ELSEVIER

Food Chemistry 78 (2002) 523–532

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, nutritional and clinical methods

Principal component analysis of the polyphenol content in young red wines

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Received 9 October 2001; received in revised form 6 February 2002; accepted 1 March 2002

Abstract

In the present study the content of 15 polyphenols was determined in 55 samples of red wines from different denominations of origin in the Canary Islands (Spain) using high performance liquid chromatography (HPLC) with UV and fluorescence detection. The most important differences in content among wines according to different categories (island, zone and denomination of origin) were established. In general, red wines from the Canary Islands had a content in polyphenols in the lower part of the range considered normal. The exception was quercetin, with a mean content (17.5 mg/l) higher than in other wines, which may be a peculiarity of these particular wines. The principal component analysis (PCA) technique was used to study the latent structure. A good differentiation among wines according to their production area was obtained using linear discriminant analysis (LDA).

Keywords: Polyphenol; HPLC; Wine; Principal component analysis; Discriminant analysis

1. Introduction

Phenolic compounds are very important components in wines for several reasons. They contribute to their sensorial properties, being responsible for red wine colour, flavour, astringency and bitterness, both directly and by interaction with proteins, polysaccharides or other phenolic compounds (Glories, 1984; Haslam, 1974; Robichaud & Noble, 1990). In addition to contributing somewhat to the olfactory profile of the wine, phenolic acids are precursors of volatile phenols, which enrich the wines with different aromas (Rapp, Bachmann, & Steffan, 1977). Likewise, they are responsible for browning reactions of the wine (Cheynier, Souquet, & Moutounet, 1989; Moutounet, Cheynier, Rigaud, & Souquet, 1989; Rigaud, Moutounet, & Cheynier, 1988) and are considered to be essential elements during the preservation and ageing (Nagel & Wulf, 1977). They have bactericidal effects and epidemiologists have observed that a diet rich in polyphenolic compounds may result in a positive health effect attributable to their antioxidant properties (Frankel, Waterhouse, & Teissedre, 1995; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993).

The polyphenolic compounds of grapes are found mainly in the skin, particularly in the epidermal cells, and in the seeds, their concentration being very low in the pulp. The polyphenolic composition of the wine depends on the grape variety, vineyard location, cultivation system, climate, soil types, vine cultivation practices, harvesting time, production process (pressing, winemaking method, skin-contact maceration period, etc.) and ageing (Shahidi & Naczk, 1995). These compounds are grouped into several families according to structure: hydroxycinnamic acids, hydroxybenzoic acids, flavanols, flavonols, etc. (Zoecklein, Fugelsang, Gump, & Nury, 1990).

These compounds are usually identified by standard HPLC techniques. Most of the published reports on wine phenols deal with the development of analytical methods for their separation (Malovaná, García Montelongo, Pérez, & Rodríguez-Delgado, 2001). Other works study the influence of the grape variety (Salagoity-Auguste & Bertrand, 1984), growing site (Brossaud,

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0308-8146/02/$ - see front matter
PII: S0308-8146(02)00206-6

Cheynier, Asselin, & Moutounet, 1999), the evolution of polyphenolic compounds during ripening (Fernández-Simón, Hernández, & Estrella, 1992) and the wine-making process (Mayén, Mérida, & Medina, 1995). Some authors have published data on *p*-coumaric acid and quercetin (Goldberg, Tsang, Karamanchiri, & Soleas, 1998), catechin and epicatechin (Carando, Teissedre, Pascual-Martínez, & Cabanis, 1999; Goldberg, Karumanchiri, Tsang, & Soleas, 1998), and myricetin and quercetin (McDonald et al., 1998) in many commercial red wines. Unfortunately very few data have been published on other phenolic compounds.

Precise data available about the phenols content in red wines from the denominations of origin of the Canary Islands are at present insufficient. Only Pazourek, González, Revilla, and Havel (2000) analysed red wines by capillary zone electrophoresis, finding 0.58–2.27 mg/l *p*-coumaric acid and 0.27–1.26 mg/l gentisic acid. The aim of this work is to quantify 15 phenolic components using high performance liquid chromatography (HPLC): Gallic, protocatechuic, vanillic, syringic, caffeic, *p*-coumaric and ferulic acids, catechin, epicatechin, quercetin, quercitrin, myricetin, kaempferol, and syringic and protocatechuic aldehydes. Fifty-five red wines from the Canary Islands were studied to compare their contents with those of other varieties and countries, and to use these data to attempt to typify wines according to their geographical origin.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. The polyphenolic compounds were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). HPLC grade methanol, acetic acid, diethyl ether, hydrochloric acid and ethanol were obtained from Merck (Darmstadt, Germany). Ultrapure water from the Milli-Q system (Millipore, Bedford, USA) with a conductivity of 18 MΩ was used in all cases. All solutions were filtered through 0.45-μm membranes (Millipore) and degassed prior to use.

2.2. Apparatus and chromatographic conditions

Separation was carried out using a Waters liquid chromatograph equipped with two pumps (Model 510), an automated gradient controller (Model 680), an injector (Rheodyne Model 7125 with a 20-μl loop), and a tuneable absorbance detector (Model 486) in series with a fluorescence detector (Model 470). Baseline Workstation 810 software (Waters) was employed for data storage and evaluation. The analytical column was a Nova-Pak C18 150×3.9 mm I.D. and 4 μm particle diameter, from Waters. A Nova-Pak C18 precolumn was employed to protect the analytical column. A model 168 diode array detector from Beckman (Beckman Instruments Inc., Fullerton, CA, USA) was used for identification purposes. Aliquots of the extracted samples were injected into the HPLC system under the following conditions: The column was initially equilibrated with methanol-acetic acid-water (10:2:88, v/v) as solvent A for 10 min. The phenolic compounds were eluted with a three stage linear gradient: from 100 to 85% of A in 15 min, from 85 to 50% of A over 10 min and from 50 to 30% of A in 9 min with a total flow rate of 1.0 ml min. A mixture of methanol–acetic acid–water (90:2:8, v/v) was used as solvent B. A wavelength of $\lambda = 280$ nm was used for the absorbance detector, while $\lambda_{ex} = 278$ nm and $\lambda_{em} = 360$ nm over 17.5 min and $\lambda_{ex} = 330$ nm and $\lambda_{em} = 374$ nm for 16.5 min was used for the fluorescence detector.

2.3. Samples

The samples used for the present study were 55 red wines produced during the 1999 harvest from five Denominations of Origin (DO) on the island of Tenerife and one on the island of Lanzarote. Five of the samples were from Abona DO, 9 from Valle de Güímar DO, 11 from Valle de La Orotava DO, 13 from Tacoronte-Acentejo DO, 11 from Ycoden Daute Isora DO, and 6 from Lanzarote DO. All samples have been elaborated as young wines, with short skin contact, 4–5 days. The red grape variety used was the indigenous "listán negro". All the samples were provided by the Denomination of Origin Certification Councils, to ensure the geographic origin of the wines. The samples were taken in the months of April and May and analysed in June and July. According to the microclimates, soils, growing systems, altitude of vineyards, etc., the different DOs can be clustered into three areas: Lanzarote, south zone of Tenerife (Abona and Valle de Güímar DOs) and north zone of Tenerife (Valle de La Orotava, Tacoronte-Acentejo and Ycoden Daute Isora DOs).

2.4. Sample preparation

Samples of wines were analysed with the following procedure: pH of wine samples was adjusted to pH = 2 by adding small amounts of 0.1 M hydrochloric acid (HCl). Then, 5 ml of wine was extracted twice with diethyl ether (5 ml) for 20 min using a Selecta Rotabit (Selecta, Barcelona, Spain) at 180 rpm. Organic phases were separated and taken to dryness with a nitrogen gas stream. The dry residue was dissolved in a methanol/water mixture (1/1) and aliquots were injected into the HPLC system. All samples were filtered through a 13 mm Stainless Swinny equipped with cellulose acetate (Millipore) 0.45-μm filter. Duplicate injections were

performed and average peak areas were used for the measurement. For calibration purposes standards were dissolved in a matrix solution (15% v/v ethanol–3 g/l tartaric acid in water) with concentrations in the range 1.8–3.6 mg/l and stored at −4 °C in the dark. Working standard solutions were prepared by diluting the stock solutions with the matrix solution.

2.5. Statistics and data presentation

Data are reported as mean ± standard deviation. Island, zone orientation, and denomination of origin grouped the wines surveyed.

2.5.1. Univariate analysis

Analysis of variance was applied to all variables studied. The mean values obtained in the different categories studied were compared by one-way ANOVA.

2.5.2. Multivariate analysis

2.5.2.1. Data analysis. Each wine sample, that constitutes an object, was considered to be a data vector of 15 variables represented by the chemical data. A data pre-treatment was made in order to avoid the differences in measurement units. Autoscaling is the most widely used scaling technique. The result is a variable with zero mean and a unit standard deviation (Kowalski & Bender, 1972). An additional advantage of autoscaling is that the covariance matrix is equal to the correlation matrix (Pardo & Barrado, 1988).

2.5.2.2. Principal component analysis (PCA). This procedure extracts the dominant patterns in the data matrix in terms of a complementary set of scores and loading plots. PCA permits us to achieve a reduction of dimensionality, a data exploration finding relationship between objects, estimating the correlation structure of the variables and investigating how many components (a linear combination of original features) are necessary to explain the greater part of variance with a minimum loss of information. When PCA is performed on autoscaled matrix data the principal component loadings are eigenvectors of the correlation matrix (Wold, Esbensen, Geladi, 1987).

2.5.2.3. Linear discriminant analysis (LDA). This classification procedure is a supervised technique where the number of categories and the samples that belong to each category are previously defined. The method supplies a number of orthogonal linear discriminant functions, equal to the number of categories minus one, that permit the samples to be classified in one or another category. The criterion used to calculate the discriminant functions is to maximise the ratio of variance between categories to variance within categories (Ramis & García-Alvarez, 2001). A variant of this method is the stepwise discriminant analysis that permits the variables with a major discriminant capacity to be selected.

2.5.2.4. Leave-one-out cross validation. During this cross validation test, a sample is removed from the data set. The classification model is rebuilt and the removed sample is classified in this new model. All the samples of the data set are sequentially removed and reclassified. Finally, a percentage of good classification is given (Ramis & García-Alvarez, 2001).

Univariate and multidimensional statistical analysis were performed by means of the statistical software package STATGRAPHICS Plus for Windows 4.0 from Statistical Graphics Corporation, and PARVUS 1.3.

3. Results and discussion

Fig. 1 shows the chromatograms using absorbance and fluorescence detectors of a red wine injected into the HPLC system after a liquid–liquid extraction. As can be seen a good resolution was obtained using both detectors. Several of polyphenolic compounds were not resolved using absorbance detector, but they were determined with the fluorescence one (Malovaná et al., 2001).

Table 1 shows the mean values and standard deviations for the different phenols according to denomination of origin (DO)—Abona, Valle de Güímar, Valle de La Orotava, Tacoronte-Acentejo and Ycoden Daute Isora, island—Tenerife and Lanzarote, and zone orientation—south and north of Tenerife. The study was carried out with the phenols grouped into five types: *hydroxybenzoic acids*—gallic, protocatechuic, vanillic and syringic acids, *hydroxycinnamic acids*—caffeic, *p*-coumaric and ferulic acids, *flavanols*—catechin, epicatechin, *flavonols*—quercetin, quercitrin, myricetin, kaempferol, and *phenolic aldehydes*—syringic and protocatechuic aldehydes. This aided in arriving at simple systematic criteria and better comparison of the results was obtained.

It can be observed that in all cases the highest contents corresponded to gallic acid, catechin, epicatechin and quercetin, which agrees with the information available in the literature (Shahidi & Naczk, 1995).

Of the *hydroxybenzoic acids* analysed, gallic (5.6–44.7 mg/l) and protocatechuic (0.1–1.4 mg/l) acids presented the highest and lowest average content among all the DOs. Vanillic (1.2–4.2 mg/l) and syringic acids (0.9–4.0 mg/l) showed a similar range of variation in content, intermediate between those of the above compounds in all the DOs. The Lanzarote DO showed the highest content in gallic and total hydroxybenzoic acids, and the Abona DO in protocatechuic, vanillic and syringic acids.

Among the *hydroxycinnamic acids*, the caffeic acid content (2.0–14.4 mg/l) was the highest in all the DOs,

followed by *p*-coumaric (0.1–6.8 mg/l) and ferulic (0.1–1.4 mg/l) acids. This was not so in Valle de La Orotava DO, where the low level of *p*-coumaric acid stood out as significantly ($P < 0.05$) smaller than in the other DOs. The Abona DO had the highest contents in total hydroxycinnamic acids.

In the *flavanols* group the catechin (9.45–38.4 mg/l) and epicatechin (5.3–31.6 mg/l) contents were determined, and the catechin mean contents were greater than those of epicatechin in all the DOs. The Lanzarote DO presented higher mean contents of both compounds than the other DOs.

Of the four compounds analysed in the *flavonols* group, quercetin (1.9–49.8 mg/l), quercitrin (0.0–6.8 mg/l), myricetin (0.0–2.5 mg/l) and kaempferol (0.0–4.1 mg/l), quercetin presented the highest average content in all the DOs, followed by quercitrin. The Tacoronte-Acentejo DO had a higher average content in quercetin, quercitrin, myricetin and total flavonols than the remainder of the DOs.

The content of two *phenolic aldehydes* was also determined: protocatechuicaldehyde (0.05–1.2 mg/l) and syringaldehyde (1.7–15.6 mg/l). All the DOs presented a much higher content in syringaldehyde, the highest being Lanzarote, while the DOs of Valle de La Orotava, Tacoronte-Acentejo and Ycoden Daute Isora, all on the north zone of Tenerife, had the highest mean contents in protocatechuicaldehyde.

An analysis of variance (ANOVA) was carried out between the three areas with environmental differences

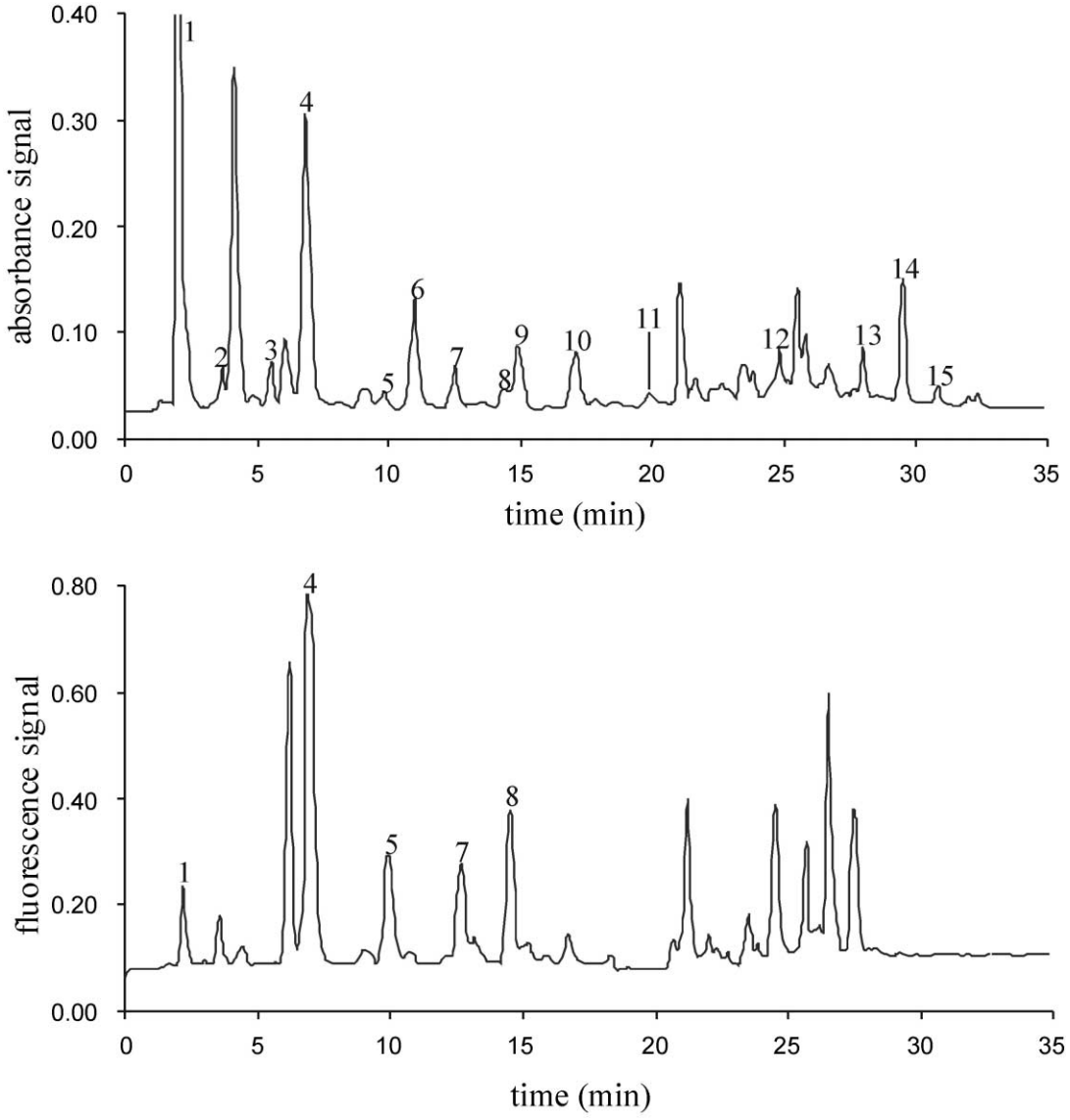

Fig. 1. Chromatograms of a red wine sample using absorbance and fluorescence detectors. Chromatographic conditions are described in 'Materials and Methods' section. Identification of compounds: Gallic acid (1), protocatechuic acid (2), protocatechuicaldehyde (3), catechin (4), vanillic acid (5), caffeic acid (6), syringic acid (7), epicatechin (8), syringaldehyde (9), *p*-coumaric acid (10), ferulic acid (11), myricetin (12), quercitrin (13), quercetin (14) and kaempferol (15).

Table 1
Mean and standard deviation values (mg/l) of the polyphenolic content according to denomination of origin, zone and island

Variables	Abona	Valle de Güímar	South Tenerife	Valle de La Orotava	Tacoronte-Acentejo	Ycoden Daute Isora	North Tenerife	Tenerife Island	Lanzarote Island
Gallic acid	15.11 ±5.09	21.15 ±1.82	19.00 ±4.36	25.07 ±11.52	18.28 ±10.64	18.57 ±8.43	20.50 ±10.47	20.07 ±9.13	27.21 ±7.42
Protocatechuic acid	0.85 ±0.15	0.48 ±0.13	0.61 ±0.23	0.50 ±0.41	0.59 ±0.27	0.44 ±0.12	0.51 ±0.29	0.54 ±0.27	0.74 ±0.44
Vanillic acid	2.99 ±0.76	2.35 ±0.58	2.58 ±0.70	1.71 ±0.37	1.90 ±0.52	2.40 ±0.63	1.99 ±0.58	2.16 ±0.66	1.85 ±0.53
Syringic acid	2.77 ±0.16	2.66 ±0.78	2.70 ±0.62	2.19 ±0.46	1.71 ±0.47	1.64 ±0.64	1.84 ±0.56	2.09 ±0.70	1.68 ±0.41
Hydroxybenzoic acids	21.73 ±4.84	26.64 ±2.40	24.88 ±4.09	29.47 ±11.61	22.48 ±10.83	23.05 ±9.69	24.85 ±10.65	24.86 ±9.21	33.92 ±11.12
Caffeic acid	7.74 ±4.37	6.57 ±2.69	6.99 ±3.27	3.74 ±1.79	6.78 ±3.78	4.44 ±1.18	5.09 ±2.87	5.63 ±3.08	5.47 ±0.61
p-Coumaric acid	2.54 ±2.26	2.70 ±1.31	2.64 ±1.62	0.22 ±0.09	2.36 ±2.11	2.52 ±2.24	1.74 ±2.03	2.00 ±1.95	2.77 ±0.43
Ferulic acid	0.74 ±0.47	0.47 ±0.28	0.57 ±0.36	0.68 ±0.15	0.47 ±0.33	0.81 ±0.34	0.64 ±0.32	0.62 ±0.33	0.51 ±0.46
Hydroxycinnamic acids	11.03 ±6.34	9.74 ±3.39	10.20 ±4.46	4.65 ±1.85	9.61 ±5.05	7.77 ±5.05	7.47 ±4.20	8.25 ±4.41	8.75 ±0.55
Catechin	17.70 ±6.18	18.68 ±4.40	18.33 ±4.89	19.20 ±7.41	19.94 ±8.49	18.37 ±5.52	19.22 ±7.14	18.96 ±6.54	30.77 ±4.25
Epicatechin	13.59 ±1.78	13.39 ±8.22	13.46 ±6.53	9.55 ±4.45	9.24 ±4.56	10.63 ±2.61	9.77 ±3.94	10.83 ±5.04	14.87 ±6.23
Catechin/epicatechin	1.30 ±0.44	1.44 ±0.58	1.39 ±0.52	2.05 ±0.90	1.96 ±0.72	1.73 ±0.29	1.92 ±0.68	1.76 ±0.68	1.90 ±0.47
Flavanols	31.29 ±6.96	32.07 ±11.66	31.79 ±9.94	28.76 ±10.72	29.18 ±11.00	29.00 ±7.82	28.99 ±9.72	29.79 ±9.77	45.64 ±9.59
Quercetin	17.07 ±6.43	18.70 ±9.77	18.12 ±8.49	17.05 ±8.72	25.57 ±11.48	12.71 ±5.85	18.85 ±10.46	18.64 ±9.86	8.45 ±3.70
Quercitrin	2.05 ±0.79	2.36 ±0.23	2.25 ±0.50	3.64 ±1.41	3.89 ±1.86	3.09 ±1.45	3.56 ±1.60	3.19 ±1.49	0.83 ±0.54
Myricetin	0.72 ±0.64	0.32 ±0.11	0.46 ±0.42	0.79 ±0.63	1.47 ±0.55	0.79 ±0.44	1.04 ±0.63	0.88 ±0.63	0.70 ±0.12
Kaempferol	0.37 ±0.33	1.72 ±1.31	1.24 ±1.24	0.96 ±0.74	1.21 ±0.92	1.29 ±0.77	1.16 ±0.81	1.18 ±0.94	0.83 ±0.21
Flavonols	20.21 ±6.33	23.10 ±10.79	22.07 ±9.27	22.44 ±9.61	32.14 ±12.55	17.88 ±7.15	24.61 ±11.65	23.88 ±10.99	10.80 ±3.73
Syringaldehyde	4.62 ±2.54	7.10 ±1.66	6.22 ±2.28	7.13 ±2.76	7.09 ±2.95	6.97 ±3.26	7.07 ±2.90	6.82 ±2.74	9.48 ±3.40
Protocatechuicaldehyde	0.40 ±0.29	0.49 ±0.19	0.46 ±0.23	0.74 ±0.24	0.68 ±0.18	0.73 ±0.30	0.71 ±0.24	0.64 ±0.26	0.35 ±0.19
Phenolic aldehydes	5.01 ±2.48	7.59 ±1.51	6.67 ±2.22	7.87 ±2.97	7.77 ±2.95	7.7 ±3.44	7.78 ±3.02	7.46 ±2.84	9.83 ±3.43

Table 2
Comparative mean values (mg/l) of the polyphenolic content according to different authors

Reference	Cheynier and Teissedre (2000)	Soleas, Dam, Carey, and Goldberg (1997)	Frankel et al. (1995)	Ribeiro de Lima and Cabanis (1998)	Simonetti, Pietta, and Testolin (1997)	Carando et al. (1999)	Vuorinen, Määltä, and Törrönen (2000)	Viñas, Lopez-Erroz, Marín-Hernández, and Hernández-Córdoba (2000)	This work
Sample number	—	21[a]	14	23	10	95	16	9	55
Country/area	—	Ontario	California	Portugal	Italy	France	Different countries	Spain	Canary Islands
Gallic acid	2–130	13.1–30.7	95	22.7					21.1
Protocatechuic acid	0.2–20			9.7					0.56
Vanillic acid	0.3–10	2.3–3.7		5.8					2.1
Syringic acid	0.3–2								2.0
Caffeic acid	0.3–26	3.15–12.95	7.1	10.6					5.6
p-Coumaric acid	0.4–15	2.6–4.5		5.4					2.1
Ferulic acid	0.1	<1							0.6
Catechin	8–400	55–213	191			114.5		71.3	20.2
Epicatechin	6–160	25–82	82			75.7		11.6	11.3
Quercetin	3–20	0.5–5.26	7.7		13.1			17.6	17.5
Quercitrin							7.2		2.9
Myricetin	2–20		8.5		3.25		8.3		0.8
Kaempferol					0.55		nd[b]–1.2		1.1

[a] Range of mean values for different cultivars.
[b] Non detected.

described in the materials and methods section: Lanzarote, and the south and north zones of Tenerife. It was found that south Tenerife had a significantly higher average content in vanillic ($P<0.01$) and syringic ($P<0.001$) acids than the other two areas. Lanzarote had significantly higher mean catechin ($P<0.001$) and total flavanol contents ($P<0.01$), but significantly lower total flavonols ($P<0.05$) than the other two areas. North Tenerife had significantly higher contents in protocatechuic aldehyde ($P<0.001$) and significantly lower epicatechin ($P<0.05$) than the other two areas. Lastly, quercitrin showed significant differences ($P<0.001$) in content in the three areas. The most important differences were in total flavanols (largely in seeds) and total flavonols (mostly in skins).

Due to the fact that these wines were elaborated using similar procedures and the same grape variety, these differences in content can be attributed to environmental differences.

Our results agree with those obtained previously by Pazourek et al. (2000) for red wines from Tenerife for *p*-coumaric acid (0.58–2.27 mg/l), using capillary zone electrophoresis.

A comparison of the content ranges reported in this paper with those reported by other authors in other wines (Table 2) showed that the Canary Island red wines were at the lower part of those ranges, with the exception of syringic and ferulic acids and quercetin, whose contents were higher.

The low flavanols (catechin and epicatechin) content of Canary Island wines may be attributed to their elaboration with short skin contact, 4–5 days. However, this does not explain the high quercetin content. Another factor that could contribute to explaining these differences may be the autochthonous grape varieties utilised.

The mean content of the red wines from the Canary Islands in *p*-coumaric acid (2.1 mg/l) was similar to that found by Goldberg, Tsang et al. (1998), which analysed *p*-coumaric acid and quercetin respectively in about six hundred and nine hundred wines from different parts of the world. In the case of quercetin the mean content in red wines from the Canary Islands (17.5 mg/l) was higher than that from other areas of the world. Goldberg, Karumanchiri et al. (1998) also analysed both catechin and epicatechin in more than eight hundred red wines from different areas of the world. The mean content in catechin of Canary Island red wines (20.5 mg/l) was lower.

Price, Breen, and Valladao (1995) pointed out that sunlight enhances the concentration of quercetin in wines, whereas McDonald et al. (1998) linked the high levels of flavonols to wines from "thick-skinned" grapes, to vines that grow in warmer sunnier climates, to the time at which grapes are picked and to the application of modern methods of winemaking. The Canary Island wines have high quercetin contents although the "listán negro" variety is a thin-skinned grape and, moreover the highest content is obtained in Tacoronte-Acentejo DO with a colder and more humid climate. On the contrary, Lanzarote, the DO with a drier, warmer and sunnier climate had half the quercetin level than the remainder of the DOs.

As regards flavanols, Goldberg, Karumanchiri et al. (1998) pointed out that the concentrations of catechin and epicatechin depend on grape genetics but these authors also detected climatic influences. This suggests that damp cool climates increase catechin concentrations in wines produced from these cultivars while dry sunny ones decreased them. In our case, the highest contents in catechin and total flavanols were obtained in the Lanzarote DO, with a drier, warmer and sunnier climate and the lowest in the north zone of Tenerife with its colder and more humid climate.

These differing conclusions regarding the influence of climate on the polyphenolic content could be due to other environmental factors that have not been considered. Thus, in the case of the Canary Islands it would not be correct to attribute differences in the content of phenols wholly to climate, since there are also differences in soils, cultivation systems, altitude of vineyards, water availability, etc. It would be worthwhile to undertake further research into the influence of environmental factors.

3.1. Multivariate analysis

Since the polyphenolic content varies from one area to another, it has been used in an attempt to distinguish the wines according to production area. This differentiation is not possible by means of univariate analysis and it is therefore necessary to resort to multivariate analysis. The multivariate techniques of data analysis

Table 3
Loadings of the features in the first four principal components

	PC1	PC2	PC3	PC4
Gallic acid	0.6368	−0.4287	−0.0381	0.0730
Protocatechuic acid	0.1408	0.4275	0.0374	0.4714
Vanillic acid	0.1939	0.4107	−0.5999	0.2139
Syringic acid	0.2047	0.2833	−0.6803	0.3121
Caffeic acid	0.1011	0.6440	0.2886	−0.1900
p-Coumaric acid	0.2202	0.6051	0.3002	−0.4239
Ferulic acid	0.1026	−0.3052	−0.3429	0.0422
Catechin	0.7678	−0.0521	0.5326	0.4066
Epicatechin	0.7290	0.1557	0.3357	0.2484
Quercitrin	−0.6167	−0.0042	0.4743	0.4811
Myricetin	−0.6313	−0.1291	0.3376	0.2786
Quercetin	−0.5675	0.0123	0.1671	0.2531
Kaempferol	0.0811	−0.3906	0.1169	−0.4552
Syringaldehyde	0.6713	−0.3858	0.2408	0.0842
Protocatechuicaldehyde	−0.1548	−0.7471	−0.0558	0.2210
% Of variance	21.84	17.77	12.60	10.18

have been used to explain wine differentiation and to obtain more information on the variables that mainly influence the sample similarities and differences (Medina, 1996; Nogueira & Nascimento, 1999).

PCA was performed on the autoscaled data (55 samples and 15 variables) using the Statgraphics software package in order to provide partial visualisation of the data set in a reduced dimension, and four principal components with eigenvalues higher than one accounting for 62.4% of total variance were obtained. From the loadings of the variables (Table 3) mainly catechin and epicatechin are the dominant features in the first principal component, accounting for 21.84% of the total variability; protocatechuicaldehyde dominates in the second principal component, representing 17.77% of the total variance and syringic and vanillic acids in the

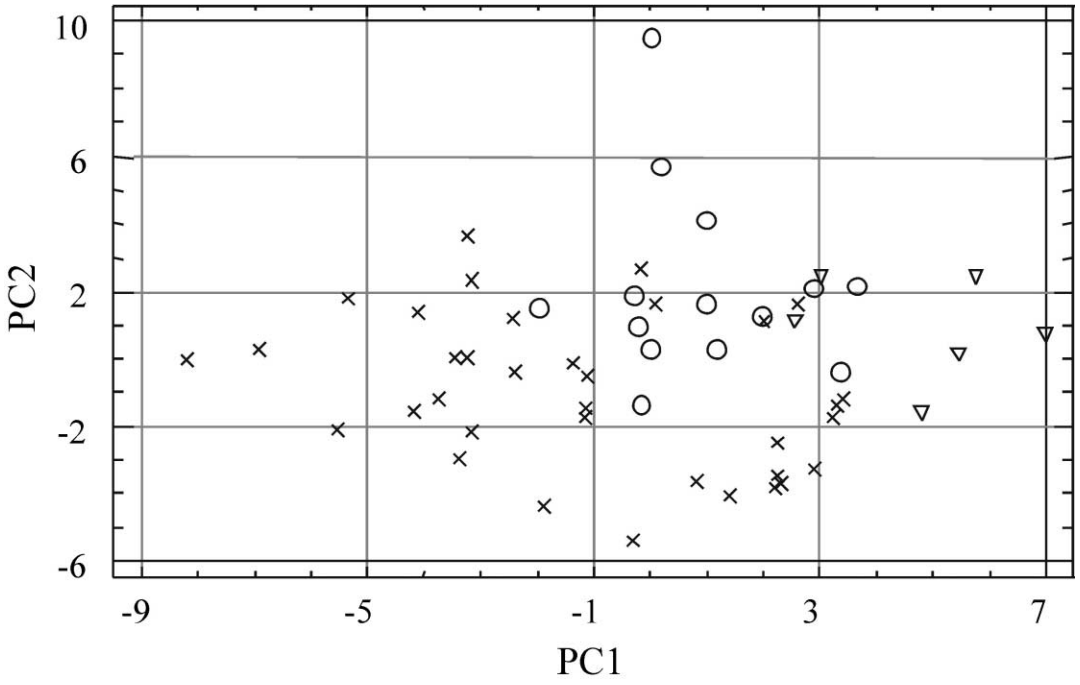

Fig. 2. Scores of the samples in the plane defined by the first two principal components: PC1 and PC2 (∇ = Lanzarote, **O** = south zone of Tenerife, **X** = north zone of Tenerife).

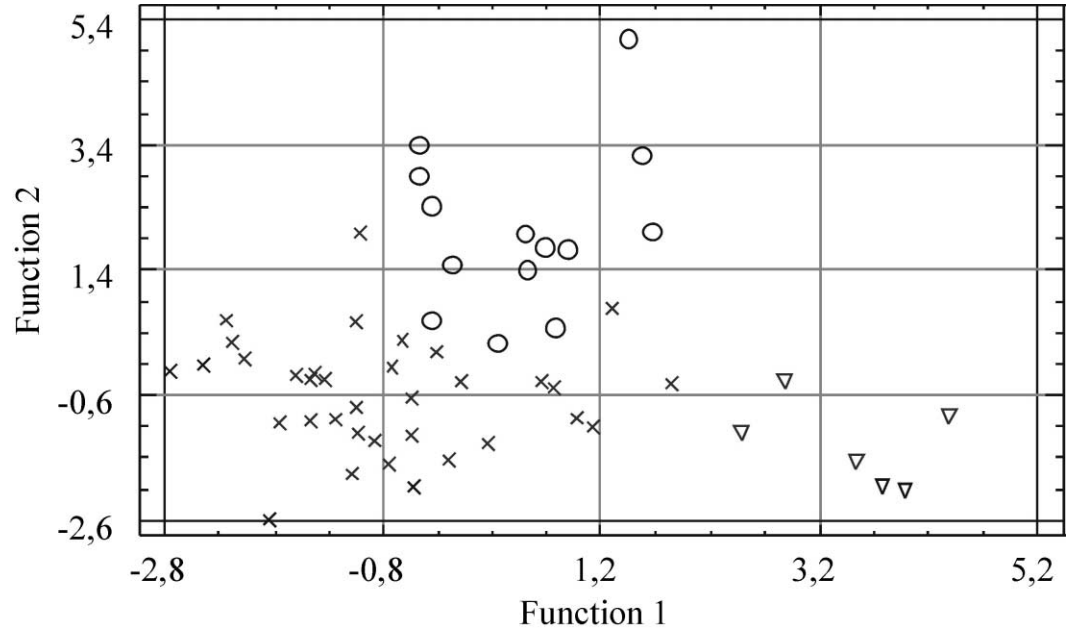

Fig. 3. Scattered plot of the 55 samples of wine according to three geographical zones in the plane defined by the two discriminant functions from five polyphenolic variables (∇ = Lanzarote, **O** = south zone of Tenerife, **X** = north zone of Tenerife).

third principal component, representing 12.6% of the total variance. In Fig. 2, when the scores of each wine sample were examined in a two-dimensional plot of the first two principal components (39.6% of the total variability) a certain separation of samples into three groups was found according to geographical areas of origin. The first, in the positive part of principal component 1, is mainly composed by samples from Lanzarote island. The second group, in the central and upper part of the graph, is mainly made up of samples from the south zone of Tenerife. The third group, mainly to the left of the principal component 1 and in the bottom part of principal component 2, is formed by samples from the north zone of Tenerife.

A supervised pattern recognition method has been applied in order to characterise the wine samples into the three mentioned classes (north and south of Tenerife, and Lanzarote). Using stepwise linear discriminant analysis, five variables: syringic acid, quercitrin, catechin, caffeic acid, and protocatechuicaldehyde were selected like the most discriminant features. When LDA was applied to the data set (55 samples and 5 features), two statistically significant ($P<0.05$) discriminant functions were obtained. The scores of the samples in these two discriminant functions are plotted in Fig. 3 and it can be seen that an acceptable differentiation of samples was obtained. Lanzarote essentially has higher concentrations of catechin and lower concentrations of quercetrin, which differentiate this island from the two zones of Tenerife. In turn, syringic and caffeic acids differentiate between the south and north zones of Tenerife. To validate the derived rules of classification and their stability for prediction leave-one-out cross-validation was used. Thus, the reliability of the classification model was studied in terms of recognition and prediction abilities. The model produced good percentages of correct recognition and prediction (Table 4). The values attained were in the 78.6–85.7% range for south Tenerife, 85.7–88.6% for north Tenerife and 100% for Lanzarote. When LDA was performed using all the features the percentages of correct recognition and prediction were slightly higher for north Tenerife than with the five features (Table 4).

Table 4
Classification results using LDA and leave-one-out cross validation

	Five variables		All variables	
	Recognition ability (%)	Prediction ability (%)	Recognition ability (%)	Prediction ability (%)
South Tenerife	85.7	78.6	85.7	78.6
North Tenerife	88.6	85.7	91.4	88.6
Lanzarote	100	100	100	100
Overall	89	85.5	90.9	87.3

Acknowledgements

We thank the councils of the denominations of origin for supplying the wine samples.

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