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ScienceDirect
Food Chemistry 107 (2008) 1413–1420
Food Chemistry
www.elsevier.com/locate/foodchem

Quality and nutritional value of strawberry fruit under long term salt stress

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Received 12 August 2007; received in revised form 26 August 2007; accepted 27 September 2007

Abstract

Modifications of fruit quality, in response to a long-term salt stress of four months, were studied in two strawberry cultivars differing in their sensitivity to salinity. The sensitive cv. Elsanta and the less sensitive cv. Korona were treated during two vegetation seasons with 0, 40 or 80 mmol NaCl/l in the nutrient solution. While mean fruit weight decreased, dry matter and contents of total soluble carbohydrates, as well as sweetness index of fruits, remained constant. Salt stress in both cultivars increased the antioxidant capacity, antioxidants pools (ascorbic acid, anthocyanins, superoxide dismutase) and selected minerals such as Na^+, Cl^-, K^+, N, P and Zn^{2+}, as well as lipid peroxidation. Furthermore, salt stress increased the contents of free and essential amino acids, especially in cv. Elsanta. The more tolerant cv. Korona was characterized by an increase of reduced glutathione and a better fruit taste. In salt-stressed fruits of cv. Elsanta, taste was significantly impaired.

Keywords: *Fragaria x ananassa*; NaCl stress; Antioxidants; Mineral nutrients; Carbohydrates; Organic acids; Free amino acids; Genotype

1. Introduction

The application of salt and/or drought stress during fruit production has been proposed to limit vegetative growth and to improve fruit quality, especially sweetness (Awang, Atherton, & Taylor, 1993; Sato, Sakaguchi, Furukawa, & Ikeda, 2006). Sensory quality of fruits, such as tomato, grown in hydroponic systems and on dry land areas is characterized by elevated sugar and acid contents and these fruits are more attractive for consumers (Zushi, Matsuzoe, Yoshida, & Chikushi, 2005). Consumer preference of fruits is related to a superior taste, firmness, and flavour. Changes of these attributes, caused by salinity and drought, are mainly due to lower water contents in fruits. In addition, fruit yield and quality can be impaired. For example, a decrease in soluble solids and a lower acceptance of strawberry fruits by consumers were observed (Keutgen & Pawelzik, 2007b).

Salt stress is known to induce the formation of reactive oxygen species and of their scavengers, enzymes or non-enzymatic low molecular mass antioxidants. However, species- and cultivar-dependent differences exist. To date, only few researchers have studied the influence of salt stress on antioxidants in fruit, and most investigations have been on tomatoes. D'Amico et al. (2003) reported a higher antioxidative capacity of tomato fruits at moderate salt stress levels, which was related to carotinoids, lycopene and ascorbic acid. Keutgen and Pawelzik (2007a) showed that moderate salt stress increased levels of superoxide dismutase (SOD) and decreased contents of glutathione (GSH) and reduced ascorbic acid in strawberry fruits.

In a saline environment, ion homeostasis can be disturbed by excessive uptake of Na^+ and Cl^-. Competitions between these and further anions and cations are well documented and may result in a reduced plant growth and yield (De Pascale, Maggio, & Barbieri, 2005; Lopez & Satti, 1996; Sharpley, Meisinger, Power, & Suarez, 1992). The effects of salinity on micronutrient contents are often equivocal and depend on species or cultivar and plant organs (De Pascale

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doi:10.1016/j.foodchem.2007.09.071

et al., 2005; Mehrota, Khanna, & Agarwala, 1986; Rahman, Vance, & Munn, 1993). Little information on the distribution of macro- and micronutrients in strawberry plants under NaCl salinity has yet been published (Awang et al., 1993; Turhan & Eris, 2005). Thus, when applying moderate salinity levels for quality improvement, it is necessary to consider changes in the pool of mineral nutrients in order to avoid negative effects of the treatment.

Salt stress may also induce changes in the composition of N-containing compounds, especially of proteins and free amino acids (FAA). Most documented is the accumulation of proline (Pro), which is often used as an indicator of salt stress or of stress tolerance (Ashraf & Foolad, 2007). An increase of FAA is frequently mentioned as an indicator of tolerance to salinity. However, changes in amino acids may also be relevant for taste. For example, glutamate is correlated with taste and has been described as 'umami' or delicious taste (Sato et al., 2006). A bitter taste can be caused by larger amounts of phenylalanine (Phe) and tyrosine (Tyr). A sweet impression may result from larger contents of alanine (Ala) or lysine (Lys). Modifications due to salt stress may also determine the acceptance and palatability of fruit, especially in strawberry, which is rated as salt-sensitive (Awang et al., 1993). It has, therefore, been the aim of the present study to investigate the differences in the influence of moderate and elevated salinity stress on fruit quality in the salt-sensitive cv. Elsanta and the less-sensitive cv. Korona, when subjected to a long-term salt stress applied during two successive growing seasons, with special attention to bioactive compounds. The results are interpreted with respect to the contribution of strawberry fruit to human health, their nutritional value and consumer acceptance.

2. Material and methods

2.1. Plant material and growing conditions

Experiments were conducted during two successive vegetation periods in 2002–2003 and 2003–2004 in Göttingen, Germany, with strawberry (*Fragaria x ananassa* Duch.) cvs Elsanta (NaCl-sensitive) and Korona (less sensitive). A single experiment of the two replicates lasted for two continuous cultivation periods with long-term salt applications and only fruits harvested in the second season were used for analyses. Commercial strawberry plantlets ('Frigo', class A+) were purchased from Kraege Beerenobst Spezialkulturen, Telgte, Germany. 'Frigo' is a designation for runner plants that are taken out in mid-November and stored frozen without leaves during winter to initiate fruit setting the following year. At the first year, end of April, plantlets were selected for similar size, cultivated in 6 l metallic Mitscherlich containers filled with quartz sand (0.7–1.2 mm grain size), and located randomly (12 plants per m^2) with ten replications per combination to ensure a statistical design and to exclude position effects. Plants were grown in a greenhouse to avoid a dilution of salt applied to the plants by rainfall. Mineral requirements of strawberry plants were covered by application of 200 ml of modified Hoagland solution per plant, twice a week (Keutgen & Pawelzik, 2007a). Three weeks after planting, salt treatment started and 100 ml of solutions containing 0 (control, $EC_e = 0.0013$ dS/m), 40 (moderate, $EC_e = 3.9$ dS/m), or 80 mmol/l of NaCl (excessive, $EC_e = 7.5$ dS/m), were supplied four times a week to each plant. Once a week, 200 ml of demineralized water were supplied to all treatments. Surpluses of solutions were allowed to pass the containers to ensure salt stress in the root medium at a given concentration, but to avoid anoxia by water logging. All of the plants received extra water when needed to avoid an additional water stress. During the salt application, which lasted from mid May to mid September, the recorded mean temperature and humidity were 17 °C (max temp. = 22.7 °C; min temp. = 11.3 °C) and 77% relative humidity. During autumn and winter, the plants were kept in a non-heated green house.

For these experiments varying NaCl concentrations of solutions (nutrient solution with or without NaCl, Hoagland solution, demineralized water) were supplied to strawberry plants growing in a sandy medium. This experimental approach of varying NaCl concentrations in the root medium was chosen, because it temporarily enhanced uptake of Na^+ and Cl^- on the one hand, but allowed leaching the soil on the other. Leaching the soil should temporarily improve the water relations of plants, but not affect salt levels within plants. In contrast to earlier studies on salinity stress effects in strawberry, this approach enabled us to minimize water stress and to focus on salt-specific effects. To improve fruit quality, runners were removed immediately.

2.2. Sample preparation

Fruits were harvested in the second year of salt stress at the optimum of fruit maturity, when about 90% of the fruit surface had reached a full red colour. The fruits were divided into sepals and fruit flesh. Only fruit flesh was used for further investigations. Fruits were frozen in liquid nitrogen immediately after harvest and stored at −30 °C prior to further analyses of entire or freeze-dried fruits (Epsilon 2-40, Christ, Germany). Dry matter contents were recorded and dried fruits were milled to a fine powder.

Ascorbic acid, glutathione, malondialdehyde and protein contents, in addition to antioxidant enzyme activities, were quantified from entire fruits. Total phenols, anthocyanins, FRAP values, carbohydrates, organic acids, amino acids, and mineral nutrients were obtained from freeze-dried material. All of the results were expressed per fruit fresh mass (FM).

2.3. Antioxidants and lipid peroxidation

For the determination of ascorbic acid and glutathione, fruits were treated with 15% *meta*-phosphoric acid

(5 ml g^{-1} fruit mass) and centrifuged (20 min, 15,000*g* at 4 °C); the supernatants were used for analyses. Reduced ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were measured titrimetrically (Nakamura & Kurata, 1997).

Contents of total glutathione, measured as reduced glutathione (GSH) plus glutathione disulfide (GSSG), were determined, based on the reaction of GSH with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) in the presence of glutathione-reductase and NADPH. Concentrations of GSSG were measured after derivatization of GSSG with 2-vinyl pyridine to GSH (Anderson, 1985).

Lipid peroxidation of fruits was measured using the modified thiobarbituric acid method according to Botsoglou et al. (1994) with 175 mmol/l of NaCl-Tris-HCl-solution, 20% trichloroacetic acid, and 0.8% butyric hydroxytoluene-solution as buffer solution (5 ml g^{-1} fruit mass). After incubation of 2.5 ml of sample extract or malondialdehyde (MDA) standard, together with 1.5 ml of 0.8% thiobarbituric acid for 30 min at 95 °C, malondialdehyde (MDA) concentrations were measured photometrically at 532 and 600 nm with a Hewlett Packard Aglient 8453 UV/VIS spectrophotometer.

To distinguish between free and cell wall-associated soluble proteins and SOD activity, two phases were extracted (Keutgen & Pawelzik, 2007a). For the free compounds, 0.1 mol/l of potassium phosphate buffer at pH 6.0 (2.5 ml g^{-1} fruit mass) was used and, for cell wall-associated constituents, double extractions with 1 mol/l NaCl solution (5 ml g^{-1} sample) were performed. Concentrations of soluble proteins were measured using a Bio-Rad Protein Assay kit with bovine serum albumin. SOD activity (EC1.15.1.1) was determined using nitro blue tetrazolium (NBT) salt (Oberley & Spitz, 1985). One unit of SOD was defined as the amount of enzyme that yielded a 50% inhibition of the reduction of NBT. The activity of SOD was determined separately for each fraction.

For the determination of total anthocyanins, total phenolic compounds and antioxidative activity (FRAP), freeze-dried strawberry fruits (0.2 g) were extracted with methanol for 10 min at room temperature three times to the final volume of 10 ml and then were centrifuged for 10 min at 10,000 rpm min^{-1} and 4 °C. Total anthocyanins content was measured using the pH differential method (Giusti & Wrolstad, 2000). Results were expressed in mg equivalents of pelargonidin-3-glucoside. Total phenolic content was measured using the Folin–Ciocalteu method (Singleton & Rossi, 1965) and the results were expressed in mg gallic acid equivalents (GAE). The antioxidative activity was measured using the FRAP (ferric reducing antioxidant power) assay (Benzie & Strain, 1996). Results are expressed in mmol Fe.

2.4. Carbohydrates and sweetness index

For carbohydrate analyses, a water extract of dried fruits (0.1 g in 10 ml) was used. Soluble carbohydrates, as well as organic acids, were determined from the membrane-filtered supernatant (pores size 0.45 μm). Carbohydrates were separated using a LiChrospher 100 NH2 (5 μm) 4 × 4 mm pre-column in combination with a LiChrospher 100 NH2 (5 μm) 4 × 250 mm separation column (Merck KGaA, Darmstadt, Germany) and an acetonitrile:pure water solution (80:20 v/v) as mobile phase (flow rate 1.0 ml min^{-1}) at 20 °C and an injection volume of 20 μl. Carbohydrates were detected with a Knauer differential refractometer 198.00 (Knauer, Berlin, Germany). The sum of glucose, fructose, and sucrose was considered as a measure of total soluble carbohydrates.

The sweetness index of fruits, an estimate of total sweetness perception, was calculated, based on the amount and sweetness properties of individual carbohydrates in strawberry (Keutgen & Pawelzik, 2007b). The contribution of each carbohydrate was calculated, based on the fact that fructose is 2.30 and sucrose 1.35 times sweeter than glucose and, hence, the sweetness index was calculated as (1.00 [glucose]) + (2.30 [fructose]) + (1.35 [sucrose]).

2.5. Organic acids

Organic acids were determined as described by Keutgen and Pawelzik (2007b). Protonated organic acids (pH of eluent = 2.2) were separated by hydrophobic interactions with the apolar stationary phase of the reversed phase column (a pre-column LiChroCART 4-4, Purospher STAR RP-8e, 5 μm, and a guard column LiChroCART 250–3, Purospher STAR RP-8e, 5μm; Merck, Darmstadt, Germany) at a flow rate of 0.4 ml/min and a temperature of 22 °C. As isocratic solution, 18 mmol/l KH_2PO_4 water solution (pH 2.0) was used as eluent. The concentrations of organic acids were detected at 210 nm (injection volume 20 μl).

2.6. Mineral nutrients content

Total nitrogen content was determined using a CN 2000 – analyzer system (Leco, Mönchengladbach, Germany), as described by Sweeney and Rexroad (1987), where gaseous N_2 and nitric oxide NO_x from burned samples were measured. The contents of macronutrients (P, K, Ca, Mg), micronutrients (Fe, Mn, Zn, Cu, Cl) and of the trace element Na were assayed after digestion with HNO_3 and H_2O_2 in a MLS-1200 microwave laboratory system (MLS GmbH, Leutkirch/Allgäu, Germany). P and Cl were evaluated photometrically (Hewlett Packard Aglient 8453 UV/VIS spectrophotometer with multi-cell sampler), P according to the P-yellow method (Wilhelm, Tegge, & Witte, 1983), and Cl using the mercury thiocyanate method analogous to EPA 325.1 and US Standard Methods, respectively. Other ions were determined with a Unicam M-Series atomic absorption and emission spectrophotometer, with a FS 95 burning oven sampler and with a GF 95 burning oven graphite tube (Spectronic Unicam, Germany), according to the manual of Spectronic Unicam.

2.7. Free amino acids

Contents and composition of free amino acids (FAA) in strawberry fruits were determined after o-phthaldialdehyde (OPA) pre-column derivatization (Knauer, Berlin, Germany). FAA were extracted using 0.1 mol/l NaOH. Diluted derivatives of amino acids were separated by reversed-phase high-performance liquid chromatography (RP-HPLC), using a pre-column, LiChroCART 4-4 Purospher STAR RP-18 5 μm, in combination with a separation column, LiChroCART 250-3 Purospher STAR RP-18 5 μm (Merck, Darmstadt, Germany). The HPLC separation was done using 50 mM sodium acetate-buffer, pH 7.0 (solvent A), and methanol (solvent B). The solvent ratio A/B was 71/29 (v/v) at the beginning, followed by a linear gradient to 20/80 (v/v) over 25 min. Amino acid peaks were detected by a fluorescence detector at wavelengths of Ex 330/Em 450 nm and a flow rate of 0.65 ml min^{-1} at 35 °C (20 μl injection volume).

Free proline (Pro) content was measured after extraction of freeze-dried material (0.4 g) with 3% of sulfosalicylic acid in water (w/w) in order to precipitate protein amino acids (Steubing & Fangmeier, 1992). The supernatant was exposed to ninhydrin under acid conditions and the pink colour complex was monitored at a wavelength of 546 nm and 25 °C after extraction with toluene using a Hewlett Packard Aglient 8453 UV/VIS spectrophotometer with multi-cell sampler.

2.8. Statistical analyses

The obtained data were analyzed with the SPSS 14.0 statistical programme (SPSS Inc.). All data sets were tested for normal distribution and variance homogeneity ($p \leqslant = 0.05$). In case of homogenous sample variances, calculated means were compared by Duncan (^{D}), and in the case of non-homogenous variances by Tamhane (^{T}) test ($p \leqslant 0.05$). Correlation and multiple regression procedures between normally distributed quality parameters were performed using Pearson-correlation coefficients.

3. Results and discussion

3.1. Yield

NaCl salinity reduced mean fresh weight of strawberry fruits, especially of the salt-sensitive cv. Elsanta. Control plants of cv. Elsanta were characterized by larger fruits (Table 1). At the elevated salt stress level of 80 mmol NaCl/l, fresh weight reductions were 26% and 46% for Korona and Elsanta, respectively. Fruit size reduction under a saline environment is explained by inhibition of water uptake and reduced water transport to the fruit (Sato et al., 2006), which could also be observed in the present experiment. However, an increase of fruit dry matter content was not observed (data not shown), indicating that carbohydrate transport to fruits was also reduced. Fruits of cv. Korona accumulated dry matter of about 120 g, and those of cv. Elsanta, 121 g kg^{-1} FM, respectively.

3.2. Antioxidants and lipid peroxidation

Antioxidants are involved in scavenging of stress-induced reactive oxygen species, such as superoxide, hydrogen peroxide, and hydroxyl radicals. Previous research has established a relationship between salt stress and antioxidant levels (Keutgen & Pawelzik, 2007a); elevated levels of AA, glutathione and enzymes, such as SOD, were found (Pirker et al., 2002). Long-term salt stress, over two vegetation seasons, altered the composition of antioxidants in strawberry fruits (Table 1), but these changes cannot be explained by lower fruit water contents.

A lower susceptibility to salt stress was associated with higher glutathione contents in strawberry fruit. Total glutathione increased sharply in fruits of cv. Korona from 247 mg 100 g^{-1} FM in control plants to 461 mg 100 g^{-1} FM (Table 1). In contrast, in fruits of cv. Elsanta, salt stress caused significant decrease at 80 mmol NaCl/l compared to controls. Changes in the glutathione accumulation were first due to alterations of GSH (Table 1). Levels of GSSG remained fairly constant in both cultivars. A higher contribution of reduced glutathione to total glutathione of over 60% is important for antioxidative defence. GSH recycles ascorbate in the Haliwell-Asada cycle and scavenges oxygen radicals. The ratio GSH/GSSG (Table 1) reflects the differences in the turnover rates of glutathione; thus salt tolerance may be related to a higher GSH/GSSG ratio. A higher ratio could be a result of a higher glutathione reductase activity, which was observed in other plant species under salinity (Keutgen & Pawelzik, 2007a; Gossett, Banks, Millhollon, & Lucas, 1996). An elevated GSH/GSSG ratio was also observed in fruits of cv. Korona exposed to salt stress while, in cv. Elsanta, the ratio remained fairly constant. According to Gossett et al. (1996), synthesis of GSH, induced by an oxidative stimulus, plays a crucial role in determining the susceptibility of cells to oxidative stress.

Fruits of cv. Elsanta were characterized by higher contents of total ascorbic acid (TAA) than those of cv. Korona with 67 mg and 37 mg 100 g^{-1} FM, respectively, and TAA contents were not affected by salinity (Table 1). These results are in line with previous studies (Hakala, Lapveteläinen, Huopalahti, Kallio, & Tahvonen, 2003). The content of dehydroascorbic acid (DHAA) increased significantly in the salt-sensitive cv. Elsanta but only in trend in cv. Korona. Thus, the redox state of TAA, defined as the ratio AA/TAA, decreased considerably, especially in cv. Elsanta (Table 1). This may indicate a higher regeneration potential of ascorbate and a lower sensitivity to salinity of cv. Korona. By contrast, Keutgen and Pawelzik (2007a) reported a stepwise decrease of TAA content, especially in cv. Elsanta. The different response compared to the present result is explained by the fact that these plants were exposed to NaCl for one vegetation period, only. This may indicate a better adaptation of older plants to salinity.

Table 1
Fruit size and fruit antioxidant parameters of strawberry cvs Korona and Elsanta differing in their sensitivity to NaCl

	Korona (less salt-sensitive)			Elsanta (salt-sensitive)		
	Control	40 mmol/l NaCl	80 mmol/l NaCl	Control	40 mmol/l NaCl	80 mmol/l NaCl
Mean fruit weight [g]^{T}	6.37 ± 1.13 b	5.86 ± 1.31 bc	4.69 ± 1.26 c	10.34 ± 2.50 a	8.14 ± 1.36 ab	5.54 ± 1.28 bc
GSH + GSSG [mg]^{T}	0.25 ± 0.08 b	0.31 ± 0.04 b	0.46 ± 0.10 a	0.20 ± 0.07 bc	0.30 ± 0.1 b	0.13 ± 0.03 c
GSH/GSSG [rel. units]^{T}	1.52 ± 0.52 bc	2.11 ± 0.97 ab	3.37 ± 1.34 a	2.20 ± 0.83 bc	2.50 ± 0.90 ab	1.86 ± 0.28 c
TAA [mg]^{T}	41.0 ± 3.44 bc	37.8 ± 6.59 c	33.0 ± 4.00 c	66.2 ± 6.16 a	66.5 ± 14.5 ab	68.3 ± 1.70 a
AA/TAA [rel. units]^{T}	0.69 ± 0.01 a	0.67 ± 0.03 ab	0.61 ± 0.05 c	0.70 ± 0.01 a	0.66 ± 0.01 b	0.65 ± 0.01 b
FRAP – value [mmol Fe]^{T}	4.66 ± 0.74 b	6.47 ± 0.95 a	7.60 ± 1.62 a	4.65 ± 1.06 b	6.44 ± 1.70 a	7.62 ± 2.06 a
Anthocyanins [mg]^{T}	39.2 ± 9.34 b	63.1 ± 9.49 a	65.4 ± 18.9 a	22.8 ± 3.46 c	44.4 ± 16.3 b	40.1 ± 5.61 b
Phenolics [mg]^{T}	276.95 ± 42.93 ab	278.86 ± 32.60 a	281.55 ± 39.48 a	236.65 ± 19.46 b	280.77 ± 28.33 a	278.12 ± 9.91 a
MDA [mmol]^{D}	5.28 ± 1.29 bc	5.99 ± 1.18 ab	7.14 ± 1.68 a	4.56 ± 0.97 c	5.33 ± 1.59 bc	6.78 ± 1.21 ab
Free + CW SOD [mg]^{D}	36.1 ± 6.51 c	49.9 ± 4.59 b	58.9 ± 11.9 ab	34.0 ± 5.59 c	54.5± 6.96 ab	61.8 ± 12.0 a
Free SOD [mg]^{D}	29.1 ± 6.51 b	40.1 ± 4.48 a	43.3 ± 11.7 a	25.6 ± 4.59 b	38.9 ± 7.30 a	44.6 ± 10.5 a
CW SOD [mg]^{D}	6.95 ± 0.54 c	9.79 ± 2.71 b	15.6 ± 1.08 a	8.43 ± 2.11 bc	15.6 ± 1.26 a	17.2 ± 1.53 a
Free + CW sol. protein [mg]^{D}	97.8 ± 4.38 c	112 ± 3.22 b	122 ± 1.43 a	93.5 ± 5.39 c	112 ± 6.69 b	114 ± 6.81 b
Free sol. protein [mg]^{D}	74.7 ± 4.09 cd	84.5± 2.54 ab	89.5 ± 0.60 a	68.9 ± 2.71 d	79.6 ± 6.83 bc	80.9 ± 6.94 bc
CW sol. protein [mg]^{D}	23.1 ± 1.05 b	27.9 ± 1.46 ab	32.3 ± 0.85 a	24.6 ± 3.94 b	32.0 ± 4.31 a	32.6 ± 4.38 a

Data are expressed per 100 g fruit fresh mass. Different letters within rows indicate significant differences by Duncan (^{D}) or Tamhane (^{T}) tests at $p \leqslant 0.05$. (GSH: reduced glutathione; GSSG: oxidized glutathione; TAA: total ascorbic acid; AA: reduced ascorbic acid; FRAP: antioxidant capacity; MDA: malondialdehyde; CW: cell wall-associated; SOD: superoxide dismutase; sol.: soluble).

Investigated strawberry fruits were characterized by a high antioxidant activity, measured as FRAP value, and values corresponded well with those reported for different strawberry cultivars (Pellegrini et al., 2003). Salinity significantly enhanced the antioxidant activity of fruits up to 64% (Table 1).

A higher accumulation of anthocyanins and their contribution to total phenolic content plays an important role in the coloration of fruits. It results in a bright red colour, as well as better acceptance by consumers. Salt stress increased the contents of anthocyanins in fruits of both cvs and the highest increase of 94% occurred in cv. Elsanta at 40 mmol NaCl/l (Table 1). Not only the contents of anthocyanins, but also their contribution to total phenolics increased with increasing salinity from 14% to 23% in cv. Korona and from 10% to up to 16% in cv. Elsanta. Cv. Korona was characterized by higher amounts of anthocyanins than cv. Elsanta.

Lipids of bio-membranes are susceptible to oxidation processes. The accumulation of their oxidation products, lipid peroxides, induces functional disturbances and pathological changes. Thus, increased levels of lipid peroxidation products are typical of abiotic, as well as of biotic, stress (Sairam, Rao, & Srivastava, 2002). When subjected to salt stress, fruits showed higher lipid peroxidation activity, measured as MDA (Table 1). Especially in the salt-sensitive cv. Elsanta, a higher accumulation of MDA was recorded; however, visible damage was not observed.

Salt stress rose the content of soluble proteins, as well as of SOD, which increased, not only in the free, but also in the cell wall-associated fraction (Table 1). Total protein contents increased by more than 20% in both cvs compared to controls and synthesis of SOD rose under NaCl salinity up to 63% and 82%, in cvs Korona and Elsanta, respectively. The increases in SOD activity and in content of soluble proteins both indicate oxidative stress and they are considered beneficial for salt tolerance. For instance, SOD is known to scavenge H_2O_2 (Gossett et al., 1996; Sairam et al., 2002).

3.3. Carbohydrates and organic acids

Increases of osmotically-active substances, such as soluble sugars and organic acids, are interpreted as active osmotic adjustment. At the same time, sugars and organic acids are key components of strawberry fruit flavour and taste. Organic acids are involved in the regulation of the pH value of the cell-sap, and stabilization of anthocyanins and, therefore, influence fruit colour. In strawberry fruit, soluble carbohydrates are the main components of dry matter and range from 8–10% of FM (Keutgen & Pawelzik, 2007b; Cordenunsi, Nascimento, & Lajolo, 2003). The major organic acid is citric acid, accounting for about 1% FM in control fruits of both cultivars, and the detected levels of total organic acids, as well as of citric acid, were higher than those recorded by Cordenunsi et al. (2003).

In the present experiments, cultivars differed in their contents of soluble carbohydrates (fructose, glucose, sucrose) and of organic acids, such as citric acid, when exposed to salinity (Table 2). In the less salt-sensitive cv. Korona, a decrease of fructose and sucrose content due to salt stress was observed, while the concentrations of glucose and total organic acids remained fairly constant. In the salt-sensitive cv. Elsanta, a considerable (up to 54%) decrease of sucrose was detected at the highest salt stress level (Table 2), while the contents of hexoses and of total organic acids, especially citric acid, increased significantly. Gallic acid rose in both cultivars due to salt stress. The increase of soluble solids in strawberry fruit as a result of restricted vegetative growth and shift of photoassimilates

Table 2
Contents of sugars, organic acids and mineral nutrients in fruits of strawberry cvs Korona and Elsanta differing in their sensitivity to NaCl

Quality parameter	Korona (less salt-sensitive)			Elsanta (salt-sensitive)		
	Control	40 mmol/l	80 mmol/l	Control	40 mmol/l	80 mmol/l
Fructose [g]	4.00 ± 0.37 c	3.52 ± 0.37 d	3.67 ± 0.34 d	4.24 ± 0.17 bc	4.52 ± 0.32 ab	4.63 ± 0.13 a
Glucose [g]	3.61 ± 0.29 bc	3.06 ± 0.48 c	3.40 ± 0.86 abc	3.87 ± 0.17 b	4.00 ± 0.49 ab	4.38 ± 0.11 a
Sucrose [g]	1.97 ± 0.15 a	1.51 ± 0.47 abc	1.28 ± 0.35 bc	1.62 ± 0.22 b	0.88 ± 0.26 cd	0.74 ± 0.05 d
TOA [g]	1.40 ± 0.10 c	1.44 ± 0.08 c	1.59 ± 0.20 bc	1.46 ± 0.17 c	1.85 ± 0.26 b	2.21 ± 0.10 a
Citric acid [g]	1.02 ± 0.13 c	1.13 ± 0.13 bc	1.26± 0.26 abc	0.95 ± 0.12 c	1.29 ± 0.13 b	1.44 ± 0.09 a
Gallic acid [mg]	0.99 ± 0.17 b	1.16± 0.16 b	1.58 ± 0.24 a	0.85 ± 0.026 b	1.34 ± 0.50 ab	1.65 ± 0.28 a
TSC/TOA [relative units]	6.81 ± 0.39 a	5.65 ± 0.78 a	5.31 ± 1.41 ab	6.79 ± 0.81 a	5.45 ± 1.08 ab	4.43 ± 0.20 b
Na^+ [mg]	1.53 ± 0.36 d	90.7 ± 7.43 c	194 ± 12.9 b	1.79 ± 0.15 d	211 ± 18.9 ab	229 ± 20.9 a
Cl^- [mg]	3.20 ± 1.80 b	20.9 ± 12.5 a	28.8 ± 9.92 a	4.12 ± 0.26 b	27.3 ± 5.05 b	32.6 ± 8.28 a
K^+ [g]	0.21 ± 0.04 b	0.27 ± 0.07 b	0.26 ± 0.05 b	0.25 ± 0.11 b	0.25 ± 0.13 ab	0.37 ± 0.05 a
P [g]	0.04 ± 0.01 c	0.05 ± 0.01 bc	0.05 ± 0.00 b	0.04 ± 0.01 bc	0.05 ± 0.01 b	0.06 ± 0.01 a
N [g]	0.12 ± 0.00 d	0.14 ± 0.01 c	0.21 ± 0.02 a	0.12 ± 0.01 d	0.18 ± 0.03 b	0.23 ± 0.01 a
Zn^{2+} [mg]	0.18 ± 0.03 b	0.24 ± 0.08 ab	0.26 ± 0.08 ab	0.09 ± 0.07 c	0.21 ± 0.03 ab	0.28 ± 0.09 a
Na^+/K^+ [relative units]	0.01 ± 0.00 c	0.35 ± 0.09 b	0.76 ± 0.13 a	0.01 ± 0.00 c	1.40 ± 1.25 ab	0.62 ± 0.10 a
Total amino acids [mg]	83.1 ± 28.6 c	89.4 ± 12.7 c	164 ± 31.7 b	148 ± 20.5 b	233 ± 63.9 ab	341 ± 11.1 a
Essential amino acids [mg]	2.39 ± 0.81 c	2.94 ± 1.04 bc	4.09 ± 0.44 b	4.20 ± 0.62 b	6.20 ± 2.22 b	11.6 ± 1.05 a

Data are expressed per 100 g of fruit fresh mass. Different letters within rows indicate significant differences by Tamhane test at $p \leqslant 0.05$ (TOA: total organic acids; TSC: total soluble carbohydrates).

to fruits, observed by Awang et al. (1993) in cv. Rapella, could not be confirmed in the present experiment.

Differences among treatments and cultivars were not detected in the case of total soluble carbohydrates and sweetness index (data not shown). Sweetness is one of the important taste parameters characterizing the acceptance of strawberry fruits by consumers. However, taste is also related to acids and volatile compounds (Cordenunsi et al., 2003). The ratio of total soluble carbohydrates (TSC) to total organic acids (TOA) is a useful tool for describing strawberry flavour and consumer acceptance (Keutgen & Pawelzik, 2007b). It should be larger than 5.3 for strawberry of good quality (Cordenunsi et al., 2003). Although the content of TOA is relatively high (>1.4% of FM) in the investigated fruits, the ratio, TSC to TOA, of control fruits of both cvs of 6.8 is larger than the value quoted in the literature for good quality strawberry fruit (Table 2). In the less salt-sensitive cv. Korona, an influence of salinity on the TSC/TOA ratio could not be detected while, in the salt-sensitive cv. Elsanta, a decrease of the ratio was observed, which could be attributed to larger amounts of citric acid at the highest salt stress level (Table 2). These fruits were not of the best quality for consumption.

3.4. Mineral nutrients

Application of salt stress caused a significant increase of Na^+ and Cl^- ions in fruits of both cultivars (Table 2). Fruits accumulated more Na^+ than Cl^-, especially those of cv. Elsanta. Accumulation of Na^+ and/or Cl^- is a major cause of the often detrimental effects of salinity, namely reduced growth and ion imbalances. However, plants delay the accumulation of toxic ions in reproductive organs such as fruits (De Pascale et al., 2005). Also, fruit contents of N and P, as well as the Na^+/K^+ ratio, rose under salinity (Table 2) and modified accumulation, transport and partitioning of N and P. Differences between strawberry cultivars were found in the accumulation of K^+ and Zn^{2+}. In berry fruits, K^+ improves visual and turgor maintenance, but may reduce fruit size. In the present experiment, K^+ content remained unaffected in the less sensitive cv. Korona or rose in the sensitive cv. Elsanta. This implicates an effective K^+ uptake system in these cultivars. By contrast, Hasegawa, Bressan, Zhu, and Bohnert (2000) reported that, in several plants, accumulation of K^+ is limited under salinity. With respect to Zn^{2+}, an increase in the salt-sensitive cv. Elsanta was found (Table 2). In summary, nutrient deficiency in strawberry fruits did not occur.

3.5. Amino acids

The accumulation of N-containing compounds, especially that of free amino acids (FAA) such as proline (Pro), is known to participate in osmotic adjustment (Ashraf & Foolad, 2007). Under NaCl salinity, concentrations of total free amino acids, as well as of free essential amino acids (threonine, valine, isoleucine, leucine, and phenylalanine), rose in fruits of both strawberry cvs, but most in cv. Elsanta (Table 2). An increase of Pro, aspartate (Asp) and asparagine (Asn) was observed in both cvs while, in the sensitive cv. Elsanta, glutamate and glutamine (Gln) rose as well (Fig. 1). According to Sato et al. (2006), the increase of amino acids such as Asp, Asn and Gln under salinity is a result of reduced water transport or represents an active physiological response to a decreasing water potential. In strawberry fruit, the second explanation is probable, because water content of fruits remained stable. Levels of Pro have been shown, in several plant species, to increase under salt or water stress as a result of inhibited Pro oxida-

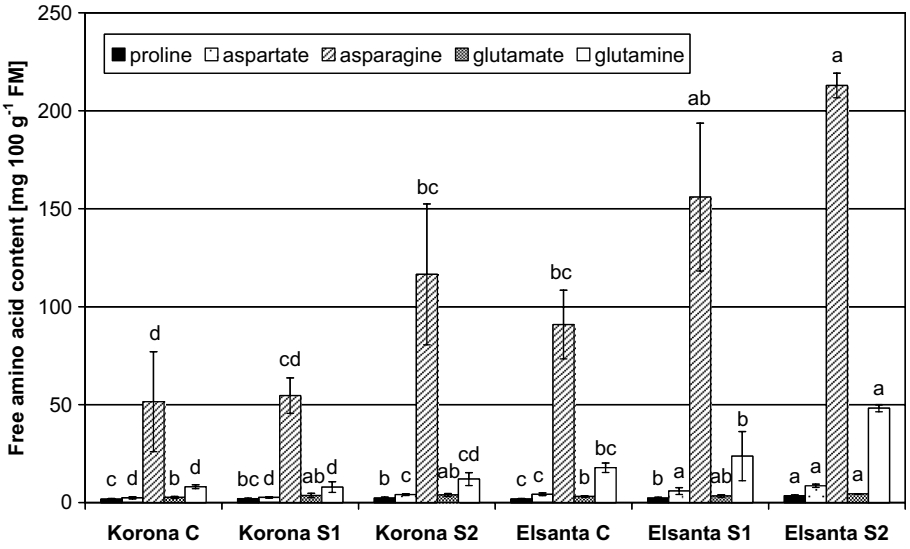

Fig. 1. Contents of selected free amino acids in fruit of strawberry cvs Korona and Elsanta differing in their sensitivity to NaCl. Different letters indicate significant differences at $p \leqslant 0.05$ for each amino acid (C: control; S1: 40 mmol/l NaCl; S2: 80 mmol/l NaCl).

tion (Ashraf & Foolad, 2007). However, it is still uncertain to which extent Pro serves as an osmoregulator, because further soluble solids, e.g. Asn, Asp or Gln, rose as well. Comparable results in tomato fruits exposed to salt stress were interpreted accordingly (Sato et al., 2006).

4. Conclusions

Strawberry cv. Korona is less susceptible to salt stress than cv. Elsanta and fruits of this cv. were characterized by a more constant fruit quality, even at the elevated salt stress level, especially with respect to taste-relevant compounds. From the economic point of view, a reduced yield under NaCl salinity stress can be at least partly compensated by improved fruit quality, as indicated by higher contents of antioxidants and soluble solids.

Acknowledgements

The authors would like to thank the co-workers of the Section of Quality of Plant Products for technical support: Bettina Egger and Gunda Jansen for plant cultivation, fruit harvest, and sample preparation; Cornelia Conradt and Marco Hanke for HPLC analyses.

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