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Effect of Sample Preparation on the Measurement of Sugars, Organic Acids, and Polyphenols in Apple Fruit by Mid-infrared Spectroscopy

Sylvie Bureau,*[,†,‡] Iwona Ścibisz,[§] Carine Le Bourvellec,[†,‡] and Catherine M. G. C. Renard[†,‡]

[†]UMR408 Sécurité et Qualité des Produits d'Origine Végétale, INRA, F-84000 Avignon, France

[‡]UMR408 Sécurité et Qualité des Produits d'Origine Végétale, Université d'Avignon et des Pays de Vaucluse, F-84000 Avignon, France

[§]Department of Food Technology, Faculty of Food Science, University of Life Science SGGW, Nowoursynowska 166, 02-787 Warsaw, Poland

ABSTRACT: The objectives of this study were (i) to test different conditions of freezing, thawing, and grinding during sample preparation and (ii) to evaluate the possibility of using mid-infrared spectroscopy for analyzing the composition of sugars, organic acids, and polyphenols in apples. Seven commercial apple cultivars were chosen for their large variability in composition (total polyphenols from 406 to 1033 mg kg^{-1} fresh weight). The different conditions of sample preparation affected only the phenolic compounds and not sugars or organic acids. The regression models of the mid-infrared spectra showed a good ability to estimate sugar and organic acid contents ($R^2 \geq 0.96$), except for citric acid. Good predictions were obtained for total phenolic, flavan-3-ols, and procyanidins ($R^2 \geq 0.94$) provided oxidation was avoided during sample preparation. A rapid and simple procedure was then proposed for phenolic compounds using sodium fluoride during sample homogenization at ambient temperature and freeze-drying before spectra acquisition.

KEYWORDS: *Malus domestica Borkh., oxidation, stabilization, ATR-FT-IR, chemometrics*

INTRODUCTION

Mid-infrared (MIR) has been shown to be a suitable technique for high-throughput analysis of the major constituents in fruit juices, homogenates, and extracts.[1−7] These studies have been aimed to assay soluble solids content, titratable acidity, and the concentrations of individual sugars, organic acids, and total phenolic compounds. It would be highly beneficial if it could also be applied to the analysis of specific metabolites such as individual polyphenols. Indeed, the quantification of these compounds requires chemicals and solvents, equipment such as high-performance liquid chromatography coupled to a diode array detector, good technical skill, and time. However, the development of high-throughput analyses has been hindered by the high reactivity of some molecules such as polyphenols. Hence, this study aimed at identifying the simplest and cheapest sample preparation for MIR application for polyphenol quantification. The fruit chosen for this methodological optimization was apple, as it is among the most consumed fruits worldwide and indeed a good source of polyphenols in the French diet.[8] A regular consumption of apples provides a large amount of polyphenolic compounds such as flavan-3-ols (procyanidins and catechins), dihydrochalcones (phloridzin), hydroxycinnamic acids (chlorogenic acid and *p*-coumaroylquinic acid), flavonols (quercetin), and anthocyanins. A large variability exists according to the studied cultivars with, for example, a variation of total phenolic content between 523 and 2724 mg kg^{-1} dry weight (DW) among 67 new and old apple varieties.[9]

At the same time, polyphenols in apple are vulnerable to degradation as substrates of polyphenol oxidases (PPO) and, therefore, are a source of enzymatic browning.[10] The browning susceptibility varies among apple cultivars, depending on the phenolic composition, notably the concentration of catechins and chlorogenic acid, and the PPO activities.[11] In the same way, concerning the carbohydrate metabolism, the ripe apples contain fructose, glucose, sucrose, and malic acid, in that starch is entirely converted to soluble sugars; the contents of these metabolites are dependent on the action of different enzymes such as invertase, sucrose synthase, phosphoenolpyruvate carboxylase, and NAD-dependent malate dehydrogenase playing an important role in determining the fruit composition.[12,13] Therefore, during sample preparation some precautions are needed to prevent the loss or degradation of studied components. The sample manipulation depends on the food and matrix. Many different procedures have been described in the literature as exemplified here for apples. Whole fruits require several steps such as crushing, freeze-drying, grinding, and then an extraction of polyphenols from the matrix using solvents. For an efficient and reproducible extraction, fruits must be reduced to small particles by crushing or grinding to obtain a homogeneous powder. Samples are then frequently stabilized with the addition of compounds preventing the oxidation of phenolics to their respective quinones, such as ascorbic acid,[14] sodium fluoride (NaF),[15] or butylated hydroxytoluene (BHT).[16] However, because freeze-dryers have become more commonplace in laboratories, it has been the method of choice for sample stabilization. Burda et al.[14] peeled apples and homogenized in a blender separately the peel and the flesh with the extraction solvent. Lamperi et al.,[15] after

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peeling apples, froze separately the flesh and the skin in liquid nitrogen; these were stored at −80 °C and then minced to obtain a fine powder for the extraction step. Alonso-Salces et al.[17] peeled the apples, and subsequently the peel was frozen in liquid nitrogen, freeze-dried, crushed in closed vials, and stored at room temperature in a desiccator until analysis. Other authors divided the flesh in aliquots and lyophilized and stored it at −20 °C until analysis.[16] Guyot et al.[18] advised a direct thiolysis on freeze-dried apple powder.

To conclude, the main variants in fruit preparation were the speed of freezing (liquid nitrogen or freezer), the temperature of storage (−20 or −80 °C), and the mode of preservation from oxidation (freezing and addition of antioxidant compounds). Then, the first aim of this study was to monitor quantitative changes in sugars, organic acids, and polyphenols involved during sample preparation of apples. Three steps were tested including the sample storage temperature, the kind of sample grinding, and the sample oxidation state. The second aim of this study was to evaluate the possibility of using Fourier transform mid-infrared spectroscopy using the attenuated total reflectance accessory (ATR-FT-IR) spectroscopy to predict these metabolite contents (sugars, organic acids, and polyphenols) in apples. The final objective was then to identify the simplest and fastest sample preparation and analysis that would preserve the studied molecules. To confirm any effect and take into account a large phenotypic variability, seven different apple cultivars (Gala, Golden Delicious, Fuji, Pink Lady, Canada, Chantecler, and Granny Smith) were studied.

MATERIALS AND METHODS

Standards and Chemicals. Chlorogenic acid, (+)-catechin, (−)-epicatechin, sucrose, glucose, fructose, and citric acid were obtained from Sigma-Aldrich (Deisenhofen, Germany). Phloretin and *p*-coumaric acid were from Extrasynthese (Genay, France). Phloridzin was from Fluka (Buchs, Switzerland) and malic acid from R-Biopharm (Darmstadt, Germany). Acetic anhydride and toluene-α-thiol were also acquired from Sigma-Aldrich. Acetonitrile was of HPLC grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA).

Plant Material. Apple fruits (*Malus domestica* Borkh. from the Rosaceae family) were purchased from a local supermarket in November 2007. Seven commercial cultivars were compared: Gala, Golden Delicious, Fuji, Pink Lady, Canada, Chantecler, and Granny Smith. For each cultivar, three replicates of five apples were analyzed. Each apple was peeled, cored, and divided as described by Renard.[19] Each apple was cut vertically into 12 equal portions, and then portions were cut in two horizontally, giving 24 pieces per apple. The 24 portions were distributed in 8 homogeneous and similar samples with a systematic repartition of top and bottom pieces and sunny and shaded face (Figure 1). The eight samples of five fruits per cultivar were pooled and submitted to different conditions of sample preparation.

Experiment. Three parameters were tested and are described in Figure 2. (1) Storage temperature at −20 or −80 °C: (i) pieces of apple flesh were frozen rapidly at −30 °C in a freezer with pulsed air and stored at −20 °C or (ii) pieces were put immediately in liquid nitrogen prior to storage at −80 °C. Samples were stored for 2 months until analysis. (2) Sample grinding: (frozen pieces were (i) ground in liquid nitrogen using a PM-400 ball grinder and more precisely a planetary ball mill (Retsch GmbH, Germany) or (ii) homogenized with Ultraturrax T-25 equipment (IKA, Labortechnik, GmbH, Staufen, Germany) after 1.5 h of thawing at 20 °C. (3) Sample oxidation during thawing for 20 h at 20 °C: (i) sample was thawed under nitrogen gas, and after homogenization, the sample was treated with sodium fluoride (100 mL of solution at 5 g L^{-1} added per kg of fruits) to inactivate polyphenol oxidases and prevent phenolic degradation (abbreviated

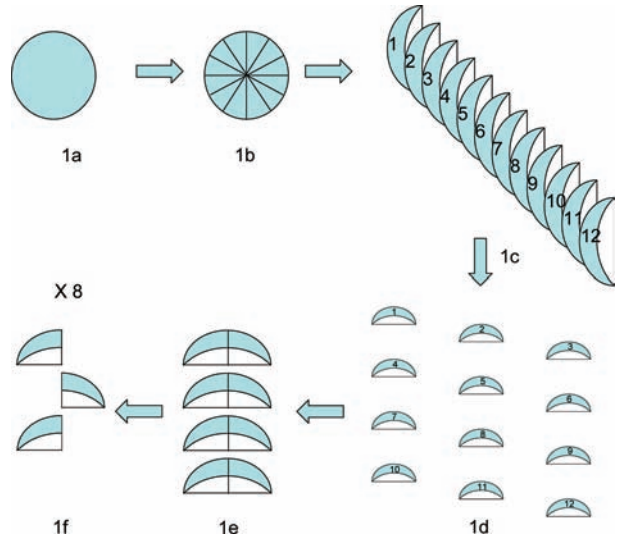

Figure 1. Scheme of fractionation of each apple to constitute eight homogeneous samples.

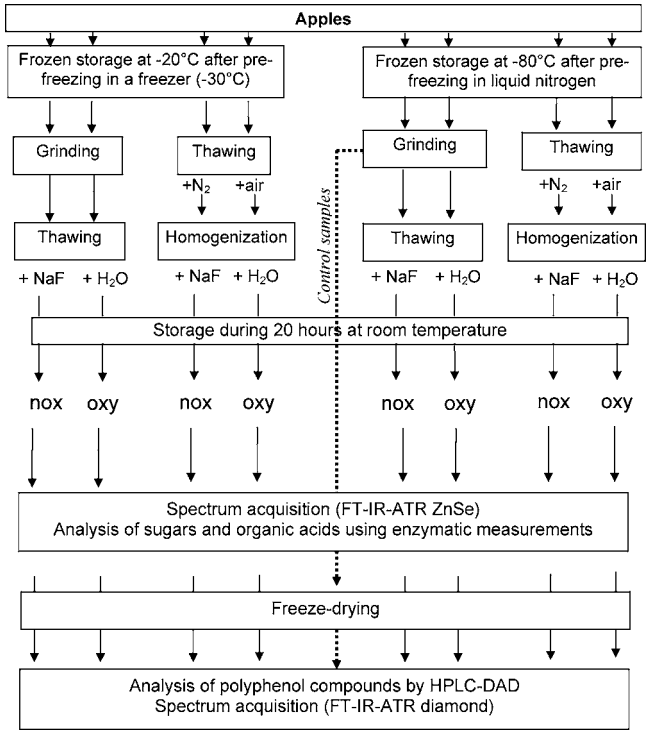

Figure 2. Experimental scheme for sample preparation. Grinding was performed in liquid nitrogen using a ball grinder, and homogenization was done at room temperature with Ultraturrax equipment after 1.5 h of defrosting at 20 °C. Nitrogen (N_2) and a solution of sodium fluoride (NaF) were added to inactivate polyphenol oxidases (PPO) and prevent phenolic degradation or, inversely, in the presence of air and water to allow phenolic oxidation. For the reference sample, after grinding in liquid nitrogen, a part of the sample was directly freeze-dried.

"nox" for nonoxidized samples) or (ii) sample was thawed in the presence of air, and water (the same quantity as in the nox samples) was added to homogenized apples allowing phenolic oxidation (abbreviated "oxy" for oxidized samples).

After treatments, all eight homogeneous samples were divided into halves. Half was directly used for the analyses of soluble solids content (SSC), titratable acidity (TA), sugars (glucose, fructose, and sucrose),

and organic acids (malic and citric acids) and for the spectrum acquisition in the MIR region (see ATR-ZnSe). The other half was freeze-dried and used for the analysis of polyphenol composition and for the spectrum acquisition in the MIR region (see ATR-Diamond).

A fraction of the sample, frozen in liquid nitrogen, stored at −80 °C, and ground in liquid nitrogen using a PM-400 ball grinder was directly freeze-dried for phenolic compound analysis and will be considered as the control (C).

Biochemical Analyses. SSC was determined with a digital refractometer (PR-101 ATAGO, Norfolk, VA, USA) and expressed in °Brix at 20 °C. TA was determined by titration up to pH 8.1 with 0.1 N NaOH and expressed in mequiv kg^{-1} fresh weight (FW) using an autotitrator (Methrom, Herisau, Switzerland). Sugars (glucose, fructose, and sucrose) and organic acids (malic acid and citric acid) were quantified using an enzymatic method with kits for food analysis (Boehringer Mannheim Co., Mannhein, Germany) and expressed in g kg^{-1} FW. These measurements were performed with an automatic analyzer BM-704 (Hitachi, Tokyo, Japan).

For polyphenols, 100 mg of freeze-dried apple powder was directly submitted to thioacidolysis, and the reaction mixture was then analyzed by high-performance liquid chromatography (HPLC)−diode array detection (DAD), as already described by Guyot et al.[18] HPLC-DAD analyses of methanolic extracts that were not submitted to thioacidolysis were also performed to separately assay monomeric catechins and procyanidins. HPLC-DAD analyses were performed using an Agilent 1050 instrument (Palo Alto, CA, USA). Chromatographic separation was carried out using a (250 × 4 mm i.d.) Licrospher PR-18 5 μm column (Merck, Darmstadt, Germany) with a guard column (Licrospher PR-18 5 μm column, Merck), both thermostated at 30 °C. The mobile phase consisted of water/acetic acid (97.5:2.5, v/v) (eluent A) and acetonitrile (eluent B). The flow rate was 1 mL min^{-1}. The elution program was as follows: 3−9% B (0−5 min); 9−16% B (5−15 min); 16−50% B (15−45 min); 50−90% B (45−48 min); 90−90% B (48−52 min); 90−3% B (52−55 min); 3−3% B (55−60 min). Twenty microliters of sample was injected. Individual compounds were quantified in mg kg^{-1} FW by comparison with external standards at 280 nm for (+)-catechin, (−)-epicatechin, phloretin xyloglucoside (quantified as phloretin), phloridzin, and (−)-epicatechin benzyl thioether (quantified as (−)-epicatechin) and at 320 nm for chlorogenic acid *p*-coumaroylquinic acid (quantified as *p*-coumaric acid) and their methylated derivatives obtained during thioacidolysis reaction quantified as their respective nonmethylated equivalents.

ATR-FT-IR Spectrum Acquisition. Two types of spectrum acquisition named in this study ATR-ZnSe and ATR-Diamond were performed according to the sample preparation. The ATR-ZnSe accessory is dedicated to liquids or purees just put on the ZnSe crystal, whereas the ATR-Diamond equipped with an ergonomic one-finger clamp mechanism that compresses samples is dedicated to freeze-dried samples.

ATR-ZnSe. As previously described,[5,7] MIR spectra were collected at room temperature with a Tensor 27 FTIR spectrometer (Bruker Optics, Wissembourg, France) equipped with a horizontal attenuated total reflectance (ATR, Bruker Optics) sampling accessory and deuterated triglycine sulfate (DTGS) detector. The homogenized samples of apples were placed at the surface of the zinc selenide crystal with six internal reflections. The samples were scanned at wavenumbers ranging from 650 to 4000 cm^{-1} and corrected against the background spectrum of air. The spectrum of each sample was obtained by taking the average of 32 scans. The crystal was cleaned between measurements with deionized water and dried with lint-free tissue. Instrument control and spectral collection were performed using OPUS software (version 5.0 Bruker Optics) supplied by the equipment manufacturer.

ATR-Diamond. The same Tensor 27 FTIR spectrometer (Bruker Optics) was used but equipped with a single-reflectance horizontal ATR cell (Golden Gate equipped with a diamond crystal, Bruker Optics). The freeze-dried homogenized samples of apples were placed at the surface of the diamond crystal and were pressed with a system press tip flap. The samples were scanned at wavenumbers ranging from 600 to 4000 cm^{-1} and corrected against the background spectrum of air. The spectrum of each sample was obtained by taking the average of 16 scans.

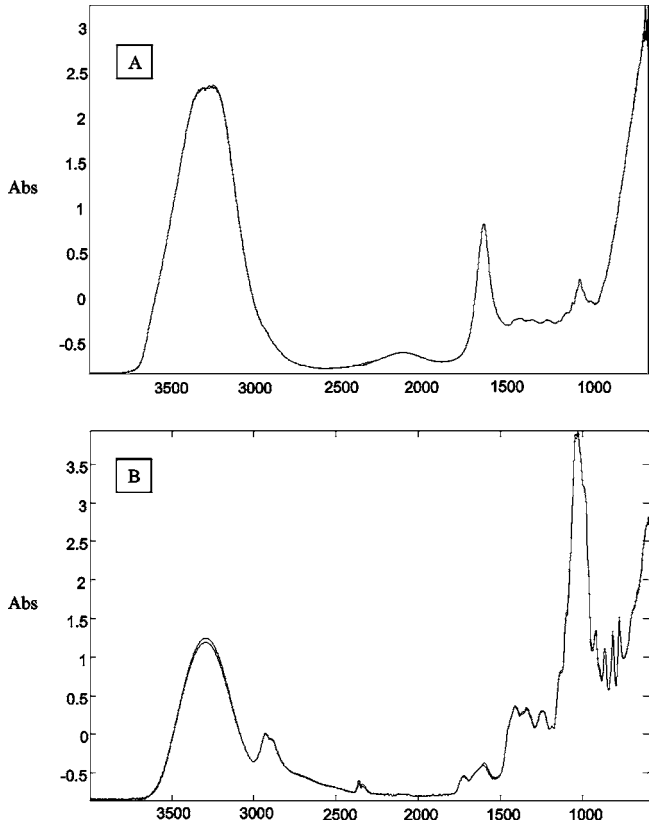

Figure 3. FT-IR spectra (4000−900 cm^{-1}) of apples: (A) apple puree recorded using a six-reflectance horizontal ATR cell equipped with a ZnSe crystal; (B) freeze-dried apple puree recorded using a single-reflectance horizontal ATR cell equipped with a diamond crystal. Oxidized apple (oxy) spectra are represented in a solid line and nonoxidized (nox) apple spectra in a dotted line.

Statistical Analyses. Results are presented as mean values, and the reproducibility of the results was expressed as pooled standard deviation values (pooled SD). Pooled standard deviations were calculated using the sum of individual variances divided by the individual degrees of freedom of each series of replicates.

Two analyses of variance (ANOVA) were performed using biochemical data. The first one concerned the analysis of the effect of sample preparation on sugars, organic acids, and phenolic compounds, and the statistical design used was as follows: 7 varieties (Gala, Golden Delicious, Fuji, Pink Lady, Canada, Chantecler, and Granny Smith) × 2 storage temperatures (−20 and −80 °C) × 2 sample grinding conditions (ball grinder with liquid nitrogen or homogenization at ambient temperature) × 2 oxidation states (oxy and nox) × 3 replicates with 167 degrees of freedom. ANOVA was carried out to determine significant differences between conditions of sample preparation using the *XL*STAT (version 2011.1.03, Addinsoft SARL, Paris, France) data analysis toolbox. The pairwise comparison between means was performed using Tukey's test ($P < 0.05$ (*), 0.01 (**), and 0.001 (***)). The second ANOVA concerned the analysis of the thawing effect on phenolic composition exclusively. This effect was observed on the same seven varieties on two types of samples: the reference samples (storage at −80 °C, grinding in the presence of liquid nitrogen, and direct freeze-drying) and the samples prepared using the conditions best preserving samples against oxidation (storage at −80 °C, grinding in the presence of liquid nitrogen, and prevention of oxidation using NaF).

Chemometrics. Spectral preprocessing and multivariate data analysis were performed with Matlab 7.5 (Mathworks Inc., Natick, MA)

Table 1. Biochemical Composition in the Flesh (Data Expressed in Fresh Weight) of Different Apple Cultivars and Different Sample Preparations[a]

variety	storage temp (°C)	sample grinding	oxidation	dry matter (%)	SSC (°Brix)	TA (mequiv kg^{-1})	fructose (g kg^{-1})	sucrose (g kg^{-1})	glucose (g kg^{-1})	malic acid (g kg^{-1})	citric acid (g kg^{-1})
Canada	−80	grinding	nox	12.4	10.6	105	46.0	24.7	11.0	7.3	0.07
			oxy	12.4	10.7	104	46.5	24.5	11.0	7.3	0.07
		homogenization	nox	12.2	10.7	104	45.4	23.7	11.0	7.2	0.10
			oxy	12.2	10.7	103	47.2	23.9	11.6	7.2	0.05
	−20	grinding	nox	12.3	10.7	102	46.0	24.5	11.0	7.2	0.05
			oxy	12.6	10.7	108	47.4	25.0	11.4	7.3	0.08
		homogenization	nox	12.3	10.7	103	45.4	24.3	11.0	7.2	0.05
			oxy	12.2	10.7	104	46.2	23.6	11.1	7.1	0.05
Chantecler	−80	grinding	nox	15.3	13.5	70	48.9	46.8	15.8	5.5	0.00
			oxy	15.5	13.4	71	49.9	46.2	15.1	5.6	0.00
		homogenization	nox	15.4	13.5	71	50.4	45.2	14.4	5.5	0.00
			oxy	15.2	13.5	71	49.2	45.4	16.0	5.5	0.00
	−20	grinding	nox	15.4	13.5	71	49.4	47.4	15.2	5.6	0.00
			oxy	15.7	13.5	72	49.3	47.5	15.8	5.5	0.00
		homogenization	nox	15.3	13.5	70	49.5	45.6	15.3	5.6	0.00
			oxy	15.2	13.3	70	49.0	45.6	15.2	5.4	0.00
Fuji	−80	grinding	nox	14.8	13.0	32	62.3	21.7	21.7	2.8	0.00
			oxy	14.7	13.0	31	60.0	22.6	22.4	2.8	0.02
		homogenization	nox	14.5	13.0	32	61.6	21.1	22.4	2.8	0.02
			oxy	14.3	13.0	31	60.5	21.7	22.1	2.8	0.03
	−20	grinding	nox	14.8	13.0	31	62.8	21.2	22.3	2.9	0.02
			oxy	15.0	13.0	32	60.7	22.1	22.7	2.9	0.00
		homogenization	nox	14.3	13.0	31	60.6	20.5	21.9	2.8	0.00
			oxy	14.4	13.0	30	62.0	19.9	23.1	2.8	0.03
Gala	−80	grinding	nox	14.2	12.3	42	56.5	26.6	16.3	3.5	0.13
			oxy	14.1	12.3	41	55.5	27.8	15.3	3.5	0.15
		homogenization	nox	14.1	12.3	42	57.0	27.4	15.6	3.6	0.10
			oxy	14.0	12.3	43	56.9	27.5	16.2	3.6	0.10
	−20	grinding	nox	14.2	12.3	41	56.8	27.8	15.6	3.6	0.17
			oxy	14.4	12.4	41	56.7	28.0	15.3	3.6	0.10
		homogenization	nox	14.1	12.4	42	58.6	23.3	17.7	3.5	0.13
			oxy	13.9	12.3	42	56.8	27.5	15.8	3.5	0.13
Golden Delicious	−80	grinding	nox	15.6	13.7	49	60.4	38.3	13.6	3.8	0.03
			oxy	15.6	13.7	46	60.5	38.5	14.0	3.7	0.07
		homogenization	nox	15.3	13.7	49	59.8	37.8	13.8	3.9	0.08
			oxy	15.4	13.7	47	60.5	37.3	13.8	3.7	0.08
	−20	grinding	nox	15.7	13.7	48	60.5	38.0	13.6	3.8	0.07
			oxy	15.8	13.7	46	61.9	37.6	13.9	3.8	0.07
		homogenization	nox	15.4	13.7	48	60.7	38.2	13.2	3.8	0.03
			oxy	15.3	13.7	48	60.6	37.7	13.7	3.7	0.05
Granny Smith	−80	grinding	nox	13.3	11.3	73	45.9	25.7	19.5	5.4	0.08
			oxy	13.3	11.2	72	46.7	25.6	19.5	5.2	0.08
		homogenization	nox	13.1	11.3	74	48.2	25.6	19.1	5.3	0.03
			oxy	13.0	11.2	74	45.6	24.9	19.8	5.3	0.08
	−20	grinding	nox	13.5	11.3	74	48.5	26.3	19.0	5.3	0.10
			oxy	13.5	11.2	74	49.0	27.1	19.3	5.3	0.18
		homogenization	nox	13.3	11.3	73	47.0	27.2	19.4	5.2	0.07
			oxy	13.0	11.3	72	46.5	26.2	19.0	5.2	0.12

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Table 1. continued

variety	storage temp (°C)	sample grinding	oxidation	dry matter (%)	SSC (°Brix)	TA (mequiv kg^{-1})	fructose (g kg^{-1})	sucrose (g kg^{-1})	glucose (g kg^{-1})	malic acid (g kg^{-1})	citric acid (g kg^{-1})
Pink Lady	−80	grinding	nox	15.4	13.2	70	45.9	54.0	8.1	6.0	0.02
			oxy	15.4	13.1	70	45.1	56.8	6.6	5.9	0.02
		homogenization	nox	15.0	13.0	70	44.8	52.6	9.6	5.7	0.00
			oxy	15.0	13.0	70	42.7	56.5	6.7	5.9	0.02
	−20	grinding	nox	15.4	13.2	71	44.8	53.8	8.2	5.8	0.03
			oxy	15.5	13.1	71	43.8	57.2	7.2	5.8	0.00
		homogenization	nox	15.1	13.2	70	46.3	53.1	8.8	5.9	0.00
			oxy	15.2	13.2	70	44.6	57.4	6.9	5.9	0.05
statistics		total mean		14.4	12.5	62.7	52.3	34.0	15.1	4.9	0.1
		pooled SD		0.3	0.2	3	1.2	1.5	0.7	0.2	0.02
F values and significance											
		variety		182.400 ***	215.320 ***	842.080 ***	253.829 ***	664.292 ***	440.377 ***	418.126 ***	31.581 ***
		storage temp		0.888 ns	0.118 ns	0.003 ns	1.453 ns	0.071 ns	0.022 ns	0.046 ns	0.736 ns
		sample grinding		18.225 ***	0.118 ns	0.116 ns	0.206 ns	4.680 *	0.585 ns	0.556 ns	1.090 ns
		oxidation		0.019 ns	0.299 ns	0.095 ns	0.811 ns	3.333 ns	0.484 ns	0.173 ns	2.084 ns

[a]Two conditions of freezing: at −80 °C after prefreezing in liquid nitrogen or at −20 °C after prefreezing in a freezer (−30 °C). Two conditions of grinding: grinding in liquid nitrogen using a ball grinder or homogenization at room temperature with Ultraturrax equipment after 1.5 h of defrosting at 20 °C. Two conditions of thawing: with addition of nitrogen (N_2) and a solution of sodium fluoride (NaF) for nonoxidative conditions (nox) and in the presence of air and water for oxidative conditions (oxy). ns, *, **, ***: nonsignificant or significant at $P < 0.05$, 0.01, 0.001, respectively.

software using the SAISIR package (SAISIR, free procedures using MATLAB for chemometrics, available at http://easy-chemometrics.fr). The samples of apples were randomly distributed among the calibration (two-thirds of the samples) and prediction (one-third of the samples) sets to perform a cross-validation test. The calibration set was used to establish the calibration models between the spectral data and the measured reference values, whereas the samples in the prediction set were used for the validation of models. The absorption band around 2400 and 2200 cm^{-1}, due to carbon dioxide, was discarded prior the calculation. Partial least-squares (PLS) regression method was performed to study the predictive ability of the calibration models. PLS regression consists of two steps. The first step was computing PLS scores, which are a linear combination of the original predictive variables (the spectral absorbances). In the second step, the regression model was calculated, using these scores as new predicting variables. In the method used in this study (PLS1), only one variable was predicted at a time, and the scores were specific of the predicted variable. The models were first validated using the full cross-validation method to determine the optimum number of the factors and detecting any spectral outliers. Then, the models were tested to predict the parameters of the apple in the independent validation set, and the best calibration models were selected on the basis of the highest determination coefficient of calibration (r^2) and determination of the coefficient of validation (R^2) and the lowest root-mean-square error of calibration (RMSEC), root-mean-square error of prediction (RMSEP), and error of prediction (RMSEP%). The percentage error of prediction was defined as percent RMSEP and was calculated with RMSEP divided by the mean values of measured quality parameters in fruits from the validation. A standard normal variate correction (SNV) was applied to all spectra. SNV corrected spectra are shown (Figure 3) for apple puree using the ATR-ZnSe cell and for freeze-dried apple puree using the ATR-diamond cell.

RESULTS AND DISCUSSION

Composition in Sugars and Organic Acids of the Flesh of Different Apple Cultivars. *Effect of Sample Preparation.* Dry matter, SSC, TA, and levels of fructose, sucrose, glucose, malic acid, and citric acid of apples with different storage temperatures, sample preparations, and oxidation states are shown in Table 1. A marked variation with a high statistical significance (see *F* values) was observed between varieties for all measured criteria. Apples from the varieties Chantecler, Golden Delicious, and Pink Lady had the highest dry matter (>15%) and SSC (>13 °Brix). The dry matter variation observed in this work was in accordance with that observed in Elstar and Gala dessert apples.[20] A very good correlation (R^2 = 0.974) was found in apples between these two criteria, dry matter and SSC. Golden Delicious had a very high fructose level, whereas Pink Lady had an unusually high level of sucrose. Pink Lady actually had higher sucrose than fructose, in contrast to the other varieties. Canada had the lowest dry matter (≤12.6%) and the lowest SSC (≤10.7 °Brix). Canada also had the highest TA (≥102 mequiv kg^{-1}) and the highest malic acid level (≥7.1 g kg^{-1}). The lowest TA (≤32 mequiv kg^{-1}) and malic acid level (≤2.9 g kg^{-1}) were observed for Fuji, which also had high levels of fructose and glucose. Values for these attributes (dry matter, SSC, TA, and levels of fructose, sucrose, glucose, malic acid, and citric acid) were well within the range reported for 175 apple varieties.[21] TA and SSC are the best predictors of acidity and sweetness in apples, and differences of 0.08% of TA and 1 °Brix are required before a trained panelist

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can detect a difference in taste.[22] In our study the variabilities in composition observed among the seven studied varieties are higher than 0.08% and 1°Brix and so must affect their taste.

The storage temperature (−20 or −80 °C) and the oxidation state (presence of nitrogen and sodium fluoride or not during the sample preparation) had no effect on these biochemical criteria. The difference was not statistically significant for all varieties (Table 1). An effect of the sample grinding, with a planetary ball mill using liquid nitrogen or with a simple homogenization at ambient temperature, was observed for two traits, dry matter and sucrose. However, this effect concerned low differences: means of 14.493% for grinding and 14.208% for simple homogenization concerning dry matter and means of 34.41 g kg^{-1} for grinding and 33.60 g kg^{-1} for simple homogenization concerning sucrose content.

These biochemical criteria were not affected in apples by the different conditions of sample preparation. The tested conditions and especially thawing steps did not conduct to the catabolism of sucrose in fruit into its monosaccharides, glucose and fructose, through the activities of enzymes such as invertase and sucrose synthase.[15,16] Lisiewska and Kmiecik[23] found no difference in sugar content for tomato cubes stored at either −20 or −30 °C for 12 months. However, these authors showed a significant increase of acidity after 9 months at −20 °C.

Estimation Using MIR Spectroscopy. The spectra obtained on apples, first on fresh puree and second on freeze-dried puree, are shown in Figure 3 after the SNV preprocessing. For sugar and acid estimation, the spectral data corresponded to those obtained directly on puree. In this work, the wavenumber range that gave the best results of prediction was 1010−1700 cm^{-1} to predict sugar and organic acid contents corresponding to the intense and characteristic bands of glucose, fructose, sucrose, malic acid, and citric acid.[5]

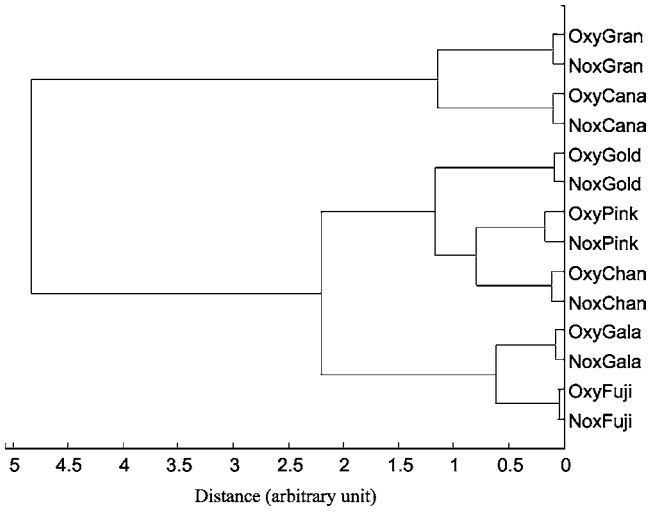

Figure 4. Dendrogram of the FT-MIR spectra using the ATR-ZnSe accessory of apple samples in the wavenumber range between 1010 and 1700 cm^{-1}.

A hierarchical cluster analysis was first performed on the basis of a distance matrix calculated using Euclidean distances to investigate the potential of MIR spectroscopy to discriminate the homogeneous apple samples. For the cluster analysis, the wavenumber range used was between 1010 and 1700 cm^{-1} (Figure 4). The clustering of spectra resulted in three main groups: (i) Granny Smith and Canada; (ii) Golden Delicious, Pink Lady, and Chantecler; and (iii) Gala and Fuji. This classification in three groups was close to the classification obtained by hierarchical clustering using the biochemical data that were SSC, TA, and contents of sucrose, glucose, fructose, malic acid, and citric acid (data not shown), except for Golden Delicious apples. Instead of having a group of Golden Delicious with Pink Lady and Chantecler using spectral data, Golden Delicious was in the same group as Gala and Fuji with the biochemical data. Otherwise, the pairs Granny Smith and Canada, Pink Lady and Chantecler, and then Gala and Fuji were the same whatever the data used. Moreover, the distance between the two types of oxidation (oxy and nox) was lower than the distance between varieties. Therefore, MIR spectroscopy allowed a good discrimination of samples per variety related to their macroconstituents, whatever the sample preparation.

In this study, we tested the ability of PLS to predict SSC, TA, and the concentration of sugars and organic acids from the MIR spectra. The spectral range, latent variables, correlation coefficients, RMSEC, and RMSEP are given in Table 2. The technique allowed a very good prediction of global criteria of apple samples such as dry matter and SSC ($R^2 \geq 0.96$ and RMSEP% $\leq 1.4\%$). For TA, the prediction was acceptable with RMSEP% of 6%. Moreover, concerning the individual sugars and organic acids, except for citric acid, the PLS regression models had a good ability to estimate each biochemical parameter on the basis of high R^2 and low RMSEP% results obtained on the independent validation set of samples ($R^2 \geq 0.97$ and RMSEP% $\leq 4.7\%$). However, the MIR technique cannot be used to estimate the citric acid, probably in relation to its very low level in apples ($\leq$0.32 g kg^{-1}). As already observed for apricot and tomato,[5,7] the estimation of compounds present in low concentrations is generally not acceptable. Moreover, these results of prediction were obtained regardless of the sample preparation, using oxidized as well as nonoxidized data. Therefore, for the prediction of sugar and organic acid contents, MIR spectroscopy was a powerful tool independent of the sample preparation. For apples and in particular apple juices, this technique has already been shown to

Table 2. Prediction Performance of PLS Regression Models Developed for Apple Composition of Sugars and Organic Acids

		calibration (n = 112)		prediction (n = 56)				
parameter	range	r^2	RMSEC	R^2	RMSEP	RMSEP%	latent variables	spectral range (cm^{-1})
dry matter (%)	11.8−16.4	0.99	0.10	0.99	0.13	0.9	2	1010−1700
soluble solids (°Brix)	10.3−14.3	0.98	0.15	0.96	0.18	1.4	3	1010−1700
titratable acidity (mequiv kg^{-1})	29−111	0.99	2.81	0.97	3.74	6.0	5	1010−1700
glucose (g kg^{-1})	6−24	0.99	0.36	0.99	0.05	3.4	5	1010−1700
fructose (g kg^{-1})	39−66	0.98	0.83	0.97	1.24	2.4	6	1010−1700
sucrose (g kg^{-1})	18−59	0.99	0.56	0.99	0.66	1.9	4	1010−1700
malic acid (g kg^{-1})	2.5−7.7	0.98	0.18	0.97	0.23	4.7	5	1010−1700
citric acid (g kg^{-1})	0.0−0.32	0.56	0.38	0.12	0.57	100.0	9	1010−1700

Table 3. Composition of Phenolic Compounds (Milligrams per Kilogram of Fresh Weight) in the Flesh of Different Apple Cultivars and Different Sample Preparations[a]

				flavan-3-ols			dihydrochalcones		hydroxycinnamic acids		
				monomers		polymers					
variety	storage temp (°C)	sample grinding	oxidation	(+)-catechin	(−)-epicatechin	procyanidins	phloretin XylGlc	phloridzin	chlorogenic acid	*p*-coumaroylquinic acid	sum of native polyphenols
Canada	*control*			*35.5*	*90.7*	*630*	*15.0*	*17.0*	*223*	*21.8*	*1033*
	−80	grinding	nox	31.5	95.2	630	14.3	19.4	226	21.6	1038
			oxy	0.0	7.6	118	5.0	8.1	48	12.3	200
		homogenization	nox	29.0	88.7	536	11.8	16.7	187	18.3	888
			oxy	0.0	2.5	64	3.0	4.9	26	9.1	109
	−20	grinding	nox	29.1	90.7	618	13.9	19.4	226	20.6	1017
			oxy	0.0	4.2	79	3.7	6.3	32	10.0	135
		homogenization	nox	26.1	77.9	586	11.8	16.0	194	18.8	931
			oxy	0.0	12.2	172	7.3	11.0	79	14.7	296
Chantacler	*control*			*0.0*	*24.8*	*428*	*4.5*	*5.8*	*171*	*5.3*	*640*
	−80	grinding	nox	2.2	22.2	359	4.7	5.6	160	3.6	557
			oxy	0.0	0.0	16	0.0	0.0	4	0.5	21
		homogenization	nox	1.5	12.6	255	3.8	5.1	126	3.3	407
			oxy	0.0	0.0	11	0.0	0.0	0	0.0	12
	−20	grinding	nox	2.3	19.4	351	4.6	6.0	153	4.1	541
			oxy	0.0	0.0	16	0.0	0.0	1	0.2	17
		homogenization	nox	1.6	14.4	314	3.6	4.9	136	3.1	478
			oxy	0.0	0.0	14	0.0	0.0	0	0.1	14
Fuji	*control*			*6.6*	*56.3*	*257*	*2.8*	*8.0*	*104*	*2.5*	*437*
	−80	grinding	nox	3.4	27.2	221	2.7	8.3	90	2.2	354
			oxy	0.0	0.0	12	0.0	0.0	1	0.2	13
		homogenization	nox	2.3	16.0	160	2.1	7.1	74	1.7	264
			oxy	0.0	0.0	9	0.0	0.0	0	0.0	9
	−20	grinding	nox	2.9	23.7	194	2.5	8.1	86	2.0	320
			oxy	0.0	0.0	13	0.0	0.0	0	0.0	14
		homogenization	nox	2.9	16.2	167	1.9	6.2	78	1.7	274
			oxy	0.0	0.0	11	0.0	0.0	0	0.0	11
Gala	*control*			*7.1*	*31.7*	*487*	*5.1*	*5.9*	*125*	*8.8*	*671*
	−80	grinding	nox	3.8	25.2	387	4.3	5.8	95	6.9	528
			oxy	0.0	0.0	19	0.0	0.0	3	0.5	22
		homogenization	nox	3.8	25.6	345	3.4	5.1	85	6.2	475
			oxy	0.5	0.0	14	0.0	0.0	0	0.1	15
	−20	grinding	nox	3.7	26.3	351	4.1	5.8	92	6.2	490
			oxy	0.0	0.0	18	0.2	0.0	1	0.3	20
		homogenization	nox	3.2	18.2	322	3.1	5.4	85	6.5	443
			oxy	0.0	0.0	15	0.1	0.1	1	0.4	16

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Table 3. continued

				flavan-3-ols			dihydrochalcones		hydroxycinnamic acids		
				monomers		polymers					
variety	storage temp (°C)	sample grinding	oxidation	(+)-catechin	(−)-epicatechin	procyanidins	phloretin XylGlc	phloridzin	chlorogenic acid	*p*-coumaroylquinic acid	sum of native polyphenols
Golden Delicious	*control*			*0.0*	*27.5*	*436*	*4.0*	*8.4*	*94*	*6.7*	*577*
	−80	grinding	nox	0.0	28.2	443	4.2	9.0	100	6.7	591
			oxy	0.0	0.0	39	0.1	0.6	5	1.4	46
		homogenization	nox	0.0	24.2	397	3.2	7.6	85	5.6	522
			oxy	0.0	0.0	34	0.0	0.4	3	0.8	38
	−20	grinding	nox	0.0	25.8	441	4.0	8.9	96	6.3	582
			oxy	0.0	0.0	32	0.0	0.2	3	0.9	36
		homogenization	nox	0.0	19.6	423	3.3	7.6	89	5.2	547
			oxy	0.0	0.0	52	0.3	1.4	12	2.2	68
Granny Smith	*control*			*18.6*	*70.0*	*611*	*4.7*	*4.4*	*94*	*1.9*	*739*
	−80	grinding	nox	11.2	40.9	590	4.7	4.2	28	1.5	680
			oxy	1.4	3.5	72	0.3	1.2	2	0.4	81
		homogenization	nox	4.9	20.7	374	2.4	2.5	16	1.0	421
			oxy	0.2	0.5	56	0.0	0.7	1	0.1	58
	−20	grinding	nox	12.1	40.9	562	4.7	4.7	27	1.4	653
			oxy	1.4	3.5	78	0.3	1.3	3	0.4	88
		homogenization	nox	7.5	25.9	475	3.0	2.4	18	1.0	532
			oxy	2.8	7.2	148	0.7	1.6	5	0.6	166
Pink Lady	*control*			*0.0*	*52.5*	*251*	*3.0*	*2.6*	*95*	*2.3*	*406*
	−80	grinding	nox	0.0	27.5	233	3.1	3.8	88	2.0	358
			oxy	0.0	0.0	15	0.0	0.7	0	0.0	16
		homogenization	nox	0.7	15.3	150	2.2	3.3	59	1.3	232
			oxy	0.0	0.2	15	0.0	0.7	0	0.0	16
	−20	grinding	nox	0.0	25.3	224	3.0	3.9	86	1.9	344
			oxy	0.0	0.0	14	0.0	0.7	0	0.0	15
		homogenization	nox	0.9	18.3	187	2.0	2.9	68	1.5	280
			oxy	0.0	0.0	16	0.0	0.7	0	0.0	17

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Table 3. continued

variety	storage temp (°C)	sample grinding	oxidation	flavan-3-ols: monomers: (+)-catechin	flavan-3-ols: monomers: (−)-epicatechin	flavan-3-ols: polymers: procyanidins	dihydrochalcones: phloretin XylGlc	dihydrochalcones: phloridzin	hydroxycinnamic acids: chlorogenic acid	hydroxycinnamic acids: *p*-coumaroylquinic acid	sum of native polyphenols
statistics		total mean		4.1	20.8	231	3.1	4.7	63	4.2	330
		pooled SD		0.8	1.8	28	0.4	0.6	5	0.5	33
F values and significance											
		variety		24.529 ***	33.545 ***	37.695 ***	112.963 ***	106.960 ***	50.269 ***	243.507 ***	46.754 ***
		storage temp		0.000 ns	0.079 ns	0.970 ns	0.283 ns	0.346 ns	0.292 ns	0.152 ns	0.718 ns
		sample grinding		0.607 ns	5.933 ns	6.504 ns	8.406 ns	5.376 ns	4.438 ns	2.215 ns	7.862 ns
		oxidation		66.931 ***	302.393 ***	796.212 ***	423.902 ***	459.979 ***	581.195 ***	218.422 ***	893.776 ***

[a]Two conditions of freezing: at −80 °C after prefreezing in liquid nitrogen or at −20 °C after prefreezing in a freezer (−30 °C). Two conditions of grinding: grinding in liquid nitrogen using a ball grinder or homogenization at room temperature with Ultraturrax equipment after 1.5 h of defrosting at 20 °C. Two conditions of thawing: with addition of nitrogen (N_2) and a solution of sodium fluoride (NaF) for nonoxidative conditions (nox) and in presence of air and water for oxidative conditions (oxy). In italics, data of control sample (direct freeze-drying). ns, *, **, ***: nonsignificant or significant at $P <$ 0.05, 0.01, or 0.001, respectively.

be adaptable to the quantification of glucose, fructose, and sucrose, using a calibration with synthetic solutions and to confirm the authenticity of processed juices and fresh extracted juices.[4]

Phenolic Composition of the Flesh of Different Apple Cultivars. *Effect of Sample Preparation.* The main phenolic compounds categorized into three classes: flavan-3-ols ((+)-catechin, (−)-epicatechin, and procyanidins), dihydrochalcones (phloretin xyloglucoside and phloridzin), and hydroxycinnamic acids (chlorogenic acid and *p*-coumaroylquinic acid), which were quantified in apples submitted to different conditions of sample preparation (Table 3 and Figure 5). The phenolic classes in the apple flesh can be listed as procyanidins > hydroxycinnamic acids > catechins > dihydrochalcones. Figure 5 summarizes the compositions obtained for the seven varieties in the reference sample, that is, directly freeze-dried. Depending on the varieties, procyanidins accounted for 59−83% of total polyphenols, hydroxycinnamic acids for 4−28%, catechins for 4−14%, and dihydrochalcones for 1−3%. In agreement with Guyot et al.,[24] procyanidins represented the major class of polyphenols in all seven varieties. The thioacidolysis reaction allows the calculation of the average degree of polymerization (DP) of procyanidins by distinguishing terminal and extension units. The DPs were relatively homogeneous, between 4.1 in Pink Lady and 5.9 in Golden Delicious (results not shown). A larger variability was observed in 67 varieties of dessert apples from 3.2 to 28.7.[9] A highly significant variability of phenolic composition was observed between the studied varieties ($P < 0.001$***). Compared to the data reported in the flesh of Golden Delicious and Granny Smith,[24] phenolic contents in this study were quite similar, little differences being probably in relation to the difference of ripening stages, years, and origins of the studied apples. Higher content of phenolic content was found in Golden Delicious and Fuji apples than already reported,[9] which was surprising because here only the flesh was analyzed. Indeed, it is well-known that the relative concentration of phenolics is several times lower in the flesh than in the skin.[14] Contents of (+)-catechin, (−)-epicatechin, phloridzin, and chlorogenic acid observed here, in apples, were in accordance with the values observed in apple juices.[21] The flesh of Canada apples had the highest concentration of all phenolic compounds (Figure 5). For procyanidins, Canada and Granny Smith apples had similar contents. Pink Lady and Fuji were the poorest in all phenolic compounds, particularly in procyanidins.

According to the *F* values (Table 3), the three conditions tested can here be listed in decreasing order as sample oxidation > sample grinding > storage temperature for their effect on phenolic compound levels. To visualize the effect of the different conditions of sample preparation, the polyphenol contents are presented for Canada (Figure 6), for catechins, procyanidins, dihydrochalcones, and hydroxycinnamic acids. The same trend was observed in the other cultivars. In Canada, only the effect of the oxidation condition was significant.

In our study the oxidation was limited by the use of liquid nitrogen during the apple grinding, by nitrogen gas during thawing, and by the addition of NaF during sample homogenization. Without these precautions, a very significant loss was observed, with mean values of total phenolic compounds being 56 mg kg^{-1} FW for apples without precaution instead of 527 mg kg^{-1} FW for apples prepared in the presence of nitrogen (gas or liquid) plus NaF. Their loss represented 89% of the total phenolic content for oxy samples

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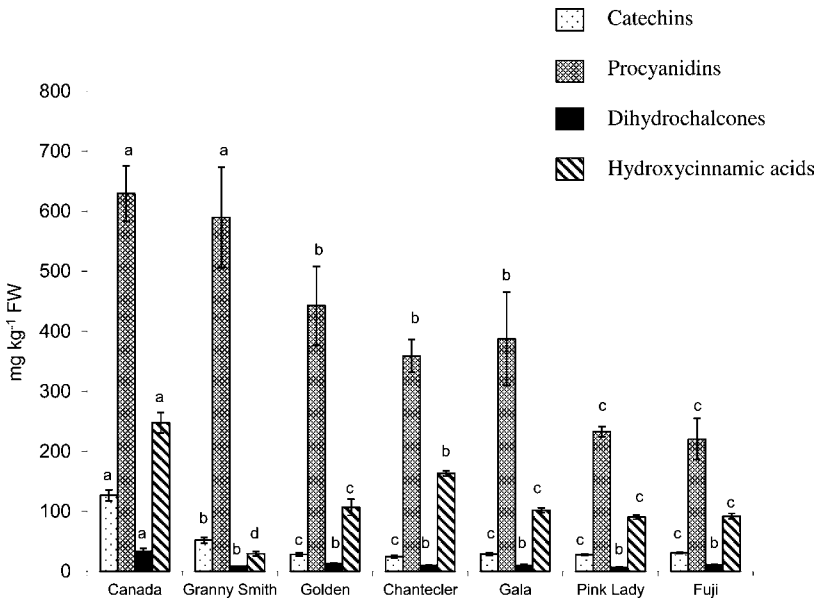

Figure 5. Characterization of phenolic compounds (catechins, procyanidins, dihydrochalcones, and hydroxycinnamic acids) in the flesh of different apple cultivars. Data corresponded to the control samples frozen at −80 °C, ground in liquid nitrogen, and directly lyophilized. Data are expressed as mg kg^{-1} of fresh weight ± standard deviation with $n = 3$. Mean values with different letters differed significantly using Tukey's test ($P < 0.05$).

compared to the nox samples and concerned all phenolic families (Table 3). Oxidation had no significant effect on the degree of polymerization of procyanidins (DP) (results not shown). In our study, polyphenol oxidation occurred during thawing steps in the absence of either nitrogen gas or NaF. The addition of NaF inhibited the polyphenol oxidase activity, as described in loquat fruits.[25] Although oxidation was allowed for 20 h on small volumes of well-homogenized samples so that ample oxygen and time were available, losses varied according to the polyphenol compounds. The susceptibility was *p*-coumaroylquinic acid (loss of 65%) < phloridzin (80%) < phloretin xyloglucoside (84%) < procyanidins (88%) < chlorogenic acid (91%) < (−)-epicatechin (95%) < (+)-catechin (96%). The most affected polyphenols were (+)-catechin and (−)-epicatechin, belonging to the class of monomeric flavan-3-ols as observed in Renard et al.[26] In apple, the main substrate of PPO is chlorogenic acid,[27] but flavan-3-ols including (+)-catechin, (−)-epicatechin, and procyandins are oxidized by coupled oxidation/reduction with chlorogenic acid quinone. Chlorogenic acid is thus generated, explaining its lesser decrease compared to (+)-catechin and (−)-epicatechin. In addition, (+)-catechin and (−)-epicatechin can be directly oxidized by PPO.[10] The class of dihydrochalcones was also reduced. Dihydrochalcones (i.e., phloridzin and phloretin xyloglucoside) are substrates of the monophenolase (cresolase) activity of PPO, but its catalytic power is much lower than the catecholase activity.[10] Moreover, dihydrochalcones are not the preferred target for the coupling reaction with *o*-quinones.[28] The less affected compound was *p*-coumaroylquinic acid; it is known to be relatively resistant to oxidation.[29] All of these results are consistent with Renard et al.[26] and Guyot et al.[28]

Moreover, sample grinding had a significant effect on the total phenolic compounds with mean values of 269 mg kg^{-1} FW with the simple homogenization at ambient temperature instead of 313 mg kg^{-1} FW with the ball grinder using liquid nitrogen. Ball grinding resulted in a finer powder and decreased particle size of the fruit tissue, which could facilitate polyphenol extraction to the solvent. However, this effect was limited compared to the effect of oxidation. The loss of phenolic compounds upon homogenization at room temperature with the addition of NaF can be acceptable taking into account the time, price, and equipment necessary for grinding with liquid nitrogen. There was also no significant difference between the storage temperatures at −20 or −80 °C. In orange and apple juices, the content of hydrolyzable and soluble polyphenols did not change at −18 or −70 °C over 10 days.[30] However, over a longer period, between the 1 and 12 months, storage at −20 °C of cauliflower induced a decrease in polyphenol content of 5−17%.[31]

Comparison between the control samples (storage at −80 °C, grinding in the presence of liquid nitrogen, and direct freeze-drying) and the samples prepared from fruit slurries in the best conditions (storage at −80 °C, grinding in the presence of liquid nitrogen, and prevention of oxidation using NaF) showed significant differences for their phenolic composition (results not shown, except for Canada in Figure 6). The control samples had the highest levels of phenolic compounds and contained in particular 24% more catechins. Three cultivars were particularly sensitive with a higher level of catechins of 47% in Pink Lady, 51% in Fuji, and 41% in Granny Smith. With regard to the other compounds, the level of hydroxycinnamic acids was 6% higher in control samples but not for procyanidins and dihydrochalcones. Although statistically significant, these variations were limited compared to the effect of thawing without control of oxidation and affected mostly one of the minor classes of phenolics. The main difference in preparation between these two types of samples was the thawing period. The control samples were directly freeze-dried after grinding, whereas the other samples were thawed in the presence of NaF. Presumably some oxidation took place before NaF could reach and inactivate all PPO molecules, causing the degradation of catechins (24%) and hydroxycinnamic acids (6%).

Estimation Using MIR Spectroscopy. For phenolic compound estimation, spectral analysis was performed on the freeze-dried puree, which allows more concentrated samples

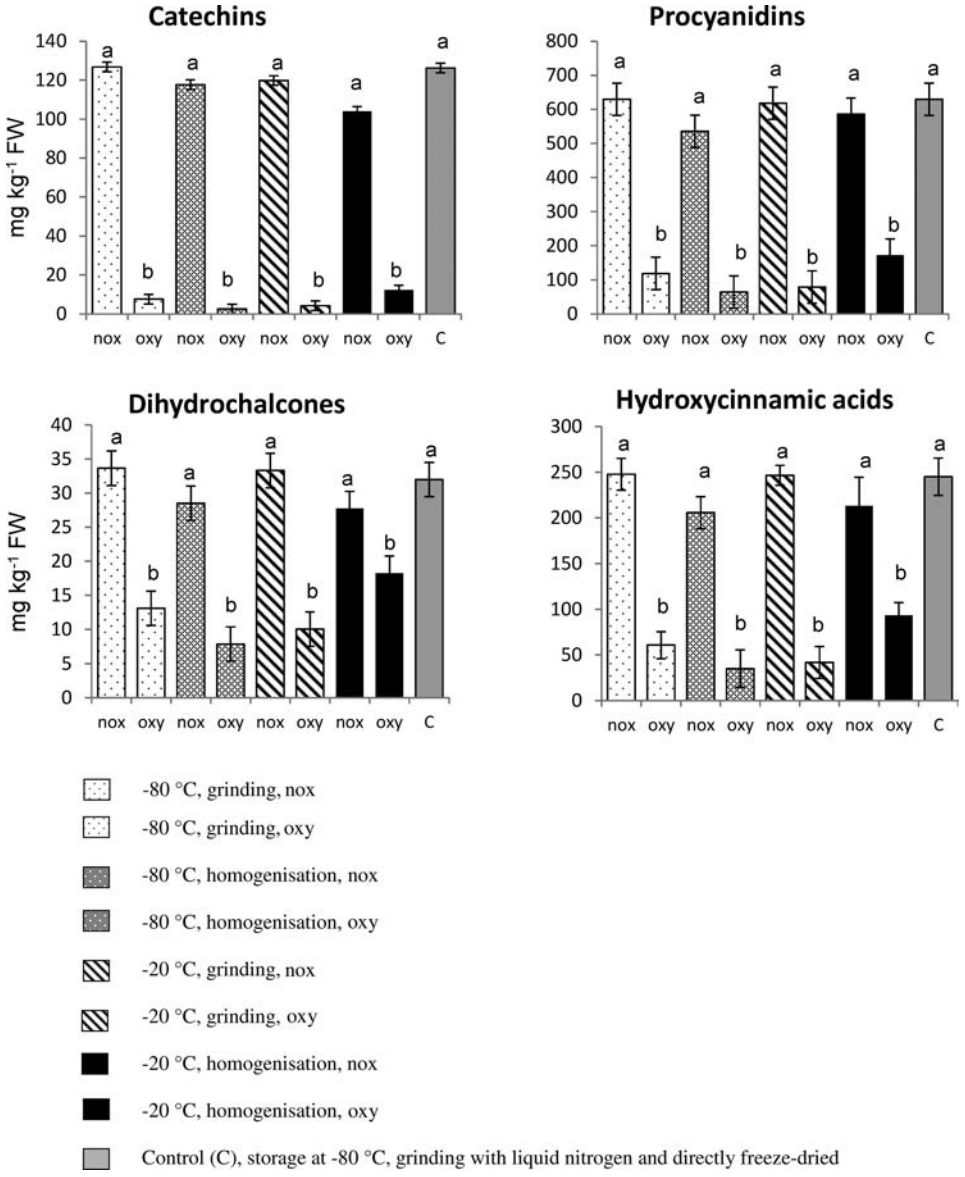

Figure 6. Characterization of phenolic compounds (catechins, procyanidins, dihydrochalcones, and hydroxycinnamic acids) in the flesh of 'Canada' apple using different conditions of sample preparations. Data are expressed as mg kg^{-1} of fresh weight ± standard deviation with $n = 3$. Mean values with different letters differed significantly using Tukey's test ($P < 0.05$). Two conditions of freezing: at −80 °C after prefreezing in liquid nitrogen or at −20 °C after prefreezing in a freezer (−30 °C). Two conditions of grinding: grinding in liquid nitrogen using a ball grinder or homogenization at room temperature with Ultraturrax equipment after 1.5 h of defrosting at 20 °C. Two conditions of thawing: with addition of nitrogen (N_2) and a solution of sodium fluoride (NaF) for nonoxidative conditions (nox) and in the presence of air and water for oxidative conditions (oxy). In solid gray, data of reference sample (−80 °C after prefreezing in liquid nitrogen, grinding in liquid nitrogen, and direct freeze-drying).

and prevents their oxidation over time. The spectral region giving the best results for phenolic compound prediction was 1010−1700 cm^{-1} (Figure 3). This area corresponds to the spectral data of catechin obtained with the same ATR technique and showing important peaks between 1700 and 1000 cm^{-1}.[32] According to Coates,[33] this area incorporates bands typical of phenolic compounds such as bands assigned to C═C—C aromatic ring stretch (1615−158 and 1510−1450 cm^{-1}), to phenol OH bend (1410−1310 cm^{-1}), to aromatic C—H in-plane bend (1225−950 cm^{-1}), and to C—O stretch of phenol (1200 cm^{-1}). The regression coefficients of the models established in this study to predict the total phenolic content showed numerous characteristic peaks in the spectral region (1010−1700 cm^{-1}), with particularly strong peaks at 1620 and 1570 cm^{-1} (data not shown). This is in accordance with the fact that the differences observed between spectral data of phenolic wine extracts were around 1605 and 1520 cm^{-1}.[1]

The results of prediction obtained for the different phenolic classes are given in Table 4. Contrary to the sugars and organic acids, a strong effect of the sample preparation was observed here. Indeed, all sample data were initially used, oxidized and not, for the model establishment (see Table 4, type of samples nox + oxy). The RMSEP% was very high, from 16.7% for dihydrochalcones to 74.0% for hydroxycinnamic acids, and appeared to be not usable. A particular wavenumber range between 1530 and 1626 cm^{-1} was also shown to be well adapted to discriminate nonoxidized samples from oxidized samples (results not shown). It was then decided to select only the nonoxidized samples to evaluate the performance of MIR (see Table 4, type of samples nox). High R^2 and low RMSEP%

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Table 4. Prediction Performance of PLS Regression Models Developed for Apple Composition of Phenolic Compounds

			calibration		prediction				
parameter (mg kg^{-1})	type of sample	range	r^2	RMSEC	R^2	RMSEP	RMSEP%	latent variables	spectral range (cm^{-1})
n = 97 (calibration) and 42 (validation)									
flavan-3-ols	nox + oxy	7−818	0.85	85.6	0.77	122.5	49.9	9	1010−1568
procyanidins	nox + oxy	7−681	0.80	92.0	0.53	143.5	64.4	9	1010−1568
dihydrochalcones	nox + oxy	0−38	0.86	3.3	0.70	4.7	16.7	9	1010−1568
hydroxycinnamic acids	nox + oxy	0−261	0.83	29.3	0.54	45.6	74.0	9	1010−1568
total polyphenols	nox + oxy	13−1798	0.85	204.4	0.73	278.3	51.7	9	1010−1568
n = 39 (calibration) and 16 (validation)									
flavan-3-ols	nox	163−818	0.97	32.7	0.95	41.6	9.3	8	1010−1568
procyanidins	nox	144−681	0.95	31.6	0.94	39.7	9.8	7	1010−1568
dihydrochalcones	nox	4−38	0.98	1.2	0.90	2.4	19.2	9	1010−1568
hydroxycinnamic acids	nox	16−261	0.93	16.2	0.48	55.8	49.3	9	1010−1568
total polyphenols	nox	375−1798	0.96	72.2	0.96	87.1	9.0	8	1010−1568

results obtained on the independent validation set of samples were observed for total phenolic compounds (R^2 = 0.96 and RMSEP% = 9.0%). Similar results were observed for flavan-3-ols, the predominant phenolic compounds in apples (R^2 = 0.95 and RMSEP% = 9.3%), and for procyanidins, the major form of flavan-3-ols (R^2 = 0.94 and RMSEP% = 9.8%). For the other polyphenol classes such as dihydrochalcones and hydroxycinnamic acids, the errors of prediction (≥19.2%) were high and not acceptable for prediction. With regard to phenolic compounds in fruits, a similar RMSEP% (9.3%) was obtained in two blueberry cultivars over two years with a spectrophotometric analysis based on Folin−Ciocalteu reaction as the reference analysis.[3] To our knowledge, this is the only time MIR spectroscopy has been used for predicting total phenolic content in fruits. In apple pomaces, after the calculation using measured and predicted values, RMSEP% was lower, 6.6%,[6] probably in relation to the higher level of phenolic content in pomace than in apple flesh. On the other hand, some authors used this technique not to quantify phenolic compounds but to discriminate and classify samples, such as dried phenolic extracts of wine.[1]

In summary, storage temperature, sample grinding, and sample oxidation did not affect the concentrations of sugars or organic acids in apple and the prediction of their concentrations based on the MIR spectra of the samples, so for their characterization, no particular precautions are required. In contrast, significant effects were observed on phenolic compounds, and the impact of tested conditions can be listed in descending order as sample oxidation > sample grinding > storage temperature according to their effect on the degradation of phenolic compounds. Analysis and MIR estimation of phenolic composition therefore demand specific sample preparation techniques that prevent oxidation. For sugars and organic acids, independent of the sample preparation, MIR spectroscopy gave good results of prediction ($R^2 \geq 0.97$ and RMSEP% ≤ 4.7%), except for citric acid, a minor compound in apple flesh. With regard to phenolic compounds, their quantification by HPLC-DAD after thioacidolysis was used as the reference method to test the ability of MIR spectroscopy to predict the different families of polyphenols. If oxidation was avoided during sample preparation, good results of prediction were obtained for flavan-3-ols and for procyanidins as well as for total phenolic compounds ($R^2 \geq 0.94$ and RMSEP% ≤ 9.8%). For dihydrochalcones and hydroxycinnamic acids, the errors of prediction were high (respectively, 19.2 and 49.3%) and not acceptable for routine use.

A procedure using sample storage at −20 °C and homogenization at ambient temperature of fruit material in the presence of NaF appears to be acceptable for the characterization of sugars, organic acids, and phenolic compounds in apple flesh. Clearly, the most important observation was to take precautions to preserve the polyphenols against oxidation. The samples can then be analyzed by MIR spectroscopy, which is a rapid and powerful tool for the prediction of SSC, TA, dry matter, and contents of glucose, fructose, sucrose, and malic acid. An additional step, freeze-drying, is required for the determination of total phenolic compounds, flavan-3-ols and procyanidins. The last is especially of interest as its analysis is complex and it is often neglected, although it is a major part of the polyphenols in fruits.[34]

AUTHOR INFORMATION

Corresponding Author

*Phone: +33 4 32 72 25 09. Fax: +33 4 32 72 24 92. E-mail: sylvie.bureau@avignon.inra.fr.

Notes

The authors declare no competing financial interest.

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