Ecological Life Sciences Status : Original Study
ISSN: 1308 7258 (NWSAELS) Received: November 2017
ID: 2018.13.3.5A0103 Accepted: July 2018

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DOI	http://dx.doi.org/10.12739/NWSA.2018.13.3.5A0103	
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DETERMINATION OF AROMATIC COMPOUNDS OF SOME PROMISING POMEGRANATE GENOTYPES

ABSTRACT

Pomegranate (*Punica granatum* L.) fruit, one of the most important antioxidant sources, is also rich in volatile component content. Four hybrid genotypes were used as material for this study. Those varieties were selected as promising varieties in breeding programme that carried out in Institute of Aegean Agricultural Research. It was aimed to determine the volatile constituents of the flavors of 'Type 18', 'Type 19', 'Type 38' and 'Type 40' by comparing of İ-1513 which is considered as standard variety. Volatile component contents of fruits of each genotype were extracted by using diethyl ether solvent for liquid-liquid extractions. The amounts and identifications of the volatile compounds of extracted samples determined with a gas chromatograph-mass spectrometer. According to the obtained results, a total of 54 volatile compounds were determined in total pomegranate aroma profiles, including 14 alcohols, 14 esters, 8 terpenes, 8 aldehydes, 5 ketones, 3 saturated hydrocarbons and 2 phenols. 'Farnesyl Acetate' and 'Limonene' were found as important volatiles in Type 19 and Type 18, respectively. Those genotypes should be considered for both of table consumption and processing for fruit juice, in terms of aroma.

Keywords: Pomegranate, *Punica granatum* L., Volatile Components, Quality, GC/MS

1. INTRODUCTION

Pomegranate, *Punica granatum* L. belongs to the *Punicaceae* family and is grown in many countries, especially in the Mediterranean region (Tunisia, Turkey, Egypt, Spain and Morocco), Iran, India, Pakistan, Afghanistan, Saudi Arabia, and in the subtropical areas of South America [1]. Turkey is one of the origins of pomegranate which is one of the oldest known edible fruits. As a result of clonal selection studies in Mediterranean and Aegean regions of Turkey, new pomegranate types were developed and registered for table and industrial purposes. Especially exported Izmir-1513 pomegranates were very popular in Turkey and EU [2]. People have improved their eating habits by consuming food not only for nutrition but also for taste and health in recent years. Therefore volatiles directly affect the sensorial quality and consumer acceptance for fresh fruits. Recent studies demonstrated that pomegranate fruit provides a rich and unique source of bioactive phytochemicals that improve human health and assist in

How to Cite:
Gündoğdu, M.A., Ekinci, N., Kaleci, N., and Şeker, M., (2018). Determination of Aromatic Compounds of Some Promising Pomegranate Genotypes, **Ecological Life Sciences (NWSAELS)**, 13(3): 142-150. DOI: 10.12739/NWSA.2018.13.3.5A0103.

disease prevention [3, 4 and 5]. Those findings, together with breeding of attractive new varieties, led to a dramatic worldwide increase in trading and consumption of pomegranate fruit and juice [6 and 7]. Production of aroma volatile compounds is an important factor determining quality of fruit produce and is directly influenced by fruit maturity [8]. Flavor is one of the most important criteria of fruit quality. The formation of flavor and aroma compounds in fruits is a highly dynamic process, volatile substances are continuously synthesized and developed during fruit growth and ripening. Volatile composition changes qualitatively and quantitatively in every step of fruit growth and development [9]. Aroma compound analysis plays an important role in the process of quality pomegranate breeding and consumer acceptance. The aroma of a fruit is the result of a complex mixture of esters, alcohols, aldehydes, terpenoid compounds, etc. [10]. They are present at very low concentrations, typically ppm or ppb levels in natural sources. Due to their dissimilar molecular characteristics and concentrations, every single aroma compound has different contributions on the final aroma of a fruit [11].

2. RESEARCH SIGNIFICANCE

Selection breeding studies for obtaining new pomegranate variety candidates are carried out by taking into consideration improving quality and consumer acceptance in Institute of Aegean Agricultural Research. Four hybrid types (I–1513xI–23) were selected as promising and were used as material for this study. It was aimed to determine the volatile constituents of the flavors of 'Type 18', 'Type 19', 'Type 38' and 'Type 40' by comparing of İzmir 1513 (İ-1513) which is considered as standard variety.

3. MATERIALS AND METHODS

3.1. Plant Materials

İzmir 1513 variety and four hybrid types (Type–18, Type–19, Type–38 and Type–40) which selected as promising types from the breeding programs were used in the research. İzmir 1513 variety is cultivated as one of the most important standard varieties and is used as one of the parents of hybrid genotypes obtained from breeding studies in Institute of Aegean Agricultural Research in İzmir, Turkey. The pomegranate variety and types used in the study were harvested at commercial ripening. Therefore İzmir 1513 cultivar and Type–38 were harvested in 25th September 2014 and Type–18, Type–19 and Type–40 were harvested in 9th October 2014 in Institute of Aegean Agricultural Research (Figure 1 and 5). The harvested fruits were transferred to the laboratories of Department of Horticulture in Çanakkale Onsekiz Mart University as soon as possible.

3.2. Sample Preparation

10 fruits of each genotype were cut with a knife, the edible arils were separated manually and placed within four layers of muslin cloth, and the juice was extracted by squeezing and collected into beakers.

3.3. Volatile Aroma Compounds Extraction Procedure

Diethyl ether is widely used for extraction of aroma volatiles in fruits, vegetables and spices. Moreover, most of the aroma compounds reported earlier in the pomegranate or its juices are readily soluble/miscible in the diethyl ether [12]. Therefore liquid-liquid extraction was performed by use of diethyl ether solvent for determining volatile constituents of pomegranate fruit juice. 100mL diethyl ether solvent was added into the Erlenmeyer flask with 100ml pomegranate fruit juice. After solvent treatment, the extract was concentrated to 1mL with a centrifuge and concentrator. Then the solvent was directly injected into a gas chromatograph for α-volatile compound [13].

Figure 1. Harvested pomegranate fruits of I-1513

3.4. Chromatographic Analyses

The amount of the aroma volatile determined with a gas chromatograph-mass spectrometer (Shimadzu QP2010 GC/MS) fitted with a DB-WAX column (30mx0.25mm ID, 0.25µm film thickness; J&W, USA). Identification of volatile content was carried out by mass spectrometry using a mass spectrometer set at 280°C of capillary direct interface temperature; the ionization energy of the mass spectrometer was programmed for 70 eV. Also the ion source temperature was set at 250°C and 40-350 amu of mass interval and 666 amu s^{-1} scan rate. WILEY and NIST libraries were used for identification of compounds. One microliter samples were injected in 1:50 split ratio (with 250°C injection temperature) by an auto injector. Firstly, the column temperature was set at 40°C for 5 min. After the column reached at 250°C by 10°C min^{-1} and held for 10 min.

Figure 2. Harvested pomegranate fruits of Type-18 (I-1513xI-23)

Figure 3. Harvested pomegranate fruits of Type-19 (I-1513xI-23)

Figure 4. Harvested pomegranate fruits of Type-38 (I-1513xI-23)

Figure 5. Harvested pomegranate fruits of Type-40 (I-1513xI-23)

4. RESULTS AND DISCUSSION

A total of 54 volatile compounds were detected and identified in pomegranate variety and types (Table 1). The compounds were consisting of 14 esters, 14 alcohols, 8 terpenes, 8 aldehydes, 5 ketones, 3 saturated hydrocarbons and 2 phenols.

Table 1. Composition* of the major volatiles in the pomegranate types and I-1513

	Aroma Volatiles	Type-18 % Area	Type-19 % Area	Type-38 % Area	Type-40 % Area	I-1513 % Area
Alcohols	1-Propanol	0.218	0.226	0.000	0.000	0.176
	2-Propanol	1.697	1.218	1.718	1.716	1.660
	Ethanol	5.746	2.653	4.191	4.186	15.651
	1-hexen-3-ol	0.124	0.163	0.000	0.322	0.000
	1-butanol	0.000	0.000	0.000	0.216	0.321
	3-Ethylbut-3-en-2-ol	0.262	0.101	0.000	0.000	0.000
	(E)-3-Octen-2-ol	0.437	0.371	0.181	0.916	0.000
	1-pentanol	0.000	0.000	0.000	0.858	0.423
	1-hexanol	0.000	0.094	0.000	0.271	0.000
	2-hexen-1-ol	0.229	0.000	0.000	0.000	0.000
	2-Ethylhexanol	0.000	0.000	0.000	0.000	0.230
	1-Penten-3-ol	0.000	0.000	0.000	0.000	0.254
	2-Nonen-1-ol	0.214	0.232	0.000	0.745	0.215
	(Z)-2-Penten-1-ol	0.000	0.000	0.000	0.000	0.210
	% Identified	8.927	5.058	6.090	9.230	19.140
Aldehydes	Hekzanal	0.000	0.139	0.000	0.000	0.000
	Tetranal	0.101	0.000	0.000	0.000	0.000
	Pentanal	0.166	0.000	0.000	0.000	0.000
	Octanal	0.498	0.466	0.000	0.529	0.000
	2-Methyl-1-Butanal	0.000	0.175	0.000	0.000	0.000
	2-Propylhexanal	0.191	0.000	0.000	0.615	0.000
	Nonanal	0.365	0.217	0.326	0.220	0.273
	4-Octadecenal	8.084	0.000	0.000	0.000	0.000
	% Identified	9.405	0.997	0.326	1.364	0.273
Ketones	3-Hexanone, 2,2-dimethyl	0.151	0.183	0.000	0.417	0.000
	2,5-Dimethyl-3-Hexanone	0.644	0.708	0.312	0.975	0.372
	2,6-Dimethylheptan-4-one	0.250	0.279	0.000	0.811	0.292
	4-Octanone	0.713	0.817	0.416	1.405	2.031
	2-Nonanone	0.000	0.111	0.000	0.000	0.000
	% Identified	1.758	2.098	0.728	3.608	2.695
Saturated Hydrocarbons	Undecane	0.368	0.388	0.265	0.923	0.438
	Tetradecane	0.396	0.326	0.240	1.215	0.952
	Hekzadecane	0.171	0.122	0.000	0.000	0.451
	% Identified	0.935	0.836	0.505	2.138	1.841
Phenols	2,6-Di-tert-butyl-4-Methylphenol	23.422	27.272	47.900	50.966	51.067
	2,4-Di-tert-Butylphenol	2.325	2.283	2.425	4.608	0.000
	% Identified	25.747	29.555	50.325	55.574	51.067
Esters	Ethyl Acetate	1.278	1.336	1.638	2.636	2.719
	Propyl Acetate	0.000	0.134	0.000	0.000	0.000
	Ethyl Butyrate	0.000	0.077	0.000	0.000	0.000
	Tert-butyl Butyrate	0.203	0.299	0.000	0.481	0.000
	Isobutyric Acid	0.000	0.081	0.000	0.000	0.000
	Butyl Glyoxylate	0.000	0.088	0.000	0.000	0.000
	Isopentyl Acetate	0.000	0.271	0.000	0.000	0.000
	Isobutyl 2-Methylbutanoate	0.091	0.303	0.000	0.726	0.572
	Allyl Propionate	0.111	0.000	0.645	0.437	0.293
	(E)-β-Ocimene	0.000	0.000	0.000	0.523	0.480
	Hexyl Acetate	0.128	0.197	0.000	0.000	0.000
	Vinyl Propionate	0.000	0.000	0.000	0.000	0.308
	Nerolidyl Acetate	0.000	0.000	0.781	0.000	0.000
	Farnesyl Acetate	0.000	19.570	0.000	0.000	0.000
	% Identified	1.811	22.356	3.064	4.803	4.372
Terpenes	Linalool	0.000	0.000	0.148	0.585	0.000
	α-Terpinol	0.000	0.173	0.000	0.000	0.000
	α-pinene	3.484	0.334	0.000	0.935	0.185
	Racemic,α-(Z)Bergamotene	0.000	0.241	0.581	0.477	0.360
	p-Cymene	0.000	0.280	0.133	0.195	0.297
	Limonene	47.933	17.620	38.100	21.091	19.770
	(Z)-Nerolidol	0.000	10.369	0.000	0.000	0.000
	β-Caryophyllene	0.000	10.083	0.000	0.000	0.000
	% Identified	51.417	39.100	38.962	23.283	20.612
% Total Identified		100.00	100.00	100.00	100.00	100.00

The number of aromatic compounds differed among the variety and types. Alcohols, aldehydes, terpenes, ketones and esters are responsible for the citrus, floral and fruity aroma [14 and 15]. Especially esters are the most important volatile compounds of the characteristic fruit aromas. Besides phenols are found in the form of tannins in many fruit and they are caused acrid flavor. Terpenoids and phenols were also found to be the major volatile in pomegranate juice additionally to alcohols, esters and aldehydes [16] which suggests that pomegranate fruit conserve some of its volatiles during differentiation from flowers to mature fruit to finally give the pleasant sweet fruity floral taste of pomegranate juice. Many investigators [21] have reported that pomegranate juice has a free radical scavenger and potent antioxidant capacity. These beneficial effects of the PJ were attributed to the anti-oxidative properties of pomegranate polyphenols and sugar containing polyphenolic tannins and anthocyanins [22]. Two phenol compounds including 2,6-di-tert-butyl-4-methylphenol and 2,4-di-tert-butylphenol were the major constituents of the volatiles. Type-40 had 55.57% of total volatiles as phenol compounds, following by I-1513 (51.07%) and Type-38 (50.33%). Especially, 2,6-di-tert-butyl-4-methylphenol compound was the highest amount of the phenol compound of pomegranate types and variety that varied from 23,42% to 51,07%. Duman et al. (2009) [17] have also reported that extracts from *P. granatum* arils possess strong in vitro antibacterial activity against many bacterial strains tested. Most of those investigations suggested that the presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major active constituents that may be responsible for these activities. Melgarejo et al. (2011), suggested that consumer liking for pomegranate juices could be associated with the presence of high levels of monoterpenes [23]. Volatile phenols such as guaiacol (2-methoxy phenol) and methyl isoeugenol give off sweet and honey-like scents and are produced in the Shikimate [27].

In this study, 8 terpenes were found. The percentages of terpenes accounted for 20.61% of total volatiles in ‘İzmir 1513’ standard variety to 51.42% of total volatiles in Type-18. The detected terpenoids are limonene, α-terpinol, α-pinene, α-(E)-bergamotene, p-cymene, β-caryophyllen and (E)-nerolidol. This group was represented by Limonene, which was identified in all types and standard variety. Type-18 and Type-19 had more limonene than the other types and variety. The sensory descriptors of limonene are: lemon, orange, citrus, and sweet [18]. Furthermore, all types and standard variety, except type-18, had α-(E)-bergamotene and p-cymene compounds. Terpenes are typical compounds in fruits, vegetables, herbs, spices and wine. The terpene group encompasses a wide range of descriptors, e.g., dill-like for α-phellandrene (impact compound in dill), peach-like for α-terpineol, or spicy for β-caryophyllene [19].

Total 14 esters were detected during the analyses. Those were ethyl acetate, tert-butyl butyrate, isobutyl 2-methylbutanoate, allyl propionate, (E)-β-ocimene, nerolidyl acetate, propyl acetate, ethyl butyrate, isobutyric acid, butyl glyoxylate, isopentyl acetate, hexyl acetate, ethyl propionate and farnesyl acetate. Type-19 had much more ester constituents than the other types and standard variety, especially farnesyl acetate. Also ethyl acetate was common ester compound of all variety and types which used in research.

Ethyl acetate known to have sweet-fruity, Anise and pineapple odor is widely reported to be an aroma component of several fruits and vegetables. The presence of this compound in pomegranate juices has been earlier reported by Vazquez-Araújo et al. (2010) [24]. In addition to this, I-1513 (2.718%) and Type-40 (2.636%) that came to the forefront were found in the highest content, followed by Type-38 (1.638%), Type-19 (1.336%) and Type-18 (1.278%), respectively. Farnesyl acetate, the major components characterized from seed oil [25], was identified just in Type-19.

14 alcohols were found during the experiment. The detected alcohols were ethanol, 1-propanol, 2-propanol, 1-hexen-3-ol, 1-butanol, 3-methylbut-3-en-2-ol, 3-octen-2-ol, 1-pentanol, 1-hexanol, 2-hexen-1-ol, 2-ethylhexanol, 1-penten-3-ol, 2-nonen-1-ol and 2-penten-1-ol. Ethanol and 2-propanol were the most abundant alcohols in types and variety. Besides İzmir 1513 (standard variety) contained more alcohols than other types.

Eight aldehydes compounds were found in surveyed genotypes. The accounted aldehydes were pentanal, hexanal, tetranal, octanal, 2-propylhexanal, 2-methyl-1-butanal, nonanal and 4-octadecenal. The percentages of aldehydes of Type-18 were more than other types and standard variety. Total percentages of aldehydes varied 9.41% of total volatiles in Type-18 to 0.27% of total volatiles in İzmir 1513. Nonanal were the most abundant aldehyde in all types and cultivar followed by octanal. Melgarejo et al. (2011) were explained that terpenes and aldehydes were the major aroma-active compounds in pomegranates of 'Wonderful' variety. These researchers also added that hexanal, limonene, (E)-2- hexenal, and (Z)-3-hexenol were the most abundant compounds in fresh pomegranate juices from Spanish and Turkish pomegranate cultivars [23 and 26].

In this study, 5 ketones were found. The detected ketones were 2,2-dimethyl-3-hexanone, 2,5-dimethyl-3-hexanone, 2,6-dimethylheptan-4-one, 4-octanone and 2-nonanone. The percentages of ketones accounted for 0.73% of total volatiles in Type-38 to 3.61% of total volatiles in Type-40. Particularly 4-octanone and 2,5-dimethyl-3-hexanone were most abundant ketones in all types and standard variety. Guler and Gul were determined that ketones in juices only at minor levels [26]. Also, researchers detected that 2-nonanone and p-menth-1-en-3-one semicarbazone in all juices of pomegranate fruits from varieties. It was detected the presence of three saturated hydrocarbons which could impart their characteristic of intense fruity and floral aromas. Saturated hydrocarbons are varied from 0.51% in Type-38 to 2.14% in Type-40. Furthermore, undecane and tetradecane are the only hydrocarbons detected in all studied types and variety.

5. CONCLUSION

According to the results obtained in the research, 14 esters, 14 alcohols, 8 terpenes, 8 aldehydes, 5 ketones, 3 saturated hydrocarbons and 2 phenols were detected on pomegranate standard variety and promising types. It was determined that alcohols, esters and terpenes were the most important component groups. Especially, the phenol derivative compound, '2,6-di-tert-butyl-p-cresol', was notable for its high level of presence and possess antioxidant properties. Besides, Type-18 had the highest terpenes; particularly 'limonene' compound that the characteristic volatile for aroma of citrus fruits. Type-19 that had 39.10% terpenes, 29.56% Phenols and 22.36% Esters, showed more proportional distribution in terms of significant volatile component groups than the other types and variety. On the other hand, it was determined that Type-38 and Type-40 which had 38.96% and 23.28% Terpenes, 50.33% and 55.57% Phenols and 3.06% and 4.80% Esters relatively, were the types with the volatile compounds closest to the standard variety İzmir 1513 which had 20.61% Terpenes, 51.07% Phenols and 4.37% Esters. This study showed that there are high variations among volatile contents of pomegranate genotypes. In the consideration of total aromatic compound profile, Type-18 and Type-19 were differed.

'Farnesyl acetate' and 'limonene' were found as important volatiles in Type 19 and Type 18, respectively. Those genotypes which previous studies have also drawn attention in terms of storage quality [20], should be considered for both of table consumption and processing for fruit juice, in terms of aroma.

ACKNOWLEDGEMENTS

The authors wish to thank Agricultural Engineer Fatih Çağır and Dr. Erol Küçük for supporting this experiment.

NOTICE

This study was presented as an oral presentation at the I. International Scientific and Vocational Studies Congress (BILMES 2017) in Nevşehir/Ürgüp between 5-8 October 2017.

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