Influence of conventional and organic agriculture practices on the total phenols and antioxidant potential of Florina apple fruits

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Abstract. The aim of the current study was to evaluate the influence of conventional and organic growing conditions on the total phenolic content, the total flavonoids content, the total monomeric anthocyanins content and the antioxidant potential in the apple fruits of Florina variety. The samples were grown and collected from the experimental fields, around Plovdiv and the village of Brestnik. The total phenolic content, the total flavonoids, the total monomeric anthocyanins content were determined. The antioxidant potential was evaluated by four different assays. The apple fruits grown under organic conditions contained higher total phenolics and they showed stronger antioxidant activity than fruits from the conventional conditions. The total phenolic content was from 3 to 5 mg gallic acid equivalent/g dry weight. The total flavonoids were below 1 mg quercetin equivalents/g dw. In the organically grown fruits, the average values for antioxidant activity varied from 30 to 100 mM Trolox® equivalent/g dw. The total monomeric anthocyanins in the organically grown apples their levels were the highest (67 mg cyn-3-gluc/100 g dw). There was a positive, high correlation between antioxidant methods and the total phenolic content and total monomeric anthocyanins. These results indicated that organically grown apples accumulated higher levels of antioxidants and bioactive compounds, in comparison to conventionally grown apples.
Key words: Florina apple, antioxidant activity, organic agriculture.

1. Introduction
In the human nutrition, apples (Malus domestica Borkh.) are popular amongst the consumers because of their organoleptic characteristics (aroma, taste, and flavour). Apples constitute an important part of the human diet, as they are a rich source of sugars, acids and various biologically active compounds. Their fruits also consist phenolic compounds, because their consumption is widespread in many countries and apples are available on the market for the whole year; therefore, they represent a major source of dietary antioxidants. The complexity of the chemical profile and the variations are caused by
growth period, growing season, environmental conditions and agriculture practices. However, the different apple orchard management affect soil dynamics and plant metabolism, resulting in differences in fruit quality. Polyphenols as secondary metabolites strongly depends on performed agriculture and soil management systems [1-3].

The main advantage of ‘Florina’ apples among other cultivars, is its scab-resistance [3]. Together with its valuable nutraceutical properties in comparison with the well known scab-susceptible apple varieties this cultivar attracts attention of consumers and farmers. However, the studies about apple quality in fruits grown in Bulgaria are still limited. Therefore, the aim of the current study was to evaluate the influence of conventional and organic growing conditions on the total phenolic content, the total flavonoids content, the total monomeric anthocyanins content and antioxidant potential in the apple fruits of Florina variety.

2. Material and Methods

2.1. Plant material and growing conditions
The research activities were realized on the training and experimental fields, spreading round the city of Plovdiv and the village of Brestnik. The study was conducted in the seven years old apple orchards with Florina cultivar – grafted on MM106 rootstock at the Agroecological Centre of Agricultural University of Plovdiv (42.133845 N, 24.807315 E) and Brestnik village. Four groups of four trees per orchard were randomly chosen as the sample trees. The apple orchards were cultivated under organic and conventional agricultural practices.

Conventional orchard: In May and during the five years orchards were treated with the fungicides Tiram 80 VG - 0.3%, Follicur 250 EV - 0.04% (broad spectrum system), Delan 700 VDG - 0.05%, Score 250 EC - 0.02%, Horus 50 EC - 0.03%, Shavit F 72 VDG - 0.2% and Bayfidan 250 EC (systemic) - 0.015%. Against cross moth, lice and mites apple plants were treated a different number of times in different years with Nurele D - 0.04%, Fury 10 EC - 0.125% (contact synthetic pyrethroid of a new generation) and Coragen 20 SC - 16 ml/ha.

Organic orchard: Shampion VP – 0. 15% (at the end of November) and Funguran ON – 0.3% (contact broad-spectrum, containing 77% cupper hydroxide) combined with Colloidal sulphur – 1:400. Trifolio S Forte 0.3% (50% vegetable oil + 50% emulsifier) and Acarzin 3% (85% mineral oil + 15% emulsifier) were used against the hibernating forms of aphids and apple fruit worms. Postblossom treatments in May was performed against apple diseases and the pests mainly target Venturia inaequalis, Podosphaera leucotricha, aphids and apple fruit worm with uses of the antifungal agent Kuore 200 g/dka (contains 10% Cu and 1.1% Zn, Colloidal sulphur – 1:400, Nimazal T/C - 200 ml/dka (a bio insecticide) + Trifolio S Forte - 0.3%. Pheromone trapping was used to control apple fruit worm.

Apples were harvested during the first week of October 2019, transported to the lab, stored at 25°C for two days before analysis.

2.2. Chemicals
All the solvents and reagents were of the analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fillab (Plovdiv, Bulgaria).

2.3. Moisture content
Moisture content (%) was determined after drying at 105°C until constant weight.

2.4. Sample preparation
Apples were washed, cleaned, seeds were removed and chopped into pieces. Then the apples were blended in a laboratory homogenized to the fine puree. The homogenized samples were weighted in 50 ml plastic tubes and extracted with 70% ethanol. The ultrasound-assisted extraction process of biologically active substances from apples were carried out in an ultrasonic bath (VWR, Malaysia) at
42 kHz frequency and 30 W power. The extraction was performed in triplicate. The obtained extracts were analyzed for the phenolic compounds and antioxidant activity.

2.5. Determination of total phenolic content
The total phenolic content in the apple samples was determined using the Folin–Ciocalteu’s reagent [4]. The analysis was performed as 0.2 ml 70% ethanol extract was mixed with 1 ml Folin–Ciocalteu reagent diluted five times and then 0.8 ml 7.5% Na₂CO₃ was added. After 20 min, the absorption was measured at 765 nm against a blank sample. The results were expressed in µg equivalent of gallic acid (GAE) per g dry weight (dw).

2.6. Determination of total flavonoids content
The total flavonoids content was determined by Al(NO₃)₃ reagent. The absorbance was measured at 415 nm. The results were presented as mg equivalents quercetin (QE) per g dw according to the calibration curve with quercetin as a standard [5].

2.7. Total monomeric anthocyanins content
Total anthocyanins content was determined using the pH differential method [6] at two wavelengths 520 and 700 nm. The results were presented as cyanidin-3-glycoside per 100 g dry weight (dw) fruits.

2.8 Antioxidant activity

2.8.1. The DPPH radical-scavenging ability. Apple extract (0.15 ml) was added to 2.85 ml freshly prepared 0.1mM solution methanol solution of DPPH. The reduction of absorbance at 517 nm was measured by spectrophotometer in the comparison to the blank containing methanol. The percent inhibition was also calculated. The results were expressed in mM Trolox® equivalents (TE)/g dw [5].

2.8.2. ABTS+ radical scavenging ability. The ABTS+ solution (2.85 ml) was mixed with 0.15 ml apple sample. After 15 min at 37°C in darkness, the absorbance was measured at 734 nm against ethanol. The percent inhibition was also calculated. The results were expressed in mM Trolox® equivalents (TE)/g dw [5].

2.8.3. FRAP assay. The FRAP reagent was prepared before analysis by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM FeCl₃·6H₂O in distilled water. FRAP reagent (3.0 ml) was mixed with 0.1 ml apple extract. After 10 min at 37°C in darkness, the absorbance of the sample was measured at 593 nm [7].

2.8.4. CUPRAC assay. Apple extract (0.1 ml) was mixed with 1 ml CuCl₂·2H₂O, 1 ml methanol solution of Neocuproine, 1 ml 0.1M ammonium acetate buffer and 1 ml distilled water. After 20 min at 50°C in darkness, the samples were cooled to 25°C and the absorbance was measured at 450 nm. The results were expressed in mM Trolox® equivalents (TE)/g dw [5].

2.9. Statistical analysis
All the experiments were performed in triplicate, and the results were expressed as mean ± SD (standard deviation). Statistical analysis was performed using and Excel 2015.

3. Results and discussion

3.1. Total phenols and total flavonoids
The phenolic content and flavonoids content in apple Florina grown under conventional and organic conditions were shown (figure 1).
Figure 1. Total phenolic content (TPC) and flavonoids (TF) in apples grown in different conditions.

The total phenolic content varied from 1.16 to 5.28 mg GAE/g dry weight. It was found that the level of total phenols was higher in the apple fruits collected from organic orchard. In comparison the apple from the organic orchard contained more than 3 times higher content of total phenols. The apple from orchard sward also accumulated more phenolic compounds in comparison with conventionally grown apple fruits. Bouayed et al. [8] reported that phenolic compounds in apples varied from 130.0 – 2.7 mg GAE/100 g fw, while Wojdylo et al. [9] reported higher values for Florina apple 3.75 mg GA/g fw. In addition, the flesh Florina apple tissue showed low total phenolic values in the range from 0.3 to 0.84 mg GAE/g fw [1].

The level of total flavonoids was below 1 mg quercetin equivalents/g dw (figure 1). It is well-known that quercetin accumulates in the apple skin. In our study, the total flavonoids dominated in the highest values in conventional growing apples – 0.96 mg QE/g dw.

3.2. Total monomeric anthocyanins content
Florina apple cultivar is characterized with red or partially dark red peels and this coloration is due to the content of cyanidin-3-galactoside and 3-glucoside [10]. Total monomeric anthocyanins content in Florina apples grown in different conditions was evaluated (figure 2). These values were in the range from 60 to 67.26 mg cyn-3-glc/100 g dw. The highest content was found in the apples grown in the organic orchard sward. In addition, the content of total monomeric anthocyanins in all other samples was around 60 mg cyn-3-glc/100 g dw. Our results were higher than reported values for cyanidin-3-glucoside - 253.8 mg/kg dw in Florina apple [9]. Moreover, the studied apple varieties Luxenbour were generally poor in anthocyanins, however the highest concentration was found in Florina (2.3 – 0.1 mg of cyanidin 3-glucoside equivalents/100 g fw) [8].

Figure 2. Total monomeric anthocyanins content in Florina apples grown in different conditions.
3.3. Antioxidant activity
The antioxidant activity of apples was evaluated by four methods based on two different mechanisms SET (single electron transfer) – FRAP and CUPRAC; and HAT-type (hydrogen atom transfer (DPPH radical-scavenging ability and ABTS$^+$ radical scavenging ability). The results were summarized in Table 1.

Table 1. Antioxidant activity of apple variety Florina, mM TE/g dw.

| Apple samples grown under different conditions | DPPH     | ABTS     | FRAP     | CUPRAC    |
|------------------------------------------------|----------|----------|----------|-----------|
| Conventional orchard                          | 12.07±2.73 | 12.85±1.48 | 8.22±0.58 | 21.62±0.37 |
| Conventional sward orchard                    | 33.40±0.69 | 33.24±1.58 | 14.77±1.96 | 56.70±3.50 |
| Organic orchard                               | 39.95±5.93 | 90.68±3.24 | 19.63±6.46 | 35.62±2.14 |
| Organic sward orchard                         | 40.73±4.35 | 103.34±4.02 | 34.06±3.62 | 46.02±3.81 |

In general, the apples from the organic sward orchards demonstrated the highest antioxidant activity by three methods (DPPH, ABTS, and FRAP assays). The highest antioxidant potential was demonstrated by ABST method for the organically grown apples (90 to 103 mM TE/g dw). The lowest antioxidant activity demonstrated apples fruits collected form the conventional orchards. However, conventionally grown apples in the sward orchards showed better metal reducing properties of CUPRAC method. Moreover, the apples grown conventionally possessed the lowest antioxidant activity, which was between 2 and 8 times lower in comparison with the organically grown apples. From the obtained results, it was clear that the performed agriculture practices and management influence on the quantities of polyphenols and antioxidant activity in apple fruits.

3.4. Correlation between antioxidant capacity and total phenolic content
The correlation between total phenolic contents, total monomeric anthocyanins, total flavonoid content and total antioxidant activity evaluated by four methods (DPPH, ABTS, FRAP and CUPRAC) were presented in Table 2. The results showed positive linear correlations between total antioxidant activities and total phenolic contents (coefficient of correlation $r^2 = 0.9464$ and 0.9210 for DPPH and FRAP values, respectively) (Table 2).

Table 2. Correlation between phenolic compounds and antioxidant activity.

|                      | DPPH     | ABTS     | FRAP     | CUPRAC    |
|----------------------|----------|----------|----------|-----------|
| Total monomeric anthocyanins | 0.6628   | 0.6661   | 0.9209   | 0.6228    |
| Total phenolic content | 0.9462   | 0.8684   | 0.9210   | 0.7031    |
| Total flavonoids content | -0.9591  | -0.7901  | -0.5970  | -0.5931   |

These results suggested that the total phenolic compounds contributed significantly to the antioxidant activity of apples. A high correlation was found between total monomeric anthocyanins and FRAP method $r^2 = 0.9209$. In addition, a similar observation about the dependence of antioxidant potential form total phenolic content were reported by other authors for Florina apple [9]. Moreover, these results suggested that antioxidant components in Florina apple could reduce oxidants (such as ferric and cupric ions) and scavenge free radicals. However, in our research no positive correlation existed between the antioxidant capacity and the total flavonoids content (Table 2). It was reported that antioxidant activity in apples due to the presence of phenolic compounds [8, 9]. Moreover, other studies also demonstrated a good relationship between the phenolic content and the antioxidant activity [3, 8, 9].
4. Conclusion
The results from the current study demonstrated that apples collected from an organic orchard sward contained the highest level of secondary metabolites as total phenols, total flavonoids and total monomeric anthocyanins. Moreover, the organically grown apples showed the highest antioxidant activity, evaluated by DPPH, ABTS and FRAP methods, especially fruits from organic orchard sward. It was found that antioxidant activity was mainly due to the presence of polyphenols in fruits. Therefore, the organically grown apple Florina cultivar from the organic orchard sward demonstrated the highest antioxidant potential and presents a rich source of phenolic compounds.

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