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Effect of Fruit Bagging on Quality of 'Conference' Pear (*Pyrus communis* L.)

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Summary

The effect of fruit bagging on the quality of 'Conference' pears (*Pyrus communis* L.) was evaluated over two growing seasons. The experiment was conducted in three treatments: bagged until harvest, where the paper bags were left on the fruit until harvest time (150 days after full bloom (DAFB)); bagged until −7 days before harvest, where the bags were removed seven days before harvest (143 DAFB); and the control – not bagged at any time. Bagged fruit were covered with paper bags in mid-June, 65 DAFB, at an early stage of fruit growth. HPLC analysis was performed for individual sugars, organic acids in whole fruit, and phenolic compounds in the skin and pulp. The year had considerable influence on observed fruit parameters. Bagging had a negative impact on sucrose and sorbitol content. The bagged fruit were brighter (higher L*) than the control fruit. The reduction of colour (increased L* parameter and hue angle around 95°) by bagging may have a negative effect on consumer appeal. In second growing season bagging reduced fruit weight to such extend, that fruit were under 110 g and therefore not marketable. Bagging of pear fruit increased some phenolic compounds in the skin. Epicatechin and caffeic acid contents in skin were highest in bagged fruit. Removing the bags seven days before harvest decreased arbutin content in the pulp and also total sugars in the whole fruit and therefore fruit may have poorer eating quality. Quercetin 3-*O*-rhamnoside content in skin decreased in both years among fruit bagged until harvest. We don't recommend the use of bagging for 'Conference' pear in European growth conditions.

Key words. bagging – carbohydrates – HPLC – organic acids – phenolic compounds – *Pyrus communis* – skin colour

Introduction

Regular annual crops with high external and internal quality are very important for the pear industry. External quality is determined by shape, colour and size of the fruit, while internal quality is determined by the texture and the content of sugars, organic acids and phenolic compounds, which contribute to the taste and aroma of fruit. Phenolic compounds play a major physiological role in fruit, especially in resistance to various stresses as well as in contributing to its sensory quality (e.g., aroma, astringency and bitterness) and colour (Lancaster et al. 2000; Davik et al. 2006).

During growth and maturation, fruit are susceptible to insect pests, birds, diseases and mechanical damage, which reduce their quality and commercial value. Bagging, a physical protection technique commonly applied to many fruit, not only improves fruit visual quality by promoting fruit coloration and reducing the incidence of fruit cracking and russet but can also change the microenvironment of fruit development, which has multiple effects on internal fruit quality (Xu et al. 2010). The practice of pre-harvest bagging has been used extensively in several fruit crops to improve skin colour and to reduce mechanical damage as well as sunburn of the skin (Noro et al. 1989; Tyas et al. 1998; Amarante et al. 2002b). Pre-harvest bagging also reduces agrochemical residues in the fruit and improves insect, disease, and bird damage control (Kitagawa et al. 1992). Several authors have reported contradictory results for the effects of pre-harvest bagging on fruit size, maturity, skin colour and flesh mineral content at harvest for various fruits. This may reflect differences in the type of bag used, the fruit stage when it was bagged, the duration of fruit exposure to natural light after bag removal (before harvesting), and/or fruit- and cultivar-specific responses (Fan and Mattheis 1998). Fruit bagging also affects internal qualities, such as fruit sweetness and acidity. Most studies indicated that fruit bagging decreased soluble solids content in Asian pear (Huang et al. 2007).

Production of European pear is very important in the world. European pear fruit has characteristics different from those of Asian pear; therefore, bagging research that is specific to European pears would be useful. Although much research has been done concerning the effects of bagging on fruit quality in various species, few of these studies have included European pear (*Pyrus communis* L.). The 'Conference' cultivar is an economically important and a high yielding cultivar, which produces many russet fruit which are small if not thinned. According to Xu et al. (2010), russeting in Asian pear cultivars can be reduce by fruit bagging early in the fruit development. The purpose of this study was to investigate the effect of fruit bagging on external (fruit dimension, skin

colour) and internal (carbohydrates, organic acids and polyphenolic compounds) quality of the 'Conference' pear cultivar (*Pyrus communis* L.).

We wanted to determine whether bagged fruit improve the appearance of fruit, especially the visual quality.

Material and Methods

Plant material

The study was conducted on 'Conference' pear trees (*Pyrus communis* L.) on quince MA rootstock, growing in sandy loam at the commercial orchard in Bistrica ob Sotli (Slovenia, 55.08° N, 10.11° E) on the same trees in 2005 and 2006. The trees were spaced 3.8 × 1.4 m. Thirty trees were randomly selected, and the bags were tied to the fruit (10 fruit per tree) after the June drop (65 days after full bloom (DAFB)). The experiment included three treatments: bagged until harvest, bagged until minus 7 d before harvest and the control. In the first treatment (bagged until harvest), we left the paper bags on the fruit until harvest (150 DAFB). In the second treatment, we removed the bags seven days before harvest (143 DAFB). The third treatment (control) was not bagged at all. For the experiment, we used triple-layer paper bags (kraft paper, 162 × 220 mm; Laiyang Yintong, Shandong, China). The inner layer was white, the middle one black and the outer layer was grey. Both bagged and non-bagged fruit were harvested on the same day at technological maturity, which was evaluated using the starch iodine test, on 7th September 2005 and 9th September 2006.

Physical characteristics such as fruit weight, fruit diameter, length and firmness were determined, and chemical analyses (soluble solids, individual sugars, organic acids and phenolic compounds) were performed at harvest on 15 randomly selected fruit (15 replication).

Fruit quality measurement

Pear skin colour was measured using a Minolta CR-300 Chroma portable colorimeter (Minolta Co., Osaka, Japan) with C illuminant. Fruit chromaticity was expressed in the L^* colour space coordinates (CIELAB). L^* corresponds to a dark/light scale (0 = black, 100 = white) and represents the relative lightness of colours, being low for dark colours and high for light colours. The hue angle (°) is calculated as $\tan^{-1}(b^*/a^*)$, where 0 ° = red, 90 ° = yellow, 180° = green and 270° = blue, and chroma (C^*) indicates the intensity or colour saturation (Lancaster et al. 1997; McGuire 1992). The colour was measured after harvest. For each fruit, two measurements were performed. The colour was measured on 15 fruit samples from each treatment. Soluble solids content was determined in the juice of each fruit using a digital refractometer (model WM-7, Atago, Tokyo, Japan) at 20 °C. Firmness was measured with a digital penetrometer (model 53205, T.R. Turoni SRL, Forli, Italy) with an 8-mm-diameter plunger.

Extraction and analysis of carbohydrates, organic acids and phenolic compounds

For the HPLC analyses of individual sugars and organic acids, 10 g of fresh fruit (flesh with skin) were used. Tissue samples were extracted with 40 ml of bidistilled water, homogenized with Ultra – Turrax T-25 (Ika-Labortechnik, Staufen, Germany) and left at room temperature for extraction; after 30 min, they were centrifuged at 12,000×*g* for 15 min. Before injection into the column, samples were filtered through a 0.45 µm Macherey-Nagel filter (Düren, Germany). Samples of 20 µl of extract were injected for the HPLC analysis of sugars and organic acids (Hudina and Stampar 2006).

The sugars (glucose, fructose, sucrose and sorbitol) and organic acids (citric, malic, shikimic and fumaric) were analyzed by HPLC. A Thermo Separation Products HPLC system (Riviera Beach, Florida, USA; pump model P1000; auto sampler model AS1000) was used. The solute elution was monitored by using the refractive index (Shodex-71RI model). Analysis of the sugars was performed isocratically, using a Rezex RCM – monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, CA) at 65 °C and a flow rate of 0.6 ml min^{-1}, with bidistilled water used as the eluent. Organic acids were analysed using an Aminex – HPX 87H column (Bio Rad Laboratories, Hercules, CA) at 65 °C with a flow rate of 0.6 ml min^{-1}. A UV detector was used with a wavelength set at 210 nm. For the mobile phase, 4 mM H_2SO_4 was used.

The samples for phenolic analysis were prepared according to the method described by Escarpa and Gonzalez (1998). The samples of 10 g of pulp or 5 g of skin were extracted with methanol containing 1 % 2,6-di-*tert*-butyl-4- methylphenol (BHT) in an ultrasonic ice bath. The samples were extracted with 10 ml of solvent for 1 h, 10 ml for 30 min, and finally 5 ml for 30 min. The three extraction fractions were combined with a final volume of 25 ml and centrifuged (Eppendorf Centrifuge 5810 R, Hamburg, Germany) at 12,000×*g* for 7 min at 4 °C. The supernatant was then filtered through the Chromafil AO-45/25 polyamide filter (Macherey–Nagel, Düren, Germany) prior to injection into the HPLC system.

The analysis of phenolic compounds was carried out using a Thermo Finnigan Surveyor HPLC system with diode array detector at 280 nm and 350 nm. The spectra of compounds were also recorded between 210 and 400 nm (flavonols according to Bakhshi and Arakawa (2006)). The elution solvents were aqueous 0.01 M phosphoric acid (A) and 100 % methanol (B). The samples were eluted according to a linear gradient described by Escarpa and Gonzalez (1998) with minor modifications: 5–50 % B (10 min), 50–70 % B (5 min), 70–80 % B (5 min) and finally 80–100 % B (for 5 min). The injection amount was 20 µl, and the flow rate was 1 ml min^{-1}. A phenomenex Gemini C18 (150 × 4.6 mm 3 µm; Phenomenex, Torrance, CA) column maintained at 25 °C was used. Identification of compounds was achieved by comparing the retention times and spectra as well as by addition of the internal standard. The concentrations of phenolic compounds were calculated with the help of a corresponding external standard.

Chemicals

For quantification of sugars and organic acids, fructose, glucose, sucrose, sorbitol, citric, fumaric and shikimic acids were obtained from Fluka (Buchs, Switzerland) and malic acid from Merck (Darmstadt, Germany). The following standards were used for quantification of individual phenolic compounds: chlorogenic acid (5-caffeoylquinic acid) and arbutin from Sigma (St. Louis, MO,

USA), caffeic acid, ferulic acid, (−)-epicatechin and p-coumaric acid from Fluka (Buchs, Switzerland) and (+)-catechin from Roth (Karlsruhe, Germany). For quantification of flavonols, rutin (quercetin 3-*O*-rutinoside) was obtained from Sigma (St. Louis, MO, USA), and quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside from Fluka.

Statistical analysis

Statistical significance was determined by one-way analysis of variance (ANOVA) using the Statgraphics Plus 4.0 program (Manugistics, Rockville, MD, USA). Two way analysis of variance (MANOVA) was used to determine the effect of bagging, year and their interaction on physical characteristics and chemical properties at a significance level of 0.05. Differences within single seasons among treatments in terms of fruit dimension, soluble solids content, firmness, colour and content of carbohydrates, organic acids and phenolics were estimated by the LSD multiple range test at $P < 0.05$. Data from each year were analysed separately.

Results and Discussion

Bagging affects the size and weight of apple, pomegranate and banana (Arakawa et al. 1994; Hasan et al. 2001). The year had considerable influence on fruit dimension, firmness and soluble solids content. Bagging had no influence on fruit size, weight, soluble solids content or firmness in 2005 (Table 1). Amarante et al. (2002a) also reported that pre-harvest bagging of 'Doyenne du Comice' pears had no effect on fruit size, weight, maturity, nor on the flesh content of N, P, K, Ca, and Mg. In the second year of our experiment, bagging reduced fruit size and weight. Bagging reduced fruit weight for 60.96 g (bagging until harvest) and 67.77 g (bagging until 7 d before harvest) compared to control in 2006. Fruit under 110 g are not suitable for selling (marketable). Lower temperature (average annual temperature was 9.3 °C, during growing season 15.8 °C) and higher precipitation (July 233 mm, August 239 mm, September 149 mm) in 2006 may reduced fruit size compared to year 2005 (average annual temperature was 10.4 °C, during growing season 16.8 °C; precipitation: July 48 mm, August 185 mm, September 62 mm). Fruit bagging resulted in higher firmness of the fruit only in 2006. Pre-harvest bagging of pears increased fruit softening during post-harvest (Amarante et al. 2002a). Soluble solids content was significantly lower in bagged fruit in 2006. In 2005, bagging had no influence on soluble solids content.

Surface colour is an important marketable quality attribute (in terms of consumer acceptance) and is a measure of L^*, chroma and hue angle. Table 1 shows that bagging improved fruit surface lightness, as L^* was higher in the bagged than in the control fruit samples; therefore, the bagged fruit were brighter. L* parameter doesn't appear to be year dependent. Amarante et al. (2002a) reported that pre-harvest bagging of pears improved skin finish, resulting in a fruit with a more attractive light green colour, without reducing blush on the exposed side of the skin. The pears bagged until harvest had a yellow tinge, with high lightness value and hue angle (Huang et

Table 1. Fruit dimension, firmness, soluble solids content and colorimetric parameters (mean ± standard error) of 'Conference' pear in response to bagging treatments (removed 7 d before technological maturity, at technological maturity, or not bagged (control)) in 2005 and 2006.

	Treatments in 2005				Treatments in 2006				T	Y	T × Y
	−7 days (143 DAFB)	Until harvest (150 DAFB)	Control	Sig.	−7 days (143 DAFB)	Until harvest (150 DAFB)	Control	Sig.			
Length (mm)	89.58 ± 3.47 a	99.30 ± 2.74 a	97.39 ± 4.27 a	NS	78.33 ± 2.64 b	79.79 ± 2.69 b	92.90 ± 1.41 a	***	**	***	*
Width (mm)	60.73 ± 1.72 a	62.89 ± 1.13 a	64.84 ± 1.62 a	NS	49.57 ± 1.27 b	51.04 ± 1.24 b	62.80 ± 0.73 a	***	***	***	***
Weight (g)	140.47 ± 11.19 a	158.59 ± 7.46 a	166.24 ± 10.68 a	NS	76.21 ± 5.96 b	83.02 ± 6.53 b	143.98 ± 4.32 a	***	***	***	**
Firmness (kg cm^{-2})	6.36 ± 0.25 a	6.33 ± 0.16 a	6.62 ± 0.16 a	NS	6.36 ± 0.28 a	6.90 ± 0.25 a	5.00 ± 0.09 b	*	***	*	***
Soluble solids (%)	11.74 ± 0.45 a	12.28 ± 0.33 a	11.69 ± 0.19 a	NS	9.28 ± 0.43 b	9.79 ± 0.39 b	10.53 ± 0.16 a	***	NS	***	NS
L*	67.26 ± 1.66 a	70.01 ± 0.67 a	57.93 ± 0.93 b	***	66.47 ± 0.81 b	70.52 ± 1.10 a	59.93 ± 0.86 c	***	***	NS	NS
Chroma (C*)	38.95 ± 0.57 a	39.36 ± 0.39 a	35.52 ± 0.69 b	***	36.02 ± 0.53 a	33.89 ± 0.56 b	36.07 ± 0.62 a	*	*	***	***
Hue angle (h°)	97.62 ± 1.17 b	98.37 ± 1.33 b	110.86 ± 0.95 a	***	98.50 ± 0.79 b	94.30 ± 0.53 c	104.83 ± 0.75 a	***	***	***	**

Values followed by different letters within each row are significantly different according to the LSD multiple-range test at $P < 0.05$.
Sig. – significance; * – significant differences at $P < 0.05$; ** – significant differences at $P < 0.01$; *** – significant differences at $P < 0.001$; NS – non-significant differences
T – Treatment; Y – Year; T × Y – Treatment × Year

al. 2009). In our experiment, Chroma (C*) in bagged fruit was significantly higher than in the control in 2005, while for fruit bagged until 7 d before harvest in 2006 there were no differences between the treatment samples and the control. The Chroma (C*) of fruit bagged until harvest had a lower intensity of colour than in the control in 2006. Hue angle was lower (yellow fruit) with the bagging treatment compared with the control in both years. Also Huang et al. (2009) reported that hue angle of control fruit of sand pears during fruit maturation period was much lower than bagged fruit. For the same bag, bag removal dates did not significantly affect the hue angle of sand pear fruit. Jia et al. (2005) stated that non-bagged peach fruit had a smaller hue angle and a higher Chroma than the bagged fruit.

The content of sugars and organic acids, which depend on the plant genotype (Hudina and Stampar 2000), is also influenced by environmental factors and by horticultural practise undertaken in an orchard (Hudina and Stampar 1999, 2009, 2010). Fructose was the most abundant sugar of all quantified ones in pear fruit (Table 2), followed by sorbitol and sucrose. We found no differences in glucose and fructose content between bagged and non-bagged fruit in 2005, while in 2006 glucose content was higher and fructose content lower than in the control fruit. Noro et al. (1989) analysed the fruit content of the main sugars and acids of bagged apples, and reported treatment effects only on fructose, which was higher in bagged apples than in the non-bagged ones. In our experiment, bagging had a negative influence on sucrose and sorbitol content in both years. The total sugar content was also significantly lower when we removed bags 7 d before harvest. Xu et al. (2010) reported that the total sugar content in the fruit bagged with single-layer white paper bags was higher than that in either the control, or in fruit bagged with two-layered paper bags with a black inner layer and a grey outer layer. Chundawat et al. (1978) showed that bagging generally reduces the sugar content of fruit, while Huang et al. (2009) reported that bagging treatments had no influence on the total soluble sugar content but did decrease the organic acid content of fruit.

Bagging had no influence on citric acid content. Among organic acids, malic acid, in particular, is formed in most pear cultivars (Hudina and Stampar 2000). We also found that malic acid was prevalent. It was higher in bagged fruit in 2006, while in 2005 the differences between bagged and control fruit were non-significant. In 2005

Table 2. Individual and total sugars and organic acids content (mean ± standard error) of 'Conference' pear in response to bagging treatments (removed 7 d before technological maturity, at technological maturity, or not bagged (control)) in 2005 and 2006.

	Treatments in 2005				Treatments in 2006				T	Y	T × Y
	–7 days (143 DAFB)	until harvest (150 DAFB)	control	Sig.	–7 days (143 DAFB)	until harvest (150 DAFB)	control	Sig.			
Glucose (g kg^{-1} FW)	11.00 ± 0.93 a	12.12 ± 0.99 a	9.77 ± 0.73 a	NS	9.61 ± 0.70 a	8.20 ± 0.75 a	5.72 ± 0.28 b	***	***	***	NS
Fructose (g kg^{-1} FW)	56.37 ± 4.78 a	65.84 ± 2.56 a	63.21 ± 3.82 a	NS	36.41 ± 2.18 b	35.82 ± 2.09 b	41.65 ± 0.98 a	*	NS	***	NS
Sucrose (g kg^{-1} FW)	14.61 ± 2.22 b	17.48 ± 1.17ab	21.57 ± 1.94 a	*	10.44 ± 1.19 b	9.89 ± 1.31 b	23.48 ± 1.17 a	***	***	*	**
Sorbitol (g kg^{-1} FW)	24.98 ± 2.53 b	31.78 ± 1.62 a	32.93 ± 1.71 a	*	17.71 ± 0.83 b	17.31 ± 0.92 b	24.55 ± 0.86 a	***	***	***	*
Total sugars (g kg^{-1} FW)	106.98 ± 8.61 b	127.22 ± 3.83 a	127.49 ± 6.28 a	*	74.15 ± 3.24 b	71.21 ± 3.68 b	95.39 ± 1.91 a	***	***	***	*
Malic acid (g kg^{-1} FW)	7.57 ± 0.36 a	8.59 ± 0.18 a	8.12 ± 0.54 a	NS	5.47 ± 0.17 a	5.76 ± 0.17 a	4.33 ± 0.10 b	***	**	***	*
Citric acid (g kg^{-1} FW)	0.48 ± 0.05 a	0.52 ± 0.03 a	0.46 ± 0.05 a	NS	0.86 ± 0.08 a	0.81 ± 0.08 a	0.71 ± 0.03 a	NS	NS	***	NS
Shikimic acid (mg kg^{-1} FW)	34.00 ± 3.00 a	36.00 ± 2.00 a	31.00 ± 2.00 a	NS	11.20 ± 1.10 a	11.10 ± 1.30 a	6.50 ± 0.30 b	**	*	***	NS
Fumaric acid (mg kg^{-1} FW)	7.60 ± 0.40 a	10.70 ± 1.00 a	10.90 ± 2.50 a	NS	0.77 ± 0.04 a	0.83 ± 0.04 a	0.63 ± 0.02 b	**	NS	***	NS
Total acids (g kg^{-1} FW)	8.09 ± 0.40 a	9.15 ± 0.20 a	8.61 ± 0.59 a	NS	6.33 ± 0.20 a	6.58 ± 0.19 a	5.05 ± 0.12 b	***	**	***	*

FW – fresh weight
Values followed by different letters within each row are significantly different according to the LSD multiple-range test at $P < 0.05$.
Sig. – significance; * – significant differences at $P < 0.05$; ** – significant differences at $P < 0.01$; *** – significant differences at $P < 0.001$; NS – non-significant differences
T – Treatment; Y – Year; T × Y – Treatment × Year

there were no differences in shikimic and fumaric acid content, but in 2006 bagging yielded a higher content of both acids. Fruit which were bagged until harvest had significantly higher total acids than control ones. Bagging treatment significantly affected the visual qualities of pear fruit but not the content of total soluble sugars. However, it did decrease organic acids content in fruit (Huang et al. 2009).

Arbutin, epicatechin and chlorogenic acids were the major phenolic compounds found in pear skin (Table 3), while in pulp these were arbutin, catechin and chlorogenic acids (Table 3). Galvis-Sanchez et al. (2003) found arbutin only in pear skin. Bagging had no influence on chlorogenic acid and catechin content, but it increased epicatechin and caffeic acid content in skin. Arbutin, ferulic acid and quercetin 3-*O*-galactioside in skin appear not to be year dependent. Arbutin content in pulp was dependent on the year. Bagging influenced higher content levels of p-coumaric acid (both baggings) and ferulic acid (bagging until harvest) in 2005, but in 2006 there were no differences between treatments. In 2005 bagging had no influence on rutin, quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside, while in 2006 bagging until harvest decreased their content in skin. Quercetin 3-*O*-rhamnoside content in the skin also decreased in both years with the bagging until harvest treatment. Xu et al. (2010) determined that total phenolic and flavonoid content was decreased by both bagging treatments. However, phenolic content in pear pulp is more dependent on year than on bagging. Arbutin content in pear pulp decreased in fruit bagged until minus 7 d before harvest in both years. We propose that bagging alters the fruit metabolism and consequently influences fruit composition.

This study has shown that bagging of European pear fruit increased some secondary metabolites (phenolic compounds) in the skin. Epicatechin and caffeic acid content in skin was significantly higher in bagged fruit. Bagging reduced sucrose and sorbitol content and therefore fruit had poorer eating quality. Surface colour is an important marketable quality attribute. The reduction of colour (increased L^* parameter and hue angle around 95°) and fruit weight by bagging may have a negative effect on consumer appeal. According to our results we don't recommend the use of bagging for 'Conference'

Table 3. Content of analysed phenolics in skin and pulp (mean ± standard error, mg kg^{-1} fresh weight) of 'Conference' pear in response to bagging treatments (removed 7 d before technological maturity, at technological maturity, or not bagged (control)) in 2005 and 2006.

	Treatments in 2005				Treatments in 2006				T	Y	T×Y
	–7 days (143 DAFB)	Until harvest (150 DAFB)	Control	Sig.	–7 days (143 DAFB)	Until harvest (150 DAFB)	Control	Sig.			
Skin											
Arbutin	20.57 ± 3.35 b	37.27 ± 4.09 a	35.37 ± 3.98 a	*	36.44 ± 2.79 b	45.96 ± 2.98 a	18.82 ± 1.47 c	***	***	NS	***
Catechin	7.32 ± 1.01 ab	9.52 ± 0.77 a	5.53 ± 0.60 b	**	2.59 ± 0.34 a	2.79 ± 0.52 a	2.61 ± 0.12 a	NS	**	***	**
Chlorogenic acid	54.83 ± 11.97 a	67.85 ± 11.83 a	62.22 ± 9.46 a	NS	13.71 ± 1.10 a	10.81 ± 0.75 b	13.45 ± 1.10 ab	*	NS	***	NS
Caffeic acid	2.78 ± 0.46 a	2.67 ± 0.32 a	1.44 ± 0.22 b	**	3.94 ± 0.21 a	3.17 ± 0.27 b	2.19 ± 0.11 c	***	***	***	NS
Epicatechin	47.98 ± 7.74 a	59.24 ± 7.16 a	28.91 ± 4.15 b	**	11.55 ± 0.53 a	11.13 ± 0.83 a	7.52 ± 0.40 b	***	***	***	**
p-coumaric acid	1.48 ± 0.19 a	1.68 ± 0.12 a	0.73 ± 0.12 b	***	2.35 ± 0.12 a	2.44 ± 0.13 a	2.16 ± 0.19 a	NS	***	***	NS
Ferulic acid	2.67 ± 0.13 b	3.62 ± 0.19 a	2.56 ± 0.24 b	**	3.12 ± 0.16 a	3.32 ± 0.23 a	2.91 ± 0.30 a	NS	**	NS	NS
Rutin	1.72 ± 0.60 a	1.11 ± 0.25 a	1.17 ± 0.40 a	NS	1.79 ± 0.39 b	1.87 ± 0.45 b	4.26 ± 0.88 a	*	NS	**	*
Q-3 galactoside[x]	2.96 ± 1.10 a	1.09 ± 0.24 a	1.83 ± 0.63 a	NS	3.01 ± 0.67 a	1.05 ± 0.15 b	3.51 ± 0.63 a	**	**	NS	NS
Q-3 glucoside[y]	6.49 ± 2.91 a	1.65 ± 0.35 a	5.61 ± 2.22 a	NS	10.72 ± 2.43 a	2.02 ± 0.36 b	13.09 ± 2.30 a	***	***	*	NS
Q-3 rhamnoside[z]	7.66 ± 3.19ab	2.67 ± 0.56 b	18.92 ± 6.92 a	*	25.56 ± 5.46 b	7.16 ± 1.80 b	94.43 ± 14.40 a	***	***	***	***
Pulp											
Arbutin	8.57 ± 2.11 b	7.22 ± 1.33 b	15.64 ± 1.67 a	**	12.22 ± 2.66 b	22.74 ± 1.58 a	26.68 ± 1.74 a	***	***	***	*
Catechin	3.62 ± 0.27 a	2.48 ± 0.49 b	2.86 ± 0.12 ab	*	1.88 ± 0.42 b	3.31 ± 0.63 ab	3.70 ± 0.60 a	*	NS	NS	**
Chlorogenic acid	3.16 ± 0.17 a	2.57 ± 0.26 b	3.21 ± 0.09 a	*	1.54 ± 0.19 a	1.66 ± 0.15 a	1.38 ± 0.20 a	NS	NS	***	*
Epicatechin	2.37 ± 0.17 b	5.23 ± 0.15 a	1.97 ± 0.09 c	***	3.86 ± 0.49 a	4.01 ± 0.49 a	4.99 ± 0.46 a	NS	***	***	***

[x] – quercetin 3-*O*-galactoside; [y] – quercetin 3-*O*-glucoside; [z] – quercetin 3-*O*-rhamnoside;
Values followed by different letters within each row are significantly different according to the LSD multiple-range test at $P < 0.05$.
Sig. – significance; * – significant differences at $P < 0.05$; ** – significant differences at $P < 0.01$; *** – significant differences at $P < 0.001$; NS – non-significant differences
T – Treatment; Y – Year; T × Y – Treatment × Year

pear in European growth conditions. Further assessments are also necessary to determine the economic viability of this practice.

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