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Boron and maturity effects on biochemical parameters and antioxidant activity of pepper (*Capsicum annuum* L.) cultivars

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Abstract: Pepper fruit (*Capsicum annuum* L.) is one of the best sources of carotenoids, carbohydrates, flavonoids, phenols, and antioxidant activity important in the human diet. The levels of bioactive compounds are variable and may be affected by cultivar, maturity stage, and boron concentration in the soil solution. A greenhouse experiment was conducted with the pepper cultivars Solario, Osho, Odysseo, and Arlequin treated with 5 boron concentrations (0.5, 1, 2.5, 5, and 10 mg B L^{-1}) for 70 days. Fruits were sampled at 40 and 70 days and the concentration of carbohydrates, carotenoids, flavonoids, total phenols, and antioxidant activity (FRAP, DPPH) were measured. The experiment included 10 plants per B treatment and cultivar, with a total of 200 plants. The results obtained in this study showed that B toxicity reduced the level of carbohydrates in four commercial pepper cultivars grown in Greece and in other countries. The levels of carotenoids, flavonoids, and phenols were a function of pepper cultivar and boron treatment. Fruit maturation increased the level of carbohydrates, phenols, flavonoids, and antioxidant capacity. The cultivar Solario had the highest phenol concentration and also the highest FRAP value among the tested cultivars. In conclusion, boron toxicity decreased carbohydrate concentration and modified antioxidant activity in pepper fruit.

Key words: Antioxidants, boron, carbohydrates, flavonoids, pepper, phenols

1. Introduction

Boron is an essential element for higher plants. The symptoms of boron deficiency in plants include cessation of root and leaf growth, necrosis of leaf primordia, necrosis of leaf and stem phloem, bark splitting, or reduced pollen germination. There is a narrow range between boron deficiency and toxicity. Typical boron toxicity symptoms occur in the marginal region of mature leaves, whose portions become chlorotic or necrotic (Ozturk et al., 2010).

Pepper (*Capsicum annuum* L.), as a fresh vegetable, is an excellent commodity containing vitamin C, α-carotene, carbohydrates, flavonoids, and phenols, and it has high antioxidant activity (Russo and Howard, 2002; Serrano et al., 2010; Silva et al., 2013). All the above substances have positive effects on various aspects of human health (Materska and Perucka, 2005). Antioxidants are very important in humans for building cell molecules, since they prevent the oxidizing and harming of cells by reactive oxygen species (Antonius et al., 2014). The antioxidant content is affected by soil management (Antonius, 2014) and the carotenoid concentration of peppers (*C. annuum* L.) is affected differently in field-grown and greenhouse-grown peppers (Keyhaninejad et al., 2012). Carotenoids serve as antioxidants and participate in the pigment/protein complex, which harvests light and transfers the energy to chlorophyll (Malkin and Niyogi, 2000), playing a determining role in photosynthesis (Guzman et al., 2010). Many researchers have worked with various aspects of carotenoids in peppers, like carotenoid composition (Collera-Zuniga et al., 2005), carotenoid accumulation in peppers of different colors at ripening (Ha et al., 2007), and carotenoid isomers (Khoo et al., 2011).

Flavonoids are ubiquitous in plants with antioxidant activity. The phytochemical changes and antioxidant activity are important dietary attributes in pepper consumption (Zhang and Hamauzu, 2003; Materska and Perucka, 2005; Navarro et al., 2006; Zhuang et al., 2012).

Phenolic compounds are important secondary metabolites (Hervert-Hernandez et al., 2010; Asnin and Park, 2013) of peppers with antioxidant properties that suppress health disorders (Clifford, 2004). Peppers contain a wide array of phytochemicals, which change due to the cultivar or maturation stage. It was found that the pepper cultivars 730F_1 and 1245F_1 had the highest

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carotenoid content (Topuz and Ozdemir, 2003). The sweet pepper cultivar Flamingo had the highest total reducing content, while Totkel and Mazurka had the highest carotene content (Deepa et al., 2006). In addition, it was shown that fresh peppers exhibited the highest phenols and flavonoids, while the *C. annuum* L. cultivars had the greatest antioxidant activity (Loizzo et al., 2015). Maturity stage, harvest time (Ghasemnezhad et al., 2011), and particularly advanced maturation increased the total antioxidant activity of bell peppers. Furthermore, it was observed that the flavonoid content of peppers increased with ripeness (Lee et al., 1995). However, there is no information available in the international literature concerning the above characteristics under the influence of various B concentrations in the cultivars Solario, Osho, Odysseo, and Arlequin even though these cultivars are greatly used for human consumption worldwide due to their high nutritional value. In this paper, the antioxidant potential of bell peppers was studied (Nadeem et al., 2011) as mature peppers are good sources of dietary antioxidants (Oboh and Rocha, 2007).

The findings will provide important information for the use of appropriate pepper cultivars as food ingredients. The phytochemical compound concentrations in the pepper fruit illustrate the importance in choosing a suitable cultivar, which, as a rich source of functional compounds, prevents the development of chronic diseases such as diabetes and cancer (Materska and Perucka, 2005). Therefore, the aim of the present study is to determine the concentration of functional materials and antioxidant activity as affected by B concentration in the nutrient solution in four *C. annuum* L. varieties of high use, with the objective to compare their bioactive profiles and use as ingredients in the human diet.

2. Materials and methods

2.1. Plant material and growth conditions

A greenhouse experiment was conducted at the farm of the Aristotle University of Thessaloniki, Greece (40°53′N, 22°99′E), from April 2013 to June 2013. Pepper cultivars Solario, Osho, Odysseo, and Arlequin (*C. annuum* L.) were included in the experiment to study the influence of 5 boron (B) concentrations (0.5, 1, 2.5, 5, and 10 mg B L^{-1}). Two plants per pot were transplanted at the fourth true leaf of growth in 8-L plastic pots filled with 1:1 sand/perlite and placed on benches in the experimental greenhouse. The conditions in the greenhouse were: relative humidity, 60%–70%; temperature, 20–30 °C; and photosynthetic photon flux density, 900 μmol m^{-2} s^{-1} measured at the top of the plants with a quantum sensor. The experimental plants were irrigated with 50% Hoagland nutrient solution (Hoagland and Arnon, 1938) modified to include 5 B concentrations (0.5–10 mg L^{-1}). Every 2 days each pot was irrigated with 300 mL of nutrient solution and every 15 days each pot was irrigated with 300 mL of deionized water to leach out any accumulated salts. The experiment included a total of 10 plants (2 plants per pot of 5 pots) per B treatment and per cultivar; this means an overall total of 100 pots and 200 plants for the entire experiment. The position of the experimental pots was changed on the bench to prevent positional effects on light intensity. Two fruit samplings were conducted, the first one at 40 days, which was the period of fruit setting, and the second one at 70 days from the initiation of the treatments (fruit maturation of cultivar Odysseo). In the fruit pericarp of all the cultivars and of the red fruits of Odysseo F_1, the carotenoids, carbohydrates, flavonoids, phenols, and total antioxidant activity (FRAP, DPPH) were measured.

2.2. Carbohydrate determination

For total carbohydrate extraction, 0.3 g of fresh fruit pepper pericarp chopped into small pieces was placed in 25-mL glass test tubes and in each tube 15 mL of 80% (v/v) ethanol was added. The tubes with the plant material were incubated in a 60 °C water bath for 30 min. The extract was filtered with Whatman No. 1 filter paper and total carbohydrates were measured with anthrone reagent using a standard curve of 0–0.2 mM (Fales, 1951).

2.3. Carotenoid determination

Carotenoid content was determined with known methods (Lichtenthaler, 1987; Porra et al., 1989; Yang et al., 1998). For the extraction, 0.5 g of fresh fruit pericarp was placed in a solution containing ethanol, acetone, and hexane in a ratio of 1.5:1.5:3 and transferred to a refrigerator until complete discoloration of the tissue, after about 24 h. After thorough homogenization of the extract, the mixture was allowed to stand for 15 min, and then 3 mL of the supernatant hexane was taken in which all the carotenoids were dissolved. The absorbance of the extract was measured at 450 nm and carotenoid concentration was calculated using the extinction coefficient (ε) of 1% = 2592 M^{-1} cm^{-1} (Rodriguez-Amaya, 1999).

2.4. Total phenols

Phenols were extracted from 0.3 g of fresh-weight (FW) tissue (fruit pericarp) in 80% methanol, assayed using the Folin–Ciocalteu reagent following standard methods and expressed as mg gallic acid equivalents (GAE g^{-1} FW), used for the standard curve with a range of 0–125 μM (Scalbert et al., 1989).

2.5. Total flavonoids

Flavonoids were extracted from 0.3 g of fresh pepper pericarp in 80% methanol and determined colorimetrically (Zhishen et al., 1999) with some modifications. Rutin was used as the standard compound for the quantification of total flavonoids. All values were expressed as mg of rutin g^{-1} FW. The reported data are the means of 5 replicates.

2.6. Antioxidant capacity

To estimate antioxidant capacity, two methods were used: ferric reducing antioxidant potential (FRAP) (Benzie and Strain, 1996) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand et al., 1995).

FRAP measures antioxidant capacity as reductants of the reduction of the Fe^{3+} 2,4,6-tripyridyl-s-triazine to a blue-colored Fe^{2+} complex at low pH, measured spectrophotometrically at 593 nm. The extracts were incubated at room temperature with the FRAP reagent and absorbance was recorded after 1 h. The reducing power is expressed as µmol ferrous sulfate ($FeSO_4$) g^{-1}. The DPPH method measures the radical scavenging activity of antioxidants against free radicals, such as the DPPH radical. Their activity was calculated based on the percentage of scavenged DPPH as follows: Scavenging activity (%) = [1 – (Absorbance of sample at 517 nm / Absorbance of control at 517 nm)] × 100. DPPH in fruits was also expressed in mM Asc. Acid g^{-1} FW.

2.7. Statistical design

The experimental layout was completely randomized and the data were analyzed with the method of ANOVA (2 or 3 factors) using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). For each treatment and biochemical analysis, five replicates were used. To compare the means, Duncan's multiple range test was applied at $P \leq 0.05$ to establish significant differences among the treatments. The significance of variability sources such as cultivar (cv), B, time (t), cv × B, cv × t, B × t, color, and B × color were calculated for carbohydrates, carotenoids, total phenolics content (TPC), total flavonoids content (TFC), FRAP, and DPPH values.

3. Results

3.1. Carbohydrates

A highly significant ($P \leq 0.001$) effect of cv, B, t, and the interaction cv × t was recorded. In addition, the interaction B × t was significant ($P \leq 0.05$). The cultivars with the highest carbohydrate concentration of fruit pericarp (Figure 1) were Solario, Osho, and Odysseo, whereas Arlequin had the lowest concentration. The maximum carbohydrate concentration at 40 days (Figure 1A) was 173 mmol g^{-1} FW and the minimum was 90 mmol g^{-1} FW. At 0.5 mg B L^{-1}, cultivars Solario, Osho, and Odysseo had similar carbohydrate concentrations, while the carbohydrate concentration in Arlequin was significantly lower compared to Odysseo. With 2.5 mg B L^{-1}, Solario, Osho, and Odysseo did not differ; however, Arlequin had significantly lower concentrations than Solario and Osho. With 5 mg B L^{-1}, Arlequin had a significantly lower carbohydrate concentration than Solario, while with 10 mg B L^{-1} Osho had a significantly greater concentration than Arlequin.

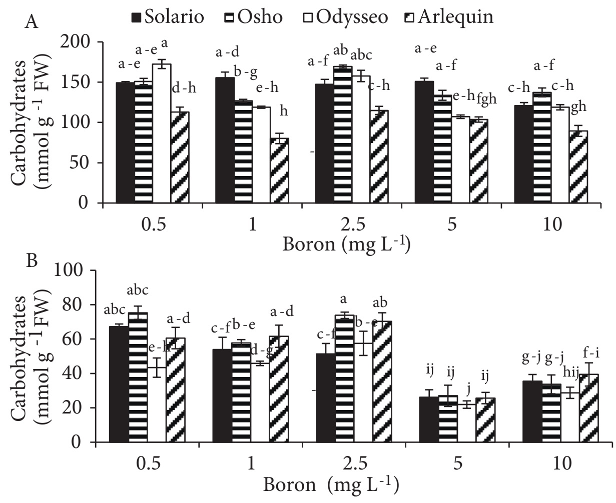

Figure 1. Concentration of carbohydrates (mmol g^{-1} FW) of fruits in four pepper (*Capsicum annuum* L.) cultivars at 5 B concentrations in the nutrient solution after 40 (A) and 70 (B) days of B treatment. Means with the same letter are not significantly different at $P \leq 0.05$ (Duncan's multiple range test, n = 5) in all figures.

In the second sampling (70 days), the carbohydrate concentration (Figure 1B) was 22–75 mmol g^{-1} FW in all the cultivars, while 5 and 10 mg B L^{-1} decreased the carbohydrate concentration by 2–3 times in comparison to 0.5–2.5 mg B L^{-1}. Therefore, the carbohydrate concentration was significantly reduced when the exposure time to B stress, and in particular at 5 and 10 mg B L^{-1}, was increased by 70%. The differences between the values at 40 days and 70 days were statistically significant (Table). A variation among the four tested cultivars was recorded concerning carbohydrate concentration, with Arlequin being affected more by B.

3.2. Carotenoids

The concentration of carotenoids was significantly affected by cv, B, t, and the interactions cv × B, cv × t, and cv × B × t ($P \leq 0.001$). The concentration of carotenoids in the four cultivars (Figures 2A and 2B) followed the descending order of Solario > Osho > Odysseo > Arlequin and in the first measurement (Figure 2A) varied from 58 to 6 µg g^{-1} FW. Cultivar Solario had the highest concentration of carotenoids at all B concentrations. In the second measurement (Figure 2B), the pepper fruits started to change color from green to yellow-red, and even though the carotenoid concentration increased, it varied from 66 to 37 µg g^{-1} FW. Among the cultivars, as in the first measurement, Solario had the highest carotenoid concentration. The differences between the values at 40 days and 70 days were statistically significant (Table).

Table. The $t_{0.05}$ values between the means at 40 and 70 days for carbohydrates, carotenoids, total flavonoids, total phenols, FRAP, and DPPH at 5 boron concentrations in 4 pepper cultivars (S = Solario, O = Osho, Od = Odysseo, A =Arlequin).

Phytonutrient, FRAP, and DPPH values	Boron concentration (mg B L^{-1})				
	0.5	1	2.5	5	10
Carbohydrates					
S	7.17	15.65	16.32	16.30	16.52
O	7.44	11.51	28.85	26.23	11.78
Od	18.87	14.37	7.73	5.43	9.14
A	5.67	5.01	6.96	14.80	5.14
Carotenoids					
S	3.07	12.09	3.274	6.78	4.65
O	18.09	16.17	18.44	34.61	19.76
Od	27.04	16.02	41.92	20.01	21.73
A	25.77	46.10	43.78	22.53	69.46
Total flavonoids					
S	2.30	1.29	0.68	1.35	2.61
O	2.63	2.68	1.37	4.47	5.30
Od	0.58	0.43	20.31	6.86	3.65
A	3.34	5.74	1.91	8.97	11.85
Total phenols					
S	2.35	2.31	21.85	30.39	10.08
O	12.29	4.63	15.32	6.77	25.63
Od	0.87	2.58	3.97	10.72	1.89
A	9.17	10.26	7.55	7.48	23.74
FRAP					
S	2.32	9.12	11.54	25.69	10.40
O	26.90	5.33	6.04	7.84	10.84
Od	1.55	3.32	4.78	13.51	8.68
A	6.81	9.33	17.50	5.67	29.03
DPPH					
S	10.62	7.28	17.10	5.77	10.46
O	3.94	2.79	9.50	5.50	13.85
Od	3.81	9.33	11.44	13.34	17.39
A	11.22	21.33	19.06	9.07	23.34

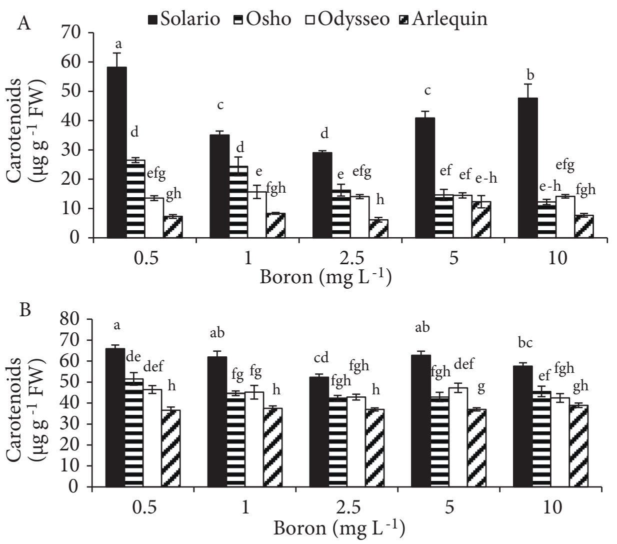

Figure 2. Concentration of total carotenoids (μg g^{-1} FW) of fruits in four pepper (*Capsicum annuum* L.) cultivars at 5 B concentrations in the nutrient solution after 40 (A) and 70 (B) days of B treatment.

3.3. Carotenoid and carbohydrate concentrations in green and red fruits of the cultivar Odysseo

The level of carotenoids in Odysseo was significantly affected by B, fruit color, and their interaction (P ≤ 0.001), while carbohydrates were significantly affected by B and fruit color (P ≤ 0.001). At 5 and 10 mg B L^{-1} (Figure 3A), carbohydrate concentration decreased. However, at all B concentrations, red fruits had the greatest carbohydrate concentration, which was 67%, 37%, 17%, 81%, and 58% greater in Odysseo red fruits than in Odysseo green fruits (Figures 3A and 3B), at 0.5, 1.0, 2.5, 5.0, and 10 mg B L^{-1}, respectively.

The concentration of carotenoids in green and red Odysseo fruits (Figure 3B) varied from 43 to 83 μg g^{-1} FW. These values were mostly not affected by B concentration in green fruits, whereas in the red fruits the higher B concentrations caused a decrease in carotenoid concentrations. The greatest carotenoid concentration of red fruits was recorded with 1 mg B L^{-1} while it decreased with 2.5, 5, and 10 mg B L^{-1}. Red fruits had 48%, 84%, 40%, 19%, and 16% greater carotenoid concentration with 0.5, 1, 2.5, 5, and 10 mg B L^{-1}, respectively. The concentration of carotenoids in red fruits as affected by B concentration in nutrient solution followed the order of 1 mg B > 0.5 mg B > 2.5 mg B = 5.0 mg B > 10 mg B. Generally, the findings showed that the carotenoid concentration in green Odysseo fruits was not affected when the levels of B were increased. However, they did rise in red Odysseo fruits.

3.4. Total flavonoids

Total flavonoid concentration was significantly affected by cv, t, cv × B, cv × t, B × t, and cv × B × t (P ≤ 0.001). In the first measurement, the concentration of flavonoids (Figure 4A) varied from 0.66 mg g^{-1} FW to 0.31 mg g^{-1} FW and was affected by both the cultivar and the B treatment. Hence, at 0.5 mg B L^{-1}, Solario had the highest flavonoid concentration, followed by, in descending order, cultivars Osho, Odysseo, and Arlequin. Increasing the B concentration in the solution to 1 mg L^{-1}, cultivars Solario, Osho, and Arlequin did not differ in total flavonoids,

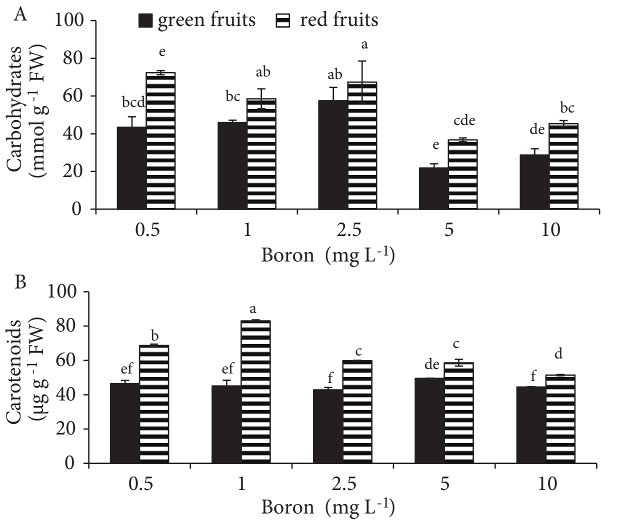

Figure 3. Concentration of carbohydrates (mmol g^{-1} FW) (A) and total carotenoids of green and red fruits (μg g^{-1} FW) (B) in cultivar Odysseo (*Capsicum annuum* L.) at 5 B concentrations in the nutrient solution at 70 days after the initiation of the experiment.

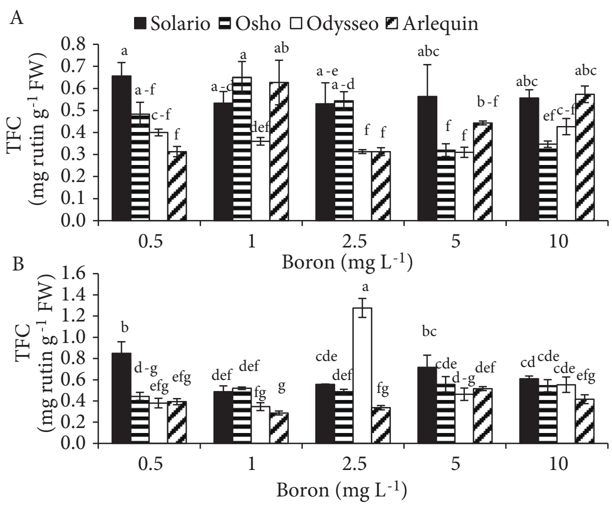

Figure 4. Concentration of total flavonoids (mg rutin g^{-1} FW) of fruits in four pepper cultivars (*Capsicum annuum* L.) at 5 B concentrations in the nutrient solution after 40 (A) and 70 (B) days of B treatment. TFC: Total flavonoid concentration.

whereas Odysseo had a significantly lower value than the other cultivars. At 2.5 mg B L^{-1} Solario and Osho had the greatest flavonoid concentrations, at 5 mg B L^{-1} Solario had a higher value than the other 3 cultivars, and at 10 mg B L^{-1} Solario and Arlequin had the highest flavonoid concentrations, while Osho and Odysseo had significantly lower values. Our data (Figure 4A) indicate that flavonoid concentration was a function of cultivar, and it appears that different B concentrations affected the flavonoid levels in the 4 cultivars differently.

In the second measurement (Figure 4B), at 0.5 mg B L^{-1} Solario had double the amount of flavonoid concentration in comparison to the other 3 cultivars. Flavonoid concentrations varied between 1.3 to 0.3 mg g^{-1} FW, meaning a 4× variation. The difference between the values at 40 days and 70 days were statistically significant (Table). With 1 mg B L^{-1}, Solario, Osho, and Odysseo did not differ in flavonoid concentration; however, Solario and Osho had significantly greater flavonoid concentrations than Arlequin. By increasing the B concentration to 2.5 mg B L^{-1} Odysseo had 3× greater flavonoid concentration compared to the other 3 cultivars. At 5 mg B L^{-1} Solario had the greatest flavonoid value in comparison to Odysseo and Arlequin. Finally, at 10 mg B L^{-1}, all the cultivars had low flavonoid concentrations, with Solario having a significantly higher difference compared to Arlequin.

3.5. Total phenols

Concerning total phenol concentration, the effect of cv, t, cv × t, and B × t was highly significant ($P \leq 0.001$); it was also significant for B and B × cv × t ($P \leq 0.01$), as well as cv × B ($P \leq 0.05$). In the first measurement (Figure 5A), at 0.5 mg B L^{-1} Solario had the highest phenol concentration, which was significantly greater (83%) than those of the other 3 cultivars. At 1 mg B L^{-1} again Solario had a greater phenol concentration compared to Odysseo and Arlequin, but it did not differ from Osho. At 2.5 mg B L^{-1} Solario and Osho had significantly greater values than Odysseo and Arlequin, of 60% and 128%, respectively. At 5 mg B L^{-1} Solario had a significantly greater value than Odysseo, but not Osho and Arlequin. Finally, at 10 mg B L^{-1}, the effect was similar in all the cultivars and phenol concentrations varied from 0.66 to 0.53 mg GAE g^{-1} FW. In the second measurement (Figure 5B), the cultivar with the highest phenol concentrations at 2.5 and 10 mg B L^{-1} was Solario, with Odysseo having the lowest. There was a significantly statistical difference in the comparison of the two sampling periods (Table). In summary, in most cases, Solario tended to have greater TPC than the other cultivars at all B levels.

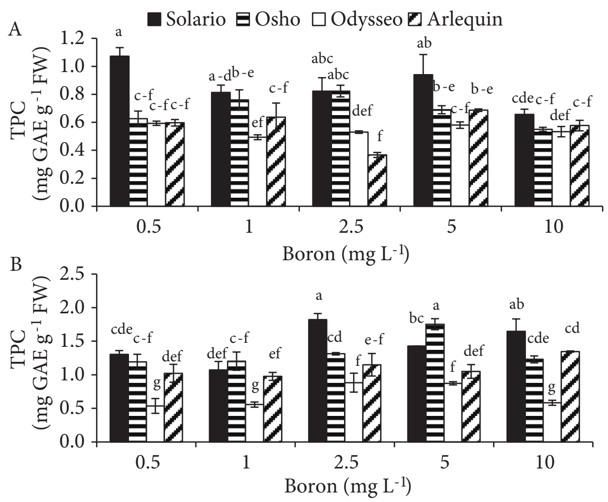

Figure 5. Concentration of total phenols (mg GAE g^{-1} FW) of fruits in four pepper cultivars (*Capsicum annuum* L.) at 5 B concentrations in the nutrient solution after 40 (A) and 70 (B) days of B treatment. TPC: Total phenolic concentration.

3.6. Effect of fruit ripening on phenol and flavonoid concentration and FRAP and DPPH values

Total phenol concentration was affected significantly ($P \leq 0.01$) only by fruit color. The effect of pepper maturation on phenol and flavonoid concentration is given in Figure 6. As can be seen in Figure 6A, B concentration and ripening did not affect phenol concentration. In green fruits, the level of phenol for all B concentrations was not significant. Ripening of red peppers increased the phenol concentration by 3 times_compared to green peppers. However, for all B concentrations, there was no statistically significant difference in phenol levels.

Total flavonoid concentration was affected significantly by B ($P \leq 0.001$) and fruit color ($P \leq 0.05$). The maximum

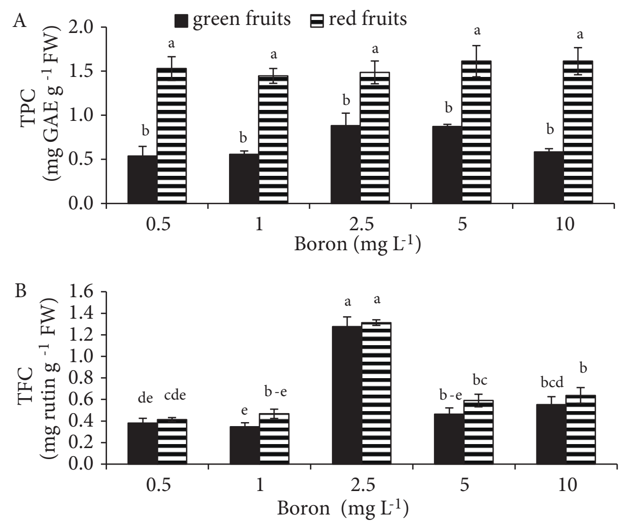

Figure 6. Concentration of total phenols (mg GAE g^{-1} FW) (A) and flavonoids (mg rutin g^{-1} FW) (B) of green and red fruits of cultivar Odysseo (*Capsicum annuum* L.) at 5 B concentrations in the nutrient solution at 70 days after the initiation of the experiment. TPC: Total phenol concentration; TFC: total flavonoid concentration.

flavonoid concentration was recorded at 2.5 mg B L^{-1}, which was 3 times greater than in the other B concentrations (Figure 6B).

The effects of B concentration and color on the FRAP and DPPH values ($P \leq 0.001$), and their interaction ($P \leq 0.01$), were significant. The FRAP value (Figure 7A) for the first four B concentrations was low in green fruits; however, at 10 mg B L^{-1}, it increased significantly (2–3 times). Ripening significantly increased the FRAP value by 6.45, 8.11, 5.13, 2.33, and 2.37 times at 0.5, 1.0, 2.5, 5.0, and 10 mg B L^{-1}, respectively. The highest FRAP increase was achieved with 1 mg B L^{-1}.

Concerning the DPPH value (% inhibition) (Figure 7B), there was no significant effect of B concentration in green peppers. It appears that in both red and green fruits the lowest DPPH value was recorded at 0.5 mg B L^{-1}. Boron concentration did not affect DPPH value (% inhibition) in green fruits, which varied from 10% to 16%, whereas in red fruits this variation was between 14% and 32%. Ripening doubled the level of the DPPH value. In red fruits B concentration affected the DPPH (% inhibition) value, and the values at 1, 2.5, 5, and 10 mg B L^{-1} were significantly greater in comparison to 0.5 mg B L^{-1}.

3.7. Total antioxidant capacity (FRAP) and DPPH value in the four pepper cultivars and 5 B concentrations

The effects of cv, t, cv × t, and B × t were highly significant ($P \leq 0.001$) while those for B, cv × B, and cv × B × t were significant ($P \leq 0.01$). Measurements were conducted in two periods (Figure 8). In the first period (Figure 8A), at 0.5 mg B L^{-1} Solario had the greatest FRAP value, which was 3 times greater than in the other cultivars. At 1 mg B L^{-1} there was no difference in FRAP value among Solario, Osho, and Arlequin; however, Odysseo had a significantly smaller FRAP value than Osho. At 2.5 mg B L^{-1} Osho had a significantly greater FRAP value than Solario and Odysseo but did not differ from Arlequin. At 5 mg B L^{-1} Arlequin had a greater FRAP value than Odysseo. Finally, at 10 mg B L^{-1} Osho had the lowest FRAP value, which differed significantly only from Solario.

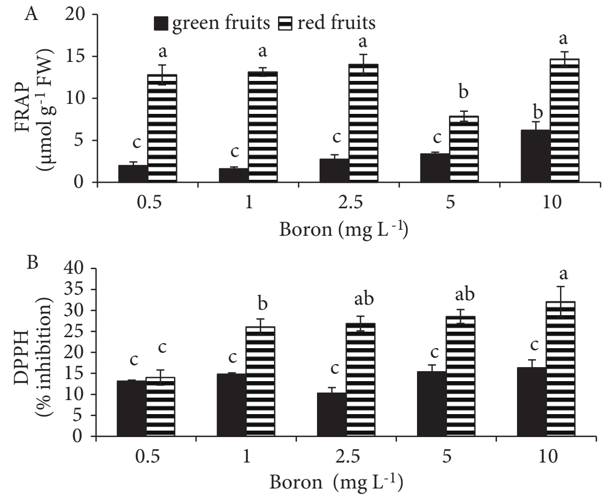

Figure 7. Total antioxidant activity FRAP (µmol g^{-1} FW) (A) and antioxidant activity DPPH (% inhibition) (B) of green and red fruits in cultivar Odysseo (*Capsicum annuum* L.) at 5 B concentrations in the nutrient solution at 70 days after the initiation of the experiment.

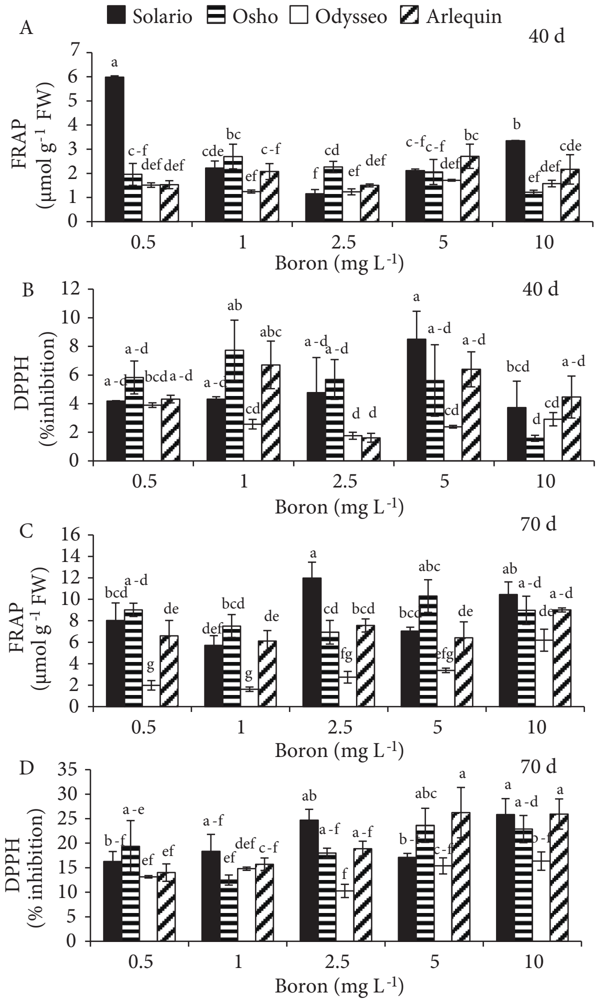

Figure 8. Total antioxidant activity (FRAP, DPPH) of fruits in four pepper cultivars at 5 B concentrations in the nutrient solution at 40 and 70 days after the initiation of the experiment: A) FRAP (µmol g^{-1} FW) at 40 days, B) DPPH (% inhibition) at 40 days, C) FRAP (µmol g^{-1} FW) at 70 days, D) DPPH (% inhibition) at 70 days.

In the case of DPPH (%), the effect was highly significant for cv, t, and B × t; it was also significant for B ($P \leq 0.01$) and B × cv × t ($P \leq 0.05$). At 0.5 mg B L^{-1} (Figure 8B) there were no differences between the cultivars. More specifically, at 1 mg B L^{-1} Osho had a significantly greater DPPH value than Odysseo, at 2.5 mg B L^{-1} Osho had a significantly greater value than Odysseo and Solario, at 5 mg B L^{-1} Odysseo showed the lowest DPPH value, and at 10 mg B L^{-1} there were no differences between the cultivars. In the second measurement (Figures 8C and 8D), the FRAP value (Figure 8C) was a function of cultivar and B concentration. The cultivar with the highest FRAP value (Figure 8C) was Solario at 2.5 mg B^{-1}, while Odysseo had the lowest FRAP value at 1 mg B L^{-1}. The DPPH value was mostly affected by all B concentrations. More specifically, at 2.5 mg B L^{-1} and 10 mg B L^{-1}, Solario had much greater values than the other cultivars. At 5 mg B L^{-1} Arlequin had 100% greater value in comparison to 0.5 mg B L^{-1} (Figure 8D). The comparison of carbohydrate concentrations between 40 days and 70 days (Table) shows there were significant differences in all the cultivars and B concentrations tested. The greatest $t_{0.05}$ values were recorded in cultivars Osho and Odysseo. The same applies to carotenoids, where the differences between 40 days and 70 days were significant for all the cultivars and B concentrations. Concerning flavonoids, the differences were not significant in Solario at 1, 2.5, and 5 mg B L^{-1}; in Osho at 2.5 mg B L^{-1}; in Odysseo at 0.5 and 1 mg B L^{-1}; and in Arlequin at 2.5 mg B L^{-1}. There were significant differences in phenols between 40 days and 70 days with the exception of Odysseo at 0.5 mg B L^{-1} and 10 mg B L^{-1}. Furthermore, the differences in FRAP and DPPH values were all significant except for the FRAP value at 0.5 mg B L^{-1} in Odysseo.

4. Discussion

All members of the genus *Capsicum* accumulate carotenoids in the pericarp with cultivar-specific abundance and composition (Guzman et al., 2010). The variations observed in Odysseo (*C. annuum* L.) were that red fruits had the highest and green fruits the lowest carotenoid concentrations. In addition, the accumulation of carotenoids in the other cultivars is affected by the ripening colors of the pepper fruits (Ha et al., 2007). Moreover, carotenoids accumulate in chloroplasts (Malkin and Niyogi, 2000) and all the cultivars in our experiments showed a relatively high carotenoid content. This is in agreement with a number of other studies that have also found red peppers to have significantly higher carotenoid content and antioxidant constituents than green ones (Deepa et al., 2007). There has been much research conducted on pungent peppers for their bioactive compounds and carotenoid content (Howard et al., 2000; Grayfeed et al., 2001; Topuz and Ozdemir, 2003), as well as a work on selected Turkish varieties (Topuz and Ozdemir, 2007) and red Lamuyo peppers (Guil-Guerrero et al., 2006). Therefore, pepper fruits are a good source of carotenoids, which might vary in concentration as a result of differences in the maturity stage (Conforti et al., 2007). Overall, carotenoid concentration in *C. annuum* L. cultivars has been extensively studied in order to select high carotenoid-producing cultivars (Hornero-Mendez et al., 2000).

Genetic and abiotic stresses, such as B, may be responsible for the overall phytochemical concentration in peppers. However, there are no data for commercial cultivars that are widely used, such as Solario, Osho, Odysseo, and Arlequin. The high or low carotenoid concentration for a given cultivar depends on various factors, such as the morphological and physiological characteristics of each cultivar, as well as certain growth factors. The carotenoid concentrations in the studied cultivars fall within the ranges reported in the literature (Hornero-Mendez et al., 2000; Russo and Howard, 2002). Out of the four cultivars in this study, Solario appears to be the most promising in terms of its high carotenoid concentration. Furthermore, the concentration of carotenoids and antioxidant content in the fruits of *C. annuum* L. varied significantly depending on soil treatments (Antonius et al., 2014) and soil nutritional factors, the stage of fruit growth, color (Ha et al., 2007; Guzman et al., 2010), and cultivar (Collera-Zuniga et al., 2005). In the present study, it was found that B affected carotenoid concentration. More specifically, a higher application of B may have the effect of decreasing the level of carotenoids in plants. In general, abiotic stresses like B toxicity generate reactive oxygen species in plant cells (Maoka et al., 2001).

Phenolic compounds are synthesized by plants as an adaptation to biotic and abiotic stress conditions. Their levels vary widely during growth and maturation, which are affected by agricultural practices, and they contribute to pungency, bitterness, flavor, and color (Perez-Lopez et al., 2007; Babou et al., 2016). It is well known that pepper fruits are a good source of phenolic compounds. These phytochemicals exhibit high antioxidant activity (Materska and Perucka, 2005) that has been linked to a reduced risk of chronic diseases. Plant maturity and fruit color are the major determinants of variation in phenolic content. In the present study, a large variability in phenol concentration in the fruits of the cultivars was observed. An increase in the total phenolic concentration in the peppers from the green to the red stage was reported (Howard et al., 2000). These concentrations were in the range of 33–250 mg GAE/100 g FW for different sweet and hot peppers (Alvarez-Parrilla et al., 2011). The red peppers of the cultivar Odysseo contained a greater level of phenolics than the green ones and showed higher scavenging activity. The genus *Capsicum* L. is a rich source of phenolics and, compared with other vegetables, ranks high in total phenolic concentration after broccoli, spinach, and onion (Chu et al., 2002). Apart from contributing to the taste and flavor of the fruit, to a certain degree (Serrano et al., 2010), phenolics also exhibit antioxidant and antiradical properties (Shetty, 2004), exerting a protective effect against lipid peroxidation (Oboh et al., 2007). Phenolic compounds are found in the pericarp, placenta, and seeds of the pepper fruit (Oboh et al., 2007). In the present study, cultivar Solario showed a significant increase in total phenolic concentration, especially at 0.5 mg B L^{-1} and 5 mg B L^{-1}. Earlier researchers also observed similar findings in the total phenolic content of different crops (Howard et al., 2000). Although in a previous study green peppers were found to have the highest activity in the phenolic extracts among three colored peppers (Zhang and Hamauzu, 2003), cultivar Odysseo in our experiments showed increased phenols in the red peppers rather than in the green, indicating a genotypic difference.

Flavonoids, a source of phytonutrients that are beneficial to human health, are produced by plants such as peppers. In the present study, total flavonoids were a function of cultivar, time of sampling, B concentration in solution, and maturity of pepper fruits. It was found that the maximum value of flavonoid concentration was greater after 70 days of B treatment in comparison to 40 days. In pepper fruits at 70 days, B toxicity reduced flavonoid concentration significantly in cultivars Arlequin, Century, Imperial, Salomon, and Blazer (unpublished data) and increased it in cultivar Odysseo, indicating a different genotypic response and a harvesting time-dependent variation (Bhanduri and Jung, 2013). This report on the high flavonoid content of Odysseo at 70 days is, to our knowledge, the first in the international literature. The high flavonoid content of Odysseo is associated with health benefits and an increase in antioxidant activity with the advance of ripening. Boron concentration seems to affect flavonoid levels. The greatest values were at 0.5 mg B L^{-1} in cultivar Solario and at 2.5 mg B L^{-1} in cultivar Odysseo (0.8–1.3 mg TFC g^{-1} FW). In our study, it appears that flavonoids play an important role in antioxidant activity. Pepper fruits are a natural source of antioxidants and as such have received considerable attention from researchers. In recent years, they have also attracted consumers, who have included peppers in their diet; being rich in bioactive compounds and vitamin C, they have contributed greatly in reducing the risk of life-threatening diseases (Materska and Perucka, 2005). Although we did not measure ascorbic acid content, boron application may increase its concentration in pepper cultivars, as was reported for radish (Maurya and Devi, 2016).

Our findings indicate that the type of cultivar and the level of B concentration have a marked effect on total antioxidant content. Therefore, the radical scavenging activity was from different antioxidants (Yasuor et al., 2015). Correlation analysis between the different phytonutrient contents (data not presented) and the antioxidant activity (FRAP) reveals that the compounds that participated most in antioxidant activity varied depending on cultivar and B concentration. Hence, in cultivar Osho, the participating compounds were carotenoids (r^2 = 0.71–0.93), phenols (r^2 = 0.69–0.90), and flavonoids (r^2 = 0.64–0.91). In Odysseo, the participating compounds were carotenoids (r^2 = 0.66–0.88), carbohydrates (r^2 = 0.46–0.64), phenols (r^2 = 0.77–0.95), and flavonoids (r^2 = 0.66–0.88). In Arlequin, they were carotenoids (r^2 = 0.62–0.83), carbohydrates (r^2 = 0.53–0.93), phenols (r^2 = 0.59–0.99), and flavonoids (r^2 = 0.68–0.99), while in Solario the participating compounds were carbohydrates (r^2 = 0.57–0.77), phenols (r^2 = 0.69–0.90), and flavonoids (r^2 = 0.64–0.91). FRAP reflects total antioxidant power involving the single-electron-transfer reaction, whereas DPPH is based on free radical scavenging activity (Ou et al., 2002). Antioxidant activity measured by DPPH radical scavenging ranged from 15% to 27% inhibition (Figures 8B and 8D) compared to 0.5 mg B L^{-1}. This variation may have been caused by differences in the concentration of reducing substances. Based on FRAP and free radical scavenging assays, cultivars Solario and Osho seem to be promising in some B concentrations, having high antioxidant activity. Recent studies have reported on the high antioxidant capacity of peppers, raising interest in this food plant (Perucka et al., 2010). Our findings show that boron applied to pepper plants modified antioxidant activity, although different cultivars seem to respond differently to B applications. Our results indicate that pepper fruits grown under B toxicity (10 mg B L^{-1}) will have lower antioxidant activity and will therefore be of lower nutritional value for human consumption, whereas moderate B treatment may significantly improve the beneficial nutritional properties of peppers. In summary, horticultural practices such as B supply may alter antioxidant activity in a specific cultivar, as well as the nutritional value of peppers. Peppers are an important crop due to their dietary value (Navarro et al., 2006), ranking first with higher total antioxidant activity than even broccoli or spinach (Chu et al., 2002). The antioxidant capacity in vegetables depends on the composition and concentration of individual bioactive compounds and their positive or negative interactions. It is, therefore, of great importance to study the phytochemicals present in pepper fruits in each commercial cultivar in order to obtain accurate and up-to-date information on their health benefits. Our results indicate that each phytonutrient has a unique pattern of accumulation during fruit growth. The differences between 40 days and 70 days of measurement indicate that the ripening of peppers is accompanied by a change in various phytonutrients and subsequent antioxidant activity. These data can be useful in predicting pepper functional values when plants are cultivated in B-rich soils, as well as providing information regarding the performance of different cultivars.

In conclusion, B toxicity significantly reduced the carbohydrate concentration of peppers. From the tested cultivars, Solario had the highest carotenoid and phenol concentration in relation to the other cultivars and constitutes a rich carotenoid and phenol source as a food ingredient. In this study we present novel data for agriculture linking phytochemicals and antioxidant activity of pepper fruits to B nutrition.

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