SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
ISSN Print: 1304-7582, Online: 2146-9377
http://www.academicfoodjournal.com

Akademik Gıda 11 (2) (2013) 6-13

Research Paper / Araştırma Makalesi

Distribution of Volatile Compounds in Organic Tomato (*Lycopersicon esculentum*) at Different Ripening Stages

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Received (Geliş Tarihi): 15.05.2013, Accepted (Kabul Tarihi): 30.06.2013
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ABSTRACT

In this study, volatile compounds of tomatoes (*Lycopersicon esculentum* cv. Sereflikochisar) at different ripening stages using solid phase micro extraction technique (SPME) were determined. Tomatoes were organically produced under open-field conditions. Forty one volatile compounds at green stage, 47 at the red stage and 33 at the over ripe stage were determined. Compounds *(Z)*-2-hexen-1-ol, 1-octen-3-ol, *(E)*-2-octen-1-ol, *(E,Z)*-3,6-nonadienol, *(Z)*-3-octen-1-ol, 2,6-dimethyl-5-heptenal, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, *(E,Z)*- 2-6-nonadienal and *(Z)*-4-decenal were identified for the first time in tomatoes. At the green stage, the main volatiles were found to be methyl salicylate, *(E)*-2-hexenal, *(Z)*-3-hexen-1-ol and hexanal in the proportions of 29.4, 23.7, 9.0 and 5.0%, respectively. At red stage, the major volatiles were 6-methyl-5-hepten-2one, 2-isobutylthiazole, 1-hexenol and *(Z)*-4-decenal in the proportions of 17.2, 12.9, 10.2 and 5%. 4-Ethyl phenol (para ethyl phenol) and 4-ethyl-2-methoxy phenol (4-ethyl guaiacol) were the most plentiful compounds in over-ripe tomato fruits. The distribution and concentrations of volatile compounds were considerably varied depending on the ripening stage.

Key Words: Volatile compounds, Organic tomato, Ripening stage

Farklı Olgunlaşma Aşamalarında Organik Domateslerdeki (*Lycopersicon esculentum*) Uçucu Bileşenlerin Dağılımı

ÖZET

Bu çalışmada katı faz mikroekstraksiyon tekniği (SPME) kullanılarak, farklı olgunlaşma aşamalarında domateslerdeki ((*Lycopersicon esculentum* cv. Şereflikoçhisar) uçucu bileşenler belirlenmiştir. Domatesler tarla şartları altında organik olarak yetiştirilmiştir. Yeşil aşamada 41 uçucu bileşen belirlerken, kırmızı aşamada 47 ve aşırı olgun aşamada 33 uçucu bileşen tespit edilmiştir. Bileşenler *(Z)*-2-hekzen-1-ol, 1-okten-3-ol, *(E)*-2-okten-1-ol, *(E,Z)*-3,6-nonadienal, *(Z)*-3-okten-1-ol, 2,6-dimetil-5-heptenal, 2,6,6-trimetil-1-siklohekzen-1-karboksaldehit, *(E,Z)*-2,6-nonadienal ve *(Z)*-4-desenal domateslerde ilk kez belirlenmiştir. Yeşil domateslerde başlıca uçucular metilsalisilat, *(E)*-2-hekzenal, *(Z)*-3-hekzen-1-ol ve hekzanal sırasıyla %29.4, 23.7, 9.0 ve %5.0'lık oranlarda tespit edilmiştir. Kırmızı aşamada ise başlıca uçucular olan 6-metil-5-hepten-2-on, 2-izobütiltiyazol, 1-hekzenal ve *(Z)*-4-desenal sırasıyla %17.2, 12.9, 10.2 ve 5.0'lık oranlarda bulunmuştur. Aşırı olgunlaşma dönemindeki domateslerde en bol bulunan bileşenler 4-etilfenol (para etil fenol) ve 4-etil-2-metoksifenol (4-etilguaikol) olmuştur. Uçucu bileşenlerin dağılımı ve konsantrasyonu olgunlaşma aşamasına bağlı olarak belirgin bir şekilde değişim göstermiştir.

Anahtar Kelimeler: Uçucu bileşenler, Organik domates, Olgunlaşma aşaması

INTRODUCTION

Tomato (*Lycopersicon esculentum*) belongs to the *Solanacea* family. The unique flavor (taste and odor) of tomato fruit is the result of a complex balance between non-volatile (sugar and acid) and volatile chemical compounds, originating from mainly carotenoids, amino acids and fatty acids [1, 4]. The C_6 aldehydes (hexanal, *cis*-3-hexenal, *trans*-2-hexenal) are known as 'green' compounds, as they impart a fresh, green character to tomato aroma, ketones (6-methyl-5-hepten-2-one, geranylacetone and β-ionone) and some esters (2-isobutylthiazole) are contributors to a fruity and bitter/pungent aroma, respectively [5]. Many factors affect tomato flavor, e.g. cultivar, growing system, maturity, harvest time, carotene content and post-harvest treatments [6]. According to [7], the flavor of commercially produced tomatoes is inferior, the biochemical and genetic complexity of the trait has made breeding for improved flavor. This may have a critical importance as lack of the characteristic flavor in marketable tomato fruits is a common consumer complaint nowadays. In general, local people prefer organic traditional tomato varieties to hybrid ones due to their excellent sensory quality. In a previous study, the traditional tomatoes showed considerably higher concentrations of several volatile compounds, such as 3-methyl butanal, hexanal, *cis*-3-hexenal, 1-hexanol and 2-isobutylthiazole [3]. However, to our knowledge, the volatile compounds of Turkish traditional tomato varieties have not been studied. It has also been no observed the changes in traditional tomato volatiles during ripening process. Of volatile extraction techniques, solid phase microextraction (SPME) is well-known and widely used technique in food. It is a simple, fast and solvent-free technique [8]. SPME is a non-quantitative extraction technique, whereas the heating of sample is minimum and the recovery of volatile compounds is closer to real value in sample than those obtained by heating techniques such as simultaneous steam-distillation extraction (SDE) and hydrodistillation (HD) [3].

There have been a large number of studies carried out on the identification of the volatile components of fresh tomatoes [1-4]. Despite these studies, there were no identified volatile compounds in traditional tomato variety (*Sereflikoçhisar*) produced organically under the open-field conditions during ripening process. This study may indicate that why people prefer organic traditional tomato variety to hybrid ones. Therefore, the first objective of the present study was to determine the volatile compounds in tomatoes at the different ripening stages. The second objective was to investigate volatiles in organic traditional tomato variety and to compare with hybrid tomato ones in previous studies.

MATERIALS and METHODS

Plant Material and Growing Conditions

Seeds of traditional tomato (*cv. Sereflikoçhisar*) were grown in the greenhouses of organic farm in Zara, Sivas (Turkey) over spring-summer cycle. The seedlings at three true leaf stages were transplanted to open-field at the beginning of June 2010. Zara has a terrestrial-climate. The experiment was set up in a complete randomized block design with three replications. Ten plants in each plot were randomly selected for data collection. Seedlings were organically grown over summer-autumn growing cycle. Seedlings were irrigated with tap-water with electrical conductivity (EC) = 0.5 dS/m and pH = 7.0–7.4. The firstly, green tomato fruits (no red color) were picked on 20 August 2010. The secondly, tomatoes were harvested on 22 September 2010, when color turned to red. Finally, over ripe tomatoes were picked on 20 October 2010. So, tomatoes harvested at about one month intervals. Tomato fruits have transferred to laboratory at 10°C for one day.

Volatile Compound Analysis

The extraction and characterization of the volatile compounds were carried out by means of headspace (HS)-solid phase microextraction (SPME)- Gas Chromatography (GC)-Mass Spectrometry (MS) analysis, which is able to detect most of the volatile compounds. Volatile samples were prepared in triplicate at each harvest stage. The each fruit was cut into small pieces and placed in a chilled mortar and ground with a pestle. After several preliminary tests to optimize solid phase microextraction (SPME) system, 10g of the homogenized flesh using a Pasteur pipette was immediately transferred in 20 mL head space vial (Agilent, USA), containing 3g NaCl, to inhibit enzyme reactions. The vials were sealed using crimp-top caps with TFE/silicone headspace septa (Agilent, USA) and immediately frozen at -20°C until use. Prior to analysis, frozen samples were thawed at 4°C overnight. At the time of solid phase microextraction analysis the vials were placed in a water bath with temperature control and stirring. Vials were kept at 45°C for 30 min, then a 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte PA/USA) fibre was exposed to the sample headspace for 15 min at 60°C. The fibre was chosen for its high capacity of trapping fruits volatile compounds [8] and a similar extraction procedure was previously carried out in tomatoes by [3]. These sampling temperatures were also chosen after preliminary trials at different temperatures. After sampling, desorption of the volatile compounds from the fibre coating was carried out in the injection port of GC at 250°C during 1 min in splitless mode. The identification and quantification of volatile compounds were carried out on Agilent model 6890 GC and 5973 N mass spectrometry (MS) (Agilent, Palo Alto, CA, USA) equipped with a HP-INNOWAX capillary column (60 m x 0,25 mm id x 0,25µm film thickness). Helium used as carrier gas at a flow rate of 1mL min^{-1}. The oven temperature program was initially held at 50°C for 1 min and then programmed from 50ºC by a ramp of 5°C min^{-1} up to 100°C and then at 10°C min^{-1} to reach a final temperature of 230ºC, which held for 5 min. The mass selective (MS) detector was operating in the scan mode within a mass range 33 to 330 m z^{-1} at 1 scan s^{-1}, with electron energy of 70eV. The interface line to MS was set at 250°C. The total analysis time was 30 min. The volatile compounds were preliminarily identified by a computer-matching of their mass spectral data supplemented with a Wiley7n.1 and Nist 02.L. GC-MS libraries, and then the identifies of most were confirmed by GC retention time (RT) and MS ion spectra of authentic standards (Sigma-Aldrich, Milwaukee, WI, USA). The retention indices were also determined for all constituents by using homologous series of *n*-alkanes C_5–C_{25}. Results from the volatile analyses were expressed as the percentage of each compounds integrated area relative to the total integration of compounds identified.

RESULT and DISCUSSION

A total of 75 volatile compounds were identified in headspace of fresh tomato extracts by solid phase microextraction technique (SPME) using GC-MS. There were qualitative and quantitative differences in the volatile composition of the tomato at the different ripening stages (Table 1). Sixty-one, 47 and 33 volatile compounds were identified at green, red and over ripe stages of tomatoes, respectively. In terms of relative proportion and number, the volatiles were mainly classified into seven chemical classes: aldehydes (20), alcohols (19), terpenes (11), ketones (5), esters (5), phenolic (7) and sulfur compounds (3). The relative proportion (percent) of the main volatile classes in green, red and over ripe tomatoes are shown in Figure 1. Only a few volatiles were detected in other chemical classes: hydrocarbon, hormon, alkoloid, furan, guanidin, spiro compounds (SHD) (Table 1).

Aldehydes, esters and alcohols were the main components, accounting for 82% of the total volatiles quantified, in tomato at green stage. However, terpenes and compounds containing sulfur, especially 2-isobuthyl thiazole as well as alcohols and aldehydes were the most abundant components, representing for 78% of the total volatile compounds quantified in tomato at red stage. Phenolic compounds, terpenes and alcohols accounted for 70% of the total volatiles in over ripe tomato. Compounds n-hexanol, *(Z)*-3- hexenol, nonanol, benzyl alcohol, hexanal, *(E)*-2-hexenal, salicyl aldehyde, α-terpinolene, *trans-β*-ionone, 6-methyl-5-hepten-2-one and napthaline were commonly detected in tomatoes at all ripening stages. Previous studies (9, 10, 5) evaluated 10 important volatile compounds (*cis*-3-hexenal, 2+3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, *cis*-3-hexenol, 1-penten-3-one, geranylacetone, 2-isobutylthiazole, methyl salicylate and *β*-ionone) for tomato aroma. Of these volatile compounds, hexanal, *(Z)*-3-hexenol, *trans- β*-ionone and 6-methyl-5-hepten-2-one, although, are detected at all ripening stages of tomatoes, 2-methylbutanol, *trans*-2-heptenal, geranylacetone and 2-isobutylthiazole have been found in red and over ripe tomatoes only. This result was in consistent with findings of [11] since they have reported that *trans*-2-heptenal, geranylacetone and 6-methyl-5-hepten-2-one were positively correlated to ripe and sweetness flavor ratings. In our study, compounds acetaldehyde, acetone, methanol, 1-penten-3-one, *cis*-3-hexenal and 1-nitro-2-phenylethane were not detected in tomatoes. This is probably due to analysis technique, cultivar and growing conditions of tomatoes since *cis*-3-hexenal was previously found in simultaneous steam-distillation extraction (SDE) and hydrodistillation (HD) techniques, but was not in solid phase microextraction (SPME) [3]. Meanwhile, acetaldehyde, acetone and methanol are volatile compounds responsible for off-flavor notes [11]. The present study is emphasized that no volatile compounds with off-flavor notes are produced in organic local tomato variety at all ripening stages. Contrary to earlier studies done by [12], [3], [7], and [4], methyl salicylate, 6-methyl-5-hepten-2-one and 4-ethyl phenol were found to be the main volatile compounds in green (29.4%), red (17.16%) and over ripe (19.62%) tomatoes, respectively, instead of C_6 aldehydes and alcohols such as *(E)*-2-hexenal, hexanal and n-hexanol.

As previously mentioned, the distributions of volatile compounds in green, red and over ripe tomatoes were different (Figure 1). Aldehydes made up 34% of the total volatiles in green tomato, followed by esters. As expected, the green tomato had considerably higher lipid-derived C_6 aldehydes and alcohols, especially *(E)*-2-hexenal, *(Z)*-3-hexen-1-ol and hexanal than red and over ripe tomatoes. These 6-carbon aldehydes and alcohols are known as green leaf volatiles (GLVs) which are synhesized from either C_{18} linoleic or linolenic via the action of 13-lipoxygenase (13-LOX) and hydroperoxide lyases [17]. Despite the precautions taken during the preparation of samples (LOX deactivation with NaCl), the possibility that this may partly be the result of tissue damage occuring during homogenization. In addition to GLVs, shikimic acid-derived volatile esters, especially methyl salicylate, are induced in response to mechanical damage or herbivore [18, 19]. Methyl salicylate (MeSa) was predominant volatile compound in green tomato and was also detected at green stage only (Table 1). Nevertheless, it seems reasonable to suppose that the high concentrations of both green leaf volatile (GLV) aldehydes and methyl salicylate are representive of the endogenous levels in green tomato since the preparation of samples was carried out under the same conditions at all stages of tomato ripening. This finding is consistent with the proposal that chloroplasts, as they develop into chromoplasts during ripening, are the major site for fatty-acid derived volatile synthesis [20]. High MeSa is retarded ripening process in tomato and is protected to tomato against chilling injury [21]. This finding was in consistent with the results of present study since the turning of green to red color has been taken for about one month. This could be attributed to high MeSa content of green tomatoes. Interestingly, monoterpenes limonen and 3-carvomenthenone (piperitone) as well as gibberellin and alkaloid lysergamide were detected in green tomato only. Gibberellin is a hormone that regulates plant growth and developmental events ranging from seed germination to the timing of flowering and senescence [22]. Lysergamide, is toxic alkaloid, may be synthesized in green tomato as a defence against viruses [23]. This study reveals that the compounds such as MeSa, limonen, lysergamide and gibberellin responsible for defence fruit against herbivores and chilling injury, and for fruit growth and development [18, 21] are synthesized in green tomatoes only. Therefore, green stage of tomatoes may be a regulation step for growth and development of fruits.

Table 1. The relative proportions (percent) of volatile compounds in traditional tomato cultivar at green, red and over ripe stages

Compounds	RT	RI	Tomatoes		
Aldehydes			Green	Red	Over ripe
Pentanal[A]	8.23	1062	ND	0.15	ND
Hexanal[A]	8.92	1099	5.01	2.99	1.18
2-methyl-butanal	9.41	1118	ND	0.83	ND
Heptanal[A]	11.51	1196	ND	0.45	ND
(E)-2-hexanal[A]	12.36	1232	23.69	3.88	0.66
Octanal[A]	13.91	1299	ND	1.9	ND
(Z, E)-2-heptenal	14.72	1340	0.38	ND	ND
(E)-2-heptenal[A]	14.75	1341	ND	0.61	0.55
2,6-dimethyl-5-heptenal	15.27	1367	ND	0.31	ND
Nonanal[A]	15.98	1403	0.67	0.72	ND
(Z,Z)-2.4-hexadienal[A]	16.33	1424	0.21	ND	ND
(E)-2-octenal	16.72	1447	0.42	1.00	1.54
Decanal[A]	17.82	1512	ND	0.27	0.51
(E,E)-2.4-heptadienal	17.86	1515	0.53	0.25	ND
Benzylaldehyde[A]	18.45	1554	0.76	ND	1.72
(Z)-4-decenal	18.47	1556	ND	5.01	ND
(E, Z)-2,6-nonadienal	19.22	1612	0.47	ND	ND
Salicylaldehyde [A]	20.71	1720	1.45	0.64	0.65
(E.E)-2.4-decadienal	22.14	1841	ND	0.41	0.55
Alcohols					
Ethanol[A]	6.02	1438	ND	0.20	ND
Cyclopentanol	10.69	1165	ND	0.44	ND
3-methyl-1-butanol[A]	11.83	1209	ND	ND	5.43
1-pentanol[A]	12.86	1254	0.64	1.38	0
Cyclohexanol	14.64	1336	0.26	ND	ND
1-hexanol	15.05	1356	3.04	10.24	2.37
(Z)-3-hexen-1-ol[A]	15.73	1390	8.95	4.87	2.1
(Z)- 2-Hexen-1-ol	16.12	1412	ND	0.51	ND
1-octen-3-ol	16.83	1453	0.33	0.23	ND
Heptanol	16.95	1460	ND	0.58	ND
2-methyl-6-hepten-1-ol	17.07	1467	ND	ND	2.98
1-octanol	18.54	1560	0.20	2.20	0.73
(E)-2-octen-1-ol[A]	19.38	1619	ND	ND	0.61
1-nonanol[A]	19.97	1663	0.66	0.49	0.89
(Z)-6-nonen-1-ol[A]	20.73	1722	2.00	ND	ND
(E,Z)-3,6-nonadienol[A]	21.13	1755	ND	ND	2.28
(Z)-3-octen-1-ol	21.72	1804	ND	ND	0.25
Delta-(4)-dodecanol	21.72	1804	ND	0.91	ND
Benzyl alcohol (Benzenemethanol) [A]	22.85	1904	2.31	0.33	0.56
Terpenes					
Limonen[A]	11.64	1201	3.82	ND	ND
p-menth-1-en-3-on (3-Carvomenthenone)	17.56	1495	0.46	ND	ND
Alfa-terpinolene[A]	18.40	1551	0.41	0.38	0.39
Z-citral(neral) [A]	20.54	1707	ND	0.64	0.19
Geranial[A]	21.17	1758	ND	1.57	ND
Geranyl acetone	22.53	1875	ND	5.05	1.17
(*E*)-ß-ionone	23.62	1979	0.74	0.38	0.3
6-metil-5-hepten-2-one[A]	14.91	1353	0.25	17.16	11.98

ND: not detected. RI= retention index based on identified compound retention times (RTs), calculated from linear aquation between each pair straight alkanes (C5-C25). [A]Compounds verified with authentic standards. All compunds were also considered to be tentative (based on the MS library Wiley7n.1/ Nist 02.L)

Table 1. The relative proportions (percent) of volatile compounds in traditional tomato cultivar at green, red and over ripe stages (continued)

Compounds	RT	RI	Tomatoes		
Ketone			Green	Red	Over ripe
3-octanone[A]	13.12	1265	ND	0.67	0.77
4-octen-3-on[A]	14.18	1313	ND	0.45	ND
3.3-dimethyl.1-2-(1-methylethyliden) cyclopentanone	19.82	1652	0.38	0.33	ND
Aceto phenone (1-phenyl-ethanone)	20.29	1687	0.79	0.18	1.8
Esters					
Salicylic acid TMS ester[A]	10.30	1151	ND	1.20	ND
3-hexenoic acid, ethyl ester	13.16	1267	0.41	ND	ND
Formic acid, octyl ester	18.54	1560	0.26	ND	ND
Methyl salicylate[A]	21.90	1819	29.41	ND	ND
Geranyl isobutyrate	22.32	1857	ND	0.18	ND
Phenolic compounds					
Synephrine	10.27	1150	1.53	ND	ND
Methoxy-phenyl-oxime	20.94	1739	2.92	ND	ND
Guaiacol (2-methoxy-phenol)	22.72	1892	2.24	ND	ND
4-ethyl-2-methoxy phenol (4-ethyl guaiacol)	24.56	1975	ND	1.76	15.57
4-ethyl phenol (para ethyl phenol)	26.07	2043	ND	ND	19.62
2.5-dicloro phenol	26.36	2056	2.77	ND	ND
2-methoxy-4-vinyl phenol (Phenol. 4-ethenyl-2-methoxy)	26.48	2061	ND	0.57	1.17
Compounds with sulfur					
5-methyl-isothiazole[A]	15.86	1397	ND	0.29	ND
2-Isobutylthiazole[A]	16.31	1421	ND	12.89	ND
4,5-dihydro-2-methyl- thiazole	17.56	1494	ND	0.38	ND
Hydrocarbone					
Naphthalene[A]	21.54	1789	1.47	3.94	4.98
Hormon					
Gibberellin	3.20	<900	0.53	ND	ND
Alkoloid					
Lysergicacidamide	17.80	1511	0.62	ND	ND
Furan			0.62		
2-pentyl- furan	12.55	1240	ND	1.5	1.44
3-(4-methyl-3-pentenyl)-furan(PERILLEN)	16.47	1432	ND	0.26	ND
Guanidine					
2-(3',5'-ditrifluorometilfenil)-1.1.3.3-tetramethylguanidin	19.45	1628	ND	2.20	ND
Flavanoid					
Catecholborane	21.82	1812	ND	0.63	ND
Spiro (SHD)					
1.1-(dimetil)spiro[2,4]hepta-4-ene	26.09	2044	ND	2.10	ND
(E)-7-metil-1,6-dioksaspiro(4,5)decane	13.90	1299	ND	ND	0.92

ND: not detected. RI= retention index based on identified compound retention times (RTs), calculated from linear aquation between each pair straight alkanes (C5-C25). [A]Compounds verified with authentic standards. All compunds were also considered to be tentative (based on the MS library Wiley7n.1/ Nist 02.L)

Apart from mainly volatiles, C_7, C_8, C_9 and C_{10} compounds detected in tomatoes, which were slightly higher in red tomato when compared to green and over ripe ones (Table 1). Of these compounds, n-octan-1-ol, 1-nonanol, (*Z,E*)-2-heptenal, (*E,E*)-2,4-heptadienal, (*E,E*)-2,4-decadienal, 3-octanone, 4-octen-3-on, cyclopentanone and acetophenone were previously found in tomato and tomato juice [13, 14]. To our knowledge, *(Z)*-2-hexen-1-ol, 1-octen-3-ol, *(E)*-2-octen-1-ol, *(E,Z)*-3,6-nonadienol, *(Z)*-3-octen-1-ol, 2,6-dimethyl-5-heptenal, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, *(E,Z)*- 2-6-nonadienal and *(Z)*-4-decenal have been identified for the first time in tomatoes. As known, C_9 compounds, especially *(E,Z)*- 2-6-nonadienal, are the main volatile component in cucumber fruit [15]. They are formed as a result of the enzymatic degradation of linolenic acid via 9-lipoxygenase (9-LOX) [16].

At the red stage, 6-methyl-5-hepten-2-one and 2-isobutylthiazole as well as 1-hexenol and *(Z)*-4-decenel increased considerably compared with green stage. Of these, 6-methyl-5-hepten-2-one and 2-isobutylthiazole were the most abundant compounds in red tomato. This result confirms that increase in these volatiles may cause the loss of green flavor and the formation of sweet flavor. Interestingly, there is a proportional balance among total alcohols (22.38%), aldehydes (19.42%) and terpenes (22.97%) at red stage. This may be important in forming the characteristic tomato flavor. The total percent of aldehydes was considerably lower in red tomato than green one. This may be due to reduction of aldehydes to alcohols depending on ripening process since alcohols increased in red tomato. However, the diversity of aldehydes increased in red tomato compared to green and over ripe tomatoes. Compound *(Z)*-4-decenal was predominant aldehyde in red tomato. It may be a significant conributer to fresh tomato aroma. Even though compound (*E,E*)-2,4-decadienal was previously found in tomato by using AEDA method as important contributor to fresh tomato aroma (24), *(Z)*-4-decenal was identified for the first time in fresh red tomato. As in other vegetable and fruit systems, this unsaturated aldehyde is most likely degradation product of unsaturated fatty acid linoleic acid through enzymatic reactions. Of lipid-derived aldehydes, *(Z)*-3-hexenal was previously reported in tomatoes, whereas it was not detected in tomatoes at any stage of ripening. *(Z)*-3-hexenal is an instable compound and then it is converted to *(E)*-2-hexenal by *cis*/*trans*-isomerase [25]. Compounds *(Z)*-3-hexen-1-ol and n-hexenol decreased and increased in red tomato, respectively, compared to green tomato. This result is not surprising since n-hexanal and *(Z)*-3-hexenal or *(E)*-2-hexenal are originated from the different unsaturated fatty acids as linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) via 13-lipoxygenase (13-LOX), respectively [16]. These aldehydes are further transformed into their corresponding alcohols (n-hexenol and *(Z)*-2-hexen-1-ol, respectively) by the action of alcohol dehydrogenase (ADH: alcohol:NAD+oxidoreductase) [26]. *Trans*-2-hexenal and hexanal were found to be the major aldehydes in green tomato (Table 1), whereas the relative proportion found for hexanal was about 5-fold lower, reflecting the ratio in which their corresponding precursors linolenic acid and linoleic acid occur in organic tomatoes without hybrid. This study reveals that the degradation of linolenic acid was much more marked at green stage of tomato due to high *(E)*-2-hexenal and C_9 compounds contents, when compared red and over ripe stages. However, C_8 and C_{10} compounds as well as *(Z)*-4-decenal and 1-hexanol, are products of degradation of linoleic acid were the most abundant in red tomato. This implies that the degradations of linolenic and linoleic acids are high at green and red stages of tomatoes, respectively.

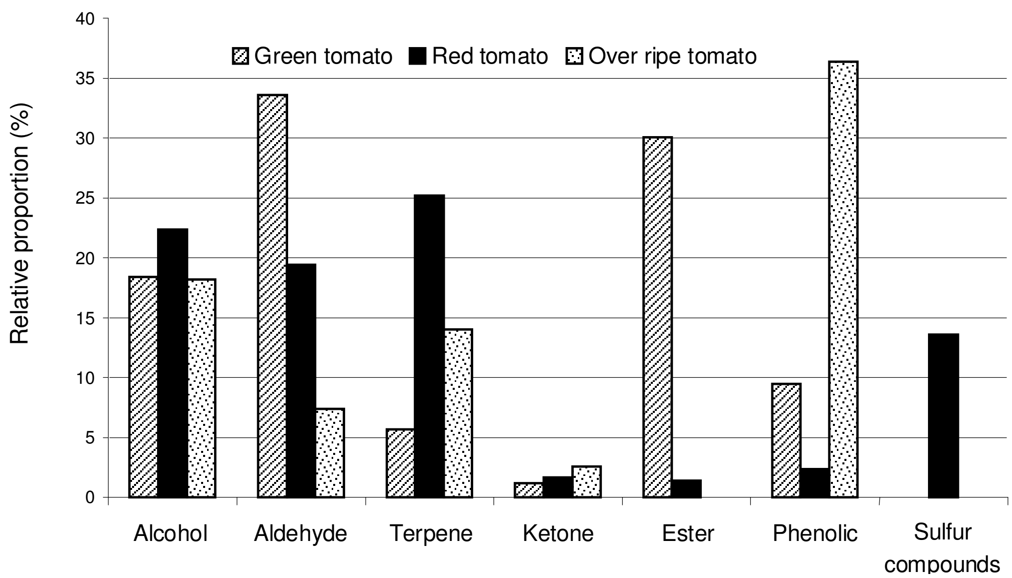

Figure 1. Relative proportion (percent) of the main classes of volatile compounds in the green, red and over ripe tomatoes

From terpenes, α-terpinolene, *trans*-β-ionone and 6-methyl-5-hepten-2-one are consistently present in tomatoes at the all stages of ripening. *Trans- β*-ionone, is oxidative breakdown product of *β*–carotene, was highest in green tomatoes. Terpen geranylacetone was determined in red tomato only. 6-methyl-5-hepten-2-one was predominant volatile compound in red tomato. This may be due to high lycopene content in tomatoes at the red stage since open chain carotenoid related volatiles such as 6-methyl-5-hepten-2-one and geranylacetone are originating from lycopene and its more saturated linear lycopene precursor *ζ*-carotene, respectively [27, 28, 7]. However, the level of 6-methyl-5-hepten-2-one decreased in over ripe tomato. This could be attributed to the reduction of 6-methyl-5-hepten-2-one to 2-methyl-6-hepten-1-ol by yeast since alcohol 2-methyl-6-hepten-1-ol was detected in over ripe tomato only. Another volatile compound responsible for fresh tomato aroma, 2+3methyl butanal was detected in red tomato at low level. Contrary to earlier studies done by [3] and [4], 2-izobutylthiazole was considerably higher than 2-methyl butanal. Both 2-izobutylthiazole and 2-methyl butanal are derived from leucine or isoleucine amino acids [2]. This may be due to cultivar since the biosynthetic pathway(s) of leucine or isoleucine in local tomato cultivar was produced to mainly compound 2-izobutylthiazole instead of methyl butanal.

As for over ripe stage, phenolic compounds increased considerably in tomatoes. The main volatile compounds in this stage were 4-ethyl guaiacol and 4-ethyl phenol which were detected in over ripe tomato only. Phenolic compounds impart a distinct flavor, frequently related to off-odours [7] and also are important defense compounds against pathogens and insects [29]. These phenolic compounds may be produced by ethylation and methylation of catechol via catechol *O*-methyl(ethyl)transferase since both ethanol and methanol did not occur in over ripe tomato. Methanol is considered as marker of the pectin degradation [30]. Both ethanol and methanol were previously found in vine-ripened and wild ripened tomato varieties at very high concentrations [30, 4]. In our study, methanol and ethanol may be used in biosynthesis of 4-ethyl phenol and 4-ethyl-2-methoxy phenol by *Brettanomyces* wild yeast which is responsible for spoliage of wine and beer [31]. On the other hand, the number (33) of volatile compounds in over ripe tomato was less than green and red tomatoes. This may be due to suppress in recovery of volatile compounds of 4-ethyl phenol and 4-ethyl-2-methoxy phenol at high levels. For example, in wine, ethanol can suppress volatile of esters. Similarly, polyphenols may interact with some flavor compounds, to alter their volatility and flavor release [32]. This finding confirmed that no ester compounds were detected in over ripe tomatoes. However, the mechanisms of these interactions and their effects on sensory perception have not been yet fully characterized.

The profile of alcohols in over ripe tomato was different from the other stages. Even though lipid derived C_6 alcohols such as n-hexanol, *(Z)*-3-hexenol are plentiful in green and red tomatoes, they decreased markedly in over ripe tomato. However, compounds *(E,Z)*-3,6-nonadienol and *(Z)*-3-octen-1-ol as well as 3-methyl-butan-1-ol and 6-methyl-5-hepten-2-ol were detected in over ripe tomato only. Three-methyl-1-butanol, is originated from leucine amino acid, was the main aromatic alcohol in over ripe tomato, which followed by 6-methyl-5-hepten-2-ol. These compounds are corresponding alcohols of 3-methyl butanal and 6-methyl-5-hepten-2-one, respectively.

Overall, the distribution of volatile compounds and their proportions varied considerably in tomatoes depending on ripening stages. This may be indicated to the diversity of biosynthetic pathways contributing to the formation of volatile compounds.

CONCLUSION

We may emphasize that linolenic acid derived aldehyde (*trans*-2-hexenal) and alcohol (*Z*-3-hexen-1-ol) and benzoic acid derived ester (methyl salicylate) were the main compounds in tomato at green stage. In contrast to, linoleic acid, lycopen and phenylalanine derived compounds (n-hexanol, geranylacetone and 6-methyl-5-hepten-2-one, and 2-isobutylthiazole, respectively) were the most plentiful in tomato at red stage. Over ripe tomatoes had high concentrations of phenolic compounds such as 4-ethyl guaiacol (4-ethyl-2-methoxy phenol) and 4-ehtyl-phenol. So, while the significant defence compounds against herbivores and green leaf aldehydes are produced in green tomato, the main compounds responsible for characteristic tomato flavor and for off-flavor are occurred in red and over ripe tomatoes, respectively. The major volatile compounds in traditional tomato cultivar were different from those in previous studies. The ripening prosess in tomatoes has been taken for a long time. This is probably due to high methyl salicylate content in green tomato depending on cultivar or growing conditions.

ACKNOWLEDGMENT

The volatile compounds analyses were carried out with the GC-MS provided by the T.R. Prime Ministry State Planning Organization (DPT) project (Project no: 02 K 120860).

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