



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

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**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 amounts of chlorogenic acid ( $30.29 \pm 0.34$  mg/100 g dw), phloridzin ( $3.12 \pm 0.01$  mg/100 g dw) and quercetin ( $11.68 \pm 0.09$  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 Božičnica ( $31.94 \pm 0.65$  mg/100 g dw), Mašanka ( $3.52 \pm 0.52$  mg/100 g dw) and Fuji ( $19.11 \pm 0.56$  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



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## 1. Introduction

Apples are generally considered “healthy food”; one of the most important features of apples is their polyphenol content, especially flavan-3-ols, phenolic acids, flavonols, dihydrochalcones 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 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

The apples 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 amounts of quercetin, chlorogenic acid and phloridzin. In Croatian traditional apple varieties, the highest amount of chlorogenic acid was detected in “Božičnica” ( $30.29 \pm 0.34$  mg/100 g dw), the highest amount of phloridzin in “Mašanka” ( $3.12 \pm 0.01$  mg/100 g dw) and the highest amount of quercetin in “Petrovnjača” ( $11.68 \pm 0.09$  mg/100 g dw), respectively (Table 1). In conventional apple varieties, the highest amount of chlorogenic acid was detected in “Granny Smith” ( $13.57 \pm 0.19$  mg/100 g dw), the highest amount of phloridzin in “Idared” ( $1.22 \pm 0.01$ ) and the highest amount of quercetin in “Golden Delicious” ( $3.34 \pm 0.13$  mg/100 g dw), respectively (Table 2).

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

| Apple Variety | Chlorogenic Acid | Phloridzin      | Quercetin        |
|---------------|------------------|-----------------|------------------|
|               | [mg/100 g dw]    |                 |                  |
| “Petrovnjača” | $14.29 \pm 0.16$ | $1.02 \pm 0.01$ | $11.68 \pm 0.09$ |
| “Kleker”      | $11.31 \pm 0.17$ | $1.22 \pm 0.02$ | $7.44 \pm 0.19$  |
| “Mašanka”     | $16.58 \pm 0.14$ | $3.12 \pm 0.01$ | $9.53 \pm 0.12$  |
| “Amovka”      | $11.94 \pm 0.22$ | $0.4 \pm 0.01$  | $7.31 \pm 0.09$  |
| “Srčika”      | $17.16 \pm 0.13$ | $1.61 \pm 0.02$ | $9.74 \pm 0.23$  |
| “Paradija”    | $23.83 \pm 0.47$ | $0.85 \pm 0.01$ | $9.58 \pm 0.06$  |
| “Kanada”      | $12.34 \pm 0.11$ | $0.99 \pm 0$    | $3.22 \pm 0.03$  |
| “Božičnica”   | $30.29 \pm 0.34$ | $0.77 \pm 0$    | $3.39 \pm 0.35$  |
| “Ivandija”    | $12.59 \pm 0.17$ | $1.1 \pm 0.01$  | $3.02 \pm 0.04$  |
| “Šampanjka”   | $12.39 \pm 0.39$ | $0.26 \pm 0.01$ | $0.82 \pm 0.28$  |

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

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

Furthermore, the highest contents of the total phenolic acids, dihydrochalcones and flavonols in conventional apple varieties were detected in “Granny Smith” and “Fuji” (Table 3). On the other hand, the highest contents of the total phenolic acids, total dihydrochalcones, and total flavonols in Croatian traditional apple varieties were detected in “Božičnica”, “Mašanka” and “Petrovnjača” (Table 4).

The amount of quercetin-3-rutinoside was also analyzed in traditional and conventional apple varieties. The highest amount was detected in “Fuji” ( $59.54 \pm 0.93$  mg/100 dw).

**Table 3.** The amount of total phenolic acids, dihydrochalcones and flavonols in five conventional apple 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, dihydrochalcones and flavonols in ten Croatian traditional apple 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           |

#### 4. Discussion

This research showed the chlorogenic acid, phloridzin, quercetin and total phenolic acids, dihydrochalcones and flavonols that were detected in Croatian traditional apple varieties. The highest amount of phloridzin and flavonols in traditional varieties, compared to commercial ones, was also reported by Ref. [6]. Many studies emphasized the health-promoting effects of different polyphenols. One 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 in Ref. [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  $\beta$ -cyclodextrin by fluorescence spectroscopy and Ferric Reducin<sup>h</sup>/Antioxidant Power Assay (FRAP) techniques. The results showed that the highest antioxidant activity was detected in quercetin, followed by rutin and chlorogenic acid. Furthermore, quercetin-3-rutinoside has been used conventionally as an antimicrobial, antifungal and anti-allergic agent, and some research has shown it had pharmacological benefits in the treatment of various chronic diseases [9]. In addition, phloridzin, quercetin and chlorogenic acid showed antimicrobial and antifungal activity, targeting 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] presented 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 a biopesticide. Phloridzin is commonly acknowledged for playing a defensive role against various kinds of pathogens, as well as being involved in resistance to various diseases. The ratios of flavonol/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 reducing *Penicillium expansum* growth and inhibiting patulin synthesis. In addition, it can be considered a natural compound to be used as alternative strategy to chemical fungicides in the post-harvest control of *P. expansum* infections [15]. In conclusion, quercetin has been reported by Nijveldt et al. [16] to have anti-inflammatory, antithrombogenic, antiviral and antioxidant properties, and it is an active ingredient in numerous commonly available dietary supplements.

## 5. Conclusions

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

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/Foods2021-10917/s1>, Poster S1: Croatian Traditional Apple Varieties: Why Are They More Resistant to Plant Diseases?

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