HPLC Analysis of Carbohydrates in Wines and Instant Coffees Using Anion Exchange Chromatography Coupled to Pulsed Amperometric Detection

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Carbohydrates (arabinose, fructose, fucose, galactose, glucose, mannose, rhamnose, ribose, sucrose, xylose, and the alditol mannitol) have been analyzed in wines and instant coffees, using anion exchange chromatography coupled to pulsed amperometric detection. Good separation and resolution of the 11 compounds were obtained using just nanopure water as eluent, although rinsing the column with 0.2 M NaOH was necessary to avoid gradual decline in column resolution. Coffee solution, red wines, and rosé wines needed a cleanup process by solid-phase extraction on C_{18} cartridges. Arabinose, galactose, glucose, xylose, mannose, fructose, and ribose were detected and quantified in red wines using melezitose as internal standard. In the case of the coffee samples mannitol, fucose, arabinose, rhamnose, galactose, glucose, sucrose, mannose, and fructose were quantified and differences between unadulterated and adulterated coffees could be detected.

Keywords: *Pulsed amperometric detection; HPLC; carbohydrates; wines; coffees*

INTRODUCTION

Accurate determinations for individual carbohydrates are gaining increasing significance as they not only provide compositional information on samples but also assist in identifying adulteration, origin, manufacturer, etc.

In some natural products certain sugars are found in low concentrations, so sensitive techniques are required for their determination. Carbohydrates can be reliably determined by using a number of high-performance liquid chromatographic (HPLC) methods based on suitable combinations of the stationary phase and detection system. The stationary phases most frequently used for this purpose include those based on octadecylsilane (Honda et al., 1989; Taverna et al., 1991) and amino groups, (Nesternko and Savelev, 1990; Sancho et al., 1990) as well as ion-exchange resins (Villanueva et al., 1987; Garleb et al., 1989; Hardy and Towsend, 1989; La Course et al., 1990; White and Widmer, 1990; Lee, 1990; Prabhu and Baldwin, 1990; Peschet and Gicalone, 1991; Del Nozal et al., 1992, 1993; Coquet et al., 1992; Mopper et al., 1992)—the latter have aroused special interest recently. Also, pulsed amperometric detection (Garleb et al., 1989; Hardy and Towsend, 1989; La Course et al., 1990; Prabhu and Baldwin, 1990; White and Widmer, 1990; Peschet and Giacalone, 1991; Mopper et al., 1992) is one of the most efficient choices for determining low carbohydrate concentrations.

Carbohydrate analysis of wines involves some interesting aspects related to pentoses. These substances, which appear in grape juice as complex combinations from which they are released during fermentation, cannot be fermented by yeast, so they are transferred to wine unchanged. Hence their analysis can be of great value in determining the grape variety used to produce the wine or in establishing differences between wines from different regions. To date, the papers published in relation to HPLC analysis of carbohydrates in wines were limited to the major carbohydrates, and detection systems with a low sensitivity as refractive index were employed (Calull et al., 1992; Kaufman, 1993; Wang et al., 1992). In a previous work we determined ribose, arabinose, rhamnose, and xylose (pentoses) and mannose, fructose, galactose, and glucose (hexoses) in wines (Del Nozal et al., 1992, 1993). We used HPLC coupled to a fluorescent postcolumn derivatization reaction; although the detector provided acceptable detection limits, the overall procedure was complex and demanded intensive control by the operator.

Some carbohydrates, such as xylose, glucose, fructose, and sucrose, and alditols, such as mannitol, have been recognized as good tracers for instant coffee adulterations. These sugars also appear in low concentration, and the methods used for their determination have been enzymatic (Berger et al., 1990) and chromatographic procedures coupled to MS or UV detectors (Blanc et al., 1989; Davis et al., 1990), but neither of them used pulsed amperometric detection.

The aim of this work is to analyze pentoses (ribose, rhamnose, xylose, and arabinose) and hexoses (glucose, fructose, mannose, and galactose) in wine samples as well as alditols (mannitol) and carbohydrates (xylose, sucrose, glucose, fucose, arabinose, and fructose) in instant coffees. For this purpose the possibilities of anion exchange chromatography coupled to pulsed amperometric detection are tested and sample treatment is discussed.

MATERIALS AND METHODS

Reagents. Sugar standards were supplied by Aldrich Química S.A. (Madrid, Spain) and used to prepare the working standards by dissolution in nanopure water.

A stock solution of 5 M NaOH (Panreac, Madrid) was used to prepare working-strength solutions daily by appropriate dilution in nanopure water.

Cellulose nitrate filters of 0.45-μm pore diameter and Sep-Pak (C_{18}, NH_2, CN, Florisil and silica) cartridges (500 mg of adsorbent) were from Waters, a division of Millipore (Milford, MA).

Apparatus and Chromatographic Conditions. The chromatographic setup used comprised the following elements:

A Model CM 4000 pump from Milton Roy (Riviera Beach, FL) was used to propel the eluent, consisting of nanopure

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Table 1. Pump Conditions

time (min)	% solvent A[a]	% solvent B[b]
0	100	0
45	100	0
46	0	100
57	100	0

[a] Solvent A, nanopure water. [b] Solvent B, 0.2 M NaOH.

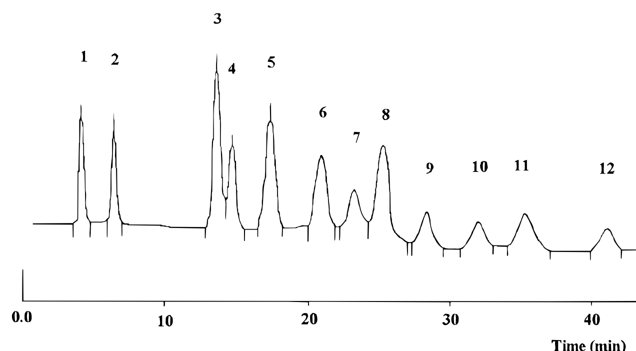

Figure 1. Chromatogram of a standard solution of the carbohydrates analyzed. Peaks: 1, mannitol; 2, fucose; 3, arabinose; 4, rhamnose; 5, galactose; 6, glucose; 7, sucrose; 8, xylose; 9, mannose; 10, fructose; 11, ribose; 12, melezitose.

Table 2. Carbohydrate Detection Limits and Retention Times

carbohydrate	detection limit (mg/L)	retention time (min)
arabinose	1.0	13.6
fructose	2.2	31.8
fucose	1.2	5.5
galactose	1.3	17.4
glucose	2.0	20.8
mannitol	1.2	4.0
mannose	2.0	29.4
melezitose	2.5	40.9
rhamnose	1.8	15.4
ribose	2.3	35.5
sucrose	2.5	22.9
xylose	1.3	25.1

water (solvent A) and 0.2 M NaOH (solvent B), according to the gradient shown in Table 1. The eluent was delivered at a flow rate of 1 mL/min and degassed with helium.

A ConstaMetric III pump, also from Milton Roy, was used to propel a 0.3 M NaOH solution at a flow rate of 0.6 mL/min after the column to boost the detector signal.

A Promis II autoinjector from Spark Holland (Emmen, The Netherlands) was furnished with a fixed loop of 20 μL.

A 4 × 250 mm Carbo-Pac PA1 column was used in conjunction with a 3 × 25 mm Carbo Pac Guard precolumn, both from Dionex Co. (Sunnyvale, CA).

A mixing tube consisted of a 50 cm long × 0.3 mm i.d. piece of Teflon tubing along which the eluate and 0.3 M NaOH were mixed to minimize baseline oscillations.

A Coulochem II 5040 pulsed amperometric detector from ESA, Inc. (Bedford, MA), was equipped with a gold working electrode that was set as follows: measurement potential, E_1 = 250 mV; measurement time, t_1 = 500 ms; delay time, t_D = 300 ms; E_2 = 700 mV; t_2 = 120 ms; E_3 = −900 mV; t_3 = 160 ms; range, 5 μA.

A Milton Roy CI 4000 recorder−integrator was used.

Sample Treatment. *Red Wines and Rosê Wines.* Forty microliters of the stock solution (10 g/L) of melezitose (internal standard) was added to a volume of 2 mL of wine and passed through a 0.45-μm filter and then through a Sep-Pak C_{18} cartridge which had previously been activated with 10 mL of methanol followed by 10 mL of nanopure water; 20 μL of the final eluate was injected into the chromatograph.

White Wines. The samples were only passed through a 0.45-μm filter and added melezitose to a final concentration of 200 mg/L; 20 μL of the final solution was injected into the chromatograph.

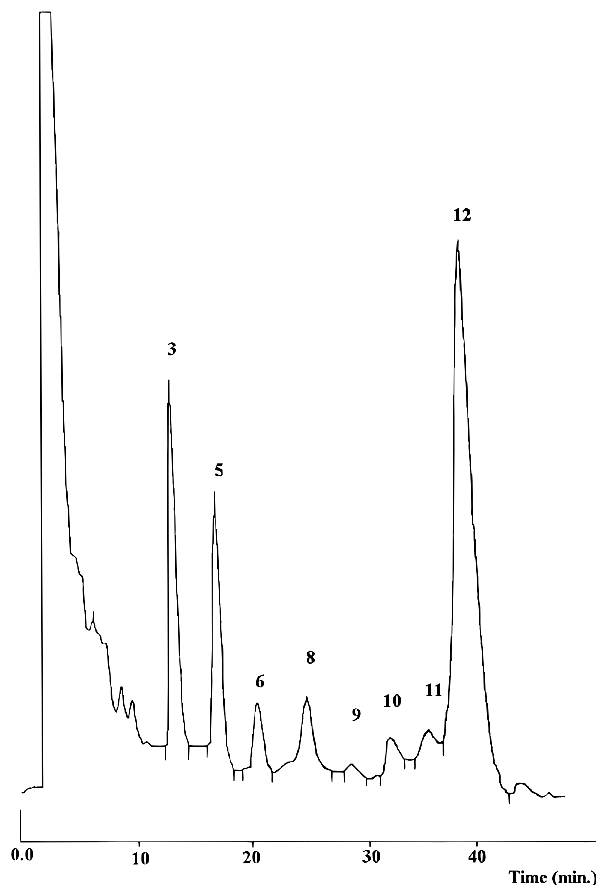

Figure 2. Chromatogram of a white wine. Peaks: 3, arabinose; 5, galactose; 6, glucose; 8, xylose; 9, mannose; 10, fructose; 11, ribose; 12, melezitose.

Table 3. Carbohydrate Percentage Retained on the C_{18} Sep-Pak Cartridge for a Red Wine Spiked with 2, 20, and 200 ppm of Each Carbohydrate

carbohydrate	ppm spiked		
	2	20	200
arabinose	23.1 ± 0.2[a]	23.2 ± 0.1	23.3 ± 0.1
fructose	26.3 ± 0.2	26.4 ± 0.1	26.5 ± 0.1
fucose	21.6 ± 0.6	21.6 ± 0.4	21.6 ± 0.4
galactose	36.4 ± 0.2	36.5 ± 0.1	36.5 ± 0.1
glucose	27.5 ± 0.3	27.6 ± 0.1	27.5 ± 0.1
mannitol	23.2 ± 0.5	23.3 ± 0.3	23.3 ± 0.3
mannose	28.4 ± 0.2	28.3 ± 0.1	28.3 ± 0.1
melezitose	23.6 ± 0.6	23.6 ± 0.4	23.7 ± 0.4
rhamnose	26.7 ± 0.6	26.6 ± 0.5	26.7 ± 0.4
ribose	25.7 ± 0.2	25.7 ± 0.2	25.7 ± 0.1
sucrose	23.6 ± 0.5	23.6 ± 0.4	23.7 ± 0.4
xylose	25.4 ± 0.5	25.4 ± 0.3	25.4 ± 0.3

[a] Results ± RSD.

Instant Coffees. For free sugars, an amount of 0.30 g of coffee was dissolved in 100 mL of nanopure water (16 °C). Then, 10 mL of the resulting solution was passed through a preactivated (10 mL of methanol followed by 10 mL of nanopure water) Sep-Pak C_{18} cartridge, and 20 μL of the eluate was analyzed.

Calibration Graphs. Stock solutions of the carbohydrates (10 g/L) were prepared in nanopure water. Standard solutions were prepared from stock solutions by sequential dilution with deionized water.

Calibration graphs for the quantitative analyses of the wines were run by using melezitose as internal standard. A fixed volume of the internal standard, to a final concentration of 200 mg/L, was added in both the standard and the samples, which were then subjected to the solid-phase extraction described above, except for the analyses of white wines. The

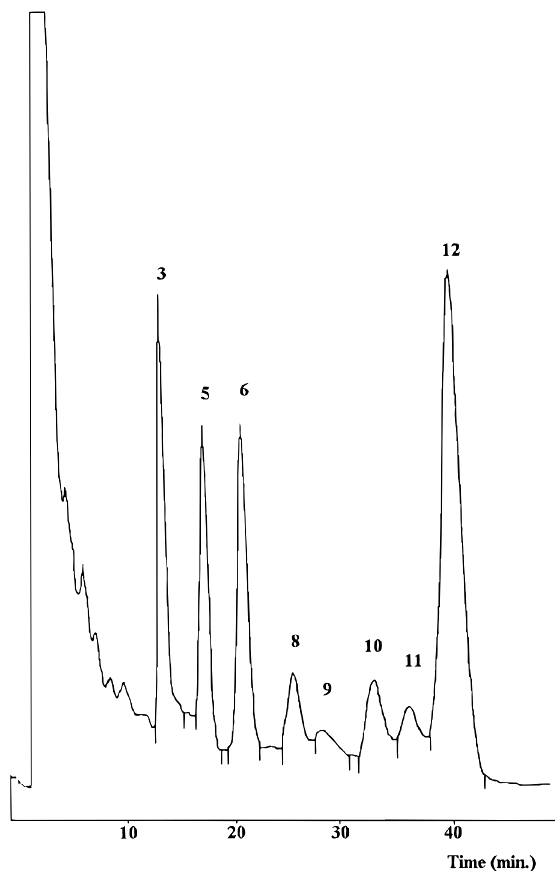

Figure 3. Chromatogram of a rosé wine. Peaks: 3, arabinose; 5, galactose; 6, glucose; 8, xylose; 9, mannose; 10, fructose; 11, ribose; 12, melezitose.

concentration ranged from 2 to 200 ppm for arabinose, galactose, and glucose and from 2 to 100 ppm for the other sugars.

The external standard method was used for the quantification of the coffee samples, and the concentration ranged from 2 to 100 ppm for each compound.

RESULTS AND DISCUSSION

Monosaccharides were resolved in isocratic conditions employing deionized water (Figure 1). Although some papers indicated the use of weak sodium hydroxide as eluent for the separation of monosaccharides, we have found that water as eluent gave the best resolution. This mobile phase was also used by Pettersen and Schwandt (1991), but only six monosaccharides were separated. Rinsing the column with 0.2 M NaOH for 10 min was necessary (Table 1) to avoid a gradual decline in column resolution, which could be noticed with the repeated use of nanopure water as eluent. The retention times and the detection limits calculated according to the IUPAC procedure (Long and Winefordner, 1983) are listed in Table 2 and ranged from 1 to 2.5 ppm, which is appropriate to determining minor sugars.

White wines only needed to be filtered by an 0.45-μm filter (Figure 2), but red wines, rosé wines, and the solutions of instant coffees required a cleanup process by solid-phase extraction because their pigment content gradually fouled the column, thereby increasing the pressure and diminishing the resolution. To circumvent this shortcoming, we tested various SPE cartridges with different stationary phases including C_{18}, Florisil, silica, NH_2, and CN, and we found that the first one provided the best results: it retained all pigments in the samples and extended the column lifetime. The optimum amount

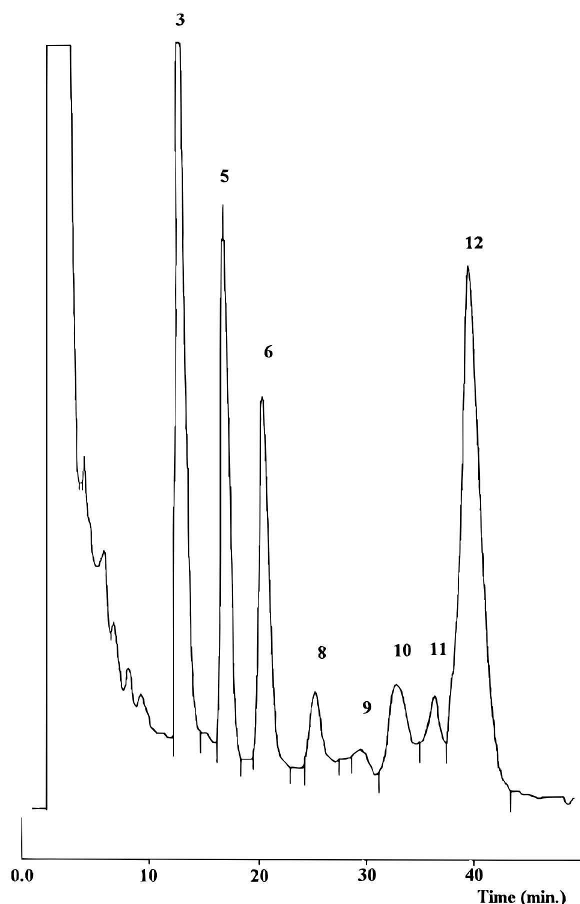

Figure 4. Chromatogram of a red wine. Peaks: 3, arabinose; 5, galactose; 6, glucose; 8, xylose; 9, mannose; 10, fructose; 11, ribose; 12, melezitose.

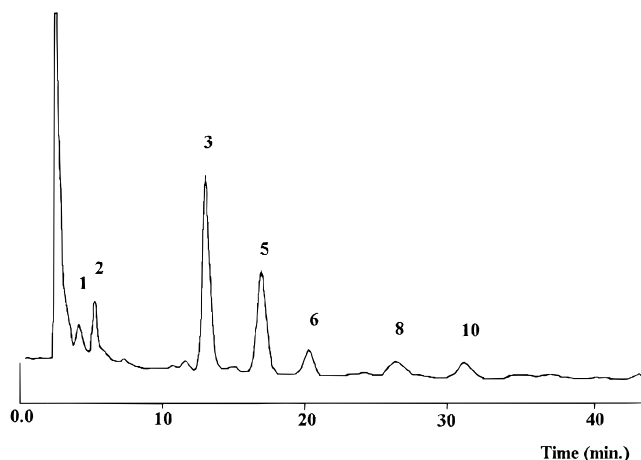

Figure 5. Chromatogram of a soluble coffee. Peaks: 1, mannitol; 2, fucose; 3, arabinose; 5, galactose; 6, glucose; 8, xylose; 10, fructose.

of sample to be passed through the cartridge was found to depend on its pigment content. Thus, the cartridge was saturated with volumes above 10 mL of coffee solution or 2 mL of wine. The quantities of carbohydrates retained on the Sep-Pak C_{18}, for a red wine spiked with 2, 20, and 200 ppm of each carbohydrate and passing 2 mL of the wine, are shown in Table 3. It should be noted that, for small sample volumes as in the case of the wines, a small fraction of carbohydrates was retained by the Sep-Pak cartridge, while when this assay was used for coffees (with 10 mL of the coffee solution passed through the cartridge), the quantity of carbohydrates retained was worthless. When the stan-

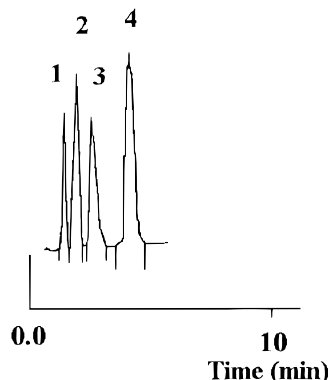

Figure 6. Chromatogram of a standard solution of 1, inositol; 2, xylitol; 3, sorbitol; 4, mannitol.

dards in nanopure water were passed through the cartridge, the results were similar: passing 2 mL, the same percentage as in the case of the wines was retained; and passing 10 mL, it was worthless. This could be explained by the fact that 10 mL is greater than the breakthrough volume for the carbohydrates on this kind of packing material. Rinsing the cartridge with deionized water produced a dilution of the wine, and the detection of minor sugars was not possible. So, due to the fact that a quantity of the carbohydrates is retained on the cartridge, the use of an internal standard (melezitose) and subjection of the samples and standards to the same treatment are necessary for the quantitative analyses of the red and rosé wines. The fact that polar compounds, such as carbohydrates, were retained on an apolar stationary phase (C_{18}) could be caused by the free OH groups from the silica particles. Figures 3, 4, and 5 show the chromatograms of a rosé wine, a red wine, and soluble coffee, respectively, after the solid-phase cleanup.

It should be noted that mannitol can be separated from other alditols such as inositol, xylitol, and sorbitol (Figure 6), although the determination of the last three in the samples was not possible because they coeluted with the peak of the matrix.

A red wine sample and a coffee sample were analyzed using the method proposed in this paper and by GC according to an AOAC method with preparation of volatile trimethylsilyl derivatives (AOAC, 1970). As can be seen in Table 4, there is a good correspondence between the results.

All of the red wines analyzed (Table 5) belong to the same Appellation D'Origin (same geographical region and the major percentage of the grape is of the same variety) and were found to be qualitatively similar with regard to carbohydrate composition. Six samples were analyzed per winemaker, and each sample was analyzed three times. They contained arabinose, galactose, and xylose, predominantly, and ribose and mannose as minor sugars. Rhamnose has not been found in any of

Table 4. Comparison of the Results Obtained with the Method Proposed and Those Obtained by GC Analysis

	wine (mg/L)		coffee (% based on dry material)	
carbohydrate	proposed method	GC	proposed method	GC
arabinose	53.1 ± 0.9[a]	53.5 ± 0.2	0.25 ± 0.02	0.26 ± 0.02
fructose				
fucose				
galactose	37.9 ± 0.9	38.1 ± 0.2	0.25 ± 0.05	0.25 ± 0.03
glucose	7.5 ± 0.9	7.9 ± 0.3		
mannitol			0.10 ± 0.04	0.11 ± 0.03
mannose	2.1 ± 0.9	2.3 ± 0.3	0.07 ± 0.02	0.07 ± 0.02
rhamnose				
ribose				
sucrose				
xylose	26.4 ± 0.9	26.6 ± 0.2		

[a] Results ± RSD.

Table 5. Carbohydrate Contents of Red Wines Analyzed

		carbohydrate concentration (mg/L)						
winemaker	time in the barrel (months)	arabinose	galactose	glucose	xylose	mannose	fructose	ribose
1	6	42.6 ± 0.9[a]	45.4 ± 0.9	145.2 ± 0.9	22.4 ± 0.9	2.6 ± 0.8	86.0 ± 0.9	
	12	53.1 ± 0.9	37.8 ± 0.9	7.5 ± 0.9	26.4 ± 0.9	2.1 ± 0.9		
2	6	58 ± 1	37 ± 1	28.5 ± 0.9	58.3 ± 0.9	11.4 ± 1	31.6 ± 0.9	3.9 ± 0.7
	12	26.9 ± 0.9	25 ± 1	13.4 ± 0.9	17.1 ± 0.9			13.5 ± 0.5
3	6	19.3 ± 0.9	27.3 ± 0.9	11.1 ± 0.9	15.3 ± 0.9	4.0 ± 0.9		
	12	33.4 ± 0.9	30.6 ± 0.9	9.3 ± 0.9	11.3 ± 0.9			
4	6	139 ± 1	40.3 ± 0.9	17.4 ± 0.9	12.7 ± 0.9		2.9 ± 0.9	
	12	179.1 ± 0.9	51.4 ± 0.9	12.6 ± 0.8	43.1 ± 0.9			28 ± 1
5	6	144.3 ± 0.9	79.7 ± 0.9	27.2 ± 0.9	37.1 ± 0.9	3.2 ± 0.9		
	12	62 ± 1	42.3 ± 0.9	5.1 ± 0.9	19.3 ± 0.9			16.4 ± 0.9

[a] Results ± RSD.

Table 6. Free Carbohydrate Contents of Soluble Coffees Analyzed (Percentage Based on Dry Material)

class	mannitol	fucose	arabinose	rhamnose	galactose	glucose	sucrose	mannose	fructose
A	0.17 ± 0.02[a]	0.148 ± 0.005	0.04 ± 0.02			2.23 ± 0.01	0.82 ± 0.02	0.30 ± 0.01	0.96 ± 0.02
B	0.15 ± 0.01	0.083 ± 0.002		0.05 ± 0.02		1.31 ± 0.02	0.74 ± 0.02		2.42 ± 0.03
C	0.15 ± 0.04	0.107 ± 0.002	0.46 ± 0.02		0.24 ± 0.04	0.11 ± 0.02		0.17 ± 0.01	
D	0.101 ± 0.003	0.107 ± 0.002	0.25 ± 0.03		0.25 ± 0.02			0.07 ± 0.01	
E	0.074 ± 0.006	0.129 ± 0.005	0.40 ± 0.01		0.29 ± 0.05	0.57 ± 0.03			0.56 ± 0.03
F	0.16 ± 0.01	0.125 ± 0.005	0.06 ± 0.02	0.05 ± 0.03		0.58 ± 0.02	0.55 ± 0.02		0.53 ± 0.3

[a] Results ± RSD.

them. The levels of glucose and fructose, as both are fermented by yeast, depend on wine age, so wines aged for 6 months in barrels contained the highest levels of these hexoses. There were also quantitative differences that can be related to the grape variety and winemaking procedure used in each case; thus, the wines from cellars 4 and 5 present the highest contents of arabinose and galactose, and the wines from cellar 3 present the lowest contents of xylose. The level of mannose, for the wines in which this carbohydrate was detected, decreased with aging and the levels of ribose increased. This leads us to believe that the evolution of mannose and ribose is related to the action of the barrel wood: mannose could be adsorbed, and ribose could be released.

The coffee samples analyzed were instant coffees sold as pure coffees from roasted Colombian (class C), Brazilian (class D), and Ecuadorian (class E) beans, as well as pure instant coffee commercialized in Spain (class F). The samples of class F were spiked with 5% of extracts of chicory (class B) and cereal blend (class A). Six samples were analyzed per class, and each sample was analyzed three times. In Table 6 the free carbohydrate contents are shown; as can be seen, xylose has not been detected in any of the samples analyzed, and it should be noted that the levels of free glucose and fructose are much higher in the adulterated coffees, which agrees with the results obtained by Berger et al. (1991). High levels of sucrose have also been found in the adulterated coffees. Although adulteration with coffee husk or parchment is not covered by the present study, it should be also detectable because very low concentrations of xylose could be determined with this technique.

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