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Aroma compounds of non-alcoholic fermented beverage: Gilaburu juice

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Abstract

The present research was planned to characterize the aroma composition of Viburnum opulus L. which is one important members of the Caprifoliaceae family. Volatile components of Viburnum opulus L. were extracted by use of the purge and trap technique with dichloromethane and analyzed by gas chromatography-mass spectrometry (GC-MS). The extraction method gave highly representative aromatic extract of the studied sample. A total of 47 aroma compounds were found including acids, alcohols, ketones, volatile phenols, aldehydes, furans, lactones, an ester a terpene and a pyranone. Among all aroma compounds, isovaleric acid was found as the most abundant aroma compound in the fermented GR juice, having a 30% of total aroma concentration. Butanoic acid, 4-methyl catechol and propanoic acid were other aroma compounds found in higher concentrations (18%, 11% and 6% respectively).

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Introduction

Within today's trends, there is a growing interest in natural food products which have the unique aroma and high nutritional value. This significant issue increases the studies of the less communal and horticultural in recent years and the family of Caprifoliaceae has found part within this interest. The family, containing about 18 generas and around 400 species all over the world (1). Turkey is one of the most important places to four native Viburnum opulus L. (Caprifoliaceae) (2). European Viburnum opulus L. commonly known as gilaburu in Turkey is a red colored fruit having special astringent taste, which is mostly grown around the Kayseri city of the country (3). Stem bark and fresh shots of this shrub have been used in traditional medicine for the treatment of various diseases (e.g. kidney disorders, menstrual, and stomach cramps) (4).

Viburnum opulus L. berries own some typical smell notes, which are rather disliked by the consumers. In the Kayseri region of Turkey, European Cranberrybush fruits are allowed to stand in plastic drums containing tap water at a dark place and room temperature approximately for 4 months to eliminate the sour, pungent flavor (4).

Like any other food products, flavor is one of the main characteristics that effect consumer perceptions (5). A complex mixture of volatiles is mainly responsible for the berries' and fruits' flavor such as that found terpenes, alcohols, ketones, esters, and acids (3). Scientific information on the structure of volatile compounds of Viburnum opulus L. is limited. Kraujalytė, Leitner and Venskutonis (1) studied the volatile compounds of the fruit and identified 41 compounds, 3-methyl-butanoic acid being the major constituent in studied European Cranberrybush cultivars. Yilmaztekin and Sislioglu (3) researched the volatile compounds of Viburnum opulus L. by liquid liquid extraction method and identified a total of 58 compounds in which 3-methyl-butanoic acid (25.4% to 66.4% of

identified volatile compounds) being the major constituent in raw, 2-, 3-, and 4-month fermented European Cranberrybush fruits, while 2-octanone was dominant in 1-month fermented sample with a 30% of the total identified volatiles.

The aim of the present research was to identify and quantify the volatile composition of Viburnum opulus L. which is cultivated in Turkey. In the present study, the aroma extraction method selected was the purge and trap technique with dichloromethane solvent. This technique is a very sensitive extraction method for many aroma compounds, especially with low boiling points. Additionally, by using this technique, it is possible to extract volatile compounds without artifacts formation with high reliability gas chromatography (GC) together with mass spectrometry (MS) and a flame ionization detector (FID) for quantification and identification of volatile compounds.

Materials and Methods

Samples and chemicals

The bunches with mature fruits of Viburnum opulus L. was gathered in October 2016 from the vicinities of Kayseri province, Turkey. Bunches with berries were picked from the bottom, middle and top parts of the plants selecting 10 different bushes (10 kg). Water used in this study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). The standard volatile compounds were purchased from Sigma-Aldrich (Steinheim, Germany). Dichloromethane, sodium sulfate and 4-nonanol were obtained from Merck (Darmstad, Germany). Dichloromethane was freshly distilled prior to use.

Fermentation procedure

After stalks and leaves were separated from the fruit, bunches from different bushes of the Viburnum opulus L. fruits were mixed, washed 3 times with tap water, and placed in plastic drums. The plastic drums containing fruits were filled with tap water to overflowing, closed tightly and allowed to ferment in a dark place at room temperature (25 °C) for 4 months. The stalks were removed and the berries were pressed by hand with a muslin cloth (3).

Extraction of volatile compounds

Volatiles of fermented juice of Viburnum opulus L. were extracted by the purge and trap system which comprises a flow-meter to control a nitrogen source and is connected to a splitter system to divide the flow into several channels in order to purge three samples at the same time. Lichrolut EN tubes obtained from Merck were used as an adsorbent which is one of the most appropriate sorbents for volatile compounds extraction with respect to the previous research (6). 5 mL samples were previously placed into a 20 mL vial; then, the sample was pre-incubated at optimized purging temperature (60 ^{O}C) for 10 min. The process was applied for 90 min with a nitrogen flow of 500 mL/min. After purging, the volatiles held in the cartridge were eluted with dichloromethane. The elute was dried by anhydrous sodium sulphate; the pooled organic extract was concentrated to 5 mL in a Kuderna Danish concentrator fitted with a Snyder column at 40 ^{O}C (Supelco, St. Quentin, France) and then to 0.5 mL under a gentle flow of nitrogen. Extracts were then stored at -20 ^{O}C in a glass vial equipped with a Teflon-lined cap until analysis. Extractions were carried out in triplicate.

GC-FID, GC–MS analysis of volatile compounds

Agilent 6890 chromatograph interfaced with a flame ionization detector (FID) and Agilent 5973-Network-mass selective detector (MSD) (Wilmington, Delaware, DE, USA) constituted the gas chromatography (GC) system. DB-Wax column (30 m length x 0.25 mm i.d. x 0.5 µm thickness, J&W Scientific, Folsom, CA, USA) were used to separate volatile compounds. An amount of 3 µL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. The mode is suitable when analyte concentrations are low for trace analysis. Injector and FID detectors were set at 270 ^{O}C and 280 ^{O}C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min-1. The conditions of the oven program of the DB-Wax column was 50 ^{O}C to 250 ^{O}C at 4 ^{O}C /min, 10 min hold. As for the mass-selective detector, the identical oven program was used. The MS (electronic impact ionization) conditions were as follows: ionization energy of 70 eV, mass range m/z of 30–300 a.m.u., scan rate of 2.0 scan s-1, interface temperature of 250 ^{O}C, and source temperature of 180 ^{O}C. The volatile compounds were analyzed in full scan mode and assigned by comparison of their retention index and their mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, NBS 75k) and the instrument's internal library made through the aforementioned laboratory researches. After identification, the internal standard method with 4-nonanol was used to determine the mean value of volatile compounds and mean values of the triplicate of GC analyses were calculated for each sample. By using n-alkane (C8–C32) series, retention indices of the compounds were calculated.

Results and Discussion

Table 1. shows the volatile compounds, their retention indices and mean values (µg/L) in Viburnum opulus L. A total of 47 volatile compounds were identified in the sample. Most volatiles detected in this study were consistent with those of previously published studies (1, 3). The main chemical group of the volatile compounds in Viburnum opulus L. sample was acids. These compounds represented over 65 % of the total volatiles in Viburnum opulus L. Yilmaztekin and Sislioglu (3) reported that acid compounds were the dominant volatile compounds in raw and 3- to 4-month fermented European Cranberrybush. Formation of acids via microorganisms are essential to the flavor of many fermented foods. Isovaleric acid, butanoic acid and propanoic acid were the main acid compounds which were detected in Viburnum opulus L. and their concentrations were found to be 2778 µg/l, 1689 µg/L, and 554 µg/L, respectively. Unlike previous studies, isovaleric acid was the most dominant

Table 1. Volatile compounds of *Viburnum opulus L.*

No	LRI*	Compounds	Concentration# (µg l^{-1})	Identification§
1	951	2-Propanol	20,74	LRI,MS,std
2	1003	2-Methyl-2-butanol	3,68	LRI,MS,std
3	1038	3-Pentanol	30,46	LRI,MS,std
4	1125	Butanol	44,73	LRI,MS,std
5	1204	3-Hexanol	14,40	LRI,MS,std
6	1212	Isoamyl alcohol	67,42	LRI,MS,std
7	1238	2-Hexanol	31,50	LRI,MS,std
8	1259	Acetoin	393,55	LRI,MS,std
9	1270	3,8-*p*-Menthadiene	230,91	LRI,MS,tent
10	1287	2-Octanone	8,07	LRI,MS,std
11	1308	Hexanol	5,95	LRI,MS,std
12	1350	Hexyl isobutyrate	17,98	LRI,MS,tent
13	1369	Nonanal	23,65	LRI,MS,std
14	1431	Acetic acid	537,40	LRI,MS,std
15	1486	Propanoic acid	554,11	LRI,MS,std
16	1531	Octanol	9,73	LRI,MS,std
17	1582	Isobutyric acid	13,40	LRI,MS,std
18	1608	(*E*)-Dihydrocarvone	122,90	LRI,MS,tent
19	1623	γ-Butyrolactone	50,66	LRI,MS,std
20	1644	Butanoic acid	1689,03	LRI,MS,std
21	1678	Furfuryl alcohol	21,08	LRI,MS,std
22	1690	Isovaleric acid	2778,11	LRI,MS,std
23	1719	*d*-Carvone	485,84	LRI,MS,tent
24	1762	3-Methyl pentanoic acid	110,72	LRI,MS,tent
25	1795	(*E,E*)-2,4-decadienal	8,29	LRI,MS,tent
26	1832	Hexanoic acid	34,03	LRI,MS,std
27	1862	Guaiacol	19,85	LRI,MS,std
28	1928	Phenylethyl alcohol	104,98	LRI,MS,std
29	1956	(*E*)-2-tridecenal	19,00	LRI,MS,tent
30	1965	Benzenemethanol	62,14	LRI,MS,std
31	1970	Heptanoic acid	19,79	LRI,MS,std
32	1992	2H-Pyran-2,6(3H)-dione	63,79	LRI,MS,tent
33	2001	Phenol	18,65	LRI,MS,std
34	2010	Pantolactone	18,69	LRI,MS,std
35	2027	4-Methoxy benzaldehyde	19,42	LRI,MS,tent
36	2046	Octanoic acid	38,46	LRI,MS,std
37	2063	*p*-Cresol	38,17	LRI,MS,std
38	2083	2-Phenoxy ethanol	12,65	LRI,MS,std
39	2141	*p*-Ethylphenol	64,96	LRI,MS,tent
40	2174	Nonanoic acid	104,73	LRI,MS,std
41	2199	2-Methoxy-4-vinylphenol	43,02	LRI,MS,tent
42	2269	Syringol	29,16	LRI,MS,std
43	2288	Decanoic acid	49,23	LRI,MS,std
44	2387	Benzoic acid	66,38	LRI,MS,std
45	2451	Dodecanoic acid	103,75	LRI,MS,std
46	2535	3-Hydroxy-β-damascone	58,85	LRI,MS,tent
47	2727	4-Methyl catechol	1042,93	LRI,MS,tent
		Total	**9306,94**	

*LRI, linear retention index calculated on DB-WAX capillary column

Concentration: Results are the means of three repetitions as µg l^{-1} dw

§ Identification: Methods of identification; LRI (linear retention index), MS tent. (tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compounds, it must be considered as an attempt of identification.

acid compounds. The compound is a natural fatty acid found in a wide variety of plants and essential oils. The compounds give the unpleasant foot odor mostly is produced by the yeast (7).

Alcohol compounds were the other important class of the aroma compounds in the Viburnum opulus L. These compounds are formed from fatty acids supplied to excised tissue, by 8-oxidation followed by reduction in two steps from acetyl-coenzyme A to aldehyde and aldehyde to alcohol. The compounds typically make a minor contribution to flavor due to the high odor threshold (8). 4-Methyl catechol was overwhelmingly the dominant alcohol compounds and followed by phenylethyl alcohol and Isoamyl alcohol with concentration of 1042 µg/L, 104 µg/L, and 67 µg/L, respectively. 4-Methyl catechol is one of the potent stimulators of endogenous nerve growth factor (NGF) which is one of an increasing number of factors that essential for the survival and maintenance of specific types of cells, synthesis both in vitro and in vivo (9).

Another important chemical group of volatiles was ketones. Mechanism for the formation of ketones via microorganisms involves the transamination and decarboxylation of free amino acids and may also be synthesized by microbial induced lipid oxidations (10). Acetoin and 2H-pyran-2,6(3H)-dione were the featured ketone compounds with the concentration of 393 µg/L and 63 µg/L respectively.

Terpenes were the other important contributor of the aroma of Viburnum opulus L. Terpene synthases are directly responsible for the production of these volatile terpenes. On the other hand, some of them are formed via modification of the main skeletons of terpene made by terpene synthases by hydroxylation, dehydrogenation, acylation, and other reactions (11). (E)-Dihydrocarvone and d-carvone were the identified terpene compounds in the sample. The basic organic chemical structure of the carvones is similar to that of the menthols and menthones. However, the organoleptic properties are not similar to menthol or menthone, nor do the carvones confer the cooling effect of menthol (12).

Conclusion

The present study was designed to characterize the aroma compounds in fermented gilaburu juice (Viburnum opulus L.). A total of 47 aroma compounds including acids, alcohols, ketones, volatile phenols, aldehydes, furans, lactones, an ester a terpene, and a pyranone were identified and quantified in sample using GC-MS and GC-FID. The purge and trap extraction method gave highly representative aromatic extract of the studied sample. Acids were determined at highest levels among the identified compounds, followed by alcohols. Within these, Isovaleric acid (2778,11 µg/l) was quantitatively a major aroma compound in Viburnum opulus L., followed by butanoic acid (1689,03 µg/l) and 4-methyl catechol (1042,93 µg/l).

Conflict of Interest Statement

The authors confirm that this article content has no conflict of interest.

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