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Product Quality of Greenhouse Tomatoes: Effect of Cultivars, Organic N-Fertilization and Harvest Time

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Summary

The aim of this project was to investigate the marketability limits of old tomato cultivars used in organic farming and to maintain their biodiversity by promoting consumption on regional markets. Four old and endangered tomato cultivars, 'Ananas', 'Auriga', 'Green Zebra' and 'Lukullus', were treated with 0 and 100 kg N ha^{-1} applied as horn splinters. Harvesting was performed at early and optimum fruit maturity. All cultivars clearly differed in colour, shape and maturity of fruits. Nutritional quality and health promoting compounds were analysed in terms of beneficial ingredients such as ascorbic acid (vitamin C) and carotenoids (lutein, β-carotene and lycopene). Significant differences in nutritional quality of cultivars were detected. The highest contents of ascorbic acid and carotenoids were determined in cv. 'Lukullus'. High amounts of soil - borne nitrogen resulted in no significant effects of N fertilization on N uptake, ascorbic acid and carotenoid contents. Harvest at the optimal ripening stage showed a higher ascorbic acid content in comparison to an early harvest.

Key words. ascorbic acid – β-carotene – harvest time – lutein – lycopene – nitrogen – organic tomato

Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the most commonly grown fresh market vegetables. The breeding of the hybrids currently dominating the market has resulted in cultivars with longer shelf-life, yet often of poor taste and flavour. Local and open-pollinated varieties are on the verge of extinction. The loss of biodiversity with valuable local germplasm has fuelled the debate on the sustainability of current intensive farming practices. Consequently, Organic Agriculture is considered as a potential solution to maintaining biodiversity. A wide range of different cultivars still exists. More and more consumers are especially interested in organic, locally grown produce as they have become aware of its value: It is fresher, often tastes better and is linked to lower fuel consumption and transportation costs. Thus, it has potential for wide adaptation by established greenhouse growers as a niche market.

Maintaining old cultivars which, compared to hybrids, have shorter shelf-life, will rely heavily on local markets. Thus, marketing locally grown organic produce is a possible strategy to maintain biodiversity. Furthermore, consumers are prepared to pay a higher price for the higher quality produce. Organically produced vegetables are preferred for their nutritional value, flavour, taste and higher content of desirable ingredients such as antioxidants (Köpke 2003). Some of these are believed to be important in the prevention of cancer and cardiovascular diseases (Dillard and German 2000). Lycopene and ascorbic acid (vitamin C) are the main antioxidants in fresh tomatoes. Lycopene accounts for the red colour of the tomato, which is one of the main traits both industry and consumers look for. Other carotenoids such as lutein and β-carotene are also thought to be health promoting ingredients with antioxidant properties. In addition to genetic factors, the antioxidant content in tomatoes can be influenced by environmental factors such as temperature, light, water and nutrient availability, especially nitrogen, the latter being managed by cultivation techniques (Auerswald et al. 1996; Veit-Köhler et al. 1999; Dumas et al. 2003; Köpke 2003).

Tomato fruits of different cultivars show variation in β-carotene and total carotenoids. The variation in vitamin C content due to cultivar is greater in comparison with the variation due to growth conditions. The antioxidant properties of old cultivars, which differ with respect to size and shape (round, pear-shaped, oval and long), have never been studied. Raffo et al. (2002) reported significant differences in the main antioxidants and the antioxidant activity of small cherry tomatoes compared with medium- to larger-sized tomato cultivars harvested at later stages of ripening. On the other hand, no significant differences in vitamin C content were observed at different ripening stages. The colour of the different cultivars varies widely and colour characteristics are under greater genetic control. A deep red colour is associated with a high level of lycopene, while a high β-carotene content is responsible for a yellow to orange colour.

More than any other nutrient, nitrogen influences plant growth, morphology and composition. It thus plays a key role for food quality. Surplus of plant-available nitrogen often results in higher nitrate contents. Increasing the level of N-fertilization especially of readily available mineral N has been proven to decrease the vitamin C content in many of those fruits and vegetables that are used as major sources of vitamin C, such as citrus fruits, tomatoes and potatoes (KÖPKE 2005).

Aside from nutrient management, the quality and biochemical composition of tomato fruits also depend on harvest time. LIN and BLOCK (1998), however, noted that tomatoes harvested at the breaker stage and room-ripened to the red stage had a similar chemical composition (soluble solids, titratable acids and aroma volatiles) and firmness to those picked vine ripe red. PETERS et al. (1998) found that fruits harvested at the green/yellow stage attaining the red stage of ripeness within 4 days showed the same quality values (reducing sugars, titratable acids, carotenoids content and deformation) as fruits harvested red, except for a slightly lower ascorbic acid content. Nevertheless, to guarantee the desired flavour, harvesting fruits at the red-ripened stage is recommended by WATZL et al. (1995).

Consequently we investigated the effects of organic nitrogen fertilization (100 kg N ha^{-1} applied as horn splinters) and harvest time (early and optimum fruit maturity) on selected beneficial ingredients of four old tomato cultivars that differed in type, shape and colour. The present investigation is part of a broader project. The aim of the project is to investigate the marketability limits of old cultivars to be used in organic farming and to maintain tomato biodiversity by promoting local consumption and regionalisation.

Materials and Methods

Experimental design

The experiment was conducted in a greenhouse located at the Wiesengut Experimental Farm, Institute of Organic Agriculture, University of Bonn, Germany (65 m a.s.l. 7 ° 17 'E, 50 ° 48 ' N) during the 2004 season. Four cultivars, 'Ananas', 'Auriga', 'Green Zebra' and 'Lukullus', were selected by screening about 100 old and endangered tomato cultivars grown in Europe and the USA. These cultivars vary in size, shape, colour and shelf-life. They also differ in their growing habits, maturity and yield (Table 1).

For the experiment, the glasshouse area of 32 x 11 m was divided into four blocks of 44.8 m^2 each. Each block was further divided into sub-blocks of 22.4 m^2 for treatment with organic fertilizer (horn splinters) at the rate of 100 kg N ha^{-1} or no fertilizer. Seeds were sown in plastic trays in the second week of March. Two weeks after sowing, seedlings were replanted to plastic pots of 11 cm in diameter containing a potting mixture consisting of 80 % peat and 20 % compost with 3 kg horn meal m^{-2} substrate and then cultivated in the pots in the glasshouse until May. The seedlings were moved to the greenhouse in the second week of May, 2004. There were 9 plants of each cultivar in each sub-block. In total, there were 36 plants of all four cultivars in the 22.4 m^2 sub-block area. Rows and plants were spaced at 100 x 40 cm. The plants were allowed to grow in a single stem system. At planting time in May, soil mineral nitrogen content amounted to 143 kg N ha^{-1} available in 0–30 cm soil depth.

The fruits of each cultivar were harvested according to their maturity period. Fruits were harvested at early and optimum stage of maturity. Early harvest means that the fruits were harvested about four days prior to the optimum stage, when they are in the hard-red stage (comparable to colour no. 8; The Greenery 2004). Optimum harvest is attained when the fruits are red ripe and soft to touch (comparable with colour no. 12; The Greenery 2004). Data were recorded from seven harvested fruits of equal and representative size. After morphological investigations, the fruits were homogenized using a household mixer and the contents of Vitamin C and carotenoids (lycopene, β-carotene and lutein) determined.

Chemical analysis

Vitamin C. For the chemical analyses 50 g of fresh homogenized tomatoes were taken and adjusted at 3.5 pH. The final volume was made up to 500 ml by adding 15 % of meta-phosphoric (H_3PO_4) acid. The samples were then filtered through a Schleicher & Schuell 595 $^1/_2$ filter paper into a 1000 ml flask. The analyses were carried out as double estimation per sample using 7 homogenized fruits per sample. For each sample, a sample blank was carried out. About 100 µl filtered sample solution was transferred to cuvets 1500 µl of double distilled water and 1000 µl of tetrazolium salt MTT [3-(4-5 dimethylthiozolyl-2)-2, 5-diphenyltetrazolium bromide] added and

Table 1. Characteristics of tomato cultivars.

Cultivar	Seed source	Plant type	Hybrid	Maturity	Fruit size	Fruit shape	Shelf life	Fruit colour
'Ananas'	Ferme de St. Marthe, France	Indeterminate	No	Late	Big	Flat-oval to heart shaped	Longer	Light yellow with red tinge in the pulp
'Auriga'	Grüner Tiger, Germany	Indeterminate	No	Early to medium	Medium	Round to high round	Longer	Orange
'Green Zebra'	Ferme de St. Marthe, France	Indeterminate	No	Medium to late	Medium to big	Round	Short	Green with light and dark stripes
'Lukullus'	Verein zur Erhaltung der Nutzpflanzenvielfalt, Germany	Indeterminate	No	Early to medium	Small	Round	Longer	Deep red

mixed thoroughly. The mixture was incubated for 6 min at 37 °C in a hot water bath. During incubation, the sample blank assay was mixed every 2 min with a spatula. After removing the ascorbate oxidase spatula, the extinction of the sample blank and the sample was measured at 578 nm with a Lamba 2 UV-VIS Spectrometer (Perkin Elmer). After first absorbance, 100 µl of PMS (5-methylphenazinium methosulfate) was added to the same cuvets, mixed well and the cuvets placed in the hot water bath at 37 °C for 15 min. The absorbance of sample blank and sample was measured immediately at the same wave length. L-ascorbic acid contents were determined in mg $100g^{-1}$of fresh matter (fm) using the Boehringer Mannheim method (ANONYMOUS 1996).

Carotenoids. The carotenoids were extracted as described by KRUMBEIN (1996). Samples were analysed with a non-aqueous reversed-phase system. A column of RP-18 Phase Lichrosphere 100 (5 µm, 250 x 4 mm) (Merck) was used for separating the carotenoids. The elution mixture contained acetonitrile, methanol and dichlormethane (75:15:10) used isocratically, the flow rate of the mixture was 1.0 ml min^{-1}. The injection volume was 20 µl and the run-time of the HPLC was set to 30 min. Wave lengths of 470 nm, 455 nm and 448 nm were used for the determination of lycopene, β-carotene and lutein, respectively. The carotenoids were identified by comparing their retention times with those of reference standards (lutein, lycopene, β-carotene, purchased from Sigma, Germany). Carotenoids were quantified from calibration curves obtained by area measurement of the reference compounds at various concentrations in mg 100 g^{-1} of fresh matter (fm).

Statistical analysis

The results for L-ascorbic acid were evaluated using analysis of variance based on a 3-factorial split-split plot design model (SAS statistical package, version 9.1, procedure GLM, factors: fertilization, cultivars, harvest time). The results for yield and carotenoids were analysed using analysis of variance based on a 2-factorial split-plot design model (SAS statistical package, version 9.1, procedure GLM, factors: fertilization, cultivars). Comparisons of means were undertaken with Tukey's-test. The data given in the figures and throughout the text are means of different treatments.

Results and Discussion

The aim of this study was to investigate the nutritional value of four endangered multi-coloured tomato cultivars treated with organic N fertilizer and harvested at different ripening stages by examining their compositional pattern with particular attention to antioxidants. The investigations were focused on vitamin C and the carotenoids lycopene, β-carotene and lutein.

An application of well-rotten cow manure of about 100 t ha^{-1} in autumn 2002, resulted in a high availability of soil-borne nitrogen and an average fruit yield of about 14 kg m^{-2}. The additional application of 100 kg N ha^{-1} as horn splinters before transplanting the tomatoes gave no significant effect on fruit yield (kg m^{-2}) produced (Table 2).

Vitamin C

Vitamin C content was clearly affected by cultivar. The cultivars 'Lukullus' (22.9 mg 100 g^{-1} fm) and 'Auriga' (22.5 mg 100 g^{-1} fm) had a higher concentration of vitamin C than 'Ananas' (14.8 mg 100 g^{-1} fm) (Table 3). The lowest concentration was determined for 'Green Zebra' (11.1 mg 100 g^{-1} fm). This is in line with RAFFO et al. (2002) who determined pronounced differences of vitamin C affected by cultivar type and size of fruits. Our results confirm the high vitamin C contents of small-sized

Table 2. Fruit yield of tomatoes. Significance of difference is denoted by different letters (Tukey's test). Figures followed by the same letter were not significantly different.

Treatment	Yield (kg m^{-2})
N_{100}	13.9 a
N_0	13.7 a
Level of significance	α=0.05
'Ananas'	13.9 a
'Auriga'	15.2 a
'Green Zebra'	13.9 a
'Lukullus'	12.2 b
Level of significance	α=0.001

Table 3. Ascorbic acid (vitamin C): Effect of organic N-fertilization, cultivars and harvest time. Evaluation with an unbalanced data set. Significance of difference is denoted by different letters (Tukey's test). Figures followed by the same letter were not significantly different.

Treatment	Ascorbic acid (mg 100^{-1} fm)
N_{100}	17.3 a
N_0	17.4 a
Level of significance	α=0.05
'Ananas'	14.8 b
'Auriga'	22.5 a
'Green Zebra'	11.1 c
'Lukullus'	22.9 a
Level of significance	α=0.001
Early harvest	16.6 b
Optimum harvest	18.0 a
Level of significance.	α=0.05

Table 4. Soil mineral nitrogen content (kg ha^{-1}) at 0–30 cm and 30–60 cm soil depth during the year 2004.

Fertilizer level	Soil depth	May	June	July	Aug.	Oct.	Nov.
N_{100}	0–30	143.3	76.0	81.6	73.0	51.9	64.0
N_0		143.3	67.4	72.0	55.4	54.8	64.0
N_{100}	30–60	55.4	49.9	44.1	40.5	52.8	45.6
N_0		55.4	47.7	48.7	41.8	53.9	56.8

Table 5. Nitrogen uptake of tomatoes (pooled sample).

Treatment	Fertilizer level	N in leave/stem (g plant^{-1})	N in fruit	Total N	Total N (kg ha^{-1})
'Ananas'	N_{100}	5.8	6.3	12.0	300.9
	N_0	5.0	6.5	11.5	287.9
'Auriga'	N_{100}	6.9	11.9	18.7	467.6
	N_0	6.1	12.5	18.6	464.6
'Green Zebra'	N_{100}	10.3	10.9	21.1	528.3
	N_0	7.9	10.2	18.1	452.0
'Lukullus'	N_{100}	3.6	6.8	10.4	260.3
	N_0	3.7	6.6	10.2	255.3
Mean ($\bar{x}$)	N_{100}	6.6	8.9	15.6	389.3
	N_0	5.7	8.9	14.6	365.0

cultivars like 'Lukullus' and 'Auriga'. In contrast to the literature, where increasing levels of N fertilization have frequently been proven to decrease vitamin C contents (Köpke 2005), in our experiment nitrogen fertilization showed no significant difference when compared with the unfertilized treatment. This result might be caused by the generally high amount of soil-borne nitrogen (Table 4) and a limited mineralisation of the horn splinters. Nitrogen availability (Table 4) and N uptake (Table 5) were not essentially different as a function of N-fertilization.

Confirming our hypothesis, optimum harvest resulted in significantly higher vitamin C contents compared with early harvest (Table 3). As the maturity advances from early to optimum (ripe) harvesting stages, the vitamin C content increased in all cultivars, as confirmed by Shi et al. (1999). These authors reported increased vitamin C content in fresh fruit from the mature-light pink to pink stage and from the mature-pink to mature red stages. Interaction between fertilization and harvest time was not significant.

Carotenoids

Clear varietal differences were determined for the 3 carotenoids (Table 6). In the present study, all tomato cultivars differed significantly in lycopene content, which mostly represented more than 80 % of the total carotenoids. The dark-red coloured 'Lukullus' showed the highest concentration (8.96 mg 100 g^{-1} fm). The ranking order of lycopene concentration, visible through the degree of redness of the skin, was 'Lukullus' > 'Ananas' > 'Auriga'. The cultivar 'Green Zebra' did not even possess traces of lycopene (detection limit was ≤ 0.06 mg 100 g^{-1}of fresh matter). This result clearly underlines the genetic influence on the lycopene content of tomatoes (Martinez-Valverde et al. 2002), but lycopene content is known to be significantly affected by agronomic practices, growth conditions, post-harvest handling and especially N-fertilization, too. No significant interactions between cultivar and fertilization were determined. Montagu and Goh (1990) found that the lycopene concentration appeared to be directly influenced by readily available N from fertilizers, whereas compost application did not show any effect in a pot experiment. All cultivars differed significantly with respect to β-carotene contents. The highest concentration was determined for cultivar 'Auriga' (4.69 mg 100 g^{-1} fm), whereas very little β-carotene was found in 'Ananas' and 'Lukullus' (Table 5). As with lycopene no β-carotene was determined for the green cultivar 'Green Zebra'. It may be elucidated that the genetic potential influences tomato composition and changes in metabolism. The high β-carotene content in 'Auriga' was associated with the orange colour of this cultivar, confirming findings of several other authors (Madhavi and Salunkhe 1998; Mar-

Table 6. Carotenoids: Effect of organic N-fertilization and cultivars. Significance of difference is denoted by different letters (Tukey's test). Figures followed by the same letters were not significantly different.

Treatment	Lycopene	β-Carotene (mg 100 g^{-1} fm)	Lutein
N_{100}	3.22 a	1.53 a	0.08 a
N_0	2.86 a	1.47 a	0.07 a
Level of significance	α=0.05	α=0.05	α=0.05
'Ananas'	2.02 b	0.44 c	dl
'Auriga'	1.17 c	4.69 a	dl
'Green Zebra'	dl	dl	0.14 a
'Lukullus'	8.96 a	0.83 b	0.10 b
Level of significance	α=0.001	α=0.001	α=0.001

dl = lower than detection limit (0.06 g 100 mg^{-1} fm)

TIN-HARNANDEZ et al. 2000; MOEHS et al. 2001). N-fertilization had no significant influence on β-carotene, again indicating no clear differentiation of N-uptake and influence on the secondary metabolites as shown for ascorbic acid under conditions of high soil fertility (i.e. high amount of available soil-borne nitrogen) and retarded or limited nitrogen mineralisation from horn splinters. Interaction between cultivar and fertilization was not significant. Only the cultivars 'Green Zebra' and 'Lukullus' showed low concentration of lutein. The N-fertilization had no significant influence on the lutein content.

Conclusion and Future Research

Our investigations have shown that contents of health-promoting compounds (vitamin C, lycopene and β-carotene) were a clear function of the cultivars chosen. Highest concentration of vitamin C, lycopene and β-carotene were determined for the cultivars 'Lukullus' and 'Auriga'. Each multicoloured cultivar has its own typical and attractive properties including, in addition to phytochemical contents, the visual aspects colour and shape. Future studies are necessary to determine whether the weaknesses of a specific cultivar can be compensated for by the strengths of another different cultivar when these cultivars are mixed and not sold separately, and whether consumer acceptance can thus be raised. Regional markets will likely be the main points of sale, and processing of cultivar mixtures will need to be encouraged through appropriate promotion measures. No clear effect of additional N applied as horn splinters has been determined for nitrogen uptake and other phytochemicals indicating a *per se* high availability of soil-borne nitrogen and/or limitations for nitrogen mineralised from horn splinters. Future studies with a broader spectrum of different amounts of nitrogen readily available or mineralised with retardation have to be performed in order to assess the effects of these N-sources on the different secondary plant metabolites.

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