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Chemometric Classification of Apple Juices According to Variety and Geographical Origin Based on Polyphenolic Profiles

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ABSTRACT: To characterize and classify apple juices according to apple variety and geographical origin on the basis of their polyphenol composition, the polyphenolic profiles of 58 apple juice samples belonging to 5 apple varieties and from 6 regions in Shaanxi province of China were assessed. Fifty-one of the samples were from protected designation of origin (PDO) districts. Polyphenols were determined by high-performance liquid chromatography coupled to photodiode array detection (HPLC-PDA) and to a Q Exactive quadrupole-Orbitrap mass spectrometer. Chemometric techniques including principal component analysis (PCA) and stepwise linear discriminant analysis (SLDA) were carried out on polyphenolic profiles of the samples to develop discrimination models. SLDA achieved satisfactory discriminations of apple juices according to variety and geographical origin, providing respectively 98.3 and 91.2% success rate in terms of prediction ability. This result demonstrated that polyphenols could served as characteristic indices to verify the variety and geographical origin of apple juices.

KEYWORDS: *polyphenol, apple juice, chemometric techniques, classification*

INTRODUCTION

Apple (*Malus domestica* Borkh.) juice is one of the most frequently consumed juices all over the world. In Shaanxi province of China, the annual harvest of apples in recent years is estimated at about 9 million tons. A large part of them are processed to juice and exported to the international market, accounting for more than one-third of the world trade volume in apple juice concentrate. Due to the favorable geographical environment of production, apples from some specific regions in Shaanxi are recognized as "protected designation of origin" (PDO) products. This designation guarantees that the quality of the product is closely linked to its territorial origin.

To confirm the safety and authenticity of the juice, determination of variety and geographical origin of the apples for juice production is required. Various kinds of adulterations can be detected more easily if the origin of the juice is ascertained. The origin identification becomes more important for juices produced from apples with the PDO label, because these juices acquire an added value and are more likely to become a target for adulteration. Ensuring the declared origin and quality is necessary to provide real protection for consumers and reliable producers.

Phenolic compounds are secondary metabolites of plants that are synthesized in the course of plant development as part of responses to several adverse effects such as infection, wounding, and UV irradiation. Plant polyphenols are multifunctional in the sense that they have antioxidant activity, free radical scavenging capacity, coronary heart disease prevention property, and anticarcinogenic ability.[1,2] Moreover, some of them could be inhibitors for microbiological growth-avoiding process spoilages.[3] Furthermore, polyphenols are associated with color and sensory characteristics, such as bitterness and astringency.[4,5] Polyphenols present in apple juices can be classified into several major classes. The flavan-3-ols are subdivided into catechins ((−)-epicatechin and (+)-catechin) and procyanidins. Procyanidins are oligomeric and polymeric catechins, consisting mainly of (+)-epicatechin units with a small proportion of (+)-catechin as a terminal unit.[6] Dimeric and trimeric procyanidins exist as well. Among the hydroxycinnamic acids, 5-caffeoylquinic acid and *p*-coumaroylquinic acid show the highest contents. The major compounds of the dihydrochalcones are phloretin glucoside and xyloglucoside. Flavonols are mainly located in apple peel, and lower levels are also present in the pulp and juice.[7] Finally, anthocyanins are essentially present in apple skin. Factors that influence the type and amount of these polyphenols in apple juice are apple variety, environment, maturity stage, and processing procedure.[6−12]

The application of chemometric techniques has been proven a versatile and valuable tool for assessing food authenticity. Thus, on the basis of various analytical data related with apple juice composition, several authors have published studies applying different chemometric techniques in the differentiation and classification of apple juices according to the apple variety and/or geographical origin.[13−18] However, little systematic work has been carried out to classify apple juice samples using phenolic compounds as characteristic indices. Furthermore, the previous studies have mainly focused on the variety-based classification, paying less attention to distinguishing apple juice samples in terms of geographical origin.

The aim of this work was to characterize and classify apple juice samples according to apple variety and geographical origin on the basis of their polyphenolic profiles. Apples for juice production belonged to five of the most widely cultivated varieties from six regions in Shaanxi province of China. Five of these regions are within the PDO districts. It should be noted that apple orchards chosen for juice production lie within a

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Table 1. Composition of Dihydrochalcones (Milligrams per Liter) in the Apple Juices[a]

sample		HPXG	HPG	PXG	PLZ	total
Fuji (n = 20)						
1	Liquan	0.54 ± 0.06	1.5 ± 0.2	5.9 ± 0.6	7.4 ± 0.4	15.3
2	Xunyi	0.31 ± 0.02	1.1 ± 0.1	3.4 ± 0.4	6.1 ± 0.5	10.9
3	Xunyi	0.31 ± 0.01	1.1 ± 0.2	3.9 ± 0.5	6.1 ± 0.8	11.4
4	Xunyi	0.42 ± 0.05	1.3 ± 0.2	4.8 ± 0.6	7.0 ± 0.5	13.5
5	Xunyi	0.29 ± 0.03	0.8 ± 0.1	3.7 ± 0.3	4.5 ± 0.8	9.3
6	Xunyi	nq	0.31 ± 0.04	3.3 ± 0.3	3.4 ± 0.5	7.0
7	Xunyi	nq	0.29 ± 0.01	1.9 ± 0.5	1.6 ± 0.1	3.8
8	Xunyi	0.46 ± 0.03	0.95 ± 0.08	3.0 ± 0.3	4.2 ± 0.6	8.6
9	Yongshou	0.40 ± 0.05	0.87 ± 0.03	3.9 ± 0.4	4.9 ± 0.8	10.1
10	Yongshou	0.79 ± 0.06	1.5 ± 0.2	7 ± 1	7 ± 1	16
11	Yongshou	0.50 ± 0.07	1.2 ± 0.2	5.6 ± 0.8	6.4 ± 0.5	13.7
12	Yongshou	0.66 ± 0.04	1.3 ± 0.1	5.7 ± 0.7	7.0 ± 0.7	14.7
13	Yongshou	0.46 ± 0.04	1.0 ± 0.2	6.1 ± 0.3	5.8 ± 0.4	13.4
14	Yongshou	0.72 ± 0.07	1.5 ± 0.2	6.2 ± 0.4	7.2 ± 0.5	15.6
15	Yongshou	0.44 ± 0.03	1.2 ± 0.1	4.5 ± 0.4	6.0 ± 0.9	12.1
16	Sanyuan	0.67 ± 0.06	1.7 ± 0.2	11 ± 1	9.0 ± 0.8	22
17	Sanyuan	0.49 ± 0.02	1.4 ± 0.1	7.3 ± 0.6	6.8 ± 0.7	16.0
18	Luochuan	0.31 ± 0.01	0.56 ± 0.05	2.9 ± 0.2	4.7 ± 0.5	8.5
19	Luochuan	0.45 ± 0.04	1.4 ± 0.2	4.8 ± 0.9	7 ± 1	14
20	Chunhua	0.65 ± 0.07	1.3 ± 0.2	7 ± 1	7.6 ± 0.5	17
mean		0.44	1.1	5	6	13
SD		0.2	0.4	2	2	4
min		nq	0.29	1.9	1.6	3.8
max		0.79	1.7	11	9.0	22
Starkrimson (n = 12)						
21	Liquan	0.38 ± 0.03	2.0 ± 0.3	2.7 ± 0.2	5.6 ± 0.5	10.7
22	Liquan	0.79 ± 0.08	3.0 ± 0.2	2.9 ± 0.2	7.0 ± 0.9	13.7
23	Liquan	1.7 ± 0.3	5.2 ± 0.7	2.9 ± 0.4	4.5 ± 0.3	14.3
24	Xunyi	0.61 ± 0.05	2.1 ± 0.2	4.4 ± 0.4	8.7 ± 0.2	15.8
25	Xunyi	1.3 ± 0.2	4.8 ± 0.3	6.1 ± 0.6	14.1 ± 0.8	26.3
26	Xunyi	1.1 ± 0.1	3.8 ± 0.4	4.1 ± 0.6	11 ± 1	20
27	Xunyi	0.43 ± 0.03	1.3 ± 0.2	4.4 ± 0.4	6.5 ± 0.2	12.6
28	Xunyi	0.34 ± 0.03	0.8 ± 0.1	3.6 ± 0.4	5.1 ± 0.2	9.8
29	Sanyuan	0.72 ± 0.06	3.2 ± 0.3	4.1 ± 0.3	7.1 ± 0.8	15.1
30	Sanyuan	0.68 ± 0.06	3.2 ± 0.4	3.7 ± 0.4	6.6 ± 0.5	14.2
31	Sanyuan	0.48 ± 0.02	2.7 ± 0.4	2.8 ± 0.1	5.9 ± 0.4	11.9
32	Sanyuan	0.30 ± 0.04	2.1 ± 0.2	2.8 ± 0.3	6.6 ± 0.7	11.8
mean		0.7	2.9	3.7	7	15
SD		0.4	1	1	3	5
min		0.30	0.8	2.7	4.5	9.8
max		1.69	5.2	6.1	14.1	26.3
Gala (n = 10)						
33	Liquan	0.36 ± 0.02	0.27 ± 0.01	6.0 ± 0.5	2.1 ± 0.1	8.7
34	Liquan	0.26 ± 0.03	0.31 ± 0.03	4.9 ± 0.5	1.5 ± 0.3	7.0
35	Liquan	0.29 ± 0.02	nd	3.8 ± 0.2	1.8 ± 0.1	5.9
36	Liquan	0.29 ± 0.04	0.31 ± 0.05	3.9 ± 0.3	1.42 ± 0.09	5.9
37	Xunyi	0.74 ± 0.06	nd	8.6 ± 0.4	1.5 ± 0.1	10.8
38	Xunyi	0.35 ± 0.02	nd	6.3 ± 0.6	1.25 ± 0.09	7.9
39	Xunyi	0.34 ± 0.06	nd	6.3 ± 0.6	1.0 ± 0.1	7.6
40	Xunyi	0.37 ± 0.03	nd	6.9 ± 0.8	1.51 ± 0.07	8.8
41	Yongshou	0.34 ± 0.03	nd	7 ± 1	1.55 ± 0.06	9
42	Luochuan	0.57 ± 0.06	nd	4.7 ± 0.4	1.0 ± 0.1	6.3
mean		0.39	0.09	6	1.5	8
SD		0.1	0.1	2	0.3	2
min		0.26	nd	3.8	1.0	5.9
max		0.74	0.31	8.6	2.1	10.8
Golden Delicious (n = 10)						

Table 1. continued

sample		HPXG	HPG	PXG	PLZ	total
43	Liquan	0.54 ± 0.07	0.65 ± 0.03	5 ± 1	6.4 ± 0.8	13
44	Liquan	0.31 ± 0.04	0.26 ± 0.04	3.5 ± 0.3	5.3 ± 0.4	9.4
45	Liquan	0.23 ± 0.03	0.51 ± 0.05	3.5 ± 0.5	5.6 ± 0.5	9.8
46	Xunyi	0.72 ± 0.08	1.07 ± 0.08	6.2 ± 0.4	7.1 ± 0.9	15.1
47	Xunyi	0.29 ± 0.05	0.44 ± 0.05	5.6 ± 0.5	6.8 ± 0.6	13.1
48	Yongshou	0.32 ± 0.05	0.50 ± 0.04	6.5 ± 0.6	6.7 ± 0.3	14.0
49	Luochuan	0.42 ± 0.04	0.49 ± 0.02	3.2 ± 0.3	3.0 ± 0.3	7.1
50	Luochuan	0.43 ± 0.04	0.54 ± 0.07	4.0 ± 0.6	4.8 ± 0.5	9.8
51	Luochuan	0.33 ± 0.03	0.68 ± 0.06	8.1 ± 0.5	8.5 ± 0.6	17.6
52	Luochuan	0.35 ± 0.02	0.63 ± 0.08	7.7 ± 0.7	8.8 ± 0.6	17.5
mean	0.39	0.58	5	6.3	12	
SD	0.1	0.2	2	2	4	
min	0.23	0.26	3.2	3.0	7.1	
max	0.72	1.07	8.1	8.8	17.6	
Jonagold (n = 6)						
53	Liquan	0.70 ± 0.09	0.55 ± 0.03	10.1 ± 0.9	5.1 ± 0.7	16.5
54	Liquan	0.51 ± 0.04	0.73 ± 0.07	6.1 ± 0.6	5.9 ± 0.5	13.2
55	Liquan	0.83 ± 0.09	0.71 ± 0.05	13 ± 1	7.5 ± 0.7	22
56	Yongshou	0.66 ± 0.07	0.71 ± 0.08	5.7 ± 0.6	5.7 ± 0.4	12.8
57	Yongshou	0.67 ± 0.04	0.58 ± 0.03	6.4 ± 0.6	4.4 ± 0.6	12.1
58	Sanyuan	0.51 ± 0.06	0.59 ± 0.05	8.1 ± 0.8	4.9 ± 0.5	14.1
mean		0.65	0.65	8	5.6	15
SD		0.1	0.08	3	1	4
min		0.51	0.55	5.7	4.4	12.1
max		0.83	0.73	13	7.5	22

[a]HPXG, 3-hydroxyphloretin-2′-*O*-xylglucoside; HPG, 3-hydroxyphloretin-2′-*O*-glucoside; PXG, phloretin-2′-*O*-xyloglucoside; PLZ, phloridzin. nd, not detectable; nq, not quantifiable.

radius of 150 km. The obtained results would contribute to the authenticity and quality control of apple juices.

MATERIALS AND METHODS

Chemicals and Standards. Standards, (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, 5-caffeoylquinic acid, quercetin-3-*O*-galactoside, and phloridzin (phloretin-2′-*O*-glucoside), were all purchased from Sigma-Aldrich Ltd. (Beijing, China). Acetonitrile, methanol, and acetic acid (HPLC grade) and ethyl acetate (analytical grade) were obtained from Chemical Reagents Co. (Yangling, China). Ultrapure water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Sample Preparation. A total of 58 apple samples belonging to 5 apple varieties (Fuji, Starkrimson, Gala, Golden Delicious, and Jonagold) were hand-picked from different orchards in 6 regions (Liquan, Xunyi, Yongshou, Sanyuan, Luochuan, and Chunhua) of Shaanxi province of China in 2011 (Table 1). These apple varieties have been chosen for the study because they are the most widely cultivated varieties in Shaanxi province, accounting for >80% of total apple production. Five of the regions (Liquan, Xunyi, Yongshou, Luochuan, and Chunhua) are within the PDO districts. Apple samples were harvested at maturity, when their starch−iodine index reached values of 4−6. The starch−iodine index was visually rated using the Cornell generic starch scale 1−8.[19] Approximately 3 kg of apples was washed and cored. Apple juice was obtained by squeezing the prepared apples with a juicer (Midea, JP351, China). Despite the fact that this extraction procedure is not used on an industrial scale by fruit juice manufacturers, it is widely used by small manufacturers and allows a suitable control of the conditions and fruits from which the juice is extracted. These processes were performed under low temperature to avoid enzymatic browning. The juice (200 mL) was immediately transferred into a glass vessel containing 0.2 g of ascorbic acid and 4 g of NaCl to prevent phenolic degradation.[20] The samples were stirred for about 1 min. Excessive pulp and foam were removed from the juice by a 100 mesh filter. Apple juice (20 mL) was adjusted to pH 7.0 and 1.5, respectively, and extracted three times with ethyl acetate (20 mL). The combined ethyl acetate phase was dried over anhydrous sodium sulfate and evaporated to dryness on a vacuum rotary evaporator.[21] The residue was dissolved in 10 mL of methanol. Three replicates of apple juice preparation were carried out. The resultant solution was filtered through a 0.45 μm membrane filter prior to HPLC analysis.

HPLC Analysis of Polyphenols. Analysis of phenolic compounds was performed on a Shimadzu HPLC system (Shimadzu LC-20AD pump, CTO-20A column oven, and SPD-M20A UV−vis detector, Shimadzu Scientific Instruments, Columbia, MD, USA) and a Waters C18 column (250 mm × 4.6 mm, 5 μm particle size) according to a method from Schieber et al.[21] The column was operated at 25 °C. Solvent A was 2% acetic acid in water (v/v), and solvent B was 0.5% acetic acid in water and acetonitrile (50:50, v/v). The gradient program was as follows: from 10 to 55% B (50 min), from 55 to 100% B (10 min), and from 100 to 10% B (5 min), with a flow rate at 0.8 mL/min. The injection volume for all samples was 20 μL. Data were collected and analyzed using LC solution software. UV−visible spectra were recorded from 200 to 600 nm. Flavan-3-ols and dihydrochalcones were determined at 280 nm, hydroxycinnamic acids at 320 nm, and flavonols at 360 nm.

Identification of polyphenols was conducted using a Thermo Accela 1250 UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA) coupled with a Thermo Scientific Q Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Solvent A was 0.5% acetic acid in water (v/v). Solvent B, chromatographic column, and separation conditions were the same as those described above. Mass detection conditions were as follows: ionization mode, negative electrospray ionization source (ESI); ion spray voltage, −2.5 kV; ion source temperature, 150 °C. Nitrogen was used as curtain and auxiliary gas. Curtain gas and auxiliary gas flows were set to 40 and 10 arbitrary units, respectively. Spectra were scanned in the mass range of m/z 100−1500.

Table 2. Standard Curves, Detection Limits, and Method Validation Data

compound	linear range mg/L)	equation[a]	R^2	LOD (mg/L)	LOQ (mg/L)	precision (RSD %) intraday Rt	intraday area	interday Rt	interday area	recovery (%)
(+)-catechin	0.60−200	$Y = 19012X - 205$	0.9997	0.20	0.60	0.92	2.81	1.17	3.15	92.3
(−)-epicatechin	0.70−200	$Y = 21125X - 4893$	0.9996	0.25	0.70	0.74	1.63	1.06	1.94	88.6
procyanidin B1	0.62−200	$Y = 24447X - 4350$	0.9997	0.25	0.62	1.12	2.19	1.41	1.88	91.6
procyanidin B2	0.65−200	$Y = 19525X - 843$	0.9996	0.23	0.65	1.16	2.74	1.70	3.34	90.4
5-caffeoylquinic acid	0.35−300	$Y = 73725X - 11212$	0.9991	0.10	0.35	0.91	1.12	1.24	1.81	103.8
quercetin-3-*O*-galactoside	0.35−200	$Y = 48004X - 8142$	0.9994	0.15	0.35	0.84	3.63	1.07	4.91	76.4
phloridzin	0.37−200	$Y = 61756X - 8684$	0.9996	0.12	0.37	1.05	2.92	1.32	3.46	95.3

[a] X, concentration (mg/L); Y, peak area.

The polyphenols were identified by their UV−vis spectra and MS as well as MS/MS information and when available, by comparison of retention time with standards. Quantification was performed by an external standard method using calibration curves. The calibration curves were made from (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, 5-caffeoylquinic acid, quercetin-3-*O*-galactoside, and phloridzin as standards. For polyphenols without standards, flavan-3-ols were quantified as (+)-catechin, dihydrochalcones as phloridzin, hydroxycinnamic acids as 5-caffeoylquinic acid, and flavonols as quercetin-3-*O*-galactoside.

Statistical Analysis. The concentrations of the phenolic compounds were given as the mean value ± standard deviation of triplicate analyses for each sample. The mean values of the obtained data set were subjected to pattern recognition analysis. The data set consisted of a 58 × 23 matrix, in which rows represented the apple juice samples analyzed (58 objects) and columns the concentrations of the individual phenolic compounds determined by HPLC-PDA (23 variables). Each sample was represented in multidimensional space by a data vector, which was an assembly of the 23 features. Data vectors were analyzed using chemometric techniques that have been described in the literature[10,22,23] to extract the main information in multivariate data and to develop classification models according to variety in the first case and geographical origin in the second case. First of all, the data set was analyzed by univariate procedures (ANOVA, Fisher index, and box−whisker plots). Then, multivariate data analyses were applied to the autoscaled data matrix. Principal component analysis (PCA) as an unsupervised technique was performed to reduce the dimensionality of the data matrix and to locate any existing clustering of juice samples based on either variety or geographical origin. Stepwise linear discriminant analysis (SLDA) as a supervised method was applied to construct classification models. In principle, SLDA determines linear discriminant functions, which maximize the ratio of between-class variance and minimize the ratio of within-class variance. In this method, a stepwise variable selection procedure is performed so that the most significant variables involved in sample differentiation are selected using a Wilks' λ as a selection criterion and an F statistic to determine the significance of the changes in λ when the influence of each new variable is evaluated. Before choosing a new variable to include, this procedure checks to see if all of the variables previously selected remain significant. If a variable selected earlier may no longer be useful, it is removed. This procedure stops when no other variables meet the criteria for entry or when the variable to be included next is one that was just removed. The leave-one-out method was used as cross-validation procedure to evaluate the classification performance. The reliability of the classification models achieved was studied in terms of recognition ability (percentage of the members of the training set correctly classified) and prediction ability (percentage of the members of the test set correctly classified by using the rules developed in the training step). All data were processed via SPSS17.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Method Validation. The linear range, calibration curve, correlation coefficient, limit of determination (LOD), and limit of quantification (LOQ) values of the standards are summarized in Table 2. These calibration curves were obtained over a relatively wide concentration range in accordance with the normal levels of these compounds found in apple juice samples. The curves were constructed using six concentration levels, each one run in triplicate. All of the components showed good linearity ($R^2 \geq 0.9991$) in the concentration range. The LODs for the standards ranged from 0.10 to 0.25 mg/L and the LOQs from 0.35 to 0.70 mg/L. LODs and LOQs were calculated as the concentrations giving signal-to-noise ratios of 3 (S/N = 3) and 10 (S/N = 10), respectively.

Six replicate analyses with the same sample on the same day were carried out to determine the intraday precision. The relative standard deviation (RSD) values were always <1.16% for the retention times and <3.63% for the peak areas (Table 2). Twelve replicate analyses with the same sample on two consecutive days were carried out to determine interday precision. The RSD values were always <1.70% for the retention times and <4.91% for the peak areas (Table 2).

To assess the recovery of the proposed method, known amounts of standards were added to a juice sample and the resulting spiked sample was subjected to the complete proposed procedure. Each compound was added at three different concentrations, and all analyses were carried out in triplicate. Recoveries were calculated on the basis of the difference between the total amount determined in the spiked samples and the amount observed in the nonspiked samples divided by the amount added. The average recoveries of these standards were between 76.4 and 103.8% (Table 2) with relative standard deviations ≤5.3%.

Polyphenolic Profiles of Apple Juices. Twenty-three well-resolved chromatographic peaks of polyphenols were observed in apple juices produced from apples of different origin and variety, belonging to four compound classes: dihydrochalcones, flavan-3-ols, hydroxycinnamic acids, and flavonols (Tables 1 and 3−5). These polyphenols exhibited notable variations even in samples from the same variety due to the impact of geographical origin. The total phenolic compounds ranged from 86 mg/L for the Golden Delicious juice in Luochuan to 305 mg/L for the Starkrimson juice in Xunyi. The most abundant polyphenols in apple juices were 5-caffeoylquinic acid, (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, 4-*p*-coumaroylquinic acid, phloretin-2′-*O*-

Table 3. Composition of Flavan-3-ols (Milligrams per Liter) in the Apple Juices[a]

sample	PB1	CAT	PB2	EC	FAT-a	FAD-a	FAT-b	FAT-c	FAD-b	total
Fuji										
1	10 ± 1	9.4 ± 0.8	46 ± 2	34 ± 2	13.8 ± 0.7	5.9 ± 0.3	nd	1.8 ± 0.3	2.3 ± 0.2	123
2	5.7 ± 0.8	6.2 ± 0.7	28 ± 1	23 ± 1	9.3 ± 0.8	2.3 ± 0.2	2.2 ± 0.1	1.43 ± 0.06	1.6 ± 0.1	80
3	6.5 ± 0.3	7.2 ± 0.5	31 ± 2	25 ± 2	10.7 ± 0.9	3.6 ± 0.2	2.1 ± 0.3	1.5 ± 0.1	1.7 ± 0.3	89
4	5.6 ± 0.3	6.4 ± 0.8	35 ± 2	29 ± 2	11.4 ± 0.8	2.6 ± 0.3	5.0 ± 0.4	1.9 ± 0.3	2.3 ± 0.3	99
5	3.6 ± 0.3	4.2 ± 0.2	24 ± 1	19 ± 1	7.4 ± 0.7	1.3 ± 0.1	4.1 ± 0.4	1.19 ± 0.03	1.3 ± 0.1	66
6	1.3 ± 0.2	5.3 ± 0.5	28 ± 1	21 ± 2	4.0 ± 0.3	0.62 ± 0.04	1.8 ± 0.2	0.63 ± 0.07	2.4 ± 0.2	65
7	1.4 ± 0.1	2.0 ± 0.2	26 ± 1	9.2 ± 0.4	2.0 ± 0.1	2.5 ± 0.2	1.0 ± 0.1	0.36 ± 0.05	0.60 ± 0.06	45
8	4.6 ± 0.2	5.3 ± 0.4	27 ± 3	22 ± 2	7.7 ± 0.4	1.22 ± 0.04	3.8 ± 0.4	1.39 ± 0.08	1.5 ± 0.1	75
9	4.9 ± 0.4	5.1 ± 0.5	24 ± 1	19.5 ± 0.9	7.9 ± 0.7	1.9 ± 0.1	4.3 ± 0.2	1.02 ± 0.07	1.3 ± 0.2	70
10	8.2 ± 0.8	7.7 ± 0.6	35 ± 2	25 ± 1	11.2 ± 0.8	3.0 ± 0.4	4.1 ± 0.1	1.40 ± 0.09	1.9 ± 0.2	98
11	6.9 ± 0.6	6.1 ± 0.6	26 ± 2	20 ± 2	8.0 ± 0.8	2.4 ± 0.1	2.7 ± 0.1	1.3 ± 0.2	1.6 ± 0.2	75
12	7.4 ± 0.7	7.6 ± 0.8	35 ± 1	28 ± 1	11 ± 1	2.8 ± 0.2	6.1 ± 0.5	1.38 ± 0.05	1.8 ± 0.2	101
13	6.7 ± 0.8	6.5 ± 0.6	34 ± 1	27 ± 2	11 ± 1	3.4 ± 0.1	3.9 ± 0.1	1.37 ± 0.08	1.9 ± 0.1	96
14	7.6 ± 0.5	7.7 ± 0.5	35 ± 2	29 ± 3	11.7 ± 0.7	2.4 ± 0.3	6.6 ± 0.7	1.6 ± 0.1	1.9 ± 0.2	104
15	6.3 ± 0.7	6.6 ± 0.4	30 ± 2	24 ± 1	10.0 ± 0.7	2.3 ± 0.2	5.5 ± 0.4	1.4 ± 0.1	1.7 ± 0.2	88
16	4.6 ± 0.2	4.7 ± 0.3	39 ± 3	32 ± 2	12.7 ± 0.9	2.6 ± 0.3	nd	1.9 ± 0.2	1.7 ± 0.2	99
17	2.2 ± 0.1	3.4 ± 0.2	38 ± 2	30 ± 1	12.3 ± 0.5	3.25 ± 0.02	1.8 ± 0.2	1.5 ± 0.2	1.2 ± 0.2	94
18	5.2 ± 0.5	5.5 ± 0.4	12.3 ± 0.8	21 ± 2	8.7 ± 0.8	3.6 ± 0.3	1.2 ± 0.1	0.98 ± 0.09	1.3 ± 0.1	60
19	8.9 ± 0.8	8.4 ± 0.7	14 ± 1	30 ± 3	13 ± 1	6.2 ± 0.3	nd	1.32 ± 0.06	1.9 ± 0.3	84
20	6.8 ± 0.6	6.9 ± 0.8	29 ± 2	22 ± 2	9.2 ± 0.6	2.4 ± 0.3	3.7 ± 0.2	1.4 ± 0.1	1.9 ± 0.2	83
mean	6	6.1	30	24	10	2.8	3.0	1.3	1.7	85
SD	2	2	8	6	3	1	2	0.4	0.4	18
min	1.3	2.0	12.3	9.2	2.0	0.62	nd	0.36	0.6	45
max	10	9.4	46	34	13.8	6.2	6.6	1.9	2.4	123
Starkrimson										
21	13 ± 1	11.7 ± 0.9	36 ± 2	24 ± 2	11 ± 1	7.2 ± 0.5	nd	1.7 ± 0.2	1.5 ± 0.2	106
22	13 ± 1	13 ± 1	35 ± 2	27 ± 1	9.6 ± 0.4	3.1 ± 0.4	1.9 ± 0.1	1.36 ± 0.06	1.45 ± 0.06	105
23	14 ± 1	17 ± 2	29 ± 2	30 ± 1	9 ± 1	2.7 ± 0.2	4.1 ± 0.4	1.2 ± 0.2	2.1 ± 0.2	109
24	12.7 ± 0.7	13.9 ± 0.9	40 ± 2	34 ± 3	14.3 ± 0.7	5.5 ± 0.6	6.2 ± 0.3	2.0 ± 0.1	2.1 ± 0.2	131
25	18 ± 2	22 ± 2	63 ± 3	50 ± 3	19 ± 1	5.4 ± 0.8	8.7 ± 0.6	1.9 ± 0.1	3.2 ± 0.4	191
26	12 ± 1	14.2 ± 0.9	37 ± 3	31 ± 2	11.5 ± 0.8	2.83 ± 0.09	6.6 ± 0.9	1.6 ± 0.2	2.09 ± 0.08	119
27	7.3 ± 0.2	8.9 ± 0.9	33 ± 2	26 ± 2	11.6 ± 0.3	4.1 ± 0.2	4.8 ± 0.1	1.3 ± 0.2	1.85 ± 0.05	99
28	5.6 ± 0.2	5.6 ± 0.3	25 ± 2	20 ± 1	9 ± 1	2.4 ± 0.2	3.6 ± 0.2	1.26 ± 0.06	1.3 ± 0.2	73
29	14.3 ± 0.9	13 ± 1	37 ± 2	27 ± 2	11.7 ± 0.9	3.5 ± 0.1	4.2 ± 0.5	1.7 ± 0.1	1.9 ± 0.2	114
30	16.3 ± 0.8	14 ± 1	46 ± 4	33 ± 3	15 ± 1	10 ± 1	nd	2.8 ± 0.1	2.42 ± 0.09	140
31	13.7 ± 0.6	12 ± 1	38 ± 2	29 ± 3	11.5 ± 0.9	3.8 ± 0.2	2.5 ± 0.1	1.73 ± 0.07	2.2 ± 0.1	114
32	14 ± 1	11.8 ± 0.8	42 ± 2	33 ± 3	12 ± 1	5.4 ± 0.1	nd	1.97 ± 0.08	2.1 ± 0.2	122
mean	13	13	38	30	12	5	3.6	1.7	2.0	119
SD	3	4	10	7	3	2	3	0.4	0.5	28
min	5.6	5.6	25	20	9	2.4	nd	1.2	1.3	73
max	18	22	63	50	19	10	8.7	2.8	3.2	191
Gala										
33	11.8 ± 0.8	10 ± 1	35 ± 2	22 ± 2	12 ± 1	6.9 ± 0.3	nd	1.5 ± 0.3	1.6 ± 0.2	101
34	11.8 ± 0.9	9.5 ± 0.9	32 ± 3	22 ± 2	11 ± 1	nd	nd	1.7 ± 0.2	1.59 ± 0.06	90
35	9.8 ± 0.6	7.8 ± 0.5	27 ± 1	16.9 ± 0.9	9.3 ± 0.5	4.8 ± 0.5	nd	1.30 ± 0.09	1.17 ± 0.05	78
36	12 ± 1	9.3 ± 0.8	35 ± 2	23 ± 2	11.3 ± 0.7	5.2 ± 0.6	nd	1.9 ± 0.1	1.6 ± 0.1	99
37	3.3 ± 0.3	7.7 ± 0.8	21 ± 2	20 ± 2	5.4 ± 0.2	nd	3.1 ± 0.2	0.78 ± 0.09	1.9 ± 0.1	63
38	8.5 ± 0.9	7.7 ± 0.6	22 ± 1	18.6 ± 0.9	9.2 ± 0.8	3.2 ± 0.2	3.9 ± 0.3	1.24 ± 0.06	1.4 ± 0.1	76
39	1.9 ± 0.1	8.5 ± 0.9	21 ± 2	21.0 ± 0.7	5.5 ± 0.6	nd	2.7 ± 0.3	0.9 ± 0.1	2.6 ± 0.3	64
40	10.1 ± 1	8.6 ± 0.7	24 ± 2	22 ± 2	10.6 ± 0.9	3.4 ± 0.1	3.9 ± 0.1	2.35 ± 0.07	1.6 ± 0.1	87
41	5.1 ± 0.6	15 ± 1	26 ± 1	32 ± 1	8.2 ± 0.9	nd	4.0 ± 0.4	1.15 ± 0.03	3.7 ± 0.2	95
42	6.1 ± 0.2	5.0 ± 0.2	15.7 ± 0.9	13.0 ± 0.5	5.8 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	0.92 ± 0.08	0.69 ± 0.04	50.6
mean	8	9	26	21	9	2.5	1.9	1.4	1.8	80
SD	4	3	6	5	3	3	2	0.5	0.8	17
min	1.9	5.0	15.7	13.0	5.4	nd	nd	0.78	0.69	50.6
max	12	15	35	32	12	6.9	4.0	2.35	3.7	101
Golden Delicious										

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Table 3. continued

sample	PB1	CAT	PB2	EC	FAT-a	FAD-a	FAT-b	FAT-c	FAD-b	total
43	7.5 ± 0.6	6.2 ± 0.5	49 ± 3	30 ± 2	14 ± 1	5.7 ± 0.3	nd	2.14 ± 0.02	2.5 ± 0.1	117
44	3.5 ± 0.3	3.6 ± 0.5	31 ± 1	18 ± 2	8.9 ± 0.4	3.3 ± 0.1	nd	1.2 ± 0.2	1.13 ± 0.09	71
45	4.7 ± 0.5	4.2 ± 0.4	34 ± 2	20 ± 2	10 ± 1	4.1 ± 0.4	nd	1.2 ± 0.1	1.6 ± 0.1	80
46	1.83 ± 0.09	2.1 ± 0.1	27 ± 2	15.3 ± 0.9	8.0 ± 0.5	2.1 ± 0.2	2.2 ± 0.2	1.85 ± 0.07	0.94 ± 0.08	61
47	2.0 ± 0.2	2.5 ± 0.1	23 ± 2	15.6 ± 0.8	7.8 ± 0.2	1.5 ± 0.1	1.1 ± 0.2	0.88 ± 0.09	0.99 ± 0.08	55
48	1.9 ± 0.1	2.0 ± 0.1	20 ± 1	14.2 ± 0.8	7.8 ± 0.9	2.3 ± 0.2	2.0 ± 0.1	0.87 ± 0.07	0.9 ± 0.1	52
49	0.45 ± 0.05	1.1 ± 0.1	11 ± 2	6.7 ± 0.4	2.6 ± 0.1	nd	nd	0.73 ± 0.09	0.40 ± 0.03	23
50	0.52 ± 0.07	1.7 ± 0.2	15 ± 1	10 ± 1	4.4 ± 0.3	1.9 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	36
51	2.3 ± 0.3	2.9 ± 0.2	14.8 ± 0.9	18.4 ± 0.9	9.5 ± 0.7	2.7 ± 0.1	1.9 ± 0.1	1.15 ± 0.06	1.4 ± 0.1	55.1
52	3.0 ± 0.4	3.3 ± 0.1	13 ± 1	20 ± 2	10.4 ± 0.8	2.5 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	1.59 ± 0.08	57
mean	2.8	3.0	24	17	8	2.6	1.0	1.2	1.3	61
SD	2	1	12	6	3	2	0.9	0.5	0.6	25
min	0.45	1.1	11	6.7	2.6	nd	nd	0.7	0.40	23
max	7.5	6.2	49	30	14	5.7	2.2	2.14	2.5	117
Jonagold										
53	3.1 ± 0.2	4.0 ± 0.4	34 ± 2	20 ± 1	10.6 ± 0.6	4.0 ± 0.5	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	81
54	3.0 ± 0.1	4.1 ± 0.4	34 ± 3	18 ± 1	10 ± 1	2.5 ± 0.2	1.8 ± 0.1	1.1 ± 0.1	1.51 ± 0.07	76
55	5.2 ± 0.5	5.4 ± 0.3	44 ± 3	22 ± 1	13.5 ± 0.6	7.0 ± 0.4	nd	1.6 ± 0.2	1.74 ± 0.06	100
56	8.2 ± 0.5	8.3 ± 0.9	42 ± 3	21 ± 2	12 ± 1	5.6 ± 0.5	6.6 ± 0.4	1.35 ± 0.09	2.3 ± 0.3	107
57	7.7 ± 0.4	7.6 ± 0.5	27 ± 1	14.3 ± 1	8.3 ± 0.5	2.9 ± 0.3	5.6 ± 0.7	0.90 ± 0.08	2.2 ± 0.3	77
58	2.8 ± 0.2	3.8 ± 0.4	31 ± 2	16 ± 1	9.9 ± 0.6	5.0 ± 0.4	1.2 ± 0.1	1.0 ± 0.1	1.38 ± 0.06	72
mean	5.0	5.5	35	19	11	4.5	2.8	1.3	1.8	85
SD	2	2	7	3	2	2	3	0.3	0.4	15
min	2.8	3.8	27	14.3	8.3	2.5	nd	0.90	1.38	72
max	8.2	8.3	44	22	13.5	7.0	6.6	1.7	2.3	107

[a]PB1, procyanidin B1; CAT, (+)-catechin; PB2, procyanidin B2; EC, (−)-epicatechin; FAT-a, procyanidin trimer a; FAD-a, procyanidin dimer a; FAT-b, procyanidin trimer b; FAT-c, procyanidin trimer c; FAD-b, procyanidin dimer b. nd, not detectable; nq, not quantifiable.

xyloglucoside, and phloridzin, which is in accordance with previous results.[18,24−27]

Four dihydrochalcones were detected in apple juices. Total dihydrochalcones ranged from 3.8 mg/L for the Fuji juice in Xunyi to 26.3 mg/L for the Starkrimson juice in Xunyi. Phloretin-2′-*O*-xyloglucoside and phloridzin were the most abundant compounds among dihydrochalcones, ranging from 1.9 to 13 mg/L and from 1.0 to 14.1 mg/L, respectively. These values are similar to the contents reported for dessert apple juices,[24,25] and they are lower than those reported for cider apple juices.[18] This agrees with the previous result that dessert apple juices show lower contents of dihydrochalcones compared to that of cider apple juices.[25] 3-Hydroxyphloretin-2′-*O*-xylglucoside and 3-hydroxyphloretin-2′-*O*-glucoside were detected in the juice samples, confirming the presence of these two hydroxyphloretin glycosides in apple.[28] Dihydrochalcones have been generally considered as specific compounds in apples, so they have been used to distinguish apple juice from a number of other fruit juices.[26,29,30] However, phloridzin has been identified in strawberry fruits, and this compound is not appropriate to guarantee genuineness of apple-derived products.[31,32]

Nine flavan-3-ols were observed in apple juices, including two monomers ((+)-catechin and (−)-epicatechin), four dimers, and three trimers. Total flavan-3-ols ranged from 23 mg/L for the Golden Delicious juice in Luochuan to 191 mg/L for the Starkrimson juice in Xunyi. Procyanidin B2 and (−)-epicatechin, ranging from 11 to 63 mg/L and from 6.7 to 50 mg/L, were the major contributors to the high total flavan-3-ols content. This result is in good agreement with literature data, where these two compounds have been reported to be the most predominant flavan-3-ols in apple juice.[24,25,27,33] The contents of procyanidin B1 and (+)-catechin were lower, ranging from 0.45 to 18 mg/L and from 1.1 to 22 mg/L, which are similar to the earlier results.[24,25]

Five hydroxycinnamic acids were identified in all samples. Total hydroxycinnamic acids ranged from 35 mg/L for the Fuji juice in Xunyi to 127 mg/L for the Fuji juice in Sanyuan. 5-Caffeoylquinic acid was the most dominant polyphenolic compound in most samples, which confirms the earlier results.[34,35] This compound reached 106 mg/L for the Fuji juice in Sanyuan. Only in a few juice samples from the Starkrimson variety was the amount of 5-caffeoylquinic acid lower than that of procyanidin B2. 4-*p*-Coumaroylquinic acid was the second most abundant hydroxycinnamic acid, ranging from 1.7 to 18 mg/L; the values are similar to the contents reported for dessert apple juices and are within the concentration range of this compound for cider apple juices.[18,25]

There are five flavonols identified. Total flavonols ranged from 2.52 mg/L for the Starkrimson juice in Liquan to 17.9 mg/L for the Jonagold juice in Liquan. With regard to individual flavonols, quercetin-3-*O*-galactoside (0.48−6.7 mg/L) and quercetin-3-*O*-rhamnoside (0.38−8.7 mg/L) were the most abundant compounds, which confirms the previous result.[21] Free quercetin was not detected as in previous literature.[25]

Univariate Data Analysis. ANOVA was employed to disclose significant differences for the individual polyphenol concentrations between apple juices according to variety and geographical origin. The Fisher index was also calculated to establish the discriminant capacity of the variables one by one.

Table 4. Composition of Hydroxycinnamic Acids (Milligrams per Liter) in the Apple Juices[a]

sample	5-CQA	3-*p*CoQA	4-CQA	4-*p*CoQA	5-*p*CoQA	total
Fuji						
1	87 ± 5	3.4 ± 0.3	nd	6.9 ± 0.4	2.7 ± 0.2	100
2	56 ± 3	6.4 ± 0.7	nd	5.0 ± 0.6	2.0 ± 0.1	69
3	65 ± 3	6.0 ± 0.4	nd	5.6 ± 0.6	2.2 ± 0.3	79
4	78 ± 4	3.6 ± 0.4	nd	5.0 ± 0.4	2.6 ± 0.1	89
5	49 ± 3	8.8 ± 0.7	nd	2.8 ± 0.3	1.4 ± 0.1	62
6	34 ± 1	3.3 ± 0.4	nd	1.8 ± 0.1	1.7 ± 0.2	41
7	31 ± 2	1.4 ± 0.1	nd	1.7 ± 0.1	1.0 ± 0.1	35
8	88 ± 6	2.5 ± 0.3	nd	5.1 ± 0.4	2.7 ± 0.2	98
9	59 ± 3	2.9 ± 0.3	nd	3.9 ± 0.5	1.8 ± 0.1	68
10	98 ± 6	4.1 ± 0.5	nd	4.6 ± 0.4	2.6 ± 0.3	109
11	81 ± 4	3.6 ± 0.3	nd	3.8 ± 0.2	2.1 ± 0.2	91
12	76 ± 3	7.5 ± 0.6	nd	5.1 ± 0.5	2.3 ± 0.2	91
13	73 ± 4	8.6 ± 0.8	nd	4.7 ± 0.3	2.4 ± 0.2	89
14	86 ± 6	5.2 ± 0.6	nd	5.6 ± 0.3	2.6 ± 0.1	99
15	71 ± 5	2.8 ± 0.4	nd	4.6 ± 0.4	2.1 ± 0.1	81
16	106 ± 7	2.3 ± 0.2	nd	16 ± 1	3.1 ± 0.3	127
17	82 ± 4	9 ± 1	nd	12 ± 1	2.7 ± 0.1	106
18	59 ± 4	2.1 ± 0.3	nd	4.4 ± 0.4	1.8 ± 0.2	67
19	76 ± 4	3.6 ± 0.2	nd	6.0 ± 0.7	2.3 ± 0.1	88
20	77 ± 3	12 ± 2	nd	4.4 ± 0.2	2.4 ± 0.2	96
mean	72	5	nd	5	2.2	84
SD	19	3		3	0.5	22
min	31	1.4		1.7	1.0	35
max	106	12		16	3.1	127
Starkrimson						
21	31 ± 1	2.1 ± 0.1	1.44 ± 0.06	8.7 ± 0.6	0.92 ± 0.08	44
22	42 ± 2	1.58 ± 0.08	1.6 ± 0.2	8.8 ± 0.4	0.68 ± 0.08	55
23	45 ± 2	0.9 ± 0.1	1.4 ± 0.2	7.6 ± 0.4	0.49 ± 0.03	55
24	50 ± 3	1.5 ± 0.1	1.6 ± 0.1	11.6 ± 0.8	1.0 ± 0.1	66
25	52 ± 3	7.9 ± 0.5	2.6 ± 0.2	18 ± 1	0.43 ± 0.04	81
26	55 ± 2	1.64 ± 0.06	1.78 ± 0.06	14.0 ± 0.9	1.3 ± 0.1	74
27	63 ± 3	1.39 ± 0.09	0.8 ± 0.2	5.7 ± 0.3	1.7 ± 0.1	73
28	64 ± 3	1.37 ± 0.05	0.36 ± 0.03	4.0 ± 0.4	1.81 ± 0.07	72
29	33 ± 2	2.6 ± 0.2	1.8 ± 0.1	10.1 ± 0.9	1.03 ± 0.08	49
30	51 ± 2	1.4 ± 0.1	1.68 ± 0.05	11 ± 1	1.86 ± 0.05	67
31	38 ± 2	1.9 ± 0.2	1.5 ± 0.2	9.5 ± 0.4	1.6 ± 0.2	53
32	34 ± 2	2.9 ± 0.1	1.53 ± 0.07	9.8 ± 0.8	1.5 ± 0.2	50
mean	47	2.3	1.5	10	1.2	61
SD	11	2	0.5	4	0.5	12
min	31	0.9	0.36	4.0	0.43	44
max	64	7.9	2.6	18	1.86	81
Gala						
33	84 ± 5	1.98 ± 0.08	nd	16 ± 1	0.85 ± 0.09	103
34	88 ± 6	1.62 ± 0.04	nd	15 ± 1	1.5 ± 0.1	106
35	62 ± 3	3.2 ± 0.4	nd	12 ± 1	0.42 ± 0.04	77
36	68 ± 4	5.1 ± 0.3	nd	15 ± 1	0.38 ± 0.05	88
37	63 ± 3	6.4 ± 0.3	nd	10 ± 1	2.0 ± 0.1	81
38	82 ± 4	2.1 ± 0.2	nd	11 ± 1	2.2 ± 0.3	97
39	46 ± 2	1.2 ± 0.2	nd	5.8 ± 0.4	1.01 ± 0.06	54
40	94 ± 6	1.6 ± 0.05	nd	12.8 ± 0.9	2.6 ± 0.2	111
41	72 ± 4	2.3 ± 0.2	nd	14 ± 1	0.78 ± 0.09	89
42	68 ± 4	1.26 ± 0.09	nd	10 ± 1	1.3 ± 0.2	81
mean	73	2.7	nd	12	1.3	89
SD	14	2		3	0.8	17
min	46	1.2		5.8	0.38	54
max	94	6.4		16	2.6	111
Golden Delicious						

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Table 4. continued

sample	5-CQA	3-*p*CoQA	4-CQA	4-*p*CoQA	5-*p*CoQA	total
43	84 ± 5	5.9 ± 0.6	nd	10.0 ± 0.9	2.1 ± 0.2	102
44	48 ± 2	4.0 ± 0.2	nd	6.1 ± 0.7	1.3 ± 0.2	59
45	52 ± 4	4.1 ± 0.3	nd	6.7 ± 0.6	1.4 ± 0.1	64
46	61 ± 2	5.1 ± 0.4	nd	7.5 ± 0.5	0.91 ± 0.08	75
47	59 ± 3	1.48 ± 0.08	nd	8.1 ± 0.8	1.30 ± 0.09	70
48	58 ± 3	1.4 ± 0.2	nd	8.0 ± 0.5	1.27 ± 0.08	69
49	40 ± 2	5.3 ± 0.5	nd	5.7 ± 0.3	0.19 ± 0.01	51
50	40 ± 3	5.3 ± 0.6	nd	5.9 ± 0.6	0.19 ± 0.02	51
51	73 ± 3	2.1 ± 0.2	nd	9.9 ± 0.9	1.6 ± 0.2	87
52	76 ± 3	1.3 ± 0.2	nd	10.2 ± 0.9	1.7 ± 0.1	89
mean	59	3.6	nd	7.8	1.2	72
SD	15	2		2	0.6	17
min	40	1.3		5.7	0.19	51
max	84	5.9		10.2	2.1	102
Jonagold						
53	63 ± 4	nd	2.9 ± 0.2	7.5 ± 0.4	1.93 ± 0.09	75
54	66 ± 3	nd	1.9 ± 0.1	7.4 ± 0.6	2.1 ± 0.2	77
55	90 ± 6	nd	2.5 ± 0.2	10.0 ± 0.5	2.3 ± 0.2	105
56	74 ± 5	nd	1.44 ± 0.06	6.7 ± 0.5	0.35 ± 0.04	82
57	55 ± 3	nd	1.44 ± 0.09	5.4 ± 0.6	0.30 ± 0.04	62
58	59 ± 4	nd	1.39 ± 0.04	12.9 ± 1	0.31 ± 0.05	74
mean	68	nd	1.9	8.3	1.2	79
SD	13	nd	0.6	3	1.0	14
min	55	nd	1.39	5.4	0.30	62
max	90	nd	2.9	12.9	2.3	105

[a]CA, 5-caffeoylquinic acid; 4-CQA, 4-caffeoylquinic acid; 3-*p*CoQA, 3-*p*-coumaroylquinic acid; 4-*p*CoQA, 4-*p*-coumaroylquinic acid; 5-*p*CoQA, 5-*p*-coumaroylquinic acid. nd, not detectable.

For variety-based classification, ANOVA and Fisher index calculation were applied to the complete data set (58 × 23 matrix). The highest Fisher values ($p < 0.001$) corresponded to (+)-catechin, 4-caffeoylquinic acid, 3-hydroxyphloretin-2′-*O*-glucoside, and quercetin-3-*O*-rhamnoside. However, the box and whisker plots of these features showed an overlap between the juices from five varieties, indicating insufficient discriminatory ability. For geographical origin-based classification, ANOVA and Fisher index calculation were performed on the 57 × 23 data matrix (Table 6). The sample from Chunhua was excluded, because there was only one sample from this region. The variables with the highest Fisher values ($p < 0.001$) were procyanidin B2 and procyanidin trimer b. Nevertheless, the box and whisker plots showed that these features did not allow us to distinguish the juices from five regions. Therefore, a multivariate data analysis was needed.

Multivariate Data Analysis. *Principal Component Analysis.* PCA was applied to the whole autoscaled data matrix to locate any existing clustering of juice samples based either on variety or on geographical origin. The three first principal components accounted for 64.20% of total system variability. From the loadings of the variables (Table 7), the most influential features on the first principal component (PC1, accounting for 32.08% of total variability) were flavan-3-ols ((+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, procyanidin trimer a, and procyanidin trimer c) and 3-hydroxyphloretin-2′-*O*-glucoside. The major contribution to the second principal component (PC2), which accounted for 21.14% of total variability, was due to flavonols (quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinoside, and quercetin-3-*O*-rhamnoside), phloretin-2′-*O*-xyloglucoside, and 5-caffeoylquinic acid. The third principal component (PC3, accounting for 10.98% of total variability) was mainly associated with 3-*p*-coumaroylquinic acid and 5-*p*-coumaroylquinic acid or was negatively related to 4-caffeoylquinic acid and quercetin-3-*O*-rhamnoside.

When the scores of the samples are represented on the two-dimensional and three-dimensional spaces defined by PC1, PC2, and PC3, respectively, a natural separation between samples according to apple variety could be obtained in the space defined by PC1 and PC3 (Figure 1), even though the juice samples of Fuji, Starkrimson, Gala, and Golden Delicious varieties were partially overlapped. However, samples from different regions could not be separated due to a notable overlapping of the clusters in the bidimensional and tridimensional plots (data not shown).

Stepwise Linear Discriminant Analysis. To develop discrimination models for an efficient classification of apple juices from different apple varieties, SLDA was applied to the autoscaled data matrix composed of 58 juice samples and 23 variables. Apple varieties were set as grouping variables. Twelve variables were retained by the stepwise procedure (F to enter = 3.84 and F to remove = 2.71) and used as input in LDA classification. A 100% recognition ability and 98.3% prediction ability were obtained (Table 8). The prediction ability of the SLDA model was evaluated by using the leave-one-out method. The eigenvalues, explained variances, and canonical correlations for the first four discriminant functions that are statistically extremely significant (Wilks' λ values) are shown in Table 9. The coefficients of the variables in the four discriminant functions (Table 9) reveal which variables have a greater influence on those.

Table 5. Composition of Flavonols (Milligrams per Liter) in the Apple Juices[a]

sample	QGal	QGlu	QXyl	QAra	QRha	total
Fuji						
1	2.2 ± 0.2	0.26 ± 0.04	0.58 ± 0.07	1.0 ± 0.1	1.1 ± 0.2	5.1
2	1.4 ± 0.2	0.22 ± 0.02	0.43 ± 0.05	0.68 ± 0.07	0.75 ± 0.09	3.5
3	1.8 ± 0.2	0.24 ± 0.01	0.49 ± 0.02	0.8 ± 0.1	0.90 ± 0.07	4.2
4	3.5 ± 0.3	0.55 ± 0.06	0.68 ± 0.08	1.6 ± 0.1	1.32 ± 0.08	7.7
5	2.5 ± 0.2	0.54 ± 0.07	0.58 ± 0.05	1.01 ± 0.08	0.8 ± 0.1	5.4
6	1.7 ± 0.1	0.37 ± 0.04	0.49 ± 0.05	0.77 ± 0.07	0.67 ± 0.05	4.0
7	1.4 ± 0.2	0.29 ± 0.04	0.30 ± 0.06	0.65 ± 0.02	0.38 ± 0.04	3.0
8	3.0 ± 0.2	0.84 ± 0.03	0.77 ± 0.09	1.3 ± 0.1	1.5 ± 0.1	7.4
9	4.0 ± 0.4	0.39 ± 0.02	0.72 ± 0.07	1.3 ± 0.1	1.07 ± 0.06	7.5
10	2.0 ± 0.2	0.62 ± 0.07	0.63 ± 0.09	0.9 ± 0.1	1.3 ± 0.1	5.5
11	1.72 ± 0.08	0.50 ± 0.08	0.52 ± 0.04	0.86 ± 0.05	1.12 ± 0.09	4.72
12	5.4 ± 0.4	0.76 ± 0.05	0.9 ± 0.1	1.8 ± 0.2	1.4 ± 0.2	10.3
13	4.6 ± 0.6	0.53 ± 0.06	0.74 ± 0.08	1.6 ± 0.2	1.3 ± 0.1	8.8
14	5.9 ± 0.3	0.8 ± 0.1	0.9 ± 0.2	1.88 ± 0.08	1.51 ± 0.09	11.0
15	4.6 ± 0.1	0.69 ± 0.06	0.69 ± 0.08	1.43 ± 0.08	1.2 ± 0.1	8.6
16	6.7 ± 0.6	1.0 ± 0.1	0.63 ± 0.09	2.4 ± 0.2	1.9 ± 0.1	12.6
17	5.4 ± 0.3	0.79 ± 0.08	0.55 ± 0.09	1.9 ± 0.2	1.6 ± 0.1	10.2
18	1.5 ± 0.1	0.23 ± 0.04	0.46 ± 0.06	0.70 ± 0.06	0.76 ± 0.08	3.7
19	1.9 ± 0.3	0.21 ± 0.01	0.65 ± 0.05	0.8 ± 0.1	0.91 ± 0.06	4.5
20	2.0 ± 0.2	0.58 ± 0.09	0.66 ± 0.08	1.08 ± 0.07	1.4 ± 0.2	5.7
mean	3.2	0.5	0.6	1.2	1.1	6.7
SD	2	0.2	0.2	0.5	0.4	3
min	1.4	0.21	0.30	0.65	0.38	3.0
max	6.7	1.0	0.9	2.4	1.9	12.6
Starkrimson						
21	1.00 ± 0.08	nd	0.42 ± 0.08	0.50 ± 0.05	0.60 ± 0.06	2.52
22	1.1 ± 0.2	nd	0.50 ± 0.03	0.58 ± 0.07	0.80 ± 0.07	3.0
23	1.3 ± 0.1	nd	0.52 ± 0.03	0.64 ± 0.07	0.84 ± 0.09	3.3
24	1.09 ± 0.08	0.36 ± 0.02	0.67 ± 0.08	0.67 ± 0.04	1.00 ± 0.09	3.79
25	2.0 ± 0.1	0.49 ± 0.06	1.02 ± 0.07	1.14 ± 0.07	1.45 ± 0.08	6.1
26	1.61 ± 0.06	0.41 ± 0.04	0.85 ± 0.08	0.93 ± 0.08	1.2 ± 0.1	5.0
27	3.2 ± 0.2	nd	0.61 ± 0.09	1.0 ± 0.2	0.84 ± 0.06	5.7
28	3.4 ± 0.3	nd	0.52 ± 0.08	1.08 ± 0.08	0.89 ± 0.09	5.9
29	1.3 ± 0.1	nd	0.51 ± 0.08	0.62 ± 0.02	0.9 ± 0.1	3.3
30	1.36 ± 0.08	nd	0.52 ± 0.06	0.60 ± 0.05	0.78 ± 0.03	3.26
31	1.2 ± 0.2	nd	0.49 ± 0.08	0.54 ± 0.06	0.69 ± 0.06	2.9
32	1.2 ± 0.2	nd	0.48 ± 0.06	0.57 ± 0.04	0.73 ± 0.09	3.0
mean	1.6	0.11	0.59	0.7	0.9	4.0
SD	0.8	0.2	0.2	0.2	0.2	1
min	1.00	nd	0.42	0.50	0.60	2.52
max	3.4	0.49	1.02	1.14	1.45	6.1
Gala						
33	1.8 ± 0.2	0.42 ± 0.03	0.44 ± 0.07	0.71 ± 0.09	1.36 ± 0.07	4.7
34	1.7 ± 0.2	0.39 ± 0.05	0.34 ± 0.05	0.7 ± 0.1	1.5 ± 0.1	4.6
35	1.20 ± 0.09	0.31 ± 0.02	0.20 ± 0.01	0.45 ± 0.06	1.0 ± 0.1	3.2
36	1.71 ± 0.07	0.39 ± 0.04	0.34 ± 0.04	0.8 ± 0.1	1.45 ± 0.08	4.7
37	1.4 ± 0.2	0.54 ± 0.05	0.53 ± 0.08	0.9 ± 0.1	1.66 ± 0.08	5.0
38	1.7 ± 0.2	0.70 ± 0.08	0.43 ± 0.03	0.71 ± 0.09	1.8 ± 0.2	5.3
39	1.5 ± 0.1	0.66 ± 0.09	0.37 ± 0.05	0.56 ± 0.07	1.55 ± 0.07	4.6
40	1.9 ± 0.2	0.91 ± 0.05	0.53 ± 0.08	0.86 ± 0.08	2.0 ± 0.1	6.2
41	2.7 ± 0.2	0.49 ± 0.02	0.45 ± 0.05	0.87 ± 0.06	1.72 ± 0.08	6.2
42	1.52 ± 0.09	0.76 ± 0.08	0.49 ± 0.06	0.56 ± 0.03	1.5 ± 0.1	4.8
mean	1.7	0.56	0.41	0.7	1.5	4.9
SD	0.4	0.2	0.1	0.2	0.3	0.9
min	1.2	0.31	0.2	0.45	1.0	3.2
max	2.7	0.91	0.53	0.9	2.0	6.2
Golden Delicious						

Table 5. continued

sample	QGal	QGlu	QXyl	QAra	QRha	total
43	1.01 ± 0.09	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	4.9 ± 0.3	9.2
44	0.53 ± 0.05	0.71 ± 0.09	0.42 ± 0.07	0.75 ± 0.05	3.1 ± 0.3	5.5
45	0.64 ± 0.05	0.62 ± 0.05	0.52 ± 0.04	0.76 ± 0.09	3.3 ± 0.3	5.8
46	1.0 ± 0.1	1.1 ± 0.1	0.69 ± 0.08	0.9 ± 0.1	3.8 ± 0.2	7.5
47	0.79 ± 0.09	0.81 ± 0.07	0.47 ± 0.07	0.8 ± 0.1	3.2 ± 0.2	6.1
48	0.9 ± 0.1	0.78 ± 0.07	0.60 ± 0.04	0.85 ± 0.09	3.1 ± 0.4	6.2
49	0.49 ± 0.05	0.8 ± 0.1	0.42 ± 0.03	0.32 ± 0.03	3.0 ± 0.4	5.0
50	0.63 ± 0.07	0.79 ± 0.09	0.48 ± 0.07	0.40 ± 0.05	3.2 ± 0.2	5.5
51	1.06 ± 0.09	1.00 ± 0.04	0.72 ± 0.05	0.58 ± 0.06	3.7 ± 0.4	7.1
52	1.04 ± 0.07	1.09 ± 0.08	0.70 ± 0.05	0.87 ± 0.09	3.8 ± 0.3	7.5
mean	0.8	0.9	0.6	0.7	3.5	6.5
SD	0.2	0.2	0.2	0.3	0.6	1
min	0.49	0.62	0.42	0.32	3.0	5.0
max	1.06	1.2	0.9	1.2	4.9	9.2
Jonagold						
53	3.6 ± 0.4	1.2 ± 0.2	1.35 ± 0.06	2.5 ± 0.2	8.3 ± 0.5	17.0
54	2.8 ± 0.2	1.08 ± 0.09	1.1 ± 0.1	2.1 ± 0.2	7.8 ± 0.7	14.9
55	3.6 ± 0.3	1.77 ± 0.09	1.35 ± 0.07	2.5 ± 0.3	8.7 ± 0.9	17.9
56	1.9 ± 0.1	0.76 ± 0.08	1.38 ± 0.09	1.1 ± 0.1	3.9 ± 0.4	9.0
57	1.5 ± 0.1	0.47 ± 0.06	1.06 ± 0.09	0.96 ± 0.09	2.9 ± 0.1	6.9
58	1.33 ± 0.09	0.32 ± 0.03	0.50 ± 0.07	1.08 ± 0.08	3.3 ± 0.3	6.5
mean	2.4	0.9	1.1	1.7	5.8	11.9
SD	1.0	0.5	0.3	0.7	2.7	5
min	1.33	0.32	0.5	0.96	2.9	6.5
max	3.6	1.77	1.38	2.5	8.7	17.9

[a]QGal, quercetin-3-*O*-galactoside; QGlu, quercetin-3-*O*-glucoside; QXyl, quercetin-3-*O*-xyloside; QAra, quercetin-3-*O*-arabinoside; QRha, quercetin-3-*O*-rhamnoside. nd, not detectable.

The presentation of the scores for each sample on the plane of the first three canonical discriminant functions is shown in Figure 2. This figure revealed a clear separation between the five varieties.

Then, SLDA was performed on the autoscaled data matrix composed of 57 juice samples and 23 variables to develop discrimination models according to geographical origin. The sample from Chunhua was excluded from analysis due to the very small number of samples from this region. The geographical regions were set as grouping variables. Sixteen variables were selected (F to enter = 2 and F to remove = 1), and the canonical discriminant analysis resulted in four discriminant functions. A satisfactory differentiation according to the five regions was achieved with a recognition ability of 98.2% and a prediction ability of 91.2% (Table 10). The eigenvalues, explained variances, canonical correlations, and coefficients of the variables for the four discriminant functions are shown in Table 11.

The graphical representation of the juices in the plane defined by the first three discriminant functions is presented in Figure 3. The juices from Luochuan and Sanyuan were clearly separated from each other and from those of Liquan, Xunyi, and Yongshou. Although the juices from Liquan, Xunyi, and Yongshou were not completely separated, the prediction abilities for juices from these three regions were 85.7, 94.4, and 81.8%, respectively, which can be considered satisfactory. Furthermore, we observed that by SLDA the juices produced from apples within the PDO region (Liquan, Xunyi, Yongshou, and Luochuan) could be distinguished from those outside the PDO region (Sanyuan).

It is accepted that biosynthesis of polyphenolic compound is strictly controlled by the genes of the corresponding enzymes involved in the relevant biosynthetic pathways. Therefore, the polyphenolic profile of a given variety reflects to a great extent its genetic character. At the same time, by regulating activities of enzymes implicated in polyphenol biosynthesis, the environmental factor also plays a critical role in affecting the polyphenolic profile. As we can see, the concentration of phenolic compounds in apple juices varied widely even for samples of the same variety, evidencing an important impact of geographical origins. For this reason, the differences of polyphenolic profiles arising from these two key parameters (genetic factor and the environmental condition) could be revealed by appropriate statistical analysis and be considered as credible indices for apple juice classification.

The stepwise linear discriminant analyses for variety- and geographical origin-based classifications revealed that two hydroxycinnamic acids, 4-caffeoylquinic acid and 4-*p*-coumaroylquinic acid, exerted an important influence on both variety- and geographical origin-based differentiations. Major flavan-3-ols, especially (+)-catechin, (−)-epicatechin, and procyanidin B1, significantly affected differentiation in terms of geographical origin, whereas they had a rather minor contribution to variety-based classification. Likewise, 3-hydroxyphloretin-2′-*O*-glucoside and phloridzin appeared to play a crucial role in geographical origin-based classification, whereas the rest of the dihydrochalcones were less important in this regard. On the contrary, flavonols including quercetin-3-*O*-glucoside, quercetin-3-*O*-arabinoside, and quercetin-3-*O*-rhamnoside were predominant variables in variety-based differentiation. It could be concluded that not only the major phenolic compounds but

Table 6. Composition (Milligrams per Liter) of Dihydrochalcones, Flavan-3-ols, Hydroxycinnamic Acids, and Flavonols in Apple Juices from Five Regions

	geographical origin																			
	Liquan (n = 14)				Xunyi (n = 18)				Yongshou (n = 11)				Sanyua (n = 7)				Luochuan (n = 7)			
polyphenol	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
dihydrochalcones																				
HPXG	0.6	0.4	0.23	1.7	0.5	0.3	nq	1.3	0.54	0.2	0.32	0.79	0.55	0.1	0.30	0.72	0.41	0.1	0.31	0.57
HPG	1.1	1	nd	5.2	1.1	1	nd	4.8	0.9	0.5	nd	1.5	2.1	1	0.59	3.2	0.6	0.4	nd	1.4
PXG	5	3	2.7	13	4.8	2	1.9	8.6	6	1	3.9	7	6	3	2.8	11	5.1	2	2.9	8.1
PLZ	4.8	2	1.42	7.5	5	4	1.0	14.1	6	2	1.55	7.2	6.7	1	4.9	9.0	5	3	1.0	8.8
flavan-3-ols																				
PB1	9	4	3.0	14	6	5	1.3	18	6.4	2	1.9	8.2	10	6	2.2	16.3	3.8	3	0.45	8.9
CAT	8	4	3.6	17	8	5	2.0	22	7	3	2.0	15	9	5	3.4	14	4.0	3	1.1	8.4
PB2	36	6	27	49	30	10	21	63	30	6	20	42	39	5	31	46	14	2	11	15.7
EC	23	5	16.9	34	23	9	9.2	50	23	6	14.2	32	29	6	16	33	17	8	6.7	30
FAT-a	11	2	8.9	14	9	4	2.0	19	10	2	7.8	12	12	2	9.9	15	8	4	2.6	13
FAD-a	4.5	2	nq	7.2	2.5	2	nq	5.5	2.6	1	nd	5.6	5	2	2.6	10	2.7	2	nq	6.2
FAT-b	0.7	1	nd	4.1	3.7	2	1.0	8.7	4.7	2	2.0	6.6	1.4	2	nd	4.2	1.0	0.8	nd	1.9
FAT-c	1.5	0.3	1.1	2.14	1.4	0.5	0.36	2.35	1.2	0.2	0.87	1.6	1.8	0.5	1.0	2.8	1.0	0.2	0.7	1.32
FAD-b	1.7	0.4	1.13	2.5	1.7	0.6	0.60	3.2	1.9	0.7	0.9	3.7	1.8	0.4	1.2	2.42	1.2	0.5	0.40	1.9
hydroxycinnamic acids																				
5-CQA	65	19	31	90	61	17	31	94	73	13	55	98	58	27	33	106	62	16	40	76
3-*p*CoQA	2.4	2	nd	5.9	3.5	3	1.2	8.8	3.5	3	nd	8.6	3	3	nd	9	3.0	2	1.26	5.3
4-CQA	0.8	1	nd	2.9	0.4	0.8	nd	2.6	0.26	0.6	nd	1.44	1.1	0.8	nd	1.8	nd			
4-*p*CoQA	10	3	6.1	16	8	5	1.7	18	6	3	3.8	14	12	2	9.5	16	7	2	4.4	10.2
5-*p*CoQA	1.4	0.8	0.38	2.7	1.7	0.7	0.43	2.7	1.7	0.9	0.30	2.6	1.7	0.9	0.31	3.1	1.3	0.8	0.19	2.3
flavonols																				
QGal	1.7	1	0.53	3.6	1.9	0.8	0.79	3.5	3.2	2	0.9	5.9	2.6	2	1.2	6.7	1.2	0.5	0.49	1.9
QGlu	0.6	0.5	nd	1.77	0.5	0.3	nd	1.1	0.6	0.1	0.39	0.8	0.3	0.4	nd	1.0	0.7	0.3	0.21	1.09
QXyl	0.6	0.4	0.20	1.35	0.58	0.2	0.30	1.02	0.8	0.3	0.45	1.38	0.53	0.05	0.48	0.63	0.56	0.1	0.42	0.72
QAra	1.1	0.7	0.45	2.5	0.9	0.3	0.56	1.6	1.2	0.4	0.85	1.88	1.1	0.8	0.54	2.4	0.6	0.2	0.32	0.87
QRha	3.2	3	0.60	8.7	1.4	0.9	0.38	3.8	1.9	1	1.07	3.9	1.4	1	0.69	3.3	2.4	1	0.76	3.8

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Table 7. Loadings of the Three First Principal Components for Apple Juice Samples

variable	PC1	PC2	PC3
procyanidin B1	0.776	−0.487	0.011
(+)-catechin	0.786	−0.484	0.015
5-caffeoylquinic acid	0.135	0.686	0.370
procyanidin B2	0.829	0.066	−0.014
3-*p*-coumaroylquinic acid	0.019	0.049	0.610
4-caffeoylquinic acid	0.633	−0.019	−0.646
(−)-epicatechin	0.891	−0.064	0.326
4-*p*-coumaroylquinic acid	0.475	−0.035	−0.237
5-*p*-coumaroylquinic acid	0.118	0.553	0.620
procyanidin trimer a	0.886	0.165	0.057
procyanidin dimer a	0.585	−0.086	−0.244
procyanidin trimer b	0.374	0.056	0.221
procyanidin trimer c	0.749	0.040	0.130
3-hydroxyphloretin-2′-*O*-xylglucoside	0.657	0.068	−0.201
procyanidin dimer b	0.667	−0.047	0.127
quercetin-3-*O*-galactoside	0.240	0.610	0.495
quercetin-3-*O*-glucoside	−0.178	0.843	−0.228
3-hydroxyphloretin-2′-*O*-glucoside	0.757	−0.338	−0.011
quercetin-3-*O*-xyloside	0.413	0.674	−0.330
phloretin-2′-*O*-xyloglucoside	0.147	0.796	−0.175
quercetin-3-*O*-arabinoside	0.279	0.865	0.068
quercetin-3-*O*-rhamnoside	−0.049	0.695	−0.652
phloridzin	0.616	0.205	0.013

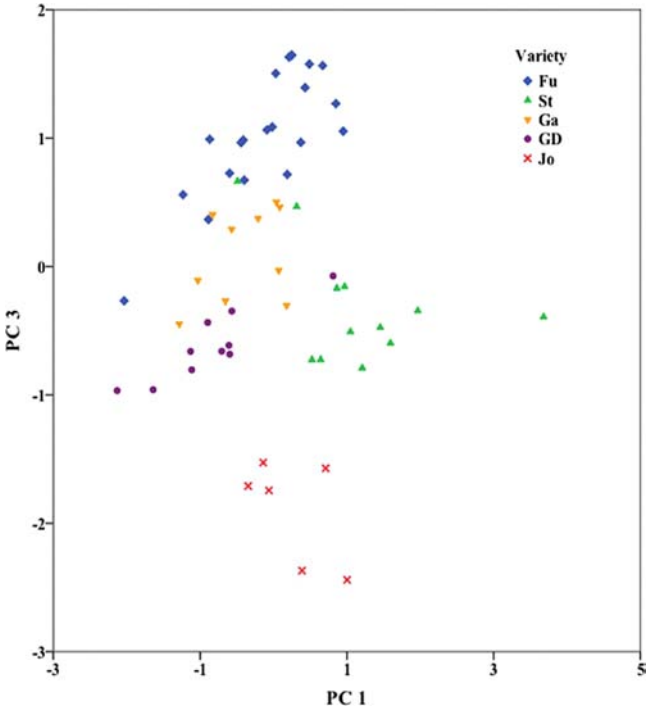

Figure 1. Scatter plot of the samples on the first and third principal component scores. Fu, Fuji; St, Starkrimson; Ga, Gala; GD, Golden Delicious; Jo, Jonagold.

also the minor ones, such as flavonols, ought to be considered as representative indices for unambiguous and reliable differentiation of apple juices from different varieties and geographical origins. This result is compatible to the report of Kallithraka et al.[36]

The information generated by phenolic compounds in combination with the support of statistical data evaluation

Table 8. Prediction Results of SLDA Model for Variety-Based Classification

	prediction ability (%)				
true category	Fuji	Starkrimson	Gala	Golden Delicious	Jonagold
Fuji	100	0	0	0	0
Starkrimson	8.3	91.7	0	0	0
Gala	0	0	100	10	0
Golden Delicious	0	0	0	100	0
Jonagold	0	0	0	0	100
	total prediction ability = 98.3%				

Table 9. Eigenvalues, Explained Variances, Canonical Correlations, and Coefficients of the Variables in the Discriminant Functions for Variety-Based Classification

function	1-1	1-2	1-3	1-4
eigenvalue	25.968	18.895	10.179	2.500
explained variance (%)	45.1	32.8	17.7	4.3
canonical correlation	0.981	0.975	0.954	0.845
(+)-catechin	−0.909	−1.520	1.484	0.437
5-caffeoylquinic acid	1.210	1.386	0.049	−1.093
4-caffeoylquinic acid	2.087	1.116	0.318	−0.438
4-*p*-coumaroylquinic acid	−1.240	0.597	0.895	0.784
5-*p*-coumaroylquinic acid	−0.090	−1.151	0.627	0.553
procyanidin trimer b	−0.115	0.765	0.389	0.335
procyanidin dimer b	0.423	0.890	−0.689	−0.501
quercetin-3-*O*-glucoside	−1.008	−1.005	0.155	−0.292
3-hydroxyphloretin-2′-*O*-glucoside	0.226	−0.217	−0.809	0.368
quercetin-3-*O*-arabinoside	1.109	−0.769	0.147	−0.965
quercetin-3-*O*-rhamnoside	−1.596	1.438	−0.579	0.881
phloridzin	0.117	−0.808	−1.367	0.314

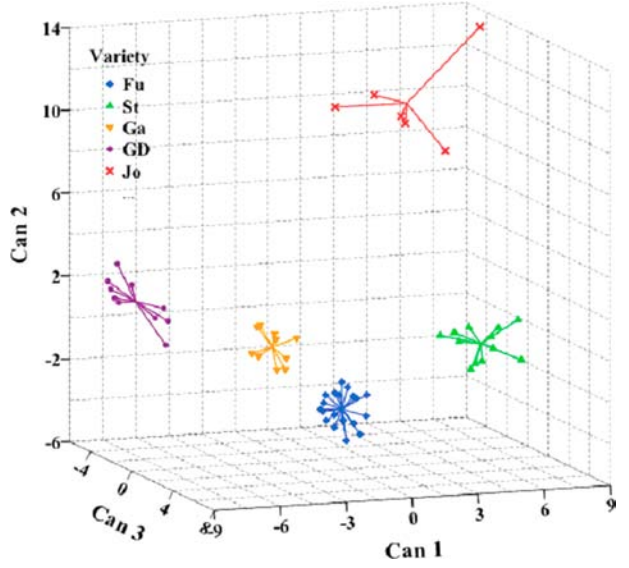

Figure 2. SLDA plot showing grouping of samples according to apple variety. Fu, Fuji; St, Starkrimson; Ga, Gala; GD, Golden Delicious; Jo, Jonagold.

techniques enabled an objective differentiation of apple juices from different varieties and geographical origins. However, it should be noted that this conclusion could not be applied to clear apple juice. In the process of clear apple juice production,

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Table 10. Prediction Results of SLDA Model for Geographical Origin-Based Classification

	prediction ability (%)				
true category	Liquan	Xunyi	Yongshou	Sanyuan	Luochuan
Liquan	85.7	0	0	7.1	7.1
Xunyi	5.6	94.4	0	0	0
Yongshou	0	18.2	81.8	0	0
Sanyuan	0	0	0	100	0
Luochuan	0	0	0	0	100
	total classification ability = 91.2%				

Table 11. Eigenvalues, Explained Variances, Canonical Correlations, and Coefficients of the Variables in the Discriminant Functions for Geographical Origin-Based Classification

function	2-1	2-2	2-3	2-4
eigenvalue	9.826	4.076	3.881	0.787
explained variance (%)	52.9	22.0	20.9	4.2
canonical correlation	0.953	0.896	0.892	0.664
procyanidin B1	1.363	1.929	−1.029	2.360
(+)-catechin	−6.631	4.069	2.613	−2.841
procyanidin B2	0.358	0.293	1.663	−0.022
4-caffeoylquinic acid	2.701	−2.765	−0.337	0.017
(−)-epicatechin	2.441	−0.875	−2.781	−1.298
4-*p*-coumaroylquinic acid	1.319	−1.405	−0.410	0.258
5-*p*-coumaroylquinic acid	0.162	−1.059	0.421	−0.248
procyanidin trimer b	−0.579	−0.925	0.779	−0.065
procyanidin trimer c	−0.247	−0.714	0.564	−0.593
procyanidin dimer b	1.143	−0.988	0.222	2.026
quercetin-3-*O*-galactoside	0.777	−0.501	−1.918	2.087
3-hydroxyphloretin-2′-*O*-glucoside	2.481	−0.862	−0.514	0.750
quercetin-3-*O*-xyloside	−1.629	−0.059	−1.654	−0.175
quercetin-3-*O*-arabinoside	−1.325	2.342	3.483	−1.250
quercetin-3-*O*-rhamnoside	0.381	2.164	−1.019	0.460
phloridzin	−2.237	0.744	0.236	0.375

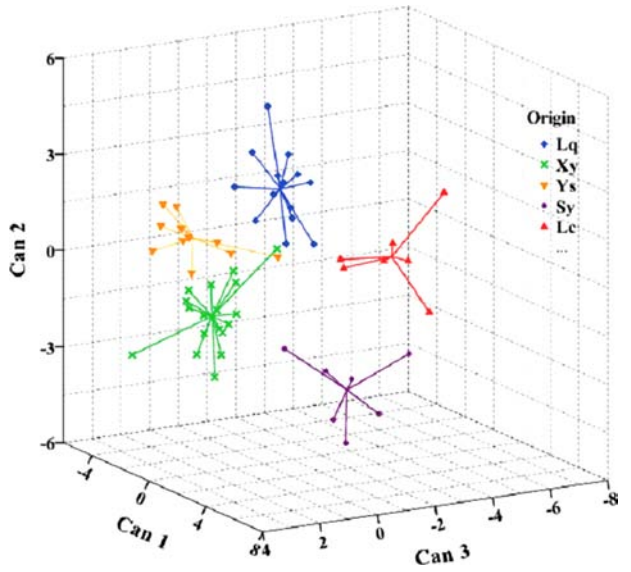

Figure 3. SLDA plot showing grouping of samples according to geographical origin. Lq, Liquan; Xy, Xunyi; Ys, Yongshou; Sy, Sanyuan; Lc, Luochua.

enzymatic clarification, ultrafiltration, and adsorption are performed to reduce the content of polyphenols and obtain a color-stable and non-haze-forming product. This process has greater influence on phenolic composition than the variation of variety and geographical origin.[9,11,37]

Although the results obtained are promising, encouraging the similar procedures to be considered in quality control of apple juices, it must be taken into account that the variety, environment, degree of ripeness, and processing procedure simultaneously affect phenolic compounds of apple juice. Among these, climatic factors are shown to play an important role. The phenolic content of apple shows year-to-year variations due to the effect of rainfall, sunshine, and temperature (the difference between day and night temperatures).[38−40] The phenolic content in apple appears to be higher with less rain and more sunshine throughout the growing season.[41] Sun irradiation and temperature can have some effect on anthocyanin pigmentation.[42] Low overnight temperatures and high levels of sunshine hours during ripening can promote the accumulation of anthocyanins in the apple skin.[43] Cyanidin 3-galactoside and quercetin 3-glycoside levels as well as the total concentration of flavonoids are shown to be higher in apple skin that has been exposed to sun radiation.[44] The state of fruit maturity at harvest is also identified as a major factor, which affects the polyphenolic profile of apple juice. In young fruit, the concentrations of total flavonoids and chlorogenic acid are relatively high, but gradually decrease during growth to a steady level during maturation and ripening.[45] Accumulation of anthocyanins shows two peaks: the first in young fruitlets during cell division and the second in fully developed apples during maturation.[45] In addition, agronomic practices, such as irrigation, fertilization, salt stress, herbicide, and/or pesticide treatment can also affect the phenolic composition of apple.[42] Polyphenols in apple fruit and apple juice are quite stable during storage. There are no losses of phenols in apple fruit during long-term storage in both normal air and controlled atmosphere.[46,47] In addition, it is found that storage of apple juice at 4 or 20 °C for up to 1 month will not lower the concentration of polyphenolic antioxidants.[48]

The actual applicability of the classification methodology proposed requires further research, extending the study to a broader number of samples, especially to samples of different harvests. Moreover, external validation of the model with a larger data set must be performed.[22] In such a way the constraints on the robustness of the classification model can be solved.

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Notes

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