ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 18(6) 2019, 39–46
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.6.4
ORIGINAL PAPER
Accepted: 15.03.2019

# THE EFFECT OF FLURIDONE ON ACCUMULATION OF CAROTENOIDS, FLAVONOIDS AND PHENOLIC ACIDS IN RIPENING TOMATO FRUIT

Wiesław Wiczkowski[1], Justyna Góraj-Koniarska[2], Marian Saniewski[2], Marcin Horbowicz[3]✉

[1] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Department of Chemistry and Biodynamics of Food, Tuwima 10, 10-748 Olsztyn, Poland

[2] Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

[3] Siedlce University of Natural Sciences and Humanities, Institute of Biological Sciences, Prusa 14, 08-110 Siedlce, Poland

## ABSTRACT

We examined the response of maturing tomato fruit exposed for 7 days to fluridone (1-methyl-3-phenyl-5--[3-trifluoromethyl(phenyl)]-4(1*H*)-pyridinone). Fluridone was applied in lanolin paste in the form of a 2–3 mm wide strip from the top to the base of the fruit. As a control, a similar stripe of lanolin was applied in the same way on the opposite side of the same fruit. The content of major carotenoids, as well as flavonoids, and free and bound phenolic acids were determined using a HPLC and HPLC-MS-MS methods. Fluridone almost completely blocked the biosynthesis of lycopene and substantial declined content of ß-carotene and lutein in the tomato fruit. The fluridone caused a decreased content of quercetin, rutin and naringenin, and increased level of epicatechin. The herbicide did not affect the content of *p*-coumaric acid, but reduced the level of caffeic acid, both free and ester form, and declined the content of free ferulic and chlorogenic acids. Changes in phenolics composition observed for the first time indicate that fluridone interferes with the biosynthesis of further products of the metabolism of *p*-coumaric acid, both flavonoids and phenolic acids.

**Key words:** fluridone, tomato fruit, ripening, carotenoid, phenolic acid, flavonoid

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) compose one of the most precious horticultural products, not only because of its economic significance but also for its sensory qualities and nutritional value. It is consumed in the form of fresh as well as processed products. More than 75% of tomatoes grown throughout the world are processed into products such as sauces, juices, ketchups, canned tomatos, stews, and soups. These all products are rich in nutrients and vitamins [Fraser and Bramley 2004, Bilalis et al. 2018, Sabijon and Sudaria 2018].

During tomato fruit ripening the colour of the pericarp changes from green to red as chlorophyll is degraded and carotenoids accumulate, and softening of fruit takes place. During these processes, chloroplasts are transformed into chromoplasts, the content of chlorophyll gradually decreases to traces, and the content of carotenoids, mainly lycopene, increases [Carrillo-Lopez and Yahia 2013, Liu et al. 2015, Su et al. 2015]. These changes are related to the increase in ethylene biosynthesis, the intensity of respiratory processes, and modulate of many enzymes activity

✉ mhorbowicz@uph.edu.pl

[Hoffman and Yang 1980, Alexander and Grierson 2002, Barry and Giovannoni 2007]. In ripe and overripe tomato fruits, ethylene production is limited due to the low activity of 1-aminocyclopropane-1-carboxylic acid oxidase [Hoffman and Yang 1980].

Tomato carotenoids are generally considered to be important health ingredients, and have therefore been the subject of hundreds biochemical and genetic studies [Moise et al. 2014, Liu et al. 2015, Su et al. 2015, Bilalis et al. 2018, Sabijon and Sudaria 2018]. Another group of compounds present in tomatoes, which are essential to our health, are phenolics [Slimestad and Verheul 2009]. Phenolic compounds have been extensively characterized in tomato fruits [Le Gal et al. 2003, Gomez-Romero et al. 2010, Barros et al. 2012, Carrillo-Lopez and Yahia 2013, Choi et al. 2014]. Like lycopene, most of them are antioxidants that remove free radicals and reactive oxygen species in the human body [Martinez-Valverde et al. 2002, Slimestad and Verheul 2009, Zanfini et al. 2017]. The accumulation of reactive oxygen species is also associated with fruit ripening and overripening of tomato fruits, and presence of elevated level of phenolics can extend its shelf life [Jimenez et al. 2002, Mondal et al. 2004, Zhang et al. 2015].

Fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4(1*H*)-pyridinone), is a systemic herbicide that strongly interferes with the biosynthesis of carotenoids by inhibiting phytoene desaturase activity, resulting in inhibition of phytofluene synthesis [Bartels and Watson 1978]. Fluridone also inhibits the biosynthesis of abscisic acid (ABA) and strigolactones [Rassmussen et al. 1997, Jamil et al. 2010]. In tomato, treatment with fluridone delayed ripening while ABA application enhances the process [Zhang et al. 2009].

Our previous studies have shown that fluridone, in addition to the strong inhibition of lycopene biosynthesis, did not affect the firmness of ripening tomato fruits and the production of ethylene and delayed degradation of chlorophyll [Góraj-Koniarska et al. 2017]. There is no information in the available literature regarding the impact of fluridone on phenylpropanoids. Therefore it seems to be important question if fluridone cause any interaction between the carotenoids and other metabolites during maturation of tomato fruit. The present research concerns the assessment of the effect of fluridone on the level of major carotenoids as well as phenolic acids and flavonoids, both free forms and chemically bound.

## MATERIAL AND METHODS

Mature green tomato fruits cv. Altadena $F_1$ (Syngenta) were used for the experiments. Tomato plants were cultivated in a greenhouse in controlled temperature, 18°C at night and 20°C during the day. The fruits were treated with fluridone (Duchefa, Netherlands) at concentrations of 1.0% w/w in lanolin paste containing 30% of water. Fluridone was applied to the fruit in the form of a 2–3 mm wide strip from the top to the base of the fruit. As a control a similar stripe of lanolin was applied in the same way on the opposite side of the same fruit. During the 7-day experiments, the tomato fruits were stored at ambient temperature (18–20°C) under natural light in greenhouse conditions. Pericarp slices from fruit halves treated with fluridone and those without the herbicide (control) were taken for chemical analysis. Before the analysis, the fruit samples were freeze-dried.

The content of particular carotenoids, flavonoids as well as free and bound phenolic acids were measured in the samples. The analysis were carried out in four or five replicates. Statistical evaluation of results were achieved with the *t*-test, and significant differences in relation to the control plants were indicated with *, $p < 0.05$ or **, $p < 0.01$. Means marked with an asterisk * and ** were considered statistically significant in comparison to control at $p < 0.05$ and 0.01, respectively. Lack of the asterisk means no significant difference in comparison to control.

The profile and content of carotenoids in tomato fruits were determined according to the modified method of Czaplicki et al. [2016]. Dried and pulverized samples were mixed with solution of hexane/acetone/absolute alcohol/toluene (10/7/6/7, v/v/v/v) and β-Apo-8'-carotenal as an internal standard. Next, the samples were saponified for 16 h with 40% methanolic KOH solution in a shaker at room temperature in darkness. After the saponification, a solution of 10% $Na_2SO_4$ was added and carotenoids were three times extracted with hexane. The hexane was evaporated under a stream of nitrogen and residue was dissolved in solution of methanol/dichloromethane (45/55, v/v). After centrifugation, to HPLC analyses a YMC C30

column (3 µm particles, 250 mm × 2.1 mm, YMC, Japan) at 30°C was used. The elution solvents were A (methanol) and B (methyl tert-butyl ether) at a flow rate of 0.2 mL· min$^{-1}$. The gradient was used as follows: 5% B for 5 min, 5–28% B in 23 min, 28–95% B in 8 min, 95–5% B in 7 min and 5% B for 20 min. The UV-Vis detector was set at the wavelength of 450 nm, and the eluted carotenoids were identified, based on retention times of available standards and the UV-Vis absorption spectra.

The profile and content of phenolic acids and flavonoids were determined according to the modified method of Weidner et al. [2000]. Briefly, a crude extract was obtained from dried and pulverized samples by extraction with sonication with 80% methanol. The extraction was repeated 5 times, and the obtained crude extracts were collected. Phenolic acids and flavonoids (free and those released from soluble esters and glycosides) were isolated from the crude extracts. Free forms were isolated with diethyl after adjusting the initial extract to pH 2 with 6 M HCl. Esters were hydrolyzed in nitrogen atmosphere for 4 h at room temperature with 4 M NaOH. Subsequently, glycosides was hydrolyzed in the residues with 6 M HCl for 1 h at 100°C. After adjusting to pH 2, phenolic acids released from glycosides were extracted with diethyl ether. The all extractions were carried out in triplicates by use of sonication and centrifugation, and the obtained ether extracts were evaporated to dryness under stream of nitrogen at 35°C. The phenolics, both free and released form bound forms, were dissolved in 80% methanol, centrifuged and subjected to HPLC-MS analysis. Aliquots of extracts were injected into a HPLC system equipped with a HALO $C_{18}$ column (2.7 µm particles, 0.5 × 50 mm, Eksigent, USA) at 45°C at a flow rate of 15 µL · min$^{-1}$. The elution solvents were A (water/formic acid; 99.05/0.95; v/v) and B (acetonitrile/formic acid, 99.05/0.95, v/v). The gradient was used as follows: 5% B for 0.1 min, 5–90% B in 1.9 min, 90% B for 0.5 min, 90–5% B in 0.2 min and 5% B for 0.3 min. For HPLC-MS/MS analysis a QTRAP 5500 ion trap mass spectrometer (AB SCIEX, USA) was used. Optimal ESI-MS/MS conditions including nitrogen curtain gas, collision gas, ion spray source voltage, temperature, nebulizer gas, and turbo gas were as follows: 25 L · min$^{-1}$, 9 L · min$^{-1}$, –4500 V, 350°C, 35 L· min$^{-1}$, and 30 L · min$^{-1}$, respectively. Qualitative and quantitative analysis were made using Multiple Reaction Monitoring (MRM) method for appropriate external standards.

## RESULTS

After seven days of treatment with fluridone the halves of the tomato fruits became yellow, while the halves without fluridone (control) turned red. The yellow color occurred both on the top layer of the tomato fruit (exocarp) but also in the deeper layer (mesocarp and endocarp). The content of lycopene in the parts of fruits treated with fluridone was 165 times lower than in the control tissues, which indicates complete inhibition of its biosynthesis. This herbicide also inhibited the accumulation of other carotenoids, ß-carotene and lutein, but this phenomenon was not as dramatic as in the case of lycopene (Tab. 1, Fig. 1).

**Table 1.** Effect of fluridone treatment on content of carotenoids (µg · g$^{-1}$ d.w. ± SD) in tomato fruit

| Analysed carotenoid | Control (lanoline) | Treated with fluridone in lanoline | Control/treated ratio |
|---|---|---|---|
| Lycopene | 3806 ±65 | 23.0 ±15.3** | 165.5 |
| ß-carotene | 1034 ±164 | 148.4 ±20.6** | 7.0 |
| Lutein | 29.4 ±2.9 | 10.2 ±1.3** | 2.9 |

Means marked with an asterisk ** were considered statistically significant in comparison to control at $p < 0.01$

In the pericarp of tomato fruit, quercetin, naringenin, rutin and epicatechin were identified (Tab. 2). Application of fluridone reduced the level of rutin and naringenin, did not significantly affect the content of quercetin and significantly increased the level of epicatechin.

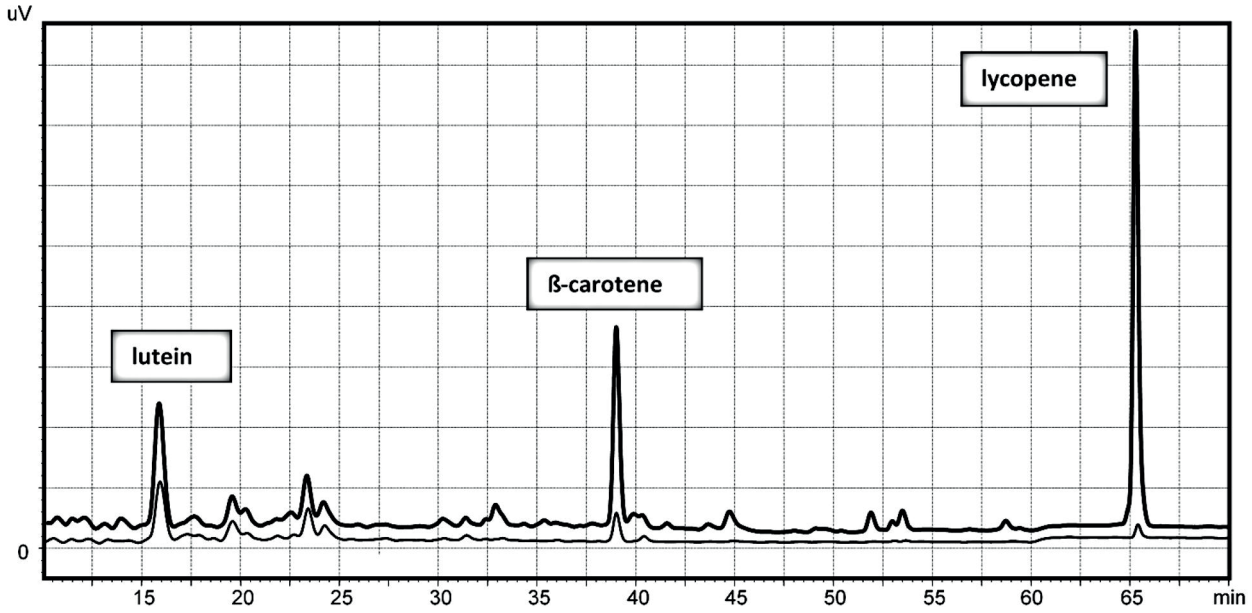


**Fig. 1.** Examples of carotenoid chromatograms in control tissue of tomato fruits (the upper bold line) and treated with fluridone (the lower narrow line)

**Table 2.** Effect of fluridone treatment on content of flavonoids (μg · $g^{-1}$ d.w. ± SD) in tomato fruit

| Analysed flavonoid | Control (lanoline) | Treated with fluridone in lanoline |
|---|---|---|
| Quercetin | 1.05 ±0.27 | 0.68 ±0.34 |
| Rutin | 1.53 ±0.14 | 0.83 ±0.06** |
| Naringenin | 2.23 ±0.21 | 1.40 ±0.21* |
| Epicatechin | 20.21 ±1.01 | 32.62 ±3.42** |

Means marked with an asterisk * and ** were considered statistically significant in comparison to control at $p < 0.05$ and 0.01, respectively. Lack of the asterisk means no significant difference in comparison to control

The presence of hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic), caffeic acid derivatives (chlorogenic and *iso*-chlorogenic acids) and protocatechuic acid have been detected in tomato fruits (Tab. 3). Among the acids, the ester forms of caffeic acid were the most abundant. In the pericarp of tomato fruit, ferulic, chlorogenic and *iso*-chlorogenic acids occurred mainly in free form. *p*-Coumaric acid occurs in tomato pericarp as free and bound forms, and fluridone did not affect the content of these compounds. Caffeic acid in tomato occurs mainly in the form of esters and in small amounts as free and glycosides (Tab. 3). Fluridone caused a reduction in the level of both the ester form and free acid. The use of herbicide slightly reduced the level of free chlorogenic acid, but did not affect the *iso*-chlorogenic and protocatechuic acid content (Tab. 3). The protocatechuic acid was found in pericarp of tomato fruit only in the form of glycosides,

**Table 3.** Effect of fluridone treatment on content of free and conjugated derivatives of phenolic acids (μg · $g^{-1}$ d.w. ± SD) in tomato fruit

| Analysed type of acid | Control (lanoline) | Treated with fluridone in lanoline |
|---|---|---|
| *p*-Coumaric (4-hydroxycinnamic acid) | | |
| Free | 12.6 ±1.3 | 11.0 ±1.3 |
| Esters | 57.0 ±2.5 | 52.5 ±2.9 |
| Glycosides | 5.05 ±0.95 | 5.45 ±1.14 |
| Caffeic (3,4-dihydroxycinnamic acid) | | |
| Free | 55.6 ±3.8 | 38.1 ±7.1* |
| Esters | 874.2 ±42.0 | 736.1 ±50.1* |
| Glycosides | 11.1 ±0.3 | 12.6 ±2.6 |
| Ferulic (4-hydroxy-3-methoxycinnamic acid) | | |
| Free | 175.0 ±18.3 | 109.0 ±9.2* |
| Esters | 13.4 ±2.0 | 14.6 ±3.4 |
| Glycosides | 1.08 ±0.25 | 0.78 ±0.05 |
| Chlorogenic (3-caffeoylquinic acid) | | |
| Free | 219.7 ±16.7 | 173.5 ±8.7* |
| Esters | 1.60 ±0.47 | 1.53 ±0.06 |
| Glycosides | 0.40 ±0.09 | 0.42 ±0.11 |
| *iso*-Chlorogenic (3,5-dicaffeoylquinic acid) | | |
| Free | 87.4 ±12.8 | 63.6 ±6.0 |
| Esters | nd | nd |
| Glycosides | nd | nd |
| Protocatechuic (3,4-dihydroxybenzoic acid) | | |
| Free | nd | nd |
| Esters | nd | nd |
| Glycosides | 46.3 ±7.5 | 41.4 ±8.7 |

Means marked with an asterisk * were considered statistically significant in comparison to control at $p < 0.05$ according to Newman-Keuls test. Analyses were carried out for each acid and its type separately. Lack of the asterisk means no significant difference in comparison to control

**Table 4.** Effect of fluridone treatment on content of total forms of phenolic acids (μg · $g^{-1}$ d.w. ± SD) in tomato fruit

| Analysed acid (chemical name) | Control (lanoline) | Treated with fluridone in lanoline |
|---|---|---|
| *p*-Coumaric (4-hydroxycinnamic acid) | 74.6 ±2.4 | 68.9 ±3.0 |
| Caffeic (3,4-dihydroxycinnamic acid) | 940.2 ±39.0 | 787.3 ±60.1* |
| Ferulic (4-hydroxy-3-methoxycinnamic acid) | 189.5 ±23.3 | 124.4 ±6.4* |
| Chlorogenic (3-caffeoylquinic acid) | 221.7 ±14.8 | 175.4 ±6.8* |
| *iso*-Chlorogenic (3,5-dicaffeoylquinic acid) | 87.4 ±12.8 | 63.6 ±6.0 |
| Protocatechuic (3,4-dihydroxybenzoic acid) | 46.3 ±7.5 | 41.4 ±8.7 |

Means marked with an asterisk * were considered statistically significant in comparison to control at $p < 0.05$. Lack of the asterisk means no significant difference in comparison to control

while *iso*-chlorogenic as unbound form. When comparing the effect of fluridone on the total content of phenolic acids, it should be noted that a clear reduction occurred in the case of caffeic acid, chlorogenic and ferulic, and affected the remain acids: *p*-coumaric, *iso*-chlorogenic and protocatechuic (Tab. 4).

## DISCUSSION

The inhibition of phytoene desaturase by fluridone caused that lycopene biosynthesis in tomato fruits was almost totally blocked [Góraj-Koniarska et al. 2017]. However, substantial decline of ß-carotene and lutein was confirmed for the first time in tomato pericarp. It is known that in the ripe tomato fruit the content of lutein and ß-carotene is much lower than lycopene [Giorio et al. 2013]. The cyclization of lycopene is a major point in the biosynthesis of further carotenoids. Route catalyzed by lycopene ß-cyclase leads to ß-carotene, zeaxanthin, violaxanthin, and neoxanthin, as well as for the synthesis of ABA and strigolactones. The alternative route as a result of lycopene ε-cyclase action leads to biosyntheis of α-carotene and lutein. The results obtained in our studies indicate that fluridone more effectively inhibits the pathway catalyzed by lycopene ß-cyclase than the latter catalyzed by lycopene ε-cyclase.

Phenolic compounds are secondary metabolites produced by plants as a part of defense mechanism against various stresses. In plants, there are two major groups of phenolic compounds, flavonoids and phenolic acids. Both groups are formed from a common substrate, *trans*-cinnamic acid, produced from L-phenylalanine by phenylalanine ammonia-lyase. According to Stewart et al. [2000] tomato skin contains more than 95% of the fruit flavonoids. The main flavonoids accumulating there are naringenin and rutin [Bovy et al. 2002]. Our results indicate that the quercetin, rutin and naringenin were present at low levels, and epicatechin in much higher concentration in the pericarp of tomato fruit. The presence of these flavonoids in tomato fruits confirm previously obtained results [Slimestad and Verheul 2009, Carrillo-Lopez and Yahia 2013, Zanfini et al. 2017]. Fluridone caused a decrease in the content of quercetin, rutin and naringenin, but at the same time a large increase in the level of epicatechin. In the flavonol biosynthesis (quercetin, rutin), dihydroflavonols are transformed by flavonol synthase (FLS). However, dihydroflavonol reductase (DFR) and anthocyanidin synthase (ANS) use the dihydroflavonols in the synthesis of anthocyanins and epicatechins as well [Muir et al. 2001]. Thus, it seems that fluridone stimulates the activity DFR and/or ANS or inhibits activity of FLS, which leads to such changes in the composition of flavonoids. However, this hypothesis requires detailed genetic studies.

Generally, in plants the free phenolic acids are rare, and are common their conjugated forms [Robbins 2003]. Our results indicate, that in the case of mature tomato fruit *p*-coumaric (4-hydroxycinnamic acid), caffeic (3,4-dihydroxycinnamic acid), and protocatechuic (3,4-dihydroxybenzoic acid) are present mainly

in bound forms as esters or glycosides. However, free forms dominate in ferulic (4-hydroxy-3-methoxycinnamic acid), chlorogenic (3-caffeoylquinic acid) and *iso*-chlorogenic (3,5-dicaffeoylquinic acid). Similar set of phenolic acids and flavonoids was found in tomato fruits by Carrillo-Lopez and Yahia [2013]. Using HPLC coupled to mass spectrometry method they identified in exocarp and mesocarp of tomato fruit at different ripeness stages several chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and rutin. Using similar method in fruits of cherry type tomato phenolic acids (gallic, *p*-hydroxybenzoic, vanillic, protocatechuic, chlorogenic, caffeic), and flavonoids (quercetin, rutin, kaempferol-3-O-glucoside, naringenin) were identified [Zanfini et al. 2017].

The metabolic pathway of phenolic acids is as follows: *trans*-cinnamic acid → p-coumaric acid → caffeic acid → ferulic acid. In the case of epicatechin, the shortened version of the pathway is as follows: p-coumaric acid → chalcone → naringenine → taxifoline → catechin. Fluridone did not affect the content of *p*-coumaric acid, an initial metabolite in the biosynthesis of both, phenolic acids and flavonoids. This herbicide significantly reduced the level the free and ester form of caffeic acid, as well as declined free forms of ferulic and chlorogenic acids. This reduction indicates that fluridone interferes with the biosynthesis of further products of the metabolism of *p*-coumaric acid, both flavonoids and phenolic acids. So far, this phenomenon has been observed and described for a first time.

## ACKNOWLEDGEMENTS

This research was financially supported by the Polish Ministry of Science and Higher Education under statutory funds (13/91/S) of the Institute of Biological Sciences, Siedlce University of Natural Sciences and Humanities, Poland

## REFERENCES

Alexander, L., Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J. Exp. Bot., 53, 2039–2055. DOI: 10.1093/jxb/erf072

Barros, L., Duenas, M., Pinela, J., Carvalho, A.M., Buelga, C.S., Ferreira, I.C.F.R. (2012). Characterization and quantification of phenolic compounds in four tomato (*Lycopersicon esculentum* L.) farmers' varieties in Northeastern Portugal home gardens. Plant Foods Hum. Nutr., 67, 229–234. DOI: 10.1007/s11130-012-0307-z

Barry, C.S., Giovannoni, J.J. (2007). Ethylene and fruit ripening. J. Plant Growth Regul. 26, 143–159. DOI: 10.1007/s00344-007-9002-y

Bartels, P.G., Watson, C.W. (1978). Inhibition of carotenoid synthesis by fluridone and norflurazon. Weed Sci., 26, 198–203.

Bilalis, D., Krokida, M., Roussis, I., Papastylianou, P., Travlos, I., Cheimona, N., Dede, A. (2018). Effects of organic and inorganic fertilization on yield and quality of processing tomato (*Lycopersicon esculentum* Mill.). Folia Hortic., 30(2), 321–332. DOI: 10.2478/fhort-2018-0027

Bovy, A., de Vos, R., Kemper, M., Schijlen, E., Almenar Pertejo, M., Muir, S., Collins, G., Robinson, S.,Verhoeyen, M., Hughes, S., Santos-Buelga, C., van Tunen, A. (2002). High-flavonol tomatoes resulting from heterologous expression of the maize transcription factor gene LC and C1. Plant Cell, 14, 2509–2526. DOI: 10.1105/tpc.004218

Carrillo-Lopez, A., Yahia, E. (2013). HPLC-DAD-ESI-MS analysis of phenolic compounds during ripening in exocarp and mesocarp of tomato fruit. J. Food Sci., 78, C1839–1844.

Choi, S.H., Kim, D.-S., Kim, D.-S., Kozukue, N., Kim, H.-J., Nishitani, Y., Mizuno, M., Levin, C.E., Friedman, M. (2014). Protein, free amino acid, phenolic, ß-carotene, and lycopene content, and antioxidative and cancer cell inhibitory effects of 12 greenhouse-grown commercial cherry tomato varieties. J. Food Comp. Anal., 34, 115–127. DOI: 10.2478/prolas-2018-0014

Czaplicki, S., Tańska, M., Konopka, I. (2016). Sea-buckthorn oil in vegetable oils stabilization. Ital. J. Food Sci., 28, 412–425. DOI: 10.14674/1120-1770/ijfs.v252

Fraser, P.D., Bramley, P.M. (2004). The biosynthesis and nutritional uses of carotenoids. Prog. Lipid Res., 43, 228–265. DOI:10.1016/j.plipres.2003.10.002

George, S., Tourniaire, F., Gautier, H., Goupy, P., Rock, E., Caris-Veyrat, C. (2011). Changes in the contents of carotenoids, phenolic compounds and vitamin C during technical processing and lyophilisation of red and yellow tomatoes. Food Chem., 124, 1603–1611. DOI: 10.1016/j.foodchem.2010.08.024

Gapper, N.E., McQuinn, R.P., Giovannomi, J.J. (2013). Molecular and genetic regulation of fruit ripening. Plant Mol. Biol., 82, 575–591. DOI: 10.1007/s11103-013-0050-3

Giorio, G., Yildirim, A., Stigliani, A.L., D'Ambrosio, C. (2013). Elevation of lutein content in tomato: a biochemical tug-of-war between lycopene cyclases. Metab.

Eng., 20, 167–176. DOI:10.1016/j.ymben.2013.10.007

Gomez-Romero, M., Segura-Carretero, A., Fernandez-Gutierrez, A. (2010). Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry, 71, 1848–1864. DOI: 10.1016/j.phytochem.2010.08.002

Góraj-Koniarska, J., Saniewski, M., Kosson, R., Wiczkowski, W., Horbowicz, M. (2017). Effect of fluridone on some physiological and qualitative features of ripening tomato fruit. Acta Biol. Cracov. Bot., 59/2, 41–49. DOI: 10.1515/abcsb-2017-0012

Hoffman, N.E., Yang, S.F. (1980). Changes of 1-aminicyclopropane-1-carboxylic acid content in ripening fruits in relation to their ethylene production rates. J. Am. Soc. Hortic. Sci., 105, 492–495.

Jamil, M., Charnikhova, T., Verstappen, F., Bouwmeester, H. (2010). Carotenoides inhibitors reduce strigolactone production and *Striga hermonthica* infection in rice. Arch. Biochem. Biophys., 504, 123–131. DOI: 10.1016/j.abb.2010.08.005

Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., Mullineaux, P. (2002). Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. Planta, 214, 751–758. DOI: 10.1007/s004250100667

Le Gal, G., Dupont, M.S., Mellon, F.A., Davis, A.L., Collins, G.J., Verhoeyen, M.E., Colquhoun, I.J. (2003). Characterization and content of flavonoids glycosides in genetically modified tomato (*Lycopersicon esculentum*) fruits. J. Agric. Food Chem., 51, 2348–2446. DOI: 10.1021/jf025995e

Liu, L., Shao, Z., Zhang, M., Wang, Q. (2015). Regulation of carotenoid metabolism in tomato. Mol. Plant., 8, 28–39. DOI: 10.1016/j.molp.2014.11.006

Martinez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicon esculentum*). J. Sci. Food Agric., 82, 323–330. DOI: 10.1002/jsfa.1035

Moise, A.R., Al-Babili, S., Wurtzel, E.T. (2014). Mechanistic aspects of carotenoid biosynthesis. Chem. Rev., 114, 164−193. DOI:10.1021/cr400106y

Mondal, K., Sharma, N.S., Malhotra, S.P., Dhawan, K., Singh, R. (2004). Antioxidant systems in ripening tomato fruits. Biol. Plant., 48, 49–53. DOI: 10.1023/B:BIOP.0000024274.43874.5b

Muir, S.R., Collins, G.J., Robinson, S., Hughes, S., Bovy, S., De Vos, C.H., van Tunen, A.J., Verhoeyen, M.E. (2001). Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. Nat. Biotechnol., 19, 470–474.

Rasmussen, R.D., Hole, D., Hess, J.R., Carman, J.G. (1997). Wheat kernel dormancy and abscisic acid level following exposure to fluridone. J. Plant Physiol., 150, 440–445. DOI: 10.1016/S0176-1617(97)80095-8

Sabijon, J., Sudaria, M. (2018). Effect of vermicompost amendment and nitrogen levels on soil characteristics and growth and yield of tomato (*Solanum lycopersicum* cv. Diamante max). Int. J. Agric. Life Sci., 2(2), 145–153.

Slimestad, R., Verheul, M. (2009). Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* Mill.) cultivars. J. Sci. Food Agric., 89, 1255–1270. DOI: 10.1002/jsfa.3605

Stewart, A.J., Bozonnet, S., Mullen, W., Jenkins, G.I., Lean, M.E., Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. J. Agric. Food Chem., 48, 2663–2669.

Su, L., Diretto, G., Purgatto, E., Danoun, S., Zouine, M., Li, Z., Roustan, J.-P., Bouzayen, M., Giuliano, G., Chervin, C. (2015). Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. BMC Plant Biol., 15, 114. DOI: 10.1186/s12870-015-0495-4

Weidner, S., Amarowicz, R., Karamać, M., Frączek, E. (2000). Changes in endogenous phenolic acids during development of *Secale cereale* caryopses and after dehydration treatment of unripe rye grains. Plant Physiol. Biochem., 38, 595–602. DOI: 10.1016/S0981-9428(00)00774-9

Zanfini, A., Franchi, G.G., Massarelli, P., Corbini, G., Dreassi, E. (2017). Phenolic compounds, carotenoids and antioxidant activity in five tomato (*Lycopersicon esculentum* Mill.) cultivars. Ital. J. Food Sci., 29, 90–99. DOI: 10.14674/1120-1770/ijfs.v316

Zhang, Y., De Stefano, R., Robine, M., Butelli, E., Bulling, K., Hill, L., Rejzek M., Martin C., Schoonbeek, H. (2015). Different reactive oxygen species scavenging properties of flavonoids determine their abilities to extend the shelf life of tomato. Plant Physiol., 169(3), 1568–1583. DOI: 10.1104/pp.15.00346

Zhang, M., Yuan, B., Leng, P. (2009). The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. J. Exp. Bot., 60, 1579–1588. DOI: 10.1093/jxb/erp026