Foods 2021

MDPI

Proceedings Paper

Croatian Traditional Apple Varieties: Why Are They More Resistant to Plant Diseases? †

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† Presented at the 2nd International Electronic Conference on Foods, 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Abstract: This study aimed to detect, quantify and compare the amounts of chlorogenic acid, phloridzin and quercetin in Croatian traditional and conventional apple varieties by HPLC-PDA. The results showed that Croatian traditional apple varieties had significantly higher amount of chlorogenic acid (30.29 ± 0.34 mg/100 g dw), phloridzin (3.12 ± 0.01 mg/100 g dw) and quercetin (11.68 ± 0.09 mg/100 g dw) detected for varieties Božičnica, Mašanka and Petrovnjača, respectively. The highest contents of the total phenolic acids, dihydrochalcones and flavonols were detected in Božićnica (31.94 ± 0.65 mg/100 g dw), Mašanka (3.52 ± 0.52 mg/100 g dw) and Fuji (19.11 ± 0.56 mg/100 g dw). These results present the beginning of the research on the resistance of Croatian traditional apple varieties to plant diseases.

Keywords: Croatian traditional apple varieties; chlorogenic acid; phloridzin; quercetin

Citation: Lončarić, A.; Kovač, T.; Gotal, A.-M.; Celeiro, M.; Lores, M. Croatian Traditional Apple Varieties: Why Are They More Resistant to Plant Diseases? *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Published: 15 October 2021

1. Introduction

Apples are generally considered "healthy food"; one of the most important featured of apples is their polyphenol content, especially flavan-3-ols, phenolic acids, flavonols, dihydrohalcones and anthocyanins [1,2]. On the other hand, apple is host to a wide range of pests and diseases, many of which are present in all apple producing regions in the world. Apple varieties with higher content of polyphenols are more resistant to plant diseases. Some authors have suggested the importance of polyphenols as resistance to plant diseases [3,4]. Therefore, the aim of this study was the to quantify, detect and compare the amounts of chlorogenic acid, phloridzin and quercetin in Croatian traditional and conventional apple varieties by high-performance liquid chromatography with diode-array detector (HPLC-PDA).

2. Materials and Methods

Apple used for the experiment were ten Croatian traditional apple varieties, 'Petrovnjača', 'Kleker', 'Mašanka', 'Amovka', 'Srčika', 'Paradija', 'Kanada', 'Božičnica', 'Ivandija', and 'Šampanjka', and five conventional apple varieties, 'Granny Smith', 'Idared', 'Golden Delicious', 'Jonagold', and 'Fuji'. This study aimed to quantify, compare and detect the amount of phloridzin, chlorogenic acids and quercetin by high- performance liquid chromatography with diode-array detectors. Furthermore, total dihydrochalcones, phenolic acids and flavonols were also determined. High-performance liquid chromatography was performed with the Jasco LC Net II, equipped with the AS-4150 autosampler, the PU-4180 pump and the MD-4010 PDA detector. JASCO Chrom NAV Version 2.01.00 (JASCO International Co., Ltd., Tokyo, Japan) controlled the system. The mobile phase consists of A

(water containing 1% formic acid) to B (methanol containing 1% formic acid). The sample of 5 µL was injected in duplicate onto the column kept at 50 °C and a flow rate of 1 mL/min. The UV-Vis absorption spectra of the standards, as well as the samples, were recorded in the range of 190 to 600 nm. Polyphenols were identified by the comparison of their retention time and UV-Vis spectra to those of pure standards, and detected at 280, 320 and 360 nm. The amount of polyphenols was expressed as mg/100 g of dw [5].

3. Results

The results showed that Croatian traditional apple varieties had significantly higher amount of quercetin, chlorogenic acid and phloridzin. In Croatian traditional apple varieties, the highest number of chlorogenic acid had 'Božičnica' (30.29 ± 0.34 mg/100 g dw), the highest amount of phloridzin had 'Mašanka' (3.12 ± 0.01 mg/100 g dw) and the highest amount of quercetin had 'Petrovnjača' (11.68 ± 0.09 mg/100 g dw), respectively (Table 1). In conventional apple varieties the highest amount of chlorogenic acids had 'Granny Smith' (13.57 ± 0.19 mg/100 g dw), phloridzin 'Idared' (1.22 ± 0.01), and quercetin 'Golden Delicious' (3.34 ± 0.13 mg/100 g dw), respectively (Table 2).

Table 1. The amount of chlorogenic acid, phloridzin and quercetin in ten Croatian traditional apples varieties.

Apple Variety	Chlorogenic Acid	Phloridzin	Quercetin
	[mg/100 g DW]		
Petrovnjača	14.29 ± 0.16	1.02 ± 0.01	**11.68 ± 0.09**
Kleker	11.31 ± 0.17	1.22 ± 0.02	7.44 ± 0.19
Mašanka	16.58 ± 0.14	**3.12 ± 0.01**	9.53 ± 0.12
Amovka	11.94 ± 0.22	0.4 ± 0.01	7.31 ± 0.09
Srčika	17.16 ± 0.13	1.61 ± 0.02	9.74 ± 0.23
Paradija	23.83 ± 0.47	0.85 ± 0.01	9.58 ± 0.06
Kanada	12.34 ± 0.11	0.99 ± 0	3,22 ± 0.03
Božičnica	**30.29 ± 0.34**	0.77 ± 0	3,39 ± 0.35
Ivandija	12.59 ± 0.17	1.1 ± 0.01	3,02 ± 0.04
Šampanjka	12.39 ± 0.39	0.26 ± 0.01	0,82 ± 0.28

Table 2. The amount of chlorogenic acid, phloridzin and quercetin in five convetional apple varieties.

Apple Variety	Chlorogenic Acid	Phloridzin	Quercetin
	[mg/100 g DW]		
Granny Smith	**13.57 ± 0.19**	0.57 ± 0.01	1.19 ± 0.04
Idared	7.9 ± 0.11	**1.22 ± 0.01**	2.9 ± 0.05
Golden Delicious	4.34 ± 0.19	0.4 ± 0	**3.34 ± 0.13**
Jonagold	5.56 ± 0.17	0.71 ± 0	1.69 ± 0.05
Fuji	6.62 ± 0.2	0.39 ± 0	2.89 ± 0.03

Futhermore, the highest content of the total phenolic acids, dihydrohalcones and flavonols in conventional apple varieties were detected in 'Granny Smith' and 'Fuji' (Table 3). On the other hand, the highest content of the total phenolic acids, total dihydrohalcones, and total flavonols in Croatian traditional apple varieties were detected in 'Božičnica', 'Mašanka' and 'Petrovnjača' (Table 4).

Table 3. The amount of total phenolic acids, dihydrohalcones and flavonols in five conventional apples varieties.

Apple Variety	Total Phenolic Acids	Total Dihydrochalcones	Total Flavonols
	[mg/100 g DW]		
Granny Smith	**14.2359**	**0.142359**	9.314
Idared	8.5623	0.085623	11.271
Golden Delicious	4.7792	0.047792	13.303
Jonagold	6.137	0.06137	9.776
Fuji	6.9878	0.069878	**19.11**

Table 4. The amount of total phenolic acids, dihydrohalcones and flavonols in ten Croatian traditional apples varieties.

Apple Variety	Total Phenolic Acids	Total Dihydrochalcones	Total Flavonols
	[mg/100 g DW]		
Petrovnjača	15.1524	1.198	**12.4689**
Kleker	11.8933	1.4164	8.2163
Mašanka	16.8465	**3.5233**	10.1015
Amovka	12.3333	0.5687	7.8828
Srčika	18.2125	1.8212	5.6331
Paradija	24.4576	1.0721	10.3979
Kanada	16.4103	1.1875	9.423
Božičnica	**31.9373**	0.8888	7.638
Ivandija	13.6652	1.3662	8.753
Šampanjka	13.9938	0.3757	4.495

The amount of quercetin-3-rutinoside was also analysed in traditional and conventional apple varieties, the highest amount had 'Fuji' (59.54 ± 0.93 mg/100 dw).

4. Discussion

This research shown chlorogenic acid, phloridzin, quercetin and total phenolic acids, dihydrohalcones and flavonols were detected in Croatian traditional apple varieties. The highest amount of phloridzin and flavanols in traditional varieties, compared to commercial ones, was also reported by [6]. Many studies emphasize the health- promoting effects of different polyphenols. First of them is chlorogenic acid, CA, playing several important and therapeutic roles, such as antibacterial, antioxidant activity, hepatoprotective, cardioprotective roles, etc. As can be seen in these results and [7], traditional apple varieties are dominated by non- flavonoids (chlorogenic acid). Alvarez-Parrilla et al. [8] reported the complexation and antioxidant activity of the major apple polyphenols: rutin, chlorogenic acid and quercetin with ß-cyclodextrin by fluorescence spectroscopy and Ferric Reducinh/Antioxidant Power Assay (FRAP) techniques. The results showed that the highest antioxidant activity had quercetin, followed by rutin and chlorogenic acid. Furthermore, quercetin-3-rutinoside, has been used conventionally as an antimicrobial, antifungal and anti-allergic agent and certain research has shown pharmacological benefits for the treatment of various chronic diseases [9]. In addition, phloridzin, quercetin and chlorogenic acid showed antimicrobial and antifungal activity targetin intracellular processes in microorganisms or inducing irreversible permeability changes in cell membrane. Antifungal activity of phloridzin and its aglycine, phloretin, was previously described [10,11]. Shim et al. [10] done the first report on the antifungal activity of phloretin against plant pathogenic fungi and investigated the influence of phloretin isolated from apple against *B. cinerea*, *F.oxysporum* and five other fungi. The results showed that phloretin could be used

as biopesticide. Phloridzin is commonly anticipated for playing a defensive role against various kinds of pathogens as well as it is involved in resistance to various diseases. Ratio of flavanol/phloridzin are mostly debated with respect to resistance against plant diseases [12,13]. Furthermore, quercetin is known as a strong antioxidant, mainly due to the presence of catechol group in ring B [14]. Sanzani et al. [15] showed that quercetin is effective in reduction of *Penicilium expansum* growth and inhibition of patulin synthesis. In addtion, it can be considered as a natural compound to be used as alternative strategy to chemical fungicides in post-harvest control of *P.expansum* infections [15]. In conclusion, quercetin has been reported by Nijveldt et al. [16] to have anti-inflammatory, antitrombogenic, antiviral and antioxidant properties and it is the active ingredient in numerous commonly available dietary supplements.

5. Conclusions

In conclusion, Croatian traditional apple varieties had significantly higher amount of phloridzin, chlorogenic acid, quercetin unlike conventional apple varieties. Furthermore, total phenolic acids, dihydohalcones and flavonols were also detected in Croatian traditional apple varieties in higher amount than conventional ones. These results present the beginning of the research on the resistance of Croatian traditional apple varieties to plant diseases.

Author Contributions: Conceptualization, A.L.; formal analysis, T.K. and M.C.; writing—original draft preparation, A.-M.G.; writing—review and editing, A.L. and M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Croatian Science Foundation (UIP-2020-02-8461).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: The authors declare no conflict of interest.

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