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ORIGINAL RESEARCH

Influence of inulin and demineralised whey powder addition on the organic acid profiles of probiotic yoghurts

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In this study, the effect of the different concentrations of inulin and demineralised whey (d-whey) powder on the chemical characteristics and organic acid profiles of probiotic yoghurts were investigated. Analysis of chemical composition was performed on the first day, while pH and organic acid profiles were carried out during storage. The results indicated that different proportions of inulin and d-whey powder significantly influenced the levels of the organic acids, produced while the duration of storage affected the orotic, pyruvic, citric, lactic and acetic acid levels. There was a direct relationship between the level of d-whey powder and organic acid (except for butyric acid) concentration in the probiotic yoghurt.

Keywords Probiotic, Inulin, Demineralised whey powder, Organic acid.

INTRODUCTION

Yoghurt plays an important role in the human diet. It is obtained from lactic acid fermentation of milk by homofermentative yoghurt starter cultures comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tamime and Robinson 1985, 1999). Recently, the use of probiotic micro-organisms such as *Lactobacillus acidophilus* and *Bifidobacterium* sp. has increased in yoghurt and other fermented dairy products, due mainly to their perceived additional health benefits beyond normal nutritional value and because they produce a less acidic aroma. The characteristic flavour of fermented dairy products is mainly the result of the metabolism of the starter cultures (Kneifel 1992; Lourens-Hattingh and Viljoen 2001a; Sodini *et al.* 2002; Gonzalez *et al.* 2011). In order to obtain a specific desired aroma profile in fermented milk products, the application of the appropriate strains and prebiotics is highly important. The composition of the product and the availability of prebiotics are also reported to affect the growth and viability of probiotic starter cultures (Dave and Shah 1998). In recent years, inulin and whey powder have probably been the most commonly used additives in fermented milks (Holzapfel and Schillinger 2002; O'Brein *et al.* 2003; Zimeri and Kokini 2003). Inulin is a good source of dietary fibre, and it consists of a mixture of oligomers and long polymer chains with a variable number of fructose molecules. When inulin is used as a prebiotic agent, it stimulates the growth and viability of the useful micro-organisms, increases calcium and magnesium absorption, stimulates the immune function and reduces the level of cholesterol (Izco and Franck 1998; Holzapfel and Schillinger 2002; O'Brein *et al.* 2003; Zimeri and Kokini 2003; Dello Stafollo *et al.* 2004; Ohr 2004). On the other hand, whey powder contains biologically active proteins or peptides, minerals and vitamins (especially vitamin B2). It has many benefits when added to yoghurt. These benefits include: textural improvement, nutritional enrichment, reduced syneresis, extended shelf life, mild flavour development and potential prebiotic and nutraceutical effects (Kailasapathy and Supriadi 1998; Antunes *et al.* 2004).

Organic acids play an important role as preservatives and contribute to the sensory characteristics of the yoghurt. The majority of organic acids are formed as a result of carbohydrate metabolism by lactic acid bacteria (lactic acid, acetic acid, pyruvic acid, propionic acid and

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formic acid), by hydrolysis of milk fat (free fatty acids, such as butyric acid), by direct addition of acidifiers (citric acid and lactic acid) or originate from natural physiological mechanisms of milk-producing animals (citric acid, orotic acid and uric acid) or bacterial growth (Tamime and Robinson 1985; Fernandez-Garcia and McGregor 1994; Tormo and Izco 2004). Production of organic acids directly influences the pH, flavour, texture and sensory properties of fermented milks. In addition, organic acids inhibit the growth of pathogenic and spoilage micro-organisms, which can cause spoilage in milk and milk products (Fernandez-Garcia and McGregor 1994; Gonzalez de Liano *et al.* 1996; Tormo and Izco 2004). Quantitative determination of organic acids is extremely important in terms of determining the activity of starter cultures in dairy products and detecting changes in product quality during storage (Gonzalez de Liano *et al.* 1996; Adhikari *et al.* 2002).

The objectives of this work were to study the effect of inulin and d-whey powder addition on the chemical composition and organic acid profiles of a probiotic yoghurt.

MATERIALS AND METHODS

Materials

Freshly milked cows' milk (50 L) was obtained from the dairy farm of Atatürk University located in the Erzurum province of Turkey, and it was transported to the pilot dairy plant in university. The milk was transported in insulated cooling tanks. Skim milk powder (SMP) was purchased from local shops, inulin (Orafti®HPX) was bought from the Artisan Food Industry Inc. (Istanbul, Turkey) and 80% demineralised whey powder (d-whey powder) was provided by Maybi Malkara Union Milk and Milk Products Inc. (Tekirdag, Turkey). Probiotic yoghurt culture (ABT-2; *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* sp.) was supplied by Chr. Hansen-Peyma, Istanbul, Turkey.

Yoghurt production

Cows' milk (milk fat 3.48 ± 0.04%, protein 3.11 ± 1.34%, total solid 12.13 ± 0.03%, pH 6.52 ± 0.15 and titratable acidity 0.19 ± 0.02%) was used for yoghurt production. After continous clarification (Model ALFA LAVAL 313T, centrifugal clarifier, 5000 g and working capacity 1000 L/h) and two-stage homogenisation (ALFA LAVAL, separations technik GMBH, Wilheim-Berger-Str. GLINDE/b.HAMBURG, pressure at 20 MPa and working capacity 350 L/h), milk (50 L) was heated to 90 °C for 10 min and then rapidly cooled to 37 °C and divided into eight equal parts (each part 5 L). After pasteurisation, the ingredients were added to each part of the milk. One batch of milk was then taken as the control (A), and the remaining batches (B, C, D, E, F, G, H) were prepared with the mixtures of inulin/d-whey powder (Table 1). The milks were then inoculated with direct vat set (DVS) ABT-2 starter culture [0.03% (w/w)], and all inoculated milks were divided into parts of approximately 200 mL (Glass jars were used for this purpose, and they were autoclaved at 121 °C, 15 min). The inoculated milks were incubated at 37 ± 1 °C until the pH reached 4.6, and then the yoghurts were stored in a refrigerator (4 ± 1 °C) for 24 h. Probiotic yoghurt samples were stored at 4 ± 1 °C for 28 days and analysed after 1, 7, 14, 21 and 28 days of cold storage. The probiotic yoghurts were manufactured in duplicate using two different batches of milk.

Chemical analysis

The total solids and ash contents of experimental probiotic yoghurts were determined using gravimetric methods (AOAC 1990/drying method were used). Fat content was measured by the Gerber method (AOAC 1990/extraction method were used) and protein content by the Kjeldahl method (AOAC 1990). Solids nonfat contents were obtained by calculation, and the pH was measured using a pH meter (model WTW pH-340-A, Weilheim, Germany) fitted with a combined glass electrode (AOAC 1990).

Organic acid concentrations

The organic acids in the probiotic yoghurts were analysed by high-performance liquid chromatography (Agilent HPLC 1100 series G 1322 A, Waldbronn, Germany) according to the modified methods described by Fernandez-Garcia and McGregor (1994) and Akalın *et al.* (2004). For the extraction of organic acids, 4 g of probiotic yoghurt sample was diluted with 25 mL 0.001 N H_2SO_4 and centrifuged at 5000 *g* for 10 min. The supernatant was filtered through Whatman No.1 filter paper and through a 0.45-μm membrane filter (PALL, Ann Arbor, MI, USA), and 2 mL aliquots were stored in HPLC vials at −20 °C until HPLC analysis. The degassed mobile phase of 0.001 N H_2SO_4 was used at a flow rate of 0.6 mL/min. The wavelength of detection was 210 nm for quantification of organic acids. Duplicate injections (approximately 10 μL) were performed for all samples. Standard solutions of orotic, pyruvic, citric, lactic, acetic, butyric and hippuric acids were prepared in distilled water to establish the elution times and calibration curves.

Statistical analysis

The experimental data were analysed statistically using the SPSS statistical software program version 13 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and Duncan's multiple range test were used to determine differences between results.

RESULTS AND DISCUSSION

General composition of the experimental probiotic yoghurts

Total solids, fat, solids nonfat, ash and protein contents of the probiotic yoghurts were analysed on the first day of

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Table 1 Skimmed milk powder and prebiotic ratios used for the probiotic yoghurt production

Probiotic yoghurt samples	*Skimmed milk powder (%)*	*Inulin (%)*	*Demineralised whey powder (%)*
A	3.0	0	0
B	0	3.0	0
C	0	2.5	0.5
D	0	2.0	1.0
E	0	1.5	1.5
F	0	1.0	2.0
G	0	0.5	2.5
H	0	0	3.0

storage in both experiments, and the mean values obtained (%) were presented in Table 2. Differences in some parameters of the samples might be explained by the proportional differences of prebiotics added to each sample, although it can be concluded that these results were as expected. The highest fat content was determined in E, while the highest protein content was found in G. This situation might be explained with the inulin (fat 0%, protein 0%) and d-whey powder (fat 1.00%, protein 8.5%) ratio in these samples.

pH

The pH values of the probiotic yoghurt samples after 24 h and during storage are shown in Table 2 and Figure 1, respectively. The highest mean pH value was observed in H (4.53 ± 0.08), while the lowest mean amount was found in B (4.41 ± 0.09), although these differences among the samples were not statistically significant. Our results were in accordance with earlier reports. Lucas *et al.* (2004) and Bakırcı and Kavaz (2008) reported that acidity production in yoghurts increased during storage. As can be seen in Figure 1, there was a fluctuation in the pH values of the experimental probiotic yoghurts, but the pH value showed an increase between the 21st and 28th days in the case of samples A, B, C and D. The obtained results also showed that d-whey powder was more effective than inulin in terms of preventing an increase in pH during storage. However, the increase of pH values at the end of storage might be explained by alkaline compounds formed as a result of proteolytic degradation, yeast and moulds activities (were measured), proportional change of prebiotics (inulin% /d-whey powder% ratios) and the buffering capacity of serum proteins in d-whey powder (Suriyarachchi and Fleet 1981; Kailasapathy *et al.* 1996; Lourens-Hattingh and Viljoen 2001b).

Organic acid profiles of probiotic yoghurts

Organic acid analysis is essential for determining the quality and safety of milk and milk products. In this study, seven different organic acids were determined in the probiotic yoghurt samples during the storage period. The organic acid profiles of samples are presented in Table 3, Figures 2a,b,c, d and 3a,b,c.

Orotic acid is an organic acid that is found in significant amounts in milk and dairy products, and it is an important factor for the growth of yoghurt starter cultures. The average concentrations of orotic acid are between 69 and 74 mg/L in fresh cows' milk, although this amount shows a significant reduction (45–48%) during fermentation (Okonkwo and Kinsella 1969; Saidi and Warthesen 1989; Fernandez-Garcia and McGregor 1994; Güzel-Seydim *et al.* 2000; Kristo *et al.* 2003). Observing Table 3, the highest mean concentration of orotic acid was found in treatment H (11.83 ± 1.02) followed by A (11.76 ± 1.65), G (11.60 ± 0.95), F (11.40 ± 1.09), E (11.27 ± 0.60), D (11.21 ± 0.41), C (10.89 ± 0.53) and B (10.34 ± 0.42). As seen in Figure 2a, A, B, C, D, E and F reached the highest value of orotic acid on 28th day, but the highest value was determined for G and H on day 21. According to statistical evaluations, the A, G and H treatments were similar to each other, while C, D, E and F could be grouped together. These sample groups were statistically different ($P < 0.01$) from each other and from sample B. The mean orotic acid value of samples decreased ($P < 0.01$) on day 7, but showed an increase ($P < 0.01$) again at the end of the 28th day of storage (Table 3).

Table 2 Chemical composition (% w/w) and pH of the experimental probiotic yoghurts

Probiotic yoghurt samples	*Total solids*	*Fat*	*Solids nonfat (SNF)*	*Ash*	*Protein*	*pH*
A	14.19 ± 0.61	3.30 ± 0.00	10.89 ± 0.61	0.87 ± 0.07	4.31 ± 0.61	4.42 ± 0.10
B	13.88 ± 0.35	3.10 ± 0.28	11.04 ± 0.07	0.64 ± 0.04	3.48 ± 0.51	4.41 ± 0.09
C	13.97 ± 0.58	3.00 ± 0.14	10.51 ± 0.10	0.65 ± 0.02	3.48 ± 0.66	4.45 ± 0.13
D	14.19 ± 0.19	3.15 ± 0.21	10.71 ± 0.20	0.69 ± 0.05	3.46 ± 0.53	4.48 ± 0.09
E	14.46 ± 0.01	3.55 ± 0.21	10.19 ± 0.20	0.70 ± 0.08	3.74 ± 0.39	4.42 ± 0.15
F	14.30 ± 0.00	3.25 ± 0.07	9.97 ± 0.40	0.70 ± 0.07	3.55 ± 0.31	4.44 ± 0.14
G	14.68 ± 0.55	3.40 ± 0.14	10.69 ± 0.20	0.74 ± 0.07	3.88 ± 0.54	4.50 ± 0.08
H	14.42 ± 0.03	3.40 ± 0.14	10.30 ± 0.50	0.73 ± 0.11	3.81 ± 0.53	4.53 ± 0.08

Mean values ± standard deviations of probiotic yoghurts manufacturing with duplicate samples.

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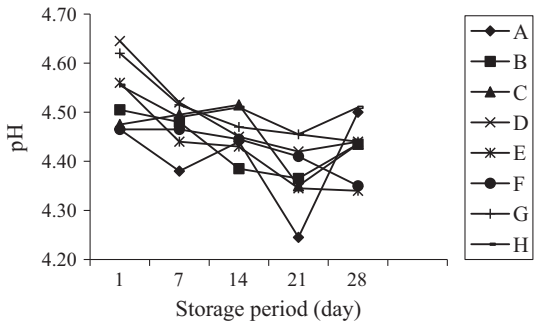


Figure 1 pH values of the probiotic yoghurt samples during storage.

Pyruvic acid occurs as an intermediate product of many reactions, in particular the metabolism of carbohydrates and proteins. At first, formation of pyruvic acid takes place in milk as a result of bacterial fermentation. Afterwards, it is converted to lactic acid and other metabolites by a series of enzymes (Fernandez-Garcia and McGregor 1994; Güzel-Seydim *et al.* 2000; Metin 2005; Ostlie *et al.* 2005). As seen in Table 3, the highest mean concentration of pyruvic acid was found in treatment G (6.90 ± 0.61 μg/g), and the lowest mean value was determined in B (5.54 ± 0.97 μg/g). On the other hand, treatments A and B were statistically similar, and C, D, E, F and H showed a similar trend with respect to pyruvic acid values. These sample groups were statistically significantly different ($P < 0.01$) from each other and from treatment G (Table 3). Generally, pyruvic acid values of the probiotic yoghurts fluctuate during storage, although the level increased in A, C, E and F on day 28, and this ariation occurring during storage was significant statistically ($P < 0.01$) (Figure 2b, Table 3).

Citric acid is the highest concentration organic acid in fresh milk, and it is found at the level of 0.2% on average (Fernandez-Garcia and McGregor 1994; Adhikari *et al.* 2002; Gueimonde *et al.* 2003; Tormo and Izco 2004; Serra *et al.* 2009). Observing Figure 2c and Table 3, the highest mean value of citric acid was found in treatment A and followed by G, H, E, F, D, C and B during storage. According to statistical evaluations, E, F, G and H were similar and B, C, D showed a similar trend, but these two groups of sample were completely different ($P < 0.01$) from each other and sample A statistically. The mean citric acid values of probiotic yoghurts decreased ($P < 0.01$) on day 7 and showed an increase ($P < 0.01$) on day 14 followed by a decrease ($P < 0.01$) until the 21st day and again increased on the 28th day of storage (Table 3).

Lactic acid is the highest concentration organic acid in fermented milks and other dairy products. Generally, it is produced on the second hour of fermentation. The formation of lactic acid is quite important for the flavour development and the protection of quality characteristics of dairy products (Fernandez-Garcia and McGregor 1994; Tormo and Izco 2004). The mean lactic acid values of probiotic yoghurt

Table 3 The mean values of organic acid properties of the experimental probiotic yoghurts and their statistical evaluations in terms of inulin%/d-whey powder% and storage period (mean ± SD)

Probiotic yoghurt samples	*Orotic acid (μg/g)*	*Pyruvic acid (μg/g)*	*Citric acid (μg/g)*	*Lactic acid (μg/g)*	*Acetic acid (μg/g)*	*Butyric acid (μg/g)*	*Hippuric acid (μg/g)*
A	$11.76 \pm 1.65^{a**}$	$5.58 \pm 1.38^{b**}$	$52.66 \pm 6.86^{a**}$	$1290.88 \pm 212.80^{a**}$	$4.89 \pm 1.12^{bc**}$	$98.38 \pm 38.93^{a**}$	$0.00 \pm 0.00^{b**}$
B	$10.34 \pm 0.42^{b**}$	$5.54 \pm 0.97^{b**}$	$43.77 \pm 2.64^{b**}$	$1045.19 \pm 74.03^{b**}$	$3.94 \pm 0.47^{d**}$	$75.49 \pm 18.22^{ab**}$	$0.00 \pm 0.00^{b**}$
C	$10.89 \pm 0.53^{ab**}$	$6.67 \pm 0.86^{ab**}$	$46.49 \pm 1.71^{b**}$	$1103.72 \pm 98.39^{b**}$	$4.61 \pm 0.28^{cd**}$	$60.79 \pm 28.28^{bc**}$	$0.64 \pm 1.06^{b**}$
D	$11.21 \pm 0.41^{ab**}$	$5.96 \pm 0.84^{ab**}$	$46.96 \pm 1.92^{b**}$	$1102.98 \pm 80.51^{b**}$	$4.68 \pm 0.18^{c**}$	$61.87 \pm 15.15^{c**}$	$0.31 \pm 0.66^{b**}$
E	$11.27 \pm 0.60^{ab**}$	$6.56 \pm 0.90^{ab**}$	$47.64 \pm 3.06^{ab**}$	$1148.83 \pm 176.52^{ab**}$	$5.02 \pm 0.50^{abc**}$	$50.40 \pm 29.61^{bcd**}$	$0.74 \pm 0.96^{b**}$
F	$11.40 \pm 1.09^{ab**}$	$6.44 \pm 0.57^{ab**}$	$47.39 \pm 4.98^{ab**}$	$1175.02 \pm 213.33^{ab**}$	$5.25 \pm 0.72^{abc**}$	$48.31 \pm 16.28^{bcd**}$	$0.32 \pm 0.68^{b**}$
G	$11.60 \pm 0.95^{a**}$	$6.90 \pm 0.61^{a**}$	$48.59 \pm 4.31^{ab**}$	$1115.08 \pm 136.33^{b**}$	$5.47 \pm 0.92^{ab**}$	$26.51 \pm 13.06^{cd**}$	$2.23 \pm 0.28^{a**}$
H	$11.83 \pm 1.02^{a**}$	$6.52 \pm 0.60^{ab**}$	$48.46 \pm 6.63^{ab**}$	$1078.78 \pm 176.26^{b**}$	$5.64 \pm 0.89^{a**}$	$16.11 \pm 6.71^{d**}$	$2.33 \pm 0.08^{a**}$
Storage period (day)							
1	$10.94 \pm 1.04^{b**}$	$7.01 \pm 0.73^{a**}$	$46.06 \pm 5.28^{b**}$	$998.63 \pm 107.08^{b**}$	$4.60 \pm 0.69^{bc**}$	41.02 ± 27.54	1.18 ± 1.12
7	$10.71 \pm 0.83^{b**}$	$6.46 \pm 0.73^{ab**}$	$45.23 \pm 3.52^{b**}$	$1038.74 \pm 118.82^{b**}$	$4.45 \pm 0.60^{c**}$	50.33 ± 32.55	0.80 ± 1.07
14	$11.37 \pm 0.79^{ab**}$	$6.19 \pm 0.92^{ab**}$	$48.56 \pm 3.89^{ab**}$	$1161.18 \pm 109.96^{a**}$	$5.08 \pm 0.82^{ab**}$	62.99 ± 35.96	0.79 ± 1.08
21	$11.52 \pm 0.92^{ab**}$	$5.82 \pm 1.03^{b**}$	$48.39 \pm 4.97^{ab**}$	$1204.62 \pm 159.02^{a**}$	$5.18 \pm 0.91^{a**}$	58.28 ± 33.59	0.67 ± 1.05
28	$11.90 \pm 1.00^{a**}$	$5.87 \pm 0.96^{b**}$	$50.48 \pm 4.95^{a**}$	$1259.63 \pm 165.14^{a**}$	$5.38 \pm 0.88^{a**}$	61.04 ± 33.66	0.66 ± 1.03

Mean values ± standart devitions of probiotic yoghurts manufacturing with duplicate samples. The letters a, b, c and d indicates means that significantly different at $P < 0.01$ level.
**$P < 0.01$.

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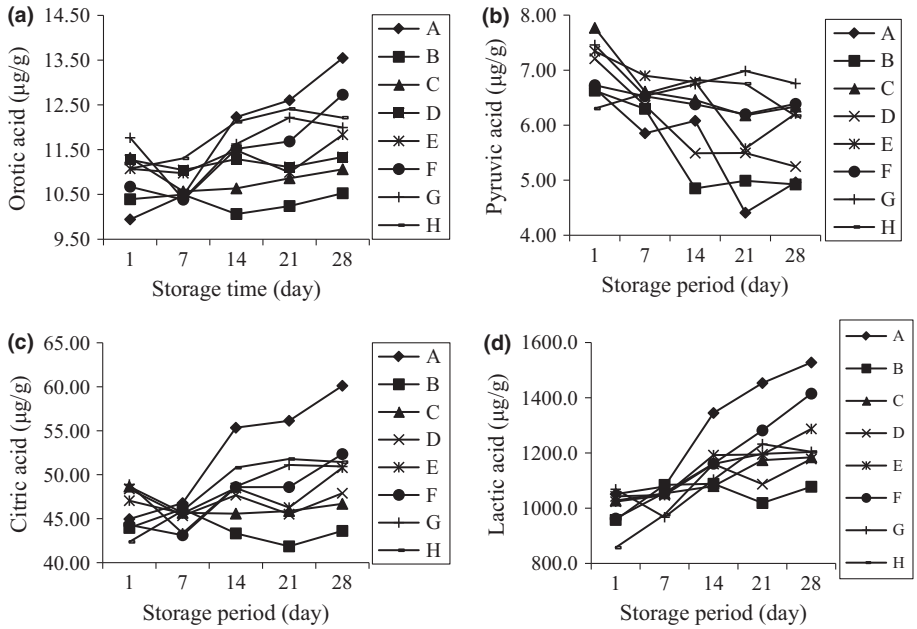


Figure 2 (a, b, c, d) Orotic acid, pyruvic acid, citric acid and lactic acid levels of the probiotic yoghurt samples during storage.

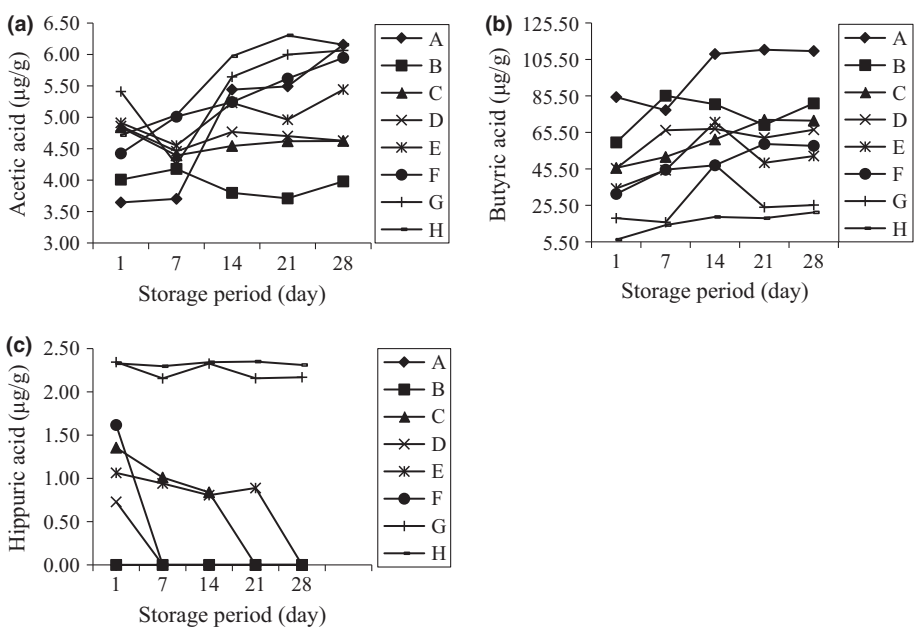


Figure 3 (a, b, c) Acetic acid, butyric acid and hippuric acid levels of the probiotic yoghurt samples during storage.

samples ranged between 1045.19 ± 74.03 µg/g (B) and 1290.88 ± 212.80 µg/g (A). Observing Table 3, B, C, D, G and H were similar, and E and F showed a similarity with respect to statistical evaluations. Contrary to this, the sample groups showed differences from each other including A at the $P < 0.01$ level during storage. On the other hand, the mean lactic concentration of probiotic yoghurt samples had a statistically significant increase during storage ($P < 0.01$) (Table 3). As can be seen from Figure 2d, A distinguished from other samples in terms of lactic acid concentration, and B had the lowest lactic acid value from the 14th day of storage.

Acetic acid is produced in milk and dairy products from citrate, lactose and amino acids (Aston and Dulley 1982). In general, it occurs in fermented milks as a result of *Bifidobacterium* sp. activity and shows a suppressing effect on the harmful bacteria due to its strong bactericidal properties. However, acetic acid gives an undesirable vinegar taste and aroma to the product when present in excess amount (Buffa *et al.* 2004). Therefore, acceptability of fermented milks shows a very rapid

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decline during storage (Gaafar 1992). As seen in Table 3, the highest mean acetic acid value (5.64 ± 0.89 μg/g) was found in H and followed by G, F, E, A, D, C and B (3.94 ± 0.47 μg/g). The acetic acid amounts of probiotic yoghurts showed a fluctuation during storage, but generally an increase was determined on the 28th day (Figure 3a). As seen in Table 3, E and F showed a similar trend with respect to statistical evaluations, but the other samples showed statistically significant differences ($P < 0.01$) from each other and these two samples. On the other hand, the mean acetic acid concentrations of probiotic yoghurts increased to the 28th day of storage, and this increase was found to be significant statistically ($P < 0.01$).

Butyric acid is found in dairy products as a result of the raw material, microbial activity, biochemical reactions, deamination of amino acids and the hydrolysis of milk fat during fermentation (Bouzas *et al.* 1993; Molkentin and Precht 1998; Metin 2005). According to Table 3, butyric acid concentration of probiotic yoghurt samples was between the 16.11 ± 6.71 μg/g (H) and 98.38 ± 38.93 μg/g (A). Observing Table 3, E and F were similar in between, but other samples showed statistically significant differences ($P < 0.01$) from each other and these two samples in terms of butyric acid values. As seen from Figure 3b, A had the highest butyric acid amount followed by B, C, D, F, E, G and H on the 28th day of storage.

Hippuric acid is defined as the nonprotein structure component of milk, and is produced at the end of lactic acid fermentation in dairy products. Afterwards, it is converted to benzoic acid by lactic acid bacteria during the fermentation process (Patton 1953; Fernandez-Garcia *et al.* 1998; Heller 2001; Qi *et al.* 2009). Complete transformation of hippuric acid to benzoic acid is extremely important for the protection of the shelf life of yoghurt (Fernandez-Garcia and McGregor 1994). Observing Table 3, the highest mean concentration of hippuric acid was found in H (2.33 ± 0.08 μg/g), and the lowest mean values (0.00 ± 0.00 μg/g) were determined in A and B. On the other hand, Torre *et al.* (2003) found the amount of hippuric acid between the 1 and 32 μg/g values in yoghurts produced using different starter cultures. As seen in Figure 3c, hippuric acid was not determined in A and B during the storage period. However, it was not determined in C from the 21st day, not identified in D and F from the 7th day and not detected in E on the 28th day of storage. The G and H had the highest hippuric acid value when they compared with the other samples, and these samples of hippuric acid contents remained almost stable during storage period. According to Table 3, there were statistically significant ($P < 0.01$) differences between the groups in terms of hippuric acid content. The A, B, C, D, E and F were similar to each other, and samples G and H showed a similar trend with respect to statistical evaluations. However, these sample groups showed differences from each other at the $P < 0.01$ level.

It was possible to say that the concentrations of orotic, pyruvic, citric, lactic, acetic and hippuric acid, but not of butryric acid, were generally increased when the proportion of d-whey powder was high. This situation may be related to the lactose and protein used by lactic acid bacteria in d-whey powder (Beshkova *et al.* 2003). Similarly, Fernandez-Garcia and McGregor (1994), Adhikari *et al.* (2002), Bruno *et al.* (2002), Desai *et al.* (2004) and Tormo and Izco (2004) reported that the concentration of organic acids produced by starter micro-organisms was dependent on prebiotic agents, their ratios and types of culture.

CONCLUSIONS

The results showed that variation in the proportions of inulin and d-whey powder added to milk affected the composition and profiles organic acid of probiotic yoghurt samples. Higher levels of organic acids, except for butyric acid, were found when the proportion of demineralised whey powder was high, except for butyric acid. Orotic acid, citric acid, lactic acid and acetic concentrations increased during storage, while pyruvic acid and hippuric acid levels decreased. Butyric acid levels fluctuated during storage. The organic acid profiles of the inulin plus demineralised whey powder combinations (Treatments C–G) followed a similar pattern to demineralised whey powder on its own (treatment H), but were different from inulin on its own (treatment B).

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