

## Influence of cultivar and fertilization treatment on bioactive content of some apple (*Malus domestica* Borkh.) cultivars

Dzevad LAVIĆ<sup>1</sup>, Mirjana RADOVIĆ<sup>2\*</sup>, Jasmina ALIMAN<sup>3</sup>, Nedim BADZAK<sup>3</sup>, Mirko KULINA<sup>3</sup>,  
Alisa HADZIABULIĆ<sup>3</sup>, Gülçe İLHAN<sup>4</sup>, Crina MUREŞAN<sup>5</sup>, Romina Alina MARC<sup>5</sup>

<sup>1</sup>Federal Institute for Agriculture, Sarajevo, Bosnia and Herzegovina

<sup>2</sup>Faculty of Agriculture, University of East Sarajevo, Bosnia and Herzegovina

<sup>3</sup>Faculty of Agro-Mediterranean, Džemal Bijedić University of Mostar, Bosnia and Herzegovina

<sup>4</sup>Faculty of Agriculture, Atatürk University, Erzurum, Turkey

<sup>5</sup>Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

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**Abstract:** The paper presents the chemical composition of the fruits of three apple cultivars (Jonagold Decosta, Red Idared and Gala Schnitzer Schniga) fertilized with three treatment: T<sub>1</sub> (control-without fertilization); T<sub>2</sub> (300 kg/ha NPK (6:18:36) + 150 kg/ha N (calcium ammonium nitrate-CAN)) and T<sub>3</sub> (foliar nutrition-mixture organic-mineral fertilizer commercially named 'FitoFert Kristal' (0.6%) (10:40:10) + 'FitoFert Kristal' (0.6%) (20:20:20) + 'FoliFertil Ca' (0.5%) (N:Ca) in Bosnia and Herzegovina during two years (2020-2021). Significant differences of contents of soluble solids, total sugars, inverted sugars, sucrose, total phenolic content, content of flavonoids and antioxidant activity were found among cultivar/treatment combinations, cultivars, treatments and years. Differences in total acids content among treatments and years were not statistically significant, but significant differences among cultivars and cultivar/treatment combinations were found. The contents of soluble solids, total sugars and sucrose were the highest in fruits of cultivar Jonagold Decosta, while the highest contents of inverted sugars, total acids, total phenolics, flavonoids and antioxidant activity were found in fruits of Red Idared cultivar. The fertilization treatment T<sub>2</sub> significantly influenced the content of soluble solids, total sugars, inverted sugars, total acids, total phenolic, flavonoids and antioxidant activity in the apple fruits. According to the results obtained, we can say that fertilization treatment T<sub>3</sub> do not have much effect on bioactive ingredients, with the exception of the sucrose content.

Among mineral elements, four were macroelements that were represented present in large amounts: potassium (K) (1266.15 mg kg<sup>-1</sup> to 1652.89 mg kg<sup>-1</sup>), calcium (Ca) (122.76-474.90 mg kg<sup>-1</sup>), phosphorus (P) (86.14-124.84 mg kg<sup>-1</sup>) and magnesium (Mg) (57.36-70.68 mg kg<sup>-1</sup>). On the other hand, four microelements were determined: boron (B) (1.06-14.25 mg kg<sup>-1</sup>); iron (Fe) (2.79-4.62 mg kg<sup>-1</sup>); manganese (Mn) (0.27-0.46 mg kg<sup>-1</sup>) and zinc (Zn) (0.15-0.48 mg kg<sup>-1</sup>). Results showed that contents of macroelements and microelements have been strongly affected by cultivar/treatment combinations, cultivars, treatments and years.

**Key words:** *Malus domestica* Borkh, cultivar, fertilization treatment, year, chemical composition, total phenolic content, flavonoids, antioxidant activity, macroelements, microelements

### 1. Introduction

Apple (*Malus domestica* Borkh.) is one of the most appreciated fruits consumed and represents the 3rd most important worldwide cultivated fruit tree (FAOSTAT, 2022). According to FAOSTAT (2022), the world apple production for the period of (2019-2021) was 90.4 million tonnes. Fruits of apple are suitable for fresh consumption and processing into different products (juice, food pastes, jellies, jams as well as in other products). Cultivar, horticultural practice and year of cropping are important factors determining the chemical composition of apples (Wicklund et al., 2021).

Soluble solid content (SSC) is the most important attribute related to the quality and price of apples (Tian et al., 2022). Soluble sugars and organic acids are important components of fruit taste, and together with aromas, they have a strong impact on the overall organoleptic quality of fruits (Borsani et al., 2009). Fruit taste depends on the content and type of soluble sugars and organic acids (Bordonaba and Terry, 2010). According to Famiani et al. (2015), soluble sugars are responsible for the sweetness of apple fruits, and organic acids determine the sourness. The content of soluble sugars and organic acids in apples vary among cultivars and some other factors such as light,

\* Correspondence: mirjana.radovic@pof.ues.rs.ba

exposure to sun, and other environmental factors could have an impact on it (Yang et al., 2021). During the apple development, the synthesis and accumulation of soluble sugars are faster than that of organic acids (Zhang et al., 2010). The major soluble sugars in apples are fructose, glucose, sucrose, and sorbitol, while the malic acid, citric acid, and tartaric acid mostly account for the organic acids (Ma et al., 2014).

According to Wu et al. (2007), apple fruits are rich in fructose, which accounts for 44%–75% of the total sugars. The right proportion of sugars as well as sucrose, fructose, and glucose attributes to the quality of the fruits (Wang et al., 2008).

Acidity has profound effects on the taste of apples. Differences in malic acid content are caused by differences in accumulation of malic acid in the vacuole. Malic acid is the dominant acid in apple fruits, accounting for up to 90% of the total organic acids (Wu et al., 2007; Zhang et al., 2010) and has an important influence on the sour taste of apples. In cultivars with low amounts of malic acid, the sweet taste becomes predominant (Veberič et al., 2009).

Phenolic compounds are biologically active substances which have antioxidant properties and positive effects on human health (Walkowiak-Tomczak, 2008). According to Boyer and Liu (2004) and Scalbert et al. (2005), phenolic compounds have different biological effects, primarily antimicrobial, antiinflammatory, antimutagenic, anticarcinogenic, anti-allergic, antiplatelet and vasodilatory actions.

Apples have been identified as one of the main dietary sources of antioxidants, mainly phenolic compounds, such as flavonoids, rather than essential vitamins, such as vitamin C, which explains only 0.4% of the total antioxidant capacity (Boyer and Liu, 2004). Cultivar, growing conditions, cultural practices, ripeness during harvest, postharvest storage conditions and processing (Imeh and Khokhar, 2002; Boyer and Liu, 2004) are important factors determining the phenolic content of apples. Previous studies of Wolfe et al. (2003) and Drogoudi et al. (2008) have shown that apple peel may contain more antioxidants and antioxidant capacity than the pulp fraction or whole fruit. This can be explained by the higher content of total phenolic compound and anthocyanins in the skin, than in the flesh.

Minerals represent a significant component of the chemical composition of apple fruit and they are responsible for the functioning of the human body and involved in the metabolism and production of carbohydrates, lipids, proteins, vitamins and enzymes. Mineral composition of any fruit is equally important as minerals are considered essential in regulation of several body functions (Cindric et al. 2012). According to Gorinstein et al., (2001) main minerals and essential trace

elements are very important in biological processes and play a vital role in normal growth and development and have also been involved in the prevention of some chronic diseases. Consumers need food sources that are rich in K, Ca, Mg, Zn and poor in Na (Francini et al., 2022). The quality and storage life of apple fruit influence calcium (Ca), potassium (K) and magnesium (Mg) and their ratios (Tagliavini et al., 2000). Fotirić Akšić et al. (2020) reported that the potassium uptake by apple trees occurs after cell division on the fruits and remains relatively high until harvest. According to Zavalloni et al. (2001), reduced leaf photosynthesis and produced fruit with less sugar may be due to K deficiency. The nutrient accumulation curves of apple trees are good indicators of nutrient requirement in each plant development stage (Nachtigall and Dechen, 2006).

Brunetto et al. (2015) recorded that those mineral contents in apples vary depending on the cultivar, production cycle and growing region. Mineral element compositions in fruits depend on pedoclimatic conditions, agronomic practices (Heimler et al., 2017) and cultivar (Badžak et al., 2021). Accumulation of nutrients into fruits depends on several factors, including the relative strengths of vegetative and reproductive sinks (Zavalloni et al., 2001). Previous finding showed that orchard nutrition affects productivity and fruit quality and has to be performed very carefully since, after harvest, fruits quality cannot be improved (Crisosto et al., 1997). Successful fertilization in the cultivation of apples and fruit crops in general requires not only definition of fertilizer application rates but also adequate use of suitable fertilization types (Murčić et al., 2012).

Fruit growers use the different types of fertilization in production, including fertilization with mineral fertilizers (conventional type), fertigation, foliar nutrition and a range of other types. In Serbia, fruit growers primarily use complex NPK fertilizer (15:15:15) and N mineral fertilizers (calcium ammonium nitrate and urea) and farmyard manure (Milošević et al., 2013). Milošević and Milošević (2015) reported that the complex NPK and manure are added to soil in the late autumn and N fertilizers in early spring.

Conventional types of fertilization lead to increased yields, but indirectly adversely affect soil and crop quality (Khan et al., 2005; Abdelaziz et al., 2007). Because of that a large part of the nutrients applied remain unused by the fruit tree, and it can cause soil and groundwater contamination. Due to the aforementioned reasons, in the cultivation of apples and other fruit crops, scientists give priority to fertigation and foliar nutrition, as well as types that significantly improved the efficiency of fertilizers, with minimum adverse environmental impact (Naseri et al., 2002; Amiri et al., 2008).

The aim of this study was to determine the influence complex NPK (6:18:36) + N (calcium ammonium nitrate-CAN) mineral fertilizer and foliar nutrition on chemical composition, antioxidant activity and mineral content of three different apple cultivars (Jonagold Decosta, Red Idared and Gala Schnitzer Schniga) under Sarajevo conditions (Bosnia and Herzegovina). Derived data can be used to establish recommendations for apple orchards fertilization in similar conditions.

## 2. Materials and methods

### 2.1. Chemical properties of soil

Soil chemical analyses were conducted prior to the experiment, i.e. in October 2019. The soil had value of pH in KCl (6.96); it contained 3.3% organic matter, 0.22% total N, 7.8 mg 100 g<sup>-1</sup> available P<sub>2</sub>O<sub>5</sub>, 38.9 mg 100 g<sup>-1</sup> available K<sub>2</sub>O, and 0.4% CaCO<sub>3</sub>. Results revealed that the soil had neutral reaction of soil according to pH value, a good supply of organic matter, low level of P<sub>2</sub>O<sub>5</sub> and high level K<sub>2</sub>O, as well as high level of total N.

### 2.2. Apple samples

The fruits were taken from the apple orchard at the experimental station 'Butmir' on the Federal Institute of Agriculture Sarajevo (Bosnia and Herzegovina). Three apple cultivars (Jonagold Decosta, Red Idared and Gala Schnitzer Schniga) grafted on M.9 rootstock were used as the plant material in 2020 and 2021. The procedure included three fertilization treatment of each cultivar: T<sub>1</sub> (control-without fertilization); T<sub>2</sub> (300 kg/ha NPK (6:18:36) + 150 kg/ha N (calcium ammonium nitrate-CAN)) and T<sub>3</sub> (foliar nutrition-mixture organic-mineral fertilizer commercially named 'FitoFert Kristal' (0.6%) (10:40:10) + 'FitoFert Kristal' (0.6%) (20:20:20) + 'FoliFertil Ca' (0.5%) (N:Ca)). Complex NPK + N mineral fertilizer was added to soil in early spring in 2020 and 2021, while the trees were treated with foliar fertilizer during both vegetation.

Every combination of cultivar/treatment was represented by 15 trees (three replication with five trees). The orchard was planted in spring 2005. The planting distance was 3 m between the rows and 0.9 m within the row. The training system was the Spindle. Standard cultural practices were applied, including drip irrigation. From every combination of cultivar/treatment, 50 fruits were randomly picketed at commercial maturity for fresh consumption. Commercial maturity was determined by fruit skin color and firmness of the fruit.

### 2.3. Preparation of the extracts and determination of chemical composition, antioxidant activity and mineral content

The soluble solids were determined using a refractometer (Pocket PAL-1, Atago, Japan). Total sugar content was determined using Luff-Schoorl method. Total acids were

determined by titration with NaOH and expressed as malic acid.

Approximately 0.5 g of apple samples were extracted with 25 mL ethanol (60%) and stirred for 1 h on a magnetic agitator at room temperature. The extraction step was repeated two more times and all supernatants were collected and evaporated to dryness under reduced pressure at 50 °C. The residue after evaporation was dissolved in a mixture of ethanol/water (60:40, v/v) to 25 mL. The total phenolic content of apple fruits was measured using the Folin-Ciocalteu colorimetric assay (Ough and Amerine, 1988). The extracts (0.1 mL) were mixed with 6 mL water, 0.5 mL Folin-Ciocalteu reagents and 1.5 mL Na<sub>2</sub>CO<sub>3</sub>. The sample was measured at 765 nm absorbance (Spectrofotometer, Shimadzu, Japan). Gallic acid was used as the standard in the concentration range of 0–500 mg L<sup>-1</sup>. The total phenolic content values were expressed as milligrams per 100 grams of fresh weight (FW).

The content of flavonoids was measured using spectrophotometric method based on color reaction of flavonoids with AlCl<sub>3</sub> (Zhishen et al., 1999). The extracts (1 mL) were mixed with 4 mL water, 0.3 mL NaNO<sub>2</sub>, 0.3 mL AlCl<sub>3</sub> and 2 mL NaOH. The sample was measured at 510 nm absorbance (Spectrofotometer, Shimadzu, Japan). Catechin was used as the standard in the concentration range of 0–100 mg L<sup>-1</sup>. The content of flavonoids was expressed as milligrams per 100 g of fresh weight (FW).

FRAP (ferric reducing antioxidant power) assay is based on the rapid reduction in ferric-tripyridyltriazine (Fe<sup>III</sup>-TPTZ) by antioxidants present in the samples forming ferrous-tripyridyltriazine (Fe<sup>II</sup>-TPTZ), a blue-colored product (Benzie and Strain, 1996). The extracts (0.11 mL) were mixed with 0.24 mL water and 2 mL FRAP (ferric reducing ability of plasma) reagents. The sample was measured at 595 nm absorbance (Spectrofotometer, Shimadzu, Japan). FeSO<sub>4</sub> × 7H<sub>2</sub>O was used as the standard in the concentration range of 0–20 Mm. The antioxidant activity was expressed as mmol Fe<sup>2+</sup> per 100 g of fresh weight (FW).

The digestion of the apple samples was performed on an Advanced Microwave Digestion System (Köttermann GmbH, Germany). For total mineralization, about 1.0 g of the wet apple samples were precisely weight with an accuracy ± 0.1 mg and mixed with 10 mL HNO<sub>3</sub> and 1 mL H<sub>2</sub>O<sub>2</sub> and then heated with a microwave to 200 °C for 20 min. After cooling and without filtration, the solution was diluted to a fixed volume into a volumetric flask of 50 mL with ultrapure water (0.05 µS/cm, Thermo Scientific, Germany). The contents of mineral elements were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies, USA) and computed as mg kg<sup>-1</sup> fresh weight (FW) of fruit. For each

digested sample, the ICP-MS measurement was carried out in triplicate  $n = 3$ .

#### 2.4. Statistical analysis

Data from the chemical composition, total phenolic content, flavonoids, antioxidant activity and mineral content presented in the tables are the mean of three replication  $\pm$  standard deviation. Duncan's multiple range test was used to detect the significance of the differences ( $p \leq 0.05$ ) between mean values. Statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY, USA).

### 3. Results

Soluble solid contents of three apple cultivars fertilized with three treatments ranged from 11.91% to 14.37% (Table 1). The highest values for soluble solids were found in Jonagold DeCosta, while the lowest values were found in Gala Schnitzer Schniga cultivar. Analyses of data showed statistically significant differences in content of soluble solids among treatments. The treatment  $T_2$  showed influence on the highest content of soluble solids (13.33%), while the treatment  $T_3$  had the influence on the lowest content of soluble solids (12.71%). Significant differences for soluble solids were found between cultivars, treatments, years and cultivar/treatment combinations. Total sugar content in apple fruits ranged from 9.39% (Gala Schnitzer Schniga/ $T_3$ ) to 12.03% (Jonagold DeCosta/ $T_2$ ) (Table 1).

Cultivars manifested significant differences in total sugar content. The highest values of total sugar content (for all treatments) were found in Jonagold DeCosta cultivar, then in Red Idared, while they were the lowest in Gala Schnitzer Schniga cultivar. The highest average total sugar content was found in fruits from trees fertilized with treatment  $T_2$  (10.96%) and the lowest was found in fruits from trees fertilized with treatment  $T_1$  (10.47%). The content of inverted sugars in the tested cultivars fertilized with three different treatments ranged from 6.58% to 9.76% (Table 1). Significant differences of inverted sugars contents among cultivar/treatment combinations were found. However, differences among cultivars, treatments and years were statistically significant. Cultivar Red Idared showed the highest amount of inverted sugars (8.74%), followed by Jonagold DeCosta (8.58%) and Gala Schnitzer Schniga (6.88%). The treatment  $T_2$  showed influence on the highest content of inverted sugars (8.71%), while the treatment  $T_3$  had the influence on the lowest content of inverted sugars (7.63%).

Sucrose content in apple fruits ranged from 1.1% to 3.24%. Differences among cultivars, treatments, years and cultivar/treatment combinations were statistically significant. Among cultivars, the highest values of sucrose content were found in Jonagold DeCosta (2.66%), and the lowest values in Red Idared (1.52%). Treatment  $T_3$

showed the highest amount of sucrose (2.55%), followed by treatment  $T_2$  (2.13%) and treatment  $T_1$  (1.83%).

The highest values of content of total acids were found in combinations Red Idared/ $T_3$  (0.35%), while the lowest values were obtained in combination Gala Schnitzer Schniga/ $T_3$  (0.22%) (Table 1). Differences in content of total acids among treatments and years were not significant, while the cultivar Gala Schnitzer Schniga had a statistically significantly lower value of content of total acids in relation to the other two cultivars. The second year of research showed influence on higher content of soluble solids, total sugars and inverted sugars, while the content of sucrose was higher in 2020.

Total phenolic content (TPC) in the apple fruits ranged from 87.16 mg 100 g<sup>-1</sup> fresh weight (FW) to 152.83 mg 100 g<sup>-1</sup> FW (Table 2). Significant differences for TPC were found among individual cultivar/treatment combinations and cultivars. As for the apple extracts, the highest values for TPC were found in Red Idared (135.42 mg 100 g<sup>-1</sup> FW), and the lowest values in Gala Schnitzer Schniga (92.78 mg 100 g<sup>-1</sup> FW). The highest average TPC values in the apple extracts were found in fruits from trees fertilized with treatment  $T_2$  (116.93 mg 100 g<sup>-1</sup> FW) and the lowest were found in fruits from trees fertilized with treatment  $T_3$  (97.36 mg 100 g<sup>-1</sup> FW). However, differences among treatments were statistically significant.

The content of flavonoids in apple fruits ranged from 43.07 mg 100 g<sup>-1</sup> FW (Gala Schnitzer Schniga/ $T_3$ ) to 113.69 mg 100 g<sup>-1</sup> FW (Red Idared/ $T_2$ ) (Table 2). The highest average content of flavonoids was found in fruits from trees fertilized with treatment  $T_2$  (74.79 mg 100 g<sup>-1</sup> FW) and the lowest was found in fruits from trees fertilized with treatment  $T_3$  (53.22 mg 100 g<sup>-1</sup> FW). Cultivars manifested significant differences in content of flavonoids. The highest values of flavonoids content (for all treatments) were found in Red Idared cultivar, then in Jonagold DeCosta, while they were the lowest in Gala Schnitzer Schniga cultivar.

The total antioxidant activity determined by scavenging FRAP assay in the apple fruit varied between 0.56 mmol Fe<sup>2+</sup> 100 g<sup>-1</sup> FW (Jonagold DeCosta/ $T_1$ ) and 1.31 mmol Fe<sup>2+</sup> 100 g<sup>-1</sup> FW (Red Idared/ $T_2$ ). Significant differences in antioxidant activity were found among cultivars, treatments and cultivar/treatments combinations. The highest values for antioxidant activity in the extracts of apple were found in Red Idared and the lowest in Gala Schnitzer Schniga cultivar. The treatment  $T_2$  showed influence on the highest antioxidant activity (0.89 mmol Fe<sup>2+</sup> 100 g<sup>-1</sup> FW), while the treatment  $T_3$  had the influence on the lowest antioxidant activity (0.66 mmol Fe<sup>2+</sup> 100 g<sup>-1</sup> FW). The second year of research showed influence on higher total phenolic content, flavonoids and antioxidant capacity of apple fruits (Table 2).

In the apple fruit, eight mineral elements were determined. The content of all nutrients is presented in



**Table 1.** Chemical composition of fruits of three apple cultivars (average 2020–2021).

Combination cultivar/treatment	Soluble solids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Total acids (%)
Gala Schnitzer Schniga /T <sub>1</sub>	12.16 ± 0.47 h <sup>†</sup>	10.44 ± 1.10 f	6.96 ± 0.98 f	1.65 ± 0.28 d	0.27 ± 0.02 ab
Gala Schnitzer Schniga/T <sub>2</sub>	12.65 ± 0.20 f	9.94 ± 0.35 g	7.09 ± 1.07 e	2.68 ± 0.68 b	0.28 ± 0.02 ab
Gala Schnitzer Schniga / T <sub>3</sub>	11.91 ± 0.40 i	9.39 ± 0.88 h	6.58 ± 0.76 g	2.69 ± 0.12 b	0.22 ± 0.02 b
Jonagold DeCosta/T <sub>1</sub>	12.86 ± 0.09 e	10.54 ± 0.61 e	8.31 ± 0.98 c	2.12 ± 0.36 c	0.30 ± 0.02 ab
Jonagold DeCosta/T <sub>2</sub>	14.37 ± 0.33 a	12.03 ± 0.88 a	9.28 ± 1.69 b	2.61 ± 0.77 b	0.31 ± 0.02 ab
Jonagold DeCosta/ T <sub>3</sub>	13.92 ± 0.22 b	11.57 ± 1.03 b	8.16 ± 1.63 d	3.24 ± 0.57 a	0.32 ± 0.04 ab
Red Idared/T <sub>1</sub>	13.38 ± 1.38 c	10.44 ± 1.10 f	8.31 ± 0.98 c	1.73 ± 0.18 d	0.31 ± 0.01 ab
Red Idared/T <sub>2</sub>	12.97 ± 1.31 d	10.91 ± 1.19 c	9.76 ± 1.29 a	1.10 ± 0.09 e	0.32 ± 0.02 ab
Red Idared/ T <sub>3</sub>	12.30 ± 1.42 g	10.58 ± 1.12 d	8.16 ± 1.63 d	1.73 ± 0.18 d	0.35 ± 0.04 a
<b>Cultivar</b>					
Gala Schnitzer Schniga	12.24 ± 0.22 c	9.92 ± 0.47 c	6.88 ± 0.52 c	2.34 ± 0.26 b	0.25 ± 0.01 b
Jonagold DeCosta	13.71 ± 0.20 a	11.38 ± 0.49 a	8.58 ± 0.81 b	2.66 ± 0.34 a	0.31 ± 0.02 a
Red Idared	12.88 ± 0.76 b	10.64 ± 0.62 b	8.74 ± 0.75 a	1.52 ± 0.11 c	0.32 ± 0.01 a
<b>Treatment</b>					
T <sub>1</sub>	12.79 ± 0.48 b	10.47 ± 0.53 c	7.86 ± 0.56 b	1.83 ± 0.16 c	0.29 ± 0.01
T <sub>2</sub>	13.33 ± 0.47 a	10.96 ± 0.52 a	8.71 ± 0.80 a	2.13 ± 0.37 b	0.30 ± 0.01
T <sub>3</sub>	12.71 ± 0.52 c	10.51 ± 0.60 b	7.63 ± 0.79 c	2.55 ± 0.25 a	0.29 ± 0.02
<b>Year</b>					
2020	11.50 ± 0.29 b	8.59 ± 0.16 b	5.33 ± 0.16 b	2.91 ± 0.21 a	0.30 ± 0.01
2021	14.39 ± 0.26 a	12.69 ± 0.21 a	10.80 ± 0.30 a	1.43 ± 0.12 b	0.29 ± 0.01

T<sub>1</sub> - (control-without fertilization); T<sub>2</sub> - (300 kg/ha NPK (6:18:36) + 150 kg/ha N (calcium ammonium nitrate-CAN)); T<sub>3</sub> - (foliar nutrition-mixture organic-mineral fertilizer commercially named 'FitoFert Kristal' (0.6%) (10:40:10) + 'FitoFert Kristal' (0.6%) (20:20:20) + 'FoliFertil Ca' (0.5%) (N:Ca)).

<sup>†</sup>Means followed by the same letter do not differ significantly according to Duncan's multiple range test at  $p = 0.05$ .

Tables 3 and 4. Among them, four are macroelements that are present in large amounts: potassium (K), calcium (Ca), phosphorus (P), and magnesium (Mg). Dominant mineral in the apple fruit was K. Its content ranged from 1266.15 mg kg<sup>-1</sup> to 1652.89 mg kg<sup>-1</sup>. It is followed by Ca (122.76–474.90 mg kg<sup>-1</sup>); P (86.14–124.84 mg kg<sup>-1</sup>) and Mg (57.36–70.68 mg kg<sup>-1</sup>). Four microelements were determined: boron (B) (1.06–14.25 mg kg<sup>-1</sup>); iron (Fe) (2.79–4.62 mg kg<sup>-1</sup>); manganese (Mn) (0.27–0.46 mg kg<sup>-1</sup>) and zinc (Zn) (0.15–0.48 mg kg<sup>-1</sup>).

Significant differences in the macroelements in the apple fruit among the cultivar/treatment combination were found. Macroelements nutrient levels in fruit were significantly affected by cultivars, fertilizer treatments and years. The significant highest average contents of K, P and Mg were found in the fruits of cultivar Jonagold DeCosta (1616.66 mg kg<sup>-1</sup> FW; 113.29 mg kg<sup>-1</sup> FW; 69.61 mg kg<sup>-1</sup> FW). On the other hand, the fruits of cultivar Red Idared had the significant highest average contents of P (113.29 mg kg<sup>-1</sup> FW). Treatment T<sub>2</sub> (300 kg/ha NPK (6:18:36)

+ 150 kg/ha N (calcium ammonium nitrate-CAN)) increased fruit content of K (1540.05 mg kg<sup>-1</sup> FW) and P (111.49 mg kg<sup>-1</sup> FW) average for the all cultivars and years, while T<sub>3</sub> promoted fruit content of Ca (301.37 mg kg<sup>-1</sup> FW), respectively, T<sub>1</sub> increased fruit content of Mg (66.47 mg kg<sup>-1</sup> FW). K, Ca, P and Mg content were year dependent. First year of researcher stored the higher level of Ca and P in average for all cultivar/treatment. On the other hand, second year promoted the higher content of K and Mg.

There were significant differences between cultivar/treatment combinations in microelements content (Table 4). The differences in B, Fe, Mn and Zn content among cultivars were significant when taking into account the average data for the all the fertilizer treatments and years. Among the cultivars, B (5.68 mg kg<sup>-1</sup> FW) and Zn (0.38 mg kg<sup>-1</sup> FW) were the highest in Red Idared, while the Mn (0.44 mg kg<sup>-1</sup> FW) content was the highest in Jonagold DeCosta. On the other hand, differences between the Red Idared and Jonagold DeCosta were not significant in

**Table 2.** Total phenolic content (mg 100 g<sup>-1</sup> FW), content of flavonoids (mg 100 g<sup>-1</sup> FW) and antioxidant activity (mmol Fe<sup>2+</sup> 100 g<sup>-1</sup> FW) of fruits of three apple cultivars (average 2020–2021).

Cultivar/treatment combination	Total phenolic content	Flavonoids	Antioxidant activity
Gala Schnitzer Schniga / T <sub>1</sub>	100.41 ± 5.48 bcd <sup>†</sup>	48.27 ± 4.85 de	0.60 ± 0.06 e
Gala Schnitzer Schniga / T <sub>2</sub>	90.76 ± 4.58 ef	43.23 ± 4.82 f	0.58 ± 0.07 ef
Gala Schnitzer Schniga / T <sub>3</sub>	87.16 ± 4.15 f	43.07 ± 3.64 f	0.57 ± 0.06 f
Jonagold DeCosta / T <sub>1</sub>	96.29 ± 1.96 de	46.06 ± 5.55 ef	0.56 ± 0.06 f
Jonagold DeCosta / T <sub>2</sub>	107.19 ± 7.23 b	67.46 ± 7.31 c	0.79 ± 0.04 c
Jonagold DeCosta / T <sub>3</sub>	99.21 ± 5.76 cd	63.97 ± 6.37 c	0.77 ± 0.04 c
Red Idared / T <sub>1</sub>	147.71 ± 8.59 a	86.99 ± 8.76 b	0.98 ± 0.09 b
Red Idared / T <sub>2</sub>	152.83 ± 15.79 a	113.69 ± 10.07 a	1.31 ± 0.13 a
Red Idared / T <sub>3</sub>	105.71 ± 4.46 bc	52.63 ± 7.73 d	0.65 ± 0.09 d
<b>Cultivar</b>			
Gala Schnitzer Schniga	92.78 ± 4.69 c	44.85 ± 2.52 c	0.58 ± 0.03 c
Jonagold DeCosta	100.89 ± 5.25 b	59.16 ± 4.21 b	0.71 ± 0.04 b
Red Idared	135.42 ± 11.30 a	84.44 ± 7.83 a	0.98 ± 0.09 a
<b>Treatment</b>			
T <sub>1</sub>	114.81 ± 8.02 a	60.44 ± 5.86 b	0.71 ± 0.06 b
T <sub>2</sub>	116.93 ± 11.68 a	74.79 ± 8.31 a	0.89 ± 0.09 a
T <sub>3</sub>	97.36 ± 4.92 b	53.22 ± 3.97 c	0.66 ± 0.04 c
<b>Year</b>			
2020	85.66 ± 2.68 b	48.51 ± 3.68 b	0.60 ± 0.04 b
2021	133.73 ± 7.09 a	77.13 ± 5.27 a	0.91 ± 0.05 a

T<sub>1</sub> - (control-without fertilization); T<sub>2</sub> - (300 kg/ha NPK (6:18:36) + 150 kg/ha N (calcium ammonium nitrate-CAN)); T<sub>3</sub> - (foliar nutrition-mixture organic-mineral fertilizer commercially named 'FitoFert Kristal' (0.6%) (10:40:10) + 'FitoFert Kristal' (0.6%) (20:20:20) + 'FoliFertil Ca' (0.5%) (N:Ca)).

<sup>†</sup>Means followed by the same letter do not differ significantly according to Duncan's multiple range test at p = 0.05.

content of Fe. Fruit microelements levels were significantly affected by fertilizer treatments, expect for content of Mn. On average, for all the cultivars and years, T<sub>3</sub> promoted the highest content of B and Zn in fruit, while the treatment T<sub>1</sub> increased fruit content of Fe. The higher contents of B, Fe and Zn, on average for all the cultivars and fertilizer treatments, were in 2021, respectively, content of Mn in 2020.

#### 4. Discussion

The quality of the fruit of apple is a combination of a large number of physical and chemical, external and internal properties of the fruit. Also, it depends on the interaction of several factors: genotype (cultivar and rootstock), pollinator, physical and chemical properties of the soil, nutrient content and water regime of the soil (Tagliavini and Marangoni, 2002; Militaru et al., 2009). According to Chun et al. (2005), the most important parameters of fruit

quality (dry matter content, content of sugar, content of total acids, organic acids, phenolic compounds) depend not only on the cultivar, but also on the agroecological conditions, growing conditions, agrotechnical and pomotechnical measures, storage. According to many authors, the key parameters that determine the quality and the acceptance of the fruit by consumers are the content of soluble solids and total acids, as well as ration between them (Nergiz and Yildiz, 1997; Crisosto et al., 2004).

The content of soluble solids was significantly different depending on the apple cultivars and in the conditions of the Chile was in the interval from 12.6% to 15.5% (Henríquez et al., 2010). In the literature, the content of soluble solids in cultivars Idared and Melrose in different fertilizer treatments was from 11.7% to 13.0% (Milošević and Milošević, 2015). The results for soluble solid content are consistent with those of Blažek and Hlušičková (2007). According to Fatih and Özcan (2010), the content of

**Table 3.** Contents of macroelements in apple fruit mg kg<sup>-1</sup> fruit weight (average 2020-2021).

Combination cultivar/treatment	Potassium	Calcium	Phosphorus	Magnesium
Gala Schnitzer Schniga /T <sub>1</sub>	1266.15 ± 33.57 h <sup>†</sup>	162.66 ± 10.81 e	86.77 ± 5.22 h	67.58 ± 6.87 b
Gala Schnitzer Schniga/T <sub>2</sub>	1616.41 ± 60.18 c	174.02 ± 10.25 d	99.73 ± 6.11 e	63.31 ± 3.51 c
Gala Schnitzer Schniga / T <sub>3</sub>	1394.84 ± 41.86 f	232.12 ± 4.95 b	89.83 ± 5.48 g	61.65 ± 4.16 cd
Jonagold DeCosta/T <sub>1</sub>	1560.66 ± 13.19 d	95.25 ± 3.67 g	95.25 ± 3.67 f	67.58 ± 6.87 b
Jonagold DeCosta/T <sub>2</sub>	1636.45 ± 23.00 b	122.76 ± 4.83 f	119.80 ± 7.50 b	70.68 ± 1.09 a
Jonagold DeCosta/ T <sub>3</sub>	1652.86 ± 29.77 a	197.10 ± 4.10 c	124.84 ± 11.77 a	70.56 ± 1.27 a
Red Idared/T <sub>1</sub>	1428.93 ± 171.42 e	125.41 ± 4.27 f	86.14 ± 2.93 h	64.23 ± 3.87 c
Red Idared/T <sub>2</sub>	1367.29 ± 183.78 g	164.13 ± 5.29 e	114.93 ± 6.39 c	59.81 ± 4.55 de
Red Idared/ T <sub>3</sub>	1394.11 ± 167.34 f	474.90 ± 117.47 a	103.04 ± 1.84 d	57.36 ± 4.28 e
<b>Cultivar</b>				
Gala Schnitzer Schniga	1425.80 ± 43.25 b	189.59 ± 8.87 b	92.11 ± 3.30 c	64.28 ± 2.81 b
Jonagold DeCosta	1616.66 ± 15.84 a	138.37 ± 10.69 c	113.29 ± 5.50 a	69.61 ± 2.24 a
Red Idared	1396.78 ± 94.82 c	254.81 ± 52.92 a	101.37 ± 3.66 b	60.47 ± 2.40 c
<b>Treatment</b>				
T <sub>1</sub>	1418.58 ± 62.19 c	127.77 ± 7.70 c	89.39 ± 2.42 c	66.47 ± 3.29 a
T <sub>2</sub>	1540.05 ± 67.88 a	153.64 ± 6.66 b	111.49 ± 4.18 a	64.59 ± 2.14 b
T <sub>3</sub>	1480.60 ± 62.32 b	301.37 ± 47.52 a	105.90 ± 5.39 b	63.29 ± 2.32 b
<b>Year</b>				
2020	1321.14 ± 52.41 b	235.28 ± 35.69 a	110.26 ± 3.96 a	58.39 ± 1.9 b
2021	1638.35 ± 29.71 a	153.24 ± 8.51 b	94.26 ± 2.99 b	71.19 ± 1.6 a

T<sub>1</sub> - (control-without fertilization); T<sub>2</sub> - (300 kg/ha NPK (6:18:36) + 150 kg/ha N (calcium ammonium nitrate-CAN)); T<sub>3</sub> - (foliar nutrition-mixture organic-mineral fertilizer commercially named 'Fitofert Kristal' (0.6%) (10:40:10) + 'Fitofert Kristal' (0.6%) (20:20:20) + 'FoliFetril Ca' (0.5%) (N:Ca)).

<sup>†</sup>Means followed by the same letter do not differ significantly according to Duncan's multiple range test at  $p = 0.05$ .

soluble solids of cultivars in the conditions of the Konya region (Turkey) was in average 16.4% (interval from 13.3% to 23.6%), while Rop et al. (2011) obtained content of soluble solids for native apple cultivar in Central Europe from 11.23% to 15.98 %. The content of soluble solids in organic cultivars of apple was 12.66%, while in conventional cultivars of apple was 12.4% (Roussos and Gasparatos, 2010). Our results for soluble solid content are in agreement with previous findings of Oztur et al. (2010) who reported that cultivar Starking Delicious had the highest content (14.32%) followed by cultivar Granny Smith (13.09%) and cultivar Golden Delicious (12.5%). Earlier it was reported that genetic background of apple cultivars. Ecology, soil type and cultural practices applied strongly affect the total soluble solid (Guleryuz et al., 2001).

According to Ertürk et al. (2006), the invert sugars of the cultivars were between 0.08 g 100 g<sup>-1</sup> and 1.25 g 100 g<sup>-1</sup>. In our study, inverted sugar content was the higher than previously established Badžak et al. (2021).

According Ticha et al. (2015), the sucrose content in the fruit of apple cultivars varied from 2.1 g 100 g<sup>-1</sup> of apple (Melrose) to 7.2 g 100 g<sup>-1</sup> of apple (Opal), while the Ertürk et al. (2006) established the sucrose quantities of the cultivars changed between 8.86 g 100 g<sup>-1</sup> and 21.28 g 100 g<sup>-1</sup>. Our findings for the content of sucrose were in agreement with data from the literature (Badžak et al., 2021).

One of the important parameters of quality of fruit is the content of total acids. Acids give the fruit a sour taste. During the ripening period, the fruits accumulate sugar and break down the total the fruits tastier. Adequate ratio of sugars and acids gives apple fruits a harmonious and refreshing taste, which is an important criterion when evaluating and consuming fruits (Mišić, 2002).

The average content of total acids in the conditions of Chile for apple cultivars was 0.25% (with variation from 0.15% to 0.4%) (Henríquez et al., 2010). The same group of author's reporter that the acidity was significantly different depending on apple cultivars. Our results are in agreement

**Table 4.** Contents of microelements in apple fruit mg kg<sup>-1</sup> fruit weight (average 2020–2021).

Combination cultivar/treatment	Boron	Iron	Manganese	Zinc
Gala Schnitzer Schniga /T <sub>1</sub>	1.06 ± 0.10 d	3.10 ± 0.64 d	0.40 ± 0.05 abc	0.16 ± 0.02 e
Gala Schnitzer Schniga/T <sub>2</sub>	1.42 ± 0.07 d	3.69 ± 0.69 c	0.39 ± 0.03 bc	0.16 ± 0.01 e
Gala Schnitzer Schniga / T <sub>3</sub>	10.89 ± 0.92 b	3.21 ± 0.62 d	0.35 ± 0.02 cd	0.23 ± 0.03 d
Jonagold DeCosta/T <sub>1</sub>	1.42 ± 0.15 d	4.11 ± 0.79 b	0.46 ± 0.02 a	0.15 ± 0.02 e
Jonagold DeCosta/T <sub>2</sub>	1.19 ± 0.23 d	3.64 ± 0.33 c	0.41 ± 0.02 abc	0.18 ± 0.03 e
Jonagold DeCosta/ T <sub>3</sub>	9.64 ± 1.61 c	3.34 ± 0.51 d	0.44 ± 0.02 ab	0.31 ± 0.00 c
Red Idared/T <sub>1</sub>	1.41 ± 0.01 d	4.62 ± 0.32 a	0.27 ± 0.02 e	0.34 ± 0.06 b
Red Idared/T <sub>2</sub>	1.38 ± 0.06 d	3.34 ± 0.06 d	0.31 ± 0.02 de	0.33 ± 0.07 bc
Red Idared/ T <sub>3</sub>	14.25 ± 3.21 a	2.79 ± 0.11 e	0.29 ± 0.01 e	0.48 ± 0.02 a
<b>Cultivar</b>				
Gala Schnitzer Schniga	4.45 ± 1.14 b	3.33 ± 0.36 b	0.38 ± 0.02 b	0.19 ± 0.01 c
Jonagold Decosta	4.08 ± 1.08 b	3.69 ± 0.32 a	0.44 ± 0.01 a	0.21 ± 0.02 b
Red Idared	5.68 ± 1.78 a	3.58 ± 0.22 a	0.29 ± 0.01 c	0.38 ± 0.04 a
<b>Treatment</b>				
T <sub>1</sub>	1.29 ± 0.07 b	3.94 ± 0.37 a	0.38 ± 0.03	0.21 ± 0.03 b
T <sub>2</sub>	1.33 ± 0.08 b	3.56 ± 0.25 b	0.37 ± 0.02	0.22 ± 0.03 b
T <sub>3</sub>	11.59 ± 1.25 a	3.11 ± 0.26 c	0.36 ± 0.02	0.34 ± 0.03 a
<b>Year</b>				
2020	4.39 ± 0.89 b	2.55 ± 0.14 b	0.34 ± 0.02 b	0.29 ± 0.03 a
2021	5.08 ± 1.32 a	4.52 ± 0.18 a	0.39 ± 0.02 a	0.23 ± 0.02 b

T<sub>1</sub>- (control-without fertilization); T<sub>2</sub>-(300 kg/ha NPK (6:18:36) + 150 kg/ha N (calcium ammonium nitrate-CAN)); T<sub>3</sub>- (foliar nutrition-mixture organic-mineral fertilizer commercially named 'FitoFert Kristal' (0.6%) (10:40:10) + 'FitoFert Kristal' (0.6%) (20:20:20) + 'FoliFertil Ca' (0.5%) (N:Ca)).

<sup>†</sup>Means followed by the same letter do not differ significantly according to Duncan's multiple range test at  $p = 0.05$ .

with Campeanu et al. (2009) who obtained similar values of total acidity. In the study of Fatih and Özcan (2010), the content of total acids was in the interval from 4.3 g kg<sup>-1</sup> to 20.3 g kg<sup>-1</sup> (average 10.0 g kg<sup>-1</sup>). Wu et al. (2007) established 2.8 g L<sup>-1</sup> to 7.3 g L<sup>-1</sup> total acids in eight apple cultivars. According to Ozturk et al. (2010), the total acidity in the fruit of apple cultivars varied from 0.409% to 0.780%. In accordance with our results, Karl et al. (2020) found that fertilization had no effect on acidity. According to Harker et al. (2002), high content of total acids is the best indicator of sour taste, while high content of soluble dry matter is an indicator of sweet taste of fruit.

Sudha et al. (2007) found that total phenolic content in different apple pomaces was 10.16 mg g<sup>-1</sup>. The amounts of total phenolic content of apple cultivars are determined as chlorogenic acid equivalent and ranged from 95 mg kg<sup>-1</sup> to 245 mg kg<sup>-1</sup>. The total phenolic content was found between 4.22 mg g<sup>-1</sup> to 8.67 mg g<sup>-1</sup> (Cetković et al., 2008). According

to Ozturk et al. (2010), the total phenolic content of the apple cultivars ranged from 25.69 mg GAE 100 g<sup>-1</sup> FW to 40.96 mg GAE 100 g<sup>-1</sup> FW. Previously finding reported that there was a wide variation in total phenolic contents among apple cultivars, ranging from 54.0 357 mg 100 g<sup>-1</sup> FW to 357 mg 100 g<sup>-1</sup> FW (Podsędek et al., 2000; Wolfe et al., 2003; Karadeniz et al., 2005). Analyzing of native apple cultivars from Central Europe, Rop et al. (2011) obtained values of total phenolic content expressed as g of gallic acid kg<sup>-1</sup> FW in interval 1.46–3.29. Fatih and Özcan (2010) established the interval of total phenolic content 95.0–245 mg kg<sup>-1</sup> FW. Our results of total phenols content were lower than in the report of Kulina et al. (2018) who studied autochthonous cultivars of apple (Petrovaca, Bjelicnik, Zelenika, Bobovec, Lijepocvjetka and Sampanjka) from the Majejica area in Bosnia and Herzegovina. Namely, previously group of authors established interval total phenols content from 247.45 mg GAE 100 g<sup>-1</sup> FW to 542.10 mg GAE 100 g<sup>-1</sup> FW.



However, the results of total phenolic content reported in the literature are obtained analyzing the flesh and skin separately, while in our research analyzing the whole fruit. Because of that, our results are not fully comparable with others. On the other hand, the phenolic content of apple fruits depends on several factors such as cultivar, climate, degree of ripeness of the fruit and growing area (Wolfe et al., 2003; Karadeniz et al., 2005).

According Francini et al. (2022), the highest antioxidant activity was measured in Mora cultivar, while the lowest was observed in Nesta cultivar. Ozturk et al. (2010) established that the highest antioxidant capacity was found in Granny Smith cultivar (79.26%), then in Starking Delicious (74.39%), while the lowest antioxidant capacity was in Golden Delicious cultivar (63.03%). Antioxidant composition of fruits varies among cultivars and genetics plays a significant role (Rop et al., 2011). Ours findings confirmed the previously statements of Ozturk et al. (2010) and Henríquez et al. (2010) that there were wide differences among the apple cultivars of total phenolic content and antioxidant activity.

Our results for the contents of K, Ca, P, Mg and Fe were higher and for Mn and Zn they were lower than those reported by Henríquez et al. (2010). In comparison with the results of Resmije et al. (2019), our values for the content of iron were similar. The obtained results for the content of the higher concentration of K in apple cultivars were in accordance with that reported by Horsley et al. (2014); Kumar et al. (2018); Preti and Tarola (2021) and Francini et al. (2022). According to Sachini et al. (2020), cultivar “Fuji Suprema” presented high K concentrations in both flesh and skin, which mineral is one with the greatest contribution to the organism with the consumption of apples in both harvest seasons. On the other hand, Fatih and Özcan (2010) have reported that dominant mineral in the apple fruit grown in Konya (Turkey) was Ca. The content of zinc recorded by us was lower than those published by Rismije et al. (2019) and Jemaneh and Chandravanshi (2021). The obtained results

for the content of Mg in apple cultivars were in accordance with that reported Jemaneh and Chandravanshi (2021), while our results for the content of Ca, Fe and Mn show lower values. On average for all the cultivars, contents of K, Ca, Mg, Fe, Zn and Mn in this study were lower than those reported by Kumar et al. (2018).

The nutrient concentration of apple fruits showed large variation influence by cultivars (Campeanu et al., 2009; Kucukyumuk and Erdal, 2011), by rootstocks (Kucukyumuk and Erdal, 2011) and year (Schweitzer et al., 2019). Results of Francini et al.’s study (2022) show that Mg, Ca and K were the most abundant elements and concentrations varied significantly among the cultivars. Several authors found greater variations in levels of nutrients in different apple cultivars, indicating strong genetically controlled traits (Nachtigall and Dechen, 2006; Nagy et al., 2006). Worthington (2001) showed that fertilization have a positive effect on the accumulation of Mg. Milošević and Milošević (2015) reported that fertilizer treatments have a significant influence on leaf nutrient levels of P, K and Mg. According Gasparatoset et al. (2011), environment, cultivation system and rootstock play an important role in nutrient status of apple trees.

Differences between ours and any results of other authors could be explain by different cultivars studied, environmental conditions, such as soil type and precipitation or cultural practices, especially fertilization and methodology used determine the mineral composition. Results in mineral content of fruit of apple may justify the variation in susceptibility to physiological disorders observed in cultivars and seasons (Schweitzer et al., 2019).

Results imply that all cultivars and fertilization treatments are adequate in order to improve bioactive content of apple fruit. Further studies should expand our knowledge about of different fertilizer applications on fruit quality of different apple cultivars. Also, further study should include increasing the number of cultivars and different fertilization treatments.

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