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RESEARCH PAPER

SPATIOTEMPORAL CHANGES IN THE CONTENT AND METABOLISM OF 9,12,13-TRIHYDROXY-10(*E*)-OCTADECENOIC ACID IN TOMATO (*SOLANUM LYCOPERSICUM* L. CV BALKONSTAR) FRUITS

J. Aghofack-Nguemezi[1] and W. Schwab[2]
[1]*Department of Plant Biology, Faculty of Sciences, University of Dschang, P. O. Box 67 Dschang, Cameroon*
aghofack@yahoo.fr
[2]*Biotechnology of Natural Products, Technische Universität München, Liesel-Beckmann-Str. 1, D-85354 Freising, Germany*

ABSTRACT
In a previous study, we showed that 9,12,13-trihydroxy-10(E)-octadecenoic acid which is probably involved in plant defense responses is synthesized in tomato fruits and subjected to metabolism. Its catabolism or conversion was thus further characterized. The endogenous level of 9,12,13-trihydroxy-10(E)-octadecenoic acid decreased in the mesocarp and endocarp during the maturation of tomato fruits. The level of this trihydroxy fatty acid in the epicarp declined till the mature-green stage and then increased during the colour change. Injection of buffered solutions of unlabeled 9,12,13-trihydroxy-10(E)-octadecenoic acid into two weeks old fruits while they were still attached to the plants led to the alteration of contents of ten metabolites. After the infiltration of buffered solutions of isotopically labeled 9,12,13-trihydroxy-10(E)-octadecenoic acid into green unripe tomato fruits which were still attached to the plant, the highest radioactivity was found in compounds much more polar than 9,12,13-trihydroxy-10(E)-octadecenoic acid and whose signals in HPLC-UV-chromatogram coincided with the citric acid peak. These results indicated that 9,12,13-trihydroxy-10(E)-octadecenoic acid could be implicated in the control of tomato fruit maturation and β-oxidation might be one of its degradation pathways.

Keywords: *Ethylene production, fruit maturation, ripening, β-oxidation, pinellic acid, Solanum lycopersicum L. cv Balkonstar*

INTRODUCTION

Fatty acid metabolites such as 9,12,13-trihydroxy-10(*E*)-octadecenoic acid (9,12,13-THODA , pinellic acid) belong to the vast and diverse family of plant secondary metabolites called oxylipins. Most oxylipins are involved in the control of plant defense against pathogens, oxidative stress and lipid peroxidation (López *et al*., 2011; Walters *et al*., 2006; Prost *et al*., 2005; Masui *et al*., 1989). Besides, 9,12,13-THODA contributed to the bitter taste of beer (Kuroda *et al*., 2002; Hamberg, 1991; Esterbauer and Schauenstein, 1977; Baur *et al*., 1977; Baur and Grosch, 1977); it also exhibited prostaglandin E-like (Ustünes *et al*., 1985) and adjuvant activities (Nagai *et al*., 2010; Shirata *et al*., 2006; Nagai *et al*., 2002).

Many studies to date have focused on the enzymatic (Kimura and Yokota, 2004; Hamberg, 1999; Hamberg and Hamberg, 1996) and chemical (Hamberg, 1987; Gardner *et al*., 1984) conversion of linoleic acid into 9,12,13-THODA. It has recently been shown that the enzyme peroxygenase is involved in the synthesis of 9,12,13-THODA in tomato fruits and that this trihydroxy fatty acid was probably further broken down or converted into other metabolites by enzymes among which at least one might be an 1-aminobenzotriazole-sensitive cytochrome P450 monooxygenase (Aghofack-Nguemezi *et al*., 2011). However, it is not yet known in which tomato fruit tissues and at which maturation stages 9,12,13-THODA is synthesized. Furthermore, no specific research work exists, to the best of our knowledge, on the catabolic aspects of the metabolism of this 9,12,13-THODA in plant tissues. The present study was thus undertaken to investigate whether the endogenous level of 9,12,13-THODA in tomato fruits varies in different tissue types and degree of maturation, test the possibility of changes in the levels of certain metabolites after the injection of buffered solutions of unlabeled 9,12,13-THODA in newly formed fruits and determine the fate of isotopically labeled 9,12,13-THODA during the fruit growth and development.

MATERIALS AND METHODS

Reagents

Most of the chemicals, salts and solvents of high purity were obtained from Fluka (Deisenhofen, Germany), Sigma-Aldrich (Deisenhofen, Germany), Roth (Karlsruhe, Germany) and Mallinckrodt Baker (Deventer, Netherlands).Water and acetonitrile of HPLC-gradient grade were from Merck (Darmstadt, Germany) and Fisher (Loughborough, United Kingdom), respectively. A [1-^{14}C]-linoleic acid solution with a specific activity of 55 mCi mmol^{-1} and a concentration of 0.1 mCi ml^{-1} was purchased from American Radiolabeled Chemicals Inc. (ARC, USA). The unlabeled 9,12,19-trihydroxy-10(*E*)-octadecenoic acid (pinellic acid) was kindly provided by Professor Mats Hamberg, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.

Production of lipoxygenase and peroxygenase proteins from transformed yeast cells

Yeast (*Sacharromyces cerevisiae*) INVSc1 host strain and the pYES2 vector were purchased from Invitron (Karlsruhe, Germany). The cloning of genes of interest (9-lipoxygenase (9-LOX) and peroxygenase (PXG)) into pYES2, preparation of competent yeast cells, transformation of pYES2 plasmid constructs into competent INVSc1 and induction of the expression of recombinant proteins were done according to protocols provided by the manufacturer. For the extraction of the 9-LOX proteins, INVSc1 cells were homogenized by centrifugation (1500 *g*, 5 min) and re-suspended in a volume of breaking buffer (50 mM sodium phosphate, pH 7.5, 1 mM EDTA, 5% glycerol, 1 mM PMSF) to obtain an OD_{600} of 50. An equivalent volume of glass beads was added to the mixture which was subsequently homogenized by vortexing for 30 sec, followed by 30 sec on ice. This procedure was repeated 10 times for a total of 10 min. Cell debris were removed by centrifugation at 13000 *g* for 2 min. PXG proteins were extracted by isolating yeast microsomes. INVSc1 cell crude extract was thus dissolved in Tris/HCl buffer (100 mM, pH 7.5) containing 1 mM PMSF, 10% (w/v) PEG4000 and 3% (v/v) of a 0.5 M NaCl solution and the mixture was centrifuged at 10000 *g* for 10 min. The precipitate was then re-suspended in Tris/HCl buffer (100 mM, pH 7.5) containing 1 mM PMSF and 0.001 % (w/v) Tween 20 and the mixture homogenized by pottering 10 times. Concentrations of LOX and PXG proteins were determined by Bradford assay (Bradford, 1976). The supernatants were aliquoted as needed and stored at -20°C.

Enzymatic *in vitro* synthesis of radiolabeled 9,12,13-trihydroxy-10 (*E*)-octadecenoic acid from [1-^{14}C]-linoleic acid

The enzymatic *in vitro* formation of 9,12,13-THODA was modeled on one known biosyn-

thetic pathway involving lipoxygenase and peroxygenase in plant tissues (Hamberg and Hamberg, 1996). The lipoxygenase reaction mixture contained 1.25 ml of sodium phosphate buffer (0.2 M, pH 7.0), 3.11 ml of distilled water, 69 µl of a solution of unlabeled linoleic acid (30 mM), 500 µl of [1-^{14}C] linoleic acid solution (0.1 mCi/ml) and 70 µl LOX-protein solution (6.77 µg µl^{-1}). This reaction mixture was allowed to stand for 4 h at room temperature. Reactions products were subsequently extracted using C_{18} cartridge. The C_{18} column was successively equilibrated with 1 ml of acetonitrile and 1 ml of distilled water. The LOX reaction mixture was then loaded onto a C_{18} cartridge which was washed with 1 ml of distilled water. Thereafter, 4 ml of acetonitrile were used for the elution. The elute was brought to dryness by speed vacuum at 40 °C and the residue resuspended in 200 µl methanol. This methanolic extract contained 9-hydroperoxy-10(*E*)-12(*Z*)-octadecenoic acid (9-HPOD) which is the substrate of peroxygenase (PXG). The PXG reaction mixture contained 0.5 ml of sodium acetate buffer (100 mM, pH 5.5), 3.1 ml of distilled water, 0.2 ml of LOX reaction product and 1.2 ml of PXG protein solution (6.3 µg µl^{-1}). This mixture was allowed to stand for 15 h at room temperature. One hundred µl of a hydrochloric acid solution (1 M) was added to adjust the pH to 2.3. The homogenate was then further incubated for 3 h at room temperature. The extraction of [1-^{14}C]-9,12,13-THODA was performed by means of C_{18} column chromatography as described above, the elute concentrated by speed vacuum at 40 °C and the residue resuspended in 300 µl of acetonitrile.

Purification of the radiolabeled 9,12,13-trihydroxy-10 (*E*)-octadecenoic acid

For the purification of radiolabeled 9,12,13-THODA, 0.28 ml of the reaction product and 1 ml of acetonitrile were mixed. This mixture was then loaded onto a C_{18} cartridge that was previously equilibrated as described above. Two ml of acetonitrile/water mixture (60/40, v/v) were used for the elution. The elute was concentrated by speed vacuum at 40 °C. The residue was then dissolved in 2 ml of a mixture containing acetonitrile and MMS medium in 1/3 volume ratio. The MMS medium was a mixture of 2.15 g of Murashige and Skoog medium, 0.97g of 2-(*N*-morpholino) ethanesulphonic acid and 10 g of saccharose in 500 ml of distilled water. The pH of the MMS medium was adjusted to 5.6 with 1 M KOH solution.

Plant material and treatments

Tomato (*Solanum lycopersicum* L. cv Balkonstar) plants were grown in a growth chamber under a 16h/8h- photoperiod with a light intensity of 70±10 µmol/m^2/s and constant temperature of 23±1 °C. Tomato fruits were harvested at different maturation stages (Fig. 1), their pericarp, mesocarp and endocarp separated and stored at -20 °C for the study of spatiotemporal variations of the endogenous level of 9,12,13-THODA. Furthermore, about two weeks old fruits of the same size were injected with solutions of unlabed and isotopically labeled 9,12,13-THODA or control solutions while they were still attached to the mother plants. Thus, 50 µl and 150 µl of solutions of [1-^{14}C]-9,12,13-THODA (total radioactivity 14.16 µCi) and unlabeled 9,12,13-THODA (2 mg in 1 ml of a acetonitrile /MMS-mixture, v/v, 1/3), respectively were injected into each treated fruit. Control fruits received 50 µl or 150 µl of MMS medium. Fruits treated with [1-^{14}C]-9,12,13-THODA and their controls were harvested at red-ripe stage. However, control and related treated tomato fruits which were injected with unlabeled 9,12,13-THODA were harvested five and twenty six days after the application of 9,12,13-THODA. The fruits were stored at -20 °C after harvest.

Extraction

Tissues (epicarp, mesocarp and endocarp) of tomato fruits at different maturation stages were separated, ground in a mortar under liquid nitrogen. One g each was homogenized with a mixture containing 1 ml of methanol and 20 µl of 4-methylumbellyferyl-β-D-glucuronide dihydrate solution (9 mg/ml methanol) as internal

Fig. 1: Maturation stages used. From left to the right: 1, ø (longitudinal diameter)= 0.7 cm; 2, ø= 2.0 cm; 3, ø= 3.0 cm; 4, mature green (ø= 4.5 cm); 5, turning; 6, pinkish; 7, red-ripe

standard. The mixture was centrifuged (5300 *g*, 30 min), 2 ml of supernatant then removed and concentrated by speed vacuum. The residue was re-suspended in 150 µl methanol and centrifuged again for 10 min at 13400 *g*. The supernatant was removed, stored at 4 °C and subsequently analyzed by LC-MS. For studies on the metabolism of radiolabeled or unlabeled 9,12,13-THODA *in vivo*, 16 g of treated or control tomato fruits were ground and homogenized with 16 ml methanol using an Ultra Turrax® (T18 basic, IKA®, Works Inc., Wilmington, NC, USA). An aliquot (320 µl) of the internal standard 4-methylumbellyferyl-β-D-glucuronide dihydrate solution (9 mg/ml methanol) was also added to extracts from controls and tomato fruits into which solutions of unlabeled 9,12,13-THODA had been injected. The mixtures were then centrifuged (4500 *g*, 30 min), the supernatants removed, stored at 4 ° C and later analyzed using LC-MS, HPLC or scintillation counter.

LC-MC

An Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump and a variable wavelength detector, and connected to a Bruker Daltonics Esquire 3000^{Plus} ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) was utilized. A C_{18} column (150 mm x 2.0 m, particle size 5 µm) held at 25 °C was used. The mobile phase was a mixture of water (A) and methanol (B) both containing 0.1% formic acid. The flow rate was 0.2 ml min^{-1}. A gradient of 100% A for 5 min, 100% A to 60% A within 20 min, 60% A to 0% A in 5 min, 100% B for 7 min, 100% B to 0% B within 3 min and100% A for 10 min was applied. The detection wavelength was 280 nm. The electron spray ionization voltage of the mass spectrometer ranged from -4000 V to – 500 V. Nitrogen was used as dry gas at temperature of 330 °C and a flow rate of 10.01 min^{-1}. The full scan mass spectra were measured in the range from *m/z* 50 to 800 with a scan resolution of 13000 *m/z* s^{-1}. The collision gas for the mass spectrometry was helium with a collision voltage of 1 V. Mass spectra were acquired in both positive and negative ionization modes. Analyses of LC-MS data were performed using the Bruker Daltonics software. Automated comparison of $[M-H]^-$ and $[M-H]^+$ was performed using the bioinformatics software R.2.13.1 (R Project for Statistical Com-

puting, version 2.13.1, www.r-project.org, accessed on 22/08/2011).

HPLC

HPLC analysis with UV detection was performed using an HPLC system equipped with a Gilson Abimed (Abimed, Langenfeld, Germany) model 231 sample injector connected to two Gilson Abimed pumps, model 303, and a Knauer variable wavelength monitor (Knauer, Berlin, Germany). A Gilson 712 HPLC controller software version 1.02 was used for data acquisition and evaluation. A C_{18}-column (GROM-SIL 80 ODS-7 AQ) and a cyano column (YMC-Pack CN, 250 x 4,6 mm,YMC, Japan) were utilized. The mobile phases were either a mixture of water (A) and acetonitrile (B) or A alone, both A and B containing 0.1% formic acid. A binary linear gradient of 100% A for 5 min, 100% A to 60% A within 25 min, 60% to 0% A in 5 min, 100% B for 2 min, 100% B to 0% B within 2 min was applied on the C_{18}-column with a flow rate of 0.5 ml min^{-1} from 0 to 5 min, and 0.8 ml min^{-1} from 5 to 44 min. An isocratic gradient of 0.1% A with a flow rate of 0.5 ml min^{-1} was also alternatively applied on a cyano column. C_{18}-column was used for the fractionation of raw extracts while the cyano column was used for the re-fractionation of elutes and for the analysis of the citric acid solution. A Knauer Eurochrom 2000 software was used for data acquisition and evaluation. The detection wavelength was 220 nm.

Determination of radioactivity

Five ml of the scintillation cocktail ULTIMA FLO™ AF (Packard Biosciences, USA) was added to the samples and the mixture was homogenized with the help of a vortex. The determination of the [^{14}C]-radioactivity was performed using a Tricarb® 2800 TR liquid scintillation analyzer (Perkin Elmer, Rodgau Hügesheim, Germany).

Statistical analysis

Group comparisons were made using One – Way Analysis of Variance (ANOVA) to see if variations among means were significantly greater than expected by chance. The Student – Newman – Keuls Test was used to compare means differences. In some few cases where differences between standard deviations of means were significant, the GraphPad Instat – software automatically proposed the use of rather the Nonparametric ANOVA and Kruskal -Wallis (as post-test).

RESULTS AND DISCUSSION

Spatial and temporal variations in the endogenous level of 9,12,13-trihydroxy-10(*E*)-octadecenoic acid

The levels of 9,12,13-THODA were measured in different fruit tissues during fruit development and maturation. The content of 9,12,13-THODA decreased in the mesocarp and endocarp of tomato fruits with increasing maturation stage (Fig. 2). Negative correlation coefficients (r= -0.769 and -0.783, respectively) could be found between changes in the level of 9,12,13-THODA in the mesocarp and endocarp, and the maturation stages of tomato fruits. This was an indication that the rate of synthesis of the trihydroxy fatty acid declined with increasing age in the tomato fruit mesocarp and endocarp. 9,12,13-THODA thus accumulated in these tissues at early developmental stages and was probably subsequently degraded or converted into new products. Moreover, the rate of degradation or conversion of this trihydroxy fatty acid may have exceeded that of its synthesis in the mesocarp and endocarp at certain stages of fruit maturation. Climacteric fruits like tomato are characterized by a very low production of ethylene until the commencement of ripening and a burst of ethylene production at the onset of ripening (Biale and Young, 1981). The present finding that the level of 9,12,13-THODA in the mesocarp and endocarp drastically decreased with increasing maturation stage of tomato fruits corroborated previous results of López *et al*. (2011) demonstrating an antagonistic relationship between products of 9-lipoxygenase (9-LOX) and ethylene pathways. 9,12,13-THODA is synthesized from 9-hydroperoxy-10(*E*),12(*Z*)- octadecadienoic acid

which is produced in a reaction catalyzed by 9-LOX (Hamberg and Hamberg, 1996).

The 9,12,13-THODA level was high in the epicarp at maturation stage 1 but declined from stages 2-4 and increased again from stage 5 onwards (Fig. 2). In fact, the epicarp is a special tissue that undergoes the most perceptible changes (e.g. color change) during the development of tomato fruits. Two different ethylene systems have been reported in plants (McMurchie *et al*., 1972; Oetiker and Yang, 1995): the system 1 is the basal low rate of ethylene production present during the preclimacteric phase of the ripening of climacteric fruits, in vegetative tissues and nonclimacteric fruits; system 2 genes are involved in the high rate of ethylene biosynthesis observed at the onset of the ripening of climacteric fruits and in certain senescent flowers. Obviously, 9,12,13-THODA differentially regulated by still unknown mechanisms the ethylene biosynthesis in the epicarp during the greening and degreening phases, thus promoting the maintenance of relatively low and high rates of ethylene production respectively during the first and second phases.

Metabolic profiling in extracts from tomato fruits after the application of buffered solutions of unlabeled 9,12,13-trihydroxy-10*(E)*-octadecenoic acid

In order to bolster the hypothesis of the degradation or conversion of 9,12,13-THODA in tomato fruits during the maturation process, buffered solutions of this fatty acid were infiltrated into green unripe full-size fruits that were still attached to the plant. Fruits extracts were then analyzed using LC-MS followed by automated comparison of ion signal intensities and statistical analysis. These studies revealed that five days after injection of 9,12,13-THODA, the endogenous levels of unknown metabolites –MS *m/z* 379, -MS *m/z* 397 and -MS *m/z* 722 were significantly higher in 9,12,13-THODA-treated than control fruits whereas extracts obtained from fruits which received only the control solution contained significantly higher levels of –MS *m/z* 119, -MS *m/z* 159 and –MS *m/z* 255 than extracts from fruits that were injected with buffered solutions of 9,12,13-THODA. Twenty six days after the infiltration of buffered solutions of 9,12,13-THODA into tomato fruits, contents of –MS *m/z* 188, -MS *m/z* 476, +MS *m/z* 497 and +MS *m/z* 519 were higher in extracts from 9,12,13-THODA-treated than control fruits (Table. 1). These results showed

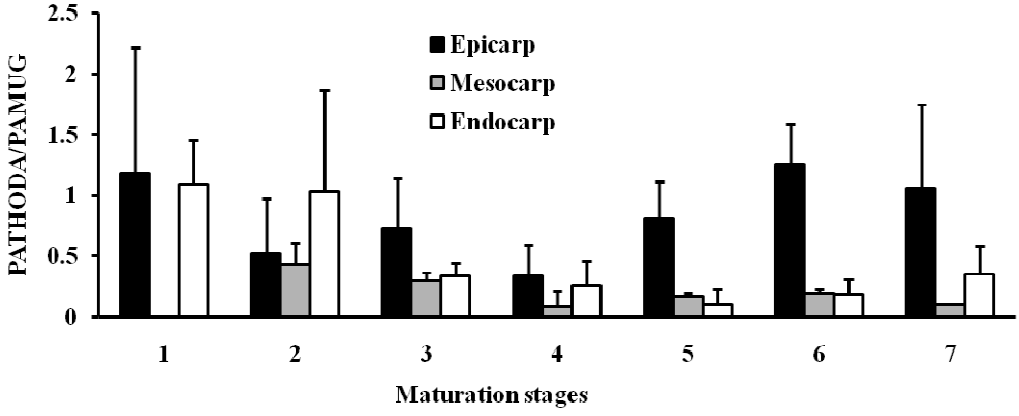

Fig. 2: **Relative content of 9, 12,13-trihydroxy-10(*E*)-octadecenoic acid in extracts from different tissues of tomato fruits at maturation stages 1 to 7 (see fig. 1). PATHODA, peak area of 9,12,13-trihydroxy-10(*E*)-octadecenoic acid; PAMUG, peak area of the internal standard 4-methylumbellyferyl-β-D-glucuronide dihydrate.**

Table 1: Bioinformatics R.2.13.1 (R Project for Statistical Computing, version 2.13.1, www.r-project.org) and statistical comparison of LC-MS-monoisotopic molecular mass signals (*m/z*) of ions ($[M-H]^+$ and $[M-H]^-$) in extracts from controls (C) and tomato fruits into which a buffered solution of 2 mg/ml unlabeled 9,12,13-trihydroxy-10(*E*)-octadecenoic acid (pinellic acid) was injected (T). RT, retention time; S, significant.

Compound	RT (min)	$[M-H]^-$ (*m/z*)	$[M+H]^+$ (*m/z*)	R 2.13.1-analysis	Statistical analysis
1	2.38	119		$C_1>T_1$	$C_1>T_1$, S ($P<0.05$)
2	2.45	255		$C_1>T_1$	$C_1>T_1$, S ($P<0.05$)
3	4.36	188		$C_2<T_2$	$C_2<T_2$, S ($P<0.01$)
4	27.71	159		$C_1>T_1$	$C_1>T_1$, S ($P<0.01$)
5	35.93	397		$C_1<T_1$	$C_1<T_1$, S ($P<0.05$)
6	36.95	379		$C_1<T_1$	$C_1<T_1$, S ($P<0.05$)
7	37.56	722		$C_1<T_1$	$C_1<T_1$, S ($P<0.05$)
8	38.36	476		$C_2<T_2$	$C_2<T_2$, S ($P<0.05$)
9	38.93		497	$C_2<T_2$	$C_2<T_2$, S ($P<0.05$)
10	38.93		519	$C_2<T_2$	$C_2<T_2$, S ($P<0.01$)

that there were temporal variations of 9,12,13-THODA breakdown or conversion products during the maturation of tomato fruits. Obviously, the increase in the endogenous level of 9,12,13-THODA after the injection of buffered solutions of this fatty acid into green unripe tomato fruits triggered reaction pathways where there was a continuous utilization on intermediate products. Further studies are needed to determine the exact chemical nature of compounds whose main ion mass signals were altered after the infiltration of buffered solution of 9,12,13-THODA into tomato fruits.

Fate of radiolabeled 9,12.13-trihydroxy-10 (E)-octadecenoic acid in tomato fruits

The isotopically labeled 9,12,13-THODA was enzymatically synthesized from [1-^{14}C]-linoleic acid and infiltrated as buffered solutions into green unripe full-size tomato fruits which were still attached to the plant. Extracts from red-ripe fruits were subsequently fractionated using HPLC and the radioactivity of each fraction determined. It appeared from these analyses that the main 9,12,13-THODA degradation or conversion products (Fig. 3B) were much more polar than 9,12,13-THODA itself (Fig. 3A). Metabolic pathways involving 9,12,13-THODA have not yet been report in plant tissues. Notwithstanding, microbial transformations of other types of trihydroxy fatty acids into tetrahydrofuranyl fatty acids and bicyclical diepoxy fatty acids have already been reported (Hou, 2009; Hosokawa *et al*., 2003; Iwasaki *et al*., 2002). Indeed, tetrahydrofuranyl fatty acids are structurally close to known anticancer compounds (Kawagishi *et* al, 1990; Stadler *et al*., 1994) and diepoxy fatty acids could be used in biochemical and chemical industrial processes.

Since it is well established that acetyl-CoA, the end-product of the β-oxidation of fatty acids, can condense with oxaloacetate to form citrate at the early step of the Krebs cycle (MacDonald

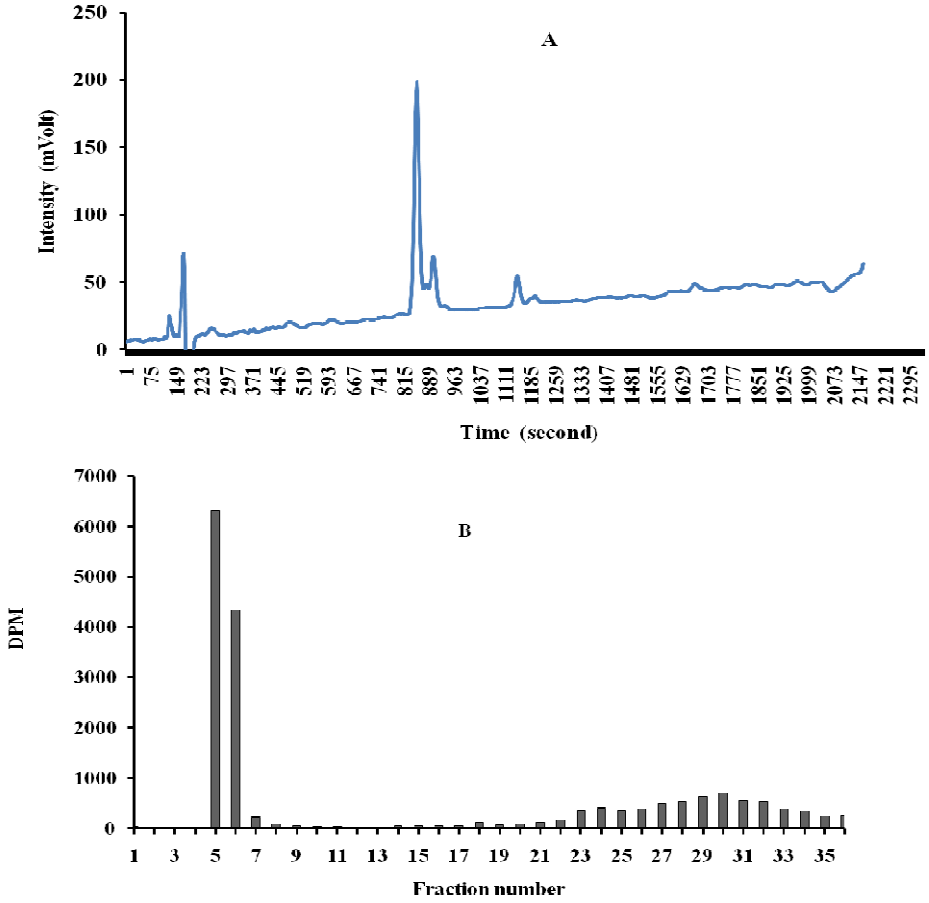

Fig. 3: HPLC-UV-chromatogram of a 2 mg/ml solution of unlabeled 9,12,13-trihydroxy-10(*E*) -octadecenoic acid (A) and the absolute radioactivity of HPLC-fractions from extracts of red-ripe tomato fruits which previously received a buffered solution of [1-^{14}C]- 9,12,13-trihydroxy -10(*E*)-octadecenoic acid (B). A C_{18}-column and a binary solvent gradient were used for the HPLC analysis. The collection of each fraction lasted 1 min. DPM, disintegrations per minute.

and Vanlerberghe, 2006), a solution of citric acid and the fraction which was supposed to contain the main product of the [1-^{14}C]-9,12,13 -THODA metabolism were further simultaneously analyzed using HPLC. The peak of the putative main 9,12,13-THODA breakdown or conversion product (Fig. 4A) coincided with the citric acid peak (Fig. 4B) on UV-chromatograms. Thus, after the completion of its yet still unknown physiological function, 9,12,13-THODA might at least partially be degraded through the β-oxidation reaction pathway and the resulting acetyl-CoA used in the Krebs cycle. However, citric acid (-MS *m/z* 191 or +MS *m/z* 215 = [M + Na^+]) was not among the metabolites whose concentration was significantly altered after the infiltration of 9,12,13 -THODA solution into tomato fruits (Table. 1).

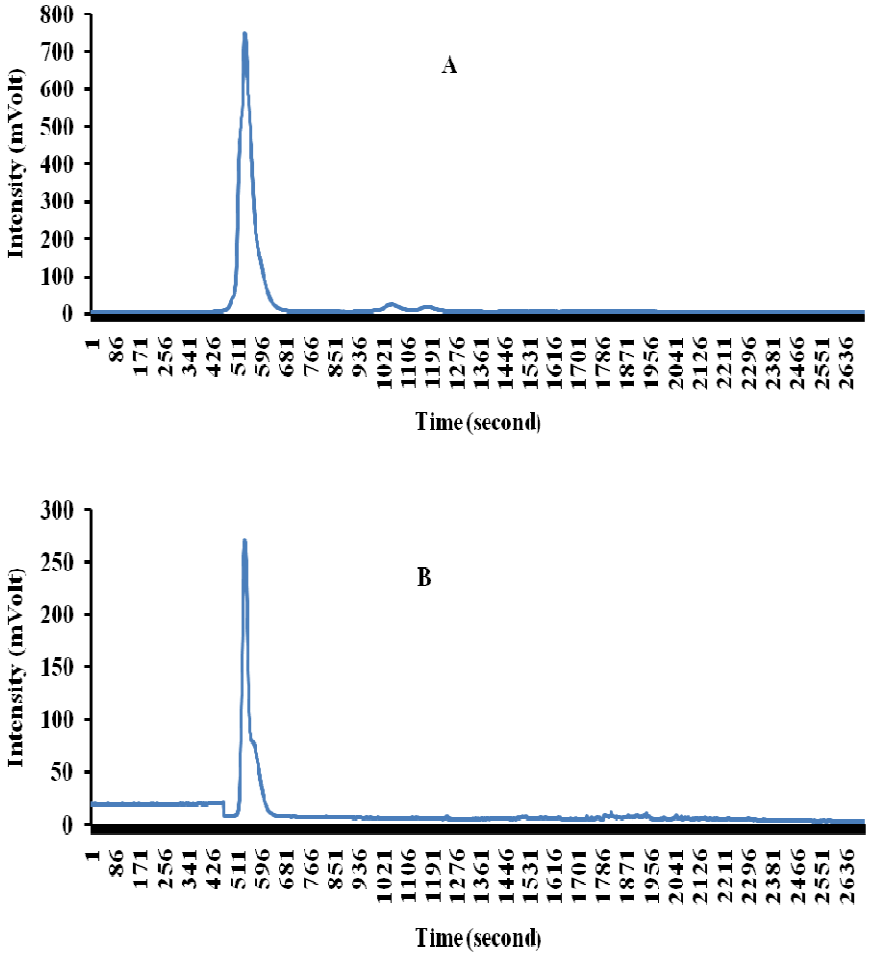

Fig. 4: HPLC-UV-chromatograms of fraction 5 (see figure 3B) (upper diagram, A) and of a 2 mg/ml solution of citric acid (lower diagram, B). A cyano column and an isocratic solvent gradient were used.

This was an indication that the amount of citric acid formed as a result of the β-oxidation of 9,12,13-THODA was insignificant in comparison to the already abundant existing endogenous pool.

CONCLUSION

The results showed that there were spatiotemporal changes in the endogenous level of 9,12,13-trihydroxy-10(*E*)-octadecenoic acid and and temporal variations of its transformation products in tomato fruits. The breakdown or conversion products of this trihydroxy fatty acid were more polar than the parent molecule. Since labeled carbons of [1-^{14}C]-THODA accumulated in citric acid it is assumed that THODA is rapidly degraded by ß-oxidation.

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