

Evolution of antioxidant activity and bioactive compounds in tomato (*Lycopersicon esculentum* Mill.) fruits during growth and ripening

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(Received November 30, 2013)

Summary

The interest in the consumption of tomato (*Lycopersicon esculentum* Mill.) is, to a large extent, due to its content of bioactive compounds and their importance as dietary antioxidants. During the growth and ripening process, there are quantitative and qualitative changes in the fruit composition which determine the nutritional quality and antioxidant potential at each stage. Two half determinate early hybrids cultivars (Prekos and Balkan) and one indeterminate mid-early hybrid cultivar (Reyana) were considered for this study. Fruits from plants grown on sandy soil in an unheated greenhouse were collected at three growth and six maturity stages. Antioxidant activity, dry matter, soluble solids, titratable acidity, ascorbic acid, lycopene, β -carotene, chlorophylls and total phenolic contents were monitored. During fruit growth, dry matter, soluble solids and titratable acidity recorded a slight decrease, polyphenols and β -carotene contents remained almost the same while ascorbic acid content and antioxidant activity increased continuously. The stage of ripening significantly influenced the content of all bioactive compounds as well as the antioxidant activity of tomato fruits. The first stages of ripening were characterized by a slight decrease of the dry matter content and by an increase of the titratable acidity, while in the last two stages of ripening these variations reversed. Ascorbic acid and total phenolics content increased as maturity progressed from mature green to pink or light red stage and decreased afterward. Lycopene started to accumulate since turning and sharply increased in the last three stages, on average 36 % of the lycopene content being accumulated in the last stage of ripening. In terms of hydrophilic antioxidant activity, depending on the cultivar, the pink or light red stages were the ones with the greatest potential. Although there were significant differences among the contents of bioactive compounds and antioxidant activity of the three cultivars studied, their patterns of variation during the nine stages were quite similar.

Introduction

Tomatoes are widely known for their outstanding antioxidant content, including their high concentration of lycopene and excellent amounts of other conventional antioxidants like vitamin C and tocopherols, additional carotenoids (β -carotene, lutein, and zeaxanthin), trace minerals (selenium, copper, manganese and zinc) and phytonutrients including flavonoids (naringenin, rutin, kaempferol, and quercetin) and hydroxycinnamic acids (caffeic, ferulic, and coumaric acid) (CAPANOGLU et al., 2010; FERNANDEZ-RUIZ et al., 2011). Recently, researchers started to identify new phytonutrients in tomatoes that help provide us with health benefits including the glycoside esculeoside A (FUJIWARA et al., 2007), the flavonoid chalconaringenin (YAMAMOTO et al., 2004; SLIMESTAD and VERHEUL, 2005) and 9-oxo-octadecadienoic acid, a fatty-acid derivative (KIM et al., 2011).

Tomato has been identified as a functional and nutraceutical food because intake of tomatoes has long been linked to health benefits (CANENE-ADAMS et al., 2005). Fresh tomatoes and tomato products have been shown to diminish the prevalence of certain types

of cancer (CAMPBELL et al., 2004) and to support the heart health by lowering total cholesterol, LDL cholesterol, and triglycerides, and the risk of atherosclerosis by preventing unwanted aggregation of platelet cells in the blood (WILLCOX et al., 2003). Furthermore, many studies have been conducted involving tomato and specific antioxidant protection of the bones, liver, kidneys, and bloodstream.

In the last years the antioxidant composition as well as antioxidant capacity has been extensively studied while development of tomato cultivars having high antioxidants content is a significant trend in plant research (FRUSCIANTE et al., 2007). Aside from the genetic potential of the cultivar, agronomic and environmental conditions, ripening stage of fruit and post-harvest storage are known to affect the chemical composition of tomatoes (DUMAS et al., 2003; HERNÁNDEZ et al., 2007).

Tomato fruit ripening is a complex process characterized by various morphological, physiological, biochemical and molecular transformations. These include degradation of chlorophylls, synthesis and storage of carotenoids (mainly lycopene and β -carotene) and aromatic compounds, alterations in organic acid metabolism, and a softening of the fruit tissue which occur in conjunction with an increase in CO₂ production (the respiratory climacteric) and an increase in ethylene production by the fruit. The amount of other important antioxidants, such as ascorbic acid and phenolics, is also variable during tomato ripening, in physiological response to various abiotic and biotic stresses (ILAHY et al., 2011; DOMÍNGUEZ et al., 2012). All these events determine major changes in texture, color, flavor, and aroma but also greatly affect the content of antioxidant compounds and the antioxidant activity of tomato fruits (DELLAPENNA et al., 1986; PÉK et al., 2010). Since tomato fruits are harvested at different ripening stages (from breaker to deep-red) depending on the consumer and market preference, the quantification of different antioxidants, as well as their variation during ripening, is of great relevance both to human health and to commercial purposes (ILAHY et al., 2011).

The aim of this study was to investigate the patterns of variation of dry matter, soluble solids, titratable acidity, and of the major antioxidant compounds (lycopene, β -carotene, phenolics, ascorbic acid) during growth and ripening of tomato fruits. As hydrophilic antioxidant activity depends upon synergistic effects among all hydrophilic antioxidants and their interaction with other constituents, its evolution was also monitored. Fruits samples from three tomato cultivars grown simultaneously on a sandy soil representative of Southern Oltenia (Romania) were analysed at nine different stages of the growth and ripening process.

Materials and methods

Plant material

Three different round-type tomato (*Lycopersicon esculentum* Mill.) cultivars were used in these experiments: Prekos and Balkan, two half determinate, early hybrids cultivars, and Reyana, an indeterminate mid-early hybrid cultivar. Plants were grown in an unheated greenhouse covered with polymeric film located at Dabuleni, Oltenia region, in Southwestern Romania (43°80' N, 24°08' E) during the spring growing season (March-July).

Seeds were sown on 20 January in alveolus plates filled with peat and transplanted into pots (12 cm diameter) after six weeks. Plants for each genotype were transplanted to the greenhouse at the end of March in a randomised complete block design with two replicates. Planting distances were 30 cm on the row and 100 cm between rows. Each tomato cultivar was arranged in two replicated plots containing 40 plants. The soil was representative of Southern Oltenia and classified as sandy soil (psamosol) with neutral to weak basic reaction and low natural fertility. Mean daily temperatures averaged between 6 °C in March and 22.7 °C in July.

The three cultivars were grown simultaneously in the same greenhouse following traditional agronomic techniques for plant nutrition and prevention of pathogens and subjected to identical cultural practices of drip irrigation and fertilization doses.

The plants were fertilized with nutrient solution of monoammonium phosphate (MAP) (12-61-0) through irrigation water until blossom, water-soluble NPK fertilizer (Polyfeed) 19-19-19 (0.5 %), three applications up to fruit formation and Multi-K potassium nitrate fertiliser from first ripening until end of the crop cycle. The conventional methods also included weed control with synthetic chemical herbicides (Fusilade S 2 l/ha and Sencor 70 WP 0.4 kg/ha) and plant pathogen control with synthetic chemical pesticides (Dithane M 45 0.2 % and Topsin M 0.1 %).

Sampling

Fruits were collected by hand at three growth and six maturity stages. The growth stages were established at 7, 17 and 27 days after pollination. According to YAMAGUCHI (1983), the stages of maturity were classified as follows: mature green (mature fruits with surface completely green, varying from light to dark green), breaker (first appearance of yellow or pink color but not more than 10 %), turning (yellow or pink color between 10 to 30 %), pink (pink or red color between 30 to 60 %), red (red color more than 60 % but less than 90 %), and deep-red (over 90 % red surface; desirable table ripeness). Fruit samples from each plot and stage were chosen randomly in four repetitions, four fruits in each repetition, for each stage selecting those samples which showed uniformity in external color, size and shape. Immediately after collection, fruits from each repetition were washed in tap water, blotted with a paper towel and cut into halves, the seeds were removed and the pericarp and mesocarp were ground to a homogeneous puree in a Waring blender for about 2 min. Part of the sample was immediately used for some analyses (dry matter content, soluble solids content and titratable acidity). The other part was frozen at -18 °C and used to determine the lycopene, β -carotene, chlorophylls, phenolics and ascorbic acid contents as well as the hydrophilic antioxidant activity. Experiments were executed in three repetitions, and the results were expressed as mean \pm standard deviation of values from four experiments performed in triplicate.

Determination of physico-chemical characteristics

Dry matter content (%) was determined gravimetrically by drying 5 g tomato homogenate in a laboratory oven (Memmert, Germany) set at 70 °C until constant weight was reached.

Soluble solids content (%) was measured with a digital refractometer (Euromex, Arnhem, The Netherlands) after tomato homogenate clarification by centrifugation (5000 \times g, 10 min). Titratable acidity (% as citric acid) was measured by titrating the water extract of tomato homogenate with a sodium hydroxide solution (0.1 N) using phenolphthalein as indicator.

Determination of antioxidant compounds

Extraction and analysis of ascorbic acid

The ascorbic acid content was determined by reversed-phase high-performance liquid chromatography (HPLC) method as described

by NOUR et al. (2010). Samples of 5 g of tomato homogenate were mixed and diluted to 100 mL with 0.1 N HCl. After 30 minutes the extraction solution was centrifuged at 5000 \times g for 10 minutes. The supernatant was passed through a 0.45 μ m pore size filter prior to HPLC analysis. The liquid chromatograph was a Finningan Surveyor Plus system (Thermo Electron Corporation, San Jose, CA, USA) equipped with a diode array detector (DAD) set at 245 nm. The separation was performed on a Hypersil Gold aQ (25 cm \times 4.6 mm id, 5 μ m) column using a 50 mM water solution of KH₂PO₄ buffer adjusted to pH 2.8 with ortho-phosphoric acid as mobile phase. The temperature of the column was set at 10 °C and the flow rate at 0.7 mL/min. Results are expressed in mg/100 g fresh weight (FW). Potassium dihydrogen orthophosphate and phosphoric acid were of analytical purity (Sigma-Aldrich, Germany). Ultrapure water was obtained from a Milli-Q water purification system (TGI Pure Water Systems, USA).

Extraction and analysis of chlorophyll and carotenoids

Determination was based on a spectrophotometric analysis following the method developed by NAGATA and YAMASHITA (1992) for the simultaneous determination of chlorophyll and carotenoids in tomato fruit. The samples were thawed in the dark in a refrigerator at 4 °C to avoid carotenoid oxidation. Samples of 1.0 g of tomato puree were extracted with 16 mL of acetone-hexane (4:6) solvent. After 15 min of shaking in a test tube and phase separation, the absorbance of the hexane layer was measured in a 1 cm path length quartz cuvette at 663, 645, 505, and 453 nm using a UV-VIS spectrophotometer (Varian Cary 50 UV-Vis, Varian Co., USA). Carotenoid and chlorophyll contents were calculated according to the equations: Lycopene (mg/100 mL) = - 0.0458 \times A663 + 0.204 \times A645 + 0.372 \times A505 - 0.0806 \times A453; β -Carotene (mg/100 mL) = 0.216 \times A663 - 1.22 \times A645 - 0.304 \times A505 + 0.452 \times A453; Chlorophyll a (mg/100 mL) = 0.999 \times A663 - 0.0989 \times A645; Chlorophyll b (mg/100 mL) = - 0.328 \times A663 + 1.77 \times A645. The results were finally expressed in mg/100 g FW.

Determination of total phenolic content

Total phenolic content was measured spectrophotometrically using the Folin-Ciocalteu colorimetric method (SINGLETON and ROSSI, 1965) with gallic acid (99 % purity, Sigma) as a calibration standard. Folin-Ciocalteu reagent (2N, Merk) and anhydrous sodium carbonate (99 % purity, Sigma) were also used. Samples (3 g of tomato homogenate) were extracted with 5 mL methanol in an ultrasonic bath for 45 min at ambient temperature. After extraction, samples were centrifuged at 5000 \times g for 5 min and supernatants were filtered through 0.45 μ m polyamide membranes. 100 μ L of each tomato methanolic extract were mixed with 5 mL of distilled water and 500 μ L of Folin-Ciocalteu reagent. After 30 sec to 8 min, 1.5 mL of sodium carbonate (20 % w/v) was added. The reaction mixture was diluted with distilled water to a final volume of 10 mL. The same procedure was also applied to the standard solutions of gallic acid. The absorbance at 765 nm of each mixture was measured on a Varian Cary 50 UV spectrophotometer (Varian Co., USA) after incubation for 30 min at 40 °C. Results were expressed as mg of gallic acid equivalents (GAE)/100 g FW.

Determination of hydrophilic antioxidant activity

The hydrophilic antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by OLIVEIRA et al. (2008), with some modifications. The extraction of samples was made according to the same protocol as described for total phenolic content. Each methanol tomato extract (50 μ L) was mixed with 3 mL of methanolic solution containing 0.004 % (v/v) DPPH. The mixture

was shaken vigorously and allowed to stand at room temperature in the dark for 30 min, at which time the decrease in absorbance at 517 nm was measured using a Varian Cary 50 UV-Vis spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated with respect to the Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which was used as a standard reference. Methanol (Merck, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Merck, Germany) were used. The radical was freshly prepared and protected from the light. A blank control of methanol/water mixture was run in each assay. All assays were conducted in triplicate. Results were expressed in mmol Trolox/100 g FW.

Statistical analysis

The results were statistically analyzed using Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). Differences among means were determined using one-way analysis of variance (ANOVA), followed by LSD multiple range test for multiple comparisons at $P < 0.05$.

Results and discussion

In the first stage of growth the average dry matter content of the three cultivars was 8 %. During fruit growth the dry matter content registered a continuous decrease (Fig. 1). This trend can be explained by the fact that dry matter content in tomatoes is mainly determined by dietary fibre and organic acid content (FRUSCIANTE et al., 2007). As regards the evolution of dry matter content during ripening, there were no significant differences among the first three stages (from mature green to turning), in the pink stage a significant decrease registered while in the red and deep red stages the dry matter content significantly increased. The final average dry matter content of the three cultivars was 6.24 %. The highest content was observed for Balkan cultivar (6.78 %) while Prekos showed the lowest value (5.84 %).

The variation of the soluble solids content of tomato fruits can be seen in Fig. 2.

There were no significant differences among the soluble solids contents of the three cultivars in the first stage of fruit growth. A slight decrease of soluble solid content was observed during fruit growth and a significant increase occurred during the last two stages of fruit ripening as reported in previous studies (HELYES and LUGASI, 2006; OPARA et al., 2012). This was due to partial breakdown of non-

reducing sugars and other polysaccharides and their subsequent inversion to reducing sugars in the course of fruit ripening.

Similarly to dry matter, in the deep red stage Balkan cultivar registered the highest soluble solids content (5.8 %), significantly higher than Reyana and Prekos between which no significant differences ($P > 0.05$) were found (5.1 % and 4.9 % respectively).

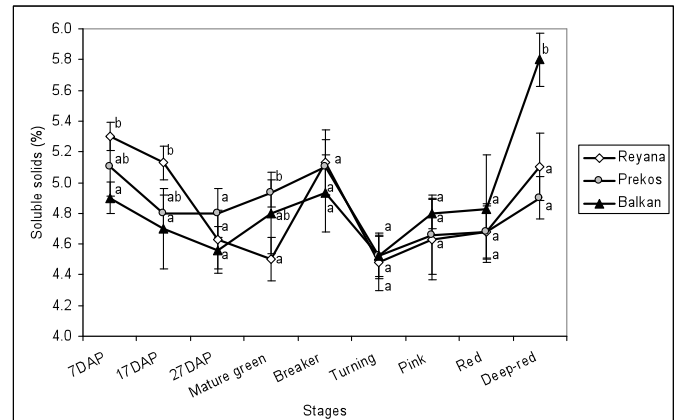


Fig. 2: Variation of soluble solids content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

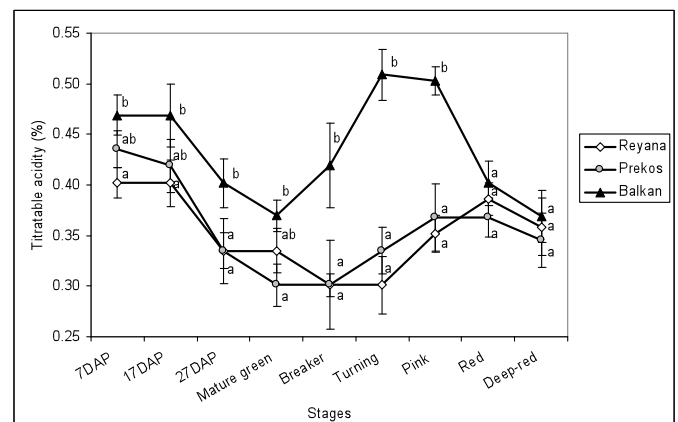


Fig. 3: Variation of titratable acidity of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

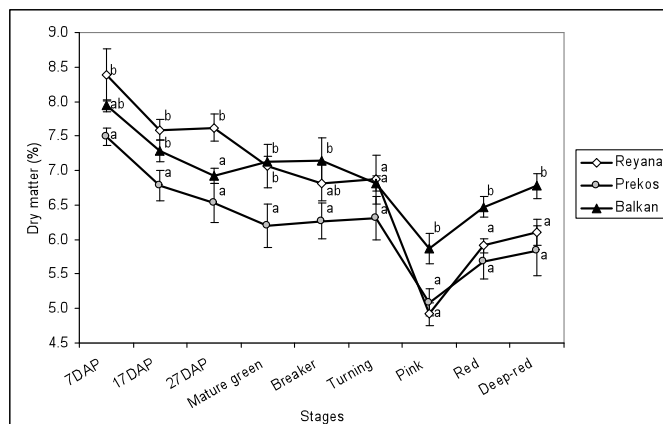


Fig. 1: Variation of dry matter content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

The level of acidity in tomato fruits is an important parameter associated with sensory attributes like flavor and astringency. According to HELYES and LUGASI (2006), general impression of flavors of tomato fruit is basically determined by the ratio of sugars and acids. The evolution of titratable acidity of tomato fruits is presented in Fig. 3. Titratable acidity recorded a decrease during fruit growth and was the lowest in the mature green stage (average 0.335 %), results in good agreement with those reported by HELYES et al. (2006). Our results however differ from previous studies concerning the evolution of the titratable acidity during ripening. In the following stages titratable acidity increased, reached a maximum value in the pink or red stage depending on cultivar and slightly decreased in the final stage of maturity, when it has reached a mean value of 0.357 %.

Malate is the predominant acid in tomato and the metabolism of malate has been a strong focus of research on tomato fruits because the acid plays an important metabolically active role. Some climacteric fruits such as tomato appear to utilize malate during the respira-

tory burst (GOODENOUGH et al., 1985; KORTSTEE et al., 2007), which explains the decrease in titratable acidity recorded in the early stages of ripening. The decline of the titratable acidity in the final stages of ripening is attributed to their use as substrate for respiration.

HELYES et al. (2006) reported that acid content remained almost the same during the ripening process (averagely 0.47 %). No significant differences were found between the final values of titratable acidity for the three cultivars.

The evolution of the ascorbic acid content of the investigated tomato cultivars at the nine different stages are shown in Fig. 4. The results showed that ascorbic acid levels were significantly different between the studied growth and ripening stages ($P < 0.05$). During fruit growth ascorbic acid content increased continuously while during ripening ascorbic acid content continues to increase as maturity progressed from mature green to pink or light red stage and decreased afterward. The decrease in ascorbic acid could be attributed to its susceptibility to oxidative destruction as impacted by the ripening environments.

Different patterns of variation were evidenced for the studied tomato cultivars during ripening. The highest amount of ascorbic acid was recorded at the pink stage in the cultivars Balkan (32.74 mg/100 g FW) and Prekos (23.2 mg/100 g FW), but at the red-ripe stage for the cultivar Reyana (23.64 mg/100 g FW). The results are in good agreement with those of other authors (GIOVANELLI et al., 2009; ILAHY et al., 2011) who reported similar variations of ascorbic acid content during ripening (increase during the early stages of ripening, followed by a decline). Other studies in tomatoes have revealed, however, that the ascorbic acid content remained practically constant in the first stages of ripening (until the pink stage), and increased slightly in the last, when the fruits were fully ripe (CANO et al., 2003). In addition to ripening stage, it is known that environmental and genotypic factors contribute to the nutritional qualities of tomato (DUMAS et al., 2003; LENUCCI et al., 2006). In our study we found also that all along the ripening process, Balkan cultivar exhibited significant higher amounts of ascorbic acid than Prekos and Reyana cultivars.

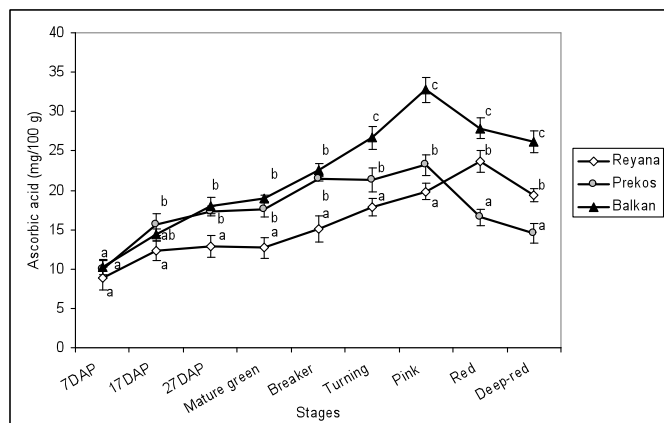


Fig. 4: Variation of ascorbic acid content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

Fig. 5 shows the evolution of total phenolics content at different growth and maturity stages. The pattern of changes of the phenolic compounds during fruit growth and ripening can vary and it depends on the species or even the variety (EGEA et al., 2009). Our results showed that although there were significant differences between total phenolics content of the three cultivars studied, the patterns of variation were quite similar both during fruit growth and ripening. Total phenolics content remained almost constant during fruit growth, continuously increased during the first stages of ripening,

reached a maximum at the pink stage and then declined significantly during the last two stages of ripening (red and deep red stages). HELYES et al. (2006) found also the lowest polyphenols content in tomato fruits from mature green stage while the highest content at the pink stage while LUGASI (2006) reported that the phenolic content of the tomato fruit does not change during ripening. ILAHY et al. (2011) observed different patterns of variation in phenolic content between the studied cultivars, with peaks in the amount of phenols reached either at the orange-red ripening stage or at the green and green-orange stages of ripening.

RAFFO et al. (2002) found also that total phenolics content showed slight, but significant, decreases at later stages of ripeness. They considered that the decrease of phenolic compounds in the fruit may be associated with the involvement of these compounds in the defense mechanisms against reactive oxygen species, which are produced in great quantities during the climacteric crisis as a consequence of the increase of the respiratory rate of the fruit.

At the deep red ripe stage the polyphenol content of the fruits ranged from 23.26 to 26.15 mg GAE/100 g while at the pink stage the total phenolic content varied between 29.39 and 38.56 mg GAE/100 g. Our data are in agreement with the results reported by OLIVEIRA et al. (2013) for immature, mature and ripe tomato fruits cultivated conventionally.

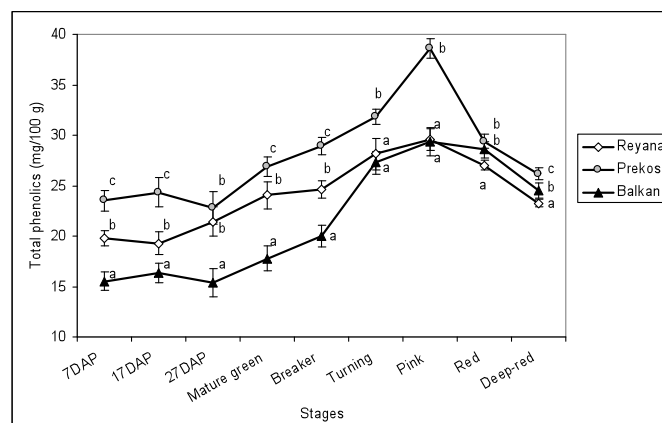


Fig. 5: Variation of total phenolics content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

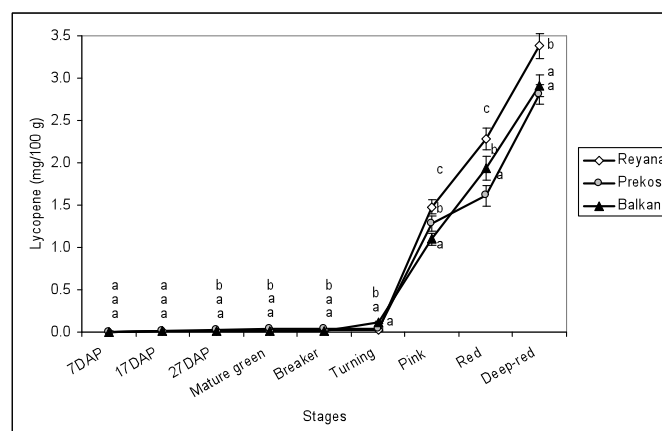


Fig. 6: Variation of lycopene content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

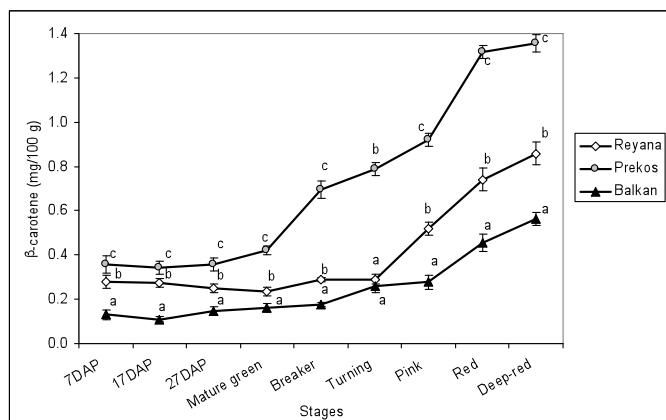


Fig. 7: Variation of β -carotene content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

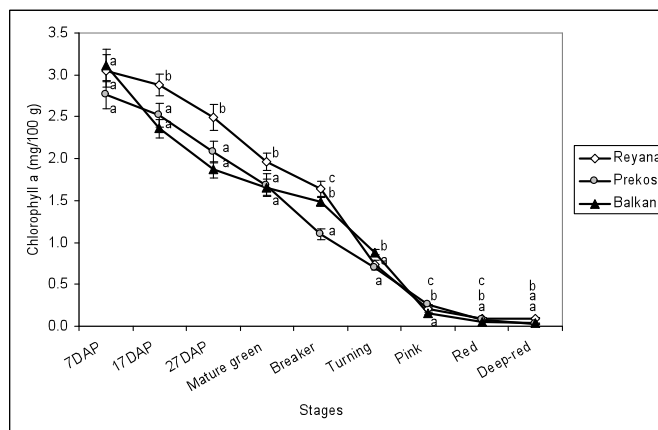


Fig. 8: Variation of chlorophyll a content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

Carotenoids are known to be unique constituents of a healthy diet and have been associated with reducing the risk of several degenerative disorders, including various types of cancer, cardiovascular or ophthalmological diseases (STAHL and SIES, 2003). In most fruits with carotenoids, like apricot, mango, orange, papaya, pepper, persimmon, tomato, etc., the ripening is accompanied by an increase of carotenoids biosynthesis. Therefore, in these fruits, the ripening degree determines the fruit carotenoid contents (EGEA et al., 2009). In tomato fruit this increase is due mainly to the accumulation of lycopene and β -carotene which is influenced by the genetic potential of the variety and environmental factors, especially temperature and light (HELYES and LUGASI, 2006). Lycopene is the most important antioxidant compound of the mature tomato fruit and the one which determines its red color. Change in the concentration of lycopene during growth and ripening of the investigated tomato cultivars is shown in Fig. 6. Up to the breaker stage the lycopene content of the fruits was nearly nil while from turning stage it increased sharply. On average 36 % of the lycopene content was synthesized and accumulated in the sixth maturity stage while HELYES and LUGASI (2006) found that almost half (46 %) of the lycopene content was synthesized and accumulated in this last stage. At the deep-red stage, Reyana cultivar showed the highest content of lycopene (3.38 mg/100 g FW) followed by Balkan (2.91 mg/100 g FW) and Prekos (2.8 mg/100 g FW). These concentrations fell well inside the interval described by other authors (HERNÁNDEZ et al., 2007; NOUR et al., 2013).

The concentration of β -carotene showed a gradual and linear increase during fruit ripening (Fig. 7). This constant increase of the β -carotene concentration was reported also by GIOVANELLI et al. (1999) while CANO et al. (2003) reported that β -carotene content increased until breaker and pink stages and decreased afterwards. EGEEA et al. (2009) asserted also that there is an old controversy regarding the accumulation of β -carotene during fruit ripening. Some studies have found a constant increase in its concentration during ripening, while others reported that β -carotene reaches its maximum concentration before the fruit reaches total ripeness, the differences being attributed to the different growing conditions and cultivars.

In the red-ripe stage the β -carotene content of Reyana and Balkan cultivars was within the literature concentration range (0.56 and 0.86 mg/100 g FW respectively) as reported by FRUSCIANTE et al. (2007) while Prekos cultivar registered a higher content (1.35 mg/100 g FW).

As previously reported (ILAHY et al., 2011) chlorophylls decrease during tomato ripening, being substituted by carotenoids, mainly lycopene (Fig. 8 and 9).

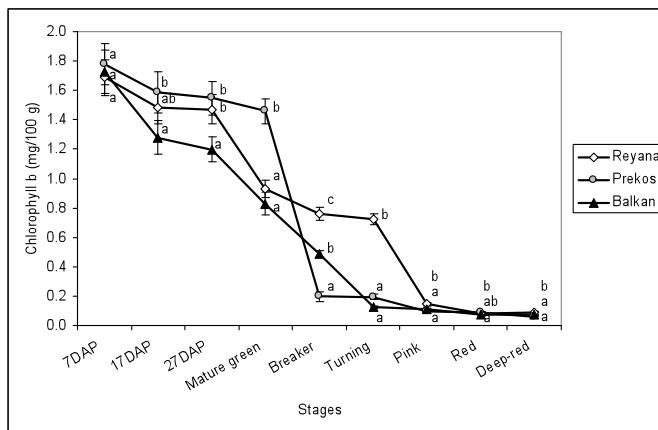


Fig. 9: Variation of chlorophyll b content of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

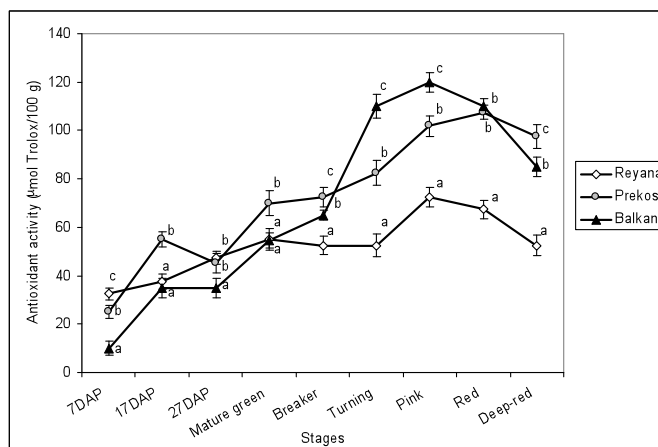


Fig. 10: Variation of hydrophilic antioxidant activity of tomato fruits during growth and ripening (mean \pm SD). Different letters within the same stage indicate significant differences ($P < 0.05$) among cultivars.

When studying the antioxidant evolution of tomato fruit during ripening, the separate study of the hydrophilic and lipophilic fractions is asserted since, as they are determined by different antioxidant compounds, they will have different evolutions. CANO et al.

(2003) established that hydrophilic antioxidant activity represented 71-85 % of the total antioxidant activity of the tomato fruit, the lowest contribution being observed in the last stage of tomato ripening, when the lipophilic antioxidant activity increased as the result of lycopene accumulation in the fruit. In our study only the hydrophilic antioxidant activity was monitored during fruit growth and ripening (Fig. 10). A continuous increase could be observed during development of tomato fruit until the pink or light red stages where, depending on the cultivar, hydrophilic antioxidant activity reached maximum values and then declined significantly.

Different results were reported by CANO et al. (2003) who found that the hydrophilic antioxidant activity and ascorbic acid content remained practically unchanged even though the phenol content increased during ripening, but also by ILAHY et al. (2011) who recorded the highest hydrophilic antioxidant activity in tomato fruits at the green stage of ripening and the lowest value at the red-ripe stage.

Hydrophilic antioxidant activity correlated well with the total phenolics content ($R = 0.77$; $P > 0.05$) and ascorbic acid content ($R = 0.78$; $P > 0.05$), which proves once more that these bioactive components contribute significantly to the hydrophilic antioxidant capacity of tomato fruits. CANO et al. (2003) found also that the levels of ascorbic acid and hydrophilic antioxidant activity were strongly correlated.

Conclusions

The experiments conducted have shown that the composition of valuable nutrients including total soluble solids, organic acids, ascorbic acid, carotenoids as well as phenolics and antioxidant activity of the tomato fruit is strongly influenced during growth and ripening stages. Total phenolics showed their highest levels at the pink stage while lycopene and β -carotene reached their highest levels in deep-red tomato fruits. Although the pink or red tomato had the greatest amount of vitamin C, the losses of ascorbic acid at the end of tomato fruit maturation were not great and the beneficial effect of ripening on the biologically-active carotenoids showed the deep-red stage to be valuable from the nutritional point of view. The hydrophilic antioxidant capacity of all investigated tomato cultivars clearly decreased during the last two stages of ripening. High correlations between hydrophilic antioxidant capacity and total phenolics as well as ascorbic acid were observed.

Acknowledgment

This work benefited from the networking activities within the European funded COST ACTION FA1106 QualityFruit.

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