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DETERMINATION OF AROMA COMPOUNDS IN BLACKBERRY BY GC/MS ANALYSIS

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The aromatic composition of five blackberry cultivars (Bursa 2, Navaho, Nessy, Chester Thornless, and Jumbo) was studied. The Im-SPME (Immersion Solid Phase Micro Extraction) extraction technique was applied and the samples were analyzed by GC/MS. Furfural and its derivatives were found to be the major aromatic compounds and 5-hydroxymethylfurfural was the most abundant compound in all the blackberry varieties.

Key words: blackberry, aroma, Im-SPME, furfural, GC/MS.

Cumulative results of epidemiological and *in vitro* research suggest that the consumption of fruits and vegetables may decrease the risk of cardiovascular disease and various forms of cancer by providing enhanced antioxidant protection in the human body [1]. The fruits of blackberries, like other berries such as raspberries, and strawberries, are good sources of natural antioxidants [2, 3] and the authors reported that extracts of berries of several cultivars of blackberries, blueberries, and black and red raspberries showed a remarkably high scavenging activity towards chemically generated superoxide radicals [3].

Fresh blackberries (100 g) consist of water (84.7 g), cellulose (4 g), protein (1.2 g), carbohydrate (8.6 g), lipid (1 g), some minerals (Na, K, Ca, Fe, and P), vitamins (Vitamin A, B complex, and C) and 48 kcal [4].

Blackberries grow in almost all parts of Turkey in the wild especially near the Black Sea region. During the last decade blackberry growing became very popular using the Thornless varieties. Thornless blackberry varieties can be propagated very easily. Primocanes can become productive in the first year after planting. In addition, the yield is high and very suitable for organic agriculture. Blackberry growing has great potential in Turkey, especially for the early season in Mediterranean coastal regions.

Marketing is easy because of different consumable forms of blackberry fruits such as fresh, frozen, jam, cake, marmalade, ice-cream, juice, wine, and liqueur. The aroma in blackberry is one of the major characters affecting quality in either fresh or processed fruits. Certainly, the balance of acid and sugar is directly related to the delicate flavours in fruits. Fruit flavor is particularly sensitive to compositional alterations. The volatile compounds responsible for the fruit flavour are biosynthesized through metabolic pathways during ripening, harvest, post-harvest, and storage and depend on many factors related to the species, variety and type of technological treatment [5]. It is sometimes important to know the typical chromatographic patterns of a fresh product in order to identify changes in the volatile composition. Nowadays, different methods have been used for aroma analysis. In these particular cases a rapid, simple, and inexpensive technique for extracting and preconcentrating fruit flavour can be very useful. One such technique is a new sample preparation method called Solid-Phase Micro Extraction (SPME) [6]. SPME is a solvent-free, inexpensive, rapid, and versatile method for the extraction of organic compounds. It consists of a fused-silica fiber, coated with a polymeric stationery phase that can be introduced into a liquid or gas sample. This method has been used by several authors for the analysis of volatile compounds in food samples, demonstrating its utility for flavour analysis [7, 8].

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TABLE 1. Aromatic Compounds Identified in Blackkberry Cultivars by GC/MS, %

R.I.	Compounds	Blackberry varieties				
		Bursa 2	Jumbo	C. Thorn.	Nessy	Navaho
3.92	Furfural	6.3	Tr.	3.1	4.5	Tr.
5.38	Citric acid	0.7	-	Tr.	-	Tr.
6.34	5-Methylfurfural	-	Tr.	Tr.	-	Tr.
8.8	4,5-Dimethylfurfural	-	Tr.	Tr.	-	-
9.40	Unknown	3.9	4.6	4.7	6.3	3.9
11.24	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	6.1	9.8	5.7	9.6	Tr.
13.35	**5-Hydroxymethylfurfural**	**81.9**	**85.6**	**85.5**	**79.7**	**96.1**

The purpose of this study was to determine the aromatic compounds of five blackberry varieties using im-SPME by GC/MS. Unknown: *m/z* 37 (5), 38 (8), 39 (18), 42 (11), 43 (100), 44 (6), 53 (9), 54 (8), 55 (36), 67 (6), 83 (7), 95 (40), 123 (11), 124 (20), 126 (77), 127 (6), 128 (5).

The aromatic compounds of the five blackberry varieties characterized by GC/MS analysis are given in Table 1. Furans were found to be the most abundant aromatic compounds, 5-hydroxymethylfurfural being the major component, consisting of 79.7–96.1% of the total aroma profile depending on the varieties. These data demonstrated that 5-hydroxymethylfurfural is the most effective compound contributing to the unique flavour of blackberries. The other main compound was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, which ranged between 5.7–9.8% in four varieties, and traces were detected in the Navaho variety. This data also showed genetic variability among the varieties.

Although the aroma of raspberries has been studied very extensively, only little attention has been paid to the flavour of blackberries (*Ribes fruitcosus* Coll.). The authors dealed with the flavor of commercial blackberry essence (var. Thornless evergreen) [9,10,11]. Twenty-three compounds were identified, of which only 3,4-dimethoxyallylbenzene was considered to contribute to the characteristic flavour of blackberries. Some of the authors studied the aromatic compounds of fresh blackberries (var.Thornless evergreen) [12].

They identified 245 compounds, and the main compounds were heptan-2-ol (43.06%), *p*-cymen-8-ol (3.72%), heptan-2-one (3.32 %), hexan-1-ol (%3.05), a-terpineol (2.38%), pulegone (2.05%), and octan-1-ol (1.83%). However, the authors did not indicate any specific blackberry-like aromatic compound. The same authors studied the volatile flavour compounds in heated blackberry juices [13]. They found that the concentrations of some aldehydes, lactones, and furan compounds increased markedly by heating. In another study [14] the same authors isolated volatile compounds of commercial blackberry juice by a simultaneous distillation-extraction procedure. Seventy compounds were identified by GC/MS (EI and CI mode), GC-IR analysis, and comparison of retention indices with those of authentic compounds. The aroma of this juice was found to be mainly due to the presence of furfural (which represents a third of the total odorous profile), 3-methylbutanol, phenylacetaldehyde, and *trans*-furan linalool oxide. In our study, the Im-SMPE technique was used. The differences between our study and the previous ones, especially the latter, may be due to different extraction techniques and the material used in the all studies. For example, in the latter study, fruit juice was used, but we used frozen fruits of five different cultivars. The juice may also have been diluted. Therefore, this is the first study to report the aromatic composition of blackberries using Im-SMPE technique. The method is reliable, detects the characteristic compounds, and reports that 5-hydroxymethyl furfural is the main specific blackberry-like aromatic compound.

Volatile compounds are ubiquitous in fruits and vegetables [15], comprising the aromatic component of flavour. Several volatile compounds are produced in plant tissue in response to mechanical and biological injury [16]. These “wound volatiles”, usually six and nine carbon aldehydes or alcohols, are formed *via* the lipoxygenase (LOX) hydroperoxide lyase enzymatic pathway, which is activated immediately following wounding. Many of the LOX products produce after wounding, as well as many other natural volatile compounds, have been identified in ripe strawberry, blackberry, grape, and other fruits [15]. In this paper, we detected LOX products as furfural, 5-methylfurfural, 4,5 dimethylfurfural, and 5-hydroxymethylfurfural.

The results of this study will provide valuable information on the biosynthesis of aromatic compounds in blackberry.

EXPERIMENTAL

Blackberries were grown in Cukurova university located in Adana province. The fruits of the Bursa 2, Jumbo, Chester Thornless, Nessy, and Navaho varieties were used for aroma analysis. The fruits were harvested at the shiny black stage and immediately treated with liquid nitrogen and stored at 80°C until analysis.

The frozen fruits were homogenized in a food processor (Braun), and 50 g homogenate was weighed and im-SPME sampling conducted. SPME fiber (Supelco) precoated with a 100 µm layer of polydimethylsiloxane (PDMS) was used. The SPME fiber was inserted into a vial for 30 min at 30°C by stirring. The SPME syringe was then introduced into the injector port of the GC/MS instrument for analysis.

Flavour compounds were analyzed on a Shimadzu QP 5050A apparatus equipped with a CP Sil 5CB (25 m $\times$ 0.25 mm *i.d.)* fused-silica capillary column. Helium (1ml/min) was used as carrier gas. The injector temperature was 250°C, set for splitless injection. Column temperature was 60°C//5°C/min//260°C - 20 min. MS were taken at 70 eV. Mass range was from *m/z* 30 to 425. Identification of the main components was carried out by comparison of their mass spectra and retention time data using the Wiley/NBS library and the in-house TBAM Library of Essential Oil Constituents.

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