Original paper

An investigation for the development of whey-based probiotic beverages

HIDAYET SAGLAM1*, TANER SARIOGLU2, AYNUR GUL KARAHAN3, ZUBEYDE ONER3

1Kilis 7 Aralik University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Kilis, Turkey
2Isparta University of Applied Sciences, Şarkikaraağaç Vocational School, Department of Food Technology, Isparta, Turkey
3Süleyman Demirel University, Faculty of Engineering, Department of Food Engineering, Isparta, Turkey

Abstract
Whey is one of the byproducts of cheese-making. Due to the high nutrient content of whey, it is being used to prepare many different products. Probiotic bacteria have been used to develop new whey containing beverages so new generation-products can be produced which influence the human health positively. The aim of this study was to define the growth and survival of probiotic microorganisms, Lactobacillus plantarum AA1–2 and Saccharomyces boulardii, in whey. The whey was inoculated and fermented with L. plantarum AA1–2, S. boulardii bacteria separately and mixture of these two bacteria was also used. Microbial counts, changes in pH values and sensory quality were analysed in the beverages. Beverages with the highest sensory scores were analysed to determine aroma compounds by using GC/MS-SPME. While pH value of reconstituted whey was 6.15, pH values of fermented beverages were diminished and ranged from 4.97 to 6.07. Yeast count was higher than 10⁵ cfu/ml, and bacteria count were between 10⁷-10⁸ cfu/ml. The best result of the sensory properties of beverages was occured at 2% inoculation of L. plantarum AA1–2. According to the results obtained with GC/MS-SPME, the highest amount of aroma compounds found in whey was acetate isopropyl.

Keywords
Whey; probiotic; beverage; GC/MS-SPME.
Introduction

Whey can be obtained as a byproduct during cheese processing precipitated either with rennin action or acid coagulation. Due to different methods of processing, serum protein can be discarded or used as byproduct to obtain whey, curd cheese, or animal food. Drainage of whey may lead to economic losses and environmental pollutions. To prevent these problems various solutions was developed. One of the most important product of whey is the drink preparation that consist of high amount of protein (YERLIKAYA & al [1]; PRAZERES & al [2]; CARVALHO & al [3]; PELEGRINE & CARRASQUERIA [4]).

1. Whey Composition

Whey can be sweet or acid according to the several parameters. These are the variety of milk (cow, goat, sheep), technology used by cheese manifacturers and type of casein coagulation. Whey includes approximately 50% of total solid of milk. It contains 7% total solids and high-quality proteins which allegedly have anticancer activities due to the sulfur amino acids constitute less than 1% of dry matter. Also, it contains vitamins, minerals, and fat. The production technology of whey affects the amount of calcium, phosphates, lactic acid and lactate it contains. In acid whey, these parameters are higher than sweet whey (Table1) (PRAZERES & al [2]; CARVALHO & al [3]; SISO [5]; PINTADO & al [6]; DJURIC & al [7]; JELICIC & al [8]).

Table 1. Typical composition (g/L) of sweet and acid whey

| Component | Sweet whey | Acid whey |
|-----------|------------|-----------|
| Total solids | 63,0-70,0 | 63,0-70,0 |
| Lactose | 46,0-52,0 | 44,0-46,0 |
| Proteins | 6,0-10,0 | 6,0-8,0 |
| Calcium | 0,4-0,6 | 1,2-1,6 |
| Phosphates | 1,0-3,0 | 2,0-4,5 |
| Lactates | 2,0 | 6,4 |
| Chlorides | 1,1 | 1,1 |

In general, beverage manufacturers have been used whey proteins as an ingredient in many different type of products such as sports drinks, smoothies, meal replacements etc. Due to the property of fresh neutral taste of whey proteins, they can be used with variety fluids and vegetables in beverages. The products consisting of plain, alcoholic, carbonated and fruit flavored have been produced and marketed (CHAVAN & al [9]).

2. Whey Beverages

Processing of whey to beverages has a very long history. It was started in 1970 and the one of the oldest beverages with whey is Rivella from Switzerland. Many types of deproteinized whey, fresh diluted whey, fermented whey, powdered whey, alcoholic whey beverages like whey beer or wine and beverages with low alcohol content (less than 1.5%) are developed. For better choices of beverages with acceptable sensory properties, manufacturers are produced fermented whey beverages. For non-alcoholic whey fermentations mainly starter and probiotic cultures of lactic acid bacteria (LAB) are used. In the case of alcoholic beverages mostly yeast species of Kluyveromyces can be used. Whey based beverages have a wide range of consumers in the world. They were used for different aims such as treating or curing to some illnesses like tuberculosis and skin and digestive tract diseases. Many institutions were founded in the 18th century for curing illnesses after the discovery of nutritional and therapeutic properties of whey in the detail. In some countries such as Switzerland, Germany and Austria, whey cures were used and they also applied for treatments of diarrhea, bile illness, skin problems, succesfully as well as urinary tract and some intoxications (SISO [5]; JELICIC & al [8]).

Whey is a valuable and rich source of bioactive nutrients for the food industry. With various procedures many highly valuable products can be provided (lactose, whey protein concentrate, whey powder, lactoalbumin, lactoglobulin, urea, galactose, glucose, syrup, beverages, alcohol and single cell proteins) due to utilization of its precious constituents which is performed by (concentration and/or fractionation and drying, fermentation or hydrolysis, etc.). Whey has an unpleasant taste due to high lactose-to-glucose ratio and excessive acidity based on the class of acid whey. Many procedures were developed to improve its characteristics for directly using in human nutrition (DJURIC & al [7]).
Because of lactose, whey is a perfect material for production of alcoholic whey beverages with low alcohol content (≤ 1.5%), such as whey beer and whey wine. For preparation of this beverages, there are many stages. These stages include deproteination of whey, concentration of whey, fermentation or addition of sucrose to reach the desired alcohol content (0.5-1%), flavoring, sweetening and bottling of beverages. Whey are fermented by yeast strains Kluyveromyces fragilis and Saccharomyces lactis. A certain amount of lactose is fermented to lactic acid which gives a refreshing sour taste to the end product. Like lactose, whey proteins are a rich source of branched chain amino acids which can metabolized directly into the muscle tissue such as isoleucine, leucine and valine. Because of this property athletes can use them as sport drinks during periods of exercise and resistance trainings. They also include lactoferrin, glycomacopeptide, phenylalanine, and alpha-lactalbumin. Owing to lactoferrin content, whey beverages can be useful for children and babies as it helps iron absorption from food and helps to keep pathogens from attaching to the intestinal walls. Also it is useful for elderly people as it may help absorption of calcium. Moreover whey beverages are useful for people suffering from phenylketonuria since it is a good source of energy micronutrients (JELICIC & al [8]; SHERWOOD & JENSINS [10]).

3. Probiotic Whey Beverages

It is very important for the development of probiotic nonalcoholic whey beverages as positive effects of probiotic strains on human health like lowering cholesterol level in blood, improving lactose metabolism, anti-diarrheal properties, lowering blood pressure, anticancerogenic properties, antimutagenic effects and immune system stimulation are known for a long period of time (SHAH [11]).

Probiotic whey beverages determines the unique flavor and texture of the final product. Lactobacillus reuteri and Bifidobacterium bifidum were used as probiotic strains for fermentation (HERNANDEZ-MENDOZA & al [12]). In some recent studies, whey was fermented by using Lactobacillus acidophilus, Lactobacillus delbrueckii sbsp. bulgaricus, Streptococcus thermophilus, Lactobacillus rhamnosus, Lactobacillus delbrueckii sbsp. bulgaricus, Bifidobacterium animalis subsp. lactis and co-culture Streptococcus thermophilus–Bifidobacterium animalis subsp. lactis. Other probiotic organisms including L. plantarum and S. boulardii have potential to be used in probiotic products (SHAH [11]; DALEV & al [13]; ALMEIDA & al [14]; PESCUMA & al [15]; AKPINAR & al [16]).

LAB that show probiotic properties are widely used for the protection of food and feed raw materials and also they improve the flavour and texture of the fermented products. One of the most important LAB is L. plantarum that is significant in the production of many fermented foods such as pickled vegetables, silage, sourdough, dry ferment sausages, fermented fish, cheese, ice cream (BASYIGIT & al [17]; BASYIGIT & al [18]; KAHALA & al [19]).

According to the definition of the World Health Organization, S. boulardii is a probiotic, i.e. “a living microorganism which, when administered in adequate amounts, confers a health benefit to the host” (SZAJEWSKA & al [20]). It can prevent and treat diarrhoea associated with among others use of antibiotics, Clostridium difficile or Vibrio cholerae infection, gastroenteritis, Crohn’s disease and irritable bowel syndrome (SURAWICZ & al [21]; HOCHTER & al [22]; MCFARLAND & BERNASCONI [23]; CETINASAURI & SIERRA BASTO [24]; POTHOULAKIS [25]; POTHOULAKIS & IM [26]). S. boulardii is not capable of fermenting lactose, but is capable of using organic acids found in milk or resulting in glucose and galactose fermentation (CZERUCKA & al [27]; VANHEE & al [28]; PARRELLA & al [29]). In association with lactic acid bacteria formation of probiotic yeast, S. boulardii, has been thought to enable the growth of the probiotic lactic acid organisms and to ensure their survival during shelf-life (REKHA & VIJAYALAKSHMI [30]).

The aim of this study was to define the growth and survival rate of L. plantarum and S. boulardii in whey for possible production of a nutritive highly valuable probiotic whey beverages.

Materials and Methods

L. plantarum AA1–2 was isolated from feces samples and some probiotic properties of it were determined by BASYIGIT [31]. The strain was also identified by 16S rRNA analysis (BASYIGIT KILIC & KARAHAN [32]) and their plasmid profiles were investigated by SAĞLAM [33]. S. boulardii was obtained from a commercial company (Reflo, France). Recomposed whey was prepared consisting 6% total solids and then sterilized. Whey was inoculated with probiotic bacteria and yeast, L. plantarum AA1–2 (%1, %2), S. boulardii (%1, %2) and mixture of L. plantarum AA1–2 (%1, %2) + S. boulardii (%1, %2). In consecutive step beverages were poured into glass jars in amount of approximately 250 ml and covered with lids. Fermentation process was carried out at temperature of 37°C for about 18 hours. After fermentation, the beverages...
were cooled to temperature of 6±1°C and stored under refrigerated conditions. After storage, sensory evaluation, pH values, microbiological analysis and content of aroma compounds were performed.

The evaluation of active acidity was completed by using pH-meter (Hanna HI 2211) according to the instruction manual. Microbiological analysis were carried out according to the KARAHAN & al [34]. It involved a determination of L. plantarum in beverages using MRS Agar and S. boulardii using Yeast-Extract Glucose Chloramphenicol Agar (YGC) Medium (Merck). All assays were conducted in 3 replicates.

Sensory evaluation included assessment of appearance, taste, smell and consistency of beverages. It was performed with the participation of 6 panelists. Sensory evaluation was performed by using scoring method and each quality factor got marks from 1 to 5, where mark 1 means very poor and mark 5 very good quality of the indicator (SKRYPLONEK & JASINSKA [35]). Beverages with the high sensory scores than 4 were analysed to determine aroma compounds by using GC/MS system consisted of an Shimadzu GCMS-QP2010 SE detector (Shimadzu, Japan). Separation were performed on fused silica columns (Restek Rx-5Sil MS 30 m, 0.25 mm, 0.25 mm). Carrier gas was helium at a flow rate of 1.61 mL/min. Oven temperature was programmed from 40°C to 250°C at a rate of 4°C/min. Ionization energy was 70 eV.

### Results and Discussion

While pH values of recomposed whey was 6.15, pH values of fermented beverages were ranged from 4.97-6.07, and after storage of them at 6°C, overnight, pH changed between 4.52-5.99. Acidic fermented whey beverages were assigned at L. plantarum (2%) + S. boulardii (2%) with pH 4.52, and less acidic ones was assigned at S. boulardii (2%) with pH 5.99. As expected, L. plantarum showed an important ability to acidify whey. Our results agree with results of PARRERLA & al [29] and ALBENZIO & al [36]. Also, it is expected that the pH will increase due to the potential use of organic acids by the yeast, whereas in the case of whey fermented with 2% yeast, the pH was found to decrease to a minimum level. It is recommended that yeast must be co-cultured with LAB bacteria during the development of the whey beverages products (Table 2). The reason why S. boulardii (2%) inoculated whey had lower acidity than the other sample is yeast does not show sufficient growth in whey. In many studies, pH values of whey that between 6-7 are sweet whey. However, with the fermentation realized, the sweet whey pH is reported to be lower than 6 (SISO [5]; CHAVAN & al [9]; LOURENHS-HATTINGH & VILJOEN [37]; DRGALIC & al [38]).

#### Table 2. pH values of fermented whey beverages

| No | Sample                  | pH value after incubation at 37°C, 18 h* | pH value after storage at 6°C, one night* |
|----|-------------------------|----------------------------------------|----------------------------------------|
| 1  | Control                 | 6.21±0.01                              | 6.25±0.01                              |
| 2  | L. plantarum (% 1)      | 5.13±0.03                              | 4.65±0.04                              |
| 3  | L. plantarum (% 2)      | 5.03±0.02                              | 4.53±0.03                              |
| 4  | S. boulardii (% 2)      | 6.07±0.05                              | 5.99±0.03                              |
| 5  | L. plantarum (% 1) and S. boulardii (% 1) | 5.12±0.08                              | 4.67±0.09                              |
| 6  | L. plantarum (% 2) and S. boulardii (% 2) | 4.97±0.04                              | 4.52±0.11                              |

* Data are the mean±standard deviation of three separate determinations.

Before the fermentation process, count of S. boulardii were ranged from 1.0×10⁴ to 6.5×10⁵; count of L. plantarum AA1-2 were ranged from 1.6×10⁶ to 1.5×10⁷ cfu/ml. After the fermentation process, yeast count was higher than 10⁶ cfu/ml, bacteria count was between 10⁷-10⁹ cfu/ml. Also, the count of the yeast were increased. So, 2% and 1% inoculation of L. plantarum AA1-2 were found that there was increase in number of bacteria as 7 times and 2 log, respectively (Table 3). ALMEIDA & al [14] found that the number of probiotic organisms that developed whey were varied considerably. According to FAO/WHO [39], the minimum level of probiotic bacteria required for functional product is 6 log cfu/ml, so, in our study L. plantarum level is sufficient to provide health benefits to consumers. L. delbrueckii subsp. bulgaricus CRL 454, S. thermophilus CRL 804 and L. acidophilus CRL 636 were developed in reconstituted whey and they were exhibited similar results like ours that the count of bacteria was less than 10⁶ cfu/ml (PESCUMA & al [15]).
An investigation for the development of whey-based probiotic beverages

Table 3. Count of colonies before and after incubation at whey

| No | Sample                        | Before incubation (cfu/ml) | After incubation (cfu/ml) |
|----|-------------------------------|----------------------------|---------------------------|
| 1  | L. plantarum (% 1)            | 3.5±0.10*10^6              | 1.3±0.08*10^8              |
| 2  | L. plantarum (% 2)            | 1.0±0.19*10^7              | 7.0±0.07*10^7              |
| 3  | S. boulardii (% 2)            | 1.0±0.22*10^4              | 4.0±0.11*10^5              |
| 4  | L. plantarum (% 1) and S. boulardii (% 1) | 1.6±0.18*10^6       | 7.5±0.21*10^4              |
|    |                               | 8.0±0.18*10^4              | 1.7±0.29*10^5              |
| 5  | L. plantarum (% 2) and S. boulardii (% 2) | 9.5±0.15*10^6           | 5.0±0.21*10^7              |
|    |                               | 6.5±0.17*10^4              | 2.5±0.12*10^5              |

* Data are the means±standard deviation of three separate determinations.

Results parameters include appearance, taste, smell and consistency of whey and fermented whey beverages. The best result of the sensory properties of beverages was occurred at 2% inoculation of L. plantarum AA1-2. Also, there were high scores at 2% inoculation of S. boulardii, and at combination of 2% inoculation of L. plantarum AA1-2 and 2% inoculation of S. boulardii (Figure 1). DRGALIC & al [38] focused on Lactobacillus acidophilus La-5, Bifidobacterium bifidum Bb-12 and Lactobacillus casei Lc-1 in reconstituted whey at 28 days of cold storage. All strains showed good survival throughout the storage period of fermented beverages. Low sensory scores were obtained at beverage fermented by the Bb-12 strain acquired than the other two fermented by the La-5 and Lc-1 strains. The best sensory score was obtained in beverages that fermented for 18 hours. The use of whey for human nutrition can be increased by the production of fermented probiotic whey drinks.

Figure 1. Sensory properties of fermented whey beverages (Data are the means±standard deviation of three separate determinations)

Because of the high sensory properties of 2% inoculation of L. plantarum AA1-2, 2% S. boulardii, and mixture of them and also whey were analysed to define aroma compounds. Results of the GC/MS-SPME showed us there are different compounds in the fermented whey beverages as given in Table 5 and their retention times as presented in Table 6. In the whey, there were 26 different compounds. That are converted to 24 different one by L. plantarum (2%), to 17 by S. boulardii (2%) and to 22 by combination of 2% of L. plantarum AA1-2 and 2% S. boulardii.

In the whey, there were number of aroma compounds, such as acetate <isopropyl->, acetic acid <1-methyl ethyl-> ester, acetoin, heptanal, benzaldehyde, heptanone and heptanol <n->. After the fermentation with 2% inoculation of L. plantarum AA1-2, those were converted to acetoin, acetate isopropyl, butanal 2 methyl, butanoate <hexyl-, 3-methyl->, butanoic acid 3-methyl, 3 hexenyl and acetate.
\textit{octyl-}. At the same conditions, those compounds are converted to acetate, isopropyl-<isopropyl->, acetoin, ethanol, isoamyl alcohol, acetic acid 1 methyl ethyl ester, heptanone and heptanol <n-> by 2% of \textit{S. boulardii}. Combination of 2% of \textit{L. plantarum} AA1-2 and 2% \textit{S. boulardii} converted to acetoin, acetate <isopropyl->, formic acid <2-methyl propyl-> ester, ethanol, acetic acid 1 methyl ethyl ester, acetate <octyl->, heptanol, heptanone, isoamyl alcohol and butanal 2-methyl. Acetate isopropyl and acetoin were found as most profound compounds in the whey and fermented whey beverages.

The compounds detected in this study like acetic, 3-methylbutanoic and hexanoic acids were also found. MAHAJAN & \textit{al} \cite{40} found that the most important acids in the sweet whey residue after cheddar cheeses product were acetic, butanoic, 3-methylbutanoic, hexanoic and octanoic acid. Acetic acid has a sharp vinegar taste, 3-methylbutanoic acid sweaty odor, and hexanoic acid produces rancid taste.

The aromatic compounds of whey were derived from whey powder production e.g. furfurol. Aldehydes are major oxidation products of unsaturated fatty acids. For example; heptanal (KARAGÜL-YÜCEER & \textit{al} \cite{41, 42}).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Name} & \textbf{Whey} & \textbf{\textit{L. plantarum} (2\%)} & \textbf{\textit{S. boulardii} (2\%)} & \textbf{\textit{L. plantarum} (2\%) \textit{S. boulardii} (2\%)} \\
\hline
Ethanol & 1.21 & 0.68 & 12.26 & 6.05 \\
Isobutyl alcohol & 0.92 & 1.59 & Nd & Nd \\
Acetic acid <1-methyl-ethyl-> ester & 5.34 & 1.84 & 5.39 & 4.02 \\
Butanal <2-methyl-> & 0.49 & 14.85 & Nd & 1.43 \\
Acetate <isopropyl-> & 62.62 & 15.53 & 45.71 & 28.94 \\
Isobutyric acid & 0.58 & 1.88 & Nd & Nd \\
Formic acid <2-methylpropyl-> ester & Nd & Nd & Nd & 10.46 \\
Valeraldehyde & Nd & Nd & Nd & 0.18 \\
Acetylpropionyl & Nd & Nd & Nd & 0.38 \\
Acetoin & 4.98 & 37.19 & 16.05 & 32.02 \\
Isoamyl alcohol & 0.88 & 5.44 & Nd & 2.46 \\
Pentanal <2-methyl-> & 0.50 & Nd & Nd & Nd \\
Toluene & 0.40 & 0.62 & Nd & Nd \\
Pentanol & 0.28 & Nd & Nd & Nd \\
Hexanal & 0.30 & 0.97 & 0.26 & 0.24 \\
Capronaldehyde & 1.11 & 0.46 & 0.37 & 0.40 \\
Furfural & 0.92 & Nd & Nd & Nd \\
Xylene & 0.81 & 0.72 & 0.83 & 0.37 \\
Hexanol <n-> & 0.76 & 0.50 & 1.63 & \\
Heptanone & 2.58 & 1.83 & 5.08 & 2.66 \\
Heptanal & 4.43 & 1.64 & 0.57 & 2.92 \\
Hexanoic acid & Nd & Nd & 0.17 & Nd \\
Benzoaldehyde & 2.66 & 0.70 & Nd & 0.43 \\
Heptanol <n-> & 2.41 & 1.30 & 2.62 & 0.75 \\
Octen-3-ol & 1.05 & Nd & 0.70 & Nd \\
Hept-5-en-2-one <6-methyl-> & 0.45 & Nd & 0.44 & 0.32 \\
Capryl alcohol & 1.04 & 0.74 & 1.61 & 0.42 \\
Heptyl methyl ketone & 1.05 & 0.69 & 0.87 & 0.50 \\
Acetate <octyl-> & 1.61 & 3.36 & Nd & 3.51 \\
Non-2 (E)-enal & Nd & 0.21 & Nd & Nd \\
Caprylic acid & Nd & 1.27 & Nd & 0.24 \\
Butanoic acid <3-methyl-, 3-hexenyl-> & 0.62 & 3.89 & Nd & 1.30 \\
Butanoate <hexyl-, 3-methyl-> & Nd & 6.75 & Nd & Nd \\
Nonanoic acid <4-methyl-> & Nd & 0.81 & Nd & Nd \\
\hline
\end{tabular}
\caption{Area (\%) of peaks of whey and fermented whey beverages}
\end{table}

*Nd: Not determined
Liquid Cheddar whey was analyzed to detect aroma compounds present in its composition and figured out that 2,3-butanedione, hexanal, 2-acetyl-1-pyrroline, methional, (E,E)-2,4-nonadienal, (E,E)-2,4-decadial, and different short-chain volatile acids could be important to the formation liquid whey aroma. Flavor quality of whey depends on cheese quality milk that’s used, whey made from various types of cheeses, the method of whey handling immediately after curd draining, the time between the drain and pasteurization (MAHAJAN & al [40]; KARAGÜL-YÜCEER & al [43]; WHETSTINE & al [44]).

Whey powder due to other steps such as concentration and spray drying is expected to have a different flavor profile than liquid one. These appropriate steps can reduce or produce flavor compounds, which can change the flavor profile of whey. WHITFIELD [45], assumed that the Maillard reaction and lipid oxidation initiated the aroma development in the whey products, but no experimental data supporting this hypothesis is available to date. The major flavor volatiles that are analyzed in the sweet whey powder are short chain fatty acids, aldehydes and ketones, lactones, sulfur compounds, phenols, indoles, pyrazines, furans and pyrroles. Some of them are obtained

| Name | Whey | L. plantarum (2%) | S. boulardii (2%) | L. plantarum (2%) | S. boulardii (2%) |
|------|------|------------------|----------------|-----------------|-----------------|
| Ethanol | 1.156 | 1.160 | 1.157 | 1.157 |
| Isobutyric acid | 1.211 | 1.214 | *Nd | Nd |
| Acetic acid <1-methyl ethyl ester | 1.468 | 1.487 | 1.486 | 1.484 |
| Butanal <2-methyl-> | 1.527 | 1.530 | Nd | 1.523 |
| Acetate <isopropyl-> | 1.612 | 1.606 | 1.609 | 1.609 |
| Formic acid <2-methyl propyl ester | Nd | Nd | Nd | 1.793 |
| Valeraldehyde | Nd | Nd | Nd | 1.899 |
| Isobutyric acid | 2.226 | 2.330 | Nd | Nd |
| Acetophenone | 2.458 | 2.553 | 2.638 | 2.661 |
| Isoamyl alcohol | 2.825 | Nd | 2.825 | 2.823 |
| Pentanal <2-methyl-> | 3.263 | Nd | Nd | Nd |
| Toluene | 3.336 | 3.338 | Nd | Nd |
| Pentanol | 3.396 | Nd | Nd | Nd |
| Hexanal | 3.695 | 4.476 | 3.704 | 3.695 |
| Capronaldehyde | 4.048 | 4.052 | 4.053 | 4.048 |
| Furfural | 4.816 | Nd | Nd | Nd |
| Xylene | 5.864 | 5.875 | 5.872 | 5.870 |
| Hexanol <n-> | 5.956 | 5.973 | 5.959 | Nd |
| Heptanalone | 6.544 | 6.550 | 6.541 | 6.532 |
| Heptanal | 6.937 | 6.946 | 6.958 | 6.935 |
| Hexanoic acid | Nd | Nd | 7.743 | Nd |
| Benzaldehyde | 8.934 | 8.953 | Nd | 8.950 |
| Heptanol <n-> | 9.408 | 9.421 | 9.415 | 9.417 |
| Octen-3-one | 9.757 | Nd | 9.769 | Nd |
| Hept-5-en-2-one <6-methyl-> | 9.916 | Nd | 9.929 | 9.924 |
| Capryl alcohol | 13.250 | 13.274 | 13.249 | 13.266 |
| Heptyl methyl ketone | 13.958 | 13.960 | 13.966 | 13.950 |
| Acetate <octyl-> | 16.166 | 16.172 | Nd | 16.154 |
| Non-2 (E)-enal | ND | 16.576 | Nd |
| Caprylic acid | Nd | 17.121 | Nd | 17.123 |
| Butanoic acid <3-methyl-, 3-hexenyl-> | 19.495 | 19.560 | Nd | 19.486 |
| Butanoate <hexyl-, 3-methyl-> | Nd | 19.747 | Nd |
| Nonanoic acid <4-methyl-> | Nd | 24.061 | Nd | Nd |

*Nd: Not determined

Table 6. Retention times of whey and fermented whey beverages
from the milk or cheese production, others are associated
to whey powder making (MAHajan & Al [40]).

The amounts of hexanal and nonanal in the two
methods differed substantially since nonanal is one of the
compounds eluted late with the WRIGHT [46] method and
exhibited a low relative abundance. These aldehydes are
important since they affect whey protein aroma and were
found by CARUNCHIA WHETSTINE & Al [47] to have the
highest relative abundance among non-acidic volatiles
in whey protein concentrate. TUNICK & Al [48], reported
that the method of detecting components present in whey
differs in the identified compounds. The compounds
obtained in our study with the compounds mentioned in
the same study are; 2-Methylbutanal, Pentanol, Hexanal,
Heptanone, Heptanol and 1-Octen-3-ol.

Hexanal, acetic, butanoic and hexanoic acid were
determined in this study. MILLS & BROOME [49] have
isolated whey protein concentrate such as ethyl acetate,
2,3-butanediol, hexanal, dimethyl trisulfide, 2-nonanone,
onanal and volatile acids that acetic, butanoic and
hexanoic acids.

KARAGUL-YUCEER & Al [43] investigated the
effects of 2,3-butanediol, 2-butanol, hexanal, 2-acetyl-1-
pyrroline, methional, (E/E) – 2,4-nonadienal, (E/E) –
2,4-decadienal, and various short-chain volatile acids have
been identified from liquid whey. Compared this study with
ours, only hexanal and some short-chain acids were found
to be similar. It is believed that the differences in the
detected compounds are caused by the difference between
liquid whey and powdered one, produced cheese variety,
and differences of acid and sweet whey. Hexanal is
associated with fat oxidation reactions in processed foods,
but also in foods such as fruits and vegetables. Hexanal
is established in a diversity of food products including
meats and processed meats, fruits, processed fruits and
milk and cereal products. Particularly, hexanal often has
been related with green/grassy aromatics in fruits and
vegetables (CHAMBERS & KOPPEL [50]).

As a product of bacterial metabolism, the degree
of acetaldehyde and other flavor compounds is a result of
beverages ingredients, the conditions of the fermentation
process and the storage and properties of the used bacterial
strains. Because of using probiotic bacteria that do not
have typical capacities to produce aroma compounds
during fermentations, they can cause low content of
that compound in studied beverages (SKRYPLONEK &
JASINSKA [35]).

Lactic acid is the main product of LAB metabolism,
while diacetyl, acetone, 2,3-butanediol, acetate, ethanol,
formate, CO2 and others are also. These substances,
which are important in the taste feeling and texture of
many fermented foods, are produced at different rates
relative to the species and/or strains, and consequently the
composition of the LAB colonies can greatly affect the
quality of the product. Glycolysis, proteolysis and citrate
degradation are considered the main metabolic pathways in
which LAB plays a role in the formation of aroma and
flavor compounds in fermented products (LAW [51];
MAURIELLO & Al [52]).

Conclusion

In the last years, probiotics have emerged as potent
adjuvants for huma health due to their many effects
including cholesterol and blood pressure lowering, improve-
ment of lactose metabolism, anti-diarrhoal properties,
anticancerogenic and antimutagenis properties as well as
immunomodulation (MIZIELINSKA, L. LOPUSIEWICZ
[53], MURESAN [54]). Among the various probiotic
sources, whey can be used. Whey is high in lactose so it is
not suitable medium to survival of yeast. So, count of
bacteria is higher than yeast. In the sensory test, the
apperance, odour and taste of beverages were examined.
So beverage with 2% inoculation of L. plantarum AA1–2
is defined as good and acceptable. Also by inoculation of
mixture of 2% of L. plantarum AA1–2 and 2% of
S. boulardii can be prepared as drinkable beverages.

The bacteria levels were enough/adequate health
benefits to consumers (SKRYPLONEK & JASINSKA
[35]). Fermentation of whey by LAB can be an interesting
alternative to increase the proper usage of whey and to
provide an extra nutrient value to consumers. The presence
of probiotic cultures in whey that we can consume as
a beverage is an indication of our goal.

References

1. O. YERLIKAYA, Ö. KİNİK, N. AKBULUT.
Peyniraltı suyunu fonksiyonel özellikleri ve peyniraltısı
suuyu kullanılarak üretilen yeni nils süt ürünleri. 
Gıda, 34 (4), 289-296 (2010).
2. A.R. PRAZERES, F. CARVALHO, J. RIVAS.
Cheese Whey: Application Management: A review. Journal of
Environmental Management, 110, 48-68 (2012).
3. F. CARVALHO, A.R. PRAZERES, J. RIVAS.
Cheese whey wastewater: Characterization and
treatment. Science of the Total Environment, 445-446,
385-396 (2013).
4. D.H.G. PELEGRINE, R.L. CARRASQUERIA.
Cheese whey in nutritional beverages enrichment:
sensorial analysis. Revista de Ciência & Tecnologia,
18, n. 36 (2015).
5. M.I.G. SISO. The biotechnological utilization of
cheese whey: A Review. Bioresource Technology, 57,
1-11 (1996).
6. M.E. PINTADO, A.C. MACEDO, F.X. MALCATA.
Review: Technology, Chemistry and Microbiology
of Whey Cheese. Food Sci Tech Int, 7, 2, 105-106 (2001).
7. M. DJURIC, M. CARIC, S. MILANOVIC, M. TEKIC, M. PANIC. Development of whey-based beverages. Eur Food Res Technol, 219:321-328 (2004).
8. I. JELICIC, R. BOZANIC, L. TRATNIK. Whey-based beverages- a new generation of diary products. Mijekarstvo, 58 (3), 257-274 (2008).
9. R.S. CHAVAN, R.C. SHRADDHA, A. KUMAR, T. NALAWADE. Whey Based Beverage: Its Functionality, Formulations, Health Benefits and Applications. J Food Process Technol, 6, Issue 10 (2015).
10. S. SHERWOOD, D. JENSINS. US Patent US 2007/0178214 A1 (2007).
11. N.P. SHAH. Functional foods and health benefits. International Dairy Journal, 17, 1262-1277 (2007).
12. A. HERNANDEZ-MENDOZA, V.J. ROBLES, J.O. ANGULO, J. DE LA CRUZ, H.S. GARCIA. Whey-Based Probiotic Product. Food Technol. Biotechnol, 45 (1) 27–31 (2007).
13. D. DALEV, M. BIELECKA, A. TROZSYNSKA, S. ZIAJKA, G. LAMPARSKI. Sensory Quality of New Probiotic Beverages based on cheese whey and sensory preparation. Polish Journal of Food and Nutrition Sciences,15/56, 1, 71-77 (2006).
14. K.E. ALMEIDA, A.Y. TAMIME, M.N. OLIVEIRA. Acidification rates of probiotic bacteria in Minas frescal cheese whey. LWT-Food Science and Technology, 41, 2, 311-316 (2008).
15. M. PESCUма, M.E. HEBERT, F. MOZZI, G.F. DE VALDEZ. Whey fermentation by thermophilic lactic acid bacteria: Evolution of carbohydrates and protein content. Food Microbiology, 25, 442-451 (2008).
16. A. AKPINAR, F. ARTEMIS TORUNOĞLU, O. YERLIKAYA, Ö. KINIK, N. AKBULUT, H.R. UYSAL. Fermented probiotic beverages produced with reconstituted whey and cow milk: sensorial and rheological properties. Agro Food Industry Hi Tech, 26, 4 (2015).
17. G. BASYIGIT, A.G., KARAHAN, M.L. CAKMACKI. Probiyotik olma özelliği taşıyan lactik asit bakterilerin dondurma üretiminde kullanılması. Gida, 30, 6, 419-424 (2005).
18. G. BASYIGIT KILIC, A.G., KARAHAN, B. KILIC. Fermente et ürünlerinde fonksiyonel starter kültürler ve probiyotikler. Türk Hijyen ve Deneysel Biyoloji Dergisi, 64, 2, 60-69 (2007).
19. M. KAHALA, V. AHOLA, E. MÄKIMATTILA, L. PAULIN, V. JOUTSOKI. The Use of Macroarray as a Simple Tool to Follow the Metabolic Profile of Lactobacillus plantarum during Fermentation. Advances in Microbiology, 4, 996-1016 (2014).
20. H. SZAJEWSKA, A. SKORKA, M. DYLAG. Meta-analysis: Saccharomyces boulardii for treating acute diarrhoea in children. Aliment Pharmacol Ther, 25, 257–264 (2007).
21. C.M. SURAВICZ, G.W. ELMER, P. SPEELMAN, L.V. MCFARLAND, J. CHINN, G. VAN BELLE. Prevention of antibiotic-associated diarrhea by Saccharomyces boulardii: a prospective study. Gastroenterology 96(4):981-8 (1989).
22. W. HOCHTER, D. CHASE, G. HAGENHOFF. Saccharomyces boulardii in acute adult diarrhea: efficacy and tolerance of treatment. Münch Med Wochenschr, 132, 188-192 (1990).
23. L.V. MCFARLAND, P. BERNASCONI. Saccharomyces boulardii: a review of an innovative biotherapeutic agent. Microb Ecol Health Dis, 6, 157-171 (1993).
24. G. CETINA-SAURI, G. SIERRA BASTO. Therapeutic evaluation of Saccharomyces boulardii in children with acute diarrhea. Ann Ped, 41, 397-400 (1994).
25. C. POTHOULAKIS. Review article: anti-inflammatory mechanisms of action of Saccharomyces boulardii. Aliment Pharmacol Ther, 30, 826-833 (2009).
26. C. POTHOULAKIS, E. IM. Recent advances in Saccharomyces boulardii research. Gastroentérologie Clinique et Biologique, 34, Suppl. 1, S62-S70 (2010).
27. D. CZERUCKA, T. PICHE, P. RAMPAL. Review article: yeast as probiotics – Saccharomyces boulardii. Aliment Pharmacol Ther, 26, 767-778 (2007).
28. L.M.E. VANHEE, F. GOEME, H.J. NELIS, T. COENYE. Quality control of fifteen probiotic products containing Saccharomyces boulardii. Journal of Applied Microbiology, 109, 1745-1752 (2010).
29. A. PARRELLA, E. CATERINO, M. CANGIANO, E. CRISCUOLO, C. RUSSO, M. LAVORGNA, M. ISIDORI. Antioxidant properties of different milk fermented with lactic acid bacteria and yeast. International Journal of Food Science and Technology, 47, Issue 12, Pages 2493-2502 (2012).
30. C.R. REKHA, G. VIJAYALAKSHMI. Biomolecules and nutritional quality of soymilk fermented with probiotic yeast and bacteria. Appl. Biochem. Biotechnol, 151(2-3), 452-63 (2008).
31. G. BASYIGIT. The probiotic properties of some lactic acid bacteria. MSc Thesis, Suleyman Demirel University, Research Project Grants, SDU-BAP-517 (2004).
32. G. BASYIGIT KILIC, A.G. KARAHAN. Identification of lactic acid bacteria isolated from the fecal samples of healthy humans and patients with dyspepsia and determination of their pH, bile and
antibiotic tolerance properties. *Journal of Molecular Microbiology and Biotechnology*, 18: 220-229 (2010).
33. H. SAGLAM. Tanınlanmış Lactobacillus plantarum suçlarına plazmid profilleri ve bunların bazı özellikle-rinin belirlenmesi. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Gida Mühendisliği Anabilim Dalı, Isparta (2013).
34. A.G. KARAHAN, B.C. ARIDOĞAN, M.L. ÇAKMAKÇI. Genel Mikrobiyoloji Uygulama Klavuzu. Süleyman Demirel Üniversitesi, Ziraat Fakültesi, Yayın No: 24, Isparta (2002).
35. K. SKRYPLONEK, M. JASIŃSKA. Fermented Probiotic Beverages Based On Acid Whey. *Acta Sci. Pol. Technol. Aliment*, 14(4), 397-405 (2015).
36. M. ALBENZIO, M.R. CORBO, S.U. REHMAN, P.F. FOX, M. DE ANGELIS, A. CORSETTI, A. SEVI, M. GOBBETTI. Microbiological and biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk, or by heating the curd in hot whey. *International Journal of Food Microbiology*, 67, 35-48 (2001).
37. A. LOURENS-HATTINGH, B.C. VILJOEN. Growth and survival of a probiotic yeast in dairy products. *Food Research International*, 34, Issue 9, Pages 791-796 (2001).
38. I. DRGALIC, L. TRATNIK, R. BOZANIC. Growth and survival of probiotic bacteria in reconstituted whey. *Lait*, 85, 171-179 (2005).
39. FAO/WHO. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. American Córdoba Park Hotel, Córdoba, Argentina, 1-4 October (2001).
40. S.S. MAHAJAN, L. GODDIK, M.C. QIAN. Aroma Compounds in Sweet Whey Powder. *J. Dairy Sci.*, 87, 4057-4063 (2004).
41. Y. KARAGÜL-YÜCEER, M.A. DRAKE, K.R. CADWALLADER. Aroma-active components of nonfat dry milk. *J Agric Food Chem*, 49 (6):2948-53 (2001).
42. Y. KARAGÜL-YÜCEER, K.R. CADWALLADER, M. DRAKE. Volatile Flavor Components of Stored Nonfat Dry Milk. *J. Agric. Food Chem*, 50 (2), pp 305-312 (2002).
43. Y. KARAGÜL-YÜCEER, M.A. DRAKE, K.R. CADWALLADER. Aroma-active Components of Liquid Cheddar Whey. *Journal of Food Science*, 68, Nr. 4, 1215-1219 (2003).
44. C. WHETSTINE, J.D. PARKER, M.A. DRAKE, D.K. LARICK. Determining flavor and flavor variability in commercially produced liquid Cheddar whey. *J. Dairy Sci*, 86:439-448 (2003).
45. F.B., WHITFIELD. Volatiles from interactions of Maillard reaction and lipids. *Crit. Rev. Food Sci. Nut*, 31:1-58 (1992).
46. B.J. WRIGHT, S.E. SEVCHAK, J.M. WRIGHT, M.A. DRAKE. The impact of agglomeration and storage on flavor and flavor stability of whey protein concentrate 80% and whey protein isolate. *J. Food Sci*, 74, S17-S29 (2009).
47. M.E. CARUNCHIA WHETSTINE, A.E. CROISSANT, M.A. DRAKE. Characterization of dried whey protein concentrate and isolate flavor. *J. Dairy Sci*, 88, 3826-3889 (2005).
48. M.H. TUNICK, S.K. IANDOLA, D.L. VAN HEKKEN. Comparison of SPME Methods for Determining Volatile Compounds in Milk, Cheese, and Whey Powder. *Foods*, 2, 534-543 (2013).
49. O.E. MILLS, A.J. BROOME. Isolation of Flavor Compounds from Protein Material. *ACS Symposium Series*, 705, Chapter 8, pp 85-91 (1998).
50. E. IV. CHAMBERS, K. KOPPEL. Associations of Volatile Compounds with Sensory Aroma and Flavor: The Complex Nature of Flavor. *Molecules*, 18, 4887-4905 (2013).
51. B.A. LAW. The formation of aroma and favour compounds in fermented dairy products. *Dairy Science Abstract*, 43, 143-154 (1981).
52. G. MAURIELLO, L. MOIO, G. MOSCHETTI, P. PIOMBINO, F. ADDEO, S. COPPOLA. Characterization of lactic acid bacteria strains on the basis of neutral volatile compounds produced in whey. *Journal of Applied Microbiology*, 90, 928-942 (2001).
53. M. MIZIELIŃSKA, Ł. ŁOPUSIEWICZ. Encapsulation and evaluation of probiotic bacteria survival in simulated gastrointestinal conditions. *Rom Biotechnol Lett*, 23(3): 13690-96 (2018).
54. A. MUREŞAN, I. SĂRBU, D. PELINESCU, R. IONESCU, O. CSUTAK, I. STOICA, T. VASSU-DIMOV. In vitro selection of some lactic acid bacteria strains with probiotic potential. *Rom Biotechnol Lett*, 23(1): 13327-39 (2018).