EFFECTS OF THE GROWING METHODS AND CONDITIONS ON THE LYCOPENE CONTENT OF TOMATO FRUITS

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Tomato is one of the most important sources of lycopene. The effect of water supply was investigated on lycopene content of Daniela F_1 tomato harvested in greenhouse. The lycopene content of fruits supplied with 50% of optimal water intake was higher (86.5 mg kg^{-1} f.w.) than that of fruits supplied with 100% of optimal water intake $(62.5 \text{ mg kg}^{-1} \text{ f.w.})$. Significantly higher lycopene content was observed in tomato harvested in greenhouse $(83.0 \text{ mg kg}^{-1} \text{ f.w.})$ than in field $(59.2 \text{ mg kg}^{-1} \text{ f.w.})$, at every harvesting time. The highest concentration of lycopene was detected in cherry tomato (77.4 mg kg⁻¹ f.w.) while Daniela F₁ with 59.2 mg lycopene per kg and Delfine F₁ with 69.6 mg lycopene per kg had significantly lower level (under field conditions).

Keywords: tomato (Lycopersicum esculentum), lycopene, greenhouse, varieties, temperature, water supply

Tomato (Lycopersicum esculentum) is the second largest group of vegetables cultivated in the world. Quantitatively the major components of tomato are water $(93–96%)$ and carbohydrates but it is rich in carotenoids, tocopherols, vitamin C, potassium and iron.

Ripe, red and sound tomatoes are desirable for customers. Colour is an important characteristic of the eating quality, for which pigments are responsible. In tomato fruit more than 21 pigments belonging to the carotenoid class have been identified. Red colour is awoken by lycopene that is the most abundant carotenoid (83%) in ripe tomatoes (LÓPEZ et al., 2001). Other sources of lycopene are watermelon, pink grapefruit, rosehips and guava, but these fruits are unimportant with respect to dietary supply. Further carotenoids found in tomato fruits are α -carotene, β -carotene, γ -carotene, phytoene, phytofluene, lutein, but their quantities are negligible (CURL, 1961). Lycopene is not utilised as provitamin A, unlike to β -carotene, but cyclization of the end groups in lycopene molecule leads to β -carotene formation in plant cells. Red tomatoes are richer sources of lycopene than the orange varieties. Deep-red colour of tomato is associated with high level of lycopene, while high β -carotene content is responsible for the orange colour (DAVIS, 1976). There are many variations in colour in different varieties and colour characteristics are under genetic control (GOODWIN,

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1986). However, in the period of fruit growing the carotenoid biosynthesis is influenced by environmental conditions. According to FARKAS (1994) lycopene production is inhibited when environmental temperature is above 32 °C.

The molecular structure of lycopene is an acyclic linear-chain unsaturated polyene hydrocarbon containing 13 double bonds, of which 11 are conjugated double bonds. Figure 1 represents the chemical structures of all-*trans* lycopene and β -carotene. Lycopene occurs in *cis* and *trans* configurations. All-*trans* variations, thermodynamically the most stable form, is found mostly (95%) in the fresh tomatoes (BARRETT $\&$ ANTHON, 2001). *Trans-to-cis* isomerisation has been observed during processing (heat treatments) and storage resulting in an unstable, energy-rich state (SHI $\&$ LE MAGUER, 2001).

Fig. 1. The molecular structure of all-trans lycopene and β -carotene

MANGELS and co-workers (1993) have focused on the antioxidant activity of lycopene such as quenching of singlet oxygen and scavenging of peroxyl radicals, which is ascribed to its molecular structure. Further abilities of lycopene are the induction of cell-to-cell communication and the cell growth control. These properties may play a role in the prevention of different cancer and heart diseases. Frequent tomato consumption could decrease the risk of carcinogenesis, atherosclerosis, cardiovascular diseases and stomach, colon, lung, prostate and ovary cancer (OLSON, 1986; MICOZZI et al., 1990; LEVY et al., 1995).

Average daily lycopene intake of about 3 mg lycopene per day per capita has been reported in the USA by SIES and STAHL (1998). The fresh fruits of common industrially produced tomato varieties contain $50-70$ mg kg⁻¹ lycopene (NIR et al., 1993), but higher amounts are found in some tomato products such as ketchup and tomato souce (up to 150 mg lycopene per kg). Lycopene intake with diet is detectable in human blood and

tissues. Lycopene level is in the range of $0-0.5$ μ mol $1-1$ plasma; tissue levels vary from 1 nmol g^{-1} wet weight in adipose tissue to up to 20 nmol g^{-1} wet weight in adrenals and testes (STAHL & SIES, 1992).

The aim of the present work was to evaluate the effects of different agricultural practices and conditions on the lycopene content of tomato fruits. In this study the effects of cultivation in greenhouse and open field stick production with respect to varieties, temperature and water supply were investigated.

1. Materials and methods

1.1. Plant material

Tomato fruits were grown and harvested on the test sites of Szent István University. The following varieties were investigated: Daniela F_1 , Delfine F_1 and cherry tomato (only on field).

In protected area (greenhouse) seeds were sown into propagation boxes on 9th January 2001. The planting of seedlings with 6–7 leaves was carried out on 27th March 2001. Plant density was about 2 plant $m⁻²$. The distance between the rows was 1.2 m and the distance between the plants was 0.4 m in the rows. Plants were arranged in randomised complete block (RBC) into four rows and from each variety ten plants got into the rows. So each variety was cultivated in four repetitions, from which two repetitions were supplied with 100% of the optimal water intake and the water supply of the others was only 50% of this quantity. The amount of water in totally and half supplied groups harvested on June 11th was 40.3 and 80.6 l per plant, while in groups harvested on July 11th was 62.8 and 125.61 per plant, respectively. Ecological conditions (temperature, relative humidity, photosynthetically active radiation PAR, expressed mol $m⁻²$) were determined by SKYE micrometeorological equipment (Fig. 2). The instrument measured the earlier mentioned factors six times per hour and calculated an average.

In the field by stick production, seeds were sown on 4th April 2001 and planted out on 8th May 2001. Seedlings were arranged in double (twin) rows with distance between the rows of 1.4 and 0.4 m and distance of 0.3 m between the plants. Each variety was cultivated in four repetitions. Moisture was quantified by Hellmann ombrometer and temperature was measured by Six type maximum minimum thermometer daily. The data are presented in Fig. 3.

In any case water and nutrients were doled out by drip irrigation during the cultivation period.

1.2. Fruit sampling

Test sites were harvested by hand. Three times during the growing season tomato fruits were randomly selected from the middle section of plants. Four samples from the picked

fruits of each variety were taken to be investigated. At least $3-4$ fruits from each repetition were washed, cut and mixed and the juice samples were stored frozen at -18 °C until analysis.

1.3. Chemicals

Lycopene was obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA), n-hexane, methanol and acetone were purchased from Merck KGaA (Darmstadt, Germany). All other chemicals and reagents were of analytical grade from Chemolab (Hungary).

1.4. Lycopene extraction and measurement

Lycopene from homogenised tomato was extracted with n -hexane-methanol-acetone (2:1:1) mixture containing 0.05% butylated hydroxy toluene (BHT). Water-free $Na₂SO₄$ was used to remove water traces of the upper part. Optical density of the hexane extract was measured spectrophotometrically at 500 nm against hexane blank (SADLER et al., 1990) by UV-VIS Spectrophotometer Lambda 3B (Perkin Elmer). Concentration of lycopene was calculated using specific extinction coefficient ($E_{1cm}^{1\%}$ 3150) (Merck & Co., 1989).

1.5. Statistical analysis

Experiments were conducted as a randomised complete block with two factors (harvesting time and water supply or harvesting time and varieties). The data were analysed by two-factor analysis of variance (ANOVA) with repetitions and the means separated using the Fischer's least significant difference test.

2. Results and discussion

Lycopene level of tomatoes ranged from 60 to 160 mg kg^{-1} , and is under genetic control, but it is effected by environmental conditions (DAVIES & HOBSON, 1981). Lycopene content is influenced by several factors such as agricultural practices (greenhouse, open field, varieties, water supply, fertilisation), soil, climate factors (temperature, moisture, solar radiation), fruit growth, harvesting date, degree of maturity and post-harvest handling. Among these factors greenhouse, open field, varieties, water supply and climate factors were investigated.

Foremost the effect of growing methods on Daniela F_1 tomato variety was analysed. Tomato plants cultivated in greenhouse received two different water doses (Table 1). At the first harvesting date the lycopene content of fruits supplied with 50% water was higher (86.5 mg kg⁻¹ f.w.) than that of fruits supplied with 100% water $(62.5 \text{ mg kg}^{-1} \text{ f.w.})$. Likewise after one month the fruits supplied with less water showed higher values (115.5 mg kg⁻¹ f.w.) than the fruits supplied with more water (90 mg kg⁻¹) f.w.). According to GROLIER and co-workers (1999) moisture stress reduced the

lycopene content in tomatoes. In spite of these findings on the basis of our results plants receiving less water tended to produce more lycopene. Between the two harvesting times there are significant differences in lycopene level. The accumulation of lycopene is puzzling, since it is an intermediate in the biosynthetic pathway of β -carotene in plants by two rings closure.

Table 1. Lycopene content (mg kg⁻¹ f.w.) of Daniela F₁ variety harvested in greenhouse by different water supply

Water supply	11 6 2001 ^x	11 7 2001 ^y	Average
50% ^A	$86.5 + 0.70^a$	$115.5 + 10.6^b$	$101.0 + 17.8$
100% ^B	$62.5 + 27.5^{\circ}$	$90.0 + 2.8^{b}$	$76.3 + 22.5$

AWater supply at "x" and "y" harvesting date was 40.3 and 62.8 l per plant, respectively. BWater supply at "x" and "y" harvesting date was 80.6 and 125.6 l per plant, respectively. a,b figures in the same row indicated with different letters are significantly different at P<0.05 probability level

Table 2. Lycopene content (mg kg^{-1} f.w.) of Daniela F₁ variety harvested in greenhouse and in the field

Greenhouse		Field by stick production	
Harvesting date	Lycopene	Harvesting date	Lycopene
11.6.2001	74.5 ± 21.0^a	14.8.2001.	63.3 ± 18.8^b
21.6.2001	$71.8 \pm 6.8^{\rm a}$	29.8.2001	$68.0 + 7.9^b$
11.7.2001	102.8 ± 16.0^a	13.9.2001	$46.3 + 5.6^b$
Average	$83.0 + 20.4^a$	Average	$59.2 + 14.7$ ^b

a, b figures in the same row indicated with different letters are significantly different at P<0.05 probability level

The tomato variety Daniela F_1 was grown in greenhouse and in the field by stick production, as well. Results are shown in Table 2. At every picking time lycopene content of tomato fruits from greenhouse was higher than that of tomatoes from field. This fact can be explained by the differences in temperature during ripening. In our correctly ventilated greenhouse the temperature varied in optimal range from 15 °C to 25 °C and the values of solar radiation were fairly low because of rainy weather during the growing period (Fig. 2). According to BAL£ZS (1989), the minimum solar radiation has to exceed 5 mol $m⁻²$ for the average and normal development of tomato. The temperature exceeded the critical 30 °C only in the middle of July. It can be explained that the growing started earlier in greenhouse thus avoiding the hotness of August. Tomato plants in the field were exposed to heat and rainfall (Fig. 3). As mentioned lycopene production is inhibited when environmental temperature is above 32 °C (GRIERSON & KADER, 1986; FARKAS, 1994) and extreme weather conditions could also adversely affect the lycopene accumulation. Environmental temperature above 30 °C and under 13 °C inhibit the setting of tomato flowers (SOMOS & HELYES, 1994). Under greenhouse conditions the lycopene level of fruits reached 83.0 mg kg⁻¹ f.w., while fruits picked from field contained 59.2 mg lycopene kg⁻¹ f.w. More than 30% higher lycopene level was found in fruits grown in greenhouse than in tomatoes produced in the field. As mentioned earlier the stress caused by rainfall or extremely high temperature may reduce the lycopene content in tomato fruits. Formation of lycopene during ripening is inhibited above 32 °C. The reason of this biochemical process could be the inhibition of a softening enzyme, namely polygalacturonase (GRIERSON & KADER, 1986). The biosynthesis of β -carotene is less temperature sensitive up to 38 °C than that of lycopene. Above 32 °C only β -carotene is produced which results in the orange colour of tomato.

Beyond the growing conditions the property of three different varieties such as Daniela F_1 , Delfine F_1 and cherry tomato was also determined. Each variety was cultivated under the same conditions in the field by stick methods. Fruits were picked three times in four repetitions. Results are presented in Fig. 4. Cherry tomato showed the highest average lycopene level (77.4 mg kg^{-1} f.w.), lower concentration was found in Delfine F_1 (69.6 mg kg⁻¹ f.w.) and Daniela F_1 produced the least amount of lycopene (59.2 mg kg⁻¹ f.w.). The order of lycopene concentrations as Cherry > Delfine F₁ > Daniela F1 was the same at every harvesting date. The statistical analysis proved a significant difference among the three varieties ($P = 0.05$). It may be elucidated that the genetic potential influences the lycopene biosynthesis. MARTINEZ-VALVEDERE and coworkers (2002) recently published lycopene concentrations in different tomato cultivars; they also found genetic-based differences among varieties. They reported markedly lower lycopene levels in Spanish tomatoes between 18.6 and 65 mg kg^{-1} , and in Daniela variety only 36.3 mg lycopene per kg fresh weight was found.

Fig. 2. The average values of temperature and radiation in greenhouse. \div : Radiation; \div : daily average temperature

Fig. 3. Temperature and rainfall during the fruit growth of tomato in the field. \parallel : Rainfall; . \equiv : minimum temperature; - : daily average temperature; -: maximum temperature

Fig. 4. The lycopene content of different varieties (field grown). \Box Daniela F₁; \Box Delfine F₁; : cherry tomato

3. Conclusions

Tomatoes (Lycopersicum esculentum) are widely consumed either raw or in processed forms and can provide a significant proportion of the total antioxidants in the diet. Indications that lycopene-rich diets are protective against some types of cancer arose from epidemiological studies. These studies have also suggested a possible role for tomato (based on its lycopene content) in the prevention of cardiovascular disease by inhibiting the formation of oxidised products of LDL cholesterol (ARAB & STEEK, 2000). Lycopene constitutes $60-74\%$ of the carotenoids present in tomato and tomato products. This compound can exist as different conformational isomers, but the predominant form found in tomatoes and tomato products (around 95%) is all-translycopene. Many factors affect the lycopene content such as maturity, cultivar, and heat treatment (STAHL & SIES, 1992, THOMPSON et al., 2000). As tomatoes develop from immature green to ripe, the increase in carotenoid content is related to the increase in lycopene content within the plastids (FRASER et al., 1994; THOMPSON et al., 2000). Concentrations were reported to increase from 0.25 mg $kg⁻¹$ in green tomatoes to values greater than 40 mg $kg⁻¹$ in fully ripe fruits.

The aim of our study was to evaluate the effect of agricultural practices and climate factors on lycopene content of tomatoes. In greenhouse tomato plants were irrigated with different water supplies. Greater water proportion resulted in decrease in lycopene content. Considerable divergence could be observed between greenhouse and open field production. In the field climatic factors cannot be controlled and plant stand is exposed

to high temperature and rainfall. Plants grown in field showed lower lycopene content than in greenhouse. Significant difference was also observed among different varieties in relation to lycopene content. Understanding the relationship between the factors by which lycopene levels are influenced and the content of other compounds with antioxidant property is necessary if the potential benefits to human health of the consumption of tomatoes are to be exploited.

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References

ARAB, L. & STEEK, S. (2000): Lycopene and cardiovascular disease. Am. J. clin. Nutr., 71, 1691S-1695S.

- BALÁZS, S. (1989): Zöldségtermesztők kézikönyve. (Handbook of the vegetable-growers.) Mezőgazda Kiadó, Budapest, pp. 207-208.
- BARRETT, D.M. & ANTHON, G. (2001): Lycopene content of California-grown tomato varieties. Acta Hortic., 542, 165-173.

CURL, A.L. (1961): The xanthophylls of tomatoes. *J. Fd Sci.*, 26, 106–111.

DAVIS, B.H. (1976): Carotenoids. -in: GOODWIN, T.W. (Ed) Chemistry and biochemistry of plant pigments. Vol. 2. Academic Press, NY, pp. 38-165.

- DAVIES, J.N. & HOBSON, G.E. (1981): The constituents of tomato fruit. The influence of environment, nutrition and genotype. CRC crit. Rev. Fd Sci. Nutr., 15, 205-280.
- FARKAS, J. (1994): Paradicsom. (Tomato.) -in: BALÁZS, S. Zöldségtermesztők kézikönyve. (Handbook of the vegetable-growers.) Mezőgazda Kiadó, Budapest, pp. 195-225.

FRASER, P.D., TRUESDALE, M.R., BIRD, C.R., SCHUCH, W. & BRAMLEY, P.M. (1994): Carotenoid biosynthesis during tomato fruit development. Plant Physiol., 105, 405–413.

GOODWIN, T.W. (1986): Metabolism, nutrition and function of carotenoids. Annu. Rev. Nutr., 6, 273-297.

- GRIERSON, D. & KADER, A.A. (1986): Fruit ripening and quality. -in: ATHERTON, J.G. & RUDICH, J. (Eds) The tomato crop. A scientific basis for improvement. Chapman and Hall, London, pp. 241-280.
- GROLIER, P., BARTHOLIN, G., CARIS-VEYRAT, C., DADOMO, M., DUMAS, Y., MEDDENS, F., SANDEI, L. & SCHUCH, W. (1999): Antioxidants in the tomato fruit. -in: Role and control of antioxidants in the tomato processing industry. European Commission Concerted Action, FAIR CT 97-3233, Seminar in Parma on 20 October 1999, pp. 9-12.
- LEVY, J., BIZIN, E., FELDMAN, B., GIAT, Y., MINSTER, A., DANILENKO, M. & SHARONI, Y. (1995): Lycopene is a more potent inhibitor of human cancer cell proliferation than either α -carotene or β -carotene. Nutr. Cancer, 24, 257-266.
- LÓPEZ, J., RUIZ, R.M., BALLESTORES, R., CIRUELOS, A. & ORTIZ, R. (2001): Color and lycopene content of several commercial tomato varieties at different harvesting dates. Acta Hortic., 542, 243-247.
- MANGELS, A.R., HOLDEN, J.M., BEECHER, G.R., FORMAN, M.R. & LANZA, E. (1993): Carotenoid content of fruits and vegetables: an evaluation of analytical data. J. Am. Diet. Assoc., 93, 284-296.
- MARTINEZ-VALVEDERE, I., PERIAGO, M.J., PROVAN, G. & CHESSON, A. (2002): Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). J. Sci. Fd Agric., 82, 323-330.

MERCK AND CO. (1989): Merck index. 11th ed., Rahway, NJ, USA, p. 884.

MICOZZI, M.S., BEECHER, G.R., TAYLOR, P.R. & KHACHIK, F. (1990): Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer. J. natn. Cancer Inst., 82, 282-288.

NIR, Z., HARTAL, D. & RAVEH, Y. (1993): Lycopene from tomatoes. $- A$ new commercial natural carotenoid. Int. Fd Ingred., 6, 45-51.

OLSON, J. (1986): Carotenoid, vitamin A and cancer. J. Nutr., 116 , $1127-1130$.

- SADLER, G., DAVIES, J. & DEZMAN, D. (1990): Rapid extraction of lycopene and β -carotene from reconstituted tomato paste and pink grapefruit homogenates. J. Fd Sci., 55, 1460-1461.
- SHI, J. & LE MAGUER, M. (2001): Degradation of lycopene in tomato processing. Acta Hortic., 542, 289-296.

SIES, H. & STAHL, W. (1998): Bioavailability of lycopene. Acta Hortic., 487, 389-393.

- SOMOS, A. & HELYES, L. (1994): Zöldséghajtatás. (Vegetable-forcing.) Lecture notes, Szent István Univ., Gödöllő, 119 pages.
- STAHL, W. & SIES, H. (1992): Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J. Nutr., 122, 2161-2166.
- THOMPSON, K.A., MARSHALL, M.R., SIMS, C.A., WEI, C.I., SARGENT, S.A. & SOTT, J.W. (2000): Cultivar, maturity and heat treatment on lycopene content in tomatoes. J. Fd Sci., 65, 791-795.