Journal of Applied Botany and Food Quality 80, 160 - 164 (2006)

Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V., Grossbeeren, Germany

Effects of environmental factors on carotenoid content in tomato (*Lycopersicon esculentum (L.) Mill.*) grown in a greenhouse*

Angelika Krumbein, Dietmar Schwarz, Hans-Peter Kläring

(Received June 26, 2006)

Summary

Tomatoes are an important source of lycopene in the human diet. The effect of temperature (15°C - 24°C), CO_2 supply (380 - 1000 ppm) and nutrient concentration measured as electrical conductivity of the nutrient solution (EC, 2 - 9 dS m^{-1}) on the content of carotenoids (lycopene, ß-carotene) were investigated in two tomato cultivars grown in a greenhouse. The cherry tomato cultivar Supersweet was characterised by higher lycopene contents than the conventional round tomato 'Counter'. The results indicated that temperature has a significant influence on the biosynthesis of lycopene and ß-carotene during ripening. A temperature above 20°C seems to be optimal for lycopene production in the investigated cultivars, whereas a decrease to 15°C diminished the lycopene content. Neither CO_2 supply nor EC increase affected the carotenoid content under the conditions investigated.

Introduction

Tomatoes are low in fat and calories but rich in minerals, vitamins, and health-promoting secondary plant compounds. Due to their high frequency in the diet, tomatoes are an important source of carotenoids. The carotenoid content increases with fruit ripening. Lycopene is the most abundant carotenoid in ripe tomatoes with about 90% of total carotenoids, followed by ß-carotene.

Epidemiological studies, in vitro, and animal studies provide evidence that carotenoids may protect humans from several kinds of cancer (STAHL and SIES, 2005). A decreased risk of contracting prostate cancer was associated with high lycopene intake through consumption of tomatoes and tomato products (GIOVANNUCCI, 2002). The protective effects of lycopene include antioxidant activity, such as the quenching of singlet oxygen and the scavenging of peroxyl radicals, induction of cell – cell communication, and growth control (SIES and STAHL, 1998). Otherwise, with respect to the prevention of cardiovascular diseases and cancer, data for ß-carotene are conflicting (CLARKE and ARMITAGE, 2002; STAHL and SIES, 2005).

The growth, yield and fruit quality of tomatoes can be influenced by their genetic potential and environmental factors like temperature, radiation intensity, CO_2 concentration, and nutrient availability.

Greenhouse vegetable plants show positive effects from CO_2 supply by increasing photosynthesis and yield (NEDERHOFF, 1994). Furthermore, it can also directly improve fruit quality. Thus, LI et al. (1999) showed that an increase in the CO_2 concentration (from 380 to 1200 ppm) may compensate the negative effect of high salinity (5.2 and 7.0 dS m^{-1}) on yield and slightly improve the tomato's quality in terms of total soluble solids, glucose, and acidity. To our knowledge, no information has yet been published on the effect of CO_2 supply on carotenoid content in tomatoes.

It is well known that increasing the nutrient solution concentration in a soilless culture increases taste components like sugar and acids in tomatoes (DORAIS et al., 2001; AUERSWALD et al., 1999), but the effects on carotenoids are still unclear. It was reported that increasing EC did not affect the content of lycopene and ß-carotene in tomatoes, but it was also found that an increase of these secondary metabolites may occur (DORAIS et al., 2001; KRAUSS et al., 2006).

Temperature has a significant influence on the growth and development of tomato fruits. Because of the shorter fruit growth period and faster initiation of new trusses at higher temperatures, early yields are higher at higher temperatures (VAN DER PLOG and HEUVELINK, 2005). Regarding carotenoids, temperatures below 12°C strongly inhibit lycopene biosynthesis, whereas temperatures above 32°C stop this process altogether (DUMAS et al., 2003). It was argued that high temperatures (35°C) specifically inhibit the accumulation of lycopene because they stimulate the conversion of lycopene into ß-carotene (HAMAUZU et al., 1998).

The aim of the present study was to determine the effect of environmental conditions such as temperature, CO_2 concentration, and nutrient solution concentration measured as electrical conductivity (EC), on the content of carotenoids (lycopene, ß-carotene) in tomatoes grown in a greenhouse. Two tomato cultivars, representing different types, were investigated: a conventional round tomato (cv. Counter) and a cherry tomato (cv. Supersweet).

Materials and methods

Plant material

The effects of the environmental conditions (temperature, CO_2 concentration, EC of the nutrient solution) on the carotenoid content of the tomato fruits were investigated in a hydroponically grown crop in five greenhouse experiments at Grossbeeren (52°N, 13°E) using the cultivars Counter and Supersweet. The combination investigated was replicated in two greenhouse compartments. Plants were grown using the nutrient film technique. A nutrient solution was prepared by mixing rainwater and stock solutions according to the recipe of DE KREIJ et al. (1997). In all experiments, variations in the environmental conditions were started when the second truss was flowering. Carbon dioxide was varied in two experiments (spring and autumn). Here, the set point for the CO_2 supply was adjusted at 1000 ppm in half of the greenhouse compartments while the other compartments were not supplied with CO_2. Each CO_2 concentration was treated with two concentrations of the nutrient solution. To this end, the EC was adjusted at 2 and 9 dS m^{-1}, where the higher concentration was achieved by a proportional increase of all nutrients. The realised EC was close to the set points, while the realised CO_2 concentration depended on the set points but also on the outside temperature and global radiation. The mean CO_2 concentrations during the daytime were 348 and 806 ppm in spring, and 393 and 997 ppm in autumn, respectively. The temperature was varied at three levels in three experiments (spring, summer and autumn). The realised mean daily temperature during the sensitive phase of the fruit ripening (the last 10 days before harvest) were 18.0, 19.9 and 22.0°C in spring, 19.9, 21.5 and 23.7°C in summer and 15.0, 17.6 and 20.3°C in autumn. Fruits were harvested when they were red-ripe, and sorted manually

* The paper was presented at the 41th meeting of the „Deutsche Gesellschaft für Qualitätsforschung (Pflanzliche Nahrungsmittel) DGQ e.V.

into the colour stage 9-10 of the colour screening scale for the tomato (CBT-scale, Anonymous, 1992).

Carotenoid analysis

Carotenoids (lycopene and ß-carotene) were determined by HPLC (KRUMBEIN, 1996).

The analyses were carried out as double estimations with 12 fruits of the cultivar Counter and 30 fruits of the cultivar Supersweet per replicate. After homogenisation, 1 g calcium carbonate, 30 g sodium sulphate and 30 ml acetone were added to15 g homogenised tomato, and the samples were homogenised for 2 minutes. The extract was then filtered under suction, and the solid materials were extracted repeatedly with acetone until the resulting filtrate was colourless. The extract was then filtered through a 0.45 mm filter for HPLC analyses. The carotenoid composition and content were determined by HPLC using a C-18 reversed-phase column Lichrosphere 100 (5 µm, 250 x 4 mm; Merck) with an isocratic eluent of 75% acetonitrile, 15% methanol and 10% methylene chloride. The analysis was carried out at a flow rate of 1 ml min^{-1}. Wavelengths of 470 nm and 455 nm were used for the determination of lycopene and ß-carotene, respectively. Contents were quantitatively determined by calibration curves of the related pure standards. The results were converted to 100 g fresh matter (fm).

Statistical analysis

The data were analysed using multifactorial analysis of variance (ANOVA) and Fisher's F-procedure. This was followed by Tukey's HSD procedure, which was carried out separately for each cultivar and each experiment. The significance level in both tests was 0.05.

Results and discussion

Effect of CO_2 supply and electrical conductivity of the nutrient solution

The predominant carotenoid detected in tomato was lycopene followed by ß-carotene. The cultivars varied significantly in their content of lycopene in the two experiments in spring and autumn (Tab. 1). The 'Supersweet' cherry tomato had higher lycopene contents (between 5.5 -8.3 mg (100 g fm)$^{-1}$) than the conventional round 'Counter' tomato, which had contents in the range of 4.9 - 5.8 mg (100 g fm)$^{-1}$ in the two experiments (Figs. 1-2). The contents of ß-carotene varied between 0.54 - 0.79 mg (100 g fm)$^{-1}$ and 0.59 - 0.68 mg (100 g fm)$^{-1}$ in 'Supersweet' and 'Counter', respectively. They were low in comparison with the detected lycopene contents and with ß-carotene contents determined in other vegetables such as spinach (approximately 2 mg (100 g fm)$^{-1}$) and leafy Asian vegetables (2 - 4 mg (100 g fm)$^{-1}$) (KRUMBEIN et al., 2005). They differed only in autumn with higher contents in the 'Supersweet' variant. The concentration of lycopene in the tomato skin is approximately three to five times higher than in the whole tomato pulp (SHI and LE MAGUAR, 2000). The greater skin to volume ratio of the cherry tomato could enhance their lycopene content.

Tab. 1: Analysis of variance for lycopene and ß-carotene in two tomato cultivars grown at two CO_2 concentrations and two EC values in spring and autumn. The probability levels of Fischer's F-procedure are given. Significant effects are marked by bold numbers.

Factor	Lycopene		ß-Carotene	
	p (spring)	p (autumn)	p (spring)	p (autumn)
Cultivar	**0.035**	**0.0096**	0.39	**0.0001**
CO_2	0.67	0.39	1.00	0.74
EC	0.27	0.72	0.23	0.74

CO_2 supply increased and EC significantly diminished the yield of tomato in the spring and autumn experiment (data not shown). Figs. 1-2 show the content of lycopene as the main carotenoid in tomato. CO_2 supply did not affect the carotenoid content either at EC 2 or EC 9 dS m^{-1} in both cultivars (Tab. 1, Figs. 1-2). Environmental stress generates oxidative stress in plants, producing free radicals and other derivatives of oxygen. The results of IDSO and IDSO (2001) indicated that CO_2 supply appears to reduce oxidative stress in plants. For this reason, they concluded that plants growing in CO_2-enriched air generally experience less oxidative stress, so that they need less antioxidant protection, which thus allows them to produce smaller quantities of antioxidants. Our results indicate that, under the investigated conditions, no oxidative stress was present. This fact is also supported by the investigations of different EC levels. The high level of 9 dS m^{-1} had no effect on the lycopene and ß-carotene concentration in comparison to EC 2 (Tab. 1, Figs. 1-2). Contrary to these findings, DE PASCALE et al. (2001) found in salt

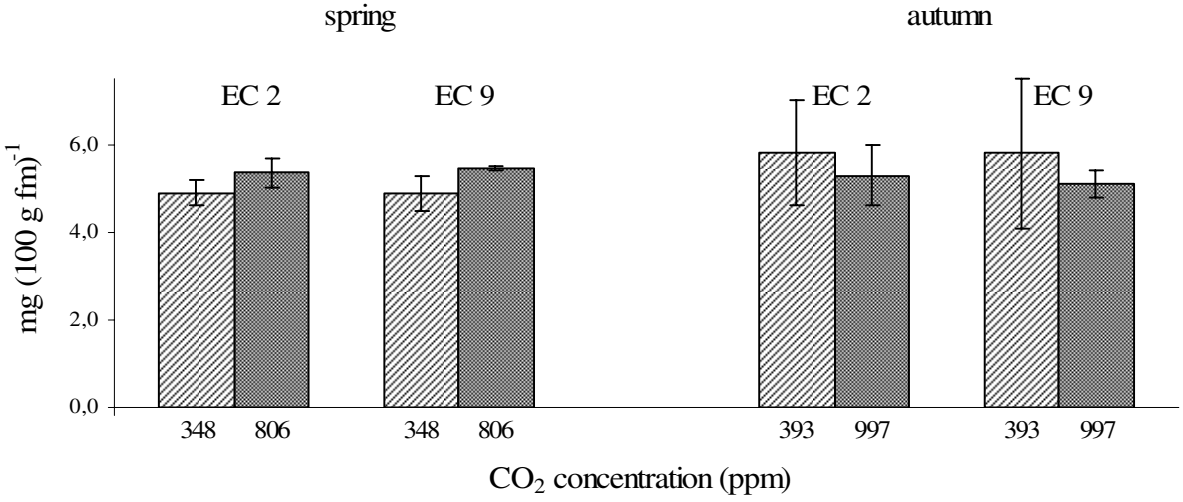

Fig. 1: Effect of CO_2 supply on lycopene content in cv. Counter grown in spring and autumn at EC 2 and EC 9. The values represent the mean of two replicates ± standard deviation.

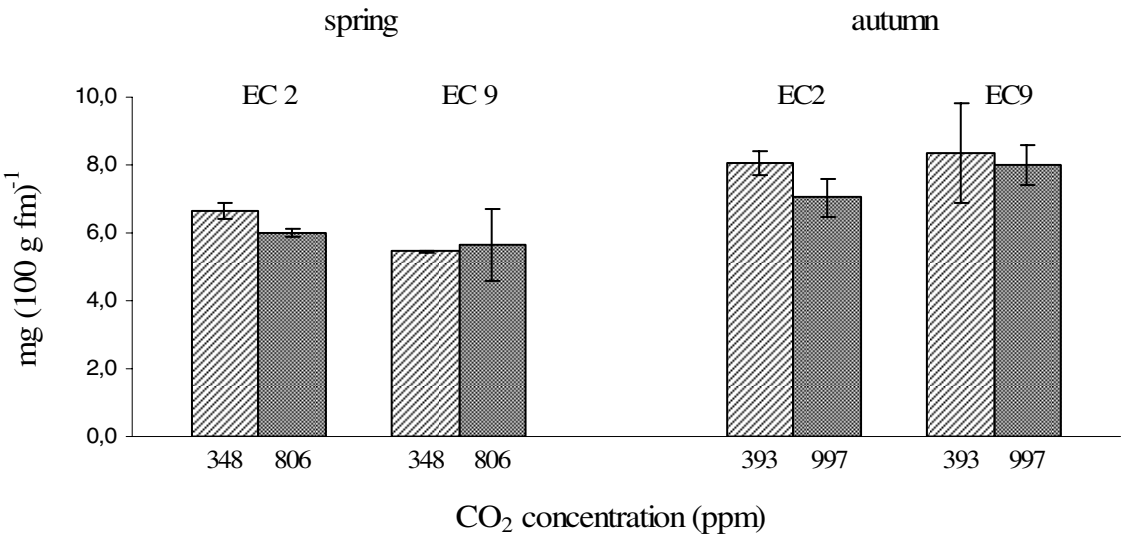

Fig. 2: Effect of CO_2 supply on lycopene content in cv. Supersweet grown in spring and autumn at EC 2 and EC 9. The values represent the mean of two replicates ± standard deviation.

grown tomatoes an increase in lycopene with increasing EC level from 0.5 - 4.4 dS m^{-1} and a decrease in lycopene with increasing EC level from 4.4 - 15.7 dS m^{-1}. They explained the increase by stress-induced upregulation of the genes' encoding for the enzymes involved in the key steps for lycopene biosynthesis. Otherwise, rising salinity levels (3, 6.5 and 10 dS m^{-1}) increased lycopene and ß-carotene content in tomato, relating to the fm basis, based on the concentration effect since plants under salinity accumulate less water and have a reduced water uptake (KRAUSS et al., 2006). Whether or not EC affects the content of lycopene and ß-carotene depends on the EC levels studied, the cultivars and the growing conditions (DORAIS, 2006).

Effect of temperature

In the three experiments with different temperature levels the tomato cultivars can be differentiated by their lycopene content (Tab. 2). Here again, the 'Supersweet' cherry tomato was characterised by higher lycopene contents than the conventional round 'Counter' tomato (Tab. 2, Figs. 3 and 4). 'Supersweet' also had slightly higher ß-carotene contents than 'Counter', with values of between 0.31 - 0.45 mg (100 g fm)$^{-1}$ and 0.26 - 0.38 mg (100 g fm) ^{1} in summer as well as 0.42 - 0.45 mg (100 g fm)$^{-1}$ and 0.29 - 0.33 mg (100 g fm)$^{-1}$ in autumn, respectively (Tab. 2).

The content of lycopene was influenced by temperature in spring and autumn (Tab. 2). Lycopene in the cultivar Counter increased significantly from 5.1 mg (100 g fm)$^{-1}$ at 18°C to 6.5 mg (100 g fm)$^{-1}$ at 22°C in spring as well as from 2.4 mg (100 g fm)$^{-1}$ at 15°C and 3.5 mg (100 g fm)$^{-1}$ at 17.6°C, up to 6.3 mg (100 g fm)$^{-1}$) at 20.3°C in autumn (Fig. 3). In the cultivar Supersweet the lycopene content increased significantly from 5.7 mg (100 g fm)$^{-1}$ at 15°C to 10 mg (100 g fm)$^{-1}$ at 20.3°C in autumn (Fig. 4). The increase of lycopene with temperature in spring was only a tendency. Increasing the temperature from 15.0°C to 20.3°C during the fruit ripening stage in autumn stimulated lycopene accumulation 2.6-fold and 1.8-fold in the cultivars Counter and Supersweet, respectively. Increasing the temperature from 18°C to 22°C in spring increased the lycopene content 1.3-fold in 'Counter'. A temperature between 20 and 24°C seems to be optimal for the biosynthesis of lycopene. This is in agreement with ROBERTSON et al. (1995), who found that the regulation of lycopene formation in a cell suspension culture of the cherry type variety VFNT showed that lycopene exhibited a broader plateau between 18 - 26°C for maximum concentration, after which the lycopene-forming activity was sharply reduced at 14°C and completely inhibited at 32 °C. The colour of the tomato fruit develops best when the ambient temperature inside the greenhouse is kept at between 12°C and 21 °C; very low (<10 °C) or very high air temperatures (>30 °C) inhibit the normal fruit ripening and the development of lycopene (DORAIS et al., 2001). The effect of low temperatures on biosynthesis has also been discussed by DUMAS et al. (2003). Lower temperature regimes (different day/night temperatures) at the ripening stage (variation of day temperature of between 25.6°C and 13.9°C as well as night temperatures of between 17.8°C and 2.8°C) decreased the content of lycopene but not that of ß-carotene. The content of ß-carotene in experiments presented here was only influenced in summer (Tab. 2). There was a slight decrease from 0.38 mg (100 g fm)$^{-1}$ at 19.9°C to 0.26 mg (100 g fm)$^{-1}$ at 23.7°C and 0.45 mg (100 g fm)$^{-1}$ at 19.9°C to 0.31 mg (100 g fm)$^{-1}$ at 23.7°C in the 'Counter' and 'Supersweet' variants, respectively. While lycopene biosynthesis is maximal above 20°C, ß-carotene biosynthesis seems to be maximal below 20°C.

Tab. 2: Analysis of variance for lycopene and ß-carotene in two tomato cultivars grown at different temperatures in spring, summer and autumn. The probability levels of Fischer's F-procedure are given. Significant effects are marked by bold numbers.

Factor	Lycopene			ß-Carotene		
	p (spring)	p (summer)	p (autumn)	p (spring)	p (summer)	p (autumn)
Cultivar	**0.00011**	**0.00000**	**0.000004**	0.14	**0.004**	**0.0004**
Temperature	**0.0027**	0.54	**0.00002**	0.10	**0.00077**	0.24

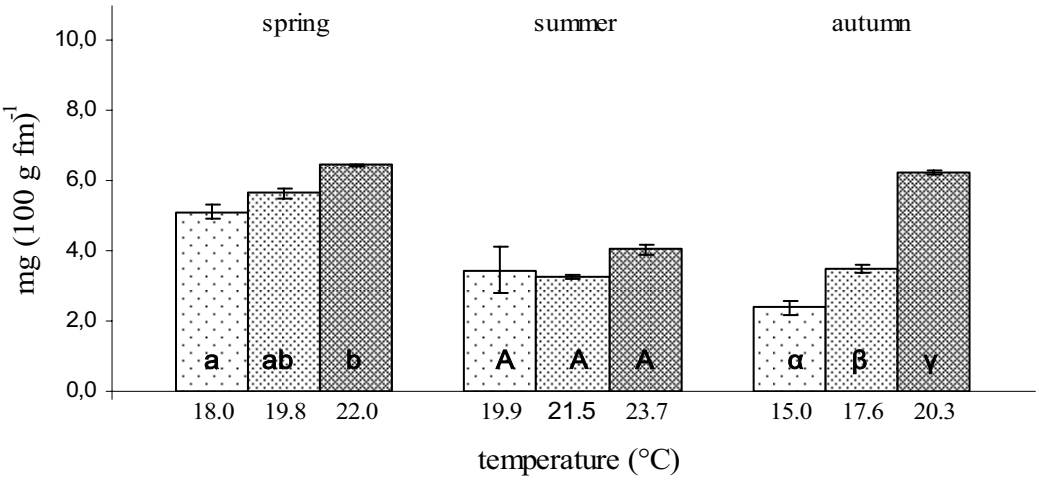

Fig. 3: Effect of the temperature during ripening (the last 10 days before harvest) on the lycopene content in cv. Counter grown in spring, summer and autumn. The values represent the mean of two replicates ± standard deviation. Different letters show significant differences within each experiment (level of significance 5%).

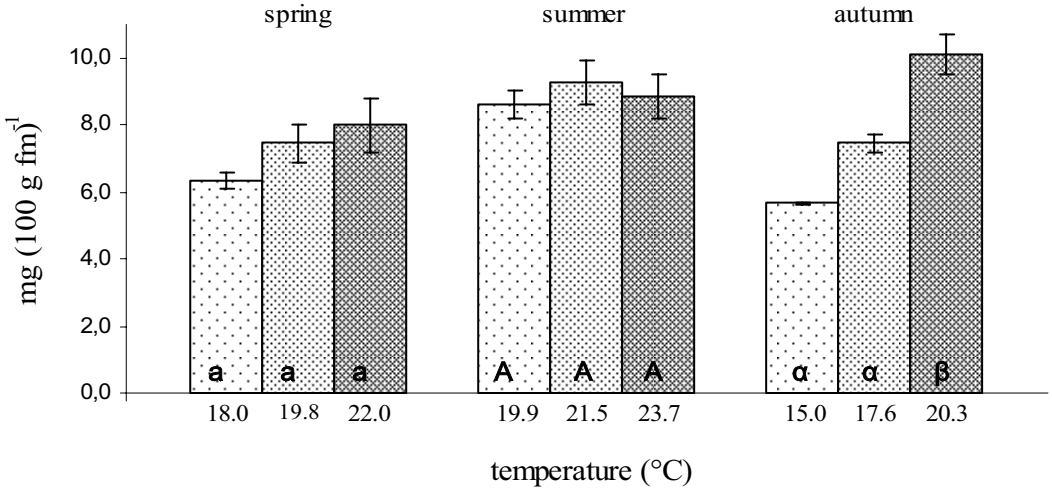

Fig. 4: Effect of the temperature during ripening (the last 10 days before harvest) on the lycopene content in cv. Supersweet grown in spring, summer and autumn. The values represent the mean of two replicates ± standard deviation. Different letters show significant differences within each experiment (level of significance 5%).

Acknowledgements

This research was supported by the German Federation, the Federal States of Brandenburg and Thuringia. We would like to thank A. Jankowsky for her carotenoid analyses and U. Zentner, A. Maikath, A. Platalla, and J. Lenk for their technical assistance.

References

ANONYMOUS, 1992: Kleur-stadia tomaten. Central Bureau van de Tuibouwveilingen in Nederland (Ed.), 2803 PE Gouda.

AUERSWALD, H., SCHWARZ, D., KORNELSON, C., KRUMBEIN, A., BRÜCKNER, B., 1999: Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution. Scientia Horticulturae 82, 227-242.

CLARKE, R., ARMITAGE, J., 2002: Antioxidant vitamins and risk of cardiovascular disease. Review of large.scale randomised trials. Cardiovasc. Drugs ther. 16, 411-415.

DE KREIJ, C., VOOGT, W., VAN DEN BOS, A.L., BAAS, R., 1997: Voedingsoplossingen voor de teelt van tomaat in gesloten teeltsystemen. Proefstation voor Bloemisterij en Glasgroente, Naaldwijk, The Netherlands VG2.

DE PASCALE, ST., MAGGIO, A., FOGLIANO, V., AMBROSINO, P., RITIENI, A., 2001: Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. J. Hort. Sci. Biotechnol. 76, 447-453.

DORAIS, M., PAPADOPOLUS, A.P., GOSSELIN, A., 2001: Greenhouse tomato fruit quality: the influence of environmental and cultural factors. Horticultural Reviews 26, 239-319.

DORAIS, M., 2006: Effect of cultural management on tomato fruit health qualities. Acta Hortic. (in press).

DUMAS, Y., DADOMO, M., DI LUCCA, G., GROLIER, P., 2003: Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Sci. Food Agric. 83, 369-382.

GIOVANNUCCI, E., 2002: A Review of the epidemiologic studies of tomatoes, lycopene, and prostate cancer. Exp. Biol. Med. 227, 852-859.

HAMAUZU, Y., CHACIN, K., UEDA, Y., 1998: Effect of postharvest temperature on the conversion of ^{14}C-mevalonic acid to carotenes in tomato fruit. J.

Jpn. Soc. Hortic. Sc. 67, 549-555.

IDSO, S.B., IDSO, K.E., 2001: Effects of atmospheric CO_2 supply on plant constituents related to animal and human health. Environ. Exp. Bot. 45, 179-199.

KRAUSS, S., SCHNITZLER, W.H., GRASSMANN, J., WOITKE, M., 2006: The influence of different electrical conductivity values in a simplified recirculating soilles system on inner and outer fruit quality characteristics of tomato. J. Agric. Food Chem. 54, 441-448.

KRUMBEIN, A., 1996: Schnelle HPLC-Methode zur Bestimmung der Carotinoide und Chlorophylle in Brokkoli. Deutsche Gesellschaft für Qualitätsforschung XXXI, 41-44.

KRUMBEIN, A., SCHONHOF, I., SCHREINER, M., 2005: Composition and contents of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected *Brassica* species (*B. juncea, B. rapa subsp. nipposinica var. chinoleifera, B. rapa subsp. chinensis* and *B. rapa subsp. rapa*). J. Appl. Bot. Food Quality 79, 168-174.

LI, Y., SAFI, M., GALE, J., VOLOKITA, M., NOVOPLANSKY, A.,1999: Response of tomato plants to saline water as affected by carbon dioxide supplementation. I. Growth, yield and fruit quality. J. Hort. Sci. Biotechnol. 74, 132-237.

NEDERHOFF, E.M., 1994: Effects of CO_2 concentration on photosynthesis, transpiration and production of greenhouse fruit vegetable crops. PhD Thesis, Wageningen, The Netherlands.

ROBERTSON, G.H., MAHONEY, N.E., GOODMAN, N., PAVLATH, A.E., 1995: Regulation of lycopene formation in cell suspension culture of VFNT tomato (*Lycopersicon esculentum)* by CPTA, growth regulators, sucrose, and temperature. J. Exp. Bot. 46, 667-673.

SHI, J., LE MAGUER, M. 2000: Lycopene in Tomatoes: Chemical and physical properties affected by food processing. Crit. Rev. Biotechnol. 20, 293-334.

SIES, H., STAHL, W., 1998: Lycopene: Antioxidant and biological effects and its bioavailability in the human. Proc. Soc. Exp. Biol. Med. 218, 121-124.

STAHL, W., SIES, H., 2005: Bioactivity and protective effects of natural carotenoids. Rev. Biochim. Biophys. Acta 1740, 101-107.

VAN DER PLOG, BY A., HEUVELINK, E. 2005: Influence of sub-optimal temperature on tomato growth and yield: a review. J. Hort. Sci. Biotechnol. 89, 652-659.

Address of the authors:
Dr. Angelika Krumbein, Dr. Dietmar Schwarz, Dr. Hans-Peter Kläring Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Theodor-Echtermeyer-Weg 1, D-14979 Großbeeren, Germany, corresponding author: krumbein@igzev.de