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ORIGINAL PAPER

EFFECT OF FLURIDONE ON FATTY ACID COMPOSITION AND OTHER PROPERTIES OF TOMATO FRUITS*

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Abstract

The effects of fluridone on the content and composition of fatty acids and some other properties of tomato fruits during a 14-day period of ripening were studied. Mature green tomato fruits were used for the experiments. Fluridone at a 1.0% concentration in lanolin paste was applied as a 2-3 mm stripe from the top to the base of a tomato fruit, and a stripe of lanolin was applied in the same way on the opposite side of the frui as control. After 14 days of the experiment, the treated (yellow) and untreated (red) halves of the tomato were separately freeze-dried and powdered before analysis. Determination of the profile and content of fatty acids was carried out in a micro-HPLC-MS/MS system including a 5600 QTOF mass spectrometer. Fatty acids were identified, based on retention times of available standards and the MS/MS spectra. The degree of lipid peroxidation, DPPH radical scavenging activity, proline and soluble protein content were determined using spectrophotometric methods. The use of fluridone did not cause significant changes in the content of unbound fatty acids, although there was a tendency to increased amounts of unsaturated acids. Concerning total fatty acids, the use of fluridone significantly increased the ratio of oleic acid to stearic, as well as the ratio of total unsaturated acids to saturated acids in the treated pericarp of tomato fruits. Fluridone had not effect on the content of proline, total phenolic acids, MDA and antioxidant activity, but inhibited soluble protein accumulation and enhanced the content of total flavonoids.

Keywords: fluridone, tomato fruit, fatty acids, proline, proteins, MDA.

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INTRODUCTION

Fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4(1*H*)-pyridinone) is used as an active ingredient in a number of herbicides which are applied to eliminate aquatic plants (Berard et al. 1978, Doong et al. 1993, Arias et al. 2005). It disrupts the biosynthesis of carotenoids by inhibiting the activity of phytoene desaturase, which is responsible for the conversion of phytoene to phytofluene (Bartels, Watson 1978, Marquis et al. 1981, Rasmussen et al. 1997). It leads to a decrease in the content of carotenes as well as the rate of photosynthesis and carbohydrate levels. Fluridone also inhibited chlorophyll biosynthesis in plant leaves (Berard et al. 1978, Fletcher et al. 1984, Popova 1998), but delayed chlorophyll degradation during the ripening of tomato fruits (Góraj-Koniarska et al. 2017). According to Fletcher et al. (1984), it is unlikely that of effects fluridone on the chlorophyll content are mediated solely by the inhibition of carotenoid synthesis, since fluridone inhibits several fundamental processes. For instance, fluridone is also used as an inhibitor of abscisic acid biosynthesis (Chen et al. 2016, Worarad et al. 2017). Prajapati et al. (2019) have recently shown that fluridone reduces total soluble solids and acidity as well as the physiological loss of weight of pepper fruits.

Like fluridone, pyridazinone herbicides (norflurazon, SAN 9789; metflurazon, SAN 6706, and SAN 9785, 2-phenyl-4-chloro-5-(dimethylamino) pyridazine-3(2H)-one) inhibit the activity of phytoene desaturase (Bartels Watson 1978). Besides, it has been shown that these herbicides inhibit the activity of desaturase, which converts linoleic acid (C18:2) to linolenic (C18:3) in wheat shoots, wheat roots and crowns and cotton roots (St John 1976, Vaisberg, Schiff 1976, Khan et al. 1979, Lem, Williams 1981). However, in embryos of olive fruit, ABA modulates desaturase gene expression, and fluridone restores desaturase transcript accumulation (Haralampidis et al. 1998). Microalgae *Spirulina platensis* and *Porphyridium cruentum* resistant to the herbicide SAN 9785 were shown to overproduce linolenic acid and eicosapentaenoic acid in the presence and absence of the inhibitor, as compared with wild-type cultures under similar conditions (Cohen et al. 1993).

Proline accumulation is a common physiological response in many plants to a wide range of biotic and abiotic stresses (Verbruggen, Hermans 2008, Lobato et al. 2010). Determination of free proline levels is a useful method to monitor the physiological status and/or stress tolerance of plants. The application of fluridone to leaves of crystal grass plants (*Mesembryanthemum crystallinum* L.) decreased the content of ABA, and increased the level of proline and MDA (Stetsenko et al. 2015). However, a positive relationship between abscisic acid and proline synthesis was confirmed in *Vigna unguiculata* leaves (Costa et al. 2011).

Data on the effect of fluridone on the composition of fatty acids and other compounds in tomato fruits are limited. In our previous research, fluridone

was found to strongly inhibit the accumulation of lycopene and to delay the degradation of chlorophyll during the maturation of tomato fruits (Góraj-Koniarska et al. 2017). These changes resulted in tomato fruits turning yellow rather than red. Overexpression of wild type tomato fruits with phytoene synthase (PSY) resulted in a decline in the content of all major fatty acids: palmitic, stearic, oleic and linoleic acid, during the maturation of fruits (Fraser et al. 2007). Therefore, the main question posed in this study was whether the inhibition of carotenogenesis by fluridone is connected with the content and composition of fatty acids in ripening tomato fruits. Another important issue was to find out if these changes affect other metabolic processes.

MATERIALS AND METHODS

Mature green tomato fruits cv. Altadena F1 (Syngenta) were used for the experiments. Tomato plants were grown from seeds in a greenhouse at the Research Institute of Horticulture in Skierniewice, at a night/day temperature of 18°C/20°C. Tomato plants were fertilized by drip irrigation. The research was designed mainly to assess the possibility of growing tomatoes on lignite. Unripe fruits picked from plants grown under these conditions but not included in that experiment were selected in order to examine the influence of fluridone. Fruits for this experiment were picked three times, from the end of August to mid-October. The weight of a fruit taken for this study was between 200 and 250 g.

Tomato fruits were treated with fluridone (Duchefa) at a concentrationsof 1.0% w/w in lanolin paste containing 30% of water. This paste was applied as a 2-3 mm stripe from the top to the base of a fruit, while a stripe of lanolin was applied in the same way on the opposite side of the fruit as control. Afterwards, the fruits were kept in the greenhouse at ambient temperature (18-20°C) and natural daylight conditions for 14 days. Every day, the fruits were turned so that the exposure to light was uniform. After the experiment, samples of the pericarp from both halves of every fruit (treated and untreated) were freeze dried separately and pulverized.

The profile and content of fatty acids was determined according to the modified method of Bromke et al. (2015). Tomato samples (100 mg) were mixed with 1 mL solution of methanol/methyl tert-butyl ether/water (1/3/1, v/v/v). After extraction in an ultrasonic bath (3 min, 4°C), 0.5 ml of the methanol/water (1/3, v/v) mixture was added, vortexed for 30 s, and the formed lipid phase was collected. This procedure was repeated twice, the collected phases were combined and next evaporated to dryness under a nitrogen atmosphere. The residue was dissolved in 0.2 mL of methanol/6% KOH (4/1, v/v) and hydrolyzed for 2 h at 60°C with continuous shaking, using a thermomixer (1400 rpm; Termomixer comfort, Eppendorf, Germany);

then 0.1 mL of NaCl (saturated solution) and 0.05 mL of 29% HCl were added. The fatty acids released from bound forms were extracted 3 times with 2 mL of chloroform/heptane (1/4, v/v) by sonication and vortexing (3 x 30 s), and the collected organic phases were combined, evaporated to dryness under a nitrogen atmosphere and stored at -80°C until analysis. The extraction of free forms of fatty acids was prepared in a similar way, excluding the hydrolysis step. Before analyses, samples were dissolved in 0.1 mL of methanol and centrifuged (4°C, 13 200 g, 20 min). Determination of the profile and content of fatty acids was carried out in a micro-HPLC-MS/MS (Sciex, USA) system, consisting of a dual-channel pump, column oven, autosampler, system controller and a 5600 QTOF mass spectrometer. Chromatographic separation was conducted in a HALO C_{18} column (2.7 µm, 0.5 x 50 mm; Eksigent, USA) at 60°C and a flow rate of 15 µL min^{-1}. The elution solvents A (water/0.5 M ammonium formate/formic acid, 98.9/1/0.1, v/v/v/v) and B (acetonitrile/0.5 M ammonium formate/isopropanol/formic acid, 68.9/1/30/0.1, v/v/v/v) were used in the following gradient patterns: 30-30-90-90-30-30% in 0-0.5-1.8-2.8-3-5 min. The MS QTOF settings were as follows: negative ionization, nitrogen curtain gas: 25 L min^{-1}, ion spray source voltage: -4500 V, temperature: 350°C, nebulizer gas 1: 35 L min^{-1}, turbo gas: 30 L min^{-1}, Q1/Q2: DP -90/-100 V, CE -25/-50 V, CES 15 V. Fatty acids were identified based on retention times of available standards and the MS/MS spectra.

In order to determine the total phenol and flavonoid content, appropriate samples were extracted in 60% methanol (v/v) for 24 h, and then the filtrates underwent spectrophotometric analyses of the total content of phenolic compounds according to the method by Singleton et al. (1999), while total flavonoids were determined according to Ordonez et al. (2006). All content volmues were calculated form the calibration curve equations prepared with chlorogenic acid and rutin for total phenols and flavonoids, respectively.

The degree of lipid peroxidation was analyzed according to the on the determination of malondialdehyde (MDA) through the reaction with thiobarbituric acid, TBA (Hodges et al. 1999).

The DPPH radical scavenging activity was measured according to method described by Brand-Williams et al. (1995).The antioxidant activity was calculated by determining the % decrease in the absorbance after adding an extract from tomato fruit to a DPPH solution with the extinction of about 1.0.

In order to analyze the content of free proline, a standard, ninhydrin-based method was applied (Bates 1973). Proline was extracted with 3% sulphosalicylic acid and the product of a reaction with ninhydrine was determined spectrophotometrically at 520 nm.

Soluble proteins were determined with the Lowry et al. (1951) method using the Folin-Ciocalteau reagent and measuring the extinction at 750 nm. The protein content was calculated from a standard curve based on bovine serum albumin.

One-way analysis of variance and the *post hoc* Tukey's test were used to verify the significance of differences between fluridone-treated and untreated fruit tissues.

RESULTS AND DISCUSSION

The tissues of green tomato fruits treated with fluridone in lanolin paste became yellow after maturation, while the fruit halves where only lanolin paste (control) had been applied were red (Góraj-Koniarska et al. 2017). This was due to the herbicide inhibiting the activity of phytoene desaturase, an important enzyme in the carotenoid biosynthesis.

Our research has shown that unbound fatty acids constitute about 1/7 of the total fatty acid content (Table 1). Saturated acids prevail, as they are four-fold more abundant than unsaturated ones. Concerning unbound acids, about 2/3 of their total content is palmitic acid (C16: 0) – Figure 1*b*. Less abundant acids, i.e. linolenic and stearic, accounted for about 1-2% of total unbound fatty acids (Figure 1*a*). The use of fluridone did not cause significant changes in the content of unbound acids, although there was a tendency towards an increase in unsaturated acids. The lack of significant differences could be attributed to the low repeatability of the analyses. This was especially evident when calculating the ratio of oleic acid to stearic (Table 1).

In the case of total fatty acids, the levels of saturated and unsaturated acids were similar (Table 1). The percentage of palmitic acid is lower, but the unsaturated acids such as linoleic (C18: 2) and linolenic (C18: 3) acids increased (Figure 2*b*). It was interesting to find out that the use of fluridone significantly increased the ratio of oleic acid to stearic acid, as well as the

Table 1

Effect of fluridone on the content of unbound and total fatty acids, and ratios of unsaturated acids to saturated in the pericarp of tomato fruits

Content or ratio	Unbound fatty acids		Total fatty acids	
	control	fluridone treated	control	fluridone treated
Content (mg g^{-1} DW)				
Total acids	30.3±8.4	34.5±2.3	221.2±39.1	217.9±19.2
Total saturated acids	24.6±8.8	27.0±2.9	118.3±34.3	102.1±9.9
Total unsaturated acids	5.71±1.81	7.42±1.72	102.9±9.3	115.8±9.5
Ratio				
Oleic to stearic acid	7.10±3.10	11.31±8.9	11.48±1.39	15.38±1.22*
Unsaturated to saturated acids	0.26±0.12	0.28±0.09	0.91±0.09	1.14±0.03*

Results (means of 5 replicates± standard deviation) marked with * are significantly different from the control; $P < 0.05$.

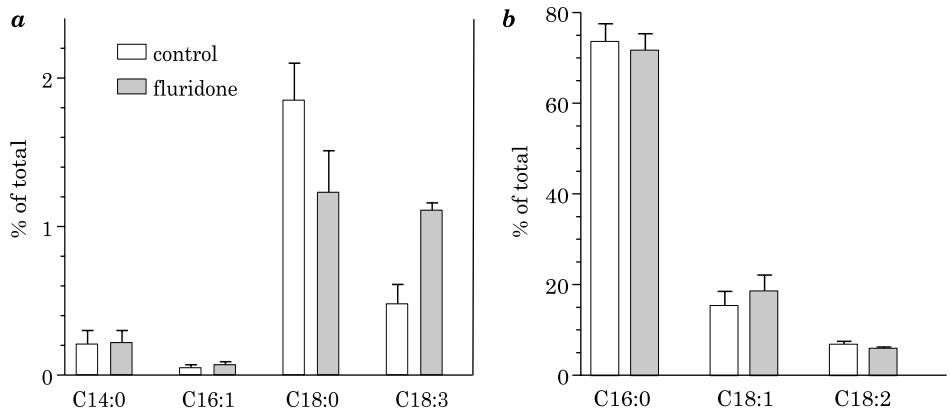

Fig.1. Effect of fluridone on composition of unbound fatty acids in the pericarp of tomato fruits: *a* – minor fatty acids: C14:0 – myristic, C16:1 – palmitoleic, C18:0 – stearic, C18:3 – linolenic, *b* – major fatty acids: C16:0 – palmitic, C18:1 – oleic, C18:2 – linoleic.
The results are means of 5 replicates + SD.
The result marked with (*) was significantly different from the control; $P < 0.05$

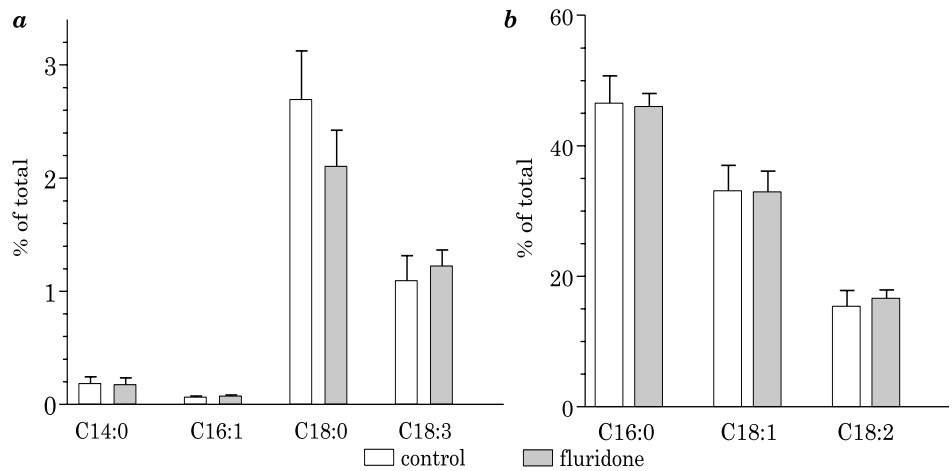

Fig. 2. Effect of fluridone on composition of total fatty acids in the pericarp of tomato fruits: *a* – minor fatty acids: C14:0 – myristic, C16:1 – palmitoleic, C18:0 – stearic; C18:3 – linolenic, *b* – major fatty acids: C16:0 – palmitic, C18:1 – oleic, C18:2 – linoleic.
The results are means of 5 replicates + SD

ratio of the sum of unsaturated acids to saturated ones. This indicates that fluridone applied to the tomato fruit probably activates the enzymes of fatty acid desaturases, especially stearate desaturase. The desaturation of stearic acid yielding oleic acid is catalyzed by stearoyl-ACP desaturase in the stroma of plastids (Slocombe et al. 1994). Then, oleic acid is transported to the cytoplasm for further possible desaturation into the lipid-bound form.

Data on the effect of fluridone on fatty acid composition are limited. There is some information on the effects of other pyridazinone herbicides

(norflurazone, metflurazone, SAN 9785) that affect the biosynthesis of carotenoids similarly to fluridone. These herbicides inhibit the activity of desaturase which converts linoleic acid (C18:2) to linolenic (C18:3) acid in tissues of several plant species (St John 1976, Vaisberg, Schiff 1976, Willemot 1977, Khan et al. 1979, Lem, Williams 1981). Concerning fluridone, the only paper available on this subject reports that this herbicide restored the accumulation of stearoyl-desaturase in olive embryos (Haralampidis et al. 1998). These authors suggest that the inhibition of ABA biosynthesis by fluridone may result in greater accumulation of unsaturated acids obtained from stearic acid. It seems that this effect has been confirmed by our results (Table 1, Figure 1*a*).

According to Fraser et al. (2007), overexpression of wild type tomato fruits with phytoene synthase (PSY) resulted in a decline in all major fatty acids, i.e. palmitic, stearic, oleic and linoleic ones, during the maturation of the fruits. This may suggest that a deficiency in phytoene desaturase activity may also affect the content of fatty acids in the tissue of tomato fruit. However, our results show that the inhibition of the activity of this enzyme has no influence on the fatty acid content, although it increased the ratio of unsaturated to saturated acids.

The use of fluridone did not affect the content of total phenols and malondialdehyde (Table 2). However, the herbicide caused an increase in the total content of flavonoids. Flavonoids are probably a small part of phenolic com-

Table 2

Effect of fluridone on some biochemical properties of the pericarp of tomato fruits

Analyzed property	Control	Fluridone treated
Total phenols (mg g^{-1} DW)	11.39±1.25	10.81±1.20
Total flavonoids (μg g^{-1} DW)	236.2±11.5	370.6±13.0**
Malondialdehyde MDA (nM g^{-1} DW)	18.94±1.34	21.13±0.62
Antioxidant capacity (% reduction of DPPH)	21.03±0.42	21.33±1.78
Proline (μg g^{-1} DW)	149.4±29.1	117.4±8.6
Soluble proteins (mg g^{-1} DW)	129.0±5.9	109.9±7.3*

Results (means of 5 replicates ± standard deviation) marked with ** and * are significantly different from the control at $P < 0.01$ and $P < 0.05$, respectively.

pounds, and their increase did not affect the antoxidant capacity. There was an insignificant decrease in the proline level under the influence of fluridone (Table 2). Our results regarding of the levels of proline and MDA are different than those obtained by Stetsenko et al. (2015). However, the cited authors applied used fluridone onto leaves of *Mesembryanthemum crystallinum* L., which decreased the content of ABA but increased the levels of proline and MDA. The authors explain that fluridone reduces the effect of ABA and acts as a prooxidant in photosynthetic tissues. A positive relationship between abscisic acid and proline synthesis was also confirmed in *Vigna unguiculata*

leaves (Costa et al. 2011). If fluridone lowers the ABA level in tissues of tomato fruit, it may be the main reason for reducing the content of proline. However, this requires further studies on the ABA content.

Lowering the proline content is probably a side effect of the significant inhibition of the accumulation of soluble proteins under the influence of fluridone (Table 2). Biosynthesis of proteins is the fundamental biochemical process, and a reduction in the protein content indicates a strong effect of fluridone on basic physiological process. The effect of fluridone on some compounds in pepper fruits has been recently studied by Prajapati et al. (2019). These authors have found that fluridone decreased the carotenoids level, respiration rate and loss of fruit weight during storage of bell pepper fruits. Besides, ABA treatment reduced the losses of ascorbic acid, total soluble solids and total carotenoid content. The above researchers indicate that the inhibition of ABA biosynthesis by fluridone is the main cause of these changes.

CONCLUSIONS

Our results indicate that use of fluridone did not cause changes in the content of unbound acids, although there was a tendency towards increasing the unsaturated acids ratio. Concerning total fatty acids, the use of fluridone significantly increased the ratio of oleic acid to stearic acid, as well as the ratio of the sum of unsaturated acids to saturated ones in the fluridone-treated pericarp of tomato fruits. Fluridone did not have an effect on the content of proline, total phenolic acids, MDA and antioxidant capacity, but inhibited the accumulation of soluble protein and enhanced the content of total flavonoids.

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