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Original paper

An investigation for the development of whey-based probiotic beverages

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Abstract Whey is the 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 10^5 cfu/ml, and bacteria count were between 10^7-10^8 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.

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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 deproteinization of whey, concentration of whey, fermentation or addition of sucrose to reach the desired alcohol content (0.5-1%), flavoring, sweetening and bootling 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 aminoacids 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, glycomacropeptide, phenylalanine, and alpha-lactalbumin. Owing to lactoferrin content, whey beverages can be usefull 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 usefull 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-diarrhoeal 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 thermophillus*, *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii* sbsp. *bulgaricus*, *Bifidobacterium animalis* subsp. *lactis* and co-culture *Streptococcus thermophillus–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]; CETINA-SAURI & 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 (Reflor, 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-SPME. The system consisted of an Shimadzu GCMS-QP2010 SE dedector (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 asidic 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 PARRELLA & 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]; LOURENS-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 means±standard deviation of three seperate determinations.

Before the fermentation process, count of *S. boulardii* were ranged from $1.0*10^4$ to $6.5*10^4$, count of *L. plantarum* AA1-2 were ranged from $1.6*10^6$ to $1.5*10^7$ cfu/ml. After the fermentation process, yeast count was higher than 10^5 cfu/ml, bacteria count was between 10^7-10^8 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 10^9 cfu/ml (PESCUMA & al [15]).

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)	$3.5 \pm 0.10*10^6$	$1.3 \pm 0.08*10^8$
2	*L. plantarum* (% 2)	$1.0 \pm 0.19*10^7$	$7.0 \pm 0.07*10^7$
3	*S. boulardii* (% 2)	$1.0 \pm 0.22*10^4$	$4.0 \pm 0.11*10^5$
4	*L. plantarum* (% 1) and *S. boulardii* (% 1)	$1.6 \pm 0.18*10^6$ $8.0 \pm 0.18*10^4$	$7.5 \pm 0.21*10^7$ $1.7 \pm 0.29*10^5$
5	*L. plantarum* (% 2) and *S. boulardii* (% 2)	$9.5 \pm 0.15*10^6$ $6.5 \pm 0.17*10^4$	$5.0 \pm 0.21*10^7$ $2.5 \pm 0.12*10^5$

* Data are the means±standard deviation of three seperate 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 *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.

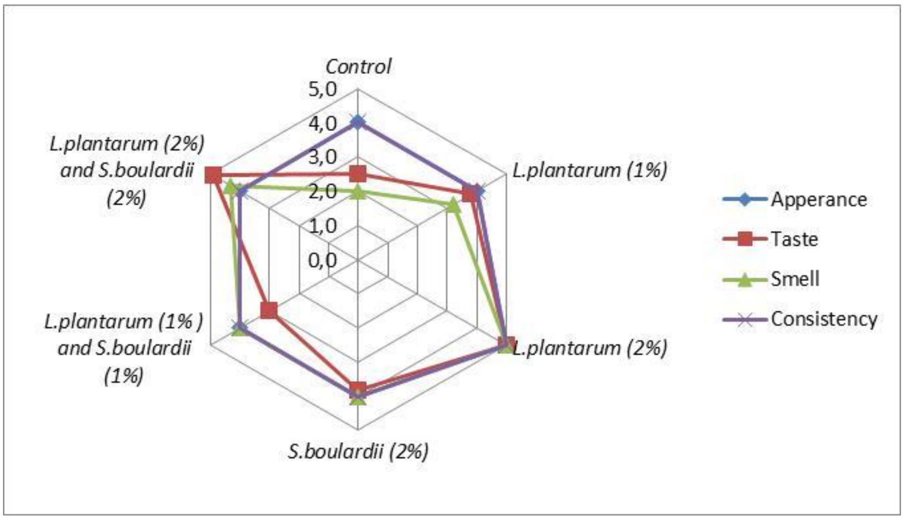

Figure 1. Sensory properties of fermented whey beverages (Data are the means±standard deviation of three seperate determinations)

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 are 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

<octyl->. At the same conditions, those compounds are converted to acetate <isopropyl->, acetoin, etanol, isoamyl alcohol, acetic acid 1 methyl ethyl ester, heptanone and heptanol <n-> by 2% of *S. boulardii*. Combination of 2% of *L. plantarum* AA1-2 and 2% *S. boulardii* converted to acetoin, acetate <isopropyl->, formic acid <2-methyl propyl-> ester, etanol, 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 & al [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 & al [41, 42]).

Table 5. Area (%) of peaks of whey and fermented whey beverages

Name	**Whey**	***L. plantarum* (2%)**	***S. boulardii* (2%)**	***L. plantarum* (2%) *S. boulardii* (2%)**
Ethanol	1.21	0.68	12.26	6.05
Isobutyl alcohol	0.92	1.59	*Nd	Nd
Acetic acid <1-methylethyl-> 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	Nd	5.44	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
Benzaldehyde	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

*Nd: Not determined

Table 6. Retention times of whey and fermented whey beverages

Name	**Whey**	***L. plantarum* (2%)**	***S. boulardii* (2%)**	***L. plantarum* (2%) *S. boulardii* (2%)**
Ethanol	1.156	1.160	1.157	1.157
Isobutyl alcohol	1.211	1.214	*Nd	Nd
Acetic acid <1-methylethyl-> 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-methylpropyl-> ester	Nd	Nd	Nd	1.793
Valeraldehyde	Nd	Nd	Nd	1.899
Isobutyric acid	2.226	2.330	Nd	Nd
Acetylpropionyl	Nd	Nd	Nd	2.314
Acetoin	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
Heptanone	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-ol	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	Nd
Nonanoic acid <4-methyl->	Nd	24.061	Nd	Nd

*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-decadienal, 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 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-butanedione, hexanal, dimethyl trisulfide, 2-nonanone, nonanal and volatile acids that acetic, butanoic and hexanoic acids.

KARAGÜL-YUCEER & al [43] investigated the effects of 2,3-butanedione, 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, CO_2 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, improvement of lactose metabolism, anti-diarrhoeal properties, anticancerogenic and antimutagenis properties as well as immunomodulation (MIZIELIŃSKA, Ł. ŁOPUSIEWICZ [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.

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