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RESEARCH ARTICLE

An Investigation of the Volatile Compound Profiles of Probiotic Yogurts Produced Using Different Inulin and Demineralised Whey Powder Combinations

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Abstract Volatile compound profiles of probiotic yogurt samples prepared using 8 different prebiotic combinations were analyzed using gas chromatography/mass spectrometry (GC-MS) with solid phase micro extraction during 28 days of storage. A total of 40 volatile compounds were identified in yogurt samples during storage consisting of 2 aldehydes, 6 ketones, 4 alcohols, 6 acids, 5 esters, 3 terpenes, 3 aromatic hydrocarbons, and 12 aliphatic hydro-carbons. Acetaldehyde, diacetyl, acetoin, 2-heptanone, ethanol, isoamyl alcohol, acetic acid, caproic acid, caprilic acid, and capric acid were the major volatile compounds in all probiotic yogurt samples.

Keywords: probiotic, yogurt, volatile, GC-MC, solid phase micro extraction

Introduction

Yogurt is a fermented dairy product with a high nutritional value and easy digestion properties. Yogurt is produced using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with lactic acid fermentation of milk (1). In recent years, an increase has been observed in consumption of probiotic fermented dairy products owing to nutritional value, therapeutic benefits, and reduced acidic aroma profiles. In order to obtain a specific desired aroma profile and textural and compositional properties in fermented milk products, use of appropriate bacterial strains and prebiotics are important (2).

Prebiotics are non-digestible dietary components that stimulate growth and/or activity of useful microorganisms (probiotics) in the colon. In recent years, inulin and whey powder have been used in fermented milk as prebiotic substances. Inulin is an indigestible carbohydrate consisting of fructooligosaccharides that is a natural prebiotic agent derived from several fruits and vegetables. However, inulin is generally obtained from chicory roots using extraction (3,4). Whey powder is another prebiotic substance that contains biologically active proteins, minerals, and vitamins (especially vitamin B_2) (5). On the other hand, whey powder provides improved flavor and nutritional value to probiotic yogurts, prebiotic effects on probiotic micro-organisms, textural improvement, and reduced syneresis of a final product when added to yogurt (6).

Flavor is one of the most important quality characteristics for determination of yogurt quality. Some aroma compounds occur naturally in milk, while other components are created during the fermentation process. The flavor of yogurt is formed by enzymatic and chemical reactions, including lipolysis, proteolysis, lipid oxidation, fermentation of carbohydrates, and catabolism of amino acids. For example, aldehydes, ketones, esters, alcohols, free fatty acids, furans, and sulfur compounds all occur as a result of numerous sequential and simultaneous biochemical reactions of milk sugar, lipids, and proteins (7,8). Acetate, acetaldehyde, ethanol, and diacetyl occur in yogurt as a result of the metabolism of lactose, lactic acid, and citric acid by microorganisms (9). However, aldehydes, alcohols, carboxylic acids, hydroxy acids, indoles, sulphur compounds, ammonia, α-keto acids, volatile amines, and phenolic compounds are formed by enzymatic degradation of amino acids. These

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processes are influenced by endogenous and microbial enzyme activities, and also by chemical reactions (10).

Volatile compounds of fermented milk have been widely studied in recent years, although there is no study regarding the effects of different whey powder and inulin combinations on the physicochemical properties and volatile compound profiles of probiotic yogurts. The determination of volatile compounds of probiotic yogurt samples was performed using a purge and trap connected to a gas chromatography with a mass spectrometer. Many volatile compounds were identified in probiotic yogurts, including aldehyde, ketone, alcohol, acid, ester, terpene, aromatic hydrocarbons, and aliphatic hydrocarbons. The 40 most important volatile compounds were identified in this study. The aim of this study was to determine the effects of inulin, d-whey powder, and different combinations of these prebiotic agents on physicochemical properties and volatile compound profiles of probiotic yogurt samples. Different ratios of inulin and d-whey powder were analyzed for effects on flavor properties of yogurt during storage.

Materials and Methods

Materials Cow milk (50 L) was supplied from the dairy farm of the Agriculture Faculty of Atatürk University in Erzurum, Turkey in March of 2010. Skim milk powder (SMP) was obtained from a local market in Erzurum in March of 2010. Inulin (Orafti®HPX, Beneo-Orafti, Oreye, Belgium) was bought from Artisan Food Industry Inc., Istanbul, Turkey and d-whey powder was provided from Maybe Malkara Union Milk and Milk Products Inc., Tekirdağ, Turkey in March of 2010. A direct-to-vat system probiotic yogurt starter culture (ABT-2; *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium* sp.) used in probiotic yogurt-making was supplied from Chr. Hansen-Peyma, Istanbul, Turkey.

Yogurt production Experimental probiotic yogurts were prepared in duplicate. Raw cow milk was strained using a cloth filter. After clarification using a Model Alfa Laval 313T, centrifugal clarifier at 5,000×*g* and a working capacity 1,000 L/h and homogenisation using an Alfa Laval, separations Technik GMBH, Wilheim-Berger-Str. Glinde/b.Hamburg, Germany at 20 MPa pressure and working capacity of 350 L/h, milk was heated to 90°C in a stainless steel tank for 10 min, and then rapidly cooled in the same tank to 37°C and divided into eight equal batches. One batch of yogurt was used as a control with no added inulin or d-whey powder, and remaining batches were prepared with mixtures of inulin and d-whey powder. Groups were A (control), B (3% w/w inulin and 0% w/w d-whey powder], C (2.5% w/w inulin and 0.5% w/w d-whey powder], D (2.0% w/w inulin and 1% w/w d-whey powder], E (1.5% w/w inulin and 1.5% w/w d-whey powder], F (1.0% w/w inulin and 2.0% w/w d-whey powder], G (0.5% w/w inulin and 2.5% w/w d-whey powder], and H (0% w/w inulin and 3.0% w/w d-whey powder]. Treated milk was inoculated with 30 g100 L of the ABT-2 starter culture. Then, each of the inoculated milk batches was transferred into 200 mL sterile glass cups and incubated at 37±1°C until the pH reached 4.6. After fermentation, probiotic yogurt samples were stored at 4±1°C for 28 days and analysed on the 1st, 7th, 14th, 21st, and 28th days of cold storage.

Physicochemical analysis Total solid, ash, titratable acidity, fat, and protein contents of probiotic yogurt samples were determined according to method by Kurt *et al*. (11). The pH was measured using a pH meter (model WTW pH-340-A; Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) fitted with a combined glass electrode (11).

Determination of volatile compounds Extraction of headspace volatile compounds of probiotic yogurt samples was performed using an SPME device (Model 57330-U; Supelco, Bellefonte, PA, USA), with 75 μm carboxen/polydimethyl-siloxane fibres (CAR/PDMS) that were conditioned once in the injection port of the GC, as indicated by the manufacturer. For analysis of volatile compounds, 5 g of each probiotic yogurt sample was weighed into a 40 mL headspace vial and sealed using a PTFE-faced silicone septum (Supelco) on the 1st, 7th, 14th, 21st, and 28th days of storage. Yogurt samples were stored in vials at −20°C for subsequent use. Each of the yogurts was dissolved in vial at room temperature for subsequent volatile compound profile analysis, and then the vial was left at 30°C in a thermo block (Supelco) for 15 min to equilibrate the headspace temperature. The fibre was exposed to the headspace with the yogurt sample at 85°C for 1 h. Volatile compounds adsorbed by fibers were identified using MS detectors. Compounds adsorbed by fibres were desorbed in the injection port in splitless mode of a gas chromatography (6890N; Agilent Technologies, Santa Clara, CA, USA) for 6 min at 250°C. Volatiles were separated using a (DB-624, 30 m×0.25 mm i.d., 1.4 μm film) capillary column (J&W Scientific Inc., Folsom, CA, USA) with helium as a carrier gas. The temperature program was started when fibres were inserted and held at 40°C for 5 min, with subsequent programming from 40 to 110°C at 3°C/min, and at a rate of 4°C/min from 150°C, then at a rate of 10°C/min from 210°C, followed by holding for another 15 min. The total run time was 56.33 min. The GC-MS interface was maintained at 240°C. Mass spectra were obtained by electron impact at 70 eV and data were acquired across a range of 50-500 uma. Peak area

identification of compounds was determined by comparison of results with mass spectra from a database developed by NIST and WILEY (12) or using standard reference molecules based on matching of retention indices with known values (12). A Supelco 44585-U (For calculating Kovats Index, Supelco 44585-U, Supelco) was used for calculation of kovats indices. Quantification was based on either a total or single ion chromatogram on an arbitrary scale (12).

Statistical analysis Obtained data were analysed statistically using the SPSS statistical software program version 13 (SPSS Inc., Chicago, IL, USA). An analysis of variance (ANOVA) and Duncan's multiple range test were used for determination of differences among the results at the levels of $p<0.05$ and $p<0.01$.

Results and Discussion

Physico-chemical characteristics of probiotic yogurt samples Total solid, ash, fat, protein, and pH values of probiotic yogurt samples ranged from 13.88 to 14.68%, 0.64 to 0.87%, 3.00-3.55%, 3.46-4.31%, and pH 4.41-4.53, respectively. Previously, Bakırcı and Kavaz (13) reported similar findings for probiotic banana yogurt at the beginning of a storage period.

Volatile compound profiles of probiotic yogurt samples The mean concentrations of volatile compounds of probiotic yogurt samples during the storage period are shown in Table 1. A total of 40 volatile compounds were identified, including 2 aldehydes, 6 ketones, 4 alcohols, 6 acids, 5 esters, 3 terpenes, 3 aromatic hydrocarbons, and 12 aliphatic hydrocarbons. These volatile compounds probably formed due to a series of chemical and biochemical reactions during the fermentation and storage period (7,9).

Aldehydes Acetaldehyde is the major aroma compound of yogurt and mainly occurs as a natural product of amino acids (especially threonine, methionine, and valine) and as a result of nucleotide and pyruvate catabolism of starter cultures (14). Acetaldehyde provides a fresh, fruity, and pungent aroma to yogurt and fermented milk (14-16).

Acetaldehyde was identified in all probiotic yogurt samples during the storage period. Yogurt sample D ranked first, followed in descending order by yogurt samples E, C, A, F, H, B, and G (Table 1). Combinations of d-whey powder and inulin were more effective than a single use of either of these prebiotics. However, Gallardo-Escamilla *et al.* (16) reported that whey powder added yogurts had higher acetaldehyde concentrations than inulin added yogurts. Similar results were also reported by Özer *et al.* (17) and Güven *et al.* (18).

Hexanal has a smell and taste similar to freshy mowed grass and fresh fruit. It is formed in milk and dairy products by β-oxidation of the n-6 fatty acids linoleic and arachidonic acids. A high hexanal concentration causes flavor deterioration and a rancid taste and aroma (19). Hexanal was present in all experimental probiotic yogurt samples during the storage period (Table 1). The highest concentration was present in yogurt sample B, followed in descending order by yogurt samples A, H, D, C, G, F, and E (Table 1). Yogurt sample E contained a large concentration of acetaldehyde and a low hexanal concentration. There were significant ($p<0.01$) differences between acetaldehyde concentrations, while the differences among the hexanal concentrations were insignificant during the storage period. However, the effect of prebiotic ratios on yogurt samples did not significantly affect yogurt volatile concentrations (Table 1). Also, the acetaldehyde and hexanal concentrations of yogurt samples (A, B, C, D, E, F, G, and H) showed irregular variations during storage. Previously, the presence of these aldehydes in yogurts was reported by Ott *et al.* (7) and Saint-Eve *et al*. (20).

Ketones Formation of ketones in milk and dairy products is associated with the pathway of β-oxidation or decomposition of β-keto acids (10,21). Identified ketones included 2,3-butanedione (diacetyl), 2,3-pentanedione, 2-butanone, 3-hydroxy (acetoin), 2-heptanone, 2-nonanone, and 2-undecanone in all probiotic yogurt samples. These compounds were formed at different concentrations during the storage period, although 2-butanone, 3 hydroxy (acetoin), 2,3-butanedione (diacetyl), and 2-heptanone were the most common. Mean concentrations of acetoin, diacetyl, and 2-heptanone were significantly ($p<0.01$) different among yogurt samples, while the concentration of 2,3-pentanedione was significantly ($p<0.05$) greater (Table 1), compared with controls. In contrast, differences between 2-nonanone and 2-undecanone were not significantly different among yogurt samples.

Acetoin, providing buttery, creamy, and pungent notes, occurs as a result of decarboxylation of α-acetolactic acid or fragmentation of diacetyl by the diacetyl reductase enzyme (1,22). The highest mean concentration of acetoin was found in yogurt sample D, followed in descending order by yogurt samples E, H, F, B, G, A, and C (Table 1). Use of equal quantities of inulin and d-whey powder were more effective for increasing the acetoin concentration than single use of these prebiotics. Acetoin levels of yogurt samples were significantly ($p<0.01$) different from each other. Mean acetoin concentrations of yogurt samples decreased significantly ($p<0.01$) until the 14th day, and increased significantly ($p<0.01$) on the 21st and 28th days of storage (Table 1), compared with controls.

Table 1. Mean peak area of volatile compounds in probiotic yoghurt samples during storage

		Probiotic yoghurt samples								Days of storage				
KI[1)]	Volatile compounds	A	B	C	D	E	F	G	H	1.	7.	14.	21.	28.
	Aldehydes													
415	Acetaldehyde	2.76± 2.39[2)]	2.08± 2.18	3.04± 3.17	3.94± 2.92	3.42± 2.76	2.65± 2.75	1.79± 2.26	2.34± 2.44	2.92± 2.12^{ab}	2.11± 2.06^{bc}	3.80± 1.91^{a}	3.31± 3.20^{ab}	1.54± 3.13^{c}
849	Hexanal	0.73± 0.51	0.78± 0.98	0.60± 0.51	0.65± 0.51	0.32± 0.50	0.40± 0.62	0.40± 0.50	0.70± 0.67	0.30± 0.30	0.58± 0.38	0.61± 0.44	0.70± 0.66	0.68± 1.04
	Ketones													
618	2,3-butanedione (diacetyl)	0.77± 1.55^{d}	1.91± 2.23^{cd}	2.29± 2.69^{cd}	5.59± 7.59^{ab}	6.23± 7.06^{a}	4.89± 5.19^{abc}	3.06± 4.02^{bcd}	3.16± 3.01^{bcd}	6.63± 6.62^{a}	2.22± 1.72^{b}	1.48± 1.77^{b}	3.49± 1.02^{b}	3.61± 5.49^{b}
780	2,3-pentanedione	0.24± 0.50^{b}	0.21± 0.40^{b}	0.10± 0.32^{b}	0.53± 0.89^{a}	0.23± 0.38^{b}	0.07± 0.33^{b}	0.04± 0.16^{b}	0.05± 0.14^{b}	0.19± 0.39^{ab}	0.31± 0.49^{a}	0.06± 0.24^{b}	0.25± 0.53^{ab}	0.10± 0.57^{ab}
790	2-butanone, 3-hydroxy (acetoin)	4.99± 2.54^{b}	5.16± 2.28^{b}	4.98± 1.94^{b}	7.57± 3.97^{a}	6.52± 3.82^{ab}	6.21± 3.68^{ab}	5.06± 2.46^{b}	6.27± 1.99^{ab}	6.93± 3.33^{a}	4.39± 1.27^{b}	3.76± 1.60^{b}	6.97± 3.22^{a}	7.19± 3.15^{a}
948	2-heptanone	1.76± 1.43^{c}	3.21± 1.89^{a}	2.10± 1.28^{bc}	2.10± 1.15^{bc}	1.98± 1.19^{bc}	2.26± 0.86^{bc}	1.74± 0.83^{c}	2.80± 1.44^{ab}	1.30± 0.89^{c}	2.07± 0.88^{b}	2.46± 1.48^{ab}	2.39± 0.95^{b}	3.04± 1.79^{a}
1169	2-nonanone	0.40± 0.23^{a}	0.46± 0.29^{a}	0.32± 0.26^{ab}	0.22± 0.26^{b}	0.31± 0.30^{ab}	0.32± 0.30^{ab}	0.31± 0.24^{ab}	0.41± 0.21^{a}	0.27± 0.28^{b}	0.46± 0.21^{a}	0.46± 0.19^{a}	0.25± 0.26^{b}	0.28± 0.32^{b}
1381	2-undecanone	0.09± 0.12	0.11± 0.15	0.05± 0.10	0.06± 0.11	0.08± 0.12	0.12± 0.16	0.09± 0.15	0.07± 0.10	ND[3)]	0.13± 0.13^{b}	0.20± 0.12^{a}	0.05± 0.11^{c}	0.04± 0.12^{c}
	Alchols													
539	Ethanol	4.61± 7.09^{ab}	0.83± 1.81^{c}	5.83± 9.60^{a}	2.02± 4.70^{bc}	3.43± 6.67^{abc}	ND	1.32± 2.70^{bc}	ND	0.30± 0.93^{b}	0.39± 1.22^{b}	1.26± 2.86^{b}	3.93± 7.45^{a}	5.25± 8.11^{a}
826	Isoamyl alchol	5.62± 6.37^{ab}	5.58± 6.45^{ab}	7.80± 5.91^{a}	5.42± 5.36^{ab}	4.80± 6.70^{ab}	2.31± 4.19^{bc}	4.27± 6.32^{abc}	1.00± 3.56^{c}	2.65± 5.40	5.10± 6.02	3.68± 4.95	5.33± 5.67	6.17± 7.05
972	Benzene methanol, 2- (2-aminopropoxy)-3-methyl	0.14± 0.46	0.01± 0.02	0.01± 0.04	0.03± 0.08	0.02± 0.07	0.03± 0.06	ND	0.01± 0.04	0.01± 0.03	0.02± 0.06	0.02± 0.05	0.03± 0.08	ND
1042	2-heptanol, 6-amino-2-methyl	0.12± 0.32^{a}	0.01± 0.04^{b}	ND	0.01± 0.03^{b}	0.06± 0.14^{ab}	ND	0.07± 0.18^{ab}	0.01± 0.04^{b}	0.10± 0.23^{a}	0.07± 0.21^{ab}	ND	ND	ND

Table 1 (continued)

		Probiotic yoghurt samples								Days of storage				
KI[1)]	Volatile compounds	A	B	C	D	E	F	G	H	1.	7.	14.	21.	28.
	Acids													
717	Acetic acid	2.68± 1.21^{abc}	1.68± 1.07^{c}	2.42± 1.83^{bc}	3.77± 1.82^{ab}	4.01± 4.41^{a}	2.94± 3.63^{abc}	2.53± 1.56^{abc}	2.43± 1.31^{bc}	5.08± 4.59^{a}	2.06± 0.86^{b}	2.06± 1.01^{b}	2.94± 2.12^{b}	1.87± 1.71^{b}
890	Butanoic acid	9.73± 3.41^{c}	12.00± 4.08^{abc}	10.34± 3.57^{bc}	12.70± 3.57^{ab}	10.95± 4.17^{abc}	11.88± 4.40^{abc}	12.40± 5.73^{abc}	13.34± 5.13^{a}	10.23± 3.65^{b}	10.30± 2.37^{b}	9.79± 3.58^{b}	13.47± 4.50^{a}	14.59± 5.17^{a}
1058	Hexanoic acid (caproic acid)	17.86± 4.83^{bc}	21.45± 5.07^{a}	17.88± 6.34^{bc}	16.18± 7.37^{c}	16.61± 5.79^{c}	19.29± 5.74^{abc}	20.04± 6.92^{ab}	22.33± 4.72^{a}	12.83±5. 05^{c}	19.83± 4.01^{b}	22.58± 5.16^{a}	19.67± 6.29^{b}	20.08± 5.51^{b}
1256	Octanoic asit (caprilic acid)	8.59± 4.03^{ab}	10.82± 4.96^{a}	8.10± 3.92^{b}	7.56± 5.51^{b}	8.28± 5.26^{ab}	9.30± 6.04^{ab}	9.28± 5.84^{ab}	8.85± 4.23^{ab}	3.34± 2.44^{d}	11.06± 4.65^{ab}	12.68± 3.27^{a}	7.19± 4.57^{c}	10.04± 3.75^{b}
1265	Benzoic acid	0.14± 0.27	0.12± 0.28	0.07± 0.23	0.08± 0.17	0.08± 0.17	0.09± 0.21	0.06± 0.16	0.05± 0.13	0.02± 0.09^{b}	0.06± 0.15^{b}	0.31± 0.32^{a}	0.04± 0.13^{b}	ND
1452	Decanoic acid (capric acid)	2.63± 2.51	3.44± 3.08	2.50± 2.63	2.17± 2.32	2.48± 2.43	2.92± 2.94	2.61± 2.75	2.71± 2.33	0.30± 0.58^{d}	2.97± 2.28^{b}	5.91± 2.13^{a}	2.00± 1.98^{c}	2.26± 1.87^{bc}
	Esters													
640	Ethyl acetate	1.28± 1.90^{ab}	0.43± 1.40^{b}	0.69± 1.84^{ab}	2.14± 4.31^{a}	0.29± 1.00^{b}	0.52± 1.78^{ab}	1.59± 2.70^{ab}	1.60± 2.98^{ab}	1.49± 3.55^{ab}	0.87± 1.88^{bc}	0.70± 1.42^{bc}	2.07± 3.22^{a}	0.21± 0.83^{c}
843	Ethyl butyrate	0.21± 0.43	ND	0.32± 0.64	ND	ND	ND	0.56± 2.51	ND	ND	ND	0.08± 0.30	0.17± 0.48	0.42± 1.99
1225	Ethyl octanoate	0.16± 0.26^{a}	0.10± 0.28^{ab}	0.10± 0.21^{ab}	0.03± 0.12^{b}	0.08± 0.23^{ab}	0.02± 0.10^{b}	ND	ND	ND	ND	0.06± 0.16b	0.04± 0.13^{b}	0.20± 0.33^{a}
1333	Thiocyanic acid, 5.alpha.-cholesteran-3.beta.-yl ester	0.11± 0.16	0.12± 0.14	0.12± 0.15	0.11± 0.13	0.13± 0.14	0.10± 0.15	0.09± 0.12	0.06± 0.10	0.13± 0.13^{a}	0.09± 0.12^{a}	0.15± 0.13^{a}	0.12± 0.16^{a}	0.02± 0.09^{b}
1399	Decanoic acid, methyl ester	0.02± 0.11	ND	ND	0.02± 0.07	0.03± 0.10	0.04± 0.12	0.02± 0.10	0.03± 0.09	ND	0.03± 0.11^{ab}	0.06± 0.14^{a}	ND	0.01± 0.07^{ab}
	Terpens													
951	á-pinene	0.33± 0.30	0.21± 0.34	0.21± 0.31	0.27± 0.31	0.18± 0.25	0.29± 0.30	0.19± 0.25	0.39± 0.40	0.15± 0.22	0.31± 0.22	0.33± 0.29	0.28± 0.36	0.22± 0.42
1054	D-limonene	0.37± 0.47^{ab}	0.46± 0.52^{a}	0.20± 0.42^{ab}	0.13± 0.28^{b}	0.25± 0.37^{ab}	0.29± 0.38^{ab}	0.28± 0.63^{ab}	0.23± 0.32^{ab}	0.17± 0.43^{b}	0.29± 0.41^{b}	0.52± 0.56^{a}	0.18± 0.35^{b}	0.24± 0.34^{b}
1056	Benzene, 1-methyl-2- (p-cymene)	0.38± 0.44^{a}	0.39± 0.41^{a}	0.18± 0.33^{b}	0.11± 0.23^{b}	0.12± 0.22^{b}	0.28± 0.40^{ab}	0.23± 0.44^{ab}	0.18± 0.35^{b}	0.57± 0.49^{a}	0.36± 0.36^{b}	0.18± 0.29^{c}	0.05± 0.16^{cd}	ND

Table 1 (continued)

KI[1)]	Volatile compounds	Probiotic yoghurt samples								Days of storage				
		A	B	C	D	E	F	G	H	1.	7.	14.	21.	28.
	Aromatic hydrocarbons													
791	Toluene	0.91± 2.30^{a}	ND	0.04± 0.16^{b}	ND	0.14± 0.34^{b}	0.16± 0.44^{b}	0.11± 0.49^{b}	ND	0.16± 0.38^{b}	ND	0.57± 1.85^{a}	0.04± 0.20^{b}	0.07± 0.40b
892	p-xylene	0.04± 0.15	0.02± 0.08	ND	ND	ND	0.02± 0.07	0.02± 0.08	ND	ND	0.01± 0.07^{b}	0.05± 0.14^{a}	ND	ND
933	Styrene	0.65± 0.34^{ab}	0.82± 0.44^{a}	0.64± 0.42^{ab}	0.53± 0.43^{b}	0.47± 0.37^{b}	0.62± 0.49^{ab}	0.54± 0.35^{b}	0.65± 0.35^{ab}	0.27± 0.26^{b}	0.67± 0.17^{a}	0.81± 0.28^{a}	0.65± 0.52^{a}	0.69± 0.48^{a}
	Aliphatic hydrocarbons													
848	2-methyl-butane (Isopentane)	0.16± 0.24^{a}	0.06± 0.17^{bcd}	ND	ND	0.11± 0.22^{abc}	0.13± 0.27^{ab}	0.05± 0.13^{bcd}	0.02± 0.10^{cd}	0.06± 0.16^{bc}	0.15± 0.25^{a}	0.11± 0.23^{ab}	ND	ND
1000	n-decane	0.21± 0.40	0.13± 0.20	0.23± 0.45	0.05± 0.09	0.10± 0.15	0.18± 0.35	0.33± 0.59	0.20± 0.56	0.22± 0.28	0.11± 0.15	0.17± 0.26	0.28± 0.62	0.11± 0.45
1032	2-methyl, decane	0.31± 0.55$_{a}$	0.14± 0.37^{b}	ND	ND	ND	ND	ND	ND	0.39± 0.58^{a}	0.05± 0.20^{b}	ND	ND	ND
1051	Dodecane, 2,2,11,11-tetramethyl	ND	0.08± 0.22	ND	ND	ND	0.06± 0.28	0.04± 0.19	ND	0.12± 0.31^{a}	ND	ND	ND	ND
1052	Heptane, 2,2,4,6,6-pentamethyl	0.07± 0.26	ND	0.05± 0.18	ND	0.11± 0.33	0.03± 0.15	0.14± 0.44	0.10± 0.33	0.16± 0.43^{a}	0.09± 0.25^{ab}	ND	0.07± 0.28^{ab}	ND
1053	Dodecane, 2, 6, 10-trimethyl	0.09± 0.21^{a}	ND	ND	ND	ND	ND	ND	0.02± 0.10^{ab}	0.04± 0.15	0.02± 0.10	ND	0.03± 0.15	ND
1057	Decane, 5,6-dipropyl	0.18± 0.36	0.17± 0.33	0.19± 0.55	0.09± 0.30	0.33± 0.79	0.40± 0.91	0.32± 0.66	0.31± 0.70	0.32± 0.39^{ab}	0.07± 0.17^{b}	0.07± 0.41^{b}	0.54± 1.05^{a}	0.24± 0.54^{ab}
1100	n-undecane	0.42± 0.60^{a}	0.24± 0.41^{ab}	0.24± 0.47^{ab}	0.06± 0.23^{b}	0.16± 0.33^{ab}	0.39± 0.71^{a}	0.08± 0.27^{b}	0.15± 0.32^{ab}	0.46± 0.68^{a}	0.16± 0.29^{b}	0.11± 0.21^{b}	0.24± 0.55^{b}	0.09± 0.25^{b}
1138	3-methyl decane	0.71± 0.99^{a}	0.26± 0.49^{bc}	0.12± 0.30^{c}	0.08± 0.24^{c}	0.22± 0.49^{bc}	0.51± 0.85^{ab}	0.49± 0.69^{ab}	0.24± 0.44^{bc}	0.86± 0.87^{a}	0.56± 0.71^{b}	0.06± 0.37^{c}	0.11± 0.30^{c}	0.05± 0.17^{c}
1198	2,3- dimethyl decane	0.12± 0.26^{a}	0.03± 0.14b	ND	ND	ND	0.04± 0.16^{b}	0.03± 0.11b	ND	0.06± 0.19	0.04± 0.17	0.02± 0.09	ND	0.02± 0.10
1200	Dodecane	0.31± 0.24	0.28± 0.32	0.16± 0.20	0.16± 0.21	0.23± 0.28	0.27± 0.32	0.26± 0.28	0.20± 0.24	0.05± 0.14c	0.41± 0.19^{a}	0.20± 0.20^{b}	0.13± 0.26^{bc}	0.37± 0.31^{a}
1600	n-hexadecane	0.71± 1.43	0.24± 0.93	0.18± 0.78	0.40± 1.01	0.74± 1.33	0.34± 1.06	0.17± 0.44	0.44± 1.17	0.53± 1.24^{ab}	0.84± 1.39^{a}	0.02± 0.13^{b}	0.42± 1.19^{ab}	0.21± 0.68^{b}

[1)]KI: Kovats Index calculated for a DB-624 capillary column (J&W Scientific: 30 m, 0.25 mm id, 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector.
[2)]Mean values SD of probiotic yogurt prepared with duplicate samples; The letters a, b, c, and d indicate the statistically significant differences.
[3)]ND: not detected.

Diacetyl is a typical aroma compound of butter and can be found in sour cream, yogurt, cottage cheese, and other fermented dairy products (23). The highest mean concentration of diacetyl was present in yogurt sample E and the lowest mean concentration was present in yogurt sample A (Table 1). The d-whey powder was more effective for increasing the diacetyl concentration than inulin, although equal amounts of these prebiotic substances were better. Yogurt samples B and C and yogurt samples G and H had similar, but not significantly different diacetyl concentrations. However, yogurt samples A, D, E, and F, and 2 yogurt sample groups (yogurt samples B and C, and yogurt samples G and H) showed significantly ($p<0.01$) different diacetyl concentrations from each other. The diacetyl concentration of probiotic yogurts decreased significantly ($p<0.01$) on the 14th day of storage, and again increased significantly ($p<0.01$) by the end of the storage period, compared with controls (Table 1).

2,3-pentanedione, like acetaldehyde and diacetyl, is a main flavor component of yogurt. It is an intermediate product of isoleucine metabolism and can be produced from a-aceto-a-hydroxybutyrate (24). Yogurt sample D contained the highest 2,3-pentanedione concentration during storage, followed by yogurt samples A, E, B, C, F, H, and G in descending order (Table 1). Significant ($p<0.05$) differences in 2,3-pentanedione concentrations between yogurt samples were present (Table 1). However, the storage period had no significant effect on the 2,3-pentanedione concentration of any yogurt sample.

Generally, 2-heptanone (fruity and cinnamon), 2-nonanone (creamy and fresh), and 2-undecanone (flower and rose) occur as a result of β-oxidation of free fatty acids or decarboxylation of β-keto acids (24). No significant differences between any yogurt samples for concentrations of 2-nonanone, and 2-undecanone existed, while the concentration of 2-heptanone was found statistically significant ($p<0.01$). Conversely, the effect of storage showed significant ($p<0.01$) differences in yogurt sample concentrations (Table 1).

Alcohols Ethanol, isoamyl alcohol, benzene methanol, 2-(2-aminopropoxy)-3-methyl, and 2-heptanol, 6-amino-2-methyl were identified in probiotic yogurt samples (Table 1). Ethanol, like acetaldehyde, diacetyl, and acetoin, provides a yogurt aroma but has little effect on the aroma profile (1,14,16). The highest mean concentration of ethanol was detected in yogurt sample C and the lowest mean concentration was identified in yogurt samples F and H.

Yogurt samples B, F, and H, and yogurt samples D and G, had similar ethanol concentrations within these groupings, but concentrations were significantly ($p<0.01$) different between the groupings. Mean ethanol concentrations of probiotic yogurt samples showed a significant ($p<0.01$) increase during the storage period, compared with controls (Table 1). Similarly, Pinto *et al.* (25) reported an increase in ethanol levels of yogurt during storage.

Isoamyl alcohol has a burning, sharp, and fruity smell and taste and provides apricot and banana-like aromas to a product (14). The highest mean concentration of isoamyl alcohol was present in yogurt sample C, followed in descending order during storage by yogurt samples A, B, D, E, G, F, and H (Table 1). Significant ($p<0.01$) differences among isoamyl alcohol concentrations in yogurt samples were present, but the effect of storage did not produce significantly different concentrations. Isoamyl alcohol concentrations in yogurt samples A, B, D, and E were similar to each other, while concentrations in yogurt samples C, F, G, and H were significantly ($p<0.01$) different from each other, and from yogurt samples A, B, D, and E.

Acids Acids are an important quality parameter of milk and dairy products. They serve as a precursor to formation of methyl ketones, alcohols, lactones, and esters (26). In this study, 6 acids were identified in probiotic yogurt samples during the storage period. The highest concentration was hexanoic acid (caproic acid), followed in descending order by butanoic acid, octanoic acid (caprilic acid), decanoic acid (capric acid), acetic acid, and benzoic acid (Table 1).

Acetic acid occurs due to lactose, citric acid, lactic acid, and amino acid catabolism of bacteria in yogurt. Formation of a high concentration of acetic acid is not desirable because it provides a pungent and vinegary flavor to the final product. (7,8). Significant ($p<0.05$) differences among yogurt samples for storage period ($p<0.05$) for acetic acid concentrations were identified (Table 1). The highest mean concentration of acetic acid was present in yogurt sample E, followed in descending order by yogurt samples D, F, A, G, H, C, and B, (Table 1). Acetic acid was previously identified in yogurt by Beshkova *et al.* (15) and Adhikari *et al.* (27).

Significantly ($p<0.01$) different caproic acid concentrations were present in probiotic yogurt samples and as a result of storage (Table 1), compared with controls. However, yogurt samples showed no significant differences with respect to butyric, caprilic, and capric acid concentrations.

Butyric acid is produced in dairy products by fermentation of lactose and lactic acid (21,28). It provides a characteristic cheese-like aroma to products (28). The highest mean concentration of butyric acid was identified in yogurt sample H, while the lowest mean concentration was identified in yogurt sample A (Table 1). The butyric acid concentrations of yogurt samples increased in relation to the d-whey powder ratio.

Short chain fatty acids, such as hexanoic acid, octanoic acid, and decanoic acid, have an effect on formation of the characteristic aroma profiles of milk and dairy products. In general, butyric acid and octanoic acid give a product a rancid taste. Hexanoic acid provides a sour, rancid, and goat-like taste, and decanoic acid provides a soapy and plastic-like flavor (20,29). The highest mean concentrations of caprilic and capric acids were identified in yogurt sample B, and the highest mean concentration of caproic acid was present in yogurt sample H (Table 1). Conversely, the lowest mean concentrations of caprilic, capric, and caproic acids were present in yogurt sample D. The mean caproic, caprilic, and capric acid concentrations of probiotic yogurts significantly ($p<0.01$) increased until the 14th day, followed by a significant ($p<0.01$) decrease on the 21st day, with a subsequent significant ($p<0.01$) increase again on the 28th day of storage, compared with controls (Table 1).

Benzoic acid occurs in dairy products as a result of degradation of phenylalanine during ripening. On the other hand, benzoic acid can occur due to oxidation of benzaldehyde by some lactic acid bacteria, or transformation of hippuric acid (30). The benzoic acid concentrations of probiotic yogurt samples were not significantly different, compared with controls, but the effect of storage on benzoic acid concentrations was significant ($p<0.01$), compared with controls.

Esters Esters have a positive effect on the flavor of dairy products at low concentrations. However, high concentrations can produce fruity, spice-like, and flower-like flavors (10). In this study, ethyl acetate, ethyl butyrate, ethyl octanoate, thiocyanic acid, 5alpha-cholesteran-3beta-yl ester, and decanoic acid methyl ester were identified in probiotic yogurt samples (Table 1).

Ethyl acetate (pineapple and fruity notes) was present in all probiotic yogurt samples during the storage period. The highest mean concentration was found in yogurt sample D, followed in descending order by yogurt samples H, G, A, C, F, B, and E. Probiotic yogurt samples showed non-significant ($p>0.05$) differences in ethyl acetate concentrations (Table 1). However, ethyl acetate concentrations of yogurt samples showed a fluctuation during storage that was significantly ($p<0.05$) different between yogurt samples (Table 1), similar to the report of Saint-Eve *et al.* (20).

Thiocyanic acid 5alpha-cholesteran-3beta-yl ester was present in all yogurt samples but concentrations were not significantly different. Conversely, ethyl butyrate, ethyl octanoate, and decanoic acid methyl ester were identified in some yogurt samples. The ethyl octanoate concentrations in yogurt samples were significantly ($p<0.05$) different, while ethyl butyrate, ethyl octanoate, and decanoic acid methyl ester concentrations in yogurt samples were not significantly different. The storage period affected the ethyl octanoate and thiocyanic acid, 5alpha-cholesteran-3beta-yl ester concentrations with significant ($p<0.01$) differences present between yogurt samples, while the effect of storage on decanoic acid methyl ester concentrations produced significant ($p<0.05$) differences between yogurt samples (Table 1). Changes in ester concentrations of yogurt samples may have been related to inulin and d-whey powder usage with different ratios causing negative microbial activities during storage (20,21).

Terpenes The presence of terpenes in milk is caused as a result of plants consumed by grazing animals and the occurrence of microbiological activity in the animal rumen (28). The 3 terpenes α-pinene, D-limonene, and benzene, 1-methyl-2- (p-cymene) were identified in probiotic yogurt samples during the storage period (Table 1).

The *p*-cymene concentrations of yogurt samples were significant ($p<0.01$) different. The α-pinene and D-limonene concentrations were not significantly different (Table 1). Conversely, the storage period significantly ($p<0.01$) affected the D-limonene and *p*-cymen concentrations, while the storage effect on the α-pinene concentration did not produce significant differences between yogurt samples. The highest mean concentration for α-pinene was present in yogurt sample H and the lowest mean concentration was present in yogurt sample E. The α-pinene concentration of yogurt samples showed irregular changes during storage (Table 1). The lowest mean concentrations of D-limonene and p-cymene were present in yogurt sample D and the highest mean concentrations were present in yogurt sample B (Table 1). On the other hand, all identified terpenes in probiotic yogurt samples showed irregular variations during storage (Table 1), similar to the report of Saint-Eve *et al.* (20).

Aromatic hydrocarbons Three different types of aromatic hydrocarbons were identified in probiotic yogurt samples. Styrene was present in all yogurt samples (B, H, A, C, F, G, D, and E, respectively), while toluene was identified in 5 yogurt samples (A, F, E, G and C, respectively). p-Xylene was present in 4 yogurt samples (A, B, G, and F, respectively) (Table 1). Addition of inulin and d-whey powder to probiotic yogurts in different proportions caused a statistically ($p<0.01$) irregular changing on the toluene concentration of samples. Effects on p-xylene and styrene concentrations were not significant. Conversely, the effect of storage period significantly) on p-xylene and styrene concentrations was irregular statistically ($p<0.01$). The storage period also had a statistically different ($p<0.05$) effect on the toluene concentration of yogurt samples. Identified aromatic hydrocarbons in probiotic yogurt samples were probably related to milk, aromatic hydrocarbons

in feed, milk transport containers, materials used during production, prebiotic agents added to milk (milk powder, d-whey powder, and inulin), and packaging materials.

Aliphatic hydrocarbons (*n*-alkanes) Aliphatic hydrocarbons occur as a secondary oxidation product of milk and dairy products under light conditions over time. On the other hand, aliphatic hydrocarbons cause bad odors and taste, loss of nutritional value, and reduction of shelf-life for dairy products (7-10). Twelve aliphatic hydrocarbon compounds were identified in probiotic yogurt samples (Table 1). 2-Methyl-butane (isopentane), 2-methyl, decane, 3-methyl decane, and 2,3-dimethyl decane concentrations in yogurt samples were significantly ($p<0.01$) different from each other. Dodecane, 2,6,10-trimethyl, and *n*-undecane concentrations in yogurt samples were significantly ($p<0.05$) different from each other.

n-Hexadecane was the most abundant aliphatic hydrocarbon in experimental probiotic yogurt samples during the storage period (Table 1). The highest mean concentration of n-hexadecane was present in yogurt sample E, followed in descending order by yogurt samples A, H, D, F, B, C, and G. Significant ($p<0.05$) differences for storage period for the *n*-hexadecane concentration, but not for the n-hexadecane concentration, between yogurt samples were present (Table 1).

In this study, 8-different probiotic yogurt samples produced using inulin and d-whey powder and a commercial probiotic starter culture (ABT-2) were analysed for volatile compound profiles during storage. The SPME GC-MS technique was used for identification of aroma compounds. Use of different ratios of inulin and d-whey powder significantly ($p<0.05$, and $p<0.01$ levels) affected the volatile compound profiles of yogurt samples. Generally, the highest mean concentrations of acetaldehyde, acetoin, 2,3-pentanedione, and ethyl acetate were present in yogurt sample D, while the highest mean concentrations of diacetyl, acetic acid, and n-hexadecane were present in yogurt sample E. Combinations of 2% inulin+1% d-whey powder (yogurt sample D) and 1.5% inulin+1.5% d-whey powder (yogurt sample E) were most suitable for formation of a desirable aroma profile. Different ratios of inulin and d-whey were more effective for volatile compound profiles than single use of these prebiotics.

Caproic, butanoic, caprilic, capric, acetic, and bezoic acids had the highest concentrations of all volatile compounds with strong aromatic properties for formation of a high quality aroma for yogurt samples. Finally, 2-butanone, 3 hydroxy (acetoin), 2-heptanone, isoamyl alcohol, ethanol, ethyl acetate, $_D$-limonene, styrene, decane, *n*-undecane, 3-methyl decane, dodecane, and n-hexadecane were present as major compounds in probiotic yogurts. They were detected in all yogurt samples during storage.

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