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Aroma Evaluation of Juniper Berry (*Juniperus drupacea* L.) Molasses (Pekmez) by Solvent-Assisted Flavour Evaporation

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Abstract

Juniper berries (*Juniperus drupacea* L.) belonging the Cupressacea family are recently gaining interest of both pharmaceutical and food industries due to their unique and dainty aroma. These fruits are generally used in the production of molasses in some regions of Turkey. There are a limited number of studies investigating the volatile compounds of juniper berries, there are no papers on the aroma of molasses made from this fruit. Therefore, the volatile composition of molasses produced from Turkish juniper berries was investigated in this study. The solvent assisted flavour evaporation (SAFE) technique was applied to extract the volatiles and the identification of compounds was carried out by the application of gas chromatography mass spectrometry. A total of 31 aroma compounds from 10 different groups were determined as a result of SAFE extraction in Juniper molasses sample. Alcohols and aldehydes (26%) were the most abundant volatile compounds in terms of variety and quantity, these compounds were followed by acids, terpenes, ketones and lactones, respectively. The highest concentrations were determined for compounds like 2,3-butanediol, furfural, hexanoic acid and *(Z)*-limonene oxide in the sample.

Keywords: Juniper berry, Juniperus drupacea L., volatile, molasses, pekmez

1. INTRODUCTION

Juniper berry *(Juniperus drupacea* L.*)* belonging the Cupressacea family comprising about 67 species all over the world is a popular fruit, mainly distributed in Eastern Mediterranean region region of Turkey and locally called "Andiz". The mature female cone of the juniper berry has long been used for flavoring agent diffferent foods, as seasoning for pickling meat products, and in alcoholic beverages. The essential oil of the juniper berry has been used in aroma formulations for perfumery as well as in folk medicine for various diseases, such as bronchitis, arthritis, and several parasitic diseases (El-Ghorab et al., 2008). However, juniper berries have long been processed into pekmez (molasses) due to their positive health effects in Turkey. Pekmez is one of the traditional food products consumed in the breakfast in Turkey and it is mostly produced from different grape cultivars and mulberry by concentration of juices up to 70–80 soluble dry matter content. It can also be made from fruits containing high levels of sugar like apple, carob, plum, watermelon, apricot, sugar beet and fig (Sengul et al., 2005).

The geographic pattern of genetic and morphological structures of *Juniperus drupacea* L. has been investigated. These authors found that the six groups of populations differed genetically including two European, three Turkish, and one Lebanese. This could imply the possible origin from another region and/or long-lasting spatial isolation, together with different reaction of populations from these parts of the species geographic range to the environmental conditions (Sobierajska et al. 2016). It is widely accepted that the quality of food products is mainly composed of the appearance, aroma, flavor, and colour, among which aroma is one of the

important parameter to determine the final overall quality and the consumer's preference. Although there have been studies on the aroma of *J. drupacea* berry, the aroma of its pekmez have not been studied. El-Ghorab et al. (2008) studied the volatiles of the dried fruit of Syrian *J. drupacea* sample, obtained by steam distillation and analyzed by gas chromatography/mass spectrometry. They found that pinene (23.73%), thymol methyl ether (17.32%) and camphor (10.12%) were the major volatile compounds in the dried fruit. Dob et al. (2008) elucidated the chemical composition of the essential oil of *Juniperus phoenicea* from Algeria. In this study, the volatile oil was isolated by hydrodistillation technique in yield 0.80% and was analyzed by GC/FID and GC/MS. A total of 103 compounds, representing 96.0% of the oil was identified, 23 being reported for the first time in *J. phoenicea* oil. Authors reported that the oil was characterized by the presence of high amounts of monoterpene hydrocarbons (62.2%) and by a lower percentage of oxygenated sesquiterpenes (8.8%). Foudil-Cherif and Yassaa (2012) investigated the enantiomeric and non-enantiomeric distribution of monoterpenes in the headspace of *Juniperus communis* L. and *Juniperus oxycedrus* needles and berries using HS-SPME technique. Large differences in chiral distribution of monoterpene compounds within the same plants and between the two junipers were detected in this study.

Although there have been various studies on the volatile contents of juniper berry fruits (Dob et al., 2008; El-Ghorab et al., 2008; Foudil-Cherif and Yassaa, 2012), no study has been found on of juniper berry pekmez aroma. Hence, the objectives of this investigation was to determine the aroma of the pekmez. To the best of authors' knowledge, this work is the first detailed study that focuses on the aroma in the juniper pekmez by using solvent-assisted flavour evaporation with gas chromatography-mass spectrometry.

2. MATERIALS AND METHODS

2.1. Materials

J. drupacea Labill berries were collected in Camliyayla-Mersin in Turkey. Pekmez sample was produced by the traditional method. The berries were cracked and smashed by a hammer, put in open stainless steel vessels filled with water and kept for 48 h in ambient temperature to extract soluble solids. Afterward, the extract was filtered for clarification. The upper phase was collected and transferred in large cauldrons hanged over an open fire, the mixture was boiled to evaporate the water, and this process continued until the desired consistency was obtained. It was stirred frequently during boiling/ heating. During boiling, any foam produced was removed. After cooling, the pekmez was filled into 500-mL glass bottles, and stored at room temperature in the darkness.

2.2. Methods

2.2.1. General chemical analysis

The pH, soluble solid content and colour analysis were performed in the samples. Colour analysis of samples was done with a Hunterlab (Model 45/0 HunterLab Color Flex, Reston, Virginia, USA) device. Calibration was done with first black then white ceramics before measurement started. Finally, the value of L* (lightness), a* (redness-greenness), and b* (yellowness-blueness) for pekmez was obtained (Sengul et al., 2005).

2.2.2. Extraction and analysis of volatile compounds

Liquid-liquid extraction/solvent-assisted aroma evaporation (SAFE) method was used for the analysis of aroma substances in pekmez sample. 50 g of sample was used for the extraction of aroma compounds. The sample was transferred to 500 mL conical flasks, and 40 µg of 4-nonanol were added as an internal standard, and extraction was carried out with stirring in a magnetic stirrer (at 4-5 ˚C) with purity dichloromethane solvent under nitrogen gas for 60 minutes in three times repeated. After the extraction, the samples were centrifuged at 6000 rpm for 15 minutes at 4˚C. After the centrifugation, samples were taken to a separatory funnel and isolated at 40°C by the SAFE technique. In this technique, nonvolatile fractions were separated

from the aromatic extract obtained by liquid-liquid extraction. The extract from SAFE was passed through sodium sulfate and concentrated to 0.5 mL thick at 40 °C in a "Vigreux" distillation column. The extract obtained in the concentrated form was directly injected into GC-FID and GC-MS systems and the flavors were determined. The extractions were performed with three replications (Sonmezdag et al., 2016).

2.2.3. GC-MS analysis of volatile compounds

The system of gas chromatography (GC) comprised an Agilent 6890 chromatograph interfaced to an FID (flame ionization detector) and an MSD (mass selective detector) (Agilent 5973, Wilmington, DE, USA). To separate the volatiles, a DB-Wax column (0.5 µm thickness × 0.25 mm i.d. × 30 m length) was utilized (J&W Scientific, Folsom, CA, USA). Extract of 3 µL was injected using splitless pulse mode at 40 psi for 0.5 min. Set temperatures of 270 and 280 ◦C were utilized for the injector and FID detectors, respectively. Flow rate of helium as carrier gas was 1.5 mL/min. Parameteras of the DB-Wax column oven program were from 50 to 250 ◦C with 4 ◦C/min and 10 min hold. These oven parameters were also utilized for the MSD. Scan rate at 2.0 scan/s, 70 eV ionization energy, mass range *m/z* of 30–300 a. m.u. and 250 ◦C of interface temperature and 180 ◦C source temperature were the parameters of the MS. Volatiles of the samples were identified based on the retention index, standard compounds and mass spectra on the DB-Wax column using a commercial database of spectra (Wiley 6, Flavor 2L, NIST 11). The volatiles were then quantified utilizing the internal standard with 4-nonanol. Response factors were computed based on the intensity ratio of each volatile to 4-nonanol and ratios of peak areas were corrected by using each constituent's response factor (Sevindik et al., 2020). Subsequently, the means and standard deviations were computed for the GC analyses in triplicate. Retention index data for the volatiles were computed by utilizing *n*-alkane series (C8–C32). All analyses were carried out in 3 replicates and presented as means ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of Pekmez samples

The chemical characteristics of pekmez are given in Table 1. As shown in Table 1, the pH and TSS (°Brix) in pekmez sample were found to be 4.99 and 71.2, respectively. These results are similar with the data reported by Akbulut et al. (2008) that *J. drupacea* pekmez has 75.2% soluble solid and 5.21 pH values. Regarding the colour properties, L*, a* and b* values were measured 14.3, 8.45 and 2.35, respectively. A high a value is not desired because it occurs as a result of excessive caramelization of sugar components. Therefore, low a* value and high L* value (brigtness) represent a good quality pekmez (Aksu and Nas, 1996).

Table 1. Standard chemical analysis

Analysis	Pekmez
pH	4.99 ± 0.00
TSS (°Brix)	71.2 ± 0.0
Colour	
L*	14.3± 0.1
a*	8.45± 0.1
b*	2.35± 0.1

± standard deviation

3.2. Aroma compounds of Pekmez

Aroma compositions of the pekmez sample extracted with SAFE method are given in Table 2. A total of 31 aroma compounds were identified and quantified in the samples including seven alcohols, six acids, four aldehydes, three terpenes, three lactones, two ketones, one ester, two pyrolles, one pyridine, one volatile phenol and one furanone compound. The dominant aroma groups in pekmez samples were alcohols, aldehydes and acids with the abundance of 2,3-butanediol (1869 µg/L), 5-hydroxymethyl furfural (1817 µg/L) furfural (1050µg/L), hexanoic acid (928 µg/L) and *(Z)*-limonene oxide (855 µg/L) (Table 2).

Seven alcohols were identified and quantified in the sample (Table 2). In the pekmez samples, a dominant part of the volatile compounds were alcohols. Similarly, these compounds have been reported to be the most dominant aroma

compounds in chokeberry juice concentrate in the previous study (Pozderovic et al., 2016). Alcohols are the compounds that directly did not affect the overall aroma of pekmez samples due to the high odour threshold values (Selli & Kelebek, 2011). As indicated in Table 2, 2,3-Butanediol was quantitatively the main alcohol compound in pekmez followed by furfuryl alcohol and 3-penten-2-ol. 2,3-Bütanediol which is also known as 2,3-butylene glycol, 2,3-dihydroxybutane, dimethylene glycol, and dimethylethylene glycol, is a chiral volatile component with a high boiling and a low freezing points, which is a colourless and odourless liquid at room temperature (Garg & Jain, 1995). Another alcohol detected in high quantity in the sample is furfuryl alcohol. This alcohol is a food component which occurs in significant levels in thermally processed food samples such as coffee, fruit juices, baked foods; in cask-stored alcoholic beverages such as wines and whiskies as a result of enzymatic or chemical reduction of furfural (Okaru & Lachenmeier 2017). In addition, Lee and Nagy (1988) reported that furfuryl alcohol was considered to be a degradation product of sugar components.

Acetic acid, butanoic acid, hexanoic acid, heptanoic acid, octanoic acid and nonanoic acid were detected as acid compound in the sample. Generally, acid compounds are not likely to have a major impact on the overall aroma of studied pekmez sample due to their high odour threshold values (Zannou et al., 2020). Among the acids (Table 2), hexanoic acid (928 µg/L) was found in the highest concentration in in the sample followed by acetic acid (299 µg/L) and nonanoic acid (118 µg/L).

Four aldehydes (furfural, benzaldehyde, 5-methyl-2-furfural and 5-hydroxymethyl furfural) were detected as aldehyde compounds in pekmez sample. This volatile group was found as quantitatively the most abundant in the studied sample. 5-Hydroxymethyl furfural was markedly the most abundant furans in the sample, the next most abundant furans was furfural (Table 2). These two aldehydes are occurred by the Maillard reaction between the sugars and the amino groups during the heat process of the *J. drupacea* pekmez.

As previously stated, terpene compounds were important aroma group in different Juniperus fruis (Chatzopoulou, & Katsiotis, 2006; Foudil-Cherif, & Yassaa, 2012). A significant part of these compounds were lost due to heat treatment during the production of pekmez. *p*-Xylene, *DL*-limonene and *(Z)*-limonene oxide were found as terpene compounds in the sample. In a previous study (Najar, Pistelli, Mancini, & Fratini, 2020), the volatile leaf and berry oils *J. oxycedrus* and *J. macrocarpa* were dominated by monoterpene hydrocarbons characterized by high amounts of α-pinene while limonene was the most abundant compound in *J. deltoids*.

γ-Butyrolactone, 5-ethyldihydro-2(3H)-furanone and pantolactone were found in the sample as lactone compounds (Table 2). The flavor characteristics of lactones were creamy, greasy, fatty, and fruity, and these compounds are produced by cyclization of the corresponding gamma-hydroxy carboxylic acids and may also be obtained from the oxidation of unsaturated aldehydes in the different food samples (Perestrelo, Fernandes, Albuquerque, Marques, & Câmara, 2006). As indicated in Table 2, the most abundant lactone was γ-butyrolactone.

3-Hydroxy-2-butanone (acetoin) and 1-hydroxy-2-propanone (acetol) were identified in the pekmez sample as ketone compounds (Table 2). Acetoin naturally occurs in different foodstuff including corn, grapes, cocoa, apples, bananas, strawberries, and also some animal tissues. Owing to its pleasant yogurt creamy odour and fatty butter taste properties, this ketone is used as food flavor enhancer in butter, cheese, coffee, yogurt, nuts, and other foods (Tian et al., 2014). The amount of acetoin was found to be 495 µg/L.

With regards to the other compounds, ester, pyrrole, pyridines, volatile phenol and furanone were also identified and quantified in the sample (Table 2).

Table 2 Aroma Compounds of Pekmez

	LRI[1]	Compounds	Concentration[2] (µg/L)	ID[3]
		Alcohols		
1	1170	3-Penten-2-ol	355±6,4	LRI, MS, Std
2	1173	4-Methyl-2-pentanol	244±5,8	LRI, MS, Std
3	1322	2- Methyl -2-buten-1-ol	81,8±3,6	LRI, MS, Std
4	1541	2,3-Butanediol	1869±1,8	LRI, MS, Std
5	1660	Furfuryl alcohol	427±6,7	LRI, MS, Std
6	1862	Benzyl alcohol	72,2±3,9	LRI, MS, Std
7	1898	Phenylethyl alcohol	28,7±0,9	LRI, MS, Std
		Acids		
8	1465	Acetic acid	299±6,7	LRI, MS, Std
9	1663	Butanoic acid	69,8±6,5	LRI, MS, Std
10	1832	Hexanoic acid	928±4,8	LRI, MS, Std
11	1930	Heptanoic acid	22,4±2,1	LRI, MS, Std
12	2050	Octanoic acid	53,1±3,9	LRI, MS, Std
13	2144	Nonanoic acid	118±3,9	LRI, MS, Std
		Aldehydes		
14	1437	Furfural	1050±2,4	LRI, MS, Std
15	1506	Benzaldehyde	88,4±5,3	LRI, MS, Std
16	1570	5-Methyl-2-furfural	71,6±0,4	LRI, MS, Std
17	2501	5-Hydroxymethyl furfural	1817±6,4	LRI, MS, Std
		Terpenes		
18	1130	*p*-Xylene	123±6,1	LRI, MS, Std
19	1195	dl-Limonene	279±5,5	LRI, MS, Std
20	1430	*(Z)*-Limonene oxide	855±8,8	LRI, MS, Tent
		Lactones		
21	1617	*γ*-Butyrolactone	599±5,8	LRI, MS, Std
22	1684	5-Ethyldihydro-2(3H)-furanone	74,7±4,0	LRI, MS, Tent
23	2034	Pantolactone	138±4,4	LRI, MS, Tent
		Ketones		
24	1259	3-Hydroxy-2-butanone	495±1,7	LRI, MS, Std
25	1290	1-Hydroxy-2-propanone	492±2,0	LRI, MS, Tent
		Ester		
26	1557	Methyl-2-furoate	352±8,8	LRI, MS, Std
		Pyrrolles		
27	1970	2-Acetylpyrolle	180±9,5	LRI, MS, Std
28	2032	2-Formylpyrolle	102±0,6	LRI, MS, Tent
		Pyridine		
29	1579	3-Methoxypyridine	154±9,0	LRI, MS, Tent
		Furanone		
30	1266	Dihydro-2-methyl-3(2H)-furanone	164±7,8	LRI, MS, Tent
		Volatile Phenol		
31	2555	Vanilline	40,4±3,6	LRI, MS, Std
		General Total	**11645,5±15,6**	

*[1]LRI: linear retention index calculated on the DB-WAX capillary column. [2]Results are the means of the three repetitions as µg/L. [3]ID: method of identification. MS tent: tentatively identified by MS; Std: chemical standard. When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification.

4. CONCLUSIONS

In the present work, volatile compounds and general chemical properties of pekmez obtained from Juniper berries were elucidated. Liquid-liquid extraction/solvent-assisted aroma evaporation (SAFE) method was used for the analysis of aroma substances in pekmez. SAFE is the most advantageous technique, due to the low extraction temperature and ability to extract the most volatile compounds in different food samples. A total of 31 volatile compounds were identified and quantified in pekmez sample, and 2,3-butanediol, 5-hydroxymethyl furfural, furfural, hexanoic acid and *(Z)*-limonene oxide in the sample.

REFERENCES

Akbulut, M., Çoklar, H., & Özen, G. (2008). Rheological characteristics of *Juniperus drupacea* fruit juice (pekmez) concentrated by boiling. Food Science and Technology International, 14(4), 321-328.

Aksu I. and Nas S. (1996). Mulberry pekmez manufacturing technique and physico-chemical properties (in Turkish). Gida 21: 83–88

Chatzopoulou, P. S., & Katsiotis, S. T. (2006). Headspace analysis of the volatile constituents from *Juniperus communis* L.'berries'(cones) grown wild in Greece. Flavour and Fragrance Journal, 21(3), 492-496.

Dob, T., Dahmane, D., & Chelghoum, C. (2008). Chemical composition of the essential oil of *Juniperus phoenicea* L. from Algeria. Journal of Essential Oil Research, 20(1), 15-20.

El-Ghorab, A., Shaaban, H. A., El-Massry, K. F., & Shibamoto, T. (2008). Chemical composition of volatile extract and biological activities of volatile and less-volatile extracts of juniper berry (*Juniperus drupacea* L.) fruit. Journal of Agricultural and Food Chemistry, 56(13), 5021-5025.

Foudil-Cherif, Y., & Yassaa, N. (2012). Enantiomeric and non-enantiomeric monoterpenes of *Juniperus communis* L. and *Juniperus oxycedrus* needles and berries determined by HS-SPME and enantioselective GC/MS. Food Chemistry, 135(3), 1796-1800.

Garg SK, & Jain A (1995). Fermentative production of 2,3-butanediol: a review. Bioresour Technol 51:103–109.

Lee, H. S., & Nagy, S (1988). Relationship of sugar degradation to detrimental changes in citrus juice quality. Food Technology, 11, 91-97

Okaru, A. O., & Lachenmeier, D. W. (2017). The food and beverage occurrence of furfuryl alcohol and myrcene—two emerging potential human carcinogens?. Toxics, 5(1), 9.

Najar, B., Pistelli, L., Mancini, S., & Fratini, F. (2020). Chemical composition and in vitro antibacterial activity of essential oils from different species of Juniperus (section Juniperus). Flavour and Fragrance Journal, 35(6), 623-638.

Perestrelo, R. M. D. S., Fernandes, A., Albuquerque, F. F., Marques, J. C., & Câmara, J. D. S. (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. Analytica Chimica Acta, 563(1-2), 154-164.

Pozderović, A., Popović, K., Pichler, A., & Jakobek, L. (2016). Influence of processing parameters on permeate flow and retention of aroma and phenolic compounds in chokeberry juice concentrated by reverse osmosis. CyTA-Journal of Food, 14(3), 382-390.

Selli, S., & Kelebek, H. (2011). Aromatic profile and odour-activity value of blood orange juices obtained from Moro and Sanguinello (*Citrus sinensis* L. Osbeck). Industrial Crops and Products, 33(3), 727-733.

Sengül, M., Ertugay, M. F., & Sengül, M. (2005). Rheological, physical and chemical characteristics of mulberry pekmez. Food Control, 16(1), 73-76.

Sevindik, O., Guclu, G., Bombai, G., Rombol´a, A. D., Kelebek, H., & Selli, S. (2020). Volatile compounds of cvs Magliocco Canino and Dimrit grape seed oils. Journal of Raw Materials to Processed Foods, 1, 47–54.

Sobierajska K, Boratyńska K, Jasińska A, Dering M, Ok T, Douaihy B, Dagher-Kharrat MB, Romo A, Boratyński A (2016) Effect of the Aegean Sea barrier between Europe and Asia on differentiation in *Juniperus drupacea* (Cupressaceae). Bot J Linn Soc 180:365–385.

Sonmezdag, A.S., Kelebek, H., Selli, S. (2016). Characterization and comparative evaluation of volatile, phenolic and antioxidant properties of pistachio (*Pistacia vera* L.) hull. Journal of Essential Oil Research, 1–9.

Tian, Y., Fan, Y., Zhao, X., Zhang, J., Yang, L., & Liu, J. (2014). Optimization of fermentation medium for acetoin production by Bacillus subtilis SF4-3 using statistical methods. Preparative Biochemistry and Biotechnology, 44(5), 529-543.

Zannou, O., Kelebek, H., & Selli, S. (2020). Elucidation of key odorants in Beninese Roselle (*Hibiscus sabdariffa* L.) infusions prepared by hot and cold brewing. Food research international, 133, 109133.