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Volatile and Odour-Active Compounds of Tuzlu Yoghurt

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The objective of this study was to identify volatile and odour-active compounds of Tuzlu yoghurt obtained by direct solvent extraction and high vacuum distillation, based on gas chromatography/mass spectrometer and gas chromatography/olfactometry. 56 Volatile compounds were identified (7 ketones, 7 aldehydes, 5 esters, 14 alcohols, 3 terpenes, 4 lactones, 4 miscellaneous and 12 free fatty acids) by gas chromatography/mass spectrometery and 6 (1 ketone, 4 aldehydes and 1 sulphurous compound) by gas chromoatography/olfactomatery. 29 Compounds were found to be odour-active. Ethyl butanoate, methional in neutral/basic fraction and ethanoic, butanoic and hexanoic acids in the acidic fractions appeared to be the most potent odour-active compounds. The detection of odour-active compounds by this study may be promising for the certification of traditional yoghurt products as Protected Designation of Origin.

Key Words: Tuzlu yoghurt, Volatiles, Odour-active compounds, Gas chromatography, Olfactometry.

INTRODUCTION

Tuzlu yoghurt (salted yoghurt) is a traditional dairy product of the Hatay province of Turkey and is different from plain yoghurt. The production of Tuzlu yoghurt requires both the process of cooking of plain yoghurt until it is two-fold concentrated and the addition of salt. Cooking enables Tuzlu yoghurt to acquire its unique characteristics of texture and smoky, slightly burnt flavour as well as a prolonged shelf life. In general, goat's milk is preferred in Tuzlu yoghurt as it yields a whiter and smoother product. However, cow's milk or a mixture of goat's and cow's milk is also used[1,2]. Since consumer preference towards goat's milk products has increased owing to their nutritional properties, there is an increasing need for characterization of odour-active compounds for such products to be certified as protected designation of origin (PDO).

To our knowledge, there is a lack of detailed study conducted to reveal odour-active compounds of Tuzlu yoghurt although volatiles of plain yoghurt have been studied extensively. Güler[2] determined 18 volatile compounds in Tuzlu yoghurt using static head space sampling technique and identified 19 free fatty acids. The researcher correlated chemical changes occurred during a 90 d storage with changes detected by sensory analysis, without the techniques of gas chromatography/olfactometry. Using dynamic head space sampling technique, Laye *et al.*[3] and Imhof *et al.*[4,5] identified 23 and 32 volatiles in plain yoghurt, respectively. Imhof *et al.*[4,5] suggested that dimethyl sulfide, 2,3-butanedione, 2,2-pentanedione, benzaldehyde, limonene and undecanal had an impact on yoghurt aroma by comparing their results with the threshold values of the components as reported in the literature. When head space sampling was combined with gas chromatography/olfactometry, Ott *et al.*[6] identified 21 aroma active compounds out of 91 volatiles in plain yoghurt and reported five compounds (1-nonen-3-one, methional, 2-methyltethrahdyrothiophen-3-one, (2E)-nonenal and guaiacol) in plain yoghurt for the first time.

This study, therefore, aims at identifying volatile compounds and key odour-active compounds of Tuzlu yoghurt, based on the techniques of high vacuum distillation, gas chromatography/olfactometry and gas chromatography/mass spectrometry.

EXPERIMENTAL

Selection of Tuzlu yoghurt samples: 12 Tuzlu yoghurt samples (1.5 kg) were collected from Antakya market (the province of Hatay, Turkey). The samples were evaluated by a panel of 20 local people at the age of 20 to 55, who are used to consuming Tuzlu yoghurt. Three samples (TY1, TY2 and TY3) selected by the panel were used for the olfactometric and chemical analyses.

Direct solvent extraction (DSE): Tuzlu yoghurt extracts were prepared according to Milo and Reneiccius[7] with some modifications as noted by Karagül-Yüceer *et al.*[8]. Each of Tuzlu yoghurt samples used for extraction was 600 g.

High vacuum distillation (HVD): Separation of volatile compounds from the yoghurt extracts was achieved by high vacuum distillation technique as detailed by Karagül-Yüceer *et al.*[8]. The apparatus used was similar to that described by Sen *et al.*[9].

Gas chromatography-olfactometry (GC/O) of solvent extracts: For the GC/O analysis, a Shimadzu 2010 (Shimadzu, Japan) gas chromatography equipped with flame ionization (FID), a sniffing port and a splitless injector was used. From each fraction of every extract, 2 μL was injected into a polar capillary column (Innowax, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness d_f, Hewlett Packard, USA). Column effluent was split 1:1 between FID and sniffing port using deactivated fused silica capillaries (1 m length × 0.25 mm i.d.). The GC oven temperature was programmed from 40 to 200 ºC at a rate of 10 ºC/min with initial and final hold times for 5 and 15 min. Three experienced sniffers evaluated the neutral/basic and acidic fractions of Tuzlu yoghurt extracts twice.

Aroma extract dilution analysis (AEDA) of solvent extract fractions was conducted using the same equipment and conditions. Solvent extracts were diluted stepwise with diethyl ether at a ratio of 1/3. Dilution procedure was carried out until no odourants were detected by the sniffers. The highest dilution was reported as flavour dilution factor[10].

Gas chromatography-mass spectrometry (GC/MS): For the GC/MS analysis of solvent extracts, HP6890 Series, GC/HP (MSD, Hewlett Packard) mass selective detector was used. Separations were done on a polar capillary column (Innowax, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness d_f, Hewlett Packard, USA). The rest of the procedure and MSD conditions were the same as described by Karagül-Yüceer *et al.*[8].

Identification of odourants: For positive identifications, retention indices (RI), mass spectra and odour properties of unknown compounds were compared with those of authentic standard compounds analyzed under the identical conditions. Tentative identifications were based on comparing mass spectra of unknown compounds with those of known compounds from the Mass Spectral Database (Wiley275/Nist02.L). For the calculation of retention indices, a *n*-alcane series was used[11].

RESULTS AND DISCUSSION

Volatile compounds detected in neutral/basic fractions: DSE-HVT led to the identification of 48 volatiles compounds in the neutral/basic fractions of Tuzlu yoghurt, including 14 alcohols, 9 ketones, 7 aldehydes, 6 esters, 4 lactones, 3 terpenes and 3 miscellaneous compounds (Table-1). The qualitative and quantitative compositions of the samples were different from one another, due probably to the production procedures such as different cooking times, milk type, starter bacteria, contaminated bacteria and feeding regime. Alcohols, ketones, esters, lactones, aldehydes and terpenes formed approximately 43.7, 26.5, 7.7, 6.4, 5.1 and 3.1 % of the total volatiles of neutral/basic fractions, respectively.

Most of the 48 compounds identified have already been reported in milk[12-14], yoghurt[3,6,15,16] and to a lesser extent in Tuzlu yoghurt[2] and the mechanisms of their formation have been mentioned in detail elsewhere[17-22]. The compounds identified for Tuzlu yoghurt for the first time in this study were phenolic compounds (phenol, *p*- and *m*-cresol), Maillard reaction products (acetophenone, acetoacetone, 2-furancarboxyaldehyde, 5-methylfufuraldehyde and

TABLE-1
RELATIVE ABUNDANCE OF VOLATILES IDENTIFIED IN NEUTRAL/BASIC FRACTIONS OF TUZLU YOGHURT

Nr	Compounds	RI*	Mean ± Standard error (µg/kg)†		
			TY1	TY2	TY3
1	Ethylbutanoate	1042	32.4±8.97	34.0±21.89	29.2±10.36
2	2-Heptanone	1181	10.9±0.03	143.3±34.06	22.7±2.02
3	3-Methyl-1-butanol	1219	128.2±31.27	514.8±29.68	996.0±10.60
4	Ethylhexanoate	1236	nd‡	29.3±4.81	152.0±0.63
5	Styrene	1252	90.6±9.79	233.9±14.89	333.9±3.58
6	2-Methyl-2-buten-1-ol	1320	7.2±4.05	13.5±0.91	9.4±0.04
7	2-Methyl-3-pentanol	1348	nd	58.8±0.64	12.1±4.26
8	2-Nonanone	1388	255.8±5.23	460.0±8.23	239.1±1.82
9	Nonanal	1392	32.2±0.37	27.0±0.26	15.5±0.32
10	Ethyloctanoate	1436	13.2±0.12	67.5±2.60	47.9±0.78
11	1-Heptanol	1468	nd	3.6±0.92	26.6±7.07
12	2-Furancarboxaldehyde	1470	25.0±0.17	8.5±0.09	4.6±4.01
13	2-Ethyl-1-hexanol	1503	100.1±0.17	49.2±7.86	44.3±6.44
14	L-Linalool	1553	0.8±0.31	10.9±0.71	6.7±0.08
15	*n*-Octanol	1568	10.0±1.23	13.4±4.39	27.4±0.24
16	α-Terpilonene	1571	3.1±3.29	1.6±0.06	19.9±9.72
17	5-Methylfurfural	1574	0.4±0.01	nd	nd
18	*trans*-Caryophyllene	1585	10.6±0.03	14.8±2.37	27.0±0.28
19	2-Undecanone	1598	214.9±5.63	246.5±3.49	169.2±0.61
20	Ethyldecanoate	1640	6.8±0.10	35.2±10.03	229.9±2.88
21	Acetophenone	1647	65.6±1.13	83.0±0.88	63.8±0.24
22	Safranal	1648	0.9±0.24	0.9±0.03	nd
23	2-Furanmethanol	1671	217.2±4.96	271.7±9.48	272.3±6.31
24	3-(methylthio)-1-propanol	1727	38.8±41.64	130.4±2.69	227.9±4.50
25	Azulene	1746	4.7±0.04	18.4±3.55	19.0±0.71
26	Acetoacetone	1792	12.7±0.49	40.4±1.26	6.3±0.33
27	2-Tridecanone	1807	132.8±0.34	180.2±0.90	100.9±0.17
28	2-Phenylethylacetate	1815	36.8±1.54	51.1±1.05	32.9±0.17
29	Dodecanal	1873	40.5±2.30	27.1±38.31	20.0±12.66
30	Benzenemethanol	1891	43.7±1.69	26.6±2.45	77.6±0.43
31	Benzenethanol	1929	107.1±23.67	174.3±6.47	497.4±12.03
32	Benzothiazole	1953	10.4 ±0.04	12.0±3.32	11.5±0.77
33	δ-Octalactone	1967	22.2±0.31	27.0±1.54	20.3±0.10
34	Phenol	2020	11.5±5.29	8.3±0.98	65.9±0.53
35	2-Dodecanone	2042	7.8±1.70	11.1±2.07	nd
36	*p*-Cresol	2079	nd	18.4±0.53	11.2±0.67
37	*m*-Cresol	2111	Nd	3.5±0.24	4.8±0.02
38	Tetradecanal	2150	14.2±7.29	23.5±0.38	nd
39	δ-Decalactone	2193	97.0±2.42	135.2±1.65	46.6±22.82
40	γ-Dodecalactone	2376	14.4±7.13	30.1±8.97	21.0±18.29
41	δ-Dodecalactone	2423	39.4±0.49	76.8±1.86	31.7±1.76
42	Indole	2451	1.8±1.71	5.0±1.70	6.1±3.83
43	5-Hydroxymethylfurfural	2537	18.7±0.99	24.6±1.75	16.4±8.55

*Retention indices were calculated from gas chromatography-mass spectrometer on Innowax column; †Mean relative abundance = concentration of internal standard × peak area of compound/ peak area of the internal standard. Data are means values of duplication; ‡nd = not determined.

5-hydroxymethylfurfuraldehyde), esters (ethylbutanoate, ethylhexanoate, ethyloctanoate, ethyldecanoate and 2-pheylethylacetate) and some of the primary constituents of essential oils of many types of grasses (l-linalool, benzenemethanol, benzeneethanol, safranal, α-terpilonene, *trans*-caryophyllene, azulene and safranal).

The presence of Maillard reaction products in casein and lactose systems such as Tuzlu yoghurt is expected due to the heat treatment of milk and cooking of yoghurt[6,17,23,24]. Identification of some constituents of essential oils of grasses in Tuzlu yoghurt appeared to reveal that goats and cows in the Hatay province are generally grazed on rangelands and thus, could be used as an indicator of feeding regime of the animals[25]. Lactic acid bacteria and yeast are most likely to be responsible for the production of esters[26]. As expected, Sahan and Say[27] reported a yeast-mould count of 1.36 × 105 and 2.80 × 108 cfu/g in Tuzlu yoghurt sold in Antakya market.

Odour-active compounds detected in neutral/basic fractions: At the sniffing port of GC/O, odour properties of the 22 compounds were described in neutral/basic fractions of the Tuzlu yoghurt samples (Table-2). Out of the entire compounds, 16 compounds and 6 compounds were identified positively and tentatively, respectively. 2,3-Butanedione, hexanal, octanal, methional, (E, Z) 2,6-nonadienal and phenylacetaldehyde were detected at the sniffing port. The AEDA results showed that ethylbutanoate and methional were the most potent odour-active compounds. Methional, the most common sulfurous compound found in cheese, can be produced by Strecker degradation and is recognized with its characteristic boiled potato odour.

The contributions of compounds to the aroma of Tuzlu yoghurt appeared to be different. Some of the characteristic aroma compounds of plain yoghurt (such as acetaldehyde) were not detected in this study. This might stem from the limitations of the DSE-HVT technique in the recovery or removal of these highly volatile compounds due to the cooking stage employed in the production of Tuzlu yoghurt.

Volatile compounds detected in acidic fractions: Acidic fraction of the yoghurt extracts included 8 aliphatic, 3 branched chain, 1 aromatic carboxylic acids and 1 organic compound (Table-3). The most abundant free fatty acids were benzoic acid (41.8 %), ethanoic acid (23.0 %), butanoic acid (10.6 %), hexanoic acid (8.6 %) and octanoic acid (6.8 %). Except for 3-methyl-2-butenoic acid and maltol, the other acids were already reported in both plain[6,28] and Tuzlu yoghurt[2]. The formation mechanisms of the aliphatic acids in plain yoghurt were already discussed elsewhere[28]. As for benzoic acid, it is a natural compound of fermented dairy products since yoghurt bacteria is capable of producing it from hippuric acid[29]. Benzoic acid is an antibacterial agent and its presence in a high amount in Tuzlu yoghurt may contribute to extension of its shelf life as was claimed by the Tuzlu yoghurt producers.

TABLE-2
ODOUR-ACTIVE COMPOUNDS IN THE NEUTRAL/BASIC FRACTIONS OF TUZLU YOGHURT BY AROMA EXTRACT DILUTION ANALYSIS

Nr	Compound[1]	Odour description[2]	RI[3]	Flavour dilution factor[4]		
				TY1	TY2	TY3
44	2,3-butandione[A]	Butter	990	9	27	9
1	Ethylbutanoate[A]	Fruity, bubblegum	1045	243	81	27
45	Hexanal[B]	Green	1081	3	3	3
2	2-Heptanone[A]	Soapy	1184	1	9	9
3	3-Methyl-1-butanol[A]	Waxy, floral	1212	9	9	3
46	Octanal[B]	Soapy	1280	9	27	9
8	2-Nonanone [A]	Green, soapy	1380	1	1	nd[5]
9	Nonanal [A]	Green, oily	1389	9	27	27
10	Ethyloctanoate[A]	Nutty, fruity	1436	1	1	1
12	2-Furancarboxaldehyde[A]	Soil, bread	1452	9	nd	nd
47	Methional[B]	Cooked potato	1465	243	81	81
13	2-Ethyl-1-hexanol[A]	Boiled, brothy	1503	1	1	nd
14	L-Linalool[A]	Brothy, Lavender	1547	nd	9	27
17	*n*-Octanol[A]	Popcorn, roasted	1567	27	9	9
18	*trans*-Caryophyllene[A]	Vitamin, rubber	1591	1	1	1
19	2-Undecanone[A]	Nutty, earthy, vitamin,	1598	3	3	1
48	(E,Z) 2.6-Nonadienal[B]	Cucumber	1618	27	9	1
22	Acetophenone[A]	Rubber, burnt	1648	1	1	1
49	Pheylacetaldehyde[B]	Cotton candy, caramel	1675	27	9	9
30	Benzenemethanol[A]	Rubber	1876	3	1	3
31	Benzeneethanol[A]	Cheesy	1922	1	3	9
32	Benzothiazole[A]	Rubber, dirty	1955	1	1	1

[1]Compounds designated with a superscript A were positively identified (retention indices, odour, mass-spectrometry); those designated with a superscript B were tentatively identified (retention indices, odour); [2]Odour descriptions at the sniffing port during gas chromatography-olfactometry; [3]Retention indices (RI) were calculated from gas chromatography-olfactometry results on Innowax column; [4]Flavour dilution factor on Innowax column; [5]nd = not determined.

Odour-active compounds detected in acidic fractions: Seven odour active compounds were detected in acidic fractions (Table-4). Ethanoic acid with its vinegar-like odour appeared to be the primary contributor to Tuzlu yoghurt aroma with the highest FD factor, followed by butanoic acid and hexanoic acid.

Conclusion

This study investigated the complex volatile composition and odour active compounds of Tuzlu yoghurt. Therefore, the volatiles and odour active compounds identified may be used for Tuzlu yoghurt to be to characterized and certified as PDO. The sensitive detection of highly volatile odour active compounds needs to be further explored based on the combination of the various sampling techniques.

TABLE-3
RELATIVE ABUNDANCE OF VOLATILES IDENTIFIED IN ACIDIC FRACTIONS OF TUZLU YOGHURT

Nr	Compounds	RI[1]	Mean ± standard error (μg/kg)[2]		
			TY1	TY2	TY3
50	Ethanoic acid	1422	2368.0±1466.6	4137.2±1401.5	5381.0±2204.6
51	Propanoic acid	1550	1281.1±42.7	147.0±16.0	579.1±2.1
52	2-Methylpropanoic acid	1580	78.9±18.6	93.3±1.8	340.2±104.1
53	Butanoic acid	1634	1387.1±394.9	1623.5±60.6	2478.0±73.5
54	3-Methyl butanoic acid	1680	215.6±10.5	111.5±157.7	450.7±637.4
55	Pentanoic acid	1751	26.4±0.5	28.5±2.4	142.9±0.9
56	3-Methyl-2-butenoic acid	1809	10.0±3.1	8.3±0.8	12.4±1.3
57	Hexanoic acid	1861	1250.6±216.9	1396.2±11.0	1810.9±47.5
58	Heptanoic acid	1968	36.6±3.1	37.3±4.2	45.2±1.7
59	Maltol	2033	15.1±3.2	17.2±0.0	66.2±0.7
60	Octanoic acid	2079	1049.8±161.1	1169.9±4.5	1285.2±16.9
61	Decanoic acid	2291	269.0±135.3	334.4±42.8	429.4±5.0
62	Benzoic acid	2443	7326.0±2782.8	7699.6±2254.4	6595.9±357.2

[1]RI calculations were made on Innowax column; [2] Mean relative abundance = concentration of internal standard × peak area of compound/ peak area of the internal standard. Data are means values of duplication.

TABLE-4
AROMA-ACTIVE COMPOUNDS IN ACIDIC FRACTIONS OF TUZLU YOGHURT BY AROMA EXTRACT DILUTION ANALYSIS

Nr	Compound[1]	Odour description[2]	RI[3]	Flavour dilution factor[4]		
				TY1	TY2	TY3
45	Ethanoic acid[A]	Vinegar	1438	27	27	81
46	Propanoic acid[A]	Sour	1552	9	3	3
47	2-Methylpropanoic acid[A]	Sour	1578	3	3	27
48	Butanoic acid[A]	Cheesy	1631	27	9	27
49	3-Methylbutanoic acid	Sweaty	1674	3	3	9
50	Pentanoic acid[A]	Sweaty	1756	1	1	1
43	Hexanoic acid[A]	Sweaty	1862	9	9	27

[1]Compounds designated with a superscript A were positively identified (retention indices, odour, mass-spectrometry); [2]Odour descriptions at the sniffing port during gas chromatography-olfactometry; [3] Retention indices were calculated from gas chromatography-olfactometry results on Innowax column; [4]Flavour dilution factor on Innowax column.

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