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Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity

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ABSTRACT

Strawberry cvs Korona and Elsanta, differing in their sensitivity to salt stress, were exposed to 0, 40, or 80 mM NaCl in the root medium from the end of April to mid-August. Although fruits of both cultivars contained comparable amounts of Na^+ and Cl^-, fruit quality was more impaired in cv. Elsanta, as indicated by the larger reductions of fruit size and sugar/acid ratios. Malondialdehyde levels started to rise significantly at 40 mM NaCl in the more sensitive cv. Elsanta, but at 80 mM in cv. Korona. Total amino acid levels, especially contents of essential amino acids, rose significantly in both cvs. Salt stress also increased contents of free proline, asparagine, and glutamine. Their increases may contribute to osmotic adjustment. The results of the present study favour the interpretation that elevated levels of proline, asparagine and glutamine are indicative of salt stress damage.

1. Introduction

The influence of NaCl salinity on nitrogen and amino acid metabolism may impair quality of foods of plant origin in two different ways. First, NaCl salinity may affect protein synthesis and, hence amino acid metabolism in general and second, it may reduce the nutritional value of the plant product. Modifications in the concentrations of nitrogen-containing compounds, such as hydrolysis of proteins and accumulation of amino acids, are well-documented responses to salt stress, although earlier studies were often inconclusive due to the severe and poorly characterized stress applied (Ashraf & Harris, 2004). Salt stress may induce production or enhance accumulation of compounds such as proline (Pro), glycinebetaine, or amines. These compounds may be involved in osmotic adjustment, free radical-scavenging, and maintenance of protein and membrane integrity (Yamamoto, Shim, Fujihara, Yoneyama, & Usui, 2003). Frequently reported is the accelerated synthesis of Pro under salt or water stress, while its oxidation is inhibited, resulting in the accumulation of Pro in stressed tissue (Mansour, 2000; Misra & Gupta, 2005). Pro accumulation is used as an indicator for the selection of tolerant species or cvs. However, some authors suggest that Pro accumulation is indicative of salt stress damage (De Lacerda, Cambraia, Oliva, Ruiz, & Prisco, 2003; Lutts, Majerus, & Kinet, 1999), because its accumulation begins after severe stress and is more closely correlated with survival than with adaptation for continued growth. Amides, such as glutamine (Gln) and asparagine (Asn), have also been reported to accumulate in plants in response to salt stress (Dubey, 1997; Mansour, 2000) and a generally higher total free amino acid level in leaves was reported in salt-tolerant species (Ashraf & Harris, 2004). In addition, amino acids, such as Pro, arginine (Arg), methionine (Met), and glutamic acid (Glu), are directly or indirectly involved in the regulation of plant responses to various environmental signals related to abiotic or biotic stress (Ashraf & Harris, 2004; Galili & Höfgen, 2002).

For the nutritional quality of foods of plant origin, so-called "essential amino acids", e.g. lysine (Lys), isoleucine (Ile), leucine (Leu), Met, valine (Val), phenylalanine (Phe), tryptophan, and cysteine, contribute significantly. In addition, some free amino acids may influence fruit taste. Best known is L-Glu, which is responsible for 'umami' or delicious taste (Lindemann, 2001). Alanine (Ala) or Lys are highly correlated with sweetness, and Phe or tyrosine (Tyr) are bitter (Belitz, Grosch, & Schieberle, 2001). The molecular taste receptor T1R1 + 3, found in humans and rodents, responds to Asn and aspartic acid (Asp) (Nelson et al., 2002).

In previous works, Keutgen and Keutgen (2003) and Keutgen and Pawelzik (2007b, 2008) demonstrated different sensitivities to NaCl stress for the strawberry cvs Korona (less sensitive) and Elsanta (sensitive). Keutgen and Keutgen (2003) were able to document an increase of soluble protein content in strawberry fruit, especially in the salt-sensitive cultivar. This increase was correlated with an increase in N content. Based on these earlier reports, the hypothesis is put forward that NaCl salinity modifies the

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concentration of further nitrogen-containing compounds, namely free and total amino acids. These concentration changes may cause alterations of fruit taste and quality. In addition, they may reflect the sensitivity of cvs. Last but not least, the study aims to add arguments in favour of a significant contribution of free amino acids to salinity tolerance of strawberry fruit. Until now, such a comprehensive investigation on strawberry fruit amino acids has not been done, probably in part, because free amino acid levels are low in non-stressed strawberry fruit.

2. Materials and methods

Experiments were conducted during two successive growth periods, in 2002 and 2003, in Göttingen, Germany, with strawberry cvs. Elsanta (NaCl-sensitive) and Korona (less sensitive) (Keutgen & Keutgen, 2003; Keutgen & Pawelzik, 2007a, 2008). Salt levels applied were moderate (40 mM NaCl) and excessive (80 mM NaCl) in addition to a control treatment (0 mM NaCl). Commercial strawberry (*Fragaria* x *ananassa* Duch.) 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. For experiments, plantlets were selected for similar size and cultivated in 6 l metallic Mitscherlich containers filled with quartz sand (0.7–1.2 mm grain size). The experiments were conducted from the end of April to mid-August. Plants were located randomly (12 plants per m^2) with ten replications per combination to ensure a statistical design and to exclude position effects. They were grown in a greenhouse at a mean ambient temperature of 17 °C (maximum temperature = 22.7 °C; minimum temperature = 11.3 °C) and a mean humidity regime of 77% in order 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. Three weeks after planting, salt treatments started and 100 ml of solutions containing 0, 40, or 80 mM NaCl 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. In summer, when transpiration was very high, additional water had to be added to the plants in the afternoon in order to avoid drought stress. At most, three times a week, 200 ml of demineralized water were supplied (in 2003).

To improve fruit quality, runners were removed immediately. Fruit were harvested at the optimum of fruit maturity, when about 90% of the fruit surface had reached a fully red colour. Fruit were divided into sepals and fruit flesh. Only fruit flesh was used for further investigations. Fruit were frozen in liquid nitrogen immediately after harvest and stored at −30 °C until freeze-dried (Epsilon 2-40, Christ, Germany). Dry matter contents were recorded and dried fruits were milled to a fine powder.

The contents of Cl^- and Na^+ were determined after digestion of fruit with HNO_3 and H_2O_2 in a MLS-1200 microwave laboratory system (MLS GmbH, Leutkirch im Allgäu, Germany). Cl^- contents were evaluated photometrically (Hewlett Packard Aglient 8453 UV/VIS spectrophotometer with multi-cell sampler) according to the mercury thiocyanate method of Merck KGaA (Darmstadt, Germany). Na^+ contents were determined using a flame photometer Elex 6361 (Eppendorf, Germany). Water contents of fruit were calculated by comparing fresh and dry masses.

For carbohydrate analyses, water extracts of dried fruit were used (Keutgen & Pawelzik, 2007a). Glucose, fructose and sucrose, as well as organic acids, were determined in the membrane-filtered supernatant (pores size 0.45 μm). Carbohydrates were separated using a LiChrospher 100 NH_2 (5 μm) 4 × 4 mm pre-column (No. 1.50966.0001, Merck KGaA) in combination with a LiChrospher 100 NH_2 (5 μm) 4 × 250 mm separation column (No. 1.50834.0001, Merck KGaA). The column temperature of 20 °C was controlled by the column thermostat Jetstream 2 (Knauer, Berlin, Germany). An acetonitrile:pure water solution (80/20 v/v) was used as mobile phase (flow rate 1.0 ml min^{-1}). Carbohydrates were detected with a Knauer differential refractometer 198.00. The sum of glucose, fructose and sucrose was considered as a measure of total soluble carbohydrates. The results were recalculated per unit fresh mass.

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, 5 μm; Merck KGaA) at a flow rate of 0.4 ml min^{-1} and a temperature of 22 °C. As isocratic solution, 18 mM 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). The amount of total organic acids was summed and expressed in g per fresh mass. The sugar/acid ratio was calculated from the amount of total soluble carbohydrates divided by the total amount of organic acids.

Lipid peroxidation of fruit was measured using the modified thiobarbituric acid method according to Botsoglou et al. (1994) with 175 mM 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, MDA concentrations were measured photometrically at 532 and 600 nm with a Hewlett Packard Aglient 8453 UV/VIS spectrophotometer.

Raw protein content was calculated on the basis of fruit nitrogen content (raw protein = N × 6.25). Total nitrogen content was determined using a CN 2000 – analyzer (Leco, Mönchengladbach, Germany) according to Sweeney and Rexroad (1987). Results were expressed per 100 g of fruit fresh mass. Total soluble protein content was calculated from free and cell wall-associated proteins (Civello, Martinez, Chave, & Añón, 1995). For the free proteins, 0.1 M potassium phosphate buffer at pH 6.0 (2.5 ml g^{-1} FM) was used and, for cell wall-associated constituents, double extractions with 1 M NaCl solution (5 mL g^{-1} sample) were performed. Concentrations of proteins were measured using a Bio-Rad protein assay with bovine serum albumin. For both fractions, independent standards were prepared, solved in either potassium phosphate buffer (0.1 M) or NaCl solution (1 M).

Free amino acids (FAA) and total amino acids (TAA – free and bound) contents and their composition in strawberry fruit were determined after *o*-phthaldialdehyde (OPA) pre-column derivatization. FAA were extracted using 0.1 M NaOH (Herbert, Barros, Ratola, & Alves, 2000) and TAA by protein hydrolysis with 6 M HCl (Nair, 1977). 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 KGaA). The HPLC separation was made 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 was 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. The separation was achieved at a flow rate of 0.65 ml min^{-1} at a temperature of 35 °C and the injection volume was 20 μl. The detection range of Asn, Asp, Gln, Glu, serine (Ser), histidine (His), glycine

(Gly), threonine (Thr), Arg, Ala, Tyr, Met, Val, Phe, Ile, Leu, and Lys ranged between 0.25 and 2.5 μM.

Free 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-coloured complex was monitored at a wave length of 546 nm and 25 °C after extraction with toluene using a Hewlett Packard Aglient 8453 UV/VIS spectrophotometer with multi-cell sampler. Pure toluene was used as a blank. Pro concentrations were calculated using a calibration curve from 0 to 150 μM. The results of all amino acids are presented in mg per 100 g of fruit fresh mass.

Experimental data were analyzed with the SPSS 12.0 statistical programme (SPSS Inc., 1989–1999). All data sets were tested for a normal distribution and variance homogeneity ($P < 0.05$). In the case of homogeneous sample variances, means were compared by Duncan tests. In case of inhomogeneous sample variances, Tamhane tests were used ($P < 0.05$). Correlation analyses were performed using the Pearson correlation coefficient.

3. Results

The application of NaCl to the root medium resulted in a significant decrease in fruit weight of 24% and 41% in cvs Korona and Elsanta, respectively, at 80 mM NaCl (Table 1). Total soluble carbohydrate content remained fairly constant in cv. Korona, while it was significantly reduced (by 19%) at 40 mM and by 35% at 80 mM in cv. Elsanta. Total organic acids remained fairly stable in both cvs, with the exception of an increase in cv. Elsanta at 80 mM NaCl (Table 1). As a consequence, the sugar/acid ratio decreased significantly in both cvs at 40 and at 80 mM NaCl.

Levels of Na^+ and Cl^- increased in fruit (Table 2), but were comparable between cvs. Na^+ contents of fruit were much higher than those of Cl^- at 40 and at 80 mM NaCl. Fruit water content was not significantly affected by NaCl stress in the root medium of plants (unpublished results). It was significantly higher in cv. Elsanta (91.0 g 100 g^{-1} FM) than in cv. Korona (89.1 g 100 g^{-1} FM).

When subjected to NaCl salinity, fruit showed higher lipid peroxidation activity, measured as MDA. In cv. Korona, MDA content was elevated at 80 mM NaCl compared to controls while, in cv. Elsanta, MDA content rose already at 40 mM (Table 1).

Generally, salt stress caused an increase of raw protein content in both cultivars (Table 2), which was more pronounced in cv. Elsanta. In this cv. the content was up to 50% higher than that in control fruit. In the case of total soluble proteins, NaCl stress also resulted in increasing contents in strawberry fruit, with cv. Elsanta showing a more distinct response (Table 2). Higher amounts of total soluble proteins were due to an increase in the free protein fraction, related to the symplast, while the contents of cell wall-associated (i.e. mainly apoplastic) proteins remained stable.

FAA, as well as TAA, contents in fruit rose with NaCl level in the root medium, irrespective of salinity level and cv. (Tables 3 and 4). Among FAA, accumulation of Asn, followed by Gln, was most distinct in both species, although the quantitative changes were different. In the more sensitive cv. Elsanta, Gln content increased by over 200% and, in the less sensitive cv. Korona, by about 100%. Contents of free Pro in fruit also rose with salt stress in both cultivars, but this increase was significant only in cv. Elsanta, where Pro content was more than twice as high at 80 mM compared to control fruit.

The content of essential FAA rose significantly (by about 50%) in cv. Korona at 80 mM NaC, while in cv. Elsanta it remained fairly stable. The increase in cv. Korona was mainly due to larger amounts of Thr and Val (Table 3). Differences in the responses to NaCl salinity between cultivars were detected for FAA such as Ala, Gly, Ile and Val. In the more sensitive cv. Elsanta a significant decrease in concentrations of these amino acids were observed at the highest salt level (Table 3). In the less sensitive cv. Korona the contents of these free amino acids remained fairly constant.

TAA contents increased significantly with NaCl salinity in both cvs (Table 4). In cv. Elsanta, increases of 30–50% in contents of total essential amino acids compared to control fruit were observed. Contents of single amino acids, such as Asp, Gly, Ile, Leu, Met, Phe, Tyr, and Val, increased with NaCl treatment in cv. Elsanta, while increases in Ile, Met, Thr, Tyr, and Val were detected in cv. Korona (Table 4). Levels of Ala, Arg, Glu, His, Lys, and Ser, remained fairly constant. Worthy of note, Asn and Gln were not detected during the analyses of TAA, despite their presence in the analyses of FAA. These amino acids were probably converted to Asp and Glu during sample preparation.

Table 1
Effects of NaCl salinity on fruit weight, total soluble carbohydrate and organic acid contents, the sugar/acid ratio and malondialdehyde generation of fresh strawberry fruit of cvs Korona (Kor) and Elsanta (Els)

	Fruit weight (g)	Total soluble carbohydrates (g 100 g^{-1} FM)	Total organic acids (g 100 g^{-1} FM)	Sugar/acid ratio	Malondialdehyde (μmol 100 g^{-1} FM)
Kor C	5.95 ± 1.05 bc	6.33 ± 0.39 a	1.21 ± 0.16 ab	5.28 ± 0.46 a	0.56 ± 0.18 c
Kor S1	5.56 ± 1.19 cd	5.86 ± 0.33 a	1.24 ± 0.11 a	4.73 ± 0.23 b	0.64 ± 0.25 bc
Kor S2	4.49 ± 0.61 d	5.33±0.44 ab	1.29 ± 0.19 ab	4.17 ± 0.49 c	1.24 ± 0.32 a
Els C	9.59 ± 2.60 a	4.76 ± 0.53 b	1.02 ± 0.06 b	4.65 ± 0.44 b	0.42 ± 0.17 c
Els S1	7.45 ± 1.16 ab	3.86 ± 0.27 c	1.03 ± 0.04 b	3.76 ± 0.22 d	0.90 ± 0.24 b
Els S2	5.66 ± 1.18 cd	3.10 ± 0.22 d	1.22 ± 0.09 a	2.55 ± 0.20 e	0.90 ± 0.25 b

Different letters indicate significant differences at $P \leqslant 0.05$ within one parameter, $n \geqslant 8$. C – 0 mM NaCl, S1 – 40 mM NaCl, S2 – 80 mM NaCl.

Table 2
Effects of NaCl salinity on Na^+ and Cl^- contents, as well as on protein content, of fresh strawberry fruit of cvs Korona (Kor) and Elsanta (Els)

	Na^+ (mg 100 g^{-1} FM)	Cl^- (mg 100 g^{-1} FM)	Raw protein (g 100 g^{-1} FM)	TSP (mg 100 g^{-1} FM)	FSP (mg 100 g^{-1} FM)
Kor C	1.17 ± 0.41 d	4.61 ± 0.70 cd	0.69 ± 0.07 c	57.3 ± 9.17 d	39.3 ± 7.35 d
Kor S1	30.5 ± 4.36 c	15.6 ± 5.13 ab	0.78 ± 0.07 b	63.3 ± 9.14 cd	48.0 ± 8.01 c
Kor S2	56.7 ± 10.87 ab	17.3 ± 3.61 a	0.76 ± 0.07 b	66.9 ± 5.89 c	47.4 ± 5.20 c
Els C	0.57 ± 0.14 e	4.58 ± 0.78 d	0.67 ± 0.10 c	62.8 ± 11.66 cd	44.2 ± 7.59 cd
Els S1	46.1 ± 11.83 b	12.0 ± 2.59 b	0.79 ± 0.07 b	78.2 ± 7.09 b	59.0 ± 4.62 b
Els S2	66.5 ± 8.03 a	22.4 ± 8.45 a	1.00 ± 0.09 a	100 ± 20.41 a	75.5 ± 7.72 a

Different letters indicate significant differences at $P \leqslant 0.05$ within one parameter, $n \geqslant 8$. C – 0 mM NaCl, S1 – 40 mM NaCl, S2 – 80 mM NaCl, TSP – total soluble protein, FSP – free soluble protein.

Table 3
Effect of NaCl stress in the root medium on the contents of free amino acids (FAAs) in fruit of two strawberry cvs differing in their sensitivity to NaCl, calculated in mg per 100 g of fruit fresh mass

Free amino acids	Korona (less salt-sensitive) (mM NaCl)			Elsanta (salt-sensitive) (mM NaCl)		
	0 (control)	40	80	0 (control)	40	80
Sum of FAA	135 ± 11.10 b	188 ± 21.12 a	224 ± 33.65 a	153 ± 35.54 b	214 ± 22.65 a	201 ± 39.43 a
Essential FAA	6.21 ± 1.04 b	7.87 ± 1.92 ab	9.27 ± 3.49 a	6.80 ± 2.10 ab	9.29 ± 1.60 a	6.80 ± 1.61 ab
Asn	64.3 ± 11.40 b	83.7 ± 8.25 b	110 ± 8.68 a	84.8 ± 25.73 b	120 ± 23.27 a	118 ± 34.59 a
Gln	23.2 ± 2.60 c	44.7 ± 9.86 a	47.2 ± 6.96 a	11.7 ± 2.95 d	31.6 ± 11.85 bc	38.1 ± 7.25 ab
Ala	13.3 ± 2.28 b	16.1 ± 4.57 ab	17.6 ± 6.56 ab	20.5 ± 3.67 a	19.7 ± 2.98 a	8.27 ± 2.44 c
Arg	0.52 ± 0.10 c	0.59 ± 0.07 c	0.50 ± 0.17 c	1.15 ± 0.27 b	1.50 ± 0.15 a	1.72 ± 0.39 a
Asp	5.36 ± 0.77 a	7.11 ± 0.93 a	7.16 ± 1.48 a	6.86 ± 1.98 a	6.58 ± 1.32 a	6.45 ± 1.42 a
Glu	5.31 ± 0.56 a	6.38 ± 1.00 a	6.85 ± 1.24 a	3.62 ± 1.47 ab	4.09 ± 0.50 b	3.21 ± 0.67 b
Gly	0.26 ± 0.11 bc	0.25 ± 0.08 bc	0.17 ± 0.06 c	0.59 ± 0.14 a	0.35 ± 0.14 b	0.16 ± 0.03 c
Ile*	0.52 ± 0.11 c	0.50 ± 0.12 c	0.59 ± 0.31 bc	0.92 ± 0.21 a	0.78 ± 0.29 ab	0.44 ± 0.08 c
Leu*	0.38 ± 0.06 ab	0.45 ± 0.10 ab	0.49 ± 0.16 a	0.33 ± 0.09 b	0.36 ± 0.10 ab	0.37 ± 0.06 ab
Phe*	0.47 ± 0.11 b	0.38 ± 0.06 b	0.28 ± 0.05 c	0.70 ± 0.08 a	0.45 ± 0.10 b	0.44 ± 0.05 b
Pro	1.68 ± 0.48 b	2.24 ± 0.84 ab	3.03 ± 1.27 ab	1.55 ± 0.16 b	2.19 ± 0.40 b	3.54 ± 0.60 a
Ser	6.82 ± 0.65 b	9.97 ± 3.73 ab	13.5 ± 4.29 ab	6.19 ± 2.33 b	11.4 ± 1.26 a	8.00 ± 3.02 ab
Thr*	3.70 ± 0.88 b	5.07 ± 1.27ab	6.49 ± 2.62 a	3.43 ± 2.01 b	6.28 ± 1.32a	4.78 ± 1.40ab
Tyr	8.43 ± 0.32 a	8.64 ± 1.31 a	7.99 ± 0.66 a	8.75 ± 1.11 a	7.37 ± 0.63 a	6.55 ± 1.22 a
Val*	1.14 ± 0.30 ab	1.47 ± 0.48 a	1.42 ± 0.44 a	1.42 ± 0.40 a	1.41 ± 0.23 a	0.76 ± 0.11 b

Different letters indicate significant differences at $P \leqslant 0.05$ within one parameter, $n \geqslant 8$. The essential amino acids are marked by an asterisk (*).

Table 4
Effect of NaCl stress in the root medium on the contents of total amino acids (TAA) in fruit of two strawberry cvs differing in their sensitivity to NaCl, calculated in mg per 100 g of fruit fresh mass

Total amino acids	Korona (less salt-sensitive) (mM NaCl)			Elsanta (salt-sensitive) (mM NaCl)		
	0 (control)	40	80	0 (control)	40	80
Sum of TAA	64.3 ± 20.13 c	86.2 ± 12.34 abc	88.9 ± 12.30 ab	77.8 ± 23.26 bc	97.1 ± 21.35 ab	108 ± 14.02 a
Essential TAA	16.8 ± 4.68 b	22.3 ± 2.15 ab	23.9 ± 2.91 ab	19.0 ± 2.28 b	21.3 ± 0.82 b	28.1 ± 3.22 a
Ala	15.2 ± 3.53 b	20.1 ± 2.35 b	20.4 ± 1.64 b	20.0± 5.12 ab	22.3 ± 4.17 ab	26.3 ± 1.67 a
Arg	6.81 ± 1.59 a	9.74 ± 0.94 a	9.57 ± 2.01 a	8.75 ± 3.50 a	12.3 ± 6.20 a	12.2 ± 2.89 a
Asp	14.0 ± 4.82 c	19.9 ± 3.82 abc	19.2 ± 5.02 bc	18.0 ± 7.16 bc	24.5 ± 6.59 ab	26.5 ± 5.58 a
Glu	7.76 ± 3.55 a	10.1 ± 2.71 a	9.53 ± 2.49 a	7.36 ± 2.86 a	11.0 ± 5.76 a	8.28 ± 1.67 a
Gly	0.84 ± 0.17 b	1.00 ± 0.10 b	1.03 ± 0.15 ab	0.85 ± 0.26 b	0.94 ± 0.20 ab	1.29 ± 0.10 a
His	0.61 ± 0.25 a	0.77 ± 0.38 a	0.71 ± 0.38 a	0.60 ± 0.41 a	0.74 ± 0.41 a	0.73 ± 0.25 a
Ile*	2.45 ± 0.82 c	3.51 ± 0.44 b	3.85 ± 0.55 b	3.15 ± 0.92 bc	3.63 ± 0.21 b	4.64 ± 0.82 a
Leu*	5.09 ± 1.48 b	6.46 ± 1.10 ab	6.76 ± 1.44 ab	5.29 ± 1.46 b	6.68 ± 0.53 ab	8.08 ± 1.59 a
Lys*	3.14 ± 0.72 a	4.31 ± 0.86 a	4.30 ± 0.98 a	3.89 ± 1.34 a	3.75 ± 0.33 a	4.62 ± 0.30 a
Met*	0.46 ± 0.23 c	0.73 ± 0.15 ab	0.74 ± 0.14 ab	0.59 ± 0.14 bc	0.70 ± 0.03 ab	0.87 ± 0.06 a
Phe*	1.18 ± 0.49 b	1.43 ± 0.24 b	1.88 ± 0.24 b	1.40 ± 0.36 b	1.75 ± 0.20 b	2.58 ± 0.07 a
Ser	0.55 ± 0.14 ab	0.60 ± 0.05 a	0.83 ± 0.26 ab	0.47 ± 0.11 ab	0.48 ± 0.05 b	0.48 ± 0.09 ab
Thr*	0.64 ± 0.18 c	0.85 ± 0.17 abc	1.04 ± 0.25 a	0.75 ± 0.23 bc	0.94 ± 0.10 ab	0.87 ± 0.08 ab
Tyr	1.64 ± 0.62 c	2.79 ± 0.28 b	2.65 ± 0.76 b	2.80 ± 1.19 b	3.45 ± 0.70 ab	4.06 ± 0.36 a
Val*	3.83 ± 1.07 c	4.82 ± 0.69 bc	5.56 ± 0.31 ab	3.96 ± 1.17 c	3.87 ± 0.71 c	6.47 ± 1.15 a

Different letters indicate significant differences at $P \leqslant 0.05$ within one parameter, $n \geqslant 8$. The essential amino acids are marked by an asterisk (*).

4. Discussion

NaCl stress in the root medium resulted in a significant increase of Na^+ and Cl^- in fruit, which was shown to impair fruit taste (Keutgen & Pawelzik, 2007b). Moreover, weight and, hence, size of fruit decreased. Apart from NaCl content, the taste of strawberry fruit depended on the ratio of total soluble carbohydrates/organic acids, the sugar/acid ratio. In both cvs, this ratio was significantly reduced, indicating a lower sweetness and/or a higher sourness of the fruit under NaCl salinity. The more salt-sensitive cv. Elsanta was characterized by a more distinct decline of this ratio.

We examined whether changes in the concentrations of FAA were also involved in modifications of strawberry taste. However, threshold values related to the tastes of amino acids (sweet or bitter) (Belitz et al., 2001) were not reached. In consequence, FAAs constitute only less significant taste-compounds of strawberry fruit. Nevertheless, it may be hypothesized that taste could, at least to a limited extent, be regarded as a vector or sum of individual free amino acids, the concentrations of which were modified by NaCl salinity and might influence strawberry taste.

In order to relate the modification of amino acid pools of strawberry fruit grown under saline conditions to nutritional quality, the essential amino acids were further investigated. Higher amounts of essential FAA were found in the less salt-sensitive cv. Korona at elevated salt levels while, in cv. Elsanta, the increasing tendency was less distinct. The increase of essential TAA was positively related to Cl^- ($r = 0.945$) and Na^+ ($r = 0.835$), with Thr, Met, Ile, and Leu contributing most to this increase. In addition, Cl^- levels were positively related to Val and Lys concentrations. Although strawberry fruit does not represent a primary source of essential amino acids, the increase of essential amino acids, by up to 50%, may be rated as a significant amelioration of the nutritional quality of strawberry. However, protein content of strawberry fruit grown under NaCl salinity remained at a very low level.

FAAs play an important role in maintaining the osmotic balance in the tissue of plants, yeasts, bacteria and animals. For example, higher FAA contents, on a fresh weight basis, which did not result from concentration effects due to water losses, were reported in tomato fruit and sorghum under salinity (De Lacerda, Cambraia, Cano, & Ruiz, 2001; Zushi, Matsuzoe, Yoshida, & Chikushi, 2005).

FAAs participate in several metabolic processes involved in salinity stress response, namely turnover, synthesis, and incorporation of N into high-molecular components, mainly proteins, or in the accumulation of Pro (Ashraf & Bashir, 2003; Ashraf & Harris, 2004; Dubey, 1997; Hartzendorf & Rolletschek, 2001; Mansour, 2000). In the present experiment, elevated levels of free amino acids, as well as of free soluble and raw protein, were found in both strawberry cultivars. Contrary to the results of other studies (Ashraf & Bashir, 2003; Gadallah, 1999), the increase of FAA was not related to a decline of raw and soluble protein content in fruit, indicating that FAA accumulation was not a result of proteolysis. This is also in line with the observation that concentrations of some amino acids did not increase. The increase of selected FAA could represent an active physiological response to a decreasing water potential in order to cope with this stress (Sato, Sakaguchi, Furukawa, & Ikeda, 2006). Nevertheless, it cannot be ruled out that FAAs accumulated partly due to inhibition of processes related to protein metabolism, indicated by the correlation of Na^+ accumulation in fruit with FAA content ($r = 0.908$).

Higher levels of Pro in plant tissues under salinity conditions have been interpreted as indicators of salt stress tolerance of select genotypes (Giridara Kumar & Matta Reddy, 2003). It is, however, important to note that Pro accumulates under various stress conditions, for example drought, temperature, and starvation while, in many salt-stressed plants, its level decreases or remains more or less constant (Yamaya & Matsumoto, 1989). Because cv. Korona accumulated less Pro than did cv. Elsanta under salinity stress, it is proposed (for strawberry) that the accumulation of Pro does not play a major role in salinity stress defence in strawberry fruit under the given experimental conditions. Instead, it is noteworthy that the elevated accumulation of Pro in the more NaCl-sensitive cv. Elsanta did not result in a better protection. Similar results have been published for rice plants (Lutts et al., 1999) and sorghum (De Lacerda et al., 2001, 2003). Hartzendorf and Rolletschek (2001) hypothesized that a strong accumulation of Pro indicates exceeding of a critical salinity level and, in consequence, tissue damage or injury. In the present experiment, free Pro contents correlated highly significantly with Cl^- ($r = 0.952$) as well as with Na^+ ($r = 0.934$), which is well in line with the argument that Pro represents a non-specific stress indicator.

Amides are said to accumulate in salinity-stressed plants to a lesser extent than other N-containing compounds (Mansour, 2000). Nevertheless, the concentrations of Asn may rise in response to salt stress, and even to higher levels than those of Pro (Dubey, 1997; Sato et al., 2006). In the present experiment with strawberry, in the case of the fruit of the less sensitive cv. Korona, Asn levels rose more than 70% at 80 mM NaCl compared to the control fruit. Furthermore, free Asn content of strawberry fruit correlated positively with Na^+ ($r = 0.883$).

In addition to Asn, also concentrations of the second amide (Gln) may be elevated under salinity stress. For instance, an accumulation of Gln has been reported in roots and leaf blades of barley (Yamaya & Matsumoto, 1989) while, in *Phragmites australis*, Gln levels rose in rhizomes (Hartzendorf & Rolletschek, 2001). In strawberry fruit, salt stress led to a considerable increase in Gln, especially in the salt-sensitive cv. Elsanta. This sharp increase occurred already at a moderate salt stress level, whereas a further increase of NaCl in the root medium did not result in a further rise of fruit Gln content. The accumulation of Gln could have been caused by both, the activation of biosynthesis from Glu and the inactivation of Gln degradation (Yoshiba, Kiyosue, Nakashima, Yamaguchi-shinozaki, & Shinozaki, 1997). While Asn content was correlated with Na^+ in strawberry fruit, Gln content was positively related to Cl^- content ($r = 0.831$).

Generally, MDA production is considered a marker of lipid peroxidation and, thus, of cell membrane damage. It is well known that salinity stress is accompanied by elevated MDA levels in strawberry (Keutgen & Pawelzik, 2008), and this was also confirmed by the results of the present study. In the less NaCl-sensitive cv. Korona, only 80 mM NaCl resulted in a significant increase in MDA production, while in cv. Elsanta, already 40 mM NaCl led to an increase in MDA emission. If the increasing contents of Pro, Asn, and Gln reflect the sensitivity of strawberry fruit to NaCl salinity, they should correlate with MDA production. Calculations of Pearson correlation coefficients indicated a significant correlation of Asn with MDA in cv. Korona ($r = 0.948$) and cv. Elsanta ($r = 0.998$), while the correlation between Pro and MDA was significant only in the case of cv. Korona ($r = 0.950$) and that between Gln and MDA only in the case of cv. Elsanta ($r = 0.972$). With respect to the present results, the accumulation of Asn, Pro, and Gln may be indicative of the stress level; however, it may also play a role in osmotic adjustment, i.e. salt tolerance.

The study revealed that salt stress-induced changes of the amino acids pool must be regarded as significant responses of strawberry to salinity levels, because they reflect the impairment of plant metabolism and the investment of nitrogen into stress-related proteins and enzymes. The accumulation of nitrogen-compounds in strawberry fruit, presumably as a protective response to NaCl stress, cannot be regarded as an improvement of strawberry fruit quality, although the enrichment in essential amino acids may be rated as an advantage for human nutrition. However, this enrichment is accompanied by a significant degradation of fruit quality, for instance fruit size and fruit taste, in addition to fruit softening and enhanced perishability (Keutgen & Pawelzik, 2008).

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