

Determination of Certain Quality Characteristics of Probiotic Yoghurts Produced with Different Prebiotic Combinations During Storage

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ABSTRACT

In this study, eight probiotic yoghurt batches were prepared with inulin and demineralised whey powder combinations (d-whey powder) and ABT-2 (*Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium* sp.) as a starter culture. The addition of inulin, demineralised whey powder and their different combinations influenced the total solid, fat, protein, ash, viscosity, syneresis, titratable acidity values, *L. acidophilus* + *Bifidobacterium* sp. counts and appearance, consistency and flavour scores at the level of $p < 0.01$, while they affected the odour scores at level of $p < 0.05$.

Key Words: Yoghurt, Probiotic starter culture (ABT-2), Inulin, D-whey powder, Storage period

Farklı Prebiyotik Kombinasyonlar ile Üretilen Probiyotik Yoğurtların Önemli Kalite Karakteristiklerinin Depolama Süresince Belirlenmesi

ÖZET

Bu çalışmada, 8 probiyotik yoğurt örneği inulin ve demineralize peynir altı suyu tozu kombinasyonları ve ABT-2 (*Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium* sp.) starter kültürü kullanılarak üretilmiştir. İnülin, demineralize peynir altı suyu tozu ve bunların farklı kombinasyonları toplam kuru madde, yağ, protein, kül, viskozite, sinerezis, titrasyon asitliği değerlerini, *L. acidophilus* + *Bifidobacterium* sp. sayıları ile görünüş, kıvam ve lezzet skorlarını $p < 0.01$ düzeyinde etkilerken, koku skorlarını $p < 0.05$ düzeyinde etkilemiştir.

Anahtar Kelimeler: Yoğurt, Probiyotik starter kültür (ABT-2), İnülin, D-peyniraltı suyu tozu, Depolama periyodu

INTRODUCTION

Probiotics are defined as live microbial food supplements and when used in adequate amounts provide health benefits to the host by improving its intestinal microbial balance. In addition, they can potentially provide beneficial effects for consumers following their consumption [1, 2]. *L. acidophilus* and *Bifidobacterium* sp. are probiotic bacteria and have several health benefits include improving the modulation of the body's immune system, lactose digestion, control

of serum cholesterol level, reduction in occurrence of diarrhoea, production of antitumor activity, control of intestinal infections and lower cholesterol in humans [3-5]. For probiotic microorganisms to provide therapeutic benefits, it has been suggested that products should contain at least 10^7 - 10^8 cfu/g bacteria [6, 7]. Also, fermented milks containing probiotic microorganisms are generally categorised as functional foods [8-10].

Prebiotics are non-digestible dietary components that electively stimulate the growth and/or activity of a limited

number of useful microorganisms in the colon and also show a beneficial effect to the host health. At the same time, they have a protective effect on probiotic bacteria and improve their survival and activity in the product during storage [11]. In recent years, inulin and whey powder are probably the most commonly used prebiotics in fermented milks. Inulin is a natural component of several fruits and vegetables; it is generally obtained from chicory roots by an extraction method. Inulin is an indigestible carbohydrate consisting of fructooligosaccharides and is also considered to have prebiotic properties such as the ability to stimulate probiotic bacteria [12-14]. Whey powder is another substance used as prebiotics that contains biologically active proteins, minerals and vitamins (especially vitamin B2) and it stimulates the growth of probiotic bacteria and improves culture viability [15]. Conversely, it has many benefits when added to yoghurt. These benefits include: improved flavour of the product, prebiotic effects on probiotics, nutraceutical health benefits, texture improvement, nutritional enrichment, reduced syneresis and extended shelf-life of yoghurt [16].

Numerous studies have been carried out on probiotic yoghurts, although there is no study about the different rates of whey powder and inulin effects on some physical, chemical, microbiological and sensorial properties of probiotic yoghurts. The aim of this study was to determine the changes in some physical, chemical, microbiological and sensory properties of probiotic yoghurts made with a commercial probiotic starter culture (ABT-2) and 2 different prebiotics (inulin and d-whey powder) during storage.

MATERIALS and METHODS

Materials

Raw cow's milk used in the manufacture of probiotic yoghurt was obtained from the dairy farm. Skim Milk Powder (SMP) was purchased from local shops, inulin (Orafti®HPX) was bought from the Artisan Food Industry Inc. and d-whey powder was provided by Maybe Malkara Union Milk and Milk Products Inc. A commercial probiotic starter culture (ABT-2) was used (Peyma-Chr. Hansen, Istanbul, Turkey) containing *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* sp.

Manufacture of Experimental Yoghurts

In this study, processed milk for probiotic yoghurts, after clarification was heated to 90°C for 10 minutes, cooled to 37±1°C and divided into eight equal parts (5 L sterile glass cups) coded with letters from A to H. One batch of milk was then taken as the control, sample A (control) [dry matter contents of sample A was standardised to 3% skimmed milk powder] and the remaining batches were prepared with 7 different ratios (inulin %/demineralised whey powder %, 0/0, 3.0/0, 2.5/0.5, 2.0/1.0, 1.5/1.5, 1.0/2.0, 0.5/2.5 and 0/3.0) inulin and demineralised whey powder (d-whey powder). Then the milks were inoculated with ABT-2 starter culture at the rate of 0.03% (w/w) and all inoculated milks were

divided into parts of approximately 200 mL. Inoculated milks were incubated at 37 ± 1°C until the pH reached 4.6 (approximately 10 hour), and were then stored for 24 hours at 4°C. The analyses were carried out after 1, 7, 14, 21 and 28 days of storage. Two trials were carried out, and the results are the means of these trials.

Physical and Chemical Analysis

Total solid and ash contents of milk, SMP, inulin, d-whey powder and experimental probiotic yoghurts were determined using the gravimetric method, fat content by the Gerber method, and protein by the Kjeldahl method as described by Kurt *et al.* [17]. The apparent viscosities of yoghurt samples were measured during storage using Visco Star-L Fungilab visco-meter equipped with a number 6 spindle. All of the measurements were taken in duplicate and the sample temperature was 4±1°C. The samples were stirred gently for 10 seconds before the viscosity measurement (cP). The readings were taken by an instrument directly at the point of the 30th second and were recorded in centipoise [18]. Syneresis was estimated using a drainage test according to Atamer and Sezgin [19]. 25 g probiotic yoghurt sample was weighed and filtered at 4 ± 1°C and after 2 hours of drainage; the volume of filtrate collected in a graduated cylinder was measured and used as an index of syneresis. The pH was measured using a pH meter (Model WTW pH-340-A, Weilheim, Germany) fitted with a combined glass electrode. The titratable acidity was determined as lactic acid percentage by titrating with 0.1N NaOH, using phenolphthalein as an indicator [17].

Microbiological Analysis

The counts of *S. thermophilus* were enumerated on M17 agar (Oxoid Ltd, Basingstoke, Hampshire, UK) by incubating the plates aerobically at 37°C for 24 ± 1 hour [20, 21]. MRS agar (Oxoid) was used for enumeration of the total viable counts of *L. acidophilus* + *Bifidobacterium* sp. [21-23]. *L. acidophilus* counts were determined using MRS-Bile agar. For this purpose, MRS agar (Oxoid Ltd) was prepared with 1.5 g/L bile (Bile, Fluka). Alternatively, selective enumeration of *Bifidobacterium* sp. was performed on MRS- cysteine agar that was prepared with 1 ml 0.5 g/L cysteine (Fluka) [21-23]. The plates of *L. acidophilus* cultures were incubated under aerobic conditions at 37°C for 72 ± 1 hour). Conversely, the plates of *L. acidophilus* + *Bifidobacterium* sp. and *Bifidobacterium* sp. cultures were anaerobically (Anerocult A system; Merck, Darmstadt, Germany) incubated at 37°C for 72 ± 1 hour [7, 24].

Sensory Analysis

A group of six panellists evaluated the experimental probiotic yoghurts by grading them on a scale of 1–5 (unacceptable/ excellent) on days 1, 7, 14, 21 and 28 day of storage [25]. The probiotic yoghurts were graded for four sensory attributes including appearance, consistency, odour and flavour. All these attributes were recorded on a 1 (poor) to 5 (excellent) point scales.

Water and bread were also given to the panel members to cleanse their mouths between samples.

Statistical Analysis

Statistical analysis was performed according to a randomised complete block design by a 2 prebiotic agent (inulin and d-whey powder) \times 8 different prebiotic agent ratios \times 5 (storage period) in factorial experiment. The data was analysed statistically using SPSS statistical software programme version 13 (SPSS, Chicago, Illinois, USA) [26]. Analysis of variance (ANOVA) and mean with significant differences were compared by Duncan's multiple range tests ($p < 0.05$,

$p < 0.01$). The data analysed is presented as the mean \pm standard deviation (mean \pm SD).

RESULTS and DISCUSSIONS

Physicochemical Characteristics of the Experimental Probiotic Yoghurts

The general compositions of cow's milk, SMP, inulin and d-whey powder used for the probiotic yoghurt production are shown in Table 1. Changes in some physicochemical properties of the experimental probiotic yoghurts are presented in Table 2.

Table 1. The mean values of some properties of raw cow's milk, SMP, inulin and d-whey powder

Samples	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	Titratable acidity (%)	pH
Cow's milk	12.13	3.48	3.11	0,66	0,19	6,52
SMP	94.79	0.20	25.85	15.40	0.21	6.16
Inulin	97.00	-	-	0.20	0.21	6.00
d-whey powder	97.00	1.00	8.50	4.00	0.20	6.20

When the dry matter content of samples were observed, the highest mean value was found in H while, the lowest mean value was determined in sample B. The differences among the samples and storage periods were significant ($p < 0.01$) statistically (Table 2). The dry matter content of probiotic yoghurt samples was different from each other statistically. This situation might be explained by the proportional differences of prebiotics added to each sample and the changes of dry matter values in yoghurts. The highest fat content was determined in sample E during storage, followed by samples H, A, G, C, F, D and B respectively. The fat content of all samples showed parallelism with the increment of dry matter, during storage. The mean protein content of the groups ranged between $4.31 \pm 0.61\%$ (A) to $3.46 \pm 0.53\%$ (B) values and differences among the samples and storage periods were found to be significant ($p < 0.01$) statistically (Table 2). Conversely, the highest ash content was determined in sample A (control) while, the lowest mean value was found in sample D. The next highest ash contents were determined in the samples that produced with d-whey powder respectively. As shown in Table 2, the ash contents of probiotic yoghurts were affected by the used prebiotics ($p < 0.01$) and storage periods ($p < 0.05$) statistically. Due to the results, it was possible to say that d-whey powder was more effective than inulin in terms of increasing dry matter, ash, fat and protein contents.

The highest mean value of viscosity (6605 ± 818.35) was found in sample A and the lowest mean value (4275 ± 439.86) was in sample D. According to statistical evaluations, samples B, C, D, E, F, G and H showed a similar trend with respect to statistical evaluations, although sample A was completely different ($p < 0.01$) from them statistically. The mean viscosity value of probiotic yoghurts increased up to the 14th day but, decreased on the 21th day and increased again on the 28th day of storage, although this situation was not significant statistically (Table 2). The apparent differences in the viscosity of samples might be due to

the used prebiotic ratios. Because, the viscosity value of probiotic yoghurts rose with the increment of total solid content. Viscosity of experimental probiotic yoghurts showed a change depending on the time. This situation might be explained by the non-Newtonian and thixotropic flow behaviour of yoghurts. Also, this increase could be attributed to the conjunction of fat globules and aggregation of some proteins at low temperatures [27, 28].

Serum separation is known as "syneresis" and it is defined as a spontaneous water release from the gel caused by gel contraction [28, 29]. The highest mean value (6.43 ± 0.57 mL/25g) of syneresis was found in sample H and the lowest mean value (4.50 ± 0.45 mL/25g) was determined in sample A. The syneresis value of probiotic yoghurts were affected significantly ($p < 0.01$) by the prebiotic ratio and storage time (Table 2). As seen in Table 2, sample A (control) was first followed by samples B, C, D, E, F, G and H, respectively during storage. According to statistical evaluations A and B yoghurts were similar to each other, and F and G yoghurts showed a similar trend with respect to statistical evaluations. Conversely, samples C, D, E and H differed from each other and the other samples ($p < 0.01$) statistically. The mean syneresis value of yoghurts decreased ($p < 0.01$) on day 7, but showed an increase ($p < 0.05$) on day 14 followed by a decrease ($p < 0.01$) until the 28th day of storage. However, the first day of storage was different from the other days statistically ($p < 0.01$) (Table 2). The obtained results also showed that inulin was more effective than d-whey powder in terms of preventing whey separation. The authors reported that inulin had a high water-binding capacity [30]. Similar results were also recorded by Staffolo et al. [31].

For the development of the desired yoghurt flavour and aroma, titratable acidity values should be between certain limits. This value is dependent on the lactose fermentation and dry matter content of yoghurts [32, 33]. In groups, the highest mean concentrations of titratable

acidity (Lactic acid%) content was found in sample A (1.10±0.10%) and the lowest values were determined in samples B and D. The obtained results were similar to Bonczar *et al.* [33], Puvanenthiran *et al.* [34]'s findings but, lower than the values determined by Kuçukoner and Tarakci [35]. There were significant differences (p<0.01) between the sample A (control) and the other probiotic yoghurts in terms of titration acidity values. The mean titration acidity values of samples showed an irregular variation during storage. The highest mean value was determined on the 28th day of storage but, this was not

significant statistically (Table 2). Generally, titratable acidity values of the probiotic yoghurt samples showed a similar changing during storage period except for sample A. This could be explained with dry matter, protein, phosphate, citrate, lactate, and mineral contents of probiotic yoghurts, different inulin%/d-PAS% ratios and tampon capacity of d-whey powder [33, 34].

Table 2. The mean values of certain physicochemical properties of the experimental probiotic yoghurts and their statistical evaluations in terms of inulin%/d-whey powder% ratios and storage period (days)

Experimental probiotic yogurts	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	Apparent viscosity (cP)	Syneresis (mL/25 g)	Titratable acidity (%)	pH
A [control 0% inulin and 0% d-whey powder]	13.92±0.32 ^{bc}	3.53±0.22 ^{ab}	4.31±0.61 ^a	0.89±0.40 ^a	6605±818.35 ^a	4.50±0.45 ^d	1.10±0.10 ^a	4.40±0.11
B [3% inulin and 0% d-whey powder]	13.47±0.30 ^d	3.26±0.22 ^b	3.48±0.51 ^b	0.68±0.03 ^d	4455±669.35 ^b	4.61±0.71 ^d	0.82±0.07 ^b	4.43±0.76
C [2.5% inulin and 0.5% d-whey powder]	13.58±0.42 ^{cd}	3.52±0.38 ^{ab}	3.48±0.66 ^b	0.69±0.03 ^d	4865±646.38 ^b	5.06±0.62 ^{cd}	0.86±0.05 ^b	4.45±0.14
D [2% inulin and 1% d-whey powder]	13.91±0.27 ^{bc}	3.40±0.19 ^{ab}	3.46±0.53 ^b	0.70±0.03 ^d	4275±439.86 ^b	5.39±0.91 ^c	0.82±0.81 ^b	4.50±0.98
E [1.5% inulin and 1.5% d-whey powder]	14.12±0.27 ^{ab}	3.63±0.20 ^a	3.74±0.39 ^{ab}	0.73±0.39 ^{bcd}	4725±443.00 ^b	5.67±0.36 ^{bc}	0.88±0.61 ^b	4.42±0.17
F [1% inulin and 2% d-whey powder]	14.08±0.37 ^{ab}	3.44±0.15 ^{ab}	3.55±0.31 ^b	0.73±0.05 ^{cd}	4635±578.82 ^b	6.16±0.65 ^{ab}	0.90±0.51 ^b	4.43±0.15
G [0.5% inulin and 2.5% d-whey powder]	14.27±0.34 ^{ab}	3.51±0.14 ^{ab}	3.88±0.54 ^{ab}	0.77±0.32 ^{bc}	4995±486.17 ^b	6.26±0.65 ^{ab}	0.89±0.62 ^b	4.50±0.89
H [0% inulin and 3% d-whey powder]	14.42±0.22 ^a	3.59±0.38 ^a	3.81±0.53 ^{ab}	0.79±0.05 ^b	4655±877.32 ^b	6.43±0.57 ^a	0.87±0.66 ^b	4.50±0.61
Storage time (days)								
1	14.26±0.38 ^a	3.27±0.22 ^c	3.28±0.60 ^b	0.72±0.08 ^b	4846.88±884.39	6.08±0.95 ^a	0.87±0.11	4.54±0.14 ^a
7	14.05±0.32 ^{ab}	3.38±0.19 ^{bc}	3.64±0.62 ^{ab}	0.75±0.07 ^{ab}	4906.25±782.07	5.42±0.72 ^b	0.90±0.12	4.47±0.10 ^{ab}
14	13.76±0.34 ^b	3.53±0.17 ^{ab}	3.92±0.22 ^a	0.75±0.08 ^a	5125.00±939.86	5.56±0.92 ^b	0.87±0.83	4.46±0.82 ^{ab}
21	13.95±0.42 ^b	3.69±0.32 ^a	3.88±0.37 ^a	0.76±0.07 ^a	4768.85±1036.80	5.43±0.86 ^b	0.92±0.12	4.38±0.12 ^b
28	13.84±0.52 ^b	3.56±0.19 ^{ab}	3.85±0.68 ^a	0.76±0.08 ^a	4921.88±961.07	5.07±0.97 ^b	0.91±0.09	4.43±0.10 ^b

Different letters indicate significant differences (p<0.01) between experiments and days of storage.

As seen in Table 2, the highest mean pH values were determined in D, G and H, and these samples followed by C, B, F, E and A, respectively during storage. It can be said that the addition of d-whey powder in yoghurts caused an increase in pH values. This situation might be explained with buffering capacity of serum proteins in d-whey powder [16]. As seen in Table 2, there were no significant differences between the groups in terms of pH, although the storage periods effect was significant (p<0.01) statistically. The pH values of samples showed fluctuations during storage and they had the lowest pH value after 2 days of storage. This observed reduction in pH could be explained by further metabolic activity of the starter cultures during storage [9]. However, the determined increase at the end of storage might be due to the alkaline compounds forming as a result of proteolytic degradation, yeast and moulds activities and the compositional characteristics of prebiotics [36].

Microbiological Characteristics of the Experimental Probiotic Yoghurts

Observing Table 4, the highest mean *S. thermophilus* count was found in F, while the lowest mean value was determined in B. Similar results were also reported by Vinderola *et al.* [7] and Dave and Shah [20]. From these results, it might be said that the highest degree of d-

whey powder with inulin combinations added to the probiotic yoghurts caused a positive effect on the *S. thermophilus* count during storage. Similarly, Dave and Shah [20]. reported that the addition of prebiotics (whey powder and protein concentrates) to ABT yoghurts stimulated the growth of probiotic bacteria. Conversely, there were no statistically significant differences among the probiotic yoghurt samples with respect to *S. thermophilus* counts. The mean *S. thermophilus* counts of probiotic yoghurts increased up to the 21th day but, decreased again on day 28 of storage, but this was not significant statistically (Table 3).

As seen in Table 3, the highest mean *L. acidophilus* + *Bifidobacterium* sp. count (7.51±0.22 log cfu/g) was found in sample A, while the lowest mean count (7.09 log cfu/g) was found in sample H. Sample A followed by F, E, C, B, D, G and H samples, respectively during storage. It was observed that a higher addition of inulin but lower d-whey powder leads to an increase in the viable counts of these bacteria. Similarly, Kailasapathy *et al.* [16] and Shah [37] also reported that the growth and survival of probiotic bacteria have also been found to be affected by the composition of milk, milk solids content and the availability of prebiotics. According to Table 3, there were statistically significant (p<0.01) differences between the groups in terms of *L.*

acidophilus + *Bifidobacterium* sp. count, although the storage period effect on log cfu/g was not significant statistically. Conversely, total viable counts of *L. acidophilus* + *Bifidobacterium* sp. decreased slightly to 21th day and increased again on day 28 of storage but, remained at sufficient levels (> 6-7 log cfu/g) until day 28. During storage, B, C and D samples were similar to each other, and E and F samples showed a similar trend with respect of statistical evaluations. Contrary to, this sample groups differed from in each other and other samples at the p<0.01 level.

It was observed that an addition of equal amounts of inulin and d-whey powder leads to an increase in the viable count of *L. acidophilus*. This bacteria count remained in all probiotic yoghurt samples at sufficient levels (> 7 log cfu/g) during the storage period for the occurrence of the therapeutic effect [16, 38]. As seen in Table 3, the highest count was determined in sample A, followed by E, H, C, F, G, D and B samples, respectively. The differences among the yoghurt batches, including the control group, were not significant statistically. Due to the obtained results, it might be said that balanced addition of inulin and d-whey powder to the probiotic yoghurts were more effective than the individual use of this prebiotics. Some authors reported that the supplementation of prebiotics might have showed a stimulatory effect on the growth of probiotic bacteria [39, 40]. Among storage periods, significant differences were observed in terms of *L. acidophilus* counts (p<0.01). The counts of *L. acidophilus* in the

experimental probiotic yoghurt samples exhibited the highest logarithmic value on the 1th day of storage and *L. acidophilus* counts of samples showed a reduction on the 21th day but, increased again on day 28 (Table 3).

Bifidobacterium sp. counts were initially present between the levels of 7.14±0.29 log cfu/g and 7.47±0.36 log cfu/g for yoghurts C and F, respectively. It was found that a higher percentage of d-whey powder with inulin in yoghurts enhanced the growth and survival of *Bifidobacterium* sp. This situation could be attributed to the sulphur amino acids in d-whey powder. These amino acids are provided to the desired anaerobic conditions in yoghurts and caused an increase in the number of viable bacteria. Because, *Bifidobacteria* sp. obligate anaerobe and it can tolerant oxygen in the presence of carbon dioxide (CO₂). Therefore, bifidogenic factors are added to the fermented milks helped to the *Bifidobacterium* sp. for reaching to the desired levels of growth during storage [41, 42]. Similarly, Lucas *et al.* [9]. and Kneifel *et al.* [43] reported that the addition of protein hydrolysed to the fermented dairy products gave rise to positive effects on the viability of probiotics. According to statistical evaluations, the effects of different levels of inulin and d-whey powder on *Bifidobacterium* sp. counts were not significant while, the effect of storage period was significant (p<0.05), statistically. Observing the Table 3, the 1th and 28th days of storage showed differences to each other and other periods statistically (p<0.05).

Table 3. The mean values of viable bacteria counts of the experimental probiotic yoghurts and their statistical evaluations in terms of inulin%/d-whey powder% ratios and storage time (days)

Experimental probiotic yogurts	<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> count (log cfu/g)	<i>Lactobacillus acidophilus</i> + <i>Bifidobacterium</i> sp. count (log cfu/g)	<i>Lactobacillus acidophilus</i> count (log cfu/g)	<i>Bifidobacterium</i> sp. count (log cfu/g)
A (control) 0% inulin and 0% d-whey powder]	7.92±0.52	7.51±0.22 ^a	7.17±.41	7.38±0.24
B [3% inulin and 0% d-whey powder]	7.65±0.72	7.27±0.20 ^{abc}	6.68±0.83	7.16±0.51
C [2.5% inulin and 0.5% d-whey powder]	7.82±0.36	7.35±0.17 ^{abc}	7.09±0.54	7.14±0.29
D [2% inulin and 1% d-whey powder]	7.71±0.53	7.25±0.21 ^{abc}	7.04±0.70	7.24±0.34
E [1.5% inulin and 1.5% d-whey powder]	7.97±0.50	7.40±0.32 ^{ab}	7.14±0.52	7.27±0.36
F [1% inulin and 2% d-whey powder]	7.98±0.49	7.44±0.34 ^{ab}	7.05±0.58	7.47±0.36
G [0.5% inulin and 2.5% d-whey powder]	7.87±0.56	7.14±0.15 ^{bc}	7.05±0.20	7.31±0.34
H [0% inulin and 3% d-whey powder]	7.72±0.53	7.09±0.20 ^c	7.11±0.26	7.24±0.18
Storage time (days)				
1	7.27±0.69 ^b	7.36±0.26	7.44±0.61 ^a	7.46±0.28 ^a
7	7.80±0.29 ^{ab}	7.32±0.31	6.91±0.40 ^b	7.21±0.30 ^{ab}
14	7.80±0.34 ^{ab}	7.20±0.22	6.89±0.70 ^b	7.21±0.20 ^{ab}
21	8.10±0.45 ^a	7.24±0.24	6.88±0.28 ^b	7.38±0.38 ^{ab}
28	8.05±0.28 ^a	7.41±0.25	7.08±0.42 ^{ab}	7.13±0.42 ^b

Different letters indicate significant differences (p<0.01) between experiments and days of storage.

Sensory Evaluations of the Experimental Probiotic Yoghurts

The mean scores of the experimental probiotic yoghurts in terms of the sensory characteristics are presented in Table 4. The addition of inulin and d-whey powder to the probiotic yoghurts in different proportions affected to the scores of appearance, consistency and flavour at $p < 0.01$ level, while their effect on the odour scores was $p < 0.05$ level. However, no significant effect was

observed on the odour scores by the addition of inulin and d-whey powder. It was also observed that the storage time effected to the all sensory parameters statistically ($p < 0.01$) except for odour scores. As seen in Table 4, the highest appearance, odour and flavour scores were determined in sample B (3% inulin/0% d-whey powder), although sample A (control) was the most popular in terms of consistency scores and it was followed by sample B, respectively.

Table 4. The mean values of sensory characteristics of experimental probiotic yoghurts and their statistical evaluations in terms of inulin%/ d-whey powder% ratios and storage time (days)

Probiotic yogurts	Appearance	Consistency	Odour	Flavour
A (control) 0% inulin and 0% d-whey powder]	4.32±0.37 ^a	4.47±0.28 ^a	4.29±0.46 ^a	4.14±0.49 ^a
B [3% inulin and 0% d-whey powder]	4.45±0.37 ^a	4.33±0.23 ^{ab}	4.35±0.26 ^a	4.17±0.35 ^a
C [2.5% inulin and 0.5% d-whey powder]	4.24±0.29 ^{ab}	4.19±0.18 ^{abc}	4.16±0.32 ^{abc}	3.94±0.39 ^{ab}
D [2% inulin and 1% d-whey powder]	4.19±0.34 ^{ab}	4.03±0.21 ^{bc}	4.19±0.31 ^{ab}	4.02±0.26 ^{ab}
E [1.5% inulin and 1.5% d-whey powder]	4.19±0.31 ^{ab}	4.07±0.32 ^{abc}	4.07±0.39 ^{abc}	3.99±0.34 ^{ab}
F [1% inulin and 2% d-whey powder]	4.09±0.40 ^{ab}	3.98±0.39 ^{ab}	4.04±0.33 ^{abc}	3.83±0.26 ^{ab}
G [0.5% inulin and 2.5% d-whey powder]	3.85±0.50 ^b	3.75±0.41 ^c	3.88±0.23 ^{bc}	3.68±0.25 ^b
H [0% inulin and 3% d-whey powder]	3.85±0.46 ^b	3.75±0.56 ^c	3.76±0.44 ^c	3.63±0.16 ^b
Storage time (days)				
1	4.47±0.25 ^a	4.38±0.27 ^a	4.12±0.29	4.08±0.33 ^a
7	4.38±0.39 ^a	4.15±0.36 ^{ab}	4.32±0.32	4.07±0.33 ^a
14	4.03±0.23 ^b	4.00±0.24 ^b	4.12±0.27	3.91±0.32 ^{ab}
21	3.98±0.45 ^b	4.00±0.38 ^b	3.99±0.32	3.90±0.38 ^{ab}
28	3.88±0.41 ^b	3.83±0.54 ^b	3.92±0.56	3.67±0.34 ^b

Different letters indicate significant differences ($p < 0.01$, $p < 0.05$) between experiments and days of storage.

CONCLUSIONS

All batches of probiotic yoghurts made with inulin, d-whey powder and a commercial probiotic starter culture (ABT-2) revealed different patterns in manufacture and storage time. The addition of inulin and d-whey powder at different levels did not have any marked effect on the pH, viable counts of *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* sp. The values of total solid, fat, ash, protein, apparent viscosity, syneresis, titratable acidity, viable counts of *L. acidophilus* + *Bifidobacterium* sp., scores of appearance, consistency, flavour and odour were significantly affected by the added inulin and d-whey powder. Conversely, the values of the total solid, ash, fat, protein, syneresis, pH, viable counts of *S. thermophilus*, *L. acidophilus*, *Bifidobacterium* sp. and scores of appearance, consistency and flavour were affected significantly from storage time. However, its effect on apparent value of viscosity, titratable acidity, viable counts of *L. acidophilus* + *Bifidobacterium* sp. and scores of odour not significant statistically. As a result, it might be said that inulin, d-whey powder and their

different combinations helped to the preserve of lower limit (10^6 - 10^7 log cfu/g) of probiotic cultures (ABT-2) necessary for the therapeutic affect during storage. Observing the results, inulin and d-whey powder combinations (especially samples C, D, and F) were found to be more useful and effective in all investigated parameters than their single use. Sample A and samples containing high amounts of d-whey powder (especially samples F, G and H) came to the fore in terms of physical, chemical and microbiological parameters during storage. Conversely, sample A and the samples produced with a high percentage of inulin (especially sample B) were more appreciated in terms of sensorial properties.

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