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Fruit Quality of Red Raspberry as Affected by Salinity

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Summary

The effect of increasing concentrations of NaCl in nutrient solution on the antioxidant capacity and other fruit quality attributes of raspberry (*Rubus idaeus* L.) was investigated. The plants were grown in perlite under protected cultivation and at pink fruit stage were supplied with Hoagland nutrient solution supplemented with 0, 10, 20, 30 and 60 mM NaCl for a period of 10 days, following another 10 d without the addition of NaCl. During application of NaCl, the antioxidant capacity, vitamin C content, total phenolics, soluble solids concentration, titratable acidity and dry matter content of fruits increased but the increase was not proportional to the salt concentration in the solution. During the post-application period these characteristics were reduced to the level measured in the non-saliniszed plants except for ascorbic acid content and total phenolics, which remained at significantly higher levels. Also antioxidant capacity was only reduced at the end of the post application period. The anthocyanin content was not influenced by the salinity treatments. Results suggest that salinity, especially at low levels, may enhance the production of antioxidant substances in raspberry fruits and therefore their potential health benefits.

Key words. antioxidants – NaCl – *Rubus idaeus* L. – soilless cultivation

Introduction

Red raspberries are highly consumable fruits rich in antioxidants with redox properties which allow them to act as reducing agents and hydrogen donors, and afford protection against active oxygen species (Ancos et al. 2000a; Deighton et al. 2002). The agents implicated in conferring health protective attributes include antioxidant compounds such as flavonoids (anthocyanins), other phenolic compounds, specific vitamins such as C and E, and carotenoids (Scalzo et al. 2004). Previous investigations have shown that the main contributors to the antioxidant capacity of raspberry are ellagitannins, anthocyanins and vitamin C (Mullen et al. 2002). Furthermore, there is a particular interest in these fruits because of increasing evidence of their anticarcinogenic effects as well as their protective role against cardiovascular and neurological disorders and oxidative stress disfunctions (Bolck 1992; Diplock 1995; Kalt et al. 1999; Anttonen and Karjalainen 2005).

The rapidly expanding interest in natural dietary components of foodstuffs possesing antioxidant activity has encouraged research targeted in maintaining or even enhancing their concentrations through cultivar development, postharvest storage, and food processing (Rommel and Wrolstad 1993a, b; Prior et al. 1998; Ancos et al. 2000a, b; hakkinen et al. 2000; Mikkonen et al. 2001; Zafrilla et al. 2001). Nevertheless, the available information regarding the influence of cultivation practices on health promoting properties of fresh fruits and vegetables is currently inadequate. Furthermore, there is an increasing interest in the cultivation of red raspberry in warmer climates, specifically in Southern Europe (Oliveira et al. 1996, 1999; Rosati et al. 1999; Neocleous et al. 2005a, b), where the salinity of the irrigation water is a major limiting factor in agricultural production. However, our knowledge regarding the impact of salinity on fruit quality and nutritional value of red raspberry is currently limited. Therefore, the objective of this study is to describe the responses of red raspberry to salinity with regard to soluble solids concentration, ascorbic acid, phenolics, anthocyanins and consequent antioxidant capacity (FRAP).

Materials and Methods

Plant material and growth conditions

The experiment was conducted at the Agricultural Research Institute of Cyprus (long. 32 °E, lat. 35 °N) during the period February–April 2006. Red raspberry plants (*Rubus idaeus* L. 'Autumn Bliss') were grown in a plastic greenhouse under soilless cultivation. The plants were planted in elevated beds (17×20×17 cm) (Ramat-Hashofet, Israel) with perlite (3.0–5.0 mm) as the planting media in 6 m rows. Rows were oriented east to west with a distance of 0.4 m between plants and 1.2 m between beds. Twelve primocanes per meter were allowed to grow. Insects and diseases were controlled using IPM practices and bumble-bees (*Bombus terrestris*) were

introduced at flowering for pollination (Bio-Bee, Israel). The average temperature was 22 °C, the mean relative humidity 51 % and the photosynthetically active radiation fluctuated from 550 to 925 μmol m^{-2} s^{-1} (Model LI-6200, LI-COR, Lincoln, Nebr., USA). The plants were pruned in order to facilitate a uniform fruiting and irrigated interchangeably with Hoagland nutrient solution (HOAGLAND and ARNON 1950) and water as needed to avoid any accumulation of salts in the substrate.

At ping fruit stage the plants were supplied with Hoagland nutrient solution containing 0, 10, 20, 30 and 60 mM NaCl for 10 days (application period), following another 10 days without the addition of NaCl (post-application period). The above salt concentrations corresponded to EC 1.57, 2.42, 3.40, 4.22 and 6.91 mS cm^{-1} respectively. The solution, including the added NaCl was applied to the plants at sufficient rates until drainage to facilitate two tasks: a) a constant build-up of NaCl salts in the rhizosphere, and b) replenishment of various elements in the root zone (data not shown). The concentrations of NaCl were arranged in a randomised complete block design with four replications. Each replication consisted of 15 sample canes. All the fruits from the sample canes were picked at a dark red stage (evenly matured) frozen in liquid N_2 and kept at −80 °C pending for further analysis. Fruits were picked at six sampling days: 4th, 7th, 10th day (NaCl application period) and 14th, 17th, 20th day (post-application period). A sample of 50 fruits per experimental treatment was used for the laboratory work.

Data were analyzed using the means procedure of SAS/ASSIST: analysis of variance (SAS Institute, Cary, NC-USA). Pearson's correlation coefficient was used to correlate FRAP results with anthocyanin, phenolic and ascorbic acid content (at $P \leq 0.001$).

Dry matter content

For the determination of dry matter, 10 g of berry sample were dried at 70 °C till a stable weight was obtained.

Ascorbic acid (vitamin C)

Frozen fruits after semi thawing at room temperature were ground by a homogeniser for 1 min. 0.5 g of the homogenized sample was added to 40 ml extracting solution (oxalic acid 1 %) and homogenized for 1 min and filtered through Watman (No.1) filter paper. The ascorbic acid content was measured with Rqflex plus (Merck) in combination with a standard solution of a known concentration of ascorbic acid (200 ppm). Ascorbic acid analytical test strips were provided by Merck (Darmstad, Germany). The test strip was inserted into the sample and transferred into the Rqflex-plus adapter for the measurement result.

Soluble solids concentration (S.S.C)

Soluble Solids Concentration (S.S.C) was determined in the homogenized sample using a GPR 11-37 refractometer (Index Instruments, Campridgeshire, England).

Titratable acidity

The titratable acidity was measured by mixing 2 g of the pulp and 50 ml distilled water with few drops of phenolphthalein indicator and titrating the mixture with 0.1 N NaOH. The titratable acidity was expressed as % citric acid equivalent to the quantity of NaOH used for the titration (RYAN and DUPANT 1973).

Total anthocyanins

For the determination of total anthocyanin content a slight modification of TORRE and BARRIT (1977) method was used. 1 g of homogenized sample was added to 50 ml of acidified ethanol and left overnight inside a refrigerator. After filtration (Watman No.1) the absorbance of the extract was determined at the maximum absorbance peak (537 nm) after scanning the samples for maximum absorbance wavelength. The absorbance values were expressed as unit's of optical density per gram fresh weight (units OD g^{-1} of FW).

Total phenolics and antioxidant capacity

Extraction. Fruit samples were freeze-dried, grounded and homogenized. Samples (1 g) were extracted with 10 ml of 50 % methanol acidified with 1.2 M HCl (VINSON et al. 2001). The mixture after shaking vigorously was stored at 4 °C for 48 h and at this time re-extracted twice. The supernatants were combined and the volume was brought up to 25 ml in a volumetric flask.

Total phenolics. The content of total phenolics was measured according to SCALBERT et al. (1989) with slight modifications. Briefly, 0.50 ml of diluted extract was mixed with 2.5 ml of 1:10 diluted Folin-Ciocalteu's phenol reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3, 75 g l^{-1}). After shaking vigorously, the mixture was incubated at 50 °C for 5 min, cooled and the absorbance measured at 760 nm. Gallic acid (G.A.) was used as standard and the results expressed as mg^{-1} of G.A. in Dry weight (DW). Triplicate determinations were made in each sample.

Antioxidant capacity. The antioxidant capacity was measured using FRAP assay (BENZIE and STRAIN 1996, 1999). Briefly FRAP reagent was freshly prepared to contain 0.3 M of sodium acetate at pH 3.6, 10 mM 2,4,6-tripyridyltriazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride corresponding to 10:1:1. A 50 μL of diluted extract was added to 3 ml of the FRAP reagent and mixed. After incubation for 4 min at 37 °C, absorbance at 593 nm was measured against a water $FRAP^{-1}$ blank. Ascorbic acid (A.A) was used as standard and the results were expressed as μmol g^{-1} in DW. Triplicate determinations were made on each replicate value.

Results and Discussion

Dry matter

The dry matter (%) content of the fruits sampled from salt-treated plants increased with salinity during the NaCl application period but the increase was not proportional to the salinity level, particularly after the first sampling day (Fig. 1). During the post-application period, the dry matter content was rapidly reduced to that measured in non-salinized plants (Fig. 1). The interaction salinity ×

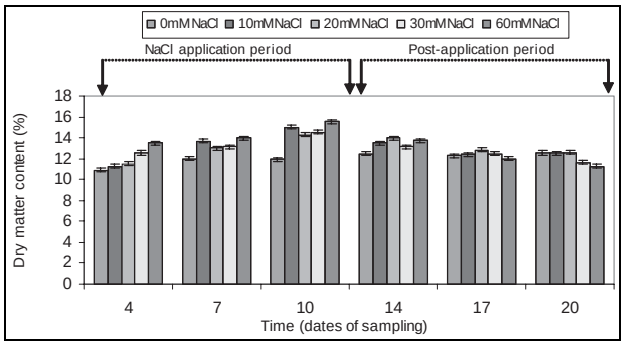


Fig. 1. The effect of salinity on dry matter content of raspberry fruits throughout the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

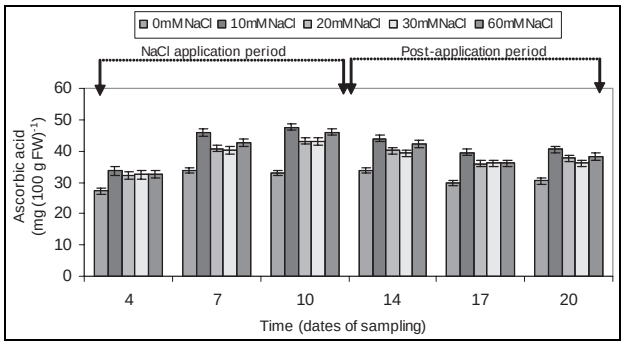


Fig. 2. The effect of salinity on ascorbic acid content (vitamin C) of raspberry fruits throughout the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

time was significant (Table 1). The highest increase was obtained at 10 mM NaCl (33 %) from the 4th to the 10th sampling day and the highest reduction at 60 mM (17.9 %) from the 17th to the 20th sampling day. These results show that salinity enhances the dry matter content in fruits but the increase is not analogous to the salinity level. In addition, the advantage is lost shortly after termination of the salt treatment. Similar results are reported in salt-treated tomato and strawberry plants (Adams and Ho 1989; Adams 1991; Awang et al. 1993; Petersen et al. 1998). The increase observed in fruit dry matter is probably due to a concentration effect depending on the reduced water content in the berries and probably associated with quality changes (Ho et al. 1987; Cuartero and Fernandez-Munoz 1999; Keutgen and Keutgen 2003).

Ascorbic acid (vitamin C)

Salinity had a marked effect on ascorbic acid content. Higher values compared to non-salinized control for all salinity concentrations were observed at all sampling dates both during the NaCl application and the post-application period (Fig. 2). However the rate of ascorbic acid increase was higher at low salinity (10 mM NaCl) and during NaCl application period. The interaction salinity × time was not significant (Table 1). Ascorbic acid in raspberries has been previously reported to range from 21.2 to 31.1 mg g^{-1} in FW (Ancos et al. 2000b). In the current study, the values determined in the control treatment were in that range whereas the salt treatments (10 mM NaCl) imposed an 1.7-fold increase. Similar results have been previously reported also for tomato (Adams 1991; Petersen et al. 1998; de Pascale et al. 2001). These results suggest that a mild salt stress in red raspberries can induce the biosynthesis of ascorbic acid (or inactivate its degradation) suggesting upregulation of the genes encoding for the enzymes involved in the key steps of the metabolic pathway.

Soluble solids concentration (S.S.C)

Soluble solids concentration increased with salinity; however this was not proportional to NaCl concentration in the solution and not consistent on all sampling dates. The fruits sampled during the NaCl application period showed a marked increase in S.S.C whereas during the post-application period the S.S.C declined to the control level (Fig. 3). The interaction salinity × time was significant (Table 1). The overall mean of S.S.C in fruits from non-salinized control in the current study was 9.13 % Brix. Similar values were found also by Ancos et al. (2000b) for the same variety in Spain (9.26 %). Noteworthy is that the salt treatments enhanced S.S.C up to 10.4–10.9 % and these values were obtained in the 10th day of sampling during NaCl application period (Fig. 3). Increased S.S.C in fruits sampled from salt-stressed plants

Table 1. Statistical significance of changes in content of dry matter, ascorbic acid, soluble solids, titratable acitidy, total anthocyanins, total phenolics and antioxidant capacity (FRAP) at different salinity concentrations[a] and exposure times[b].

Factor	F probability						
	Dry matter	Ascorbic acid	Soluble solids	Titratable acidity	Total anthocynanins	Total phenolics	Antioxidant capacity
Time	**	**	**	**	**	**	**
Salinity	**	**	**	**	ns	**	**
T × S	**	ns	**	**	ns	ns	ns

[a]Salinity concentrations: 0, 10, 20, 30 and 60 mM NaCl.
[b]Dates of sampling: 4th, 7th, 10th, 14th, 17th and 20th day.
Significance of F was as follows: ns = not significant, ** = significant at P≤0.001.

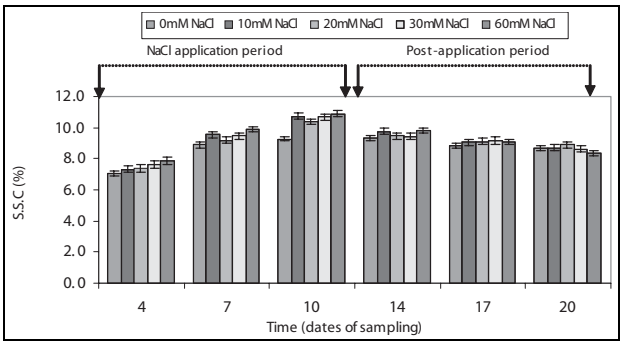


Fig. 3. The effect of salinity on soluble solids concentration (S.S.C) of raspberry fruits throughput the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

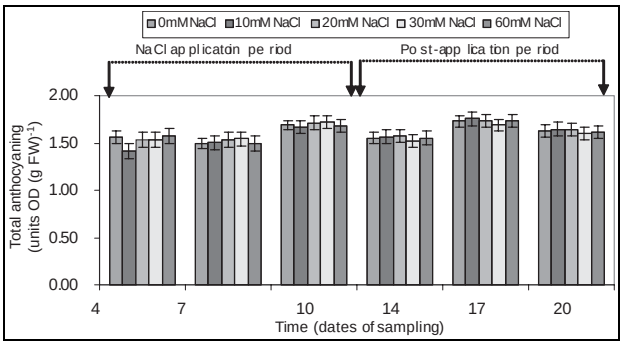


Fig. 5. The effect of salinity on total anthocyanins of raspberry fruits throughout the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

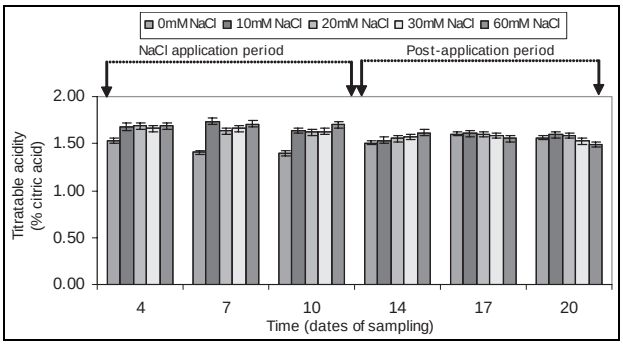


Fig. 4. The effect of salinity on titratable acidity of raspberry fruits throughout the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

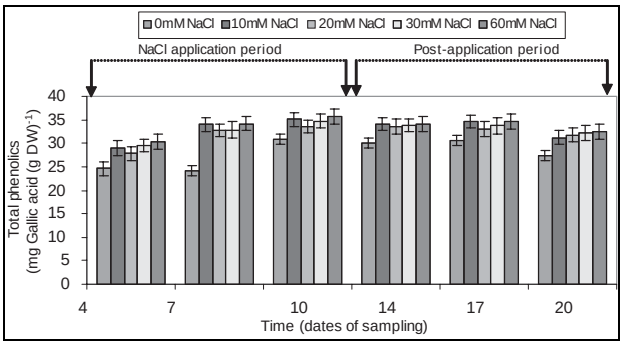


Fig. 6. The effect of salinity on total phenolics of raspberry fruits throughout the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

was most probably caused by a concentration effect and an active accumulation of solutes, mainly ions and organic molecules that are typically produced, aimed at osmotic adaptation in salt-stressed plants (HASEGAWA et al. 2000). Salinity-related increases of soluble solids and acid content of fruits has been observed also in other fruit and vegetable crops, including strawberries and tomato (MIZRAHI et al. 1988; ADAMS 1991), guava (WALKER et al. 1979), sweet pepper (JANSE 1989) and cucumber (CHARTZOULAKIS 1992).

Titratable acidity

Increased acidity, calculated as citric acid, in fruits of salt stressed plants during NaCl application period was also observed (Fig. 4). However, the increase was not proportional to the salt concentration in the solution and disappeared rapidly after termination of the NaCl-treatments (Fig. 4). The interaction between salinity and time was significant (Table 1). The highest values were obtained during the NaCl-application period. According to data reported by HAFFNER et al. (2002), nearly 90 % of the organic acids contained in the fruits of various raspberry cultivars was found to be citric acid; in absolute terms the concentration of citric acid in that study ranged from 17 to 23 mg g^{-1} of fresh fruit juice. In the current experiment, the mean values of acidity fell in the lower part of that range (Fig. 4), presumably due to environmental or genotypical differences. In accordance with our results, increased acidity in salt stressed vegetables crops has been reported for tomato (KAFKAFI et al. 1982; FEIGIN et al. 1987; MARTINEZ and CERDA 1989), cucumber (MARTINEZ and CERDA 1989), eggplant (SAVVAS and LENZ 1996) and melon (FEIGIN et al. 1987) and has been associated with enhanced concentrations of organic acids (DE PASCALE et al. 2001).

Total anthocyanins

The salinity treatments did not affect the total anthocyanin content in the raspberry fruits. This was true in all sampling days both during application and post application period (Fig. 5). Moreover, the interaction salinity × time was not significant (Table 1). It seems that in our study, the salt stress did not influence the genes encoding for the enzymes involved in anthocyanin biosynthesis. The red colour of the fruits is closely correlated with anthocyanin biosynthesis (ANCOS et al. 2000a) and therefore, maturity at harvest may has a major influence on total anthocyanin content (BARRIT and TORRE 1975; PRIOR et al. 1998; HAFFNER et al. 2002) and consequent antioxidant activity (LIU et al. 2002). MULLEN et al. (2002) have identified 11 anthocyanins in raspberries, of which cyanidin-3-sophoroside and cyanidin-3-glucoside have been characterized as major compounds (ANCOS et al. 1999). Although anthocyanins mainly contribute to the antioxi-

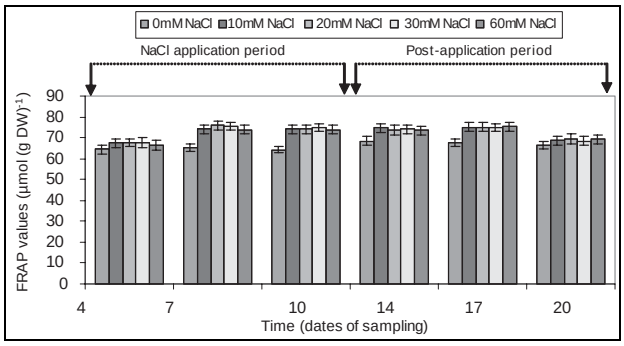


Fig. 7. The effect of salinity on antioxidant capacity (FRAP values) of raspberry fruits throughout the experimental period (NaCl application and post-application period). Bars represent ± SE values (n=6).

dant capacity (Mullen et al. 2002; Wang and Lin 2000) of raspberry fruit, other health benefits seem unclear (Anttonen and Karjalainen 2005). Liu et al. (2002) reported that other metabolites than anthocyanins were responsible for inhibition of tumour cells and play a major role in antiproliferative activity of raspberries.

Total phenolics

Salinity affected the concentration of phenolics in berries. The increase of total phenolic content in the fruits sampled from plants exposed to salinity was evident at all salt concentrations not only during the salt treatment but also during the post-treatment period (Fig. 6). However the increase was not proportional to the salt concentration level (Fig. 6). The interaction salinity × time was not significant (Table 1). The above referenced results suggest that even a mild salt stress can activate the genes encoding for the enzymes involved in the key steps of secondary metabolism, which is responsible for the biosynthesis of phenolic compounds. According to Sellappan et al. (2002) the concentration of phenolics in berries is influenced by several factors, including the growing season, variety, environmental and climatic conditions, plant diseases, soil type, geographic locations, and stage of maturity. In strawberries, higher temperatures (day and night) increased the flavonol and anthocyanin content (Wang and Zheng 2001). Furthermore, high levels of carbon dioxide in the air have also been found to lead to higher concentrations of phenolic compounds and antioxidant capacity in plants (Wang et al. 2003). The major class of phenolic compounds in berries of the *Rosaceae* family, namely, raspberry (*Rubus idaeus* L.), arctic bramble (*Rubus arcticum* L.), cloudberry (*Rubus chamaemorus* L.) and strawberry (*Fragaria* × *ananassa* Duch.), is represented by hydrolysable tannins (gallo- and ellagitannins). The anthocyanins form the second most abundant class in pigmented berries, while hydroxycinnamic acids, flavonols, flavon-3-ols and proanthocyanidins are the minor ones (Maatta-Riihinen et al. 2004). Moreover, ellagic acid, a dimeric derivative of gallic acid, corresponded to 88 % of the total phenolic content found in raspberry fruits (Hakkinen et al. 1999). There is a particular interest in the amount of ellagic acid in these fruits because of the increasing evidence of its anticarcinogenic and antioxidant effect (Ancos et al. 2000a) and its significant contribution to the antioxidant activity of raspberry (Mullen et al. 2002). Total phenolics, expressed as gallic acid, in raspberries have been previously reported to range from 11.4 to 21.1 mg g^{-1} in DW (Prior et al. 1998; Ancos et al. 2000a, b; Anttonen and Karjalainen 2005). In the current study, the values determined for total phenolics were even higher (24.6–33.7 mg g^{-1} in DW). These differences could be associated not only with the salt effect, but also with other factors that may influence the concentration of phenolics in berries, as mentioned earlier.

Antioxidant capacity (FRAP)

Fruits from salt-treated plants had a higher antioxidant capacity (FRAP) compared to that measured in fruits from the non-salinized control, in four out of six sampling dates (Fig. 7). In the first and the last sampling date, no significant differences in antioxidant capacity were observed. Additionally, no significant differences were found between the tested salinity levels both during the NaCl-application and the post-application period (Fig. 7). The interaction salinity × time was not significant (Table 1). A considerable body of data suggests that a higher content of total phenolics, flavonoids and anthocyanins in red raspberry fruits enhances their antioxidant activity (Wang and Lin 2000; Liu et al. 2002). Although vitamin C has been considered as an important antioxidant, it accounts only for 6 % of the total antioxidant activity in red raspberry (Anttonen and Karjalainen 2005) and the majority of antioxidant capacity may come from total phenolics and flavonoids (Wang et al. 1996; Liu et al. 2002). Stewart et al. (2001) reported that soluble antioxidant capacity in raspberry consists of vitamin C (15 %), anthocyanins (20 %), catechins (5–10 %) and the unknown fraction is probably phenolic and most likely glycosylated phenolics. In our experiment, the increase of antioxidant capacity imposed by salinity was presumably the result of raised vitamin C (correlation coefficient, R=0.76**) and total phenolics levels (correlation coefficient, R=0.85**) but not anthocyanins (correlation coefficient, $R=0.24^{ns}$). Hence, our results suggest that salinity enhances the levels of total phenolics in red raspberry fruits, and this is probably the major reason for increased antioxidant activity, with vitamin C being a secondary one.

Conclusion

In summary, our results suggest that the production of antioxidant substances and therefore the potential health benefits expected from the consumption of red raspberry fruits may be stimulated by a mild salt stress (10 mM NaCl). Salt stress may also improve other desirable characteristics (i.e. dry matter, acidity, soluble solids concentration). However, the favourable salinity effects may disappear after discontinuation of the treatment and more extensive measurements are needed to assess long-term potential hazards associated with irrigation using saline water.

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