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To cite this article: Valentina Picchi, Roberto Lo Scalzo, Elisabeth Kurze, Marta Fibiani, Eivind Vangdal & Wilfried Schwab (2019) Impact of year of harvest, genotype and cultivation method on bioactives and Pru d 1 allergen content in plums, International Journal of Food Sciences and Nutrition, 70:6, 688-700, DOI: [10.1080/09637486.2018.1557606](https://doi.org/10.1080/09637486.2018.1557606)

To link to this article: <https://doi.org/10.1080/09637486.2018.1557606>



Published online: 01 Feb 2019.



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Impact of year of harvest, genotype and cultivation method on bioactives and Pru d 1 allergen content in plums

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ABSTRACT

The present work studied the effect of the year of harvest, the genotype and the cultivation method on the nutritional quality and the allergen content of three plum cultivars. The common quality parameters and the phytochemical content strongly varied with the year and the cultivar, while the system of cultivation had a minor influence. In particular, ascorbic acid greatly decreased in 2016 compared to 2015, while polyphenols were higher in 2016. The health-promoting compounds, and particularly phenolics, were significantly correlated with the antioxidant capacity. Finally, the allergen content was strongly dependent on the content of flavan-3-ols, suggesting that this class of phenolics is determinant in influencing the allergen content in plums. Results showed that the major factor affecting the quality and the concentration of natural metabolites of plum, in addition to the diversity among genotypes, is the year-to-year variation, whereas the system of cultivation plays a marginal role.

ARTICLE HISTORY

Received 13 September 2018
Revised 23 October 2018
Accepted 6 December 2018

KEYWORDS

Plum; phytochemical; organic farming; antioxidant capacity; allergen

Introduction

Plum (*Prunus domestica* L.) is a fruit rich in bioactives, mainly phenolics. At molecular levels, the potential effects on human health of these compounds are currently under investigation (Rice-Evans et al. 1997). The content of bioactives in a fruit can be influenced by several factors, among which the genotype and the environment. As regards to the genotype, Slimestad et al. (2009) found large cultivar differences in phenolic content in Norwegian plums, and the similar variations were observed for other quality indexes (soluble solids and organic acids content) (Vangdal et al. 2007). In addition, the year of harvest may deeply influence the content of bioactive compounds, mainly through variations of climatic conditions (e.g. rainfall, humidity and mean temperature) (Hornick 1992; Woese et al. 1997; Bourn and Prescott 2002). Several studies have compared the nutritional quality of vegetables and fruits obtained with different systems of cultivation, particularly focussing on the effect of organic management on the phytochemical content. The nutritional superiority of organic raw material is not clearly supported by the studies on the topic, that

reported contrasting results (Gaśtoł and Domagała-Świątkiewicz 2013). The production of vitamins is mainly depending on variety, ripeness of fruits and crop size (Bernacchia et al. 2016). Some studies underlined a positive effect of organic farming on vitamin C content, while others did not found a consistent relation (Rembalkowska 2007). Analogously, polyphenols, which are involved in the plant defensive mechanism against biotic and abiotic stress, were found to be both increased and decreased in organic products compared to samples produced with conventional (Conv) methods (Bernacchia et al. 2016). Recently, it was suggested that the interaction between cultivation practices and genotypic characteristics, plays a major role in determining the final content of bioactive compounds (Lo Scalzo et al. 2013). As regards plums, previous works have demonstrated that Conv and organic (Org) products differ only marginally in macronutrients whereas the concentration of some antioxidant vitamins and of secondary metabolites (phenolics) could be markedly influenced by the system of cultivation (Lombardi-Boccia et al. 2004).

Besides health beneficial compounds, plums also contain allergenic proteins (i.e. compounds that can

Table 1. Mean monthly temperatures and total monthly rainfall during the period May – September in the Hardanger area (Western Norway) in 2015 and 2016.

	Temperature (°C)			Rainfall (mm)		
	2015	2016	(10.2)	2015	2016	(50)
May	8.7	11.8	(10.2)	140	73	(50)
June	11.9	16	(13.8)	135	29	(64)
July	14.4	14.9	(15.0)	85	74	(75)
August	15.1	14.3	(14.1)	73	138	(92)
September	12.3	14.3	(10.5)	64	160	(157)
Harvest date	22-set	06-set				

The historical mean temperatures and rainfall are indicated in parenthesis. For each year of study, the harvest date is reported.

cause food allergies). In plums, allergens from different protein families have been identified, including the thaumatin-like protein Pru d 2, the lipid transfer protein Pru d 3 and the profilin Pru d 4 (Pastorello et al. 2001). Pru d 1, a member of the pathogenesis-related (PR) protein 10 (PR-10) family, was also identified due to its similarity with the major birch pollen allergen Bet v 1 (El-Kereamy et al. 2009). Plants synthesise the PR proteins as response to various stress conditions, such as pathogen attack, chemical application, wounding or environmental factors (Sinha et al. 2014). Homologous PR-10 proteins are widely distributed in the plant kingdom. Because of their high homology of amino-acid sequence, IgE antibodies can cross-react with similar epitopes from different plant origin (Hoffmann-Sommergruber 2000). Thus, due to cross-reaction of antibodies with Bet v 1 and related PR-10 proteins in fruit, birch-pollen related food allergies occur. Despite this potentially important effect, little information is available about the variation of Pru d 1 content in plums due to genotypic characteristics, weather variability and type of cultivation system.

In the present work, we analysed the effect of the year of harvest, the genotype and the cultivation method on the bioactives and the Pru d 1 allergen content of three plum cultivars commonly grown in Norway. This study contributes to elucidate the effect of organic cultivation on plum quality, a matter that was so far was investigated.

Material and methods

Plant material and cultivation

The experiment was conducted using three cultivars of *P. domestica* L.: Jubileum, Victoria and Reeves. The Victoria cultivar was selected as one of the most commonly cultivated in Norway, whereas Jubileum and Reeves were selected because of their interesting characteristics as reported in a previous work (Vangdal

et al. 2007). Plums were cultivated in two consecutive years (2015 and 2016) with either Org or Conv systems. Fruits were collected from three farms located in the Hardanger area (Western Norway) having comparable climatic and soil conditions. In particular, Conv plums were picked in the same farm, whereas Org Victoria were supplied from a second farm (9 km from the conventional farm) and Org Jubileum and Reeves were picked in a third farm (6 km from the conventional farm). The orchards had been grown as Org or Conv since they were established (at least 6 years). The trees were 6–8 years old and grafted on St. Julien A rootstock. Planting space was 1.25–1.75 × 4.5 m and the trees were vertical axis shaped. A 1 m wide strip in the tree row was mechanically kept weed free, and management practices followed to standard procedures in the area with fertilisers and plant protection programme for Conv and Org growing, respectively. No fungicides or rain protection systems were used in the orchards. Plums were harvested 2–4 d before they were fully ripe as advised in commercial orchards (Vangdal and Flatland 2010). After harvest, plums were cold stored (4 °C) overnight before being shipped by aeroplane to Italy the day after. Plums arrived in Milan in the evening and were then cold stored (3 °C) for a night. The day after, three replicates of fruits of uniform size (2.5 kg each sample) for each combination of cultivar × year × cultivation were randomly selected for further processing. Plums were cut into two halves and rapidly frozen at −50 °C in an air-forced tunnel and then lyophilised using a Dura-Stop tray dryer, combined with a Dura-Dry condenser module (FTS Systems, Stone Ridge, NY).

Weather data

In the two sampling years (2015 and 2016), the daily mean temperature and rainfall from 1 May until the date of harvest were measured in the area (i.e. NIBIO Ullensvang Research Centre) by the Norwegian Meteorological Institute (www.met.no). The data were processed to obtain the monthly mean temperature and cumulative rainfall and were compared with the climatology computed over historical series (period 1960–1990) (Table 1).

Common quality parameters

Dry matter (DM) was determined as the percentage of the lyophilised weight with respect to the fresh weight (FW). Soluble solids content (SSC) and titratable

acidity (TA) were measured in aqueous extracts obtained by adding 27 ml of distilled water to 3 g of lyophilised powder. SSC was measured with a bench-top refractometer (RFM 91, Bellingham/Stanley Ltd., Atlanta) and expressed as °Bx on a FW basis. TA was determined by automatic titration with 0.1 N NaOH until pH 8.2 (682 Titrprocessor, Metrohm Ltd., Herisau, Switzerland) and expressed in g malic acid Eq/100g FW.

Polyphenol content

Monomeric phenols

The monomeric phenols were determined by RP-HPLC-DAD following the method of Vangdal et al. (2017). The extract was prepared from 800 mg of lyophilised powder dissolved in 10 ml of a 1:1 mixture of EtOH and 0.06 N HCl. The mixture was vortexed for 30 s and vigorously shaken for 60 min at room temperature, then centrifuged at $25,000 \times g$ at 4 °C. The supernatant was filtered through a 0.45 mm filter and analysed by HPLC. Phenolic acids were detected at 320 nm, flavonols at 350 nm and anthocyanins (ATHs) at 520 nm. The components of plum extracts were identified by their UV-VIS spectra and according to the literature (Hong and Wrolstad 1990; Slimestad et al. 2009). The quantification was made by external standard calibration response, using chlorogenic acid (CGA) for both Neo-chlorogenic acid (Neo-CGA) and CGA, *p*-coumaric acid, rutin, cyanidin-3-glucoside and cyanidin-3-rutinoside.

Total flavan-3-ols

Total flavan-3-ols were quantified by the vanillin reaction, following the method by Price et al. (1978). The analysis was performed on an acidified aqueous EtOH extract, as previously described for monomeric phenols. The reaction solution consisted of 1.5 ml of EtOH, 1.0 ml of 12 N HCl, 0.3 ml of plum extract and 0.5 ml of a freshly prepared vanillin solution (5% w/v in EtOH). The solutions were kept at room temperature for 45 min, then the absorbance was read at 500 nm, 1 cm light path, 20 °C. For each sample, a blank was prepared by substituting the vanillin solution with pure EtOH. Calibration was carried out with (+)catechin solutions at known concentrations (0.1–3.0 mg/ml), treated in the same way as the samples, so the results were in mg catechin equivalents (CE) per 100 g dry weight (DW) of fruit.

Total ascorbic acid (AsA) content

Total AsA was determined after extract reduction with Tris-carboxyethyl phosphine (TCEP), following the method described by Wechtersbach and Cigić (2007). Briefly, 15 mL of cold 6% aqueous solution of metaphosphoric acid was added to 1 g of lyophilised powder, vortexed for 30 s and then centrifuged for 15 min at $25,000 \times g$. Extracts were then treated with 100 mmol/L TCEP in 1 M HCl. Reduced extracts were then threefold diluted with 0.02 M orthophosphoric acid and immediately analysed by HPLC. The analytical column was a 250×6 mm i.d., Inertsil ODS-3, maintained at 40 °C. The isocratic elution was performed using 0.02 M orthophosphoric acid at a flow rate of 0.7 mL/min. Samples were measured at 254 nm. The concentration of AsA was calculated from the experimental peak area by analytical interpolation in a standard calibration curve.

Polyphenol oxidase (PPO) activity

The PPO (EC 1.10.3.1) activity was assayed as described by Fujita and Tono (1988). In brief, 70 mg of lyophilised fruit was dissolved in 1.2 mL of Mc Ilvaine buffer (pH 5.0). CGA served as substrate in Mc Ilvaine buffer at pH 7.0 at 25 °C, following the increase in absorbance within 1 min of reaction and considering the increase in absorbance in the linear portion of the curve (Concellòn et al. 2004). The results were expressed as U/g of DW, with 1 U corresponding to 0.1 absorbance unit increase per minute, at 420 nm and 20 °C.

Antioxidant capacity

Folin-Ciocalteu index (FCI)

The FCI was measured on the same extracts used for ATHs and phenolics with the Folin-Ciocalteu reagent, as described in Slinkard and Singleton (1977) with slight modifications. An aliquot of 50 µl of sample was diluted with 3 ml distilled H₂O and 0.5 mL of Folin-Ciocalteu reagent and left at room temperature for 5 min. The solution was then treated with 1 mL of 15% (w/v) Na₂CO₃ and incubated in the dark for 1 h. The absorbance of the blue coloured solution was read at 730 nm. The FCI was estimated using a standard curve obtained with gallic acid.

DPPH• and superoxide anion scavenging

The antioxidant capacity was measured using 1,1-diphenyl-2-picrylhydrazil (DPPH•) radical quenching

and superoxide anion ($O_2^{\bullet-}$) radical scavenging, according to a previously validated method described in Picchi et al. (2012). To measure the electronic paramagnetic resonance (EPR) we used a MiniScope MS200 Magnettech (Berlin, Germany). For sample preparation, 500 mg of lyophilised powder was extracted with 15 ml of a 1:1 mixture of EtOH and 0.06 N HCl, homogenised and centrifuged at $25,000 \times g$ for 10 min at 4 °C.

The reaction solution for the DPPH $^{\bullet}$ scavenging experiment consisted of 0.2 ml methanol, 0.05 ml extract solution and 0.05 ml of a 0.5 mM DPPH $^{\bullet}$ ethanolic solution. These solutions were accurately mixed in a glass tube assay and successively placed in a capillary for EPR measurements. The experimental settings of the spectrometer were as follows: field set, 3350 G; scan range, 50 G; scan time, 60 s; modulation amplitude, 3000 mG; microwave attenuation, 7 dB; and receiver gain, 7 E10. The system was calibrated with known concentrations of AsA (range 0.009–0.360 mg/mL, $y = 14.979 \ln(x) + 80.266$, $R^2 = 0.984$) and the results were expressed as mg equivalents of AsA per 100 g DW.

The antioxidant capacity towards $O_2^{\bullet-}$ was based on the spin trapping of generated radicals by potassium superoxide (KO_2) in DMSO with the addition of 18-crown-6 ether to complex K^+ . The spin trap reagent was 5,5-dimethylpyrrolidine-*N*-oxide (DMPO). Under these conditions, a typical DMPO-OOH adduct (1:1:1:1) was observed. The reaction solution contained 0.1 ml of a 50 mM DMPO phosphate buffer solution (PBS 0.1 M pH 7.4), 0.1 ml of superoxide solution and 50 μ l of plum extract. EPR recording conditions were as follows: field set, 3500 G; scan range, 50 G; scan time, 45 s; modulation amplitude, 1500 mG; microwave attenuation, 4 dB; receiver gain, 2×100 . The system was calibrated with known concentrations of AsA (range 0.01–0.20 mg/mL, $y = 24.146 \ln(x) + 126$, $R^2 = 0.975$) and the results were expressed as mg equivalents of AsA per 100 g DW.

Allergen content of plums

Protein extraction

Protein extraction from freeze-dried plum samples was performed according to Björkstén et al. (1980) and prepared in duplicates. Briefly, lyophilised plum powder was homogenised with extraction buffer (10 mM KH_2PO_4 ; 10 mM K_2HPO_4 ; 10 mM Na-DIECA; 2 mM EDTA; 2% PVPP w/v) containing 0.5 mM phenylmethylsulfonyl fluoride and protease

inhibitor cocktail (Complete Protease Inhibitor Cocktail, Roche) in a ratio of 1:4 (w/v) and incubated for 1 h at 4 °C. After centrifugation for 15 min at $5000 \times g$ at 4 °C the supernatant was dialysed against PBS (pH 7.4) at 4 °C for 20 h. A second centrifugation step was performed to remove remaining precipitates. Extracts were directly used for allergen quantification by enzyme linked immunosorbent assay (ELISA).

Indirect competitive ELISA

Recombinant Pru d 1 was heterologously expressed in *Escherichia coli* strain BL21 (DE3)pLysS (Novagen, Darmstadt, Germany) as a fusion protein with a C-terminal His-tag and purified using Profinity IMAC Resin according to manufactures instructions. The recombinant allergen was further used for the indirect competitive ELISA to determine the Pru d 1 content of dried plum samples. The ELISA was performed according to Sancho et al. (2006) with minor modifications. Microtiter plates were coated with 0.1 ng/ μ l recombinant Pru d 1 in PBS pH 7.4. After three washing steps with washing buffer [0.05% (v/v) Tween 20 in PBS] blocking was performed with 2% BSA in PBS for 2 h at room temperature. Dialyzed plum extracts were incubated with a specific polyclonal Pru d 1 antibody (Davids Biotechnologie, Regensburg, Germany) for 4 h at 4 °C for competition reaction. After another washing step, an Anti-Kanin-HRP secondary antibody (Carl Roth, Karlsruhe, Germany) was used to detect the primary antibody and incubated for 1 h at room temperature. After a final washing step, 1-Step Ultra 3,3',5,5'-tetramethylbenzidine substrate was used as substrate for the enzymatic reaction, which was stopped after 10 min with 2 M sulphuric acid. Absorbance was detected at 450 nm and 620 nm as reference wavelengths with CLARIOstar plate reader (BMG Labtech, Germany). The quantification of Pru d 1 allergens in plum extracts was determined based on the standard curve with recombinant Pru d 1 protein. Serial dilutions (0.0001–25 μ g/ml) of recombinant Pru d 1 were pipetted as “free” allergen and followed by the same procedure as the plum samples. The MARS software (BMG Labtech, Ortenberg, Germany) was used for data analysis. Pru d 1 allergen content was expressed as μ g Pru d 1/g dry weight.

Statistical analysis

The experimental design was a completely randomised block design. All the analyses were conducted using three biological replicates for each combination of cultivar $\times$ year $\times$ cultivation. Results were expressed as

Table 2. Significance of ANOVA for each measured quality parameter, phytochemical, PPO, antioxidant capacity (Folin-Ciocalteu Index, DPPH[•] and Superoxide anion scavenging capacity) and Pru d 1 content, relating to their value in the three plum cultivars harvested across two cultivation years (2015 and 2016) and two cultivation systems (conventional and organic).

	Year (Y)	Cultivar (C)	System of cultivation (S)	Y × C	Y × S	C × S	Y × C × S
Common quality parameters							
DM	***	**	**	***	ns	***	ns
SSC	***	***	**	***	ns	ns	ns
TA	***	***	***	***	**	***	***
Phytochemicals							
Neo-CGA	***	***	*	***	ns	**	ns
Coumaryl derivative	**	***	ns	*	ns	ns	ns
CGA	***	*	ns	**	*	*	ns
Rutin	ns	**	*	ns	ns	ns	ns
ATH	***	***	*	*	ns	ns	ns
TP	***	***	*	***	ns	*	ns
Flavan-3-ols	***	***	ns	***	*	***	***
AsA	***	**	ns	***	*	***	***
PPO activity	**	***	*	***	ns	**	**
Antioxidant capacity							
Folin-Ciocalteu Index	ns	***	ns	**	ns	**	ns
DPPH [•]	***	***	*	**	ns	**	*
Superoxide	***	***	ns	*	ns	ns	ns
Pru d 1 content	***	***	ns	***	ns	***	***

ns: not significant.

* $p < .05$.** $p < .01$.*** $p < .001$.

mean of three replicates. Data were subjected to three-way analysis of variance (ANOVA) to test the effect of the year, cultivar and system of cultivation. The mean values associated with the main factors as well as their interactions were evaluated using Tukey's test and statistically significant differences were accepted at the probability level $p < .05$. Pearson's correlation coefficient was used to determine the correlation among variables. Data of Pru d 1 content were analysed using the nonparametric Kruskal–Wallis test. Dunn's Test was used to calculate median values and statistical significance levels between the variable groups. For p -value adjustment, Bonferroni method was applied. p values of <0.05 were considered as significant. To obtain a comprehensive characterisation of the samples, data were finally subjected to principal component analysis (PCA). The statistical analyses were performed using R (The R Foundation for Statistical Computing, R Core Team, Vienna, Austria, R version i386 3.5.0) and Statgraphics v.7 (Manugistic Inc., Rockville, MD).

Results and discussion

Weather during the two seasons

Compared to local climatology (i.e. the historical series of meteorological data), the year 2015 was characterised by lower temperatures and higher rainfall from May to July (Table 1). Year 2016 was characterised by normal temperature and rainfall with the exception of

the month of June that was exceptionally dry. These different climatic conditions caused the harvest to be carried on at different timings. The recommended standard ripeness was in fact reached in 2015 more than 2 weeks later than in 2016. As a result, plums were harvested the 22nd and 6th September, in 2015 and 2016, respectively.

Common quality parameters

The results of multi-factorial ANOVA indicated that the factor year significantly affected the quality parameters (Table 2), with higher values of DM and SSC in 2015 compared to 2016 particularly in Jubileum and Victoria, while lower values of TA were recorded in 2015 compared to 2016, apart from Jubileum variety (Table 3). These findings may relate to the lower than usual temperatures that characterised year 2015 and caused the delayed in the harvest. In fact, the accumulation of sugar content in two out of three varieties is in accordance with the finding that late-harvested plums tend to have higher SSC in comparison with the early mature ones (Sahamishirazi et al. 2017). DM, SSC and TA were influenced by the system of cultivation ($p < .01$, Table 2). For DM and TA, the interaction “cultivar × system of cultivation” was also significant ($p < .01$, Table 2). Jubileum and Victoria showed significantly lower values of DM when cultivated with the Org system, whereas Org Reeves had higher DM compared to Conv samples. As for SSC content, it was found high in all samples (ranging

Table 3. DM, SSC and TA of conventional and organic Jubileum, Reeves and Victoria plums in the 2 years of cultivation (2015 and 2016).

Cultivar	System of cultivation	Year	DM (%)		SSC ($^{\circ}$ Bx FW)		TA ^a (g/100 g FW)	
Jubileum	Conv	2015	16.9 $\pm$ 0.8	a	13.3 $\pm$ 0.7	a	1.33 $\pm$ 0.07	a
		2016	13.9 $\pm$ 0.4	bc	10.2 $\pm$ 0.6	b	1.12 $\pm$ 0.02	b
	Org	2015	15.7 $\pm$ 1.0	a	12.1 $\pm$ 0.9	a	1.37 $\pm$ 0.08	a
		2016	12.3 $\pm$ 0.7	d	8.5 $\pm$ 0.8	c	1.11 $\pm$ 0.09	b
Reeves	Conv	2015	12.7 $\pm$ 0.9	cd	9.6 $\pm$ 0.7	bc	0.84 $\pm$ 0.06	d
		2016	13.0 $\pm$ 0.1	bcd	10.4 $\pm$ 0.3	b	0.83 $\pm$ 0.01	d
	Org	2015	14.0 $\pm$ 0.8	bc	10.6 $\pm$ 0.5	b	0.87 $\pm$ 0.04	d
		2016	14.1 $\pm$ 0.3	bc	10.7 $\pm$ 0.4	b	1.07 $\pm$ 0.05	bc
Victoria	Conv	2015	17.1 $\pm$ 1.8	a	12.8 $\pm$ 1.6	a	1.11 $\pm$ 0.14	b
		2016	14.1 $\pm$ 1.0	b	10.0 $\pm$ 1.1	b	1.28 $\pm$ 0.14	a
	Org	2015	14.2 $\pm$ 0.2	b	10.3 $\pm$ 0.4	b	0.95 $\pm$ 0.03	cd
		2016	12.3 $\pm$ 0.7	d	8.5 $\pm$ 0.6	c	1.04 $\pm$ 0.07	bc

Results are presented as mean of three replicates $\pm$ standard deviation. Different letters within each column indicate means significantly different according to Tukey's test ($p < .05$). DM: dry matter; SSC: soluble solids content; TA: titratable acidity; Conv: conventional; Org: organic.

^aData are expressed as g malic acid equivalents.

from 8.5 to 13.3 $^{\circ}$ Bx), with higher values in Conv compared to Org in Jubileum and Victoria plums in both years. TA was lower in Reeves with respect to the other two varieties. Victoria showed lower TA in Org than Conv in both years, while in Jubileum the TA was unaffected by the organic cultivation. The results show that the system of production differently influenced the common quality parameters, particularly decreasing DM and SSC in Jubileum and Victoria but not in Reeves. The literature did not show a clear trend depending on the cultivation method (Woese et al. 1997; Gąstoł and Domagała-Świątkiewicz 2013) and the evolution of the above parameters could greatly depend on the assessed variety.

Polyphenol content

Three compounds were detected at 320 nm in plum extracts: Neo-CGA (Rt 12.4 min), CGA (Rt 19.6 min), and an ester of *p*-coumaric acid (coumaryl derivative, Rt 16.1 min), probably the 3'-hydroxycoumaroyl quinic acid, in agreement to the results of Simestad et al. (2009). Additionally, rutin was detected at 350 nm (Rt 25.2 min) whereas the two major ATHs characteristic of plums were identified at 520 nm as cyanidin-3-glucoside (Rt 16.4 min) and cyanidin-3-rutinoside (Rt 18.2 min).

Neo-CGA was the most important phenolic in plums, responsible for more than 80% of total plum monomeric polyphenols (Table 4), in accordance with previous data (Kim et al. 2003; Tomás-Barberán et al. 2001). The level of Neo-CGA was strongly influenced by the year and the cultivar but to a lesser extent by the system of cultivation (Table 2). As regards to the year, Neo-CGA showed significantly ($p < .001$) higher values in 2016 compared to 2015, varying on average between 185.2 and 553.9 mg/100g DW, in 2015 and

2016, respectively. Recently, Sahamishirazi et al. (2017) observed that lower radiation and less sunshine hours from July to October determined higher mean values of phenolic content in plums. Similarly, in our study, the higher rainfall observed in 2016 before and during the harvest time could have reduced the incident radiation and avoided water stress conditions, which in turn may have influenced the transpiration and photosynthetic activities, consequently resulting in higher phenolic content, as previously observed by Sahamishirazi et al. (2017). Similarly, the same authors found that CGA derivatives and flavans of plum cultivars that received higher radiation and more sunshine hours contained lower free phenolic acids. This observation is in accordance with the reported inverse correlation between phenolic concentrations and air temperature (Miletić et al. 2012; Xu et al. 2011), particularly during winter when plants are exposed to sunlight (Grace and Logan 2000).

A clear difference in Neo-CGA content was also evidenced among the varieties, with the highest concentration observed in Victoria (635.7 mg/100 g DW on average) and the lowest in Reeves (162.8 mg/100 g DW on average). Differences among the three cultivars were already reported by other authors (Simestad et al. 2009) and confirmed the genotypic difference with regard to the polyphenol content. In general, organic management resulted in a lower content of Neo-CGA compared to the conventional management, and this result was particularly evident in Victoria (Table 4). Although higher contents of secondary metabolites in organically grown compared with conventionally grown food products have been observed in many studies (Caris-Veyrat et al. 2004; Hallmann 2012; Lombardo et al. 2017), other studies found that the differences between cultivation systems are not consistent (Dangour et al. 2009; Worthington

Table 4. Neo-CGA, p-Coum, CGA, Rut, ATH, TP of conventional and organic Jubileum, Reeves and Victoria plums in the 2 years of cultivation (2015 and 2016).

Cultivar	System of cultivation	Year	Neo-CGA	p-Coum	CGA	Rut	ATH	TP
Jubileum	Conv	2015	212 ± 15 ef	21 ± 5 bc	33 ± 10 ef	22 ± 8 abcd	22.2 ± 2 b	309 ± 39 fg
		2016	411 ± 30 cd	30 ± 3 ab	41 ± 5 cde	11 ± 3 d	35.9 ± 1 a	530 ± 35 cd
	Org	2015	161 ± 47 fg	19 ± 13 bc	19 ± 8 g	14 ± 8 cd	20.0 ± 8 bc	234 ± 80 gh
		2016	456 ± 54 c	40 ± 3 a	47 ± 5 bcd	16 ± 2 bcd	22.3 ± 1 b	581 ± 53 c
Reeves	Conv	2015	86 ± 8 gh	7 ± 6 cd	22 ± 8 g	19 ± 9 abcd	1.9 ± 0.2 f	137 ± 15 hi
		2016	238 ± 23 ef	5 ± 1 d	36 ± 6 de	27 ± 0.1 a	17.1 ± 1 c	323 ± 30 fg
	Org	2015	68 ± 19 h	5 ± 4 d	20 ± 6 g	12 ± 6 d	2.3 ± 0.1 ef	107 ± 14 i
		2016	260 ± 4 e	5 ± 1 d	52 ± 2 bc	19 ± 6 abcd	6.7 ± 1 d	342 ± 2 ef
Victoria	Conv	2015	350 ± 111 d	31 ± 19 ab	25 ± 10 fg	29 ± 11 a	4.3 ± 2 def	438 ± 151 de
		2016	1040 ± 55 a	42 ± 3 a	64 ± 7 a	26 ± 0.2 ab	4.8 ± 1 def	1177 ± 66 a
	Org	2015	235 ± 38 ef	22 ± 11 b	18 ± 5 g	25 ± 8 abc	2.5 ± 1 ef	302 ± 55 fg
		2016	919 ± 32 b	36 ± 1 a	56 ± 5 ab	20 ± 1 abcd	6.2 ± 1 de	1037 ± 38 b

Results are presented as mean of three replicates ± standard deviation. Data are expressed in mg/100g DW. Different letters within each column indicate means significantly different according to Tukey's test ($p < .05$). Neo-CGA : neo-chlorogenic acid; p-Coum: *p*-coumaric acid; CGA: chlorogenic acid; Rut: rutin; ATH: anthocyanins; TP: total polyphenols; Conv: conventional; Org: organic.

Table 5. AsA content, Flavan-3-ols content, PPO activity, FCI of conventional and organic Jubileum, Reeves and Victoria plums in the 2 years of cultivation (2015 and 2016), expressed on a dry weight (DW) basis.

Cultivar	System of cultivation	Year	AsA	Flavan-3-ols ^a	PPO ^b	FCI ^c
			(mg/100 g DW)	(mgCE/100 g DW)	(U/g DW)	(mg GAE/100 g DW)
Jubileum	Conv	2015	11.5 ± 3 a	882 ± 373 e	43 ± 2 ab	593 ± 23 cd
		2016	3.6 ± 1 ef	2064 ± 156 cd	53 ± 17 ab	508 ± 39 de
	Org	2015	4.2 ± 1 ef	1112 ± 152 de	56 ± 10 ab	471 ± 34 ef
		2016	2.9 ± 0.1 f	1388 ± 161 d	54 ± 22 ab	459 ± 106 ef
Reeves	Conv	2015	4.9 ± 0.1 de	521 ± 75 f	59 ± 15 a	411 ± 42 ef
		2016	5.0 ± 2 de	2463 ± 313 b	8 ± 3 de	376 ± 43 f
	Org	2015	6.1 ± 1 cd	766 ± 148 ef	23 ± 2 cd	434 ± 51 ef
		2016	4.1 ± 1 ef	4018 ± 170 a	4 ± 2 e	518 ± 22 de
Victoria	Conv	2015	9.0 ± 3 b	2004 ± 183 d	38 ± 5 bc	665 ± 66 bc
		2016	4.6 ± 1 de	2490 ± 184 b	51 ± 1 ab	841 ± 26 a
	Org	2015	11.1 ± 0.4 a	1416 ± 95 e	42 ± 12 abc	657 ± 133 bc
		2016	7.2 ± 0.3 c	2353 ± 122 bc	18 ± 6 de	733 ± 23 b

Results are presented as mean of three replicates ± standard deviation. Different letters within each column indicate means significantly different according to Tukey's test ($p < .05$). AsA: ascorbic acid; PPO: polyphenol oxidase; FCI: Folin-Ciocalteu Index; Conv: conventional; Org: organic.

^aData are expressed as mg catechin equivalents (CE).

^bOne unit (U) of enzyme activity was defined as the amount of enzyme that causes an increase in absorbance of 0.1 per minute per ml, monitored at 420 nm, at 25 °C.

^cData are expressed as mg gallic acid equivalents (GAE).

2001), while others reported higher phenolics in conventionally produced fruit. Specifically, in a three-year study on conventionally and organically grown plums, Lombardi-Boccia et al. (2004) found that the total polyphenol content and Neo-CGA were significantly higher in conventionally grown plums. These findings indicate that the modulation of phenolic biosynthesis and metabolism depends on many factors, among which the different Carbon to Nitrogen (C/N) balance between Conv and Org cultivation could be one of the causes (Rembalkowska 2007; Hallmann 2012). Compared to monomeric phenolics, lower amounts were found for flavonols and ATH, in agreement with a previous report (Kim et al. 2003). As regards to flavonols, plums are mainly rich in quercetin-derivatives and here rutin was the most predominant, with values ranging from 11 to 29 mg/100g DW (Table 4). Similar findings were reported by Kim et al. 2003. The level

of this compound was not influenced by the year of cultivation, while it varied mostly with the cultivar, showing higher values in Victoria compared to the other two cultivars. Finally, the Conv system seems to positively affect rutin content.

ATHs, which are mainly responsible for the pigmentation of plum skin, were higher in 2016 than 2015, similarly to Neo-CGA that also showed an increase (Table 4). In both years, the Conv system generally determined higher values compared to the Org system. There was a great varietal difference and the highest ATH content was detected in Jubileum, in accordance to what was previously reported (Vangdal et al. 2017).

Flavan-3-ols are abundant in plums (Nunes et al. 2008; Venter et al. 2013), particularly as dimeric and trimeric form of catechin (Tomás-Barberán et al. 2001). In the present study, the level of flavan-3-ols

(Table 5) showed a high variability, ranging from 500 to 4000 mg CE/100g DW. In particular, flavan-3-ols were significantly lower in 2015 (1117.1 mg CE/100g) compared to 2016 (2462.6 mg CE/100g), following the same temporal variation of monomeric phenols. Among the three cultivars, Jubileum showed the lowest flavan-3-ols level (1361.5 mg CE/100g) compared to Reeves (1942.3 mg CE/100g) and Victoria (2065.8 mg CE/100g). The highest variation between years was observed in Reeves, where in 2016 the flavan-3-ols were more than fourfold higher in Conv samples and more than fivefold in Org ones.

Total ascorbic acid

Gil et al. (2002) reported that plums do not contain high AsA levels. In their work, in fact, AsA ranged from 2.5 to 10.2 mg/100g fruit. However, this antioxidant metabolite is of great importance in determining the antioxidant potential of a fruit. In our study, we observed that the year strongly influenced the AsA level (Table 2) and particularly in Jubileum and Victoria, that showed higher AsA values in 2015 compared to 2016 (Table 5). Among the three varieties, Victoria, which showed the highest AsA content, was subjected to a strong decrease of this metabolite in 2016, compared to 2015. This result could be related to the earlier harvest date in 2016, and is line with the finding that some early-ripening apple cultivars had lower AsA content compared to those harvested late in the season (Davey and Keulemans 2004). The interactions “year $\times$ cultivation system” and “cultivar $\times$ cultivation system” were significant, as well as the interaction between the three factors, indicating that the cultivation system differently affected the AsA level depending on the year and the cultivar. For example, Jubileum, showed significantly lower AsA levels in Org plums than Conv in 2015, but not in 2016. On the contrary, Victoria showed higher AsA content in Org plums compared to Conv in 2015, but not in 2016.

PPO activity

The PPO activity was significantly affected by the three factors (Tables 2 and 5). On average, plums harvested in 2015 had higher PPO activity (43.6 U/g DW) compared to those of 2016 (31.3 U/g DW). The variation of the PPO activity between the two years was particularly evident in Reeves, which showed very low values in 2016 compared to 2015 (Table 5). On the other hand, Jubileum distinguished for the highest

values in both years. Even if to a lesser extent, the system of cultivation significantly affected the PPO activity, being higher in Conv compared to Org samples. Interestingly, a significant negative correlation was found between PPO activity and total flavan-3-ols ($r = -0.58$, $p < .001$), indicating that samples with higher value of flavan-3-ols had lower PPO activity. This observation is in agreement with previous results by other authors who suggested that these compounds – when highly polymerised – act as PPO inhibitors even in low concentration (Le Bourvellec et al. 2004).

Antioxidant capacity

In the present paper, the level of reducing substances (FCI), ranged from 375.6 to 555.5 mg/100g DW, which corresponded to 53.3–118.7 mg/100g FW. Kim et al. (2003) reported a wider range in plums from California (125.0–372.6 mg/100g FW), whereas Slimestad et al. (2009) reported for Norwegian plums a range (18–44 mg/100g FW) similar to the one we observed. The FCI (Tables 2 and 5) was not influenced by sampling year and systems of cultivation ($p > .05$), but it varied with the variety ($p < .001$, Table 2). In particular, Victoria distinguished for the highest FCI level (724.1 mgGAE/100g DW), while Reeves had the lowest one (434.5 mgGAE/100g DW), in accordance with the study of Slimestad et al. (2009), which reported low TEAC values for Reeves. This result is also in line with the positive relationship found between FCI and Neo-CGA content ($r = 0.73$, $p < .001$).

On the other hand, the year of cultivation strongly influenced the other two measured indexes, DPPH $\bullet$ and superoxide scavenging (Table 2). Interestingly, two opposite effects were found, with DPPH $\bullet$ scavenging increasing in 2016 with respect to 2015 (Figure 1, top), while the superoxide scavenging was comparable for two out of three varieties while in the case of Victoria it was higher in 2015 than 2016 (Figure 1, bottom). Similarly to FCI, Victoria had a significantly higher DPPH $\bullet$ and superoxide anion scavenging capacity compared to the other cultivars. In plums, Gil et al. (2002) measured a DPPH $\bullet$ scavenging capacity in the range of 27.4–61.1 mg AsAeq/100g FW, while in the presented study DPPH $\bullet$ varied between 4.5 and 22.0 mg AsAeq/100g, when reported on FW basis.

As expected, the health-promoting compounds, and particularly phenolics, were significantly correlated with the measured antioxidant indexes, in accordance with previous findings (Gil et al. 2002; Kim et al.

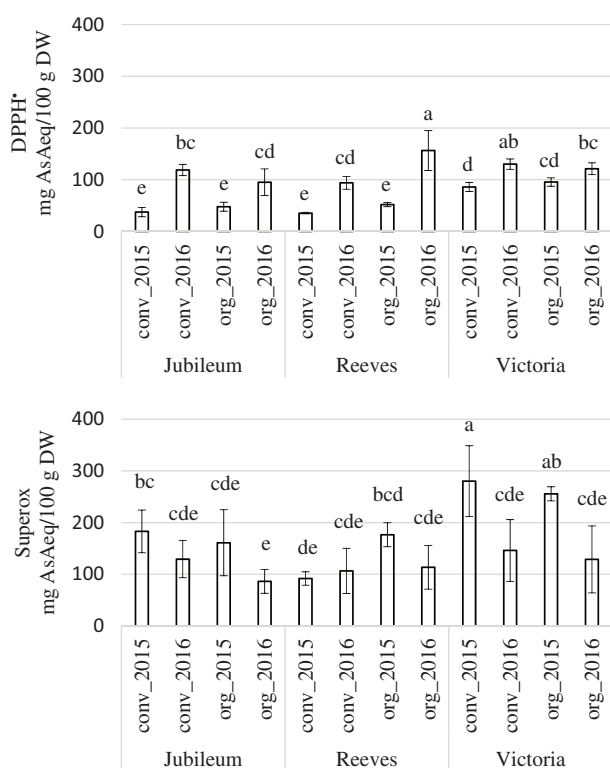


Figure 1. Antioxidant activity measured as DPPH• (top) and superoxide anion (Superox, bottom) scavenging capacity of conventional (conv) and organic (org) Jubileum, Reeves and Victoria plums in the 2 years of cultivation (2015 and 2016). Error bars represent the standard deviation. Different letters above bars indicate means significantly different according to Tukey's test ($p < .05$). ^aData are expressed as mg of ascorbic acid equivalents (AsAeq)/100 g dry weight (DW).

2003; Slimestad et al. 2009). The linear regression analysis showed that flavan-3-ols were primarily responsible for the DPPH• scavenging activity ($r = 0.86$, $p < .001$), while AsA content was positively related to superoxide anion quenching capacity ($r = 0.66$, $p < .001$). The high antioxidant capacity of flavan-3-ols was demonstrated in the work of Venter et al. (2013) on *P. salicina*, where the post-column on-line coupling of the ABTS•⁺ scavenging assay with HPLC-DAD revealed that flavan-3-ols had the greatest antioxidant response.

Allergen content

Allergies to food, especially to fruits from *Rosacea* plants such as apple, peach, strawberry or plum are often accompanied with birch pollen allergies due to cross-reacting antibodies. Previous studies showed, that the allergenicity of apples and tomatoes is cultivar dependent (Dölle et al. 2011; Sancho et al. 2006). The influence of the cultivar, growing conditions and seasonal effect on the Pru d 1 allergen content of plums was studied by indirect competitive ELISA. Comparing fruits grown under the same conditions, we observed that the three varieties differed to some extent in their allergen level.

According to the Kruskal–Wallis test, five significantly different groups (letters a–e) were detected with 5% of significance level (Figure 2). The significantly lowest level of Pru d 1 was found in the cultivar

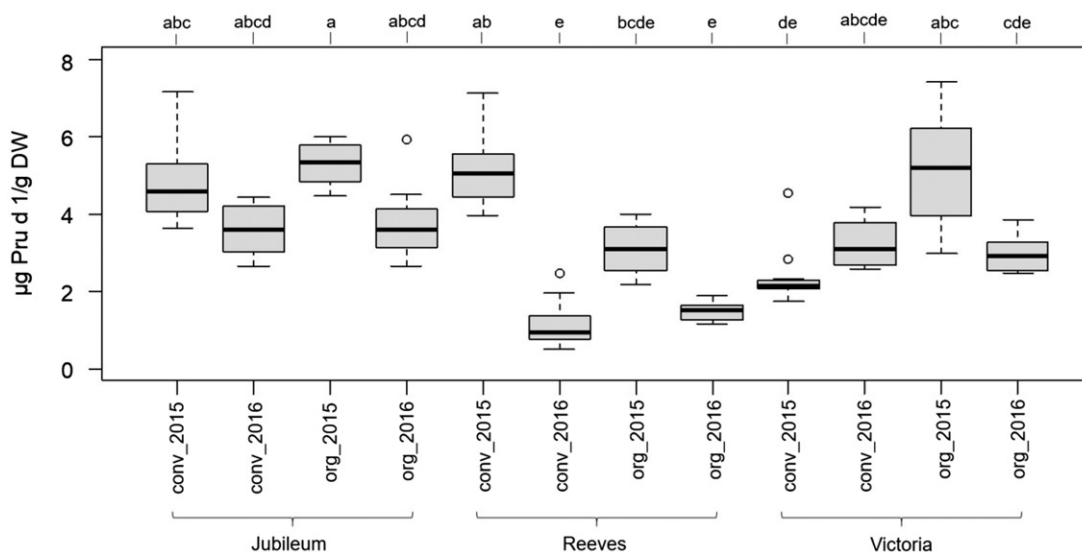


Figure 2. Box-and-whisker plots describing the allergen content expressed as µg Pru d 1/g dry weight (DW) of conventional (conv) and organic (org) Jubileum, Reeves and Victoria plums in the 2 years of cultivation (2015 and 2016). Medians, quartiles, and extreme values are given. Whiskers extend to the most extreme data point if it is within a distance of $1.5 \times$ inter-quartile range from the box, otherwise to the $1.5 \times$ inter-quartile range and outliers are shown. Different letters above bars indicate medians significantly different ($p < .05$).

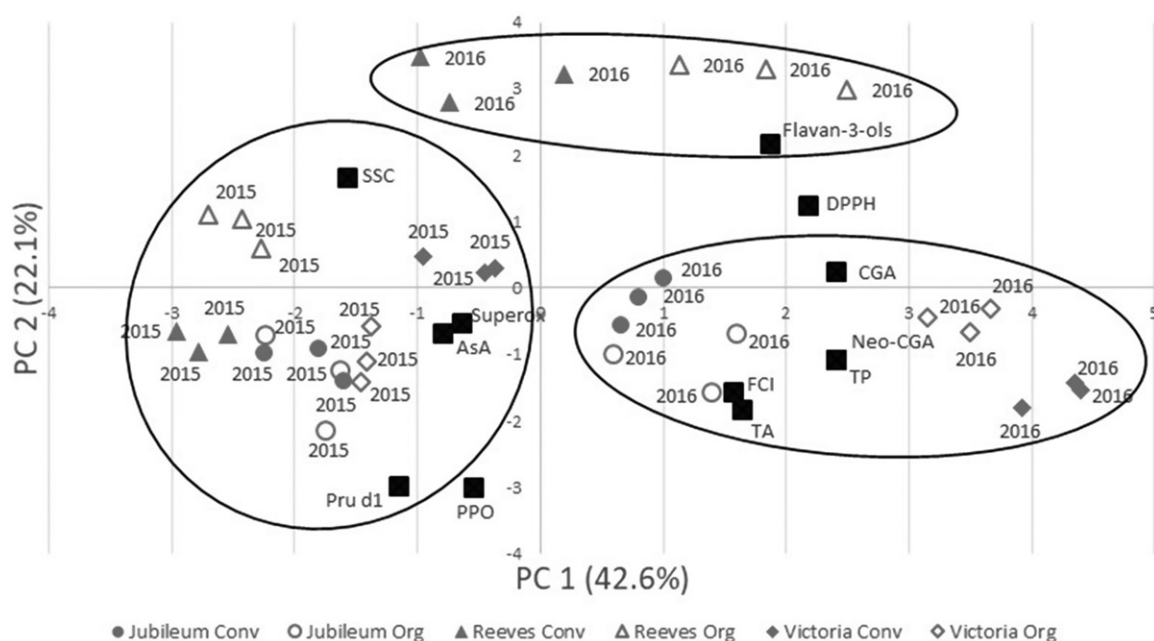


Figure 3. Results of PCA: biplot of PC1 versus PC2 showing the loadings of variables (black squares) and the scores of Jubileum (circle), Reeves (triangle) and Victoria (diamond) plums from conventional (full symbols) and organic (open symbols) farming. The selected parameters used in PCA were common quality parameters, phytochemicals, PPO activity and antioxidant capacity, which clearly separates the 2 years of cultivation (2015 and 2016). The percentage of total variance explained from each principal component is shown in parentheses. SSC: Soluble solids content; TA: titratable acidity; Neo-CGA: neo-chlorogenic acid; CGA: chlorogenic acid; TP: total polyphenols; AsA: ascorbic acid; PPO: Polyphenol oxidase activity; FCI: Folin-Ciocalteu Index; DPPH: DPPH scavenging capacity; Superox: superoxide anion scavenging capacity; Pru d1: allergen content.

Reeves grown conventionally in year 2016 with $1.15 \mu\text{g Pru d 1/g DW}$ (mean value $0.954 \mu\text{g Pru d 1/g DW}$), whereas the significantly highest concentration was determined in the cultivar Jubileum organically grown in 2015 with $5.29 \mu\text{g Pru d 1/g DW}$ (mean value $5.328 \mu\text{g Pru d 1/g DW}$).

The results of the statistical analysis indicated that the cultivation method had no significant effect on the allergen content. Only in Victoria in 2015 the Pru d 1 level changed with the cultivation system. In fact, the Pru d 1 level was higher in Org compared to Conv in Victoria. When comparing the two consecutive years, all plum samples from 2016 showed lower allergen content than samples harvested in 2015 (Table 2; Figure 2), and a cultivar effect was observed, with Jubileum showing significant higher values compared to Victoria and Reeves. Varying weather conditions (precipitation and temperature) thus appear to have an important influence on the allergen content in plums.

The linear regression analysis evidenced that Pru d 1 content was negatively related with flavan-3-ols content ($r = -0.73$, $p < .001$) and positively related with PPO activity ($r = 0.72$, $p < .001$). This result indicates that lower Pru d 1 values were observed in those

samples with higher flavan-3-ols content and higher PPO activity, and these two parameters were in turn inversely related. In fact, in 2015, when flavan-3-ols were significantly lower, the values of Pru d 1 were significantly higher. The relationship between the allergen content and total phenols as well as PPO activity was previously studied on Mal d 1 (Kiewning et al. 2013; Schmitz-Eiberger and Matthes 2011). In line with our results, Rudeschko et al. (1995) associated the lower allergenicity of apple to a higher polyphenol content and the assumed oxidative reactions that took place between apple phenols and the allergen. Similarly, as regards to the cherry allergen Pru av 1, Gruber et al. (2004) observed a decrease in allergenic potential of the protein in the presence of polyphenols and PPO. Kiewning et al. (2013) found a high correlation between Mal d 1 and the PPO activity of two apple cultivars, that is, Elstar and Diwa, while the correlation of these factors was moderate for fruits of the cultivar Boskoop. The latter cultivar showed the highest amount of total phenols, which was nearly twice as high as in Diwa. The authors concluded that the activity of PPO is more important than the content of total phenols to reduce the Mal d 1 level and if there is a high PPO activity, Mal d 1 could be

reduced even if the total phenol concentration is low. On the contrary, in the present study, we observed a tendency towards higher Pru d 1 level in Jubileum, which also showed the highest PPO activity and the lowest level of flavan-3-ols, thus suggesting that flavan-3-ols content is probably more important than the activity of PPO to reduce the Pru d 1 level in plums. Conv and Org cultivation showed minor effects on the Pru d 1 level, and our results indicated that the allergen content of plums might be cultivar dependent. A wider range of cultivars should be analysed to clarify which cultivars constantly express low amount of Pru d 1.

Principal component analysis

PCA was performed to have a more comprehensive view of the relationships among the analysed variables and the three factors (year, cultivar and system of cultivation). As expected, the PCA (Figure 3) clearly separated plums cultivated in 2015 (left side) from those cultivated in 2016. Polyphenols (Neo-CGA and CGA), TA and FCI had the most positive loading for PC1, and best distinguished the 2 years of sampling. Among plums harvested in 2016, cultivar Reeves grouped towards the more positive region of the PC2, since it has the greatest flavan-3-ols content compared to other two genotypes. Plums harvested in 2015 distinguished for their higher SSC, AsA and Superoxide scavenging capacity, with respect to those cultivated in 2016. Additionally, also Pru d 1 content and PPO activity were higher in samples grown in 2015.

Conclusion

The presented data clearly indicate that the main factor that affects the quality parameters in plum, besides the diversity among assayed genotypes, is the year-to-year variation, whereas the system of cultivation plays a marginal role. New and interesting results were reported regarding the relationship between the allergen content of plum and specific class of phenols, as well as the involvement of PPO enzymes. Further studies are needed on a wider range of genotypes, in order to better clarify the influence of the system of cultivation on the quality of plum fruit.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

This research was supported by the ERA-NET action “CORE Organic Plus” [Contract no. 618107] in the framework of the “FaVOr-DeNonDe” Project (<http://coreorganic-plus.org/researchprojects/favor-denonde/>).

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