Determination of Phenolic Compound Profiles in Maple Products by High-Performance Liquid Chromatography

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A high-performance liquid chromatography method, using ultraviolet and electrochemical detectors, was developed for the analyses of phenolic and furfural compounds in maple products. The concentrations of compounds were calculated using external standards that conformed to linear behavior. Most of compounds identified in saps, concentrates, and syrups were related to lignin derivatives. Statistical analyses of data showed that 5-(hydroxymethyl)-2-furaldehyde (HMF) concentrations and phenolic profiles were significantly different as related to harvest time and maple products. Although HMF concentrations were not significantly different as related to the producers, a highly significant difference was observed for phenolic profiles. An increase in the relative proportion of phenolic acids and a decrease in that of aldehydes and alcohols were observed during the reverse osmosis of maple sap. The thermal evaporation resulted in an increase in the amount of HMF, ferulic acid, vanillin, and syringyl aldehyde with a concomitant drastic decrease in sinapic acid.

Keywords: *Maple products; HPLC; UV-diode array; electrochemical detector; ANOVA*

INTRODUCTION

Maple syrup is one of the most important plant product in Québec, Canada, and represents 72% of the world production (Dumont et al., 1993). The distinctive flavor of maple syrup has kept this product selling at premium prices for many years. Maple syrup is the characteristic product resulting from thermal processing of maple sap, the exudate tapped from the trunk of mature sugar maple trees (*Acer saccharum* Marsh). The initial maple sap represents a solution in which sucrose is the major component (Naghski and Willits, 1957). In addition, minor quantities of reducing sugars (Jones and Alli., 1987; Kallio, 1988), organic acids (Mollica and Morselli, 1986; Kallio, 1988), minerals (Kuentz et al., 1976), and nitrogenous compounds (Morselli and Whalen, 1986) have been reported to be present in maple sap.

Phenolic compounds are widely distributed in plants, many being essential metabolites, and contribute to the sensory properties associated with food quality such as color and aroma (Macheix et al., 1990a). In addition, Huang and Ferraro (1992) reported that some phenolic compounds may have potential health benefits, including the reduction of cancer risk. Filipic and Underwood (1964) reported the presence of phenolic-related compounds such as vanillin, coumarin, syringaldehyde, coniferaldehyde, and 2,6-dimethoxybenzoquinone at concentrations lower than 1 ppm in chloroform extracts of maple sap as well as an ether insoluble lignin. Recently, Potter and Fagerson (1992) reported on the identification of phenolic lignin monomers and related flavor compounds in dichloromethane extracts of maple syrup. The source of vanillin and syringaldehyde in maple syrup has been suggested by Underwood and Filipic (1964) to be lignin or lignin fragment. Bound vanillin was also reported to be present in maple sap as a precursor of vanillin in maple syrup (Belford et al., 1992).

The separation and quantitative analyses of phenolic compounds in plant extract were achieved by high-performance liquid chromatography (HPLC) equipped with an ultraviolet (UV) detector (Wilson, 1981; Spanos et al., 1990) as well as an electrochemical (EC) detector (Nagels and Creten, 1985; Roston and Kissinger, 1981; Joerg and Sontag, 1993). The literature indicated that the limit of detection of some phenolic compounds is higher with the electrochemical detector compared to that of UV detector (Hayes et al., 1987; Galetti et al., 1990; Kermasha et al., 1994).

The objectives of this study were to develop a HPLC analytical method for the separation and characterization of phenolic and furfural compounds in maple products extracts, using a combination of UV diode array and EC detectors as well as to analyze the effects of harvest time, technological processes, and producers on the profiles of these compounds in maple products.

MATERIAL AND METHODS

Reagents and Standards. All chemicals used throughout this study were of ACS reagent grade or better. Phenolic standards (*p*-coumaric and ferulic acids) were purchased from Sigma Chemical Co. (St. Louis, MO). Sinapic (3,5-dimethoxy-4-hydroxycinnamic acid), syringic (4-hydroxy-3,5-dimethoxybenzoic acid), coumaric (4-hydroxycinnamic acid), vanillic (4-hydroxy-3-methoxybenzoic acid), and homovanillic ((4-hydroxy-3-methoxyphenyl)acetic acid) acids, coniferol (4-hydroxy-3-methoxycinnamyl alcohol), coniferal (4-hydroxy-3-methoxycinnamyl aldehyde), syringal (4-hydroxy-3,5-dimethoxybenzaldehyde), vanillin (4-hydroxy-3-dimethoxybenzaldehyde), and 5-(hydroxymethyl)-2-furaldehyde (HMF) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Chemical structures of phenolic compounds are reported in Figure 1.

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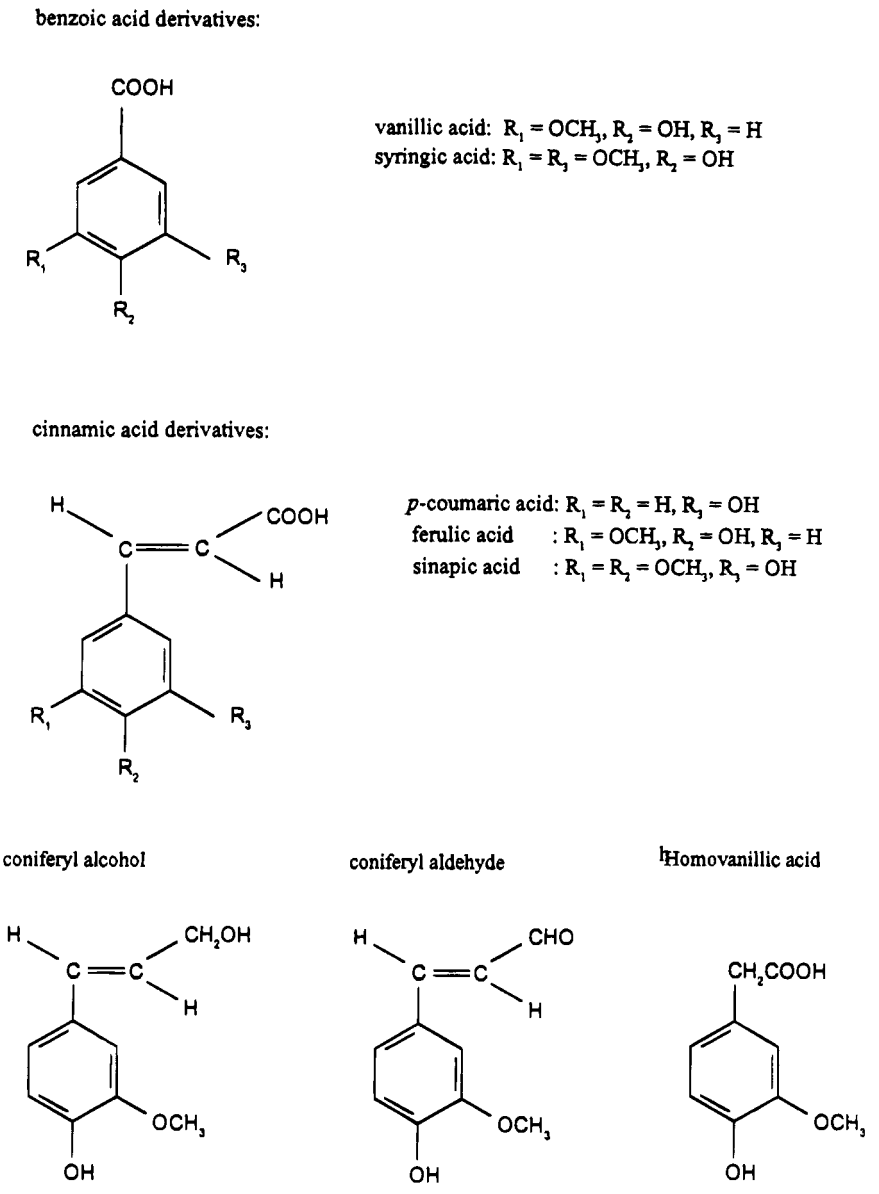

Figure 1. Structures of phenolic compounds studied.

Maple Products Samples. Maple products were obtained from three different Quebec producers identified as ML, AT, and LL. Maple saps, reverse osmosis concentrated saps, and syrups were provided by both producers ML and AT, whereas producer LL provided only saps and syrups.

Reverse osmosis concentration was performed by producers ML and AT at 10 °C using, respectively, a Lapierre system (St. Ephren, Québec) equipped with two membranes Filmtech (Filmtech Corp., Minneapolis, MN) and a Dominion Grim system (Montréal, Québec) equipped with a Seprotec high-performance membrane (Ottawa, Ontario) both set at 3000 KPa and 2700 L/h. Thermal evaporation was performed by producers ML, LL, and AT at boiling point of the solution until 66 °Brix, using, respectively, an oil burner evaporator 1.65 m × 4 m (Small Brothers, Durham, Québec), a wood-heated evaporator 2.0 m × 5.3 m (Dominion Grim, Montréal, Québec), and an oil burner evaporator 2 m × 4 m (Waterloo, Waterloo, Québec).

Maple saps, concentrates, and syrups were sampled in triplicate for each harvest day over the season 1993. The pH and Brix degree (°Brix) values were determined for each sample. The degree Brix was defined as the refractometric dry substance at 20 °C. In accordance with Québec regulations (Gouvernement du Québec, 1983), maple syrups had 66% of refractometric dry substance at 20 °C.

Extraction of Phenolic and Furfural Compounds. Different methods of extraction of phenolic and furfural compounds were developed. The standard solution, containing 1 μg/mL of each phenolic and furfural standard compounds, was concentrated 25 times by all methods of extraction.

Lyophilization. The standard solution (25 mL) was concentrated by lyophilization using a Labconco (Labconco, Kansas City, MO) freeze dryer set at −50 °C with a vacuum of 10 μm of Hg. The resulting residue was redissolved in 1 mL of methanol and filtered throughout a 0.20 μm filter. The filtrate was subjected to HPLC analyses.

Ethyl Acetate Extraction. The extraction of phenolic compounds was carried out according to a modification of the method of Mahler et al. (1988). The standard solution (25 mL) was adjusted to pH 2 with 6 N HCl and the compounds of interest were extracted successively with 60, 30, and 30 mL of ethyl acetate. The fractions were then pooled and dried with anhydrous Na_2SO_4, and the solvent was removed at room temperature under a gentle stream of N_2. The resultant residue was dissolved in 1 mL of methanol.

Diethyl Ether Extraction. The extraction of phenolic compounds was performed according to a modification of the method described by Krygier et al. (1982). The extracted phenolic and furfural compounds were prepared as described above.

Sep-Pak. The extraction of phenolic and furfural compounds using Sep-Pak C18 column (Millipore, Bedford, MA) was performed according to the method described by Jaworski et

al. (1987). The extracted phenolic and furfural compounds were prepared as described above.

Supelclean. The extraction of phenolic and furfural compounds using Supelclean column (Envichrom-P-SPE, Supelco, Oakville, Ontario) was performed according to the method described previously (Anonymous, 1993). The extracted phenolic and furfural compounds were prepared as described above.

The best recovery was obtained with the ethyl acetate extraction of phenolic and furfural compounds; this method was used for the extraction of saps and concentrates as well as syrups diluted 40 times with distilled water.

High-Performance Liquid Chromatography Analyses of Phenolic and Furfural Compounds. The extracted phenolic and furfural compounds were separated by a gradient elution using a HPLC system (Beckman Model 126, Beckman Instruments Inc., San Ramon, CA) equipped with a UV diode array (UV) detector (Beckman, Model 168) and an electrochemical (EC) detector (Coulochem II, Esa Inc., Bedford, MA) assembled in series and computerized integration with data handling. A Beckman analog interface Model 406 was used to transfer data from the EC detector to the HPLC system. The UV detection was performed at two different wavelengths, 280 and 320 nm. Scanning from 200 to 400 nm was monitored at 1 s interval. The EC detector was set at an output of 1 V, and the detection was performed at 200 and 600 mV, at 10 μA. Automatic injection (Varian, Autosampler 9095, Varian Associates, Inc., Walnut Creek, CA) was carried out with a 20 μL loop onto an Econosil C18 column (150 × 4.6 mm i.d., pore size 5 μm) (Alltech Associates, Inc., Deerfield, IL). The elution (47.5 min) was performed at room temperature and at a flow rate of 0.75 mL/min, using methanol (Omnisolv grade, BDH Inc., Poole, United Kingdom) as solvent A and an aqueous solution of 0.2% trifluoroacetic acid as solvent B, with a linear gradient of 2 to 40% solvent A.

Identification and Quantitation. Initial identity assignments of phenolic and furfural compounds were based on comparison retention data obtained with UV and EC detectors for standard compounds and sample components. Comparison of spectral characteristics (scans from 200 to 400 nm) of standards and sample components provided confirmation of the initial identity assignment. Additional confirmation was provided by the comparison of EC characteristics of standards and sample components.

Calculation of concentrations of compounds of interest was based on the external standard method. Dilutions of aqueous solutions containing 50 ng/mL of all standards were used to fit a standard curve (area versus concentration in nanograms per milliliter), with a linear regression for each compound. Concentration (C) of each compound was calculated from peak area (A) by using the equation

$$C = \alpha + \beta A$$

where α is the curve intercept and β is the curve slope.

The concentrations of phenolic and furfural compounds in maple products were determined in triplicate and the average concentrations were standardized per degree Brix of initial solution and expressed as nanogram per milliliter per degree Brix of solution.

Statistical Analyses. Statistical analyses were performed using StatGraphics software version 5.2 (STSC, Inc., Rockville, MD). Analyses of variance (ANOVA) of variable "HMF concentrations" (147 samples) were performed with three factors, i.e., day of harvest, maple products, and producers. ANOVA of variable "phenolics concentrations" (1470 samples) were performed with four factors, i.e., type of phenolic compound, day of harvest, maple products, and producers. Hypothesis of a nonsignificant effect was made for all ANOVA.

RESULTS AND DISCUSSION

Extraction of Phenolic and Furfural Compounds. The results (Table 1) indicate that the mean percentage of recovery for all phenolic and furfural compounds, using different methods of extraction, was obtained in a decreasing order and as follows: ethyl acetate (87.6%) > Sep-Pak (82.2%) > lyophilization (62.9%) > ether (44.3%) > Supelclean (41.8%). Additional work on ethyl acetate extraction (data not shown) indicated that the mean standard of deviation for all phenolic and furfural compounds of 10 replicates of extraction was 3.081 with a mean coefficient of variation of 7.7%; these findings may indicate a very good reproducibility. On the basis of these results, the ethyl acetate method of extraction was used throughout this study.

Table 1. Percentage of Recovery of 5-(Hydroxymethyl)-2-furaldehyde and Phenolic Compounds Using Different Methods of Extraction

compound	recovery[a] (%) lyophilization	diethyl ether	ethyl acetate	Sep-Pak	Supelclean
5-(hydroxymethyl)-2-furaldehyde	48.6	64.7	85.8	85.7	28.6
vanillic acid	86.0	53.9	97.1	110.8	22.1
syringic acid	0.0	43.9	84.1	104.5	43.0
homovanillic acid	84.6	49.8	85.5	99.1	19.7
coniferyl alcohol	91.3	0.0	87.2	14.6	63.2
vanillin	0.0	58.6	115.8	90.3	44.2
p-coumaric acid	80.7	58.9	88.7	95.7	52.3
syringaldehyde	78.3	45.4	99.1	94.7	52.3
sinapic acid	92.4	0.0	36.0	51.3	58.2
ferulic acid	78	45.2	97.1	86.0	53.6
coniferylaldehyde	52	66.4	87.3	71.6	22.4
av recovery	*62.9*	*44.3*	*87.6*	*82.2*	*41.8*

[a] The relative recovery was calculated as percentage of mean of peak area obtained from triplicate HPLC injections of extract of standard compounds divided by the mean of peak area obtained from triplicate HPLC injections of standard compounds but without extraction. Coefficients of variation of the values reported were from 0.9 to 2.0%.

Optimization of HPLC Analyses. Preliminary trials, carried out for the optimization of HPLC analyses, indicated that the retention times of standard phenolic acids were mostly pH dependent, whereas those of furfural compounds were mostly dependent on acetonitrile concentration. Hence, a gradient elution solvent system, consisted of 2−40% acetonitrile and 98−60% of an aqueous solution of 0.2% trifluoroacetic acid, was developed to provide a chromatogram of well-separated and high-resolution peaks (Figure 2). Scan analyses of standard compounds indicated that the detection of phenolic and furfural compounds was optimum at 280 and 320 nm. Preliminary work for the optimization and selection of the most appropriate potential values for setting the electrode of EC detector indicated that both sensitivity and stable baseline were obtained for the analyses of phenolic compounds at 200 and 600 mV. Typical retention times are reported in Table 2.

The EC analyses provided a dramatic increase in the limits of detection of all phenolic compounds compared with those obtained by UV analyses. The results (Table 2) demonstrate that the limits of detection obtained with EC analyses were 100 (coniferol and homovanillic acid), 50 (vanillin and sinapic acid), 40 (vanillic acid), and 20 (syringic, *p*-coumaric, ferulic, and coniferal) times higher than those obtained with UV analyses. The detection limits (Table 2) are of the order of previous work on UV/EC comparison (Hayes et al., 1987; Galetti et al., 1990).

Identification of Phenolic and Furfural Compounds in Maple Products. Typical chromatograms of HPLC analyses of phenolic and furfural compounds are reported for maple sap (Figure 3), concentrate (Figure 4), and syrup (Figure 5). The literature indi-

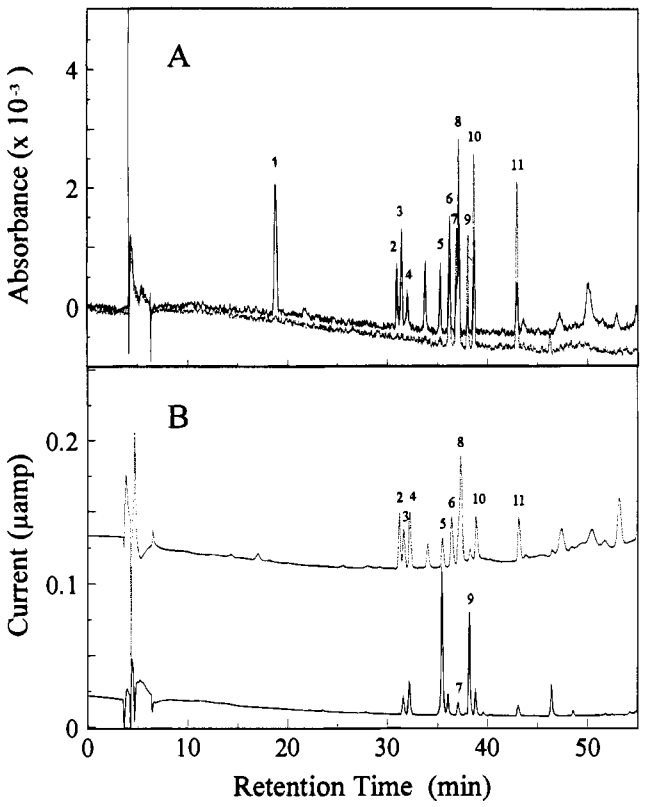

Figure 2. Chromatograms of HPLC analyses of a mixture of standard phenolic and furfural compounds using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks are indicated as follows: (1) 5-(hydroxymethyl)-2-furaldehyde, (2) vanillic acid, (3) syringic acid, (4) homovanillic acid, (5) coniferol, (6) vanillin, (7) syringal, (8) *p*-coumaric acid, (9) sinapic acid, (10) ferulic acid, and (11) coniferal.

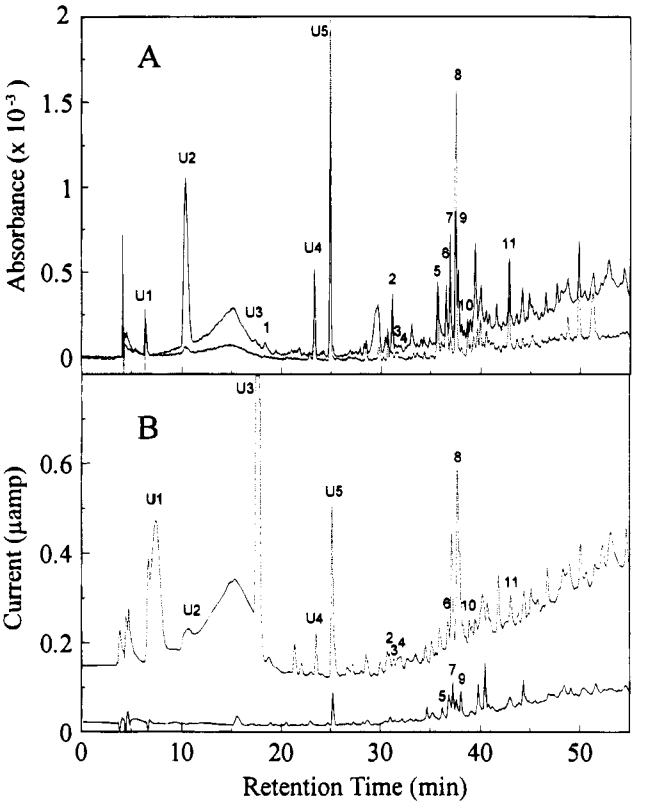

Figure 3. Chromatograms of HPLC analyses of maple sap ethyl acetate extract using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks are indicated as follows: (1) 5-(hydroxymethyl)-2-furaldehyde, (2) vanillic acid, (3) syringic acid, (4) homovanillic acid, (5) coniferol, (6) vanillin, (7) syringal, (8) *p*-coumaric acid, (9) sinapic acid, (10) ferulic acid, and (11) coniferal, (U1, U2, U3, U4, U5) unknown compounds.

Table 2. Limit of Detection and Retention Times of 5-(Hydroxymethyl)-2-furaldehyde and Phenolic Compounds Using Ultraviolet and Electrochemical Detectors

		detection limit[a] (ng/mL)			
		ultraviolet diode array		electrochemical	
compound	retention time (min)	280 nm	320 nm	200 mV	600 mV
HMF[b]	18.5	5.00	50.00	*c*	*c*
vanillic acid	30.7	10.00	*c*	50.00	0.25
syringic acid	31.3	5.00	750.00	1.00	0.25
homovanillic acid	31.8	25.00	*c*	1.00	0.25
coniferyl alcohol	35.0	10.00	50.00	0.10	5.00
vanillin	35.7	5.00	5.00	1.00	0.10
p-coumaric acid	37.2	5.00	5.00	*c*	0.25
syringaldehyde	36.7	25.00	5.00	1.00	5.00
sinapic acid	37.9	25.00	5.00	0.10	5.00
ferulic acid	38.3	5.00	5.00	1.00	0.25
coniferylaldehyde	43.0	25.00	5.00	5.00	0.25

[a] Detection limit is the minimum detectable concentration of phenolic and furfural compounds calculated on the basis of a 3:1 of signal/noise ratio and expressed as nanograms of standard compound per milliliter. [b] 5-(Hydroxymethyl)-2-furaldehyde. [c] Not detectable at 750 ng/mL.

cates that spectral (Bartolomé et al., 1993) and EC (Joerg and Sontag, 1993) characteristics could be used to assign standard compounds to unknown sample components. Roston and Kissinger (1981) indicated that the comparison of EC responses of standards and sample components could provide a confirmation of the initial identity assignment, obtained with retention time and UV data. Hence, by matching retention time data and spectral and electrochemical characteristics of the corresponding peaks in maple products HPLC analyses with those of standards, the results (Figures 3–5) indicate that peaks 2, 3, 4, 8, 9, and 10 correspond, respectively, to vanillic, syringic, homovanillic, *p*-coumaric, sinapic, and ferulic acids. Similarly, the presence in maple products, of HMF (peak 1), coniferol (peak 5), vanillin (peak 6), syringal (peak 7), and coniferal (peak 12) were confirmed by retention times and spectral and electrochemical data. Our results are in agreement with those reported by Potter and Fagerson (1992) who identified the presence of vanillin, homovanillic, syringic, and vanillic acids as well as coniferal and coniferol in maple syrup.

Spectral characteristics of five major unknown peaks U_1, U_2, U_3, U_4, and U_5 (Figures 3–5) did not allow the identification of these compounds. In order to identify these major peaks, preparative purification was performed. A maple sap sample (100 mL) was extracted and subjected to preparative HPLC, using the same conditions as for the analytical analyses. The five separated fractions exhibited a positive response with the 0.2 N Folin–Ciocalteu reagent (Sigma), a specific test of phenolic compounds (Singleton and Rossi, 1965); hence peaks U_1 to U_5 were tentatively identified as phenolic-related compounds.

Effect of Harvest Time, Processing, and Producer on Concentration of HMF in Maple Products. The results (data not shown) indicate that HMF (0–155.52 ng/mL/°Brix) was detected in the majority

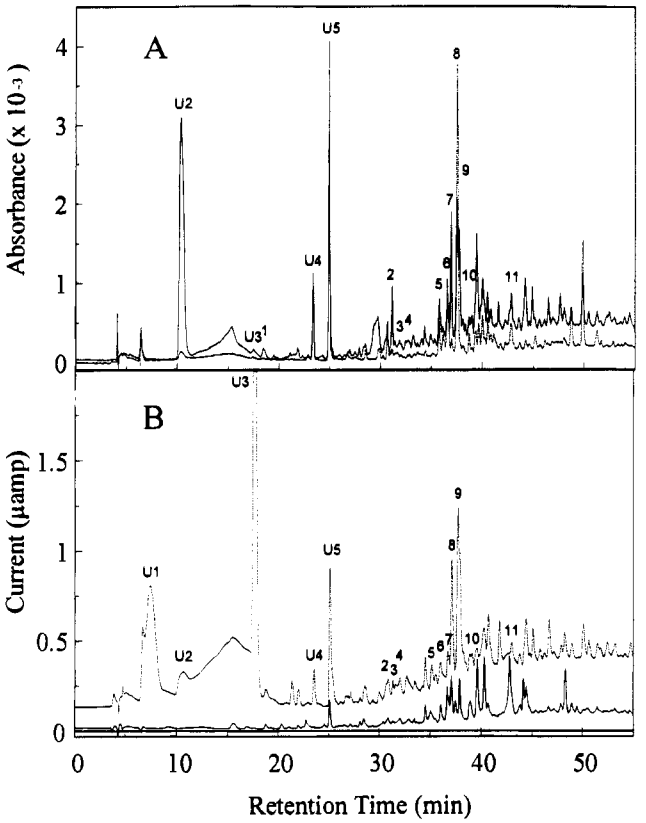

Figure 4. Chromatograms of HPLC analyses of maple concentrate ethyl acetate extract using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks: see Figure 2.

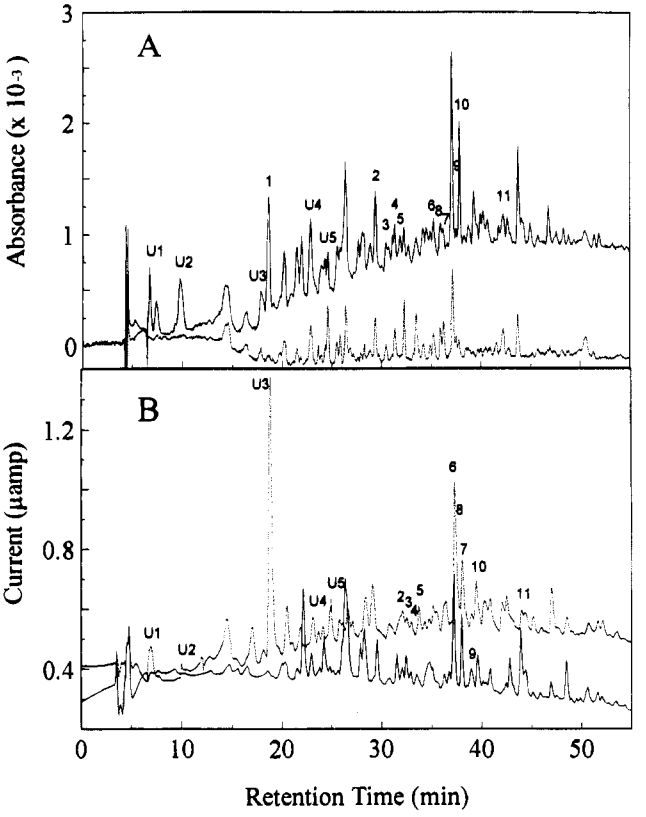

Figure 5. Chromatograms of HPLC analyses of maple syrup ethyl acetate extract using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks: see Figure 2.

of maple products. An ANOVA at three factors of the variable "HMF concentrations" in maple products was performed. The results (Table 3) indicate a significant day effect at the level of 0.05, a highly significant maple product effect, and a nonsignificant producer effect.

Graphic representations of ANOVA, i.e., means plots, are reported on Figure 6. The results (Figure 6A) indicate a trend toward a slight seasonal increase of HMF in maple products, that may be related to the increase of the temperature during the season.

The results (Figure 6B) also indicate that the syrups exhibited the highest concentration of HMF, compared to that present in saps and concentrates. Alfonso et al. (1980) reported that the most common product of dehydration of ketopentose, particularly in acid or high-temperature environments, was HMF. The presence of HMF in maple saps and concentrates, with mean pH values of, respectively, 7.06 ± 0.48 and 7.02 ± 0.58, may suggest that the formation of HMF could occurred in neutral or slightly basic conditions. The drastic increase of HMF during heating could be related to the thermal processing, in agreement with the study of Underwood (1971) who reported an increase of HMF peak height by 250% and 800% after heating times of 1.5 and 4 h, respectively.

Although the ANOVA indicate that there is no significant difference in HMF concentration between the producers, the results (Figure 6C) suggest that the concentration of HMF in maple products of producer LL is higher than that of the two other producers.

The concentration of HMF in maple syrups (data not shown) up to 10.26 ppm can be related to the implication of HMF in maple syrup flavor. HMF has been described by Filipic et al. (1969) to be a major constituent of high-flavored maple syrup.

Effect of Harvest Time, Processing, and Producer on Phenolic Compound Profiles in Maple Products. An ANOVA at four factors of the concentrations of 10 phenolic compounds in maple products was performed. The results (Table 4) indicate a highly significant phenolic compound effect, a harvest time effect at the level of 0.05, a highly significant maple product effect, and a highly significant producer effect. Corresponding means plots are reported on Figure 7.

Phenolic Compounds Effect. The intervals of factor means for the level of phenolic compounds in all samples (Figure 7A) show the presence of four different homogeneous groups: group 1 with coniferyl alcohol at the lowest concentration, group 2 with vanillic and syringic acids, group 3 with homovanillic, coumaric and ferulic acids, as well as vanillin, syringaldehyde and coniferaldehyde, and group 4 with sinapic acid with the highest concentration.

Harvest Time Effect. The periods of harvest were from March 26 to April 16, March 27 to April 19, and March 24 to April 11 for the producers ML, AT, and LL, respectively. The results (Table 4) demonstrate the significant effect of harvest time on the concentration of total phenolic compounds present in saps, concentrates, and syrups.

Figure 7B indicates a trend toward a slight seasonal increase of phenolic compounds in maple products. It appears that the highest contents of phenolic compounds occurred at the end of harvest time. In addition a seasonal increase of unknown phenolic-related compounds U_1, U_2, U_3, U_4, and U_5 was observed (data not

Table 3. Analysis of Variance of Concentrations of (Hydroxymethyl)furfural in Maple Products

source of variation	sum of squares	degrees of freedom	mean square	F ratio[a]	significant level[b]
main effects					
A: concentration[c]/day[d]	1254.43	22	57.02	1.73*	0.0321
B: concentration/maple product[e]	6644.78	2	3322.39	101.01**	0.0000
C: concentration/producer[f]	24.28	2	12.14	0.37	0.6921
residual	3946.94	120	32.89		
total	11870.43	146			

[a] All F ratios are based on the residual mean square error. [b] Effect A is significant at the level 0.05, effect B is nonsignificant, and effect C is highly significant at the level 0.01. [c] Concentrations of (hydroxymethyl)furfural are expressed as ng/mL/°Brix. [d] Concentrations of (hydroxymethyl)furfural were determined each day during the harvest time. [e] Concentrations of (hydroxymethyl)furfural were determined in maple saps, concentrates, and syrups. [f] Concentrations of (hydroxymethyl)furfural were determined for three different producers. *Significant at the 0.05 level. **Significant at the 0.01 level.

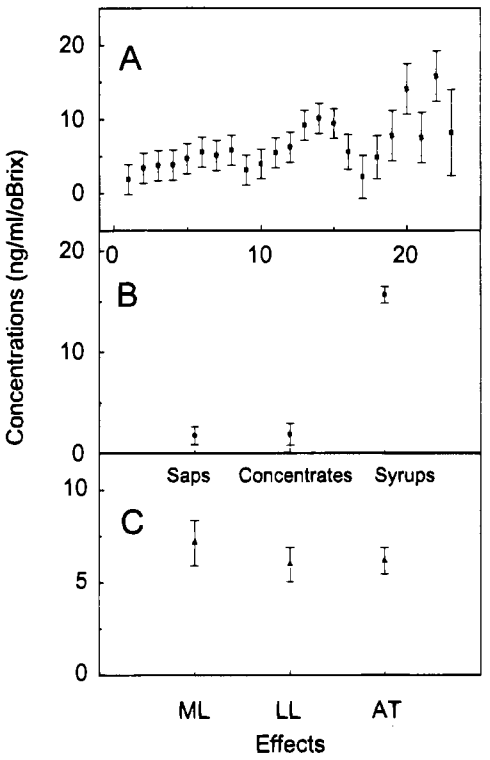

Figure 6. Means plots with 95% confidence for the variable concentration of (hydroxymethyl)furfural concentrations (A) day effect, (B) maple product effect, and (C) producer effect.

shown). These results are in agreement with those of Laing et al. (1971) who reported a slight seasonal increase of phenol-reacting compounds in maple saps. It is probable that different factors, including genetics and climatic and soil conditions, combined to provide variations in qualitative and quantitative profile of phenolic compounds in maple products (Belford et al., 1992); these authors reported that variations in vanillin glycosides concentrations were associated with harvest time during the season of collection.

Maple Products Effect. The results (Figure 7C) indicate that the saps exhibited the lowest concentration of phenolic compounds, whereas there were no significant differences in phenolic concentrations between concentrates and syrups.

Analyses of the interaction between phenolic compound and maple product sources of variations (Table 4) show a highly significant effect. These results indicate that the proportion of each phenolic compound is different as related to the maple products. ANOVA for saps, concentrates, and syrups were performed separately and the results (data not shown) demonstrate that there were significant differences between phenolic compound concentrations for each ANOVA. Corresponding mean plots are reported in Figure 8. The results (Figure 8) show that the relative importance of each phenolic compound is different for saps, concentrates, and syrups. The results (Figure 8) show the presence of four different homogenous groups in the decreasing importance order for saps (group 1, sinapic acid; group 2, vanillic, homovanillic, and *p*-coumaric acids, coniferal, syringal, and vanillin; group 3, syringic and ferulic acids; group 4, coniferol), concentrates (group 1, sinapic acid; group 2, homovanillic acid and coniferal; group 3, vanillic, syringic, and *p*-coumaric acids, syringal, and vanillin; group 4, ferulic acid and coniferol), and syrups (group 1, ferulic acid; group 2, syringal and vanillin; group 3, syringic, homovanillic, *p*-coumaric, and sinapic acids and coniferal; group 4, vanillic acid and coniferol). Thus, sinapic acid is the major phenolic compound identified in saps, and sinapic and homovanillic acids and coniferal are the major phenolics in concentrates, whereas ferulic acid, syringal, and vanillin are the major phenolic compounds identified in maple syrup.

The analyses of the relative proportion of each phenolic in percentages of total phenolics results (Figure 9) indicate that the effect of concentration by reverse osmosis of maple sap has the same trend on the relative composition of phenolic compounds for the producers ML and AT which is an increase of the relative proportions of phenolic acids and a decrease of the relative proportions of aldehyde and alcohol. The loss of aldehydes could be related to the oxidation of the sap in the reverse osmosis system. Chou et al. (1991) reported a substantial reverse osmosis processing loss of aldehyde compounds in apple juices due to the evaporation and membrane capture. In addition, Sheu and Wiley (1983) showed that the retention of some apple juice aldehyde components was dependent on the type of membrane used.

The results (Figure 9) also indicate that the thermal evaporation process resulted in a dramatic increase of ferulic acid and moderate increases of vanillin and syringal, with a concomitant drastic decrease of sinapic acid.

Vanillin and syringal have been previously reported and ascribed to degradation of ligneous material present in maple sap (Underwood et al., 1964). Recently, Belford et al. (1992) reported the presence of a bound vanillin fraction that could be hydrolyzed by β-glucosidases. Macheix et al. (1990b) reported that ferulic acid can be found in plants linked by ester bonds to various polymers, such as lignin derivatives. Hence, the increase in the concentrations of vanillin and ferulic acid during the thermal evaporation process (Figure 9) could be related to the hydrolysis of bound forms of these phenolic compounds.

Table 4. Analysis of Variance of Concentrations of Phenolic Compounds in Maple Products

source of variation	sum of squares	degree of freedom	mean square	F ratio[a]	significant level[b]
main effects					
A: concentration[c]/phenolics[d]	5164.77	9	5738.64	48.61**	0.0000
B: concentration/day[e]	10274.42	22	487.47	4.13**	0.0000
C: concentration/maple product[f]	2979.69	2	1489.85	12.62**	0.0000
D: concentration/producer[g]	6773.62	2	3386.81	28.69**	0.0000
interactions					
A/C	60161.51	18	3342.31	28.31**	0.0000
A/D	21.34	18	1185.67	10.04**	0.0000
residual	165048.70	1398	118.06		
total	318677.75	1469			

[a] All F ratios are based on the residual mean square error. [b] All effects and interactions are highly significant with a the level 0.01. [c] Concentrations of phenolic compounds are expressed as ng/mL/°Brix. [d] Concentrations of 10 phenolic compounds were determined. [e] Concentrations of phenolic compounds were determined each day during the harvest time. [f] Concentrations of phenolic compounds were determined in maple saps, concentrates, and syrups. [g] Concentrations of phenolic compounds were determined for three different producers. **Significant at the 0.01 level.

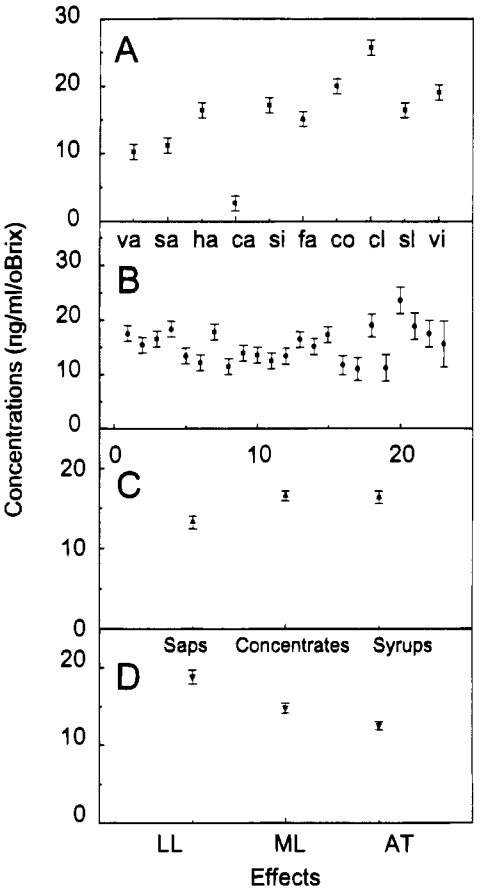

Figure 7. Means plots with 95% confidence for the variable concentration of phenolic compounds (A) phenolic compound effect (vanillic acid, va; syringic acid, sa; homovanillic acid, ha; *p*-coumaric acid, ca; sinapic acid, si; ferulic acid, fa; coniferol, co; coniferal, cl; syringal, sl; vanillin, vi), (B) day effect, (C) maple product effect, and (D) producer effect.

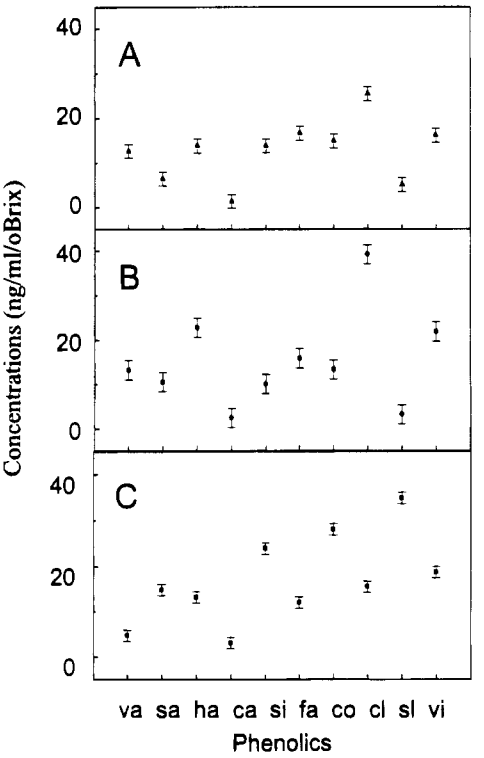

Figure 8. Means plots with 95% confidence for the variable concentration of phenolic compounds (see Figure 7) for the analyses of variance as related to maple products (A) saps, (B) concentrates, and (C) syrups.

The results (data not shown) indicate that vanillin is present in maple syrups at concentrations from 11.05 to 62.02 ng/mL/°Brix which correspond, for syrups of 66 °Brix, to 0.73 to 4.09 ppm, respectively. Vanillin has been described as the most important compound derived from ligneous material with respect to flavor contribution in maple syrup (Filipic et al., 1969). Vanillin is known to have extremely low flavor threshold of 0.69 ppm (Fazzalari, 1978). The results (data not shown) indicate concentrations of ferulic acid in maple syrups from 1.61 to 2.80 ppm. Fazzalari (1978) reported that the flavor threshold of ferulic acid is 90 ppm; in addition, Huang and Ferraro (1992) suggested an anticarcinogenic effect of ferulic acid.

Although there was an evidence of some variations in identified phenolic compound concentrations of maple products, there was not a pronounced variation in unknown phenolic related compounds U_1, U_2, U_3, U_4, and U_5 as a result of the reverse osmosis concentration of maple sap. However, the thermal evaporation process resulted in decrease of major unknown phenolics U_1, U_2, U_3, U_4, and U_5 (data not shown).

Producers Effect. The results (Figure 7D) indicate that the concentrations of phenolic compounds were significantly different among the three producers and that there were, in decreasing order LL > ML > AT.

Analyses of the interaction between phenolic compound and producer sources of variations (Table 4) show a highly significant effect. These results indicate that the proportion of each phenolic compound is different as related to the producer. ANOVA for producers ML, AT, and LL were performed separately and the results (data not shown) demonstrate that there were significant differences between phenolic compound concentra-

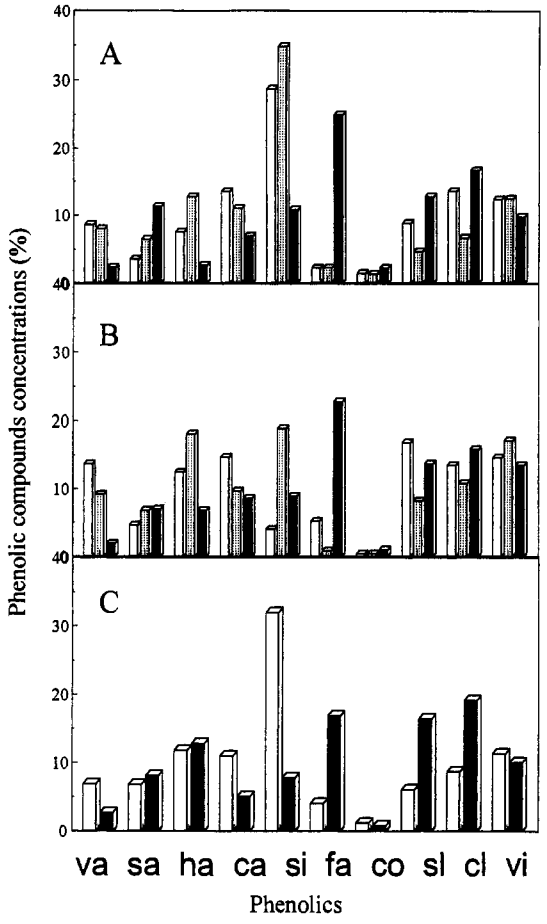

Figure 9. Distribution of phenolic compounds expressed (see Figure 7) as percentage of total phenolics present in maple products from the producers (A) ML, (B) AT, and (C) LL. The bars are differentiated as follows: open, sap; dotted, concentrate; and filled, syrup.

tions for each ANOVA. Corresponding mean plots are reported in Figure 10. The results (Figure 10, parts A–C) show the presence of three similar homogeneous groups for producers ML and LL (group 1, sinapic acid; group 2, vanillic, homovanillic, *p*-coumaric, syringic, and ferulic acids, coniferal, syringal, and vanillin; group 3, coniferol), whereas the proportion of each phenolic is different for producer AT (group 1, homovanillic, *p*-coumaric, sinapic, and ferulic acids, coniferal, syringal, and vanillin; group 2, vanillic and syringic acids; group 3, coniferol).

Those differences between producers may be related to harvest and processing of maple products as well as climatic and soil conditions.

CONCLUSION

The results gathered in this study demonstrated that the optimization of the HPLC analyses using UV and EC detectors allowed the identification and quantification of phenolic and furfural compounds in maple sap, concentrate, and syrup. The present work indicated that HMF concentrations and phenolic profiles of maple products were significantly different as related to harvest time and technological process used to manufacture maple syrup. Highest contents of phenolic compounds occurred at the beginning and at the end of harvest time for all producers. Variations of the quantitative phenolic profile were also observed between the different producers. An increase in the relative proportion of phenolic acids and a decrease in the relative

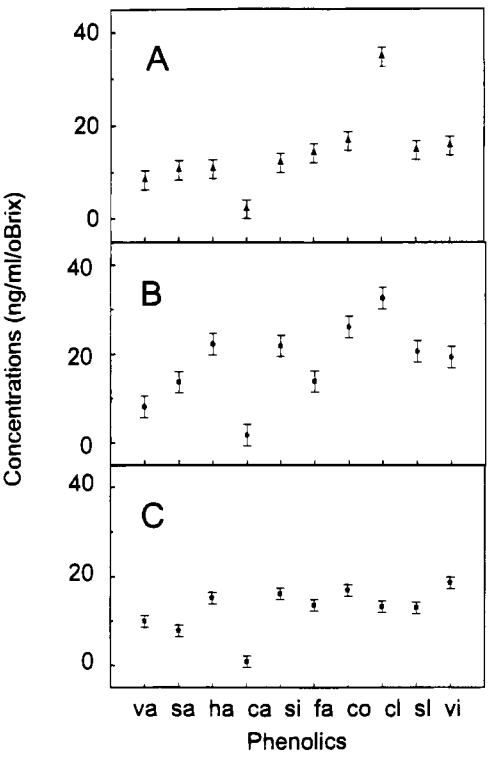

Figure 10. Means plots with 95% confidence for the variable concentration of phenolic compounds (see Figure 7) for the analyses of variance as related to producers (A) ML, (B) LL, and (C) AT.

proportions of aldehyde and alcohol was observed during the reverse osmosis processing of maple sap. The thermal evaporation of maple sap or concentrate resulted in an increase of ferulic acid, HMF, vanillin, and syringal and a concomitant drastic decrease of sinapic acid.

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