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Opportunities of Reduced Nitrogen Supply for Productivity, Taste, Valuable Compounds and Storage Life of Cocktail Tomato

Lilian Schmidt ^{1,2,*} and Jana Zinkernagel ²

¹ Soil Science and Plant Nutrition, Hochschule Geisenheim University, Von-Lade-Strasse 1, 65366 Geisenheim, Germany

² Vegetable Crops, Hochschule Geisenheim University, Von-Lade-Strasse 1, 65366 Geisenheim, Germany; Jana.Zinkernagel@hs-gm.de

* Correspondence: Lilian.Schmidt@hs-gm.de

Abstract: Vegetable production requires high nutrient input for ensuring high quality and high yield. As this is ecologically disadvantageous, it is necessary to determine if nitrogen (N) fertilization can be reduced without negative effects on productivity. For quality reasons, the effects of reduced N supply on taste, valuable compounds and storage life must be elucidated in parallel. This study examines whether reducing the N supply of cocktail tomatoes by 50% to recommendations affects the yield and quality of tomato fruits. Three varieties with different skin colors, yellow-orange ('Apresa'), red ('Delioso') and brown ('Bombonera'), were grown in soil in a greenhouse and harvested at the red-ripen stage. Quality parameters were assessed at harvest and after eight-day storage. Total yield decreased exclusively with 'Bombonera' due to reduced fruit weight. Firmness of the fruit pulp, concentrations of minerals, soluble solid contents, total acidity, total phenolics and liposoluble pigments of fruits were not influenced. However, storage affected chemical compositions positively, as shown by increased antioxidants. Descriptive sensory analyses revealed no impact of reduced N supply. From the perspective of the yield, quality and shelf life of fruits, reducing the N supply by 50% offers opportunities for the three cocktail tomato varieties in soil cultivation.

Keywords: tomato; product quality; nitrogen; shelf life; carotenoids; antioxidants; taste



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1. Introduction

Nitrogen (N) is the most important mineral element for plants and, as a constituent of amino acids and proteins, often limiting for their growth processes [1]. In the context of plant production, N influences the appearance and internal quality of plant products, their yield and their shelf life. The supply of N is therefore decisive for the quality of food crops; the application of fertilizer in crop cultivation is essential for profitable plant production. In particular, the intensive cultivation of vegetable crops involves high amounts of N fertilizer (as well as other nutrients) and often results in an excessive N supply [2]. This implies that vegetable production often does not meet the requirements of environmental legislations for good ecological water status (e.g., the EU Nitrates Directive or the EU Water Framework Directive). Vegetable production is, like no other, faced with the trade-off between ecological and economic demands. This adds to the importance for human health and global diet. Increasing the efficiency of nutrient supplies, especially nitrogen, is therefore essential for sustainable food production.

There is no general optimum for the nitrogen supply of vegetable crops; it depends on, among others, the production target (e.g., plant organ, quality). Consequently, the potential for a reduction in the N supply also varies among species and production systems. Generally, low N availability is known to decrease photosynthetic activity and thus plant growth [1]. Besides this, there are impacts on the secondary metabolism which can result

in decreased synthesis of N-based compounds such as alkaloids and increased synthesis of C-based secondary metabolites [3]. A limited N supply may consequently increase crops' quality, especially in terms of phytonutrients that are potentially beneficial for human health. In fact, a low N supply of plants is one possible way to achieve enrichment of secondary metabolites, as shown for several vegetable crops such as lettuce [4], and for medicinal plants such as chamomile [5]. The enrichment of secondary metabolites while maintaining high yield and quality may be particularly important for crops that are consumed in large quantities. Tomato offers great potential in this regard, as it is the most widely grown and consumed vegetable in the world [6]. A serving of tomatoes (defined as around 200 g of fresh tomatoes) covers the recommended dietary allowances of several mineral elements and, to a larger extent, those of vitamin C [7]. Due to the high consumption and concentration of many valuable compounds such as carotenes and polyphenols, tomato fruits are important sources of further secondary plant metabolites and can improve the intake of antioxidants in the human diet [8]. Carotenes such as lycopene and β -carotene are strong antioxidants and may reduce the risks for some cancers and coronary heart diseases, as well as those for diabetes and osteoporosis [9–11]. Polyphenols (such as flavonoids) are known to have antioxidant effects as well. Several studies suggest that polyphenols may protect against coronary heart diseases, counteract cancer development and prevent neurodegenerative diseases [12]. Additionally, tomato fruits are relevant sources of other antioxidants, such as ascorbic acid and vitamin E. An increase in the concentration of these secondary compounds in tomato fruits is therefore favorable for human health.

The internal quality and the taste of tomato fruits can be improved by agronomic strategies such as moderate salinity stress or modulated mineral nutrition [13]. When subjected to repeated short-term nitrogen limitation, tomato leaves contain increased flavonol glycoside concentrations [14]. Evidence for the transferability from tomato leaves to the fruits is still pending. Reduced N supply is known to cause an increase in soluble sugars and a decrease in acid concentrations of tomato fruits [15]. This may improve tomatoes' taste as sugars and acids are the major constituents of the taste experience [16]. Moreover, the N supply may alter other flavor components such as the volatile organic compounds of tomato fruits [17].

In tomato seedlings grown under N deficiency, shikimate and soluble sugars increased in the xylem sap, which suggests that the biosynthesis of phenolic substances might be favored [18]. In fact, increased concentrations of phenolic compounds in fruits resulted from reduced N supply at transplant or anthesis of greenhouse-grown tomatoes, albeit there were little effects on the carotenoid concentration [19].

However, the positive effects of reduced N supply on the secondary metabolism have not been found reliably. Bénard et al. [15] showed little effects of reduced N supply on secondary metabolites such as phenolic compounds, ascorbic acid and carotenoids in tomato fruits. Studies with two lycopene-rich tomato varieties revealed no impacts of reduced N supply on yield, firmness, valuable compounds and sensory properties of the fruits [20]. In contrast, Wang et al. [17] observed close relationships between N application rates and flavor parameters such as titratable acidity, soluble sugars, soluble solids, volatile compounds and fruit firmness in cherry tomatoes. Nevertheless, studies on quality and sensory properties of tomatoes grown with reduced N supply are rare, especially with the focus on shelf life.

This study aims at closing this gap in the knowledge. In a one-year trial, it was tested whether a reduction in the N supply by 50% of the recommended amount [21] impacts the yield and quality of tomato fruits. Fertilizers' reduction was performed at the beginning of fruit ripening to minimize the impact on fruit yield while maximizing the impact on fruit quality. Three varieties of cocktail tomato, differing in skin color, were chosen for the experiments since small-fruited and highly aromatic tomatoes are increasingly demanded by consumers. Quality parameters of the tomatoes were assessed after harvest and after eight-day storage.

We hypothesized that the reduced N supply (i) has no impact on the yield, firmness and color of tomato fruits, (ii) results in better taste due to the altered sugar-to-acid ratio of the fruits and (iii) enhances the nutritional quality of the fruits by increasing their antioxidant content.

2. Materials and Methods

2.1. Experimental Design and Plant Material

Three one-factorial experiments with the factor “N supply” and two factor levels, control (con) and reduced (Nred) N supplies, were performed for three cocktail tomato varieties separately in a greenhouse in Geisenheim, Germany. The experiments were set up in a randomized block design with four repetitions. Each repetition (plot) included ten plants. Subsequently, a storage experiment was carried out with the harvested fruits. Quality parameters were measured immediately after harvest and after storage. Hypotheses were tested with two-factorial analysis with the factors “N supply” and “storage”.

For the greenhouse experiment, seeds of the tomato varieties ‘Delioso’ (red color; Rijk Zwaan Welter GmbH, Welter, Germany), ‘Aprisa’ (orange color; Hild Samen GmbH, Marbach am Neckar, Germany) and ‘Bombonera’ (green-brownish color; Uniseeds Select B.V., Bleiswijk, The Netherlands) were sown into the substrate (Floradur A; BayWa AG, München, Germany) at the beginning of April 2012. The seedlings were picked and transferred into Ø 10 cm pots containing “Einheitserde ED Koko 1+1” (Einheitserdewerke Werkverband e.V., Sinntal-Altengronau, Germany) 14 d later. The transplants were watered and fertilized (Ferty 2 Mega, Planta Düngemittel GmbH, Regenstauf, Germany) as required. At the beginning of May 2012, the transplants (30 days old, BBCH stage 103) were planted into the soil (sandy loam) in the greenhouse with 2.8 plants m^{-2} . Plants of each tomato variety were planted onto eight randomly arranged plots (9.6 m^2 each with 10 plants per plot).

2.2. Cultivation and N Supply

The “con” treatment received weekly N supplies of 3 g N m^{-2} , according to the recommendations for fertigated tomatoes [21]. For the “Nred” treatment, the weekly N supply was reduced by 50% at the onset of fruit ripening, yielding 1.5 g N m^{-2} per week.

Until the differentiated N application, the fertilizer “Ferty 2 Mega” was applied (1 g N m^{-2} per week; Hauert HBG Dünger AG, Grossaffoltern, Switzerland). Starting mid-May 2012, the N dose was increased to 2 g N m^{-2} per week until early June 2012. At the breaker stage, when the fruits started to change color (early June 2012), the N control plots received 3 g N m^{-2} per week (composed of Ferty Basis 1, calcium nitrate and ammonium sulphate; Planta Düngemittel GmbH). The reduced N treatments received 50% less N, yielding 1.5 g N m^{-2} per week (composed of Ferty Basis 1 and calcium nitrate; Beiselen GmbH, Ulm, Germany). In total, plants received 360 (con) and 210 (Nred) kg N ha^{-1} throughout the growing season. Albeit this represents a reduction in total N application of approximately 42%, this is further regarded as a 50% reduction.

The fertilizer was applied with the irrigation water as fertigation. Each plant was supplied by drip irrigation (two drippers per plant, with 0.85 L h^{-1}), if soil tension fell below -9 kPa (measured at 20 cm soil depth by tensiometers). The application of water and fertilizer was controlled by the KLIWADU[®] software (developed by Michael Beck, Hochschule Weihenstephan-Triesdorf, Germany).

Pollination of the flowers was ensured by regular placement of bumblebees (re-natur GmbH, Stolpe, Germany) in the greenhouse. Plant protection was carried out by application of beneficial insects (*Amblyseius cucumeris* and *Encarsia formosa*, all from re-natur GmbH) when required.

The radiation inside the glass-covered greenhouse reached 724.7 kW m^{-2} during the cultivation period. The temperature regime throughout the cultivation season in the greenhouse is listed in Table 1.

Table 1. Temperature regime throughout the cultivation season.

Cultivation Stage	Minimum Day Temperature (°C)	Minimum Night Temperature (°C)	Temperature Set Point for Ventilation (°C)
Sowing till germination	24	20	26
Germination till planting	22	18	26
First week after planting	24	20	26
From second week after planting	20	16	22

2.3. Harvest

Mature tomato fruits were harvested at the fruit ripening stage “eating ripeness”, which is chemometrically described as “red ripe” for red cultivars [22]. The different colored varieties were harvested analog to this. The harvest was carried out twice a week lasting from the end of June 2012 (week 26, corresponding to BBCH stage 801) to early September 2012 (week 36). The tomato fruits from each experimental plot were sorted into marketable and non-marketable fractions (broken, blossom end rot, green shoulder, soft, fissured, unfertilized, others). Fresh matter of tomato fruits was determined per plant and plot.

2.4. Storage

At least 12 marketable, similarly sized fruits from each experimental treatment were kept in a cold storage at 12 °C in the dark (CS 0300 T, Viessmann Kühleysteme GmbH, Hof/Saale, Germany). The fruits were stored in plastic boxes in single layers and covered with plastic film to prevent water loss. After eight days, the fruits were removed from the cold storage for further analysis and sensory evaluations. During the course of the harvesting season, the storage experiment was repeated five times.

2.5. Spectroscopic Measurements

Non-invasive spectroscopic measurements were performed with the Multiplex® 3.6 device (Force-A, Orsay Cedex, France). Three marketable fruits from each plot were randomly chosen. Measurements were conducted in configuration 4 using red, blue, green and UV excitation at the bottom of the fruit. Parameters of interest were SFR_G (indicating the chlorophyll content), FLAV (indicating the concentration of flavonoids) and ANTH_RG (indicating the anthocyanin content) [23]. Averages of the indices of the fruits per plot were calculated for each date separately.

2.6. Firmness of the Fruit Pulp

For assessing the firmness of the fruit pulp, a single measurement was taken with the HHP-2001 device (Heinrich Bareiss Prüfgerätebau GmbH, Oberdischingen, Germany). A 0.25 cm² test anvil was pressed against the bottom of the fruit and after one second, resistance readings were taken. Average values per plot and measuring date were calculated.

2.7. Sampling and Chemical Analyses

After the spectroscopic measurements and the determination of fruit pulp firmness, the fruits were weighed, cut and dried at 60 °C. After three days, the dry matter was determined. The dried tomato fruits were sampled for analysis of minerals. For other chemical analyses, an aliquot of approximately 1000 g of fruits per plot was sampled and stored at −20 °C until further analyses.

Dried tomato samples were ground to a fine powder. For determination of mineral concentrations, an aliquot of the sample was extracted according to the Kjeldahl procedure (TURBOTHERM, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). The extracts were filtered, and the content of N, P K, Ca, Mg, Fe, Zn, Mn and Cu was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SPECTRO ARCOS, SPECTRO Analytical Instruments, Kleve, Germany).

For further analyses, the frozen fruit material was thawed at room temperature for 12 h and then homogenized with commercial kitchen blenders. Aliquots of this homogenate were taken for carotenoid analysis. The remaining homogenate was centrifuged at 4500 rpm for 10 min (ROTANTA 460 RS, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The supernatant was filtered, and the resulting juice was kept deep-frozen at -20°C until analysis of its composition.

The total content of liposoluble pigments (mainly carotenoids) from the tomato homogenate was extracted in acetone/hexane with addition of sodium sulphate and calcium carbonate (both of Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The extracts were filtered (syringe filter ROTILABO PTFE 0.2 μm , Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and the concentration of the liposoluble pigments was determined spectrophotometrically at 450 nm as equivalents of β -carotene. Calibration curves were conducted using β -carotene (Merck KGaA, Darmstadt, Germany) as a standard.

The juice was thawed for 3 h at room temperature prior to analysis of its composition. The following parameters were assessed according to the standards of the International Fruit Juice Union (Paris): soluble solids (refractometric analysis), conductivity (conductometric analysis), pH (potentiometric analysis), total acidity (expressed as citric acid, potentiometric analysis after titration to pH 8.1 with 0.3 M NaOH; Titroline alpha, Schott, Mainz, Germany), concentration of ascorbic acid (automatic potentiometric titration with SCHOTT TitroLine alpha plus, SI Analytics, Mainz, Germany). The contents of total phenolics were estimated by the Folin–Ciocalteu assay, and the antioxidative capacity was estimated by a photometric assay (as Trolox equivalent antioxidative capacity, TEAC). The color space parameters a^* and b^* were assessed by reflection readings of the juice against a white standard (CR 200, Konica Minolta Sensing Europe B.V., Bremen, Germany) and the “chroma” value was calculated according to D’Souza et al. [24].

All chemical analyses were performed as duplicates.

2.8. Sensory Evaluation

The effects of reduced N supply and the variety on the sensory attributes taste (sweetness, sourness, typically tomato, foreign taste, watery), consistency (firmness of fruit pulp, firmness of the peel, mealiness), aftertaste (intensity, foreign) and overall liking were assessed by quantitative descriptive sensory evaluation. The intensity of the attributes was rated on a scale from 1 (non-existent) to 9 (very strong). For assessing possible differences between the treatments before and after storage, extended triangle tests (as forced choice tests) were used. An additional question was posed concerning the preference of one sample. All samples were coded with three-digit random numbers by the “FIZZ Data Acquisition und Calculation” software (Biosystèmes, Couternon, France). For sensory evaluations, the tomato fruits were cut into four parts. Each sample consisted of six quarters from six different tomato fruits per treatment. The samples were presented in random order to the panelists. The sensory panel comprised 15 to 17 trained people that were asked to take bits from each tomato piece to obtain sensory impressions of mixed samples. The panelists received water and matzo (Dr. Schär AG/SPA, Burgstall, Italy) to clear their palates between the sensory evaluations of two samples.

2.9. Statistics

The impact of N supply, cold storage and their interaction was assessed by two-way-ANOVA for each tomato variety separately, if the conditions of normal distribution (tested with the Shapiro–Wilk test) and variance homogeneity (tested with the Levené test) were met. In case of no interactions between the factors N supply and storage, differences between the treatments were analyzed by Tukey pairwise comparison. If the data were not normally distributed or homogeneity of variances was not observed, the Kruskal–Wallis test followed by Mann–Whitney pairwise comparison was performed.

Impacts of the N supply on fruit yield data and on the content of mineral elements in the fruits were assessed by a *t*-test. All statistics of yield and quality data were performed (with $\alpha = 0.05$) using the PAST software (version 3.01, [25]).

The sensory data were analyzed by calculating averages for the single attributes and testing for significant differences with the Friedman test using the “FIZZ Data Acquisition und Calculation” software. When significant differences were observed, the means were separated by using least significant difference at $\alpha = 0.1$. The triangle tests were analyzed by comparing the percentage of correct answers to the probability of the corresponding binomial law.

3. Results

3.1. Yield

The total yield (=marketable and non-marketable fruits) was not significantly impacted by the N supply for the varieties ‘Apresa’ and ‘Delioso’ (Table 2). In contrast, ‘Bombonera’ had lower total fruit yields when supplied with reduced N amounts. Nevertheless, there were tendencies of a larger proportion of marketable fruits in the Nred treatment of ‘Bombonera’ ($p = 0.054$). For ‘Delioso’ and ‘Bombonera’, the fruits of the control treatments were significantly heavier compared to the Nred treatment (Table 2). This was not observed for the variety ‘Apresa’.

Table 2. Total yield, proportion of marketable fruits and weight of single fruits in three tomato varieties with normal (con) and reduced (Nred) N supplies. $N = 4 \pm \text{SD}$. Asterisks in the “Nred” line indicate significant differences between the “con” and the “Nred” treatment for the respective tomato varieties (*t*-test at $\alpha = 0.05$).

Variety	N Supply	Total Yield (kg m ⁻²)	Proportion of Marketable Fruits (%)	Single Fruit Weight (g)
‘Delioso’	con	4.27 \pm 0.32	98.87 \pm 0.51	39.67 \pm 1.00
	Nred	3.98 \pm 0.38	98.14 \pm 0.86	36.75 \pm 1.42 *
‘Apresa’	con	3.13 \pm 0.51	78.40 \pm 0.71	33.88 \pm 1.31
	Nred	2.91 \pm 0.04	82.72 \pm 2.68 *	32.77 \pm 0.97
‘Bombonera’	con	4.10 \pm 0.07	36.59 \pm 4.94	51.97 \pm 1.18
	Nred	3.40 \pm 0.23 *	50.31 \pm 10.39	48.67 \pm 1.71 *

3.2. External Quality Parameters

The firmness of the fruit pulp did not differ between the control and the Nred treatments of the three tomato varieties, both before and after storage (Table 3). However, tendencies of differences in the fruit pulp firmness due to different N supplies were observed in ‘Bombonera’ immediately after harvest ($p = 0.08$). In all varieties and N supply treatments, the fruit pulp firmness decreased significantly after storage.

The spectroscopic parameters FLAV, ANTH_RG and SFR_G are indices for the contents of different pigments of the fruit skin. Immediately after harvest, exclusively ‘Bombonera’ showed differences due to the N supply, with lower ANTH_RG indices in Nred compared to control fruits (Table 3). After storage, ‘Apresa’ fruits that were grown with reduced N supply had lower FLAV indices as compared to fruits grown under control conditions.

Fruits of the varieties ‘Delioso’ and ‘Bombonera’ did not show differences in the FLAV index between control and Nred treatments after storage. All indices were decreased after the storage in fruits of ‘Delioso’ grown under control and Nred conditions. The FLAV index was decreased after the storage in ‘Apresa’ control and Nred fruits but was not affected in ‘Bombonera’ fruits grown under both N supplies. In contrast, ANTH_RG was increased after storage in both control and Nred fruits of ‘Bombonera’ but was not affected in ‘Apresa’. The index SFR_G was lowered after storage in ‘Apresa’ grown under reduced N supply, while there was no impact for ‘Apresa’ that was grown under control conditions.

Table 3. External quality parameters assessed by non-invasive measurements of tomato fruits of three cocktail varieties grown with normal (con) and reduced (Nred) N supplies, immediately after harvest (before storage) and after 8 days of storage at 12 °C (after storage). Means of $N = 22$ to 36 for firmness, and $N = 31$ to $58 \pm SD$ for FLAV, ANTH_RG and SFR_G. Different letters indicate significant differences due to storage within a variety and N supply. Asterisks indicate significant differences due to N supply within a variety and storage (two-way ANOVA with Tukey pairwise comparison, or Kruskal–Wallis test with Mann–Whitney pairwise comparison; at $\alpha = 0.05$).

Parameter (unit)	Storage	‘Delioso’ con	‘Delioso’ Nred	‘Apresa’ con	‘Apresa’ Nred	‘Bombonera’ con	‘Bombonera’ Nred
Firmness (kg cm ⁻²)	Before	50.8 ± 4.3 a	50.7 ± 6.2 a	43.8 ± 4.8 a	43.9 ± 8.2 a	61.9 ± 6.0 a	58.3 ± 7.8 a
	After	46.1 ± 5.8 b	46.9 ± 4.2 b	40.9 ± 4.7 b	40.4 ± 5.4 b	53.7 ± 7.6 b	53.4 ± 8.0 b
FLAV	Before	1.48 ± 0.12 a	1.51 ± 0.15 a	1.62 ± 0.11 a	1.60 ± 0.10 a	2.19 ± 0.14 a	2.19 ± 0.06 a
	After	1.27 ± 0.16 b	1.26 ± 0.13 b	1.48 ± 0.12 b	1.44 ± 0.12 b *	2.19 ± 0.06 a	2.17 ± 0.07 a
ANTH_RG	Before	1.42 ± 0.07 a	1.40 ± 0.10 a	1.21 ± 0.12 a	1.20 ± 0.10 a	1.15 ± 0.16 a	1.22 ± 0.14 a *
	After	1.34 ± 0.14 b	1.33 ± 0.12 b	1.23 ± 0.07 a	1.20 ± 0.06 a	1.32 ± 0.13 b	1.34 ± 0.10 b
SFR_G	Before	1.26 ± 0.07 a	1.24 ± 0.07 a	1.35 ± 0.09 a	1.36 ± 0.11 a	1.28 ± 0.16 a	1.23 ± 0.12 a
	After	1.21 ± 0.08 b	1.20 ± 0.07 b	1.33 ± 0.08 a	1.31 ± 0.08 b	1.27 ± 0.14 a	1.26 ± 0.15 a

3.3. Chemical Composition of Tomato Fruits

The concentrations of the elements N, P, K, Mg, Fe, Zn, Mn and Cu differed between the varieties (data not shown) but were not influenced by N supply (Table 4).

Table 4. Mineral composition of tomato fruits at harvest. The concentrations of the mineral elements are expressed on a fresh weight base. Mean of $N = 4 \pm SD$. No significant differences between the con and the Nred treatment for the respective tomato varieties were observed (t -test at $\alpha = 0.05$).

Mineral	‘Delioso’ con	‘Delioso’ Nred	‘Apresa’ con	‘Apresa’ Nred	‘Bombonera’ con	‘Bombonera’ Nred
N (g kg ⁻¹)	2.90 ± 0.09	2.71 ± 0.23	3.15 ± 0.20	3.07 ± 0.35	2.87 ± 0.20	2.73 ± 0.07
P (g kg ⁻¹)	0.44 ± 0.02	0.44 ± 0.04	0.55 ± 0.02	0.54 ± 0.03	0.42 ± 0.04	0.40 ± 0.01
K (g kg ⁻¹)	5.05 ± 0.22	4.90 ± 0.41	6.36 ± 0.59	5.86 ± 0.46	5.29 ± 0.44	5.16 ± 0.28
Ca (g kg ⁻¹)	0.22 ± 0.03	0.21 ± 0.02	0.27 ± 0.06	0.21 ± 0.01	0.27 ± 0.09	0.20 ± 0.01
Mg (g kg ⁻¹)	0.19 ± 0.00	0.19 ± 0.02	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.02	0.21 ± 0.01
Fe (mg kg ⁻¹)	5.88 ± 0.58	6.11 ± 0.67	7.78 ± 0.83	7.82 ± 1.75	5.91 ± 1.03	6.10 ± 0.73
Zn (mg kg ⁻¹)	3.60 ± 0.29	3.85 ± 0.53	4.40 ± 0.17	4.04 ± 0.40	5.34 ± 3.39	3.47 ± 0.09
Mn (mg kg ⁻¹)	1.12 ± 0.07	1.20 ± 0.09	1.41 ± 0.07	1.35 ± 0.10	1.29 ± 0.16	1.32 ± 0.09
Cu (mg kg ⁻¹)	1.79 ± 0.18	1.79 ± 0.26	2.16 ± 0.26	1.91 ± 0.26	1.46 ± 0.18	1.56 ± 0.08

The concentrations of total phenolics and total contents of liposoluble pigments were not affected by the N supply, both before and after storage, in all tomato varieties. Nevertheless, the eight-day storage of the fruits of ‘Delioso’ and ‘Apresa’ significantly increased their total content of liposoluble pigments (Table 5).

Ascorbic acid concentrations were higher in ‘Delioso’ fruits grown with reduced N supply both before and after storage. No changes in the ascorbic acid contents were observed in the fruits due to storage.

The antioxidant capacity (as TEAC) was higher in ‘Delioso’ fruits grown with reduced N supply before storage, but no differences between control and Nred fruits were observed after storage.

In general, the content of soluble solids, the pH and the total acidity of tomato fruits have impacts on their taste. The three parameters were not affected by the N supply before and after storage in fruits of all varieties here (Table 6). Storage had no effect on the content of soluble solids in all tested tomato samples but increased the pH in fruits of ‘Delioso’ and ‘Apresa’ grown under control and Nred conditions. There were no effects on the total acidity, except ‘Delioso’ under Nred conditions, where a decrease in total acidity was

observed after storage. The fruits of ‘Bombonera’ showed no changes in the content of soluble solids, total acidity and pH due to N supply and storage.

Table 5. Mean and standard deviation of the antioxidant status of fruits of three cocktail tomato varieties grown with normal (con) and reduced (Nred) N supplies, immediately after harvest (before storage) and after 8 days of storage at 12 °C (after storage). The antioxidant parameters are expressed on a fresh weight base. $N = 3-4$. Different letters indicate significant differences due to storage within a variety and N supply. Asterisks indicate significant differences due to N supply within a variety and storage (Two-Way ANOVA with Tukey pairwise comparison, or Kruskal-Wallis test with Mann-Whitney pairwise comparison; at $\alpha = 0.05$).

Parameter (unit)	Storage	‘Delioso’ con	‘Delioso’ Nred	‘Apresa’ con	‘Apresa’ Nred	‘Bombonera’ con	‘Bombonera’ Nred
Total phenolics (mg kg ⁻¹)	Before After	355.5 ± 32.5 a 351.4 ± 24.8 a	370.5 ± 35.3 a 382.5 ± 33.6 a	410.3 ± 14.2 a 413.5 ± 20.6 a	411.4 ± 17.7 a 420.5 ± 36.5 a	343.3 ± 85.2 a 346.7 ± 5.4 a	369.0 ± 54.1 a 355.9 ± 13.5 a
Total content of liposoluble pigments (mg kg ⁻¹)	Before After	4.60 ± 0.20 a 5.75 ± 0.48 b	4.45 ± 0.25 a 6.18 ± 0.26 b	3.31 ± 0.24 a 4.19 ± 0.19 b	3.00 ± 0.21 a 4.05 ± 0.16 b	7.28 ± 0.15 a 7.05 ± 0.20 a	7.04 ± 0.42 a 7.11 ± 0.13 a
Ascorbic acid (mg kg ⁻¹)	Before After	170.3 ± 19.9 a 185.8 ± 15.6 a	203.1 ± 12.0 a * 216.4 ± 15.9 a *	203.1 ± 64.1 a 186.8 ± 17.8 a	191.2 ± 26.1 a 188.1 ± 23.1 a	173.1 ± 26.6 a 157.3 ± 2.8 a	167.8 ± 30.7 a 147.9 ± 7.2 a
TEAC (mmol kg ⁻¹)	Before After	3.10 ± 0.08 a 3.28 ± 0.31 a	3.35 ± 0.10 a * 3.57 ± 0.19 b	3.40 ± 0.27 a 3.79 ± 0.17 b	3.55 ± 0.30 a 3.91 ± 0.28 a	3.63 ± 0.32 a 3.36 ± 0.06 a	3.67 ± 0.31 a 3.55 ± 0.32 a

Table 6. Composition and internal color (expressed as “chroma”) of tomato fruits of three cocktail varieties grown with normal (con) and reduced (Nred) N supplies, immediately after harvest (before storage) and after 8 days of storage at 12 °C (after storage). Mean of $N = 3-4 \pm SD$. Different letters indicate significant differences due to storage within a variety and N supply (two-way ANOVA with Tukey pairwise comparison, or Kruskal-Wallis test with Mann-Whitney pairwise comparison; at $\alpha = 0.05$).

Parameter	Storage	‘Delioso’ con	‘Delioso’ Nred	‘Apresa’ con	‘Apresa’ Nred	‘Bombonera’ con	‘Bombonera’ Nred
Soluble solids (°Brix)	Before After	5.40 ± 0.50 a 5.26 ± 0.37 a	5.44 ± 0.40 a 5.38 ± 0.49 a	5.73 ± 0.18 a 5.68 ± 0.23 a	5.42 ± 0.46 a 5.48 ± 0.37 a	5.53 ± 1.23 a 5.64 ± 0.07 a	5.88 ± 0.76 a 5.74 ± 0.18 a
pH	Before After	4.15 ± 0.02 a 4.24 ± 0.01 b	4.16 ± 0.02 a 4.25 ± 0.03 b	4.18 ± 0.02 a 4.25 ± 0.01 b	4.16 ± 0.03 a 4.23 ± 0.03 b	4.22 ± 0.05 a 4.22 ± 0.01 a	4.18 ± 0.03 a 4.19 ± 0.04 a
Total acidity (g kg ⁻¹)	Before After	4.13 ± 0.38 a 3.52 ± 0.27 a	4.09 ± 0.21 a 3.57 ± 0.12 b	4.96 ± 0.47 a 4.62 ± 0.27 a	5.03 ± 0.22 a 4.31 ± 0.39 a	4.08 ± 0.88 a 4.01 ± 0.03 a	4.01 ± 0.54 a 3.95 ± 0.13 a
Chroma	Before After	22.14 ± 0.33 a 19.10 ± 11.18 a	21.85 ± 1.83 a 19.82 ± 11.92 a	24.34 ± 1.31 a 4.83 ± 3.05 b	25.37 ± 1.01 a 4.47 ± 2.93 b	14.16 ± 4.97 a 10.33 ± 5.83 a	10.28 ± 6.85 a 19.02 ± 1.58 a

The color index “chroma” was not significantly affected by both the N and storage treatments in all varieties of cocktail tomatoes (Table 6). Exclusively, ‘Bombonera’ grown with reduced N tended to have higher “chroma” values after storage compared to the control ($p = 0.067$). Due to storage, ‘Apresa’ grown under controlled and reduced N supplies showed a significant decrease in “chroma”.

3.4. Sensory Evaluation

Descriptive sensory evaluation revealed differences in the attributes sweetness, watery, foreign aftertaste, firmness of the fruit pulp and the peel, typically tomato and overall liking in the three varieties (Figure 1a, statistical data not shown). Differences due to N supply were not observed for all attributes.

In triangle tests, the sensory panel was not able to discriminate significantly between controls and Nred samples immediately after harvest (Figure 1b). However, there was a trend for sensory discrimination between control and Nred treatments in ‘Bombonera’ fruits ($p = 0.08$). After storage, a significant difference between controls and Nred fruits was detected for ‘Bombonera’. However, there was no preference for either controls or Nred fruits.

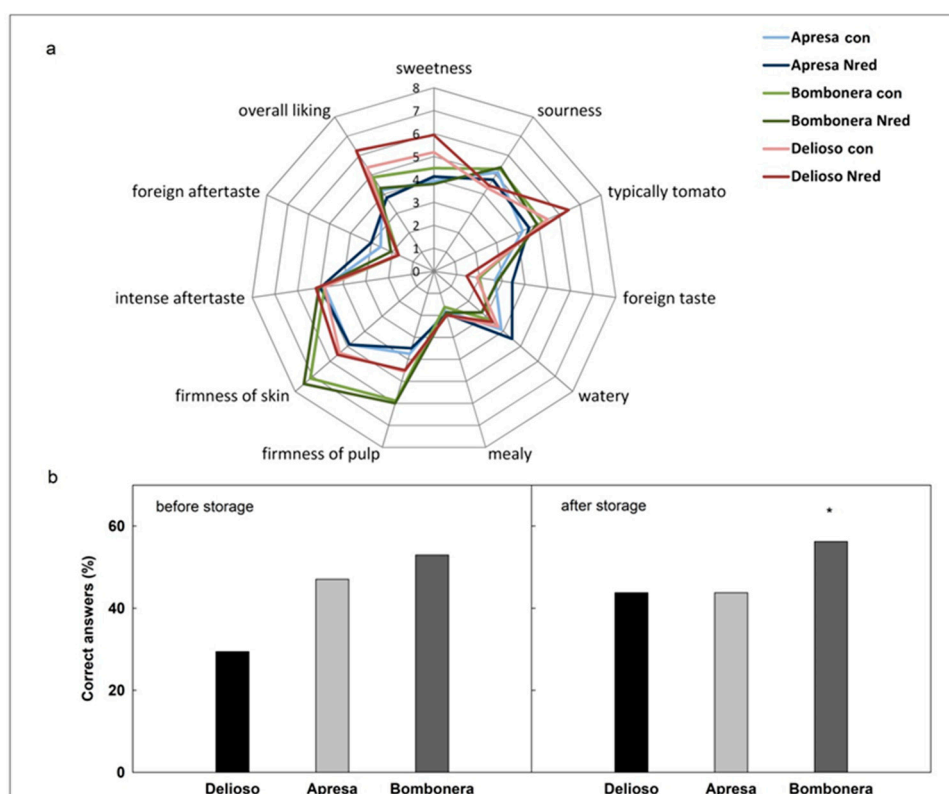


Figure 1. Sensory attributes of three tomato varieties grown with normal (con) and reduced (Nred) N supplies (a) and results of triangle tests (b). In (b), the “correct answers” indicate the relative abundance of correct distinctions between Nred and control samples. Asterisks in (b) show significant sensory distinctions between controls and Nred samples. $N = 16$ for (a). $N = 16$ to 17 for (b).

4. Discussion

Reducing the nitrogen supply in vegetable production aims at lowering the nitrate leaching into the groundwater and thus negative impacts to the environment. However, a sub-optimal nitrogen supply is known to alter the metabolism of plants and thereby their allocation of resources. As a consequence of this abiotic stress, photosynthetic and growth processes might be impeded, which could lower the crop yield [1]. In order to achieve environmentally sound fertilization while maintaining yield and quality, this experiment examined for tomato crops, cultivated in soil in a greenhouse, whether this balance could be achieved by reducing the N supply by 50% at the onset of fruit ripening. In fact, the total yield of fruits of the cocktail tomato varieties ‘Delioso’ and ‘Apresa’ (Table 2) was not reduced, being in accordance with studies using similar N levels for soil-grown tomato plants to our experiment (210 vs. 360 kg N ha⁻¹) [20,26–30].

The lower single fruit weight did not affect the total yield of ‘Delioso’, whereas this was not the case for ‘Bombonera’ (Table 2). Lower yields due to lower fruit weight with reduced N supply were reported by Qu et al. [31] and Wang et al. [32], but they occurred at lower N levels than in our experiment. It can be concluded that ‘Bombonera’ is more sensitive to reduced N availability under sub-optimal growing conditions as there were only around 37% marketable fruits in the control treatments (Table 2). Therefore, the reduction in the N supply was even beneficial for ‘Bombonera’ as the trend of increased fractions of marketable fruits resulted in comparable marketable yields of control and reduced N-grown fruits [33]. This positive effect of reduced N supply on the marketable fruit fraction was even significantly larger in ‘Apresa’ (Table 2) and was also observed in other studies, for example, due to lower numbers of rotten fruits [26,34].

Similarly to the marketable fruit yield, there was no impact of N reduction by 50% at the onset of fruit ripening on the firmness of the tomato fruits (Table 3). Albeit the

fruit firmness decreased after storage in all varieties and treatments (as reported in other studies [35–38]), which—as consequence of decreased turgor and water loss by transpiration [39]—is a commonly known phenomenon of fruit ripening and senescence, there was no effect of the reduced N supply. This is surprising as N limitation is known to enhance the senescence processes of plants [1], but this is probably not true for fruit softening, as several other studies also report little effects on the firmness of tomato fruits [15,20,40–42].

Besides degradation of the cell wall polysaccharides, the ripening of tomato fruits is characterized by breakdown of chlorophylls in the peel [35] and concomitant increases in pigments such as carotenoids. These pigments possess antioxidative properties and scavenge reactive oxygen species which occur increasingly at the onset of fruit ripening [43], thereby accelerating senescence and thus being detrimental for the shelf life of tomatoes [44]. The concentrations of carbon-based carotenoids and flavonoids are known to increase in plant tissues with low N availability due to accumulation of carbohydrates that could not be used for growth processes [3]. This is supported by several studies that observed increasing concentrations in phenolic substances (i) in vegetative tissue [14,45–47], (ii) in fruits as a result of strongly reduced N supply (e.g., [19]), (iii) in soilless cultivation systems such as hydroponics (e.g., [7]) and (iv) at earlier developmental stages (at transplant, see [19]).

However, N supplies of 210 and 360 kg ha^{−1} did not impact the concentration of soluble solids and acidity after harvest in our experiment (Table 6), which is in accordance with other studies [20,26,32,42,48]. The fact that sweetness and acidity are the major constituents of the tomato taste experience, along with volatile aroma compounds and texture parameters [17,49], explains the lack of significant differences in the strength of selected attributes assessed by the sensory panel (Figure 1a).

As expected from the similar soluble solid contents of fruits of the control and reduced N treatments, suggesting that excess carbohydrates did not occur in the Nred fruits, neither differences in the concentrations of total phenolics and total contents of liposoluble pigments in the whole tomato fruits (Table 5), in the inner color index “chroma” (Table 6), nor in the index FLAV were found between control and Nred tomato fruits. The lack of differences between the index SFR_G of control and Nred fruits (Table 3) further suggests that the reduced N supply did not enhance chlorophyll breakdown and thus ripening and senescence processes. This is in accordance with other reports on the coloration of tomato fruits showing no effects of reduced N supply [15,41,50,51]. Similar to our findings, no impact of reduced N fertilization of tomato plants was observed on the content of the most important carotenoids in tomato fruits, lycopene, β -carotene and lutein [7,13,15,20,50,52].

In accordance with the very low effects of reduced N supply on phenolic substances (assessed by [19,20]), there were no impacts on the antioxidative capacity of water-soluble compounds (assessed as TEAC) of the fruits (Table 5), except ‘Delioso’ fruits. They showed higher TEAC values probably due to the higher contents of ascorbic acid (Table 5). Other studies showed that reductions in the N supply up to one third of the optimal level often had no effect on the vitamin C content of the tomato fruits [2,7,15,20,29,32]. Moreover, other results suggest that the antioxidative capacity of tomato fruits is little affected by the N supply [20,50,51].

Overall, there were very small effects of the reduced N supply on tomato fruit yield and quality. Even the N content of the fruits (Table 4) was not affected by the N supply, in the same way as the other mineral elements (Table 4), which is in accordance with other studies with a comparable or even more severe N reduction than in our experiment [26,29,40,42,53–55]. As proposed by Stefanelli et al. [3], reduced N contents in plants would be expected if the N supply to plants was low. However, the N content of the tomato leaves was not affected by the reduced N supply (data not shown), similarly to other reports of the N content in plants [40,53,54,56]. In other studies, there were no impacts of low N fertilizer levels on photosynthetic activity [57,58] and very little effects on the height and the above-ground biomass [27,40,53,56] of soil-grown tomato plants. This suggests that the reduced N supply in our experiment (210 kg N ha^{−1}) was not a limiting factor for the tomato plants in soil cultivation.

In fact, Hartz and Bottoms [28] stated that a “seasonal N rate of 200 kg ha^{−1} appeared adequate to maximize fruit yield”, which is supported by various studies of tomatoes [29,34,41,48,56,59]. Moreover, Zotarelli et al. [27] proposed that N application rates above 176 kg ha^{−1} are not beneficial for tomato fruit yields but increase the leaching of nitrate. According to the classification of Hartz and Bottoms [28], our control treatments (360 kg N ha^{−1}) received excessive amounts of N and the tomato plants in our Nred treatments still had adequate N supplies, which might explain the lacking or minimal effects on tomato yield and quality.

These conclusions apply to tomato plants grown in soil, while soilless cultivation of tomato plants seems to increase their sensitivity towards reduced N supply as several studies report decreased photosynthetic activity [2], plant biomass [14,15,46,60] and fruit mineral contents [7]. However, it is possible to overcome N deficiency and ensure high fruit yields of soilless-grown tomato plants by supplying N levels larger than 4.5 mmol L^{−1} [2,15,19].

The very little effects of the N supply reduction by 50% at the onset of ripening were even less apparent after the storage. Exclusively, the higher ascorbic acid concentrations in ‘Delioso’, the lower FLAV index of ‘Apresa’ and the different taste observations in ‘Bombonera’ after the storage period could be ascribed to low N supply. All other storage effects, such as reduced firmness, decreased acidity and concomitant increase in pH and increases in liposoluble pigments such as carotenoids, are common phenomena that occur during ripening and senescence of tomato fruits [38]. However, in contrast to other observations [38,55,61], no increases in total soluble solid contents were observed in the three cocktail tomato varieties of our study.

Nevertheless, there was a trend (before storage) or even the significant perception (after storage) of different sensory properties between fruits of ‘Bombonera’ grown with either optimal or reduced N supply (Figure 1b). This sensory discrimination between the N supply levels can be less attributed to the soluble solid content and the total acidity than to the volatile organic compounds. It is possible that the concentration and composition of these compounds in tomato fruits were altered during the storage [62] and thus resulted in significantly different sensory properties of control and Nred-grown fruits of ‘Bombonera’ (Figure 1b).

Fruit storage at 12 °C for eight days had no effects on the content of ascorbic acid of all tomato varieties and treatments (Table 5) but increased the antioxidative capacity, significantly or in trend, in fruits of ‘Apresa’ and ‘Delioso’ (Table 5). This suggests that storage at 12 °C for eight days contributed to maintaining or even enhancing the potential nutritional quality of the tomato fruits, regardless of the N supply during their cultivation.

5. Conclusions

Reducing the N supply by 50% upon the onset of fruit ripening had little or no effects on the yield and external quality of three cocktail tomato varieties grown in soil. Contrary to our expectation, the products of secondary metabolism were not elevated, presumably due to the only moderately perceived stress. Reduced N supply thus did not result in increased nutritional quality and improved taste at harvest. However, fruit quality showed no deterioration with storage due to the lower N supply. The good shelf life was partly accompanied by increases in antioxidants. Consequently, the amount of nitrogen fertilizer can be reduced for several tomato varieties in soil cultivation while maintaining the yield, quality, taste and shelf life of tomato fruits.

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