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Analytical Methods

Characterisation of Italian commercial apricot juices by high-performance liquid chromatography analysis and multivariate analysis

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Abstract

The modern fruit juice industry needs their products to be characterized by high-quality attributes to meet consumers' expectation. In this view, the composition of 26 Italian commercial apricot juices obtained from organic, integrated and conventional agriculture was analysed for carbohydrates, organic acids, amino acids, phenolic compounds and furanic compounds by high-performance liquid chromatography (HPLC). The content of 5-hydroxymethylfurfural in apricot juices (range 0.1–18 mg/l) was within the regulatory limit of 20 mg/l. The lack of furanic compounds in apricot fresh fruits confirmed their importance as quality markers of heating condition during processing and storage of fruit juices. Univariate analysis disclosed some significant differences among the composition of the apricot juices in terms of glucose, fructose, malic acid, glycine, chlorogenic acid, rutin, and a^*-parameter (redness). Principal component analysis on chemical composition of apricot juices resulted in two principal components (PCs) that accounted for 66% of the total variance. Organic apricot juices showed some separation from the other juices, whereas a lack of distinction between integrated and conventional juices appeared.

Keywords: Apricot juice; Compositional data; HPLC; Organic foods; PCA

1. Introduction

Apricot is the third most widely grown stone fruit crop with a world production of ca. 2.8 Mt in 2005. The production is mainly located in the Mediterranean countries which collectively account for 40% of global production. Italy is the third largest apricot producing country in the world with about 180.000 tons/year, and around 40% of the Italian production is located in the Emilia–Romagna region, north of Italy (Mennone, Mattatelli, & Pirazzini, 2005). Moreover, fruit juices constitute an important sector of the local food industry and several major fruit processing companies are located in this region as well (Lunati, 2005). The modern apricot industry needs commercial juices characterized by high-quality juice attributes to meet consumers' expectation and for regulatory purposes.

The common concern of consumers about food quality and nutrition has led to an increasing attention in the market of foods with natural ingredients, including food products from organic and integrated agriculture. Organic agriculture differs from the conventional type by its avoidance of synthetic chemicals for soil preparation, fertilization and pest control (Council Regulation ECC No. 2092/91). Integrated agriculture allows production methods in between the organic and the conventional types. Numerous claims are made about the benefits of organic foods, in order to justify the premium price that consumers have to pay. Although products from organic origin are believed to be 'nutritionally superior', healthier', and also 'tastier' than the corresponding conventional foods, a clear evidence supporting this assumption requires further and specific research (Brandt & Mølgaard, 2001; Fillion & Arazi, 2002; Woese, Lange, Boess, & Bögl, 1997).

With regard to fruits and juices, it is well known that varietal, geographical, seasonal and maturity differences, as well

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as processing conditions generally affect their composition. Carbohydrates, organic acids (Bartolozzi, Bertazza, Bassi, & Cristoferi, 1997; Gurrieri, Audergon, Albagnac, & Reich, 2001; Wills, Scriven, & Greenfield, 1983), amino acids (Katona, Sass, & Molnár-Perl, 1999) and phenolic compounds (Fernández de Simón, Pérez-Ilzarbe, Hernández, Gómez-Cordovés, & Estrella, 1992; Garcia-Viguera, Bridle, Ferreres, & Tomas-Barberan, 1994; Trifirò, Risi, Cocconi, & Bolzoni, 2006) are among the major constituents of apricot fruits and juices. These compounds are useful to monitor the quality of fruits during ripening, processing and storage, and they contribute to the nutritional and sensory attributes of both fresh fruits and juices. Carbohydrates are the main source of energy for humans, being highest for glucose, followed by sucrose and fructose (Miller, Colagiuri, & Brand, 1986). Fructose has been reported to be 1.8 times sweeter than sucrose (Doty, 1976), whilst glucose is less sweet than sucrose (Pangborn, 1963). The content of organic acids in fruit juices not only influences their flavour but also their stability, nutrition, acceptability and keeping quality. In particular, malic and citric acids are correlated to the perception of sourness in peach juices (Esti et al., 1997) and a sensory contribution is expected to occur in apricot juices also (CoSeteng, McLellan, & Downing, 1989; Noble, Philbrick, & Boulton, 1986). Phenolic compounds may have potential health benefits due to their antioxidant properties (Clydesdale, Kolasa, & Ikeda, 1995; Johnson, Williamson, & Musk, 1994; Rice-Evans, Miller, & Paganga, 1996), thus fruit juices with a high content of bioactive compounds may represent a specialty products/ingredients in the design of functional juices. Furanic compounds have been identified as degradation products associated with non-enzymatic browning in model solution and fruit juices, and are considered as markers for heat abuse during processing and storage of fruit juices (Lee & Nagy, 1996). The amino acid proline is considered a marker for the authenticity of apricot juices (RSK-value, 1987). While data on the composition of apricot fruit cultivars grown in Italy are available, information on commercial apricot juices is limited.

The aim of this work was to analyse the composition of carbohydrates, organic acids, amino acids, phenolic compounds and furanic compounds by high-performance liquid chromatography (HPLC) of 26 commercial apricot juices produced in Italy. This work focused on apricot juice – from organic, integrated and conventional agriculture – due to the economic interest importance of this product in Italy.

2. Materials and methods

2.1. Samples

Twenty-six commercial apricot juices were purchased from local markets and stored at room temperature before analysis. Samples were representative of the major Italian fruit juice companies and consisted of five organic (ORG), five integrated (INT), and 16 conventional (CON) juices. Juices were diluted at a fixed soluble solid content of 10°Brix (digital refractometer PR-101, Atago, Tokyo, Japan), centrifuged at 3000g × 15 min at 4 °C and filtered through a 0.45 μm cellulose-acetate membrane (Orange Scientific, Dasit, Milano, Italy) before HPLC analysis. To verify the origin of the furanic compounds, fully ripe apricot fruits were sampled, freshly squeezed, then analysed by HPLC and the result compared with commercial juices.

2.2. Standards

Furanic compounds: 5-Hydroxymethylfurfural (HMF), 3-hydroxy-2-pyrone (HPYR), furfural (FUR), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) and 2-acetylfuran (AcFUR); *Carbohydrates*: Glucose, fructose, sucrose, and sorbitol; *Organic acids*: Malic acid, citric acid and ascorbic acid; *Amino acids*: Arginine, aspartic acid, asparagine, glutamic acid, threonine, alanine, proline, tyrosine, γ-aminobutyric acid, valine, phenylalanine, isoleucine, methionine, leucine and hystidine; *Phenolic compounds*: Catechin, chlorogenic acid and rutin were from commercial source (Sigma, Milano, Italy).

2.3. Chromatographic apparatus

A HPLC system (Jasco, Tokyo, Japan) equipped with a pump system (PU980), a refractive index detector (RI830) for carbohydrate analysis, and a diode array detector (MD1510) monitored at 210 nm, 263 nm, 280 nm and 350 nm, for the analysis of organic acids, amino acids, furanic compounds and phenolic compounds, respectively. Data were acquired and processed using Borwin-PDA version 1.50 software (JMBS Developments, Grenoble, France). Juices were injected with a 20 μl loop using a 7125 valve (Rheodyne, Cotati, CA).

2.4. Analysis of carbohydrates

Carbohydrates were simultaneously analysed onto an Aminex HPX-87Ca column (300 × 7.8 mm) protected with a pre-column (30 × 4.6 mm) filled with the same stationary phase (Bio-Rad) and kept at 80 °C. The analytical conditions used were as follows: Flow 0.6 ml/min, eluent 0.045 N H_2SO_4 with 6% acetonitrile (v/v).

2.5. Analysis of organic acids

Malic and citric acid were simultaneously analysed onto an Aminex HPX-87H column (300 × 7.8 mm) protected with a pre-column (30 × 4.6 mm) filled with the same stationary phase (Bio-Rad) and kept at 45 °C. The analytical conditions used were as follows: flow 0.5 ml/min, eluent 0.045 N H_2SO_4 with 6% acetonitrile (v/v) (Castellari, Versari, Spinabelli, Galassi, & Amati, 2000). Ascorbic acid was analysed by capillary electrophoresis according to a method previously described (Versari, Mattioli, Parpinello, & Galassi, 2004).

2.6. Analysis of amino acids

Amino acids were analysed according to the methods previously described in details (Fabiani, Versari, Parpinello, Castellari, & Galassi, 2002). Briefly, Amino acids were derivatized (FMOC–AA) at room temperature using a precolumn procedure. An aliquot of 300 μl of fruit juice (or a standard solution of amino acids) was added with 600 μl of 200 mM borate buffer (pH 10.0). Then, 600 μl of 15 mM FMOC-Cl (in acetonitrile) was added to the fruit juice and the derivatization occurred. After 5-min, the reaction was stopped by the addition of 600 μl of 300 mM ADAM (water–acetonitrile, 1:1, v/v), and the reaction lasted for 1-min to form the FMOC–ADAM complex. Then, the sample was filtered and analysed by HPLC using a Purospher RP-18 column (250 × 4-mm) protected with a guard column of the same material (Merck, Darmstad, Germany). The column operated at 25 °C with a flow rate of 1.0 ml/min using 50 mM acetate buffer (pH 4.2) as eluent A and acetonitrile as eluent B. Amino acids were separated with the following linear gradient elution conditions (min/A%): 0/72, 3/72, 27/55, 32/0, 37/0, 39/72, 47/72.

2.7. Analysis of phenolic compounds

A method for the analysis of phenolic compounds in apricot was developed using an Inertisil ODS2 RP-column (250 × 4 mm) kept at 25 °C. The analytical conditions used were as follows: flow 0.4 ml/min, eluent (A) H_2O/CH_3COOH (98/2, v/v), eluent (B) H_2O/CH_3COOH/CH_3OH (34/1/60, v/v/v), with a simple gradient elution (min/A%): 0/100; 50/20; 60/20; 65/100; 75/100.

2.8. Analysis of furanic compounds

The separation of furanic compounds was performed with an Aminex HPX-87H column (300 × 7.8 mm) protected with a pre-column (30 × 4.6 mm) filled with the same stationary phase (Bio-Rad Laboratories, Hercules, CA). Column and precolumn were thermostated at 45 °C by a heater (Jones Chromatography, Mid Glamorgan, UK). The isocratic elution was run with a flow rate of 0.5 ml/min, using 0.01 N H_2SO_4 with acetonitrile (810:190, v/v) as mobile phase. The UV spectra (200–350 nm) of each compounds was recorded on-line with a resolution of 4 nm. Precision (CV%) was calculated by four repeated analysis of a sample containing DMHF (CV = 5.7%), HMF (0.4%), and FUR (0.7%). Accuracy (recovery%) was evaluated in triplicate using the apricot juices spiked with a known amount of HMF (94–98%), and FUR (95–101%).

2.9. Peak identification and quantification

Peak identification was based on the retention times (t_R), spiking technique and spectral overlay, whereas peak quantification was based on the external standard calibration method using linear regression analysis ($r > 0.999$) (Statistica 5.0, StatSoft, Tulsa, OK). The quantification of selected compounds in commercial Italian apricot juices was carried out in duplicate.

2.10. Color measurement

The CIE$L^*a^*b^*$ color parameters were measured in the reflectance mode with D_{65} illuminant and 2° observer angle (Chroma Meter CR-300, Minolta, Ramsey, NJ). Samples were measured against a white ceramic reference plate ($L^* = 97.43$, $a^* = -0.13$, $b^* = +1.68$).

2.11. Statistical analysis

The range, mean and standard deviation (mean ± SD) of the three types of apricot juices (organic, integrated, and conventional) were calculated. Analysis of variance (ANOVA) and mean comparisons by Tukey Honest Significant difference (HSD) test for unequal number of samples at 5% level was performed using Statistica 5.1 (StatSoft, Tulsa, OK). To gain insight the data structure a multivariate analysis called principal component analysis (PCA), a pure display method, was performed to detect the most important factors of variability and to describe the relationship between variables and observations (Lewi, 1992).

3. Results and discussion

3.1. Analysis and composition of fruit juices

There is an ongoing interest in developing a reliable and rapid methods to asses the quality of foods for nutritional and regulatory purposes. Fig. 1 shows a typical chromatographic pattern of carbohydrates (A), organic acids (B), furanic compounds (C), phenolic compounds (D) and amino acids (E) found in commercial apricot juices. HPLC analysis of apricot fruit juices was satisfactory in terms of main peak separation and identification. Besides the identified compounds, the chromatographic profiles showed further information due to the presence of minor non-identified peaks. The diode array detector has led to considerable improvements in the HPLC analysis, as not only the retention time but also the UV spectrum can be used for routine identification purposes. In this view, commercial apricot juices showed the presence of HMF ($t_R = 22.1$ min), FUR ($t_R = 31.6$ min), together with an unknown peak ($t_R = 23.2$ min) (Fig. 1C). Additional information can be derived from the UV absorption spectra of HMF [$\lambda_{max} = (228)$, 284 nm], FUR [$\lambda_{max} = (228)$, 276 nm], HPYR [$\lambda_{max} = (231)$, 294 nm], and the unknown peak eluting at $t_R = 23.2$ min [$\lambda_{max} = (232)$, 292 nm] which clearly resembled that one of HPYR. The definitive identification of the peak eluting at $t_R = 23.2$ min requires further investigation.

Table 1 summarizes the composition of the apricot juices. The mean values for glucose, fructose, malic acid, glycine, chlorogenic acid, rutin and a^*-parameter showed a significant difference among apricot juices, being always

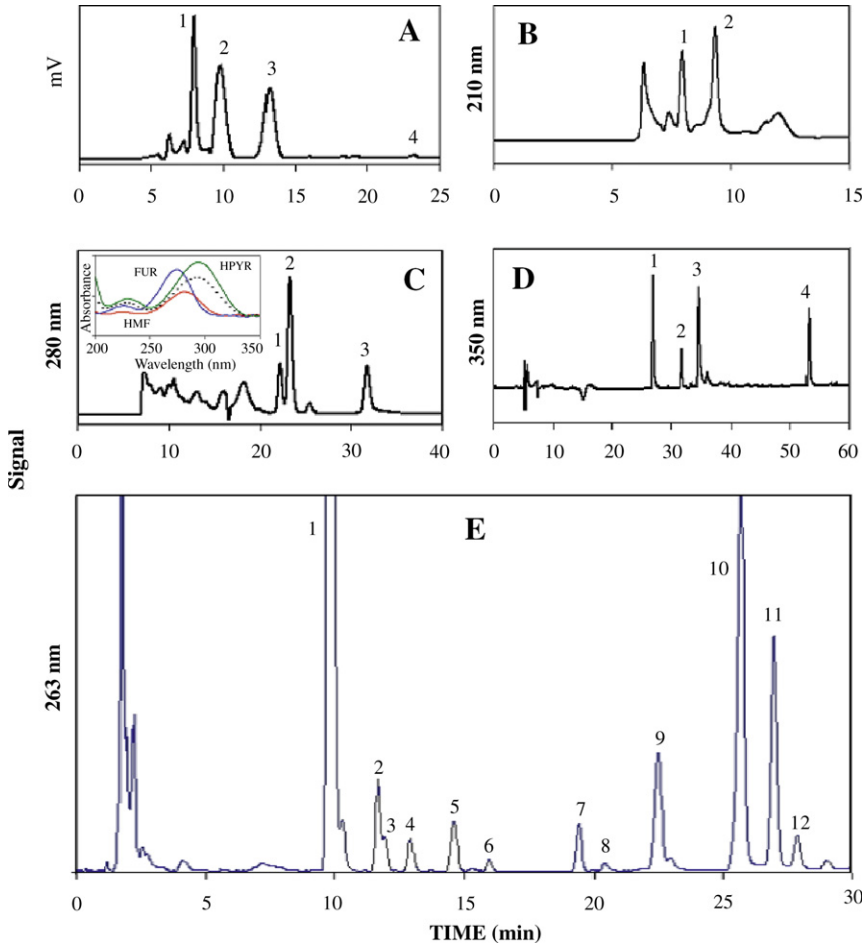

Fig. 1. HPLC chromatogram of commercial apricot juices. Legend: carbohydrates (A), organic acids (B), furanic compounds (C), phenolic compounds (D) and amino acids (E). For analytical conditions see Section 2. Legend A. Peaks: 1. Sucrose; 2. Glucose; 3. Fructose; 4. Sorbitol. Legend B. Peaks: 1. Citric acid; 2. Malic acid. Legend C. Peaks: 1. HMF; 2. Unknown; 3. FUR. In the window, UV spectra of Furfural (FUR-blu); 5-Hydroxymethylfurfural (HMF-red); 3-Hydroxy-2-pyrone (HPYR-green); and unknown peak at $t_R = 23.2$ min (–––). Legend D. Peaks: 1. Chlorogenic acid derivative; 2. Rutin derivative; 3. Chlorogenic acid; 4. Rutin. Legend E. Peaks: 1. Asparagine; 2. Serine; 3. Aspartic acid; 4. Glutamic acid; 5. Threonine; 6. Glycine; 7. Alanine; 8. Tyrosine; 9. Proline; 10. fmoc (derivatization reagent); 11. Methionine + NH_4OH; 12. Valine.

higher in organic juices except for the glycine content. The general composition of apricot juices was consistent with data from the literature (Bartolozzi et al., 1997; Fernández de Simón et al., 1992; Garcia-Viguera et al., 1994; Gurrieri et al., 2001; Katona et al., 1999; Trifirò et al., 2006), whereas a lack of information on furanic compounds in apricot juices occurred. Thus, to verify the origin of the furanic compounds, fully ripe apricot fruits were sampled, then analysed by HPLC and the result compared with commercial juices. A lack of HMF, FUR and the unknown peak was found in apricot fruits, thus confirming the importance of the furanic compounds as markers of heating condition during processing and storage of apricot juices. Although the organic juices showed a high content of both HMF (range 1–18 mg/l) and FUR (0.1–2.3 mg/l) (Table 1), the overall level of HMF in apricot juices was lower than the regulatory limit of 20 mg/l (A.I.J.N., 1996). Based on this result, a lack of sensory contribution of the furanic compounds in apricot juices is expected, taking into account the taste thresholds reported in the literature for HPYR, FUR and HMF in orange juice, being 30 mg/l, 80 mg/l, and >200 mg/l, respectively (Shaw, Tatum, Kew, Wagner, & Berry, 1970).

The analysis of carbohydrates and amino acids of apricot juices provided additional information related to the occurrence of furanic compounds. A high correlation between HMF with the a^*-parameter ($r = 0.75$) and fructose ($r = 0.72$) was found. The color variables are commonly used to predict both chemical and sensory changes in food products. On peach juice the a^*-value (redness) increases by increasing heating time and temperature (Garza, Ibarz, Pagan, & Giner, 1999). These findings suggest that fructose play a major role in HMF formation. Apricot juice is characterized by a similar content of fructose (range 9–33 g/l) and glucose (range 13–31 g/l). Both sugars may contribute to the browning of juice depending on the pH value. At the pH value of apricot juices (range 3.12–3.60) fructose degrades more rapidly than glucose (Shallenberger & Mattick, 1983). Asparagine was the main amino acid found in apricot juice (range 820–1570 mg/l), representing

Table 1
Composition of Italian commercial apricot juices and significance level for statistical evaluation

Compounds	Apricot juices[a] (mean ± SD)				
	ORG ($n = 5$)[b]	INT ($n = 5$)	CON ($n = 16$)	p-Level[c]	Overall ($n = 26$)
Glucose (g/l)	23 ± 5^{A}	18 ± 3^{B}	19 ± 3^{B}	0.0410	19 ± 4
Fructose (g/l)	22 ± 8^{A}	14 ± 3^{B}	14 ± 3^{B}	0.0006	16 ± 5
Sucrose (g/l)	21 ± 17	24 ± 6	25 ± 7	n.s.	24 ± 9
Sorbitol (g/l)	4 ± 4	2 ± 1	3 ± 2	n.s.	3 ± 2
Malic acid (g/l)	3.2 ± 0.7^{A}	1.8 ± 0.4^{B}	2.0 ± 0.4^{B}	0.0001	2.2±0.7
Citric acid (g/l)	1.8 ± 0.8	1.4 ± 0.3	1.4 ± 0.3	n.s.	1.5 ± 0.4
Ascorbic acid (mg/l)	138 ± 61	187 ± 183	215 ± 104	n.s.	194 ± 116
Asparagine (g/l)	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.3	n.s.	1.1 ± 0.3
Proline (mg/l)	61 ± 35	50 ± 14	52 ± 23	n.s.	53 ± 24
Glycine (mg/l)	20 ± 6^{A}	31 ± 5^{B}	25 ± 5^{A}	0.0038	25 ± 6
Chlorogenic acid (mg/l)	26 ± 10^{A}	18 ± 4^{B}	18 ± 4^{B}	0.0011	20 ± 6
Rutin (mg/l)	10 ± 2^{A}	9 ± 1^{B}	9 ± 1^{B}	0.0008	9 ± 1
HMF[d] (mg/l)	6 ± 7	3 ± 1	2 ± 1	n.s.	3 ± 3
Peak $t_R = 23.2$ min[e] (mg/l)	7 ± 3	4 ± 1	6 ± 2	n.s.	6 ± 2
FUR[f] (mg/l)	1.2 ± 0.8	0.7 ± 0.3	1.2 ± 0.5	n.s.	1.1 ± 0.5
pH	–	3.24 ± 0.09	3.29 ± 0.03	3.30 ± 0.11	n.s.
a^* value	–	14.0 ± 1.3^{B}	11.8 ± 0.2^{A}	12.9 ± 0.6^{A}	0.0211

[a] Fruit from: organic (ORG), integrated (INT), and conventional (CON) agriculture.
[b] Number of samples.
[c] ANOVA and mean comparisons by Tukey HSD test for unequal number of samples at 5% level.
[d] Hydroxymethylfurfural (HMF).
[e] Peak $t_R = 23.2$ min (unknown) quantificated as 3-Hydroxy-2-pyrone derivative.
[f] Furfural (FUR).
[A–B] Means within rows with changed letter are significantly different according to the Tukey HSD test ($P \leqslant 0.05$).

up to 78% of the total amino acid content (Fig. 1E). Amino acids did not appear to have a strong relationship with the accumulation of the furanic compounds in commercial apricot juices ($r \leqslant 0.47$). Among the different amino acids, asparagine, the main amino acid of apricot juice, is considered to produce only an 'intermediate extent of browning', whereas the most reactive amino acids are lysine, glycine, tryptophan and tyrosine (Kaaname & Labuza, 1989). The chemical reactions responsible for non-enzymatic browning in foodstuffs are complex. Sometimes the main reactants are aldoses and amino acids, but in fruit juices fructose and organic acids are also involved. In system containing low ascorbic acid content, *e.g.* apricot juice, the formation of non-enzymatic browning is mainly due to the acid-catalyzed dehydration of sugars (Burdurlu, Koca, & Karadeniz, 2006; Roig, Bello, Rivera, & Kennedy, 1999).

Sorbitol is an important index for detection of adulteration of apricot juice with sweeteners. The analysed apricot juices showed a range between 0.8 and 11 g/l, with one sample (0.8 g/l) falling outside the RSK guide values range of 1.5–14 g/kg.

3.2. Principal components analysis

PCA was used as unsupervised method to examine the similarity among the three types of apricot juices. PCA on chemical composition of apricot juices resulted in two principal components (PCs) with eigenvalues >1 ($PC_1 = 4.9$; $PC_2 = 1.7$) that accounted for 66% of the total variance (Fig. 2). The PC_1 (49%) was associated with HMF, fructose, malic acid, chlorogenic acid, and a^*-parameter. The PC_2 (17%) was associated with asparagine and threonine. A clustering of apricot juices partially occurred. In fact, organic apricot juices showed some separation along the first factor (positive half), whereas a lack of distinction between integrated and conventional juices appeared. The organic apricot juices were separated on the PC_1 axis because of their high fructose, malic acid, chlorogenic acid, HMF content and a^*-parameter, while the dispersion of conventional apricot juices along the PC_2 axis was a function of asparagine and threonine content. Integrated juices were located close to the centre of the plot and did not appear to be dominated by any of the parameters.

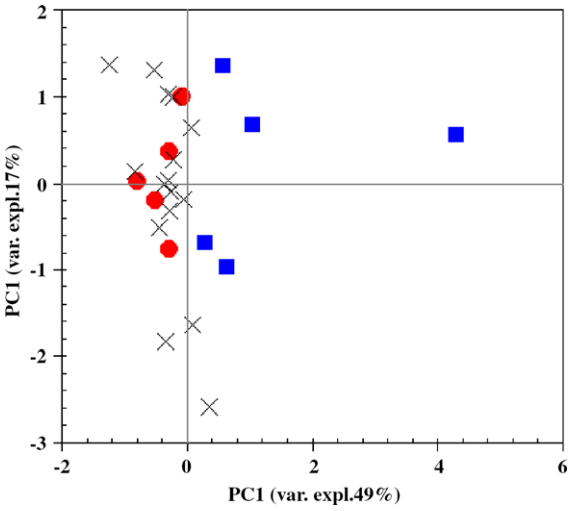

Fig. 2. Positions of the PC scores on the first two PC axes for the apricot juices from organic (■), integrated (●), and conventional agriculture (×).

In conclusion, the modern fruit juice industry needs their products to be characterized by high-quality attributes to meet consumer expectations. In this view, the analytical control of fruit juice composition is becoming an important issue, and HPLC provides an important tool for research as well as for routine analysis. The commercial apricot juices showed some difference in their composition especially in terms of glucose, fructose, malic acid, glycine, chlorogenic acid, rutin and a^*-parameter. These compositional data are helpful in the development of future commercial juices to target specific consumer requirements. A further long-term study is required to set up a reliable authentic database to be used to analyse unknown test samples. In this view, it is important to get more insight the interaction between the genetic and the environmental variability, the growing condition and the processing on the composition of apricot fruits and juices.

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