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IMPROVEMENT OF PRODUCTION PERFORMANCE OF FUNCTIONAL FERMENTED WHEY-BASED BEVERAGE

Article Highlights

- Effect of culture mixing and different fermentation temperature on fermentation time, titratable acidity, total cell count, aroma and storage stability of functional whey-based beverage were investigated
- Culture mixing contributes to the aroma improvement
- Temperature has significant influence on the fermentation dynamic and cell viability during the storage of produced beverage
- A beverage produced by mixed culture of *Lactobacillus helveticus* ATCC 15009 and *Streptococcus thermophilus* S3 at 42 °C achieved high storage stability with a shelf life of 22 days

Abstract

The aim of this study was improvement of the performances for the production of whey-based beverages with highly productive strains of *Lactobacillus*. Individual or mixed cultures containing *Lactobacillus helveticus* ATCC 15009, *Lactobacillus delbrueckii* ssp. *lactis* NRRL B-4525 and *Streptococcus thermophilus* S3 were studied. The scientific hypothesis was that production performances, especially aroma and viable cell count, are positively affected by the strains combination and temperature. Based on the results, beverages obtained by mixed cultures *Lb. helveticus* ATCC 15009 - *S. thermophilus* S3 and *Lb. delbrueckii* ssp. *lactis* - *S. thermophilus* S3 had higher aroma values than beverages obtained by individual strains. The symbiosis of tested strains had a positive impact on the aroma of produced beverage. In addition, the temperature had significant effects on cell viability during storage and fermentation dynamics. The beverages produced by mixed cultures *Lb. helveticus* ATCC 15009 - *S. thermophilus* S3 and *Lb. delbrueckii* ssp. *lactis* - *S. thermophilus* S3 at 42 °C achieved higher storage stability (19 to 22 days) than beverages produced at 37 and 45 °C (13 to 19 days). Subsequently, at 42 °C fermentation time for both mixed cultures was 1.5 h shorter, compared to the time achieved at 37 °C.

Keywords: whey, functional beverages, probiotics, *Lactobacillus*, fermentation, stability.

Whey is a major by-product of the cheese industry often disposed as waste, causing high environmental contamination because of high COD (57–75 g/L) [1] and BOD₅ (35–40 g/L) [2] values, which is completely at odds with the nutritional potential that this material possesses. Considerable efforts have

been made over the past years to find new outlets of whey utilization in terms to reduce environmental pollution [3–5]. Whey itself has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral and chelating agent [6]. Thus, in recent years the bioconversion of whey has become an interesting process from the viewpoint of human nutrition, especially for therapeutic purposes, in regard to economy, and with advantage for reducing pollution [7].

As main nutritive components, whey contains: 0.50–0.55% of beta-lactoglobulin (source of essential and branched chain amino acids), 0.20–0.25% of

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alpha-lactoalbumin (primary protein found in human breast milk also of essential and branched chain amino acids), 0.10-0.15% of immunoglobulins (primary protein found in colostrum with immune modulating benefits), 0.01-0.02% of lactoferrin (antioxidant, antibacterial, antiviral and antifungal agent which promotes growth of beneficial bacteria), 0.005% of lactoperoxidase (inhibits growth of bacteria), 0.05-0.10% of bovine serum albumin (large protein which is source of essential amino acids) and 0.10-0.15% of glycomacropeptide (source of branched amino acids that lacks aromatic amino acids such as phenylalanine, tryptophan and tyrosine) [6].

The fermentation of whey by lactic acid bacteria allows the production of beverages with significantly improved characteristics. Fermented whey contains: a) lactic acid and possibly antimicrobial compounds important for maintaining of intestinal microflora; b) flavor compounds (*e.g.*, acetaldehyde in yoghurt and cheese) and other metabolites (*e.g.*, extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; c) free amino acids and vitamins which improve the nutritional value of whey; d) substances that provide a special therapeutic or prophylactic effect against cancer and control of serum cholesterol levels [8-12].

Numerous strains of *Lactobacillus* genera are already known as highly productive in lactic acid fermentation [13]. In addition to their role in fermentation processes, some of these lactic acid bacteria have been studied as dietary sources of substances destined to promote a positive impact in the host by improving the health benefits. The strains *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis*, beside high fermentation productivity, have recently been considered as important bacteria for human health. Therefore, the main effect attributed to strain *L. helveticus* includes the production of whey hydrolysates that contains potent angiotensin I-converting enzyme (ACE) inhibitory peptides, with a high inhibition rate [14,15]. The strain *Streptococcus thermophilus* S3 is also marked as a good producer of exopolysaccharides [16], which could be considered as prebiotics because of their positive impact on human gut microflora. Also, these exopolysaccharides can enhance the viability of probiotic bacteria in cases when they are present in beverages. High productivity of these strains is very important in terms of profitability of the beverage production process. The use of highly productive strains shortens the fermentation time, which can significantly decrease the beverage production costs and valorize whey from cheese production what is important in term of production generated income.

In addition, from the consumer's point of view, the manufacture of whey-based beverages through lactic fermentation must provide desirable sensory profiles of product [17,18]. This is not always the case, especially when the fermentation is performed with highly productive strains that produce high level of lactic acid and substances with unacceptable odour and taste. Combining different species or strains can lead to a significant improvement of production performance due to symbiotic interaction between microorganisms [19]. There are many combinations of microorganisms that can provide production of beverages with satisfactory sensory characteristics, and the necessary beverage production criteria such as low cost of production, functionality and storage stability. However, this area has not been fully explored.

The aim of this study was improvement of the performances for the production of functional whey-based beverages with highly productive strains of *Lactobacillus* genera. The scientific hypothesis was that whey-based beverage performances, especially aroma and viable cell count are positively affected by the strain combination and appropriate temperature. Influence of fermentation temperature on the stability of viable cell count during the storage of whey-based beverage was also investigated.

MATERIALS AND METHODS

Microorganisms and media

The strains *L. helveticus* (ATCC 15009), *L. delbrueckii* ssp. *lactis* (NRRL B-4525) and *S. thermophilus* (S3) used in this work were obtained from the Culture Collection of Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Belgrade, Serbia. Stock cultures were stored at -20 °C in 3 mL vials containing MRS broth and 50% (v/v) glycerol as a cryoprotective agent. For the preparation of laboratory cultures, a drop of stock culture were transferred in 3 mL of the MRS broth and incubated for 18 h under anaerobic conditions at the optimal growth temperature (37 °C). All working cultures were pre-cultured twice in an MRS broth prior to experimental use.

Whey fermentation

Sweet whey powder (Lenic Laboratories, Belgrade, Serbia), with following composition: proteins 12.11%, fat 1.0%, and carbohydrates 69.62%, was reconstituted to contain 8% of dry matter. A volume of 300 mL of the reconstituted whey with pH 6.2 was poured into sterile glass bottles of 500 mL. Samples were pasteurized at 60 °C for 60 min, cooled at fer-

mentation temperature (37, 42 and 45 °C) and immediately inoculated by adding 2% (v/v) of individual strains or mixed cultures. For the preparation of mixed cultures highly productive strains *L. helveticus* and *L. delbrueckii* ssp. *lactis* were mixed with strain *S. thermophilus* in ratio 1:1. The fermentations were carried out until pH 4.6 was attained. During the incubation time samples were withdrawn every 1 h for determination of pH value. When pH 4.6 was reached, fermentations were stopped by quick cooling. The resulting beverages were distributed in sterile plastic bottles in triplicates and stored at 4 °C for 35 days. Viable cell count (log CFU/mL) was determined every 5 days of the storage.

Chemical and microbiological analysis

Acidity of whey samples was analyzed as pH and titratable acidity. The pH was measured by a pH meter (WTW pH 720), and titratable acidity (°SH) by the Soxhlet-Henkel method [20].

The fermented samples were analyzed for viable cell count by pour plate technique on MRS agar [21].

Sensory evaluation

Aroma as the most pronounced sensory characteristic was analyzed by a panel group of 5 sensory analysts and evaluated with grades from 1 to 5. For aroma evaluation, the following scale was used: 1 - on sauerkraut, 2 - on sourdough, 3 - on whey, 4 - on mild yogurt, 5 - on yogurt [22].

The experiments were done in triplicate, and the results are shown as average values.

RESULTS AND DISCUSSION

Effect of culture mixing on beverage production performances

In order to investigate the possibilities of improvement of beverage production performances the fermentation with individual and mixed cultures was performed. Fermentations were carried out at 37 °C, statically. Production performances were evaluated by determining the fermentation time (h), titratable acidity (°SH), viable cell count (log CFU/mL) and aroma value.

As shown in Figure 1, both assayed strains showed relatively short fermentation time between 5.0 and 6.5 h. Short fermentation time can substantially decrease the beverage production costs and valorize whey from cheese production. The fermentation with *L. helveticus* was 1.5 h shorter than the fermentation with *L. delbrueckii* ssp. *lactis*. Based on the obtained results, it could be said that both strains are highly productive, which is very important in terms of production costs. Beside shorter fermentation time, strain *L. helveticus* showed higher titratable acidity (11.4 °SH) and viable cell count (8.0 log CFU/mL) than the strain *L. delbrueckii* ssp. *lactis* (10.4 °SH and 6.6 log CFU/mL). It is interesting to note that the strain *L. delbrueckii* ssp. *lactis* showed very low cell growth in addition to high amount of produced lactic acid. This

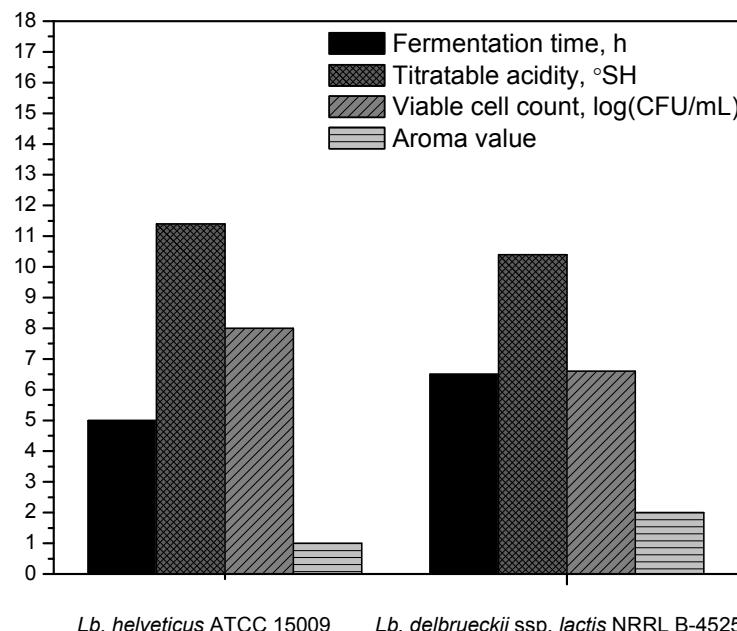


Figure 1. Fermentation time, titratable acidity, total cell count and aroma value of whey fermented by individual strains *Lactobacillus helveticus* ATCC 15009 and *Lactobacillus delbrueckii* ssp. *lactis* NRRL B-4525.

could be due to differences in lactic acid production abilities and growth characteristics, which, in the case of this strain, are independent of each other. Both strains produced beverages with unpleasant aroma and exhibited low aroma values. Strain *L. helveticus* exhibited value 1 for aroma which was on sauerkraut, while strain *L. delbrueckii* ssp. *lactis* exhibited value 2 for aroma which was on sourdough. This could be because the tested strains do not produce diacetyl, which has long been known as a major contributor to aroma in fermented dairy products [23]. In addition, both strains produced beverages without presence any type of precipitate and sour-salty taste because of high amount of present lactic acid.

To improve production performances, individual strains were mixed with strain *S. thermophilus*. Since both mixed cultures were prepared by adding the same amount of *S. thermophilus*, a proportional change in fermentation time was expected. But, as shown in Figure 2, the fermentation time with mixed culture *L. delbrueckii* ssp. *lactis*-*S. thermophilus* did not change, while the fermentation with mixed culture *L. helveticus*-*S. thermophilus* was longer for 1.5 h than the fermentation with individual strain. Different impact of the culture mixing on the fermentation time is probably a consequence of different proteolytic activity of used *Lactobacillus* strains. According to Pescuma *et al.* [24], species *L. delbrueckii* have high

proteolytic activity, which provides amino acids that are a growth factor of many microorganisms including *S. thermophilus*. On the other hand, *L. helveticus* requires the presence of certain amino acids for growth and fermentation activity [25] that cannot be provided by *S. thermophilus*. It could be assumed that *L. delbrueckii* ssp. *lactis* has higher proteolytic activity than *L. helveticus* and probably was able to provide the amino acids corresponding to strain *S. thermophilus*. Therefore, the symbiosis of strains *Lb. delbrueckii* ssp. *lactis* and *S. thermophilus* proved to be better. The reduced amount of *L. delbrueckii* ssp. *lactis* in the inoculum can be compensated by the addition of *S. thermophilus* so that the fermentation time remains the same. On the other hand, the symbiosis of strains *L. helveticus* and *S. thermophilus* gives mixed culture with lower fermentation activity which leads to a prolongation of the fermentation. In contribution to this assumption, a higher titratable activity (13°SH) was achieved in the sample fermented with mixed culture *L. delbrueckii* ssp. *lactis*-*S. thermophilus*. This also could be due to presence of amino acids that promote the metabolism of *S. thermophilus* strain.

The sensory characteristics are a very important factor for product placement. As shown in Figure 2, the aroma value was higher (value 5 - on yogurt) in both mixed cultures compared with the values shown

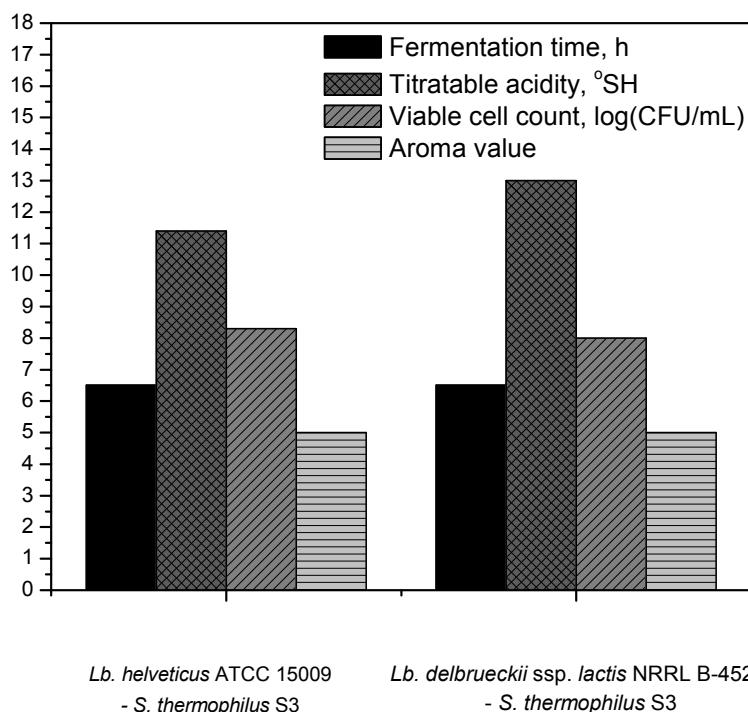


Figure 2. Fermentation time, titratable acidity, viable cell count and aroma value of whey fermented by mixed cultures *Lactobacillus helveticus* ATCC 15009-*Streptococcus thermophilus* S3 and *Lactobacillus delbrueckii* ssp. *lactis* NRRL B-4525-*Streptococcus thermophilus* S3.

in Figure 1 obtained by individual strains. This is probably due to presence of *S. thermophilus* S3 that is marked as a good producer of exopolysaccharides and probably diacetyl. Also, produced exopolysaccharides possibly induce *L. helveticus* and *L. delbrueckii* ssp. *lactis* metabolism to produce diacetyl, a major contributor of flavor and aroma. In this regards, it could be said that the culture mixing in both cases contributes significantly to improvement of sensory characteristics.

It must be considered that the national consumer preferences could also play an important role in sensory analysis. There are not too many whey-based drink products in the Serbian market and there is no tradition of drinking whey and whey-based beverages in Serbia. These products are more widely available and accepted by people in countries such as Germany, Austria, and Switzerland where there is a longer tradition of whey consumption [26]. In this respect, this study favors the beverages with sensory characteristics that are similar to the fermented products present on Serbian market to which the consumers are already accustomed.

Effect of incubation temperature on production performances and beverage storage stability

In order to improve the beverage production performances, the influence of various incubation temperatures on fermentation parameters and storage stability was investigated. Fermentations were performed at three different temperatures by using mixed cultures, statically. The data on the effect of temperature of 37, 42 and 45 °C on fermentation time (h), titratable acidity (°SH), viable cell count (log CFU/mL) and aroma value of the beverage are presented in Tables 1 and 2. Table 1 compares fermentation time, titratable acidity, viable cell count and aroma value of whey fermented by mixed culture *L. helveticus*-*S. thermophilus* at different temperatures. Table 2 compares fermentation time, titratable acidity, viable cell count and aroma value of whey fermented by mixed culture *L. delbrueckii* ssp. *lactis*-*S. thermophilus* at different temperatures.

As shown in Tables 1 and 2, for both mixed cultures the fermentation time at 42 and 45 °C was decreased for 1.5 h, compared to the time achieved at 37 °C. Therefore, the temperature increase has a significant influence on the fermentation dynamics. Reduced fermentation time reduces costs of the production and valorizes obtained product. Subsequently, the temperature increase leads to decrease of titratable acidity and increase of viable cell count in both samples. Temperature of 40–45 °C is optimal for

S. thermophilus which is probably one of the reasons for faster whey fermentation. Both samples had excellent aroma values at all temperatures. With respect to viable cell count as a main fermentation parameter, the best results were observed at a temperature of 45 °C for both mixed cultures.

*Table 1. Effect of incubation temperature on fermentation time, titratable acidity, viable cell count and aroma value of beverage fermented by mixed culture *Lactobacillus helveticus* ATCC 15009 - *Streptococcus thermophilus* S3*

Parameter	Incubation temperature, °C		
	37	42	45
Fermentation time, h	6.5	5.0	5.0
Titratable acidity, °SH	11.4	11.2	11.0
Viable cell count, log(CFU / mL)	8.30	8.38	8.60
Aroma value	5	5	5

*Table 2. Effect of incubation temperature on fermentation time, titratable acidity, viable cell count and aroma value of beverage fermented by mixed culture *Lactobacillus delbrueckii* ssp. *lactis* NRRL B-4525-*Streptococcus thermophilus* S3*

Parameter	Incubation temperature, °C		
	37	42	45
Fermentation time, h	6.5	5.0	5.0
Titratable acidity, °SH	13.0	11.4	11.2
Viable cell count, log(CFU / mL)	8.00	8.25	8.36
Aroma value	5	5	5

To examine the effect of incubation temperature on storage stability of the fermented product, the beverages were kept in the refrigerator at 4 °C for 35 days. Stability was evaluated after 0, 5, 10, 15, 20, 25, 30 and 35 days of the storage by determining the pH value, titratable acidity, viable cell count and aroma values. Data for pH, titratable acidity and aroma values are not presented because the most interesting aspect of our study was to determine bacterial survival during the storage and the influence of fermentation temperature on total bacterial count during the storage. Figure 3 compares the effect of incubation temperature on viable cell count during the storage of whey fermented by a mixed culture *L. helveticus*-*S. thermophilus*. Figure 4 compares the effect of incubation temperature on viable cell count during the cool storage (4 °C) of whey fermented by a mixed culture *L. delbrueckii* ssp. *lactis*-*S. thermophilus*.

As shown in Figure 3, the viable cell count \geq 6 log units has been held until the 23rd day of the storage in the sample fermented by *L. helveticus*-*S. thermophilus* at 42 °C. In samples fermented at 37 and 45 °C, the viable cell count was less than 6 log units already after 15 and 20 days of storage, respect-

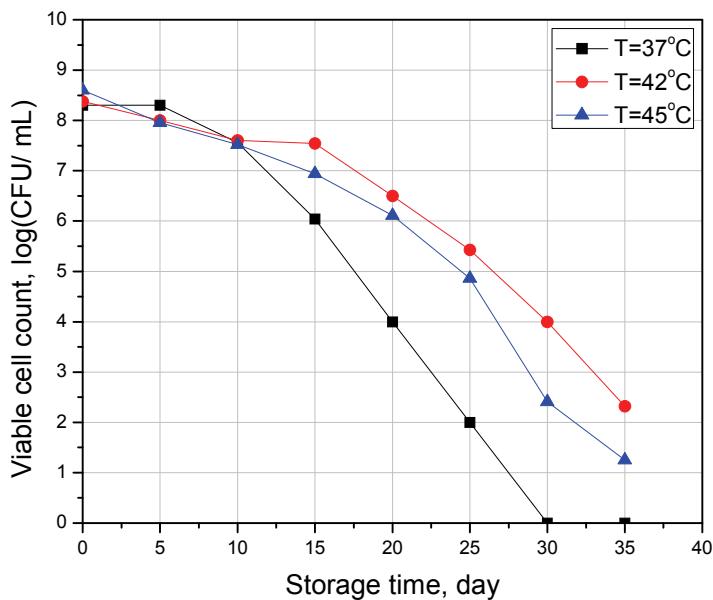


Figure 3. Effect of incubation temperature on viable cell count during the cool storage ($4\text{ }^{\circ}\text{C}$) of beverage fermented by mixed culture *Lactobacillus helveticus* ATCC 15009-*Streptococcus thermophilus* S3.

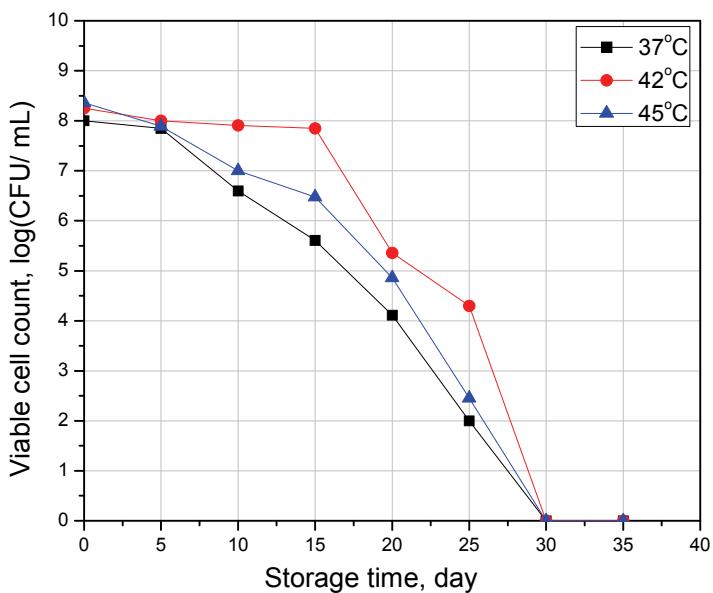


Figure 4. Effect of incubation temperature on viable cell count during the cool storage ($4\text{ }^{\circ}\text{C}$) of beverage fermented by mixed culture *Lactobacillus delbrueckii* ssp. *lactis* NRRL B-4525-*Streptococcus thermophilus* S3.

ively. From the comparison of the viable cell count on the 23rd day of storage (Figure 3) in samples fermented at different temperatures, it can be concluded that the temperature of $42\text{ }^{\circ}\text{C}$ had a positive impact on the stability of viable cell count during the storage period.

As shown in Figure 4, temperature $42\text{ }^{\circ}\text{C}$ showed positive effects on the stability of the viable cell count of mixed culture *L. delbrueckii* ssp. *lactis*-*S. thermophilus* during the storage period. The viable cell count ≥ 6 log units was held until about 20 days of storage.

In samples fermented at 37 and $45\text{ }^{\circ}\text{C}$, the viable cell count was less than 6 log units already after 13 and 15 days of storage, respectively.

As shown in Figures 3 and 4, after 20 days of storage viable cell count in sample fermented by *L. helveticus*-*S. thermophilus* was 6.5 log CFU/mL while in sample fermented by *L. delbrueckii* ssp. *lactis*-*S. thermophilus* was 5.36 log CFU/mL. Based on these results, it can be concluded that the mixed culture *L. helveticus*-*S. thermophilus* was more stable than

mixed culture *L. delbrueckii* ssp. *lactis*-*S. thermophilus* after the fermentation at 42 °C.

Regardless of the temperature, both mixed cultures had excellent aroma values at the end of the storage period (value 5). Titratable acidity increased in all samples for 20 days (about 0.5-1.0 °SH) of storage, regardless of temperature. After 20 days, the titratable acidity was stable to the end of the storage period. A significant pH decrease (around 0.5 pH units) was noticed in both samples fermented at 42 and 45 °C after 15 days of storage, while samples fermented at 37 °C showed pH decrease (around 0.4 pH units) after 5 days of storage. The pH value was stable until the end of the storage period.

CONCLUSIONS

Whey fermentation by mixed cultures *L. helveticus*-*S. thermophilus* and *L. delbrueckii* ssp. *lactis*-*S. thermophilus* showed very similar characteristics. Symbiosis of testing cultures contributes to the increase of the aroma values, with negligible prolongation of fermentation time, which can be avoided by increasing the fermentation temperature.

Based on the presented results, for both mixed cultures maximal viable cell count (about 8.5 log CFU/mL) was achieved at 45 °C, but during the storage period this count was decreased significantly faster than in samples fermented at 42 °C. Subsequently, the temperature of 42 °C is estimated as optimal for the fermentation of whey by both mixed cultures. Samples fermented at this temperature showed longer stability of viable cell count during the storage period than samples fermented at 45 °C. Viable cell count \geq 6 log units in the sample fermented at 42 °C by *L. helveticus* ATCC 15009-*S. thermophilus* S3 has been held until 22nd day, while in the sample fermented by *L. delbrueckii* ssp. *lactis*-*S. thermophilus* until 19th day of storage. Subsequently, the mixed culture *L. helveticus*-*S. thermophilus* was more stable during the storage and had a longer shelf life.

Acknowledgements

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NAUČNI RAD

UNAPREĐENJE PERFORMANSI PROIZVODNJE FUNKCIONALNOG FERMENTISANOG NAPITKA NA BAZI SURUTKE

Postoji veliki broj sojeva iz roda *Lactobacillus* koji su već poznati kao visoko produktivni u procesu mlečno-kiselinske fermentacije. Primena ovih visoko produktivnih sojeva skraćuje vreme trajanja fermentacije, smanjuje troškove proizvodnje napitaka na bazi surutke i valorizuje surutku nastalu tokom procesa proizvodnje sira. Cilj ovog istraživanja je bio unapređenje performansi procesa proizvodnje napitaka na bazi surutke primenom visoko produktivnih sojeva iz roda *Lactobacillus*. Proučavane su pojedinačne ili mešane kulture koje sadrže sojeve *Lactobacillus helveticus* ATCC 15009, *Lactobacillus delbrueckii* ssp. *lactis* NRRL B-4525 i *Streptococcus thermophilus* S3. Polazna naučna hipoteza je bila da na performanse procesa proizvodnje napitaka, posebno na aromu i ukupni broj ćelija, pozitivno utiče kombinovanje sojeva i primenjena temperatura fermentacije. Takođe je ispitivan uticaj temperature fermentacije na preživljavanje primenjenih sojeva tokom procesa skladištenja napitaka. Na osnovu dobijenih rezultata, simbioza ispitivanih sojeva doprinosi unapređenju arome. Napici dobijeni primenom mešanih kultura imaju vrlo prihvratljivu aromu što je veoma važno za njihovo uključivanje u ishranu ljudi. Rezultati pokazuju da temperatura ima veoma značajan uticaj na dinamiku procesa fermentacije kao i na preživljavanje primenjenih sojeva tokom procesa skladištenja. Napitak proizveden pomoću mešane kulture sastavljene od sojeva *Lactobacillus helveticus* ATCC 15009 i *Streptococcus thermophilus* S3, fermentacijom na temperaturi 42 °C ispoljava visoku stabilnost tokom skladištenja sa rokom trajanja od 22 dana.

Ključne reči: surutka, funkcionalni napici, probiotici, *Lactobacillus*, fermentacija, stabilnost.