.063103		
Arabinose					
Regression	19.81592	2	9.907961	134.0503	0.000000
Residuals	1.25651	17	0.073912		
Lack of fit	0.53313	7	0.076162	1.0529	0.454826
Pure Error	0.72337	10	0.072337		
Total	21.07243	19	1.109075		
Galactose					
Regression	103.3420	2	51.67102	177.4430	0.000000
Residuals	4.9504	17	0.29120		
Lack of fit	0.9394	7	0.13420	0.3346	0.920233
Pure Error	4.0110	10	0.40110		
Total	108.2924	19	5.69960		
Glucose					
Regression	5584.670	2	2792.335	313.1800	0.000000
Residuals	151.573	17	8.916		
Lack of fit	32.895	7	4.699	0.3960	0.884213
Pure Error	118.678	10	11.868		
Total	5736.243	19	301.908		
Xylose					
Regression	19.10171	2	9.550853	110.0718	0.000000
Residuals	1.47508	17	0.086769		
Lack of fit	0.59079	7	0.084399	0.9544	0.509276
Pure Error	0.88429	10	0.088429		
Total	20.57678	19	1.082989		
Mannose					
Regression	195.1420	2	97.57099	174.7631	0.000000
Residuals	9.4912	17	0.55830		
Lack of fit	1.9616	7	0.28023	0.3722	0.898726
Pure Error	7.5296	10	0.75296		
Total	204.6331	19	10.77017		

$$\text{Mannitol} = \underset{(\pm 0.055)}{-0.006\ \text{coffee}} + \underset{(\pm 0.055)}{0.736\ \text{husk}} + \underset{(\pm 0.055)}{0.055\ \text{corn}} \quad \text{Eq. 1}$$

$$\text{Arabinose} = \underset{(\pm 0.142)}{2.099\ \text{coffee}} + \underset{(\pm 0.142)}{3.960\ \text{husk}} + \underset{(\pm 0.142)}{0.314\ \text{corn}} \quad \text{Eq. 2}$$

$$\text{Galactose} = \underset{(\pm 0.282)}{8.074\ \text{coffee}} + \underset{(\pm 0.282)}{2.587\ \text{husk}} - \underset{(\pm 0.282)}{0.095\ \text{corn}} \quad \text{Eq. 3}$$

$$\text{Glucose} = \underset{(\pm 1.562)}{1.127\ \text{coffee}} + \underset{(\pm 1.562)}{3.960\ \text{husk}} - \underset{(\pm 1.562)}{54.137\ \text{corn}} \quad \text{Eq. 4}$$

$$\text{Xylose} = \underset{(\pm 0.153)}{-0.087\ \text{coffee}} + \underset{(\pm 0.153)}{3.354\ \text{husk}} + \underset{(\pm 0.153)}{0.777\ \text{corn}} \quad \text{Eq. 5}$$

$$\text{Mannose} = \underset{(\pm 0.391)}{9.979\ \text{coffee}} + \underset{(\pm 0.391)}{0.262\ \text{husk}} - \underset{(\pm 0.391)}{0.131\ \text{corn}} \quad \text{Eq. 6}$$

Standard error estimates are presented in parenthesis directly below their corresponding model coefficients. Bold-faced coefficients indicate those that are significant at the 95% confidence level. In Equation 1, it can be observed that only the husk contains mannitol, the terms of the Equation for coffee and corn are not significant. It is possible to observe, by Equation 2, that the presence of husk in coffee significantly increases the level of arabinose. In Equation 3, it can be observed that both coffee and husk contribute to the increase in the level of galactose, this increase being more significant in coffee. Corn contributed to the increase in the level of glucose, Equation 4. For xylose (Equation 5), the most significant term is of the husk, while for mannose (Equation 6) is only for coffee. These results show that the increase in the addition of husk to coffee results in an increase in the levels of mannitol and xylose. The addition of corn to coffee increases the level of glucose. In the mixture of the three, the concentration of arabinose increases. Greater concentrations of mannose are obtained for pure coffee, while the mixture of coffee and husk increase the concentration of galactose. The predominance of each carbohydrate in relation to the proportions of coffee, husk, and corn can be observed by the response surfaces which are presented in Figure 8.

Conclusions

The analysis of total carbohydrates using HPAEC–PAD for roasted and ground coffee was shown to be efficient in determining the level of each monosaccharide present in coffee and adulterants studied in this paper, husk and corn, based on the predominance of polysaccharides constituting the matrixes described in literature. The results followed similar tendencies to the profiles related for soluble coffee of high levels of galactose and mannose, for pure coffee, of mannitol and xylose for coffee husk, and of glucose for corn. The application of statistic design enabled the acquisition of linear models, which presented the influence of adulterants incorporated to coffee in the levels of carbohydrates. Factor analysis enabled the differentiation of four distinct groups according to the composition of the mixtures of raw material used, confirmed by the hierarchical analysis.

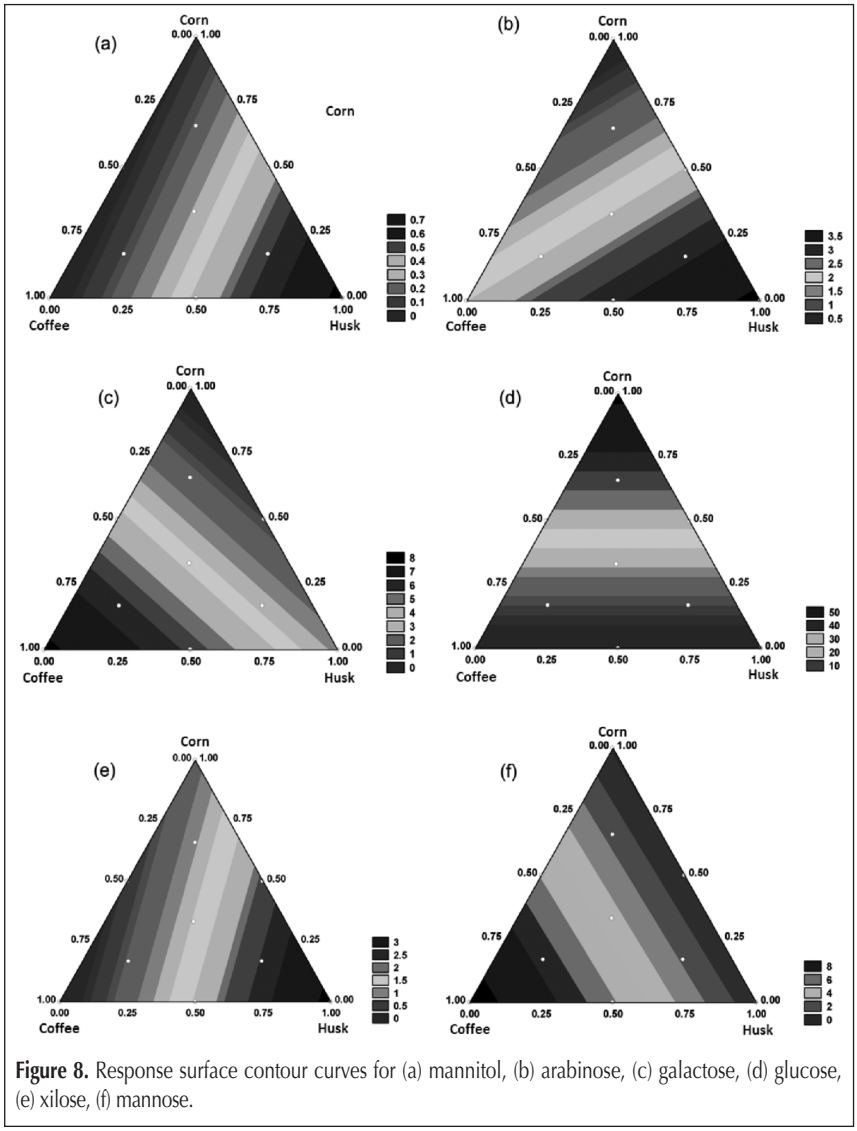

Figure 8. Response surface contour curves for (a) mannitol, (b) arabinose, (c) galactose, (d) glucose, (e) xilose, (f) mannose.

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