Available online at www.sciencedirect.com

SCIENCE DIRECT

Food Chemistry 100 (2007) 419–427

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Distribution of metals and phenolic compounds as a criterion to evaluate variety of berries and related jams

M. Plessi *, D. Bertelli, A. Albasini

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Modena e Reggio Emilia, Via Campi 183, 41100 Modena, Italy

Received 8 June 2004; received in revised form 12 September 2005; accepted 25 September 2005

Abstract

Polyphenols, anthocyanins, phosphate, iron, zinc, manganese, calcium, potassium, magnesium and sodium were evaluated both in berries belonging to the genera *Rubus* and *Ribes* and in related jams by means of spectrophotometric techniques, to verify a possible differentiation on the basis of these parameters. The phenolic and anthocyanins higher contents were detected for black currant fruits and jams. Regarding the total phenolic content, in 11 cases it is possible to observe a higher content in jams with respect to the related row fruits. All samples contain a high amount of potassium, calcium and magnesium but small quantities of sodium. Phosphate is abundant especially in currants. As regard jams all results about metals are lower than those obtained for fruits, this is due to the dilution during the preparation of the jams. Multivariate statistical analysis shows that on the basis of obtained analytical results it is possible differentiate not only fruit but also the derived jams.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: *Rubus*; *Ribes*; Polyphenols; Anthocyanins; Metals

1. Introduction

Since the cultivation and transformation of berries belonging to *Rubus* and *Ribes* genera is particularly important in mountains communities, we are currently involved in the study of the composition of these fruits and their transformation products. Particularly, we are interested in macro and microelements, essential for the physiological equilibrium of the organism, and phenolic substances with their well known antioxidant proprieties.

As well known, the chemical characterization with chemometric interpretation of analytical data is currently used, i.e., for the differentiation and classification of food products according to geographical origin or for the chemotaxonomic approach to botanical classification; some recent application are reported in references section (Benedetti, Mannino, Sabatini, & Marzaccan, 2004; Muik, Lendl, Molina-Diaz, Ortega-Calderon, & Ayora-Canada, 2004; Penza & Cassano, 2004; Terrab, Hernanz, & Heredia, 2004). Nowadays, food products have to satisfy numerous quality criteria before commercialization, especially in industrialized countries, where there is a need to have food products of high quality with well-defined characteristics. Among these quality criteria, the authenticity and traceability of wild berries and their product of transformation are very important. In order to assure these criteria the chemometric approach can be efficiently used. (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Plessi, Bertelli, Rastelli, Albasini, & Monzani, 1998).

Anthocyanins are the largest group of water soluble pigments in plant kingdom and are responsible for most of the red, purple and blue colors exhibited by most attractive, colorful, flavorful fruits and berries. Their presence in abundant amount in berries is well established as well the high total phenolic content (Benvenuti et al., 2004; Costantino, Albasini, Rastelli, & Benvenuti, 1992; Kahkonen et al., 1999; Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Rotundo et al., 1998; Skrede, 1997; Wang & Lin, 2000).

* Corresponding author. Tel.: +39 59 205 5147; fax: +39 59 205 5131.
E-mail address: Plessi.Maria@Unimore.it (M. Plessi).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.09.018

The elements characterization of berries is very interesting because their concentration can influence the quality of fruit, including organoleptic characteristics and stability, as well as the health status of consumers.

In a our previous work the contents of macro- and microelements, anthocyanins and total polyphenols in fruits of *Ribes*, *Rubus*, *Vaccinium* genera were studied (Plessi et al., 1998). Therefore, to confirm these results and to verify if a differentiation on the basis of the same parameter is possible, not only for fruits but also for their main product of transformation, the jams, we have analyzed samples of two species of *Rubus* (*R. fruticosus* L. and *R. idaeus* L.) and two of *Ribes* (*R. rubrum* L. and *R. nigrum* L.) and the jams obtained from each variety of fruit.

In fact, during the production of jam the fruits undergo to a long heating. Above all, the extended heating could influence in meaningful way the content in phenolic substances (Garcia-Viguera, Zafrilla, & Tomas-Barberan, 1999; Mazza & Miniati, 1994; Mikkelsen & Poll, 2002), mineral element composition is also influenced by heating, but especially by dilution with sugar.

In the present paper the content of phosphate, Zn, Fe, Mg, Mn, Ca, Na, K, the total phenolic content and anthocyanins have been determined by using microwave acid digestion followed by ICP-AES for minerals and well established spectrophotometric methods for phenolics. The chemical composition and the nutritional value of berries is influenced not only by cultivar, but also by many other factors such as production area, soil and climate, agricultural practices, quality of the irrigation water and eventually the storage and commercialization conditions. To avoid the influence of these factors, the samples were collected from only one farm during only one harvest. Monovariate and multivariate statistical analysis techniques have been applied to classify fruits and jams according to their metal and phenolic contents.

2. Experimental

2.1. Material

(a) Ultrapure water, obtained by a MilliQ Plus 185 system from Millipore (Milford, Mass., USA).
(b) Nitric Acid >69.5% (TraceSelect®), Folin–Ciocalteu reagent, Gallic acid pure reference compound (Fluka) (Buchs, Switzerland).
(c) Working standard solutions for metals were prepared by diluting with water Baker INSTRA Stock solutions (1000 ppm) (J.T. Baker, Milan, Italy).
(d) pH 1 buffer: dissolve 1.49 g of KCl into 100 ml of ultrapure water. Mix 25 ml of the KCl solution with 67 ml of 0.2 N HCl solution. Adjust to pH 1.0 ± 0.1 if necessary.
(e) pH 4.5 buffer: dissolve 1.64 g of sodium acetate in 100 ml of ultrapure water. Adjust to pH 4.5 ± 0.1 with HCl.

All reagents used to obtain buffers are of analytical grade and were purchased from Fluka (Buchs, Switzerland).

2.2. Sampling

Six blackberry (*Rubus fruticosus* L.) cultivar: Black Diamond, Black Satin, Darrow, Dirkensen, Hull Thornless and Smoothstem, four of cultivated and two of spontaneous raspberry (*Rubus idaeus* L.): Glen Moy, Heritage, Scepter, September, Wild 1°, Wild 2°, six of red currant (*Ribes rubrum* L.): Junifer, Jonkheer V.T., Rolan, Rosetta, Perfection and Red Lake, six of black currant (*Ribes nigrum* L.): Baldwin, Ben Lomond, Black Down, Tenah, Cumax and Titania were collected from only one farm during only one harvest. After the harvesting, fruits were immediately frozen and preserved at −18 °C until use. Each analysis was performed in triplicate on subsamples prepared to avoid unripe, damaged or overripe fruits.

2.3. Preparation of jams

To obtain jams, 100 g of fruit were heated and after some minutes heating, 70 g of sucrose were slowly added. The mixture was heated under stirring until the jam reached the appropriate density.

2.4. Extraction of phenolic compounds

Twenty grams of frozen berries or jams were accurately weighed into a beaker and added of 50 ml of a methanol/HCl (98:2, v/v) solution. One hour later the mixture was homogenized with Ultra Turrax T25 (IKA) for one minute at 11,000 rpm and then centrifuged for 15 min at 3000 rpm. The supernatant was filtered in a 100 ml volumetric flask. The extraction was repeated and the reunited extracts were filled up to 100 ml with methanol/HCl (98:2, v/v), if necessary.

2.5. Total polyphenols determination

The method used was based on the Folin–Ciocalteu reagent and the spectrophotometric determination, results are expressed as g/100 of gallic acid equivalents.

One ml of methanol/HCl (98:2, v/v) extract was diluted in a 100 ml volumetric flask with 70 ml of ultrapure water, 5 ml of Folin–Ciocalteu reagent and, after one minute, 10 ml of hot saturated solution of Na_2CO_3 were added. Then the solution was filled up to volume. After 2 h, the absorbance at 760 nm was measured using gallic acid as calibration standard (Singleton & Rossi, 1965).

2.6. Total anthocyanins determination

Anthocyanins exist in a pH-dependent equilibrium among at least four different structural forms. The well known red or purple color is due to the oxonium or flavilium form, which is present only at low pH in aqueous solution. By rising the pH above 4, yellow or colorless carbinol form is produced.

The determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5. The difference in absorbance at 510 nm is proportional to the anthocyanins content (Giusti & Wrolstad, 2001).

One ml of each methanol/HCl (98:2, v/v) extract was filled up to volume with buffer at pH 1 in a 100 ml volumetric flask. Another 1 ml was filled up at the same volume with buffer at pH 4.5. After 30 min the absorbances at 510 nm of the two solutions were measured.

Even though cyanidin-3-glucoside is not the only anthocyanin in the analyzed berries, it was used to express the results because of its historical usage for similar assays.

To calculate the concentration of total anthocyanins, expressed as g of cyanidine/100 g of fruit, the following formula was applied :

$$\%\mathrm{w/w} = (\Delta A * \mathrm{MW} * \mathrm{DF} * V * 100)/\varepsilon L * \mathrm{Wt},$$

where $\Delta A = \mathrm{Abs}\ (\mathrm{pH}\ 1) - \mathrm{Abs}\ (\mathrm{pH}\ 4.5)$, ε = Cyd-3-glu molar absorbance (26,900), MW = cyanidine molecular weight (449.2), DF = dilution factor, V = final volume (mL), Wt = sample weight (g), L = cell path length (usually 1 cm).

2.7. Metals and phosphate determination

For the mineral content determination, fruits and jams were dried at 110 °C until constant weight then grinded. To avoid the risk of metal contamination all laboratory glassware were scrupulously soaked in 1 M HNO_3, rinsed with ultrapure water, filled with 0.1 M EDTA, kept for 1 night and again rinsed with ultrapure water.

An aliquot of 0.3 g of dried fruit or jam was digested with 3 ml of nitric acid (69.5%). Digestion was carried out by a programmable Milestone 1200 microwave (Milestone) digestion system, equipped with MDR vessel. The digestion conditions were: 2 min at 250 W; 2 min at 0 W; 6 min at 250 W; 5 min at 400 W and finally 5 min at 600 W.

After cooling, the content of each vessel was transferred in a 50 ml flask an diluted to volume with ultrapure water. The phosphate, iron, manganese, zinc, calcium, potassium, magnesium and sodium contents were determined by Inductively Coupled Plasma Atomic Emission Spectrophotometry using a ICP-AES Spectro Flame D (Spectra Analytical Instruments). Calibration was performed with standard solutions from Baker. The analytical conditions are reported in Table 1.

2.8. Statistical analysis

In this paper, several monovariate and multivariate statistical methods were performed, aiming to explore the relationship between the mineral and phenolic composition of fruits and genera and to verify if a differentiation on the basis of these chemical parameters is possible not only for the berries but also for their main transformation product, the jams.

Table 1
ICP-AES instrumental conditions

Metal	λ (nm)	Calibration range (ppm)
PO_4	185.94	20–50
Fe	259.94	0.5–2
Mn	293.93	0.5–2
Zn	213.86	0.1–5
Ca	422.67	1–50
K	766.49	20–100
Mg	285.21	1–50
Na	589.59	0.5–2

Two data matrix (one for fruits and one for jams) of 24 rows (berries cultivar) and 10 columns, which corresponded to the mentioned analytical descriptors, were built for use in the chemometric calculation.

Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were used to evaluate the statistical significance of measured analytical differences between fruits and between jams. To evaluate the most important variables which discriminate between the two genera fruits, post hoc test was performed using the Tukey "Honest Significant Difference test" (HSD). For all these monovariate tests the P level was set at 0.05. In order to achieve a more reliable differentiation between different fruits and genera and between jams, pattern recognition and supervised pattern recognition procedures were applied to the data matrix. The most important use of these chemometric methods is to represent the n-dimensional data set in a smaller number of dimensions, usually two or three. This allows the observations of groupings of cases, which can define the structure of the data set; therefore, Principal Component Analysis (PCA), Cluster Analysis (CA) and Canonical Discriminant Analysis (CDA) were used in the attempt to classify fruits and jams according to their metal and phenolic contents. Another type of information that can be obtained, in particular from PCA, is which are the most discriminating of the studied descriptors. All analyses was performed using Statistica 6 for Windows (StatSoft® Italia, Vigonza, Italy).

3. Results

Total polyphenolic content reaches the maximum value in black currant, as regards the anthocyanins the higher content in fruits and jams was obtained for black currant, the lowest for red currant (Table 2). Comparing the total phenolic content in fruits and jams in 11 cases the measured content in jams are higher of those in fruits; this fact should be probably due to the best extractability of phenolics caused by heating, as demonstrated in studies on various grape processing techniques (Netzel et al., 2003; Vrhovsek, Vanzo, & Nemanic, 2002).

The measured differences between various fruits resulted statistically significant when analyzed by ANOVA and MANOVA for a P level of 0.05. The mean concentrations

Table 2
Contents of natural antioxidants in berry fruits and in derived jams

	Fruits		Jams	
	Total phenolics[a]	Anthocyanins[b]	Total phenolics[a]	Anthocyanins[b]
Blackberry				
Black Diamond	0.556	0.102	0.494	0.059
Black Satin	0.476	0.130	0.368	0.072
Darrow	0.337	0.073	0.310	0.032
Dirkesen	0.462	0.100	0.358	0.077
Hull Thornless	0.405	0.118	0.519	0.063
Smoothstem	0.402	0.066	0.360	0.048
Average	*0.440 ± 0.076*	*0.098 ± 0.025*	*0.402 ± 0.08*	*0.058 ± 0.016*
Raspberry				
Glen Moy	0.320	0.016	0.361	0.007
Heritage	0.251	0.052	0.279	0.027
Scepter	0.316	0.078	0.315	0.016
September	0.255	0.039	0.289	0.018
Wild berry no. 1	0.271	0.053	0.253	0.021
Wild berry no. 2	0.276	0.025	0.218	0.014
Average	*0.282 ± 0.030*	*0.043 ± 0.022*	*0.286 ± 0.049*	*0.017 ± 0.007*
Red currant				
Junifer	0.353	0.022	0.356	0.012
Jonkheer V.T.	0.337	0.036	0.425	0.023
Rolan	0.267	0.021	0.256	0.016
Rosetta	0.299	0.017	0.338	0.009
Perfection	0.207	0.017	0.251	0.008
Red Lake	0.418	0.016	0.371	0.006
Average	*0.314 ± 0.073*	*0.022 ± 0.008*	*0.333 ± 0.068*	*0.012 ± 0.006*
Black currant				
Baldwin	0.657	0.240	0.684	0.146
Ben Lomond	0.653	0.230	0.893	0.150
Black Down	0.795	0.235	0.621	0.170
Tenah	0.797	0.251	0.575	0.157
Cumax	0.764	0.342	0.806	0.203
Titania	0.910	0.273	0.842	0.163
Average	*0.763 ± 0.970*	*0.262 ± 0.042*	*0.737 ± 0.128*	*0.165 ± 0.021*
Total average	*0.449 ± 0.206*	*0.106 ± 0.099*	*0.439 ± 0.198*	*0.063 ± 0.064*
P(ANOVA)	*<0.05*	*<0.05*	*<0.05*	*<0.05*
P(MANOVA)	*<0.05*			

[a] Expressed as g gallic acid/100 g FW (fresh weight).
[b] Expressed as g of cyanidin-3-glycoside/100 g FW.

are generally in good agreement with previously published data (Benvenuti et al., 2004; Costantino et al., 1992; Kahkonen et al., 1999; Moyer et al., 2002; Rotundo et al., 1998; Skrede, 1997; Wang & Lin, 2000).

The mineral content of fruits, expressed as mg/100 g on a dry basis, is shown in Table 3. As expected potassium is the more abundant metal with higher values in fruits of *Ribes* genera and an average content of 1556.8 mg/100 g. All considered samples contain a high amount of calcium and magnesium, but little quantities of sodium. Phosphate is also abundant especially in red and black currant samples. Also for iron, zinc, and manganese the results are in sufficient accord with previously published data (Plessi et al., 1998; Varo, Lahelma, Nuurtamo, Saari, & Koivistoinen, 1980). Analysis of variance shows that the differences between the fruits are statistically significant for a P level <0.05 for phosphate, iron, manganese, potassium, magnesium and sodium. Multiway ANOVA also shows the significance of differences between the various fruits.

As regards jams, results are reported in Table 4, obviously all results are lower than those obtained for fruits, in relation to the dilution of fruit pulp with sugar during the jam preparation. In these products all the measured differences are statistically significant except for iron.

On all these data the post hoc Tukey "HSD" test was performed and its results are reported in Table 5. As regards fruits, the polyphenols and anthocyanins contents are not fundamental to discriminate between genera; for metal content the variables responsible of main difference between *Rubus* and *Ribes* genus fruits are phosphate,

Table 3
Metal contents in berry fruits expressed as mg/100 g dried weight

	PO_4	Fe	Mn	Zn	Ca	K	Mg	Na
Blackberry								
Black Diamond	796.67	4.95	3.10	2.02	207.17	981.67	165.17	4.36
Black Satin	624.29	3.79	2.04	1.96	175.71	772.55	135.27	8.89
Darrow	859.19	7.95	1.83	2.33	153.65	1016.1	144.64	7.54
Dirkesen	634.17	8.45	1.33	2.95	232.83	931.67	165.67	9.80
Hull Thornless	619.00	4.73	2.87	2.19	198.33	753.83	141.00	9.80
Smoothstem	635.77	5.56	1.71	2.32	191.01	906.82	153.74	7.20
Average	*694.85 ± 105.15*	*5.91 ± 1.87*	*2.15 ± 0.69*	*2.30 ± 0.35*	*193.12 ± 27.08*	*893.76 ± 108.24*	*150.92 ± 12.74*	*7.93 ± 2.07*
Raspberry								
Glen Moy	819.13	8.49	1.08	3.29	198.17	1347.7	180.37	5.36
Heritage	683.18	8.96	1.35	2.85	199.87	977.64	162.66	3.35
Scepter	604.17	8.22	1.61	2.99	267.83	946.67	173.50	3.92
September	731.61	14.16	3.11	2.97	165.39	881.86	129.96	3.69
Wild berry no. 1	823.27	7.02	2.10	2.83	216.41	1378.8	156.89	4.17
Wild berry no. 2	790.46	7.49	1.17	2.87	153.15	1209.5	152.32	7.73
Average	*741.97 ± 86.56*	*9.06 ± 2.+60*	*1.74 ± 0.77*	*2.97 ± 0.17*	*200.14 ± 40.66*	*1123.70 ± 216.29*	*159.28 ± 17.73*	*4.70 ± 1.63*
Red currant								
Junifer	1120.0	4.32	0.82	3.04	154.50	1585.0	82.95	16.78
Jonkheer V.T.	986.67	4.18	0.93	2.25	129.67	1503.3	79.35	11.85
Rolan	900.17	3.90	0.80	1.47	181.20	1748.8	79.18	9.75
Rosetta	794.03	5.10	1.03	1.73	228.99	1426.0	89.06	9.74
Perfection	896.97	5.65	0.90	2.02	121.04	1718.9	83.86	7.85
Red Lake	1073.0	5.23	1.10	2.12	204.93	1671.1	97.63	15.21
Average	*961.81 ± 121.79*	*4.73 ± 0.69*	*0.93 ± 0.12*	*2.11 ± 0.54*	*170.06 ± 42.64*	*1608.85 ± 127.06*	*85.34 ± 7.02*	*11.86 ± 3.48*
Black currant								
Baldwin	999.33	3.41	0.88	3.16	169.50	1446.4	95.76	7.74
Ben Lomond	1030.6	3.22	0.87	1.79	202.63	1461.9	109.72	7.07
Black Down	1048.1	3.59	0.71	2.15	207.78	1512.0	107.98	7.43
Tenah	901.20	4.04	0.83	2.23	214.45	1570.4	101.97	8.24
Cumax	1118.5	5.20	1.10	4.95	236.89	1714.5	114.36	5.91
Titania	1122.6	5.00	0.95	2.73	198.53	1635.6	110.59	6.52
Average	*1036.7 ± 82.45*	*4.08 ± 0.84*	*0.89 ± 0.13*	*2.84 ± 1.14*	*204.96 ± 22.01*	*1556.8 ± 104.37*	*106.73 ± 6.73*	*7.15 ± 0.84*
Total average	*858.84 ± 174.16*	*5.94 ± 2.51*	*1.42 ± 0.73*	*2.55 ± 0.72*	*192.07 ± 34.73*	*1295.78 ± 334.39*	*125.57 ± 33.21*	*7.61 ± 3.35*
P (ANOVA)	*<0.05*	*<0.05*	*<0.05*	*0.101*	*0.327*	*<0.05*	*<0.05*	*<0.05*
P (MANOVA)	*<0.05*							

potassium and magnesium, while zinc and calcium seem to be absolutely not related with genetic origin. As regards jam, post hoc "HSD" test shows that jams obtained from the two different genera of fruits considered can be really differentiate only for their phosphate and potassium contents and partially for manganese.

In Fig. 1 the plot of the first two principal components obtained from the metal content data of fruit samples is reported. As evident, four groups of samples are identifiable, even if not completely separated, corresponding to the four considered berries, the classification is more evident considering the two genera (*Rubus* and *Ribes*). The PCA allows to explain the 80% of the total variance with 3 components (PC1 50%, PC2 18%, PC3 12%), the analytical variables more related with the first component are phosphate, magnesium and potassium, confirming that these variables are the main responsible of differentiation between *Rubus* and *Ribes* genera, as it was found also by HSD test. The second component is mainly related with zinc and calcium and seem to be more able to discriminate between species of the two different genera. Introducing in the analysis the phenolic substances data, PCA explain the 78% of total variance with three components (PC1 42%, PC2 15%, PC3 11%). The plot reported in Fig. 2 shows a better classification only for samples belonging to *Ribes* genera. The phenolic variables appear to be particularly related to the second component.

As regard jams, little differences were observed with respect to fruits. PCA on metal content data (Fig. 3) is able to discriminate between sample of the *Ribes* and *Rubus* genera, but not completely between species of the same genera. The first three component explain the 74% of

Table 4
Metal contents in jams expressed as mg/100 g dried weight

	PO_4	Fe	Mn	Zn	Ca	K	Mg	Na
Blackberry								
Black Diamond	161.95	2.05	0.92	0.70	61.31	240.59	39.74	3.29
Black Satin	137.30	1.31	0.54	0.36	32.82	141.98	31.05	7.54
Darrow	183.56	1.37	0.46	0.43	42.65	237.08	34.29	4.07
Dirkesen	120.80	2.66	0.36	0.74	54.78	187.19	35.37	5.91
Hull Thornless	114.01	0.95	0.65	0.54	41.96	148.64	29.03	3.60
Smoothstem	123.00	1.30	0.65	0.52	44.40	214.83	31.17	3.93
Average	*140.10 ± 27.27*	*1.61 ± 0.63*	*0.60 ± 0.19*	*0.55 ± 0.15*	*46.32 ± 10.15*	*195.05 ± 43.04*	*33.44 ± 3.86*	*4.72 ± 1.66*
Raspberry								
Glen Moy	175.16	2.10	0.28	0.95	50.08	224.44	42.22	2.80
Heritage	111.65	1.20	0.66	0.78	36.29	148.92	29.18	1.71
Scepter	149.17	2.03	0.28	0.91	48.45	219.83	34.80	1.75
September	164.00	2.68	0.65	1.76	43.13	172.51	30.85	2.12
Wild berry no. 1	169.27	1.83	0.42	0.77	43.96	241.77	35.42	2.91
Wild berry no. 2	152.75	2.03	0.48	1.34	31.01	202.66	30.83	2.57
Average	*153.67 ± 22.80*	*1.98 ± 0.48*	*0.46 ± 0.17*	*1.08 ± 0.39*	*42.15 ± 7.28*	*201.68 ± 34.9*	*33.88 ± 4.76*	*2.31 ± 0.52*
Red currant								
Junifer	338.34	1.74	0.25	0.93	53.77	338.34	31.21	7.73
Jonkheer V.T.	229.49	1.53	0.22	0.66	34.89	278.19	21.05	4.75
Rolan	273.50	1.57	0.22	0.47	55.77	341.00	26.30	3.59
Rosetta	177.28	2.00	0.26	0.34	55.54	241.83	20.13	4.08
Perfection	165.33	1.20	0.14	0.32	28.13	274.17	16.68	2.70
Red Lake	177.39	1.33	0.20	0.41	38.25	275.09	16.18	3.79
Average	*226.89 ± 68.27*	*1.56 ± 0.29*	*0.22 ± 0.04*	*0.52 ± 0.23*	*44.39 ± 12.12*	*291.44 ± 39.64*	*21.92 ± 5.83*	*4.44 ± 1.74*
Black currant								
Baldwin	250.83	1.54	0.24	0.57	52.64	350.07	26.25	3.48
Ben Lomond	271.21	1.39	0.25	1.74	52.73	383.53	27.02	3.56
Black Down	276.13	1.29	0.26	0.58	66.25	324.90	31.77	3.18
Tenah	242.01	1.23	0.21	0.98	54.08	381.66	26.17	4.34
Cumax	362.03	2.04	0.37	0.63	82.50	454.62	39.19	3.67
Titania	259.73	1.35	0.24	0.57	52.64	355.34	28.25	2.62
Average	*276.99 ± 43.52*	*1.47 ± 0.30*	*0.26 ± 0.06*	*0.84 ± 0.47*	*60.14 ± 12.18*	*375.02 ± 44.66*	*29.78 ± 5.06*	*3.47 ± 0.57*
Total average	*199.41 ± 70.21*	*1.66 ± 0.46*	*0.0.39 ± 0.2*	*0.75 ± 0.39*	*48.25 ± 12.23*	*265.80 ± 84.29*	*29.76 ± 6.71*	*3.74 ± 1.52*
P(ANOVA)	*<0.05*	*0.245*	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*
P(MANOVA)	*<0.05*							

Table 5
Results of HSD test: the same letter in the same column indicates no significant differences ($P < 0.05$)

	TP[b]	Ant[c]	PO_4	Fe	Mn	Zn	Ca	K	Mg	Na
Fruits[a]										
BB	B	B	A	A	B	A	A	A	C	A
RB	A	A	A	B	AB	A	A	A	C	A
RC	A	A	B	A	A	A	A	B	A	B
BC	C	C	B	A	A	A	A	B	B	A
Jams[a]										
BB	B	A	A	A	C	AB	AB	A	B	B
RB	A	A	A	A	BC	B	A	A	B	A
RC	A	A	B	A	A	A	AB	B	A	B
BC	C	B	B	A	AB	AB	B	C	AB	AB

[a] BB, blackberry; RB, raspberry; RC, red currant; BC, black currant.
[b] TP, total phenolics.
[c] Ant, anthocyanins.

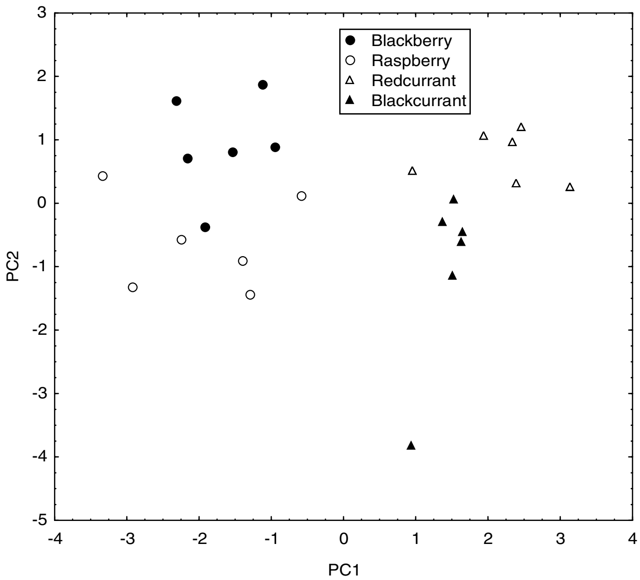

Fig. 1. Plot of first two PCs for metal contents in fruits.

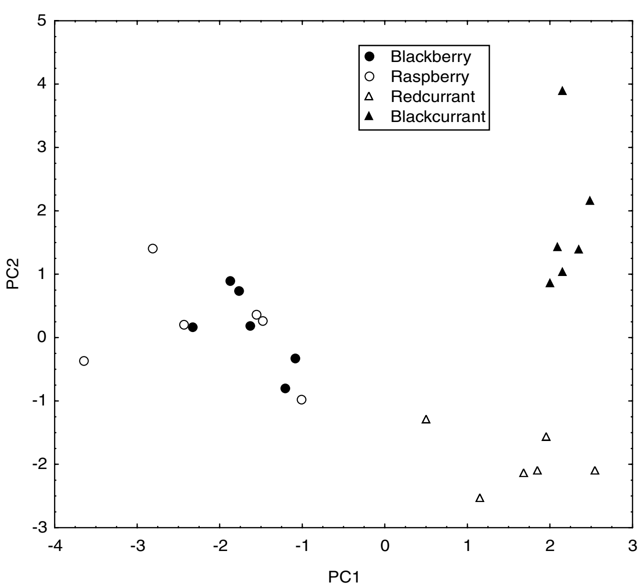

Fig. 2. Plot of first two PCs for metal and phenolic contents in fruits.

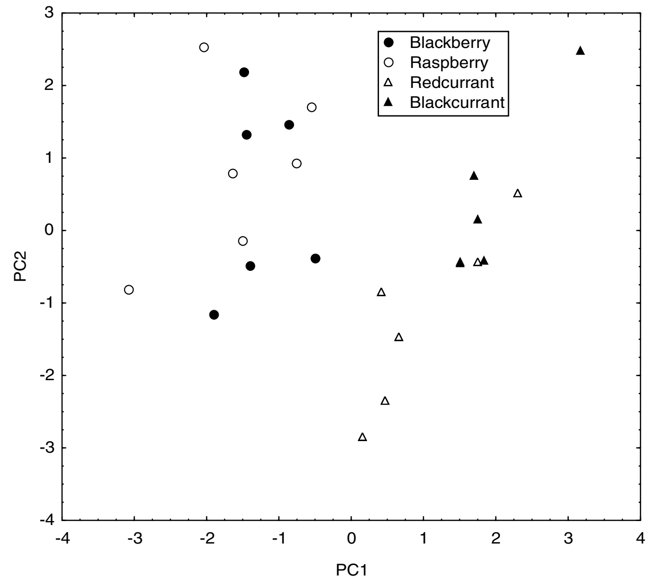

Fig. 3. Plot of first two PCs for metal contents in jams.

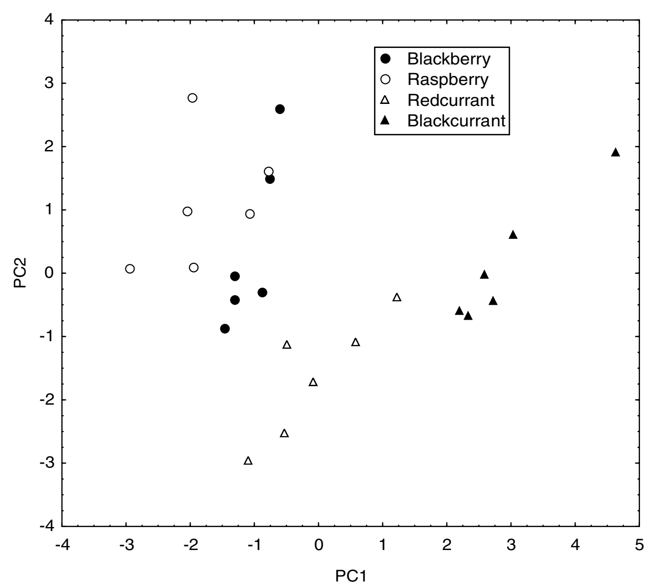

Fig. 4. Plot of first two PCs for metal and phenolic contents in jams.

the total variance (PC1 35%, PC2 25%, PC3 14%). In this case, the variables most related with the first component are phosphate, manganese and potassium, confirming the results of HSD test on jams. Introducing polyphenols data is more evident the discrimination between black and red currant samples, the first three component explain the 71 % of the total variance (PC1 38%, PC2 21%, PC3 12%) with polyphenols and anthocyanins contents particularly related with the first component (Fig. 4). In conclusion PCA allows to discriminate between the two considered genera of fruit and between the related jams. Including in the analysis the data about the total phenolic and anthocyanins contents, the discrimination capacity, especially for red and black currants fruits and jams, results improved.

Performing cluster analysis on data, the best result is obtained using complete linkage as clustering method and squared Euclidean distance as similarity measure on metal and phenols content of fruits. In fact this method is able to classify correctly all cultivar of *Ribes* and *Rubus* genera except Glen Moy and Wild berry no. 1 raspberry cultivar.

Applying this method to the data from jams the result obtained by this technique is poorer, in fact looking at the posteriori probabilities, jams of three cultivar of red currant are badly classified with *Rubus* genera jams.

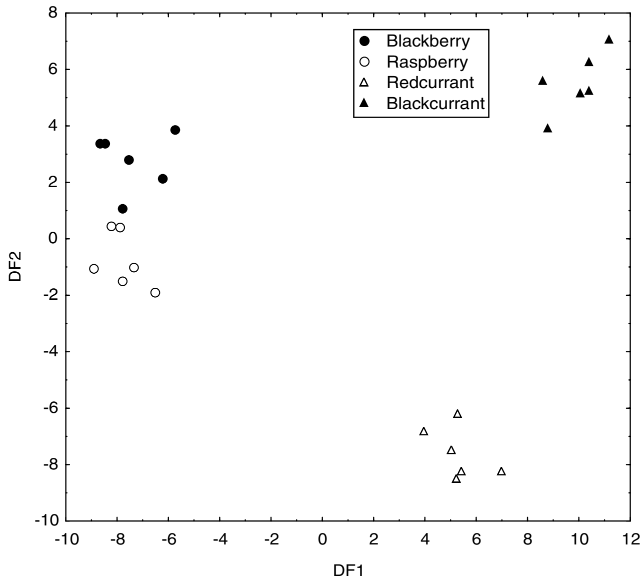

Fig. 5. Plot of two canonical discriminant functions obtained for metal and phenolic contents of fruits.

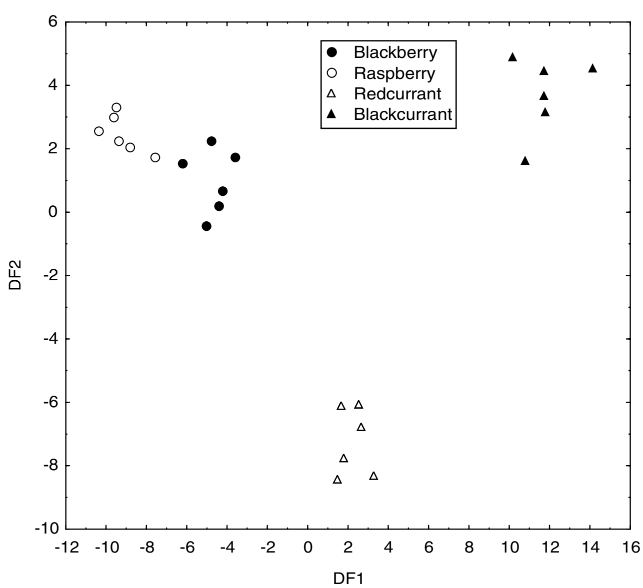

Fig. 6. Plot of two canonical discriminant functions obtained for metal and phenolic contents of jams.

As regard canonical discriminant analysis, the results are graphically reported in Fig. 5 for fruits and Fig. 6 for jams, the analysis was performed on all analytical variables. As evident, the method is able to discriminate not only between different genera but also between different species of berries and jams, especially in the case of black and red currant.

In the case of fruit the first two discriminant function explain the 98.2% of variance (DF1 70.2%, DF2 28.1%), in the case of jams the 97.4% (DF1 75.3%, DF2 22.1%). The first two calculated discriminant function for fruits and jams are reported below:

$$\begin{aligned}
&\text{Fruits :}\\
&\text{DF1} = 0.84\text{PO}_4 - 0.25\text{Zn} + 0.03\text{Fe} - 1.82\text{Mg}\\
&\qquad - 1.17\text{Mn} + 1.06\text{Ca} - 0.55\text{Na} + 0.20\text{K}\\
&\qquad + 0.51\text{TP} + 0.23\text{Ant}\\
&\text{DF2} = 0.08\text{PO}_4 - 1\text{Zn} + 0.4\text{Fe} + 1.07\text{Mg} - 0.18\text{Mn}\\
&\qquad - 1.02\text{Ca} - 0.15\text{Na} + -0.32\text{K} + 0.08\text{TP} + 1.48\text{Ant}\\
&\text{Jams :}\\
&\text{DF1} = 0.78\text{PO}_4 - 0.31\text{Zn} - 0.38\text{Fe} - 2.22\text{Mg} - 0.36\text{Mn}\\
&\qquad + 0.96\text{Ca} - 0.3\text{Na} + 0.96\text{K} + 0.71\text{TP} + 0.41\text{Ant}\\
&\text{DF2} = -0.6\text{PO}_4 + 0.98\text{Zn} - 0.35\text{Fe} + 1.79\text{Mg} - 0.3\text{Mn}\\
&\qquad + 0.84\text{Ca} - 0.86\text{Na} - 0.53\text{K} + 0.95\text{TP} - 0.23\text{Ant}
\end{aligned}$$

Looking at the posteriori probabilities, all samples were correctly classified. To evaluate the classification performance the leave-one-out method (Henrion & Henrion, 1994) was used as a validation procedure. A prediction ability of 100% was obtained for fruit and of 95.8% for jams.

The data obtained from fruits, all in the same field and therefore cultivated in the same conditions, have confirmed that the genome is the principal factor that determines the contents of the metals and phenolic compounds in the considered berries. This results confirm the ones obtained in an other our published work (Plessi et al., 1998) and shows that these fruits could be differentiable on the basis of their metal, anthocyanins and polyphenols contents. The employed chemometric techniques also allowed to perform the classification on derived jams.

References

Benedetti, S., Mannino, S., Sabatini, A. G., & Marzaccan, G. L. (2004). Electronic nose and neural network use for the classification of honey. *Apidologie, 35*(4), 397–402.

Benvenuti, S., Pellati, F., Melegari, M., & Bertelli, D. (2004). Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus, Ribes* and *Aronia*. *Journal of Food Science, 69*(3), 164–169.

Costantino, L., Albasini, A., Rastelli, G., & Benvenuti, S. (1992). Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Medica, 58*, 342–344.

Garcia-Viguera, C., Zafrilla, P., & Tomas-Barberan, F. A. (1999). Influence of processing and storage conditions in strawberry jam color. *Food Science and Technology International, 5*(6), 487–492.

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV–Visible spectroscopy. In R. E. Wrolstad, T. E. Acree, H. An, E. A. Decker, M. H. Pennere, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, & P. Sporns (Eds.), *Current protocol in food analytical chemistry* (pp. F1.2.1–F.1.2.13). New York: Wiley.

Henrion, R., & Henrion, G. (1994). Uberwachte klassifikation. In R. Henrion & G. Henrion (Eds.), *Multivariate datenanalyse* (pp. 71–73). Berlin: Springer-Verlag.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*(10), 3954–3962.

Mazza, G., & Miniati, E. (1994). *Anthocyanins in fruits, vegetables and grains*. Boca Raton FL: CRC press.

Mikkelsen, B. B., & Poll, L. (2002). Decomposition and transformation of aroma compounds and anthocyanins during black currant (*Ribes nigrum* L.) juice processing. *Journal of Food Science, 67*(9), 3447–3455.

Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium, Rubus* and *Ribes*. *Journal of Agricultural and Food Chemistry, 50*, 519–525.

Muik, B., Lendl, B., Molina-Diaz, A., Ortega-Calderon, D., & Ayora-Canada, M. J. (2004). Discrimination of olives according to fruit quality using Fourier transform Raman spectroscopy and pattern recognition techniques. *Journal of Agricultural and Food Chemistry, 52*(20), 6055–6060.

Netzel, M., Strass, G., Bitsch, I., Konitz, R., Christmann, M., & Bitsch (2003). Effect of grape processing on selected antioxidant phenolics in red wine. *Journal of Food Engineering, 56*, 223–228.

Penza, M., & Cassano, G. (2004). Chemometric characterization of italian wines by thin-film multisensors array and artificial neural network. *Food Chemistry, 86*(2), 283–296.

Plessi, M., Bertelli, D., Rastelli, G., Albasini, A., & Monzani, A. (1998). Fruits of *Ribes*, *Rubus*, *Vaccinium*, and *Prunus* genus. Metal contents and genome. *Fresenius Journal of Analytical Chemistry, 361*, 353–354.

Rotundo, A., Bounous, G., Benvenuti, S., Vampa, G., Melegari, M., & Soragni, F. (1998). Quality and yield of *Ribes* and *Rubus* cultivars grown in Southern Italy hilly location. *Phytotherapy Research, 12*, S135–S137.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolibdic-phosphotungstic reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Skrede, G. (1997). Evaluation of colour quality in blackcurrant fruits grown for industrial juice and syrup production. *Norwegian Journal of Food Science, 1*, 67–74.

Terrab, A., Hernanz, D., & Heredia, F. J. (2004). Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. *Journal of Agricultural and Food Chemistry, 52*(11), 3441–3445.

Varo, P., Lahelma, M., Nuurtamo, M., Saari, E., & Koivistoinen, P. (1980). Mineral element composition of Finnish foods. VII. Potato, vegetables, fruits, berries, nuts and mushrooms. *Acta Agriculturae Scandinavica*(Suppl. 22), 89–113.

Vrhovsek, U., Vanzo, A., & Nemanic, J. (2002). Effect of red wine maceration techniques on oligomeric and polymeric proanthocyanidins in wine, cv Blaufrankisch. *Vitis, 41*(1), 47–51.

Wang, S. Y., & Lin, H. S. (2000). Antioxidant activity in fruit leaves of blackberry, raspberry, and strawberry varies with cultivar and development stage. *Journal of Agricultural and Food Chemistry, 46*(10), 4113–4117.