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Effect of whey fractions on microbial and physicochemical properties of probıotıc ayran (drinkable yogurt)

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Abstract

The purpose of this study was to improve the functional properties of ayran that are important for nutrition of Turkish society. For this purpose, concentrated whey (normal and demineralized) and whey protein powder were added to the milk of ayran. *Lactobacillus acidophilus* was used as probiotic starter culture in samples. Whey concentrates significantly affected the physicochemical and probiotic properties of ayran. The dry matter, protein and ash contents in whey concentrates added ayran samples showed significant increases. Again, the amounts of mineral matter of the samples increased significantly. *L. acidophilus* counts in ayran samples produced with 4% normal or demineralized whey concentrates were the highest ones. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* counts in treated ayran samples were also higher than that of control group. It can be said that addition of concentrated whey and probiotic culture improved the functional properties of ayran. Produced ayran samples have got to probiotic and functional food properties.

Introduction

The ayran is traditionally manufactured with separated of fat from water-added yoghurt in churns by hand. Ayran is separated from other fermented milk beverages being a yoghurt drink with salt and without any fruit flavoring. The Shelf life of ayran is reported as 10–15 days at 4°C by the manufacturers. Ayran have probiotics properties, depending on the culture used in the production (Aysal, 2008). Probiotics, which are regulated as dietary supplements and foods, consist of bacteria or yeast. Probiotic products may contain a single microorganism or a mixture of several species. The most widely used probiotics include lactic acid bacteria, specifically *Lactobacillus* and *Bifidobacterium* species (Williams, 2010). However, probiotic strains may be used with a support culture, like *Str. thermophilus* or another yoghurt culture. Also FAO/WHO has adopted the definition of probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". *L. acidophilus* nonpathogenic and a member of the normal intestinal microflora is widely used in fermented dairy products and is of considerable industrial (Deraz *et al*., 2007).

The probiotic product must have counts vary from 5.00 to 7.00 log CFU.g^{-1} of probiotic bacteria at use-by date. Properties such as growth rate, metabolic rate proteolytic activity and flavour promotion of several probiotic strains are species specific and success in industrial applications may be obtained through careful management of these factors and tailored manufacturing processes. Production of high-quality fermented milk products containing *Bifidobacterium* spp. and *L. acidophilus* is, according to Gomes and Malcata (1999), a challenge to dairy plants owing to the sensitive character of the microorganisms.

Three additional components of whey have been found to beneficially affect prebiotic formulation. The first is a protein called glycomacropeptide (GMP). GMP is derived from the partial enzymatic breakdown of kappa-casein during cheese production, becoming a component of whey. GMP has been shown to support the growth of bifidobacteria. The second whey-derived prebiotic is lactoferrin, which has been shown to support the growth of bifidobacteria and lactobacilli. Interestingly the third whey component with prebiotic potential is a mineral that is found in high amounts within all quality whey protein formulations (Splechtna *et al*., 2007).

As today greater attention is focused on a healthy lifestyle and functional and organic food, the attention should be drawn to the natural whey. To enrich whey and therefore increase its nutritional value, probiotic microorganisms are one of the best choices for production of a fermented whey beverage. Developing probiotic food and feed is a key research and development area for functional food markets.

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Considering its potential, the aim of this study was to define the growth and survival of probiotic bacteria in ayran and the influence of addition of whey concentrate on it, for possible production of a nutritive highly valuable drink.

Material and Methods

Material

L. delbrueckii subsp. *bulgaricus* Bulk Set FYE 41 LYO 100 l, *Str. thermophilus* Bulk Set Y 572 LYO 500 l and *L. acidophilus* NCFM LYO 10 DCU (Danisco Deutschland GmbH Germany/Alemanha) cultures were originally obtained from Chr. Hansen's Lab (Denmark). The starter cultures were activated in sterile non-fat dry milk according to the manufacturer's recommendations. The cow milk used in production of ayran samples was provided from stockbreeding pilot management in the Animal Science Department of the Agriculture Faculty of Selcuk University. Whey concentrates (Normal Whey Concentrate (NWC) and 50% Demineralized Whey Concentrate (DWC)), and Whey Protein Powder (WP) has been provided from ENKA Dairy Co., Konya, Turkey.

Ayran Production

The formula for ayran samples are given in Table 1. The raw cow's milk was used in the production of ayran samples. Whey fractions were added to the milk. The milk is homogenized and heated to 90°C for 10 min, and cooled to 45°C. It is then inoculated with 40 $g.kg^{-1}$ of a mixed lactic starter (*Str. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus*) (Table 1). Then, samples were placed in 100 ml polystyrene cups and the experiment was realized in triplicate. The inoculated milk was incubated at 45°C until a pH of 4.6 was attained in approximately 3.5 h. After incubation, the ayran samples were cooled at 20°C. 20 $g.kg^{-1}$ water and 4 $g.kg^{-1}$ salt was added in ayran samples. Samples were mixed for mixing of salt and water and were stored at 4°C temperature during all period of post-acidification (for 12 days).

Method

Ayran samples stored at 4°C were analyzed in 1, 4, 8 and 12 days. Total solids, crude protein, fat, titration acidity as lactic acid and ash contents of yoghurt samples were determined according to the standard methods of AOAC (2005). The pH of samples was measured at 6°C using a pH meter. Water activity (a_w) of the samples was measured using a commercially available a_w meter, measuring system (Model AQUALAB 3TE, Washington). The water holding capacity was determined by a procedure adapted from Guzman-Gonzalez *et al.* (1999).

Table 1. The experimental design, starter cultures and WC used in the manufacture of ayran samples (%)

Ayran Samples	A Culture Group (% 4)	B Culture Group (% 5)	A + B Culture Group (%2+%2)	NWC	DWC	WP
C	*Lactobacillus delbrueckii subsp. bulgaricus* + *Str. thermophilus*	*Lactobacillus acidophilus*	*Lactobacillus delbrueckii subsp. bulgaricus* + *Str. thermophilus* + *Lactobacillus acidophilus*	0	0	0
WP1				0	0	1
WP2				0	0	2
DWC1				0	2	0
DWC2				0	4	0
NWC1				2	0	0
NWC2				4	0	0

C: Control, NWC: Normal Whey Concentrate, DWC: Demineralized Whey Concentrate, WP: Whey Protein

Mineral contents of ayran samples were determined as following: A 500 mg sample was weighed into crucibles, and incinerated in a microwave furnace (MARS 5, CEM Corporation) at 200°C, 170 psi for 30 min. The ashes were dissolved with a few drops of nitric acid (650 $g.kg^{-1}$) (Sigma, USA) and diluted to 50 ml with deionized water. Concentrations of minerals were quantified by ICP-Atomic Emission Spectroscopy (VARIAN-CCD Simultaneous ICP-AES, Australia) with an auto-sampling system.

Aerobic mesophilic bacteria were enumerated on pour plates of PCA (Oxoid, Basingstoke, Hampshire, England) incubated at 30°C for 48-72 h. Yeast and moulds were identified on Yeast Glucose Chloramphenicol Agar (Oxoid) with plates incubated at 25°C for 5 days. Coliforms were identified on VRB Agar (Oxoid) after incubation at 30°C for 24 h (Harrigan and Mccance, 1993). M17 agar (Difco Laboratories) was used to enumerate streptococci in ayran samples. *L. acidophilus* also grew on this medium; therefore, after enumeration of all colonies on M17 agar, count of *Str. thermophilus* was determined by subtracting the count of *L. acidophilus* (MRS agar with oxgall) from the total count. Plates were incubated in an aerobic incubator at 37°C for 72 h. Acidified (to pH 5.2) MRS agar (Difco Laboratories) was used for enumeration of *L. delbrueckii* subsp. *bulgaricus*. Plates were incubated under anaerobic conditions at 37°C for 72 h. MRS agar (Difco Laboratories, Detroit, MI) with oxgall (2 $g.kg^{-1}$) (Difco Laboratories) was used for count of *L. acidophilus* (Marshall, 1992). Plates were incubated aerobically at 37°C for 72 h.

Data were statistically analyzed repeated measure ANOVA using SPSS for Windows 6.0. Separation of the means ($P < 0.01$) was accomplished by Duncan's multiple range test. All experiments and analyses were replicated three times (Minitab, 1991)

Results and Discussion

Physicochemical properties

The dry matter, protein, fat and ash contents of the ayran samples are presented in Figure 1. It was obvious that the WC affected the chemical composition of the ayran samples significantly ($P < 0.01$). There are numerous factors which affect chemical composition of ayran, mainly the methods of fortification used to increase the solid content (Aysal, 2008; Guzman-Gonzalez *et al*., 2000). The dry matter content ranged from 105.30 g.kg^{-1} in control to 125.80 g.kg^{-1} in WP2 sample, with significant differences ($P < 0.01$) among the samples. The protein content was in the range from 25.43 to 38.47 g.kg^{-1}, and the highest value was found in WP samples. WP significantly increased the amount of protein in ayran. There were significant differences in fat content ($P < 0.01$), which varied from 25.08 to 28.20 g.kg^{-1}. The ash content was in the range from 9.78 to 13.35 g.kg^{-1}, with significant differences ($P < 0.01$) among the samples.

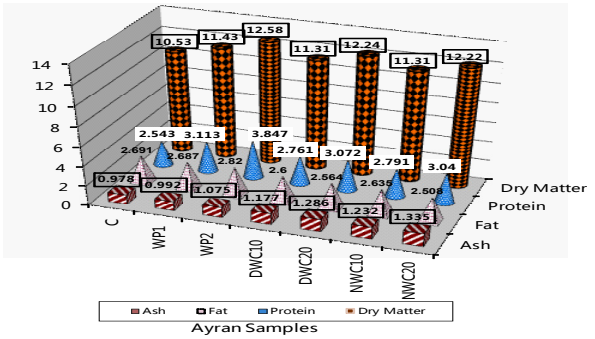

Figure 1. The some chemical properties of ayran samples

The contents of Ca, Fe, K, Mg, Na, P, S and Zn determined in ayran samples can be seen in Table 2. Ca, Fe, K, Mg, Na, P, S and Zn contents were significantly increased with the addition of WC and WP Powder ($P < 0.01$). The amounts of mineral matter showed wide intervals in WC added ayran samples (Ca 691-1076, Fe 3.329-5.299, K 448-857, Mg 75-166, Na 517-983, P 1159-1882, S 248-380, Zn 3.158-6.704 mg.kg^{-1}). NWC added ayran samples had the highest content of mineral components. This would indicate that fortified dairy products can be produced with additives abundant in minerals such as whey concentrates. This supply can represent an advantage from a nutritional point of view as a source of essential nutrients in diet in comparison with other dairy products. Moreover ayran could act as an alternative source of mineral for sufferers of lactose intolerance.

The some physicochemical properties of the ayran samples are presented in Table 3. The acidity in the WC added ayran samples was found to be higher than that of the control sample ($P < 0.01$). In contrast to the acidity, the pH values were lower in the WC added samples. Total acidity of the samples showed significant increases during storage. A consistent rise in titratable acidity was observed for all the ayran samples during storage. However, the rates were greatly influenced by storage atmosphere and a_w. a_w values of samples were determined between 0.985 and 0.988. The lowest a_w has been detected in NWC2 and DWC2 ayran samples. The highest a_w values were found in control and WP samples. Low a_w value influenced the acidity of the samples. a_w reflects a combination of water-solute and water-surface interactions plus capillary forces. The nature of a hydrocolloid or protein polymer network can thus effect on a_w, crosslinking reducing the activity (Chaplin, 2009).

The highest amount of water holding capacity was determined in the DWC2 ayran sample. The lowest water holding capacity was determined in control sample (Table 3). The water holding capacity may be effective in the less-developed acidity in *L. acidophilus* added ayran samples. The capacity of water holding increased with increasing amount of WC. The water holding capacity was the highest in *L. acidophilus* culture added ayran sample.

The microbiological characteristics

The yeast and moulds and coliform bacteria were not detected in samples. The counts of total bacteria in the ayran samples ranged from 8.071 log CFU.ml^{-1} (WP2) to 9.250 log CFU.ml^{-1} (DWC2) (Table 5). *L. acidophilus* added samples had the highest counts of total bacteria. The low acidity and any other lactic acid bacteria of ayran samples in this group have increased the number of the total bacteria. The increasing amount of WC has led to an increase in the counts of total bacteria.

L. delbrueckii subsp. *bulgaricus* counts in WC added ayran samples ranged from 7.787 log CFU. ml^{-1} (WP2) to 8.894 log CFU.ml^{-1} (DWC2) and in the control samples was 7.374 log CFU.ml^{-1} (Table 5). *L. delbrueckii* subsp. *bulgaricus* counts decreased continuously and significantly ($P < 0.01$) during storage of ayran samples (from 8.069 to 8.009 log CFU.ml^{-1}). Investigations carried out by Beal *et al.* (1999) revealed that the survival of milk fermentation bacteria could fluctuate in the range of 40 to 75% and, as a rule, more bacteria from the *Lactobacillus* genus survive than those from the *Streptococcus* genus. In the present study, Figure 2 shows that the counts of *L. delbrueckii* subsp. *bulgaricus* in 40 g.kg^{-1} normal WC added ayran samples was the highest out, and has remained the highest during storage.

Table 2. The mineral matter contents of ayran samples (mg/kg)

Sample	Ca	Fe	K	Mg	Na	P	S	Zn
C	661±19.61	3.091±0.257	484±19.99	75±3.92	644±33.81	1312±84.48	236±27.02	3.143±0.231
WP1	691±19.18	3.329±0.368	448±19.72	75±4.41	517±74.11	1159±44.81	248±40.58	3.158±0.339
WP2	826±36.12	3.352±0.374	512±47.40	97±4.66	761±22.10	1425±99.12	344±27.49	4.631±0.155
DWC1	811±21.63	3.681±0.132	620±17.16	105±5.29	522±18.98	1416±74.27	275±44.20	4.210±0.143
DWC2	930±35.07	3.945±0.175	786±37.47	108±7.87	551±16.00	1418±45.14	264±24.07	4.368±0.139
NWC1	967±32.30	4.610±0.467	669±41.33	150±7.13	755±15.39	1470±180.19	328±31.16	4.422±0.275
NWC2	1076±76.41	5.299±0.395	857±27.44	166±10.43	983±44.62	1882±162.02	380±30.22	6.704±0.268

Table 3. The some physicochemical properties of ayran samples

Sample	Acidity (% Lactic Acid)	pH	a_w	Water Hold Capacity (%)
C	0.166±0.019 f*	4.59±0.23 a	0.988±0.004 a	24.89±1.04 f
WP1	0.210±0.020 d	4.39±0.12 f	0.988±0.003 ab	26.55±1.90 e
WP2	0.217±0.033 c	4.48±0.21 d	0.988±0.003 ab	28.38±2.96 ab
DWC1	0.210±0.028 d	4.53 ± 0.16 b	0.987±0.003 b	28.33±4.04 b
DWC2	0.235±0.020 b	4.48±0.10 d	0.986±0.004 c	28.47±2.40 a
NWC1	0.215±0.024 c	4.49±0.18 c	0.987±0.004 ab	27.19±1.86 d
NWC2	0.242±0.024 a	4.44±0.11 e	0.985±0.003 c	28.19±1.81 c

*: Means in the same columns of physicochemical properties with different superscripts are significantly different (P < 0.01) among applications.

Table 4. The effect of cultures on the physicochemical properties of ayran samples

Group	Acidity (% Lactic Acid)	pH	a_w	Water Hold Capacity (%)
A	0.227±0.025 a	4.32±0.08 c	0.982±0.004 c	25.56±1.55 c
B	0.195±0.035 b	4.64±0.15 a	0.985±0.003 a	29.68±3.23 a
A+B	0.213±0.026 c	4.44±0.09 b	0.983±0.003 b	27.08±2.01 b

*Means in the same columns of physicochemical properties with different superscripts are significantly different (P < 0.01) among applications.

Table 5. Microbiological properties of ayran samples produced by addition different whey concentrates (log CFU.ml^{-1})

Samples	Total aerob mesophilic bacteria	*Str. thermophilus*	*L. delbrueckii* subsp. *bulgaricus*	*L. acidophilus*
C	8.120±0.548g*	7.516±0.110 g	7.374±0.064 h	7.870±0.234 h
WP1	8.217±0.520 e	7.798±0.676 e	7.787±0.583 f	8.246±0.206 f
WP2	8.071±0.443 h	8.048±0.754 d	8.049±0.760 d	8.454±0.358 c
DWC1	8.686±0.240 c	8.254±0.611 c	8.203±0.546 c	8.331±0.480 d
DWC2	9.250±0.450 a	8.984±0.082 a	8.894±0.049 a	8.985±0.354 a
NWC1	8.421±0.601 d	7.784±0.122 f	7.812±0.084 e	8.306±0.635 e
NWC2	9.030±0.787 b	8.819±0.188 b	8.753±0.153 b	8.861±0.228 b

*Means in the same columns of microbiological properties with different superscripts are significantly different (P < 0.01) among applications.

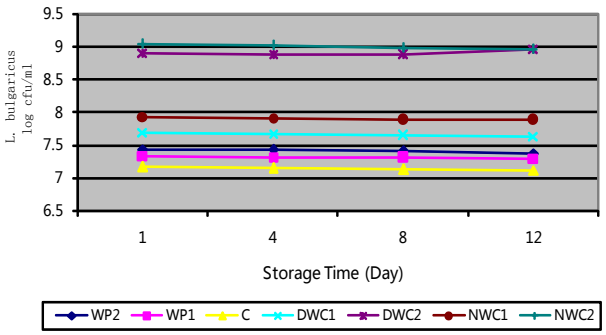

Figure 2. The change of counts of *L. delbrueckii* subsp *bulgaricus* in ayran samples

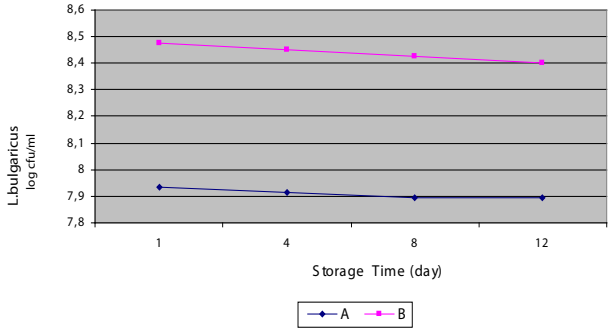

Figure 3. The change of counts of *L. delbrueckii* subsp. *bulgaricus* in ayran samples produced using different cultures during storage

While the counts of *L. delbrueckii* subsp. *bulgaricus* in samples produced by using of only yoghurt cultures were 7.841 log CFU.ml^{-1}, the counts in yoghurt culture and *L. acidophilus* added samples was 8.236 log CFU.ml^{-1}. As shown in Figure 3, the supplementation of *L. acidophilus* in ayran samples, significantly increased the development of *L. delbrueckii* subsp. *bulgaricus* (P < 0.01) (Table 5).

Bulgaricus benefited from the whey as a source of protein, and found a more effective development environment. The environmental acidity, resulting from the growth of microflora deliberately introduced during the manufacturing process, determines not

only the degree of survival of individual strains in the course of further yoghurt storage, but later on, leads to changes in the yoghurt structure and viscosity as well as its sensitivity to syneresis (Radtke-Mitchell and Sandine, 1984). The quantities of technological additives or their mixtures, at carefully balanced proportions, provides its dry matter with essential quantities of lactose and amino acids necessary for the growth of microflora (Birollo *et al.*, 2000).

The lowest number of *Str. thermophilus* was determined in control sample, the highest thermophilus number, as well as *L. delbrueckii* subsp. *bulgaricus* were determined in ayran produced with 40 g.kg^{-1} DWC. *Thermophilus* counts in ayran samples showed significant reduction similarly with *bulgaricus* counts during 12 day storage period ($P < 0.01$) (Figure 4).

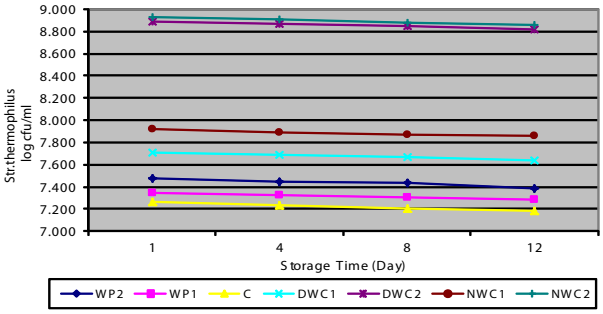

Figure 4. *The change of counts of Str. thermophilus* in ayran samples produced by adding of different whey concentrates during storage

Again, the counts of *Str. thermophilus* in ayran samples produced with *acidophilus* and yoghurt culture was significantly higher. *Thermophilus* counts of 40 g.kg^{-1} DWC and NWC added samples were found to be significantly higher than that of other samples during storage (Figure 4). Oliveira *et al.* (2002) reported similar results for counts of *Str. thermophilus* in fermented lactic beverages containing probiotic bacteria. However, Vinderola *et al.* (2002) reported that lactic bacteria were inhibited by the probiotic bacteria. These authors stressed the existence of lactic bacteria strains which were not inhibited and the necessity of a careful restriction of the results obtained to the strains studied. As shown in Figure 5, *Str. thermophilus* counts in ayran samples produced together with both of A and B group cultures were found to be significantly higher than that of samples produced with only A culture during storage.

L. acidophilus counts in ayran samples produced with different whey concentrates showed significant differences ($P < 0.01$). The number of *L. acidophilus* in WP1 ayran sample was 8.246 log CFU.ml^{-1}, while in DWC2 sample was identified as 8.985 log CFU.

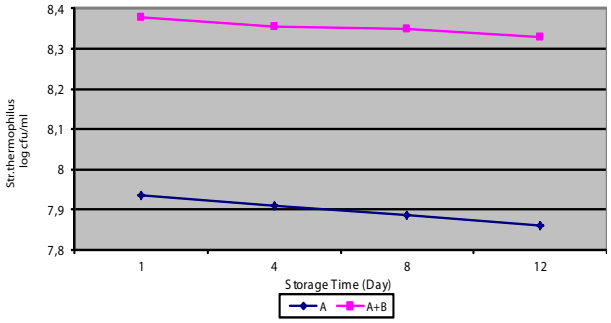

Figure 5. The change of counts of *Str. thermophilus* in ayran samples produced using different cultures during storage

ml^{-1}. *L. acidophilus* in control samples were counted as 7.870 log CFU.ml^{-1}. The number of *L. acidophilus* in whey concentrates added ayran samples was significantly higher than that of the control sample (Table 5). It could be caused by the preservative used in WP that led the culture bacteria count in WP added ayran samples was lower than that of whey concentrate was added to the other ones. In addition, the WC is more proper as a food component than WP. Because, in the production of cheese milk was not heated to high temperatures and loss of nutrient elements in whey less occur. The temperature of evaporation is also lower than that of WP production. In a study, survival of *Lactobacillus*, physiochemical and sensory properties of probiotic yoghurts were evaluated every 7 days to 21 days. The results showed that, increasing the total solid concentration of milk increased the survival of *L. acidophilus*. However, the survival of probiotic *Lactobacillus* decreased throughout the storage period at 4°C (Božanić *et al.*, 2004).

The number of *acidophilus* such as other yoghurt cultures also showed a decrease from 8.415 to 8.354 log CFU.ml^{-1} during storage and reached the lowest number at the end of storage (Figure 6). The number of *L. acidophilus* in samples added as alone culture were higher than in yoghurt cultures and *L. acidophilus* added samples. *L. acidophilus* better develop in the presence of proteins and peptides. They also aid in the digestion of proteins which is important for the production of essential enzymes made in the body. In this way, yoghurt cultures in the same environment with *acidophilus* may further increase (Matar *et al.*, 2003). During storage, the number of *L. acidophilus* in DWC2 and NWC2 ayran samples were higher counted than that in other samples. The number of *acidophilus* in control sample and WP added samples were found as the lowest values (Figure 6). Others researchers have reported that ultrafiltered WPC can positively stimulate the rate of fermentation and the growth of *L. acidophilus* (Marshall *et al.*,

1982). Previous studies have reported that the most important contributing factors for loss of cell viability are decreasing of pH during product storage (post-acidification) and the accumulation of organic acids as a result of growth and fermentation (Dave and Shah, 1997).

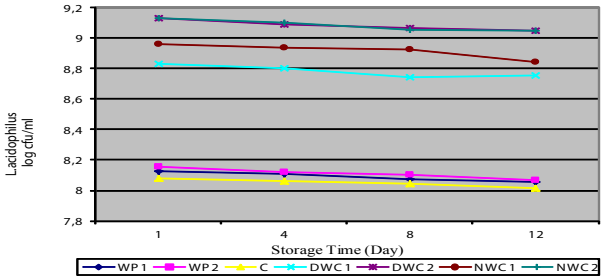

Figure 6. The change of counts of *L. acidophilus* in ayran samples

Acidophilus culture in ayran samples produced only with *L. acidophilus* was significantly more growth. The development of acidophilus in ayran samples produced with both of yoghurt culture and *L. acidophilus* was significant less. In conjunction with yoghurt culture, while slowly of acidophilus was growth, yoghurt cultures were more growth (Table 6 and Figure7).

Table 6. Microbiological properties of ayran samples produced using different culture

Culture Group	Total aerob mesophilic bacteria	*Str. thermophilus*	*L. delbrueckii* subsp. *bulgaricus*	*L. acidophilus*
A	8.403±0.926 b*	7.847±0.679 b	7.841±0.634 b	ND
B	8.943±0.226 a	ND	ND	8.578±0.474 a
A+B	8.139±0.360 c	8.316±0.595 a	8.236±0.611 a	8.189±0.479 b

A: *Lactobacillus delbrueckii* subsp *bulgaricus*+ *Streptococcus thermophilus*
B:*Lactobacillus acidophilus*
ND: Not determined
*Means in the same columns of microbiological properties with different superscripts are significantly different (P < 0.01) among applications.

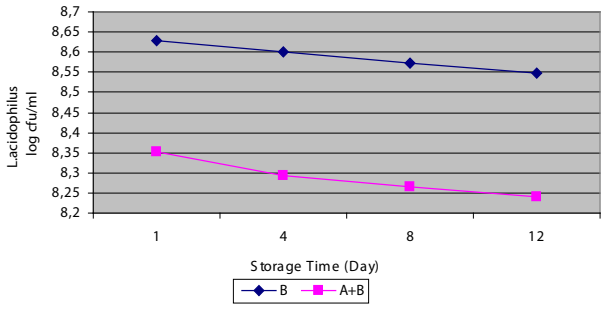

Figure 7. The change of counts of *L. acidophilus* in ayran samples during storage

Conclusions

The results of the study showed that the *L. acidophilus* count significantly increased with addition of WC, and survive in refrigerated conditions for 12 day in a number of greater than 7.00 log CFU. ml^{-1} for all ayran samples. This is essential if a product should have probiotic properties. It can be said that WC have prebiotic effect on *L. acidophilus* added in ayran. According to the results, all samples possessed excellent stability during 12 day of storage, and spoilage was not noticed in any sample. The adding of WC and *L. acidophilus* to ayran, the probiotic properties gained to it and it will make a significant contribution to adequate and healthy diet of the consumers. The usage of whey in human nutrition can be enhanced by production of probiotic ayran.

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