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Influence of Sodium Bicarbonate, an Anti-Apple Scab Agent, on Quality Parameters of 'Golden Delicious' Apples

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Summary

Experimental studies in the past years recommend the use of sodium bicarbonate (SBC) as a fungicidal agent in prevention and control of apple scab disease. Generally, the research aim of previous studies was investigating the effects of SBC on spore germination and germ tube elongation of *Venturia inaequalis* [(Cooke) G. Wint.] fungus. However, this study monitors the accumulation of sugars and organic acids as well as individual and total phenolic compounds in apple fruit at technological ripening stage as affected by the use of SBC against *Venturia inaequalis*. Three different treatments were established: water-treated control, fungicide treated and 1 % SBC solution. The use of SBC demonstrated a reduction in the disease symptoms on apple fruits and leaves. The fruit quality parameters (mass and fruit firmness) did not differ among treatments. On the other hand, fruit treated with SBC contained lower amounts of total phenolics, flavonols and flavan-3-ols compared to fruit from the control treatment. SBC treated fruit also contained higher amounts of sugars than fruit from the control treatment.

Key words. agricultural practice – alternative fungicides – *Malus domestica* (Borkh.) – natural substances – primary metabolites – phenolics – *Venturia inaequalis*

Introduction

Prevention and control of apple scab, caused by *Venturia inaequalis* [(Cooke) G. Wint.], is often very difficult, especially due to an increasing resistance of pathogens to chemicals used in treatments (McHardy 1996). There are a considerable number of chemicals used and recommended to control this disease that vary greatly in their effectiveness. Successful control and prevention of apple scab dispersal requires from 10 to 22 fungicide applications per season, depending on weather condition. Moreover, control strategies based on the massive use of fungicides may face problems of resistance to some active ingredients and might disturb the ecosystem and pollute the environment (Köller 1994; Kelderer et al. 2008).

Other options for controlling apple scab may be the use of natural substances that act as fungicides and have no known adverse effects on the environment and human health. Selected organic and inorganic salts are active antimicrobial agents and are widely used as food additives (Miyasaki et al. 1986). Bicarbonates are one of several control options that are now attracting attention. They are common food additives allowed in many applications under European and North American regulations (Horst et al. 1992). Among these, sodium bicarbonate (SBC) is used for controlling pH levels, and maintaining the taste and texture of various food products. It also exhibits a broad-spectrum antifungal activity that has been demonstrated in several studies against different plant pathogens (Horst et al. 1992), and recently also against apple scab (Schulze and Schönherr 2003; Ilhan et al. 2006; Jamar and Lateur 2007; Jamar et al. 2007). Ilhan et al. (2006) reported that SBC effectively inhibited spore germination and germ tube elongation of *Venturia inaequalis in vitro*.

Apple, like other fruits, varies greatly in its chemical composition, even within the same cultivar; depending on maturity, location of production, and agricultural process, as well as on numerous environmental factors (Lee et al. 2003). Furthermore, every cultivar has its typical chemical composition comprising of various primary and secondary metabolites; the latter including the all important group of phenolic compounds. These are naturally occurring plant secondary metabolites which determine the outer and inner quality parameters of fruits such as appearance, flavour and health-promoting properties (Lattanzio 2003). The content levels of primary metabolites (sugars and organic acids) in fruits may be influenced by fruit type (species and cultivars), cultivation conditions (environmental and cultivation techniques), climatic conditions, growing seasons, storage conditions, and industrial processing (Chun et al. 2005). The balance between the organic acids and sugars is very important in achieving a harmonized taste of fruits (Hudina and Stampar 2000).

The aim of our study was to compare the effectiveness against apple scab, phytotoxicity and the effects of SBC on outer (mass, colour, firmness) and inner (organic acids and sugars, individual and total phenolic compounds) fruit quality parameters with control and fungi-

cide treatments. Literature provides data firstly on the SBC treatment in inhibiting the germination and germ tube elongation of conidia of *Venturia inaequalis*, and secondly of the SBC phytotoxicity and its effects on yield. However, there is a lack of information on fruit quality parameters as affected by the SBC treatment. Therefore, in our study we focused on the effect of SBC treatment on the most important quality parameters in the high scab-susceptible apple cultivar 'Golden Delicious'. These data are of high importance in considering the use of SBC as a possible standard practice against apple scab prevention and suppression.

Materials and Methods

Chemicals

The following standards were used for the quantification of sugars and organic acids: sucrose, glucose, fructose and sorbitol; and citric, malic acids from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: chlorogenic acid (5-caffeoylquinic acid), phloretin and rutin (quercetin-3-*O*-rutinoside) from Sigma-Aldrich, ferulic acid, (−)-epicatechin, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, *p*-coumaric acid, procyanidin B2 and phloridzin dihydrate from Fluka Chemie (Buchs, Switzerland), quercetin-3-*O*-arabinofuranoside and quercetin-3-*O*-xyloside from Apin Chemicals (Abingdon, UK) and (+)-catechin from Roth (Karlsruhe, Germany). Methanol for extraction of phenolics was acquired from Sigma-Aldrich. The chemicals for the mobile phases were HPLC-MS grade acetonitrile and formic acid from Fluka. Water for mobile phase was twice distilled and purified with the Milli-Q system (Millipore, Bedford, MA). For the total phenolic content, Folin-Ciocalteu phenol reagent (Fluka), sodium carbonate (Merck, Darmstadt, Germany), gallic acid and methanol (Sigma-Aldrich) were used.

Plant material and experimental design

The study was performed on apple trees (*Malus domestica* Borkh.), cv. 'Golden Delicious', a scab-susceptible cultivar, in the growing season 2009. Fruits were harvested from 9-year-old trees grafted on the M9 rootstock growing in the University experimental orchard in Ljubljana (latitude: 46° 2', longitude: 14° 28').

The experimental treatments and the application protocol for the treatments were as followed: (1) water-treated control, (2) fungicide treated in accordance with the integrated pest management guidelines (Anonymous 2009), and (3) 1 % SBC (Merck). The first application of the treatments was on April 22nd. The treatments were repeated every 10 days until harvest and 14 applications were completed. Spray solutions were applied to the trees with a hand gun sprayer and each tree received 1 L of water/fungicide/SBC solution per application.

For the severity assessment of leaf phytotoxicity, 10 shoots per tree were monitored during the whole growing season and 10 fully developed leaves per shoot were surveyed. The following leaf phytotoxicity levels were recorded: 0 = no damage; 1 = 0 to 2 % damage; 2 = 2 to 5 % damage; 3 = 5 to 20 % damage; and 4 = >20 % of the leaf surface damaged. The leaf damage severity was evaluated using the grading scale described by Parisi et al. (1993): score 0 = no visible symptoms; score 1 = 0 % < percentage of scabbed leaf surface (sls) ≤ 1 %; score 2 = 1 % < sls ≤ 5 %; score 3 = 5 % < sls ≤ 10 %; score 4 = 10 % < sls ≤ 25 %; score 5 = 25 % < sls ≤ 50 %; score 6 = 50 % < sls ≤ 75 %; score 7 = sls > 75 %.

Fruit sampling was performed at technological maturity, which was determined by the use of the starch iodine test. The harvesting date was September 23rd. Five apples in five repetitions for each treatment were peeled as thin as possible and immediately shock-frozen (peel and pulp separately) in liquid nitrogen and stored at −20 °C until analysis of the samples. Disease assessment on fruit was made on harvesting date and disease severity was quantified using a scale developed by Tomerlin and Jones (1983). The scale consisted of five categories; (0) no visible lesion; (1) < 10 % fruit surface infected; (2) 10–25 % fruit surface infected; (3) 25–50 % fruit surface infected; (4) > 50 % fruit surface infected.

Physical fruit characteristics

Fruit firmness, colour and mass at technological maturity were evaluated in order to determine the effect of SBC treatments on outer quality parameters. The measurements were made on 25 randomly selected fruits for each treatment. Firmness was measured on four peeled sides of fruit with a penetrometer (tr Italy, TR Turoni, Forli, Italy) equipped with a 10 mm tip.

Peel color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka; Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In CIE $L^*a^*b^*$ system of color representation, the L^* value corresponds to a dark-bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). The a^* and b^* values extend from −60 to 60: a* negative is for green and a* positive is for red and b* negative is for blue and positive for yellow. The hue angle (h°) is expressed in degrees from 0 to 360 (0° = red, 90° = yellow, 180° = green and 270° = blue). Color was measured in the middle of each fruit (two repetition per fruit on different sides) to ensure equal measurement conditions.

Analysis of individual sugars and organic acids

The samples were analyzed for their content levels of sugars (sucrose, glucose, fructose and sorbitol) and organic acids (malic, citric). Apples were halved, cut into small pieces and 10 g of the fresh mass was immersed in 50 mL of twice distilled water and homogenized with a T-25 Ultra-Turrax (Ika-Labortechnik, Staufen, Germany). The samples were left for extraction for half an hour at room temperature with frequent stirring, and the extracted samples were centrifuged at 10 000 rpm for 7 min at 10 °C (Eppendorf Centrifuge 5810R, Hamburg, Germany). The supernatants were filtered through a 0.45 µm cellulose ester filter (Macherey-Nagel, Düren, Germany), transferred to a vial and analyzed according to the method described by Sturm et al. (2003) using high performance liquid chromatography (HPLC; Thermo Scientific, Finnigan Spectra System, Waltham, MA, USA). For each analysis 20 µL of sample was used. Analysis of sugars was carried out using a RezexRCM-monosaccharide column

(300 × 7.8 mm; Phenomenex, Torrance, CA) with a flow of 0.6 mL min^{-1} and column temperature maintained at 65 °C. For the mobile phase, twice distilled water was used, and an RI detector for identification. Organic acids were analyzed using a RezexROA-organic acid column (300 × 7.8 mm; Phenomenex, Torrance, CA), and the UV detector set at 210 nm with a flow of 0.6 mL min^{-1} maintaining the column temperature at 65 °C. For the mobile phase, 4 mM sulfuric acid (H_2SO_4) was used. The concentrations of carbohydrates and organic acids were calculated with the help of corresponding external standards. The concentrations were expressed in g kg^{-1} fresh weight (FW).

Extraction and determination of individual phenolic compounds

The apples were peeled with a mechanical peeler, and the peel was separated from the pulp. The extraction of fruit samples (peel and pulp separately) was done as described by Mikulic Petkovsek et al. (2007), with some modification. Fresh apple samples were ground to a fine powder in a mortar chilled with liquid nitrogen. The samples of 10 g pulp or 5 g peel were extracted with 10 ml methanol containing 1 % (w/v) BHT in a cooled ultrasonic bath (0 °C) for 1 h. The treated samples were centrifuged for 7 min at 10000 rpm. The supernatant was filtered through a Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) and transferred to a vial prior to injection into a HPLC system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (hydroxycinnamic acids, dihydrochalcones and flavan-3-ols), 350 nm (flavonols). A Phenomenex (Torrance, CA) HPLC column C18 (150 × 4.6 mm, Gemini 3 µ) protected with a Phenomenex security guard column operated at 25 °C was used. The injection volume was 20 µl and the flow rate maintained at 1 ml min^{-1}. The elution solvents were aqueous 1 % formic acid (A) and 100 % acetonitrile (B). Samples were eluted according to the linear gradient described by Marks et al. (2007): 0–5 min, 3 % to 9 % B; 5–15 min, 9 % to 16 % B; 15–45 min, 16 % to 50 % B; 45–50 min, 50 % isocratic; and finally washing and reconditioning of the column. Identification of compounds was achieved by comparing retention times and their UV-VIS spectra from 200 to 600 nm, as well as by the addition of an external standard. Compounds were identified and quantified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionisation (ESI) operating in negative ion mode. The analyses were carried out using full-scan data dependent MS^n scanning from *m/z* 115 to 2000. Quantification was achieved according to the concentrations of a corresponding external standard.

Concentrations of phenolic compounds were calculated from the peak areas of the sample and the corresponding standards. The concentrations were expressed in mg 100 g^{-1} fresh weight (FW) for both fruit peel and pulp. For the compounds lacking standards, similar compounds as standards for the quantification were used. Thus, 4-*O*-*p*-coumaroylquinic acid was quantified in equivalents of *p*-coumaric acid, phloretin-2-*O*-xylosylglucoside in equivalents of phloridzin, quercetin-3-*O*-arabinopyranoside in equivalents of quercetin-3-*O*-arabinofuranoside. Procyanidins, which in our extracts were found to be represented by dimers and trimers, were quantified in equivalents of procyanidin B2.

Determination of total phenolic content

The extraction of fruit samples for the determination of total phenolic content (TPC) was made according to the same protocol as for individual phenolics, with the difference that no BHT was added. TPC of the extracts was assessed using the Folin-Ciocalteu phenol reagent method (Singleton and Rossi 1965). Six ml of twice-distilled water and 500 µl of Folin-Ciocalteau reagent were added to 100 µl of the sample extracts and left to stand between 8 s and 8 min at room temperature; 1.5 ml sodium carbonate (20 % w/v) and 1.9 ml twice-distilled water was than added. The extracts were mixed and allowed to stand for 30 min at 40 °C before measuring absorbance at 765 nm on a Lambda Bio 20 UV/VIS spectrophotometer (Perkin Elmer, Waltham, MA). A mixture of water and reagents was used as a blank. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg 100 g^{-1} fresh weight of fruit peel or pulp. Absorptions were measured in three replications.

Statistical analysis

The data was analyzed using the Statgraphics Plus 4.0 program (Manugistics. Inc.; Rockville, Maryland, USA). Data from all the analyses was tested for any differences among treatments using one-way analysis of variance (ANOVA). Significant differences among means were determined by the least significant difference (LSD) with a significance level of 0.05.

Results and Discussion

The 1 % SBC treatment was effective in reducing the disease severity on leaves during all samplings. Moreover, 1 % SBC did not cause phytotoxic symptoms on apple leaves. Leaves were considered healthy where no visible symptoms of scab infection were apparent (score 0), while the infection rate for infected leaves during the growing season quickly increased. At the end of the growing season the disease severity on leaves in the control treatment reached score 7, while in SBC and fungicide treatment the score was 5 and 3, respectively.

Physical fruit quality parameters

The disease severity which caused visual apple scab symptoms on fruit was reduced with applications of 1 % SBC treatments in comparison to the control. The control reached the total value in categories from 3 to 4, fungicide treatments from 0 to 1 and SBC treatments from 1 to 2 (Fig. 1). Also, a reduced apple scab severity on fruit as affected by the use of 1 % SBC was previously reported by Ilhan et al. (2006). Quality evaluations of technologically mature fruit indicated that treatments with SBC have a positive effect on the fruit mass. Inhibition of apple scab with 1 % of SBC may be the main reason for the higher fruit mass of technological mature fruit compared to the control (Table 1). Statistically lower firmness of fruit from fungicide and SBC treatments was detected compared to control (Table 1). The reason for lower firmness

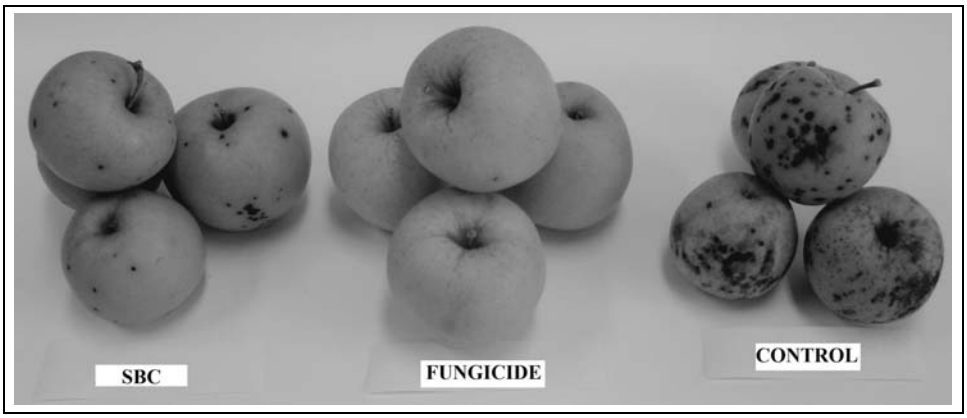


Fig. 1. Apple fruit from different treatments.

Table 1. The effect of sodium bicarbonate (SBC) treatment on quality of harvested fruit (means ± SE).

Treatments	Mass (g)	Firmness (kg cm^{-2})	CIE/Lab parameters			
			a	b	h	L
Control	106.29 ± 5.20 a*	8.14 ± 0.16 b	−18.90 ± 0.22 b	42.28 ± 0.30	114.10 ± 0.29	61.91 ± 0.42 a
Fungicide	154.67 ± 3.99 b	7.29 ± 0.12 a	−19.92 ± 0.85 ab	42.49 ± 0.22	114.98 ± 1.01	66.73 ± 0.41 b
SBC	142.41 ± 6.09 b	7.27 ± 0.11 a	−21.45 ± 0.37 a	43.09 ± 0.24	116.41 ± 0.42	61.25 ± 0.90 a

* Different letters (a–c) in rows denote statistically significant differences by LSD test at $P \leq 0.05$.

of fruits of SBC treatment compared to the control can be mostly attributed to the smaller mass of control fruits. The control fruits were of poor quality due to strong infection with with *Venturia inaequalis* which hindered their normal development (Fig. 1).

At harvest time, SBC treatment affected CIE parameter a*; a statistically lower value was measured indicating a greener fruit colour. The CIE coordinates b* and h* were not statistically different among treatments and the fruit did not show any visible discoloration of the skin (Table 1 and Fig. 1).

Sugars and organic acids in fruit

Glucose, fructose, sucrose, sorbitol and total sugars content levels of different treatments are presented in Table 2. Generally, fructose was found in highest concentration, followed by sucrose, glucose and sorbitol. This is in accordance with the results of Mikulic Petkovsek et al. (2007), who report somewhat higher values for sucrose (59.10 g kg^{-1} FW) and sorbitol (3.14 g kg^{-1} FW) for the same cultivar and maturity stage. Fungicide treatment caused a significantly higher content of fructose and

Table 2. The effect of sodium bicarbonate (SBC) treatment on content of organic acids and sugars of harvested fruit (mean ± SE in g kg^{-1} FW).

Treatments	Fructose	Glucose	Sucrose	Sorbitol	Total sugars
Control	50.82 ± 1.21 a*	21.85 ± 0.64 b	21.37 ± 2.78	1.14 ± 0.03 a	92.31 ± 3.20 a
Fungicide	67.23 ± 0.77 c	21.40 ± 0.51 b	26.46 ± 1.69	1.45 ± 0.08 b	118.90 ± 3.21 c
SBC	55.80 ± 1.32 b	19.09 ± 0.67 a	25.25 ± 0.74	1.09 ± 0.03 a	101.24 ± 2.16 b

	Citric acid	Malic acid	Total org. acids	Sugars/acid ratio
Control	0.29 ± 0.03 b	6.06 ± 0.22	6.61 ± 0.32	14.97 ± 1.14 a
Fungicide	0.20 ± 0.03 a	5.87 ± 0.36	6.13 ± 0.39	20.97 ± 1.57 b
SBC	0.16 ± 0.02 a	5.61 ± 0.21	5.56 ± 0.27	18.39 ± 1.14 ab

* Different letters (a–c) in rows denote statistically significant differences by LSD test at $P \leq 0.05$.

sorbitol compared to control treatment. In contrast, SBC treatment caused a significantly lower content of glucose compared to control (Table 2). The content of total sugars in different treatments is comparable to reported values of Hofer et al. (2005) in range from 115 to 183 g kg^{-1} FW. Statistically higher contents of total sugars were noticed in fungicide treatments compared to the other treatments. The reason could be a lesser leaf infection and damage caused by apple scab and thus increased photosynthesis.

In apple fruit two organic acids were determined, the predominant malic acid, followed by citric acid (Table 2). Mikulic Petkovsek et al. (2007) obtained similar results. No statistical significance among treatments was found in the content of malic acid and total organic acids.

The ratio between the analysed sugars and organic acids in apple is a common quality index and a good indicator of internal fruit quality. Optimal ratio is crucial for a harmonious taste. Although organic acids in apple are present in lower concentration than sugars, their effect on the fruit flavour is considerable. The higher the ratio, the sweeter the fruits and the lower the ratio, the more acidic they taste (Colaric et al. 2005). The highest sugar/organic acid ratio was calculated for fungicide treatments (Table 2), while the ratio of fruit from SBC treatments did not significantly differ from other treatments.

Phenolic compounds in the fruit pulp and peel

In the fruit pulp and peel the content of the following hydroxycinnamic acids was determined: chlorogenic, 4-*O*-*p*-coumaroylquinic and caffeic acid (Table 3). In both peel and pulp chlorogenic acid was prevailing (6.32 to 16.50 mg 100 g^{-1} FW in skin, 3.93 to 4.76 mg 100 g^{-1} FW in pulp). Control had significantly higher content levels of all analyzed hydroxycinnamic acids compared to apples of fungicide or SBC treatments (Table 3). The results show that the pulp from control treatment had significantly higher content levels of caffeic acid compared to other treatments. In analyzed fruit pulp a similar proportion of hydroxycinnamic acid among different treatments was noticed (Table 4). The increased content of hydroxycinnamic acids in control treatment compared to the other treatments is possibly caused by the stronger infection with the fungus *Venturia inaequalis*, which induces stress conditions in the plant. Similarly, in tissue infected with fungus *Venturia inaequalis* increased amounts of hydroxycinnamic acids were reported by Mikulic Petkovsek et al. (2009) and Slatnar et al. (2010) as a result of stress conditions.

In addition to phloridzin (phloretin 2'-*O*-*β*-*D*-glucoside), dihydrochalcone phloretin 2-*O*-xylosylglucoside was also determined in apple peel. Its content ranged from 7.52 to 9.87 mg 100 g^{-1} FW in apple skin and from

Table 3. The content of individual phenolic compounds (mean ± SE in mg $100g^{-1}$ FW), resp. total phenolic content (mean ± SE in mg GAE $100g^{-1}$ FW) in peel of different type of treatment.

	Control	Fungicide	SBC
4-*O*-*p*-Comaroylquinic acid	4.34 ± 0.17 b	3.97 ± 0.32 ab	3.29 ± 0.07 a
Caffeic acid	1.51 ± 0.09 b	1.10 ± 0.09 a	0.94 ± 0.09 a
Chlorogenic acid	16.50 ± 1.07 b	7.91 ± 0.40 a	6.32 ± 0.15 a
Hydroxycinnamic acids	**22.35 ± 1.14 b**	**12.66 ± 0.49 a**	**10.61 ± 0.41 a**
Phloretin-2-*O*-xyloside	9.87 ± 0.33 b	9.34 ± 0.38 b	7.52 ± 0.59 a
Phloridzin	11.30 ± 0.14 b	10.82 ± 0.18 b	9.50 ± 0.44 a
Dihydrochalcones	**21.17 ± 0.38 b**	**20.16 ± 0.32 b**	**17.61 ± 1.12 a**
Catechin	12.96 ± 1.11 b	6.52 ± 0.97 a	7.06 ± 0.80 a
Epicatechin	13.30 ± 0.56 b	12.32 ± 0.62 b	10.22 ± 0.57 a
Procyanidin B2+procyanidin trimer	14.07 ± 0.99 NS	16.18 ± 0.89 NS	13.63 ± 1.07 NS
Procyanidin dimer	2.16 ± 0.10 b	1.01 ± 0.04 a	2.17 ± 0.10 b
Flavan-3-ols	**42.51 ± 1.97 b**	**36.53 ± 1.68 a**	**33.61 ± 2.06 a**
Q-3-*O*-arabinofuranoside	14.19 ± 0.07 b	14.77 ± 0.39 b	11.08 ± 0.44 a
Q-3-*O*-arabinopyranoside	1.28 ± 0.02 b	1.67 ± 0.14 c	0.81 ± 0.05 a
Q-3-*O*-galactoside	25.14 ± 0.37 b	29.76 ± 1.79 c	20.34 ± 0.92 a
Q-3-*O*-glucoside	4.65 ± 0.23 a	10.89 ± 2.25 b	3.98 ± 0.46 a
Q-3-*O*-rhamnoside	14.27 ± 0.32 b	15.36 ± 0.29 b	11.13 ± 0.50 a
Q-3-*O*-rutinoside	1.60 ± 0.13 a	7.82 ± 2.25 b	1.65 ± 0.35 a
Q-3-*O*-xyloside	10.17 ± 0.18 b	10.71 ± 0.37 b	7.80 ± 0.36 a
Flavonols	**71.30 ± 0.43 b**	**96.36 ± 5.94 c**	**56.79 ± 2.96 a**
TPC[a]	**132.82 ± 1.41 b**	**129.44 ± 4.66 b**	**105.49 ± 5.53 a**

GAE: gallic acid equivalents
Different letters (a–c; NS – not significant) among different treatments denote significant differences (LSD test, $P \leq 0.05$).
[a] TPC: total phenolic content determined by Folin Ciocalteu method in mg GAE $100g^{-1}$ FW.

Table 4. The content of individual phenolic compounds (mean ± SE in mg 100g^{-1} FW), total phenolic content (mean ± SE in mg GAE 100g^{-1} FW) in pulp of different type of treatment.

	Control	Fungicide	SBC
4-*O*-*p*-Comaroylquinic acid	0.89 ± 0.10 NS	0.72 ± 0.04 NS	0.67 ± 0.07 NS
Caffeic acid	0.32 ± 0.03 b	0.22 ± 0.02 a	0.22 ± 0.02 a
Chlorogenic acid	4.76 ± 0.48 NS	4.67 ± 0.12 NS	3.93 ± 0.31 NS
Hydroxycinnamic acids	**5.97 ± 0.61 NS**	**5.62 ± 0.11 NS**	**5.14 ± 0.26 NS**
Phloretin-2-*O*-Xyloside	0.30 ± 0.02 ab	0.34 ± 0.03 b	0.23 ± 0.02 a
Phloridzin	0.85 ± 0.03 NS	0.86 ± 0.04 NS	0.75 ± 0.02 NS
Dihydrochalcones	**1.15 ± 0.06 ab**	**1.19 ± 0.06 b**	**0.99 ± 0.04 a**
Catechin	0.50 ± 0.06 b	0.35 ± 0.02 a	0.28 ± 0.04 a
Epicatechin	1.16 ± 0.07 NS	1.09 ± 0.03 NS	0.99 ± 0.08 NS
Procyanidin trimer	0.65 ± 0.08 NS	0.47 ± 0.04 NS	0.45 ± 0.05 NS
Procyanidin dimer X	0.43 ± 0.07 NS	0.35 ± 0.07 NS	0.37 ± 0.02 NS
Procyanidin B2	1.40 ± 0.09 NS	1.39 ± 0.10 NS	1.10 ± 0.06 NS
Procyanidin Dimer Y	0.19 ± 0.02 NS	0.16 ± 0.01 NS	0.14 ± 0.02 NS
Flavan-3-ols	**3.71 ± 0.28 b**	**3.34 ± 0.14 ab**	**2.77 ± 0.10 a**
Q-3-*O*-arabinofuranoside	0.02 ± 0.003 NS	0.02 ± 0.003 NS	0.02 ± 0.001 NS
Q-3-*O*-galactoside	0.02 ± 0.003 NS	0.02 ± 0.005 NS	0.02 ± 0.002 NS
Q-3-*O*-glucoside	0.03 ± 0.003 NS	0.02 ± 0.001 NS	0.02 ± 0.002 NS
Q-3-*O*-rhamnoside	0.22 ± 0.02 b	0.23 ± 0.01 b	0.15 ± 0.01 a
Q-3-*O*-xyloside	0.04 ± 0.002 NS	0.03 ± 0.002 NS	0.03 ± 0.003 NS
Flavonols	**0.30 ± 0.02 b**	**0.32 ± 0.01 b**	**0.24 ± 0.01 a**
TPC[a]	**13.51 ± 1.40 b**	**10.18 ± 0.31 a**	**8.20 ± 0.68 a**

GAE: gallic acid equivalents
Different letters (a–b; NS – not significant) among different treatments denote significant differences (LSD test, $P \leq 0.05$).
[a] TPC: total phenolic content determined by Folin Ciocalteu method in mg GAE 100g^{-1} FW.

0.23 to 0.34 mg 100 g^{-1} FW in apple pulp; according to previously published results (Treutter 2001; Chinnici et al. 2004). SBC treatment caused significantly lower content levels of all analyzed dihydrochalcones compared to apples from fungicide or control treatments (Table 3). In the pulp, the content was statistically higher in fungicidal treatment, whereas no statistically significant differences were established between the control and the SBC treatment (Table 4). Chinnici et al. (2004) reported that the phloridzin content in apple peel was higher in the organic fruit production compared to integrated fruit production. However, our results show that the content of individual phenols as well as their sum does not show any differences between the control and fungicidal treatment, while in SBC treatment their content is lower.

The following flavanols were determined in apple fruit: catechin, epicatechin, procyanidin B2, and, additionally, in the pulp the two unknown procyanidin dimers ([M-H$^-$] at *m/z* 577, MS^2 fragment at *m/z* 289) and an unknown procyanidin trimer ([M-H$^-$] at *m/z* 865, MS^2 fragment at *m/z* 577, MS^3 fragment at *m/z* 289). The latter was also present in peel. The highest content level in this group was represented by procyanidin B2 (in the pulp) and procyanidin B2+procyanidin trimer (in the skin), procyanidin dimer was only present in traces (pulp and skin) (Table 3 and 4). In apple peel the control had significantly higher content levels of total flavan-3-ols than apples from fungicide or SBC treatments (Table 3). Also, in the pulp the control had significantly higher content levels of total flavan-3-ols than apples from SBC treatments (Table 4). It is known that a rapid accumulation of catechins and oligomeric proanthocyanidins on the infection site restricts the fungal spread (Mayr et al. 1997). Similar results are confirmed in our study; the highest content levels of flavanols are present in extremely infected fruit from the control treatment.

In the group of flavonols seven quercetin-glycosides were identified (quercetin-3-*O*-rutinoside (skin), quercetin-3-*O*-xyloside (skin and pulp), quercetin-3-*O*-ramnoside (skin and pulp), quercetin-3-*O*-glucoside (skin and pulp), quercetin-3-*O*-galactoside (skin and pulp), quercetin-3-*O*-arabinofuranoside (skin and pulp) and quercetin-3-*O*-arabinopyranoside (skin). Among quercetin-glycosides, the most abundant individual compound in the apple peel was quercetin-3-*O*-galactoside, followed by quercetin-3-*O*-arabinofuranoside and quercetin-3-*O*-rhamnoside (Table 3). In comparison to the SBC treated apple skin and pulp, control and fungicide treated apple skin and pulp contained statistically higher amounts of flavonols (Table 3 and 4). Fungicide treated peel contained more than 1.7 fold more total flavonols compared to SBC treated peel. Chinnici et al. (2004) report a higher content of total quercetins in integrated production in comparison with organic production for the 'Golden Delicious' cultivar. We obtained similar results as we can compare organic production with our SBC treatment.

As reported by several authors (Chinnici et al. 2004; Mikulic Petkovsek et al. 2007), apple peel was richer in

single phenolic compounds as well as in total polyphenolics compared to pulp. The content levels of phenolics were less affected by the treatment in apple pulp than peel. Apple peel contained 8 to 12.8 fold more total phenolic than pulp. In both peel and pulp SBC treated fruit had significantly lower content levels of TPC compared to apples from the control (Table 3). Also, CHINNICI et al. (2004) report that integrated apple peel is richer in phenols than organically produced apples.

Conclusions

We can conclude that the SBC treatment provided positive results in decreasing apple scab infection without influencing the appearance of fruits and leaves. Good leaf condition is crucial for a normal fruit development as well as general conditions of the tree that affect the next growing season. Fruit quality parameters show comparable results to other treatments and in consideration of a significantly decreased burden for the environment by pesticide residuals SBC can be an effective treatment against apple scab. Moreover, the use of SBC did not cause any damage, such as discoloration on the skin of the fruit. The content of primary metabolites using SBC was even higher than in the control. The content of TPC, flavonols, flavan-3-ols, hydroxycinnamic acids (only in the skin) and dihydrochalcones (only in the skin) was lower in the fruits where the SBC was used. Since the phenolics are often synthesized as a response to stress conditions, we can assume that the fruits of the SBC treatment were probably exposed to less stress than the ones in the control or in some cases even in the fungicidal treatment.

In our future research we aim to evaluate the effectiveness and influence of different bicarbonate salts on the quality parameters of apple cultivars and apple scab prevention. Simultaneously, it would also be interesting to determine the possibility of using some bicarbonate salts as fertilizers.

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