Aroma composition of shalgam: a traditional Turkish lactic acid fermented beverage

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Abstract Shalgam, a traditional red, cloudy and sour soft beverage, is produced by lactic acid fermentation of black carrot, sourdough, salt, bulgur flour, turnip and adequate water. The present study was designed to characterize the volatile compounds of shalgam obtained from different methods. The aroma compounds of shalgams produced by traditional and direct methods, and addition of Lactic acid bacteria (LAB) cultures were examined. Volatile components of shalgam samples were extracted by liquid–liquid extraction technique with pentane/dichloromethane and analyzed by gas chromatography-mass spectrometry (GC–MS). Sixty aroma compounds were identified in shalgam samples including 20 terpenes, 9 esters, 9 alcohols, 5 volatile acids, 6 volatile phenols, 5 lactones, 3 naphthalenes, 2 carbonyl compounds and 1 C13-norisoprenoids. It was found that the aroma profiles of shalgams were quite similar. However, the total volatile content of the shalgam samples increased with addition of *Lb. plantarum*.

Keywords Shalgam (Salgam) · Fermented beverage · Aroma · Production methods · Autochthonous starter culture · *Lb. plantarum*

Introduction

Fermented beverages have an important part of our beverage consumption. These products are produced in different regions of the world in distinct methods. Many of these (wine, beer, and dairy products etc.) are done into the large-scale industrial manufacture. These products are of great commercial significance worldwide (Waites et al. 2001). Conversely; some fermented beverages such as ayran, kefir, kanji, koumiss, tarhana, boza and shalgam are minor fermented products produced in different countries. Those products are also produced mercantile in some territories (Erten et al. 2008).

Shalgam is a traditional fermented Turkish product. It is a cloudy, reddish coloured, and sour soft beverage. It is manufactured from the lactic acid fermentation of black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.), bulgur flour, sourdough, commercial unrefined salt (rock or sea salt), turnip (*Brassica rapa* L.) and adequate water. For shalgam production, black carrot is used as the main raw material. It plays a significant role for the formation of organic acid (lactic acid) as the predominant end product during the fermentation of sugar and for the red shalgam colour (Canbas and Deryaoglu 1993; Erten and Tanguler 2016). That colour of shalgam beverage derives from anthocyanin pigments present in roots of black carrots (Erten and Tanguler 2016). For the manufacture of shalgam, bulgur (bulghur) flour (bran) is a minor raw material. During the first stage of shalgam fermentation in the traditional production method as well as in the direct

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production method, it provides nutrients to microorganisms (Erten et al. 2008). Rock salt (sodium chloride), which is an significant ingredient in shalgam production, serves several functions during the shalgam fermentation and gives salty taste to the ready-to-drink shalgam. It inhibits some microorganisms such as pathogenic and spoilage microorganisms depending on its concentration. In addition, rock salt favoring the lactic acid bacteria (LAB) growth. In addition, turnip and sourdough are minor raw materials of shalgam production (Erten and Tanguler 2016).

Shalgam production is mainly carried out at home or at home-scale grade, however it is also manufactured on large scale and small scale in Industry. A standard manufacturing technique for shalgam production is not available. The processing method is different from one plant to another. For industrial production of fermented shalgam, there are two basic processing methods: the traditional method and the direct method (Erten et al. 2008; Tanguler and Erten 2012). Shalgam is very popular in some provinces of Southern Turkey, particularly in the cities of Adana, Mersin and Hatay and its popularity has also increased all over Turkey (Erten and Tanguler 2016; Canbas and Deryaoglu 1993). Newly, that fermented beverage is also marketed in some European capitals (Tanguler and Erten 2013). Mostly, shalgam is consumed with food (kebab, pita etc.) and as a refreshing drink (Erten et al. 2008).

Until today, there is no commercial starter cultures used for shalgam production (Erten and Tanguler 2016). Most suitable starter cultures are autochthonous microorganisms which isolated from traditional fermented foods and beverages. Autochthonous microorganisms are generally well concerted in the fermentation medium. On the other hand, they are significant to obtain aspired fermentation parameters specific for the type of fermented product (Drosinos et al. 2005). The usage of autochthonous starter cultures in the fermentation may help to prolong shelf-life of fermented products which also protect acceptable nutritional value and sensorial characteristics (Di Cagno et al. 2011). Unfortunately, no previous study related with the use of autochthonous LAB as starter culture in the production of fermented shalgam with the exception of our previous study (Tanguler et al. 2015).

Aroma compounds are low molecular weight compounds which are less than 300 Da. These compounds easily vaporise at room temperature (Angerosa 2002). Aroma is one of the critical quality parameters that influence consumer acceptance and the market value of products. Aroma compounds of shalgam mainly come from raw materials, mainly black carrot, and fermentation. The analysis of aroma compounds tends to be one of the main parameters to take into account in the development of a novel and particular fermented food. Raw materials, fermentation, processing and storage conditions affect the

flavour of fermented foods in a various ways. Generally, the most evident change in lactic acid fermentation is acid production and lowering pH. These changes result in an increase in sourness of the product (McFeeters 2004).

As far as it is known, there are some studies on shalgam. All published papers dealing with the fermented shalgam beverage have considered anthocyanin content of shalgam (Turker et al. 2007), identification of LAB in shalgam fermentation (Tanguler and Erten 2012; Tanguler et al. 2015), effects of addition of black carrot in various quantities on the chemical, physical, microbiological, and sensory characteristics (Tanguler et al. 2014) and selection of potential autochthonous starters (Tanguler and Erten 2013). As far as it is known, there is no publication on volatile compounds of shalgam. The main objective of this present study was to determine and compare the aroma compounds of shalgams produced by traditional and direct methods, and by the addition of autochthonous LAB starter cultures isolated from shalgam fermentations.

Materials and methods

Raw materials and reagents

Black carrot was obtained from Eregli-Konya province of Turkey. Other raw materials were supplied from market. The water used in the study was purified by a Milipore-Q system (Milipore Corp., Saint-Quentin, France). The reference aroma compounds were obtained from Merck (Darmstadt, Germany). Dichloromethane was freshly distilled prior to use. Starter cultures of *Lactobacillus* (*Lb.*) *plantarum*, *Lb. paracasei* subsp. *paracasei* and *Lb. fermentum* were previously isolated and identified from fermented shalgam samples produced at laboratory and in industry and selected among 447 strains by Tanguler (2010), Tanguler and Erten (2012). They stored in MRS broth as liquid cultures with glycerol at -20°C until used.

Shalgam production

Production methods of shalgams are given in Figs. 1 and 2. They were coded as TM (Traditional method) and DM (Direct method). In addition, shalgam production with the addition of starter cultures is given in Fig. 1 and they were coded as LP (addition of *Lb. plantarum*), LF (addition of *Lb. fermentum*) and PA (addition of *Lb. paracasei* subsp. *paracasei*).

The starter cultures of *Lb. plantarum*, *Lb. fermentum* and *Lb. paracasei* subsp. *paracasei* were propagated in pasteurized black carrot juice as described our previous study on microbial, chemical and sensory properties of shalgam (Tanguler et al. 2015). Black carrot juice was achieved

Fig. 1 The production of shalgam by traditional method

Fig. 2 The production of Shalgam by direct method

according to Demir et al. (2006). Fresh black carrots were washed and blended with Juice Extractor (F172 Felix Juicy). Black carrot juice obtained was pasteurized at 85 °C for 5 min and cooled down to 25 °C. Pure cultures for each strain were inoculated into 800 mL of pasteurized carrot juice which contain 1.2% unrefined rock salt in a 1 L sterile conical flask for 48 h with orbital shaking at 160 rev min⁻¹. Later on, each starter culture was centrifuged at 4000 rev min⁻¹ for 10 min at 4 °C, and washed with sterile cold water (Tanguler 2010).

Shalgam production by traditional method

Shalgam production as described our previous study (Tanguler et al. 2015) was carried out according to Erten et al. (2008) by traditional method.

Traditional method consisted of two stages. First (dough) fermentation and second (carrot or main) fermentation. The first fermentation was done for the enrichment of LAB and yeasts. At that stage, dough was obtained by mixing bulgur flour (30 g L⁻¹), rock-salt (2 g L⁻¹), sourdough (2 g L⁻¹) which was made by the incubation of baker's yeast at 30 °C for 24 h and adequate water and then kneading. The dough was left for fermentation in a tank for 3 days at 25 °C. After

3 days dough fermentation, water was added into fermented mixture and extracted for 15 min. The extraction procedure was performed four times, and obtained extracts were combined to perform the carrot (second) fermentation of shalgam. Second fermentation was done with the addition sorted and chopped black carrots (150 g L⁻¹), sliced turnip (10 g L⁻¹), and rock-salt (10 g L⁻¹) in 10 L glass jars. Later on, adequate amount of water was added to fill the glass jars,

Shalgam production by direct method

Shalgam production by direct method was carried out according to Erten et al. (2008). Dough fermentation is not performed in direct production method. As mentioned in our previous work (Tanguler et al. 2015), root of black carrots were sorted out and sliced into pieces. The sliced black carrots (150 g L⁻¹), bulgur flour (30 g L⁻¹), rock-salt (12 g L⁻¹), sliced turnip (10 g L⁻¹), sourdough (2 g L⁻¹) and adequate drinkable water were transferred to a container and allowed to carry out the fermentation.

Shalgam production with the addition of starter cultures

For the production of shalgam beverage with the addition of starters, traditional production method was used (Fig. 1). Fermentation of dough and extraction were performed in the same way as explained in traditional method. Then, the extracts obtained were combined to perform the second fermentation with sorted and chopped black carrots (150 g L⁻¹), rock-salt (10 g L⁻¹), sliced turnip (10 g L⁻¹) in a 10 L glass jars. Starter LAB cultures (5%) were inoculated into the tank at this stage.

All fermentations were performed in duplicate. Fermentations were carried out at 25 °C and followed daily by measuring total acidity as lactic acid and pH. After the completion of fermentation, shalgam was removed from the vessel and stored at 4 °C.

Chemical analysis

The total titratable acidity was assessed by titration up to pH 8.1 with sodium hydroxide (0.1 N) using digital pH meter and expressed as lactic acid (as grams/L). The pH value was measured using a digital pH meter (Inolab WTW, Weilheim, Germany) (Demir et al. 2006). All measurements were performed in duplicate.

Extraction of the aroma compounds

Liquid–liquid extraction

Extraction of aroma compounds was performed in pentane/dichloromethane (2/1, v/v), which was the most

suitable solvent for isolating volatiles (Selli 2007). Before extraction, 40 mg of 4-nonanol as internal standard and 40 mL of pentane/dichloromethane (2/1, v/v) were pipetted into a 500 mL flask containing 100 mL of sample. The content was stirred at 4 °C for 30 min under nitrogen gas. The mixture was then centrifuged at 4 °C (9000 g, 15 min). After the dehydration by anhydrous sodium sulphate, the pooled organic extract was reduced to 1 mL using a Vigreux column at 37 °C. The whole process was repeated three times. The extracts were subsequently stored at −20 °C in a glass vial equipped with Teflon-lined cap before the analysis. Each sample was extracted in triplicate and the concentration of volatiles, as 4-nonanol equivalents was obtained as a means of three repetitions (Selli 2007, Selli and Kelebek 2011).

GC-FID-MS analysis of aroma compounds

The gas chromatography (GC) system consisted of Agilent 6890 chromatograph equipped with flame ionisation detector (FID) and Agilent 5975-Network-mass selective detector (MSD) (Wilmington, USA). Aroma compounds were separated on DB-Wax (30 m length × 0.25 mm i.d. × 0.5 µm thickness, J&W Scientific Folsom, USA) column. 3 µL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. This mode was chosen to minimize artifact formation by thermal degradation of analytes in injection port. Injector and FID detectors were set at 270 and 280 °C, respectively. The flow rate of carrier gas (helium) was 1.5 mL min^{−1}. The oven temperature of the DB-Wax column was increased from 50° to 250 °C at a rate of 4 °C min^{−1} with a final hold at 250 °C for 10 min. The same oven temperature programs were used for the mass-selective detector. 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 °C, and source temperature of 180 °C.

The aroma compounds were identified by comparing their retention index and their mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, NBS 75 k) and of the instrument's internal library created from the previous laboratory studies. Some of the identifications were confirmed by the injection of the chemical standards into the GC–MS system. Retention indices of the compounds were calculated by using an *n*-alkane series. Quantification and identification of volatile compounds were made using GC-FID and GC–MS, respectively.

Statistical analysis

Analysis of variance (ANOVA) was performed by SPSS 22 software. Mean comparisons were performed by Duncan's multiple range test at $p = 0.001$, 0.01 and 0.05, when appropriate.

Results and discussion

The concentration of total acidity expressed as lactic acid was the highest in shalgam produced by the addition of autochthonous *Lb. plantarum* and the lowest in shalgam obtained by DM. Their amounts were found as 9.27 and 6.36 g L^{−1}, respectively. pH value was found the lowest with the addition of autochthonous *Lb. plantarum* (3.43) and the highest with *Lb. paracasei* subsp. *paracasei* (3.56). According to the Turkish Shalgam Standard, total acidity in shalgam beverage must be at least 6.0 g L^{−1} (as lactic acid) and pH between 3.3 and 3.8 (Anon 2003). Our results for pH and total acidity were in good agreement with the Turkish Shalgam Standard (Anon 2003) and previous studies (Canbas and Deryaoglu 1993; Tanguler and Erten 2012).

Aroma compounds of shalgam samples

As far as it is known, there is no previous work associated with aroma compounds of shalgam. Therefore, shalgams produced with different production methods were compared for aroma compounds. The aroma compounds identified in the shalgam samples and linear retention index values on the DB-Wax column for these compounds are presented in Table 1. Different class of aroma compounds, including terpenes, esters, alcohols, volatile acids, volatile phenols, lactones, C-13 norisoprenoids, naphthalenes, carbonyl compounds (aldehyde and ketone) were identified in the samples. It was observed that the aroma profiles of all samples were similar, but total volatile content of the shalgam samples were found different. The total concentration of aroma compounds in the LP (4917.63 µg L^{−1}) was greater than the DM (3523.31 µg L^{−1}), LF (3502.5 µg L^{−1}), PA (3432.34 µg L^{−1}) and TM (3162.24 µg L^{−1}) samples. Comparing to carrot volatiles, a number of new compounds appeared in the fermented products because of lactic acid fermentation. Higher alcohols quantitatively represent the main group of the aroma fraction in shalgam samples. All the samples have higher alcohol contents ranging from 1728.06 to 3031.0 µg L^{−1}. After higher alcohols, terpenes were clearly the dominant constituents (Table 1).

Terpenes

Terpenes qualitatively represent the main group of aroma compounds in the shalgam samples. A total of 20 terpenes was determined in the samples. A significant increase in the total amount of terpenes were observed in the LP samples reaching in maximum levels (862.6 µg L^{−1}), followed by TM (740.4 µg L^{−1}), DM (684.2 µg L^{−1}), LF (561.3 µg L^{−1}) and PA (495 µg L^{−1}).

Table 1 Aroma compounds of shalgams ($\mu\text{g L}^{-1}$)

Compounds	RI ^r	ID ^{id}	TM	LP	LF	PA	DM	F
Terpenes								
α -Pinene	1013	B	26.73 ^c	31.64 ^b	47.92 ^a	48.64 ^a	44.25 ^a	*
β -Pinene	1108	B	3.67 ^b	2.5 ^c	3.1 ^{bc}	2.44 ^c	9.33 ^a	**
β -Myrcene	1145	B	18.25 ^b	13.11 ^c	24.32 ^a	26.16 ^a	14.48 ^c	*
DL-Limonene	1213	A	11.21 ^b	8.18 ^c	11.3 ^b	6.22 ^c	30.59 ^a	*
γ -Terpinene	1220	B	35.89 ^{ab}	44.65 ^a	12.18 ^c	9.26 ^d	27.06 ^b	*
α -Terpinolene	1232	B	212.9 ^a	257.56 ^a	92.92 ^c	43.18 ^d	146 ^b	*
(Z)-Linalool oxide	1424	A	22.01 ^a	16.36 ^c	22.86 ^a	19.48 ^b	23.51 ^a	*
(E)-Linalool oxide	1453	B	61.99 ^c	77.31 ^b	91.89 ^a	94.86 ^a	84.46 ^{ab}	*
Vitispirane	1507	B	3.42 ^a	3.58 ^a	3.66 ^a	1.58 ^c	2.21 ^b	**
Linalool	1536	A	13.05 ^{bc}	25.39 ^a	15.49 ^b	11.86 ^c	26.19 ^a	*
4-Terpineol	1581	B	9.48 ^a	10.83 ^a	5.67 ^c	5.74 ^c	8.63 ^b	**
(E)-Pinocarveol	1626	B	6.29 ^c	8.84 ^{ab}	10.96 ^a	4.41 ^d	8.21 ^b	**
α -Caryophyllene	1656	B	6.26 ^b	9.31 ^a	3.78 ^c	2.39 ^d	8.32 ^{ab}	**
Lavandulol	1659	B	1.34 ^c	2.22 ^{ab}	2.01 ^b	1.27 ^c	2.77 ^a	***
1,8-Menthadien-4-ol	1663	B	16.57 ^{ab}	20.02 ^a	10.38 ^c	15.04 ^b	12.82 ^c	*
1-Borneol	1677	A	146.06 ^a	139.4 ^a	85.19 ^b	81.7 ^b	138.33 ^a	*
(Z)- γ -Bisabolene	1725	B	16.43 ^b	27.63 ^a	7.2 ^c	4.89 ^d	16.37 ^b	**
(E)- γ -Bisabolene	1753	B	44.55 ^b	82.95 ^a	36.73 ^{bc}	31.38 ^c	26.17 ^d	*
Myrtenol	1769	B	42.15 ^a	42.4 ^a	42.68 ^a	48.88 ^a	24.65 ^b	*
Geraniol	1832	A	42.16 ^a	38.69 ^a	31.06 ^b	35.61 ^{ab}	29.81 ^b	*
Total			740.41 ^{ab}	862.57 ^a	561.3 ^{bc}	494.99 ^c	684.16 ^b	*
Esters								
Butyl acetate	1064	A	4.3 ^a	3.01 ^b	2.7 ^c	2.7 ^c	3.17 ^b	**
Isoamyl acetate	1118	A	16.52 ^a	18.51 ^a	15.97 ^{ab}	12.89 ^b	11.28 ^b	*
Bornyl acetate	1561	B	1.79	1.96	2.19	2.68	2.36	ns
Diethyl succinate	1650	B	6.74 ^{ab}	8.88 ^a	4.57 ^b	1.91 ^c	1.6 ^c	**
Methyl salicylate	1727	B	2.63 ^c	3.28 ^b	3.05 ^{bc}	3.34 ^b	5 ^a	***
2-Phenylethyl acetate	1778	B	10.12 ^c	18.45 ^a	13.54 ^b	11.8 ^c	14.4 ^b	*
Ethyl hexadecanoate	2268	A	67.3 ^a	63.81 ^a	33.6 ^b	33.32 ^b	27.72 ^c	*
Ethyl-9-hexadecanoate	2293	B	10.02 ^b	15.75 ^a	14.29 ^{ab}	16.9 ^a	9.11 ^b	**
Benzyl benzoate	2594	A	13 ^d	39.5 ^a	15.04 ^c	22.59 ^b	33.48 ^a	*
Total			132.42 ^{ab}	173.15 ^a	104.95 ^b	108.13 ^b	108.12 ^b	*
Higher alcohols								
2-Butanol	994	A	9.86 ^d	11.12 ^c	6.94 ^c	36.07 ^a	18.68 ^b	*
1-Propanol	1036	A	32.9 ^a	28.19 ^{ab}	30.49 ^a	8.35 ^b	30.73 ^a	*
2-Methyl-1-propanol	1090	A	12.58 ^{bc}	19.39 ^a	14.22 ^b	13.45 ^b	11.17 ^c	**
2-Pentanol	1099	A	110.06 ^c	285.52 ^a	175.78 ^b	154.56 ^{bc}	177.42 ^b	*
1-Butanol	1137	A	24.67 ^c	47.95 ^a	18.53 ^d	29.74 ^b	20.22 ^c	*
Isoamyl alcohol	1209	A	928.58 ^c	2016.11 ^a	1336.32 ^b	1247.62 ^b	1180.07 ^{bc}	*
6-Methyl-5-hepten-2-ol	1449	B	2.63 ^b	3.56 ^a	1.88 ^c	2.54 ^b	0.85 ^d	***
2-Phenyl ethanol	1868	A	403.04 ^a	485.19 ^a	325.5 ^b	306.69 ^b	315.37 ^b	*
2-Methoxy-phenyl ethanol	1989	B	203.74 ^a	133.97 ^c	168.31 ^b	221.99 ^a	183.46 ^{ab}	*
Total			1728.06 ^c	3031.0 ^a	2077.97 ^b	2021.01 ^b	1937.97 ^b	*
Volatile acids								
Butanoic acid	1597	B	0.78 ^c	1.48 ^c	5.4 ^b	7 ^a	6.96 ^a	**
Isovaleric acid	1638	B	2.56 ^d	28.55 ^a	9.17 ^b	4.1 ^c	0 ^e	*
Pentanoic acid	1705	A	2.55 ^a	2.28 ^a	1.46 ^{bc}	1.27 ^c	1.71 ^b	***

Table 1 continued

Compounds	RI ^r	ID ^{id}	TM	LP	LF	PA	DM	F
Hexanoic acid	1817	B	5.61 ^d	109.15 ^a	70.74 ^b	53.57 ^c	64.26 ^{bc}	*
Heptanoic acid	1923	B	144.64 ^a	21.87 ^b	1.85 ^c	3.32 ^d	13.95 ^c	*
Total			156.14 ^a	163.33 ^a	88.62 ^b	69.26 ^c	86.88 ^b	*
Volatile phenols								
3-Methylacetophenone	1731	B	4.43 ^b	3 ^c	4.17 ^b	6.52 ^a	3.66 ^{bc}	**
4-Methyl-2,6-di-tert-butylphenol	1899	B	5.16 ^c	45.03 ^b	52.18 ^a	55.57 ^a	48.85 ^{ab}	*
Eugenol	2124	A	33.06 ^a	28.58 ^b	38.32 ^a	25.97 ^b	20.11 ^c	*
p-Vinyl guaiacol	2146	B	16.62 ^{bc}	17.38 ^b	14.34 ^c	36.75 ^a	0 ^d	*
Acetovanillone	2582	A	4.49 ^c	7.31 ^a	5.73 ^b	6.81 ^a	6.13 ^{ab}	**
Propiovanillone	2644	A	7.66 ^c	16.22 ^a	8.2 ^c	8.08 ^{bc}	9.08 ^b	***
Total			71.42 ^b	117.52 ^a	122.94 ^a	139.70 ^a	87.83 ^b	*
Lactones								
γ-Butyrolactone	1565	B	1.28 ^d	26.66 ^b	17.81 ^c	15.55 ^c	64.51 ^a	*
γ-Hexalactone	1647	A	5.27 ^c	7.73 ^a	6.31 ^{ab}	4.41 ^c	7.05 ^a	**
γ-Nonalactone	1992	A	76.76	73.72	86.88	95.63	79.87	ns
δ-Dodecalactone	2414	A	8.76 ^c	8.92 ^c	12.38 ^b	15.93 ^b	35.14 ^a	*
Massoilactone	2446	B	15.75	16.73	17.86	19.81	11.14	ns
Total			107.82 ^c	133.76 ^b	141.24 ^b	151.33 ^{ab}	197.71 ^a	*
C13-Norisoprenoids								
3-OH-β-damascenone	2498	B	44.8 ^b	69.17 ^a	40.07 ^{bc}	37.52 ^c	42.61 ^b	*
Total			44.8 ^b	69.17 ^a	40.07 ^{bc}	37.52 ^c	42.61 ^b	*
Naphthalenes								
Naphthalene	1685	A	3.36 ^c	169.35 ^b	201.68 ^a	214.39 ^a	187.44 ^{ab}	*
2-Methyl-naphthalene	1842	B	5.32 ^a	2.14 ^c	3.51 ^b	3.94 ^b	2.14 ^c	**
2-Ethyl-naphthalene	1915	B	137.45	141.84	123.18	156.04	148.95	ns
Total			146.13 ^b	313.33 ^a	328.37 ^a	374.37 ^a	338.53 ^a	*
Carbonyl compounds								
Heptanal	1182	B	2.19 ^c	4.06 ^a	1.26 ^d	3.33 ^{bc}	2.04 ^c	***
4-Hydroxy-4-methyl-2-pentanone	1310	A	32.85	49.74	35.78	32.7	37.46	ns
Total			35.04	53.8	37.04	36.03	39.5	ns
General Total			3162.24	4917.63	3502.5	3432.34	3523.31	

r: RI, Retention index based on a series of n-hydrocarbons on DB-WAX column, id: ID, Identification: A = GC retention and MS data in agreement with that of pure compound available in the lab; B = GC retention and MS data in agreement with spectra found in the library. TM (Traditional method), DM (Direct method), LP (addition of *Lb. plantarum*), LF (addition of *Lb. fermentum*) and PA (addition of *Lb. paracasei* subsp. *paracasei*). F: Significance ***, ** and * display the significance at 0.1, 1 and 5% by LSD, respectively. a–d Values not sharing the same superscript letter within the horizontal line are different according to Duncan test

ns not significant

Terpenes generally caused a harsh or bitter flavour sensation in carrot cultivars (Simon et al. 1980) and α-pinene along with β-caryophyllene, limonene and terpinolene are key aroma compounds in raw carrots (Kreutzmann et al. 2007). Many studies have suggested that characteristic flavour of carrots was mainly due to the terpenes (Alasalvar et al. 2001; Kjeldsen et al. 2001; Kreutzmann et al. 2008). It is interesting to note that the pattern of terpene compounds did not change markedly during the different production methods. The terpenes of carrots were also present in shalgam samples. α-

Terpinolene and 1-borneol were the quantitatively main terpenes in all shalgam samples. The production method of shalgam samples significantly ($p < 0.05$) affect the amount of these compounds. Previous studies on black carrot flavour state that terpinolene (Simon et al. 1980) and 1-borneol (Rakic 2008) are the main terpenes in black carrots.

Esters

Esters are significant aroma components on many foods, and most of them have a fruity aroma that contributes to

the strong fruity and floral odour of foods. A total of nine esters were identified and quantified in all shalgam samples. Except for bornyl acetate, the amount of esters are significantly ($p \leq 0.05$) affected by shalgam production methods (Table 1). The total amount of ester compounds generally increased in LP ($173.15 \mu\text{g L}^{-1}$) and TM ($132.42 \mu\text{g L}^{-1}$) samples. As can be seen in Table 1, the total concentration of esters in LF, PA and DM samples did not vary significantly between those samples. Ethyl hexadecanoate was the most abundant esters in the samples, followed by benzyl benzoate and isoamyl acetate. Among all esters, bornyl acetate was only determined in raw carrot cultivars (Kjeldsen et al. 2001; Seljasen et al. 2001). The amount of bornyl acetate was quite low in the samples between 1.79 and $2.68 \mu\text{g L}^{-1}$. The occurrence of ester components in shalgam were probably attributed to the role of microorganisms during fermentations of mainly LAB and yeasts. These compounds are synthesised enzymatically by yeast during ethyl alcohol fermentation (Molina et al. 2007) and some LAB are also able to synthesize esters via a transferase reaction in which fatty acyl groups from glycerides were transferred to alcohols (Liu et al. 2003).

Higher alcohols

Nine higher alcohols (2-butanol, 1-propanol, 2-methyl-1-propanol, 2-pentanol, 1-butanol, isoamyl alcohol, 6-methyl-5-hepten-2-ol, 2-phenyl ethanol and 2-methoxy phenyl ethanol) were identified in the samples (Table 1). Higher alcohols in shalgams quantitatively represent the main group of aroma compounds. These compounds showed an increase after fermentations. The total concentration of higher alcohols increased in shalgam samples produced with the addition of starter cultures especially in LP sample ($3031.0 \mu\text{g L}^{-1}$). Abundant higher alcohols of yeast fermentation are isoamyl alcohol, 2-phenyl ethanol and 2-pentanol in all samples. As indicated in Table 1, isoamyl alcohol was the main higher alcohol that is responsible for 66.52% of LP, 64.31% of LF, 61.73% of PA, 60.9% of DM and 53.74% of TM of the total higher alcohols analysed. Within the higher alcohols, 1-propanol was found in stored raw carrot by Alasalvar et al. (1999).

Volatile acids

A total of five volatile acids including butanoic, isovaleric, pentanoic, hexanoic and heptanoic acids were identified in shalgam samples. Formation of acids by LAB mainly results from the action of α -keto acid dehydrogenase. This enzyme produces aldehydes that then these aldehydes either oxidized to acids or reduced to alcohols, probably depending on the redox potential inside the cell or in the

medium, since lactobacilli exhibited both aldehyde and alcohol dehydrogenase activities (Helinck et al. 2004). In the acid components, straight-chain fatty acids were the main components in shalgam juices. Hexanoic acid (ranged from 5.61 to $109.15 \mu\text{g L}^{-1}$) and heptanoic acid (ranged from 1.85 to $144.64 \mu\text{g L}^{-1}$) were the most abundant ones (Table 1). The production method of shalgam samples significantly affected the amount of volatile acids ($p < 0.05$). Acid compounds do not seem to contribute to the overall aroma of orange juice because of their high odour threshold values (Kelebek and Selli 2011).

Volatile phenols

Six volatile phenols were identified and quantified in the shalgam juices. PA ($139.70 \mu\text{g L}^{-1}$) shalgam juices contained more volatile phenol components than the others. Volatile phenols detected in fermented beverages can originate from p-coumaric and ferulic acids by enzymatic or thermal decarboxylation (Nykanen 1980). In addition, production method of shalgams were found significantly important with regard to the amount of volatile phenols ($p < 0.05$). Eugenol and p-vinyl guaiacol were the main volatile phenols in all shalgams (Table 1). Eugenol has been identified as a potent aroma-active compound in fresh apricots, providing a clove-like odour with a low threshold value of $6 \mu\text{g L}^{-1}$ in water (Greger and Schieberle 2007).

Lactones

γ -Butyrolactone, γ -hexalactone, γ -nonalactone, δ -dodecalactone and massoilactone were found in shalgam samples as lactone compounds. As can be seen in Table 1, levels of those compounds formed with the exception of γ -nonalactone and massoilactone levels were found statistically important ($p < 0.05$). On the other hand, γ -nonalactone was found in the highest amount in the shalgams. The total lactone content of the DM ($197.71 \mu\text{g L}^{-1}$) was higher than the others. Lactones are cyclic esters of primarily γ - and δ -hydroxy acids, and they are ubiquitously found in food, contributing to taste and flavour nuances such as fruity, coconut-like, buttery, creamy, sweet or nutty (Longo and Sanroman 2006).

Naphthalenes

With regard to naphthalene compounds, naphthalene, 2-methylnaphthalene and 2-ethylnaphthalene were determined in the shalgam samples. These compounds could be produced from the degradation of plant materials by microorganisms. Naphthalene and 2-ethylnaphthalene were found in the highest amount in PA sample (214.39 and $156.04 \mu\text{g L}^{-1}$), whereas 2-methylnaphthalene in the TM

sample ($5.32 \mu\text{g L}^{-1}$). The production method of shalgam samples significantly affect the amount of naphthalene and 2-methylnaphthalene ($p \leq 0.05$). Similarly, naphthalene has been found in fermented bamboo shoots by Fu et al. (2002).

The other group of volatile compounds identified in the shalgam samples were C13-norisoprenoids (3-hydroxy- β -damascenone) and carbonyl compounds including aldehyde (heptanal) and ketone (4-hydroxy-4-methyl-2-pentanone). 3-Hydroxy- β -damascenone has an important sensorial impact on food aroma as they have very low olfactory perception thresholds (Sefton et al. 1989) and are thought to arise from photochemical and enzymatic oxidation of carotenoids (Kanasawud and Crouzet 1990). The total amount of these compounds was significantly ($p \leq 0.05$) affected by shalgam production methods.

Conclusion

In the present study, aroma profiles of shalgams obtained from different production methods (traditional production method, direct production method, and also using three autochthonous LAB starters) were determined. As far as it is known, this first report on aroma compounds of shalgam showed that the aroma profiles of the samples were quite similar, but total aroma compounds of the samples were affected by the different production methods. In general, terpenes, esters and alcohols were found as the main types of aroma components of all shalgam samples. The results of this study also showed that shalgam LP produced by addition of *Lb. plantarum* was high in terpenes, esters, higher alcohols, volatile acids, volatile phenols and carbonyl compounds, leading to possible usage of this bacterium as a starter culture in industry. However, further studies are required to confirm the limited knowledge of shalgam aroma.

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