INFLUENCE OF STARTER CULTURES ON THE ACIDITY AND SENSORY CHARACTERISTICS IN PROBIOTIC YOGHURT

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Abstract
Probiotic yoghurt belongs to a group of products known as functional foods and has an increasing importance in human nutrition nowadays. The chemical and physical properties of raw cow's milk for production of probiotic yoghurt had the following average values: milk fat (3.70%), proteins (3.25%), lactose (4.45%), non-fat dry matter (8.37%), freezing point (-0.517 °C), titratable acidity (6.12 °SH) and pH (6.65). After the standardization of milk fat and addition of skim milk powder, the milk was with the following parameters: milk fat (1.00%), proteins (3.47%), lactose (4.51%), non-fat dry matter (9.00%), freezing point (-0.520 °C), titratable acidity (6.32 °SH) and pH (6.64). The paper examined the dynamics of titratable and active acidity, as well as the sensory properties of probiotic yoghurt using 3 types of starter cultures (ABT 6, ABT 10 and ABT 21), produced in Chr. Hansen, Denmark. These starter cultures, were composed of bacteria Lactobacillus acidophilus LA-5, Bifidobacterium ssp.BB-12 and Streptococcus thermophilus ST, in different ratios. The studies were conducted on the 1, 7, 14 and 21 day. The titratable acidity of the yoghurt ranged in intervals between 28.55-29.18 °SH on the first day of the trials and reached values of 34.29-36.10 °SH after a 21 day storage. The pH value ranged between 4.45-4.48 (1 day) and reached 4.04-4.14 units (21 days). The lowest post-acidification and best sensory properties of the final product were determined in the variant yoghurt produced with the starter culture ABT 10.

Key words: probiotics, ABT culture, bacteria, acidity, quality

Received: 25.06.2018 Reviewed: 10.09.2018 Accepted: 28.09.2018

1. INTRODUCTION

Fermented dairy products are a very important segment in human nutrition, not only because of their sensory characteristics, but also because of the high nutritional values and positive health effects. They received the greatest publicity in the early 20th century, when Russian scientist Ilya Mechnikov (1845-1916) placed the thesis that, with the consumption of sour milk, the lactic acid bacteria found in its composition, inhibited the growth of harmful proteolytic bacteria in the digestive system, and prolongs human life (Samaržija, 2015).
The use of probiotic bacteria in the production of fermented milk is of increasing importance nowadays because of their role in the treatment of gastrointestinal disorders and diarrhea associated with the use of antibiotics (Mazahreh and Ershidat, 2009).
Probiotic bacteria stimulate the immune system, act anti-cancerous and antimicrobial to pathogens, improve lactose metabolism, and lower blood cholesterol levels (Frece et al., 2005). Probiotics, also have therapeutic properties against numerous allergies, *Helicobacter pilory* infections, liver diseases, urinary infections, and other health problems with humans (Ejtahed et al., 2011).
In addition to numerous health effects, probiotics have the ability to form low molecular weight components such as conjugated linoleic acid (CLA), gamma aminobutyric acid (GABA) and bacteriocine (Gobbetti et al., 2010). An important factor that should be emphasized, is that microorganisms to be classified as probiotics, must survive the transition through the stomach where a low pH environment prevails and demonstrate their metabolic activity in the intestine (Hyun and Shin, 1998).
The loss of viability of probiotic bacteria used in fermented milk is due to the adverse effects of acids on the bacteria (Shah, 2000). The minimum daily therapeutic dose of probiotic

cells to have a beneficial effect on the consumer is thought to be 10^8-10^9 CFU ml^{-1} or CFU g^{-1}, which corresponds to the injection of 100g product containing 10^6-10^7 CFU ml^{-1} or CFU g^{-1} per day (Lourens-Hattingh and Viljoen, 2001). This amount of live probiotic bacteria of a minimum 10^7 CFU/g product during consumption is recommended by the International Dairy Federation (IDF).
According to Tamime et al. (2005), higher doses are recommended to compensate for the loss of probiotic cells during the passage through the upper and lower parts of the gastrointestinal tract.
The most common dairy products where probiotic bacteria are applied are yoghurt, cheese, ice cream and frozen dairy desserts (Soccol et al., 2010).
According to Saarela et al. (2000), the criteria for the selection of probiotic microorganisms used in the dairy industry are: human origin, clinical validation and documented health effects, antagonism towards pathogenic bacteria, antimicrobial production, survival in the human intestinal tract, and safe use in food. The authors also point out that these probiotic microorganisms must retain their functionality during storage as frozen or freeze-dried starter cultures, without the production of off-flavors characteristics when used in the dairy industry.
Parvez et al. (2006), note that the most common bacteria in probiotic preparations are lactic acid species: *Lactobacillus sp.*, *Bifidobacterium sp.*, *Enterococcus sp.*, and *Streptococcus sp.* In addition, other bacterial species are used, for example: *Bacillus sp.*, yeast: *Saccharomyces cerevisiae* and *Saccharomyces boulardii*, filamentous fungi - *Aspergillus oryzae* etc.
Lactobacillus acidophilus and *Bifidobacterium animalis subsp. lactis* are the most commonly used lactic acid bacteria as probiotics. These species slowly grow and slowly proliferate in milk due to their poor proteolytic activity, and for these reasons in the starter cultures they are combined with *Streptococcus thermophilus* (Casarotti et al., 2014). The combination of these three bacterial strains in the dairy industry is known as ABT culture, while the same combination with the addition of *Lactobacillus bulgaricus* is known as ABY culture.
The aim of our research was to determine the impact of three types of ABT culture that have the same bacterial composition, in different ratios, on the dynamics of acidity and sensory characteristics in the probiotic yoghurt. This will allow the dairy industry to recommend a starter culture that gives the best organoleptic characteristics of yoghurt, a small post-acidification, and consequently a greater survival of the probiotic bacterial species in the gastrointestinal tract of the consumers.

2. MATERIALS AND METHODS

For the production of the probiotic yoghurt, raw milk from the collection area in dairy factory in the Bitola region (Republic of Macedonia), was used. Raw milk is filtered, deaerated to 55 °C, then milk fat is separated at 60 °C, and it is bactofuged at 62-68 °C to improve its microbiological quality. Standardization of milk fat (1%) and non-fat dry matter (minimum 8.50%) is carried out for the production of yoghurt with standard characteristics, by adding skim milk powder up to a maximum of 0.70%. Then the milk is homogenized at 150 bar/65 °C and pasteurized at 78 °C/16 sec. After the first pasteurization, prebiotics oligofructose in the amount of 1.5% is added to the milk in order to improve the textural, sensory and nutritional properties of yoghurt. Then the second homogenization of the milk is carried out at a pressure of 200 bar/55 °C, pasteurization at 95 °C/5 min. and cooling at 37 °C.
The inoculation was performed at 37 °C with 3 types of DVS (Direct Vat Set) starter cultures: ABT 6, ABT 10 and ABT 21, production of Chr. Hansen, Denmark. These starter cultures were composed of bacteria: *Lactobacillus acidophilus LA-5*, *Bifidobacterium ssp. BB-12* and *Streptococcus thermophilus ST*, in different ratios.

After completing fermentation, yoghurt is mixed with stirrers in the fermentation tanks, packaged in cardboard packaging from Tetra Pak, and the samples cooled to 4-6 °C.
The examination of the amounts of milk fat, proteins, lactose and non-fat dry matter in raw milk, was performed using the Milcoscan Minor apparatus, freezing point using the thermistor-cryoscopic method (ISO 5764), titratable acidity (according to the Soxhlet-Henkel method) and the active acidity with pH meter Mettler-Toledo (Carić et al., 2000). Determination of the presence of antibiotics from the group of penicillins, cephalosporins and tetracyclines was performed using the Twin Sensor test, while the presence of aflatoxins M1 with the Afla sensor test.
Chemical trials of the three variants of yoghurt were performed on the 1, 7, 14 and 21 day in 10 repetitions.
The active acidity of yoghurt was determined by pH meter Mettler-Toledo. The titratable acidity was measured according to the Soxhlet-Henkel method.
Sensory analysis (flavour, consistency, odor, color and general appearance) was carried out by a panel commission composed of 5 members using a scoring system with weighted factors in the 20-points' scale. Factors of significance for each sensory characteristic individually were: flavour (2.0); consistency (1.2); odor (0.4); color (0.2) and general appearance (0.2) (FIL-IDF, 1997).
Statistical data processing was performed using the Excel program (Microsoft Office, 2007), whereby the arithmetic mean (X), standard deviation (SD) and coefficient of variation (CV) were calculated.

3. RESULTS AND DISCUSSION

Considering the fact that probiotic yoghurt has a refresing effect on consumers, there is also a health benefit, for its production it is necessary to use raw milk with the highest quality. Milk quality is estimated according to chemical composition, physical properties and hygiene parameters. Table 1 presents the basic chemical-physical properties of the raw milk and standardized milk with the addition of skim milk powder.
From the table it can be concluded that the percentage of milk fat (3.70%) and proteins (3.25%) are within the normal values for the cow milk. Lactose has a certain decrease from the usual value of 4.70%, indicating the possible occurrence of infections of the mammary gland in dairy animals. This observation is in accordance with the examination of Bruckmaier et al. (2004).
To improve the strength and viscosity of the gel structure and to obtain probiotic yoghurt with standard quality, standardization of milk

Table 1. Chemical composition of raw and standardized milk for production of probiotic yoghurt (n = 10)

Parameter	Type of milk	X	SD	CV
Milk fat (%)	raw	3.70	0.0310	0.8395
	standardized	1.00	0.0082	0.8257
Proteins (%)	raw	3.25	0.0391	1.2048
	standardized	3.47	0.0216	0.6225
Lactose (%)	raw	4.45	0.0439	0.9873
	standardized	4.51	0.0156	0.3476
Non-fat dry matter (%)	raw	8.37	0.0527	0.6306
	standardized	9.00	0.0385	0.4287
Freezing point (^{o}C)	raw	-0.517	0.0019	0.3738
	standardized	-0.520	0.0008	0.3738
Titratable acidity (^{o}SH)	raw	6.12	0.1316	2.1512
	standardized	6.32	0.0339	0.5378
Active acidity (pH)	raw	6.65	0.0211	0.3184
	standardized	6.64	0.0149	0.2245

fat at 1% and enrichment of the non-fat dry matter is carried out. Standardization of milk fat is performed because it has been scientifically proven that yoghurt with high fat content has an inhibitory effect on probiotic cultures, especially to *Bifidobacterium bifidum* (Vinderola et al., 2000).

In our research, the correction of the non-fat dry matter was made, from an average of 8.37% to 9% with the addition of skim milk powder. According to Samaržija (2015), the addition of this dry milk ingredient increases titratable acidity, because its basis is casein and thus shortens the formation time of the gel structure.

In our study, we found that the titratable acidity increased from 6.12 °SH in raw milk to 6.32 °SH in standardized milk, which is in accordance with the previous conclusion. The freezing point ranged from -0.517 °C (raw milk) to -0.520 °C (standardized milk), which is a sign that the appearance of deliberate addition of water by farmers has been reduced to a minimum.

In the active acidity-pH, we did not find more significant changes after the standardization of the milk. Low values of standard deviation and coefficient of variation, indicate that raw milk had constant quality, good control of technological operations, and consequently, a standard product was obtained.

With the rapid screening tests Twin sensor and Afla sensor, no presence of antibiotics and aflatoxins in raw milk was detected, which slow down or completely stop the fermentation of yoghurt.

Titratable acidity is a very important factor, which affects the storage period and the acceptability of fermented dairy products (Yilmaz-Ersan and Kurdal, 2014).

The titratable acidity dynamics of the three variants of probiotic yoghurt is shown in Figure 1. On the first day of the trials it ranged in the range of 28.55-29.18 °SH. After 21 day storage of yoghurt, it was found that the lowest titratable acidity from 34.29 °SH had a variant of yoghurt produced using the starter culture ABT 10, then the variant ABT 6 with an acidity of 35.49 °SH, and the highest value showed the variant ABT 21 with value 36.10 °SH.

Our results are consistent with the studies of Khosravi-Darani et al. (2015), which found titratable acidity between 82-164 °D and pH value 3.98-4.47 in probiotic yoghurt. Similar results are presented by Vinko et al. (2011), where the titratable acidity of the fermented dairy products with mesophilic culture after 28

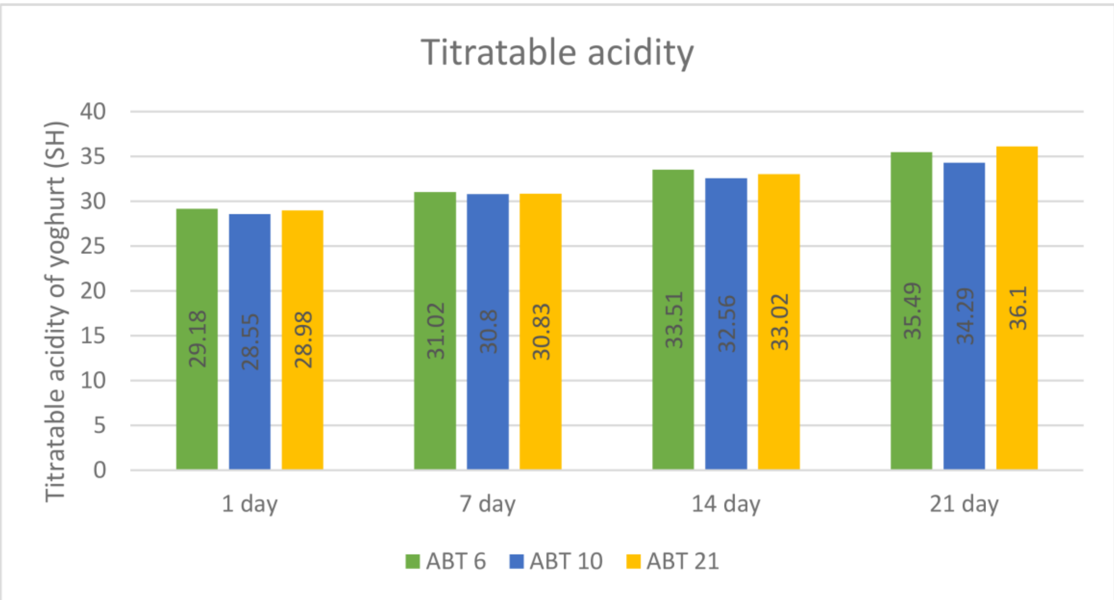

Figure 1. Dynamics of titratable acidity (°SH) in the probiotic yoghurt during storage

days of storage, was ranged from 34.07 to 37.60 °SH.

From the analyzes carried out it can be concluded that the activity of the used starter cultures was good, and the created lactic acid successfully inhibits the development of undesired microorganisms in yoghurt. The probiotic bacteria that were used in our study are more slowly evolving compared to the thermophilic bacteria used in commercial yoghurt. According to Donkor et al. (2006), this is due to the fact that milk is not a natural environment of bifidobacteria, which are part of starter cultures. The same remark is noted by Shah (2000), who states that probiotic bacteria have a weaker proteolytic activity

In Figure 2, the active acidity (pH) dynamics is shown in the three variants of probiotic yoghurt. From the figure it can be determined that there is a constant decrease in pH value during the whole period of the examinations. Active acidity on the first day was fairly equal in all three variants and ranged in the range of 4.45-4.48 units. After 21 days of storage of the yoghurt, the lowest pH value of 4.04 units was determined in the variant produced by ABT 21, followed by ABT 6 with acidity of 4.06, and the weakest acidity showed the ABT 10 variant with a pH value of 4.14.

Our results are similar to that of Stijepić et al. (2011), which used a mixed probiotic culture composed of bacteria: *Streptococcus thermophilus* (70%), *Lactobacillus bulgaricus* (10%), *Lactobacillus acidophilus* (10%) and *Bifidobacterium spp.* (10%). In their trials, the pH value of the probiotic yoghurt ranged from 4.38 (1 day) to 4.15 (21 days), which is approximately up to our values.

The speed of the acidification process and the desired level of bacterial cells at the end of the fermentation and in the storage period is greatly influenced by the amount of inoculum (Tamimme and Robinson, 2007).

With the application of skim milk powder, the lactose content in the milk increases, the acidification is stimulated and the fermentation time is reduced. The composition of starter cultures for probiotic yoghurt in our studies, has allowed the creation of a significant amount of lactic acid and a reduction in pH values over a short period of time, consistent with examinations of Sodini et al. (2002). Slightly higher values of ours are presented by Yilmaz-Ersan and Kurdal (2014), which determined the pH value of bio-yoghurt with different starter cultures in the range of 4.10-4.31 after 25 days of storage.

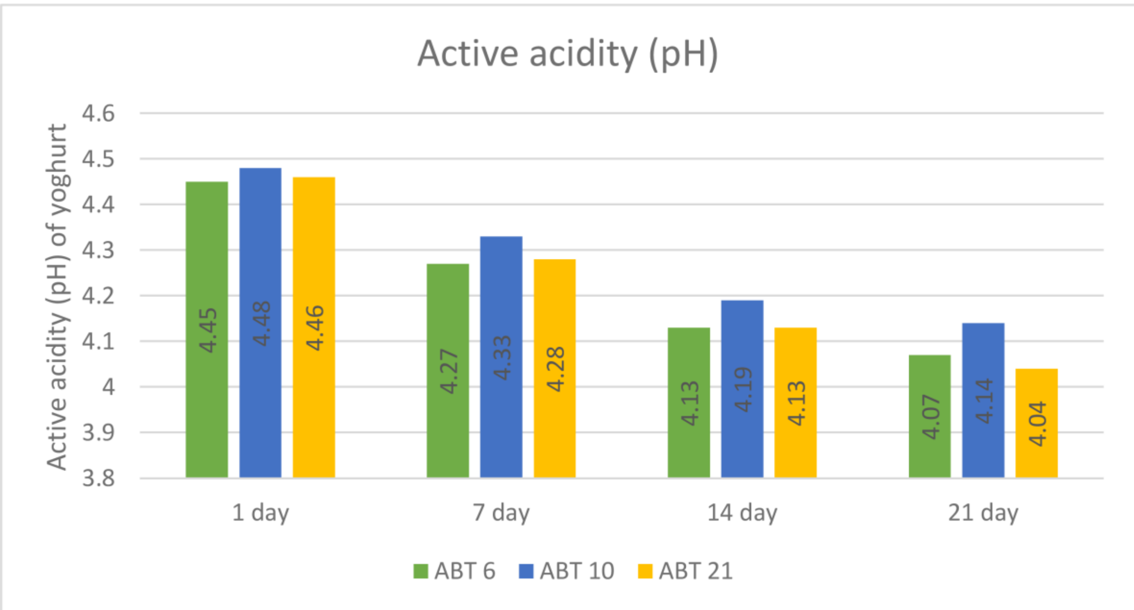

Figure 2. Dynamics of active acidity (pH) in the probiotic yoghurt during storage

Table 2. Sensory evaluation of probiotic yoghurt

Parameter	ABT 6	ABT 10	ABT 21
Flavour (10)	8.00	9.20	7.20
Consistency (6)	4.56	5.76	4.32
Odor (2)	1.84	1.92	1.76
Color (1)	1.00	1.00	1.00
General appearance (1)	0.88	0.96	0.80
Total score (20)	**16.28**	**18.84**	**15.08**

The quality of the probiotic yoghurt is evaluated according to the physico-chemical, microbiological and sensory characteristics of the final product. The sensory analysis in our research is shown in Table 2 and it included the evaluation of flavour, consistency, odor, color and general appearance. The main objective of the sensory analysis is to determine the deviation from the desired product characteristics and the expectations of the consumers.

Individually, the best results for the flavour of the assessors received the variant produced with the culture ABT 10, or 9.20 points out of a possible 10 points. This variant had a more pronounced mouth thickness compared to the other two variants. It also had weaker titratable and active acidity, as well as weaker post-acidification in relation to the other two variants. According to El-Kadi et al. (2016), this is especially important for this type of yoghurt, because supplemental and excessive acidity adversely affects the stability of bifidobacteria during storage.

Also, in the consistency of the product, the better firmness of the gel had the ABT 10 variant in comparison with ABT 6 and ABT 21. Scores of flavour for ABT 10, ABT 6 and ABT 21 were 1.92; 1.84 and 1.76 points respectively.

The color of the three variants was uniformly white and no significant differences were found between them. The general appearance of the ABT 10 variant was in the first place with a smooth structure, while the weakest grades were given to the ABT 21 variant where the appearance of the grain structure was determined, which is certainly a negative feature for this fermented product.

The sensory analysis of the 3 examined variants of starter cultures coincides with their manufacturing specifications from the manufacturer Chr. Hansen. According to the sensory commission, the variant probiotic yoghurt produced using the starter culture ABT 10 won the highest grades, i.e 18.84 points from a maximum of 20 points. The ABT 6 variant was placed in the second place, which received 16.28 points from the assessors. The lowest results were obtained for the starter culture ABT 21 with 15.08 points.

The general assessment is that the use of ABT cultures improves the flavour and odor of the yoghurt, which is in line with the claim of El-Kadi et al. (2016), but produces a product with a weaker consistency and texture than classical yoghurt.

4. CONCLUSIONS

ABT cultures are widely used in the dairy industry, not only for the production of new types of fermented milk, but they contribute to improving human health as well. Analyzes in this paper have shown that starter culture ABT 10 achieves the best acidification and sensory characteristics in the production of probiotic yoghurt.

5. REFERENCES

[1]. Bruckmaier R.M., Ontsouka C.E., Blum J.W. (2004). Fractionized milk composition in dairy cows with subclinical mastitis. Wet. Med.-Czech, 49, 2004 (8): 283-290.

[2]. Carić M., Milanović S., Vucelja D. (2000). Standardne metode analize mleka i mlečnih proizvoda. Prometej, Novi Sad.

[3]. Casarotti S.N., Monteiro D.A., Moretti M.M.S., Penna A.L.B. (2014). Influence of the combination of probiotic cultures during fermentation and storage of fermented milk. Food Research International, 59, 67-75.

[4]. Donkor O.N., Henriksson A., Vasiljević T., Shah N.P. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. Int. Dairy J. 16, 1181-1189.

[5]. Ejtahed H.S., Mohtadi-Nia J., Homayouni-Rad A., Niafar M., Asghari-Jafarabadi M., Mofid V., Akbarian-Moghari A. (2011). Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. Journal of Dairy Science, 94(7), 3288-3294.

[6]. El-Kadi S.L., Ismail M.M., Hamad M.F., Zidan M.S. (2016). Chemical and microbiological characterizations of bio-yoghurt made using ABT culture, cow milk and coconut milk. EC Microbiology 5.3: 109-124.

[7]. FIL-IDF Bulletin (1997). Sensory evaluation of dairy products by scoring - reference methods. Standard 99C. Bruxelles.

[8]. Frece J., Kos B., Beganović J., Vuković S., Šušković J. (2005). In vivo testing of functional properties of three selected probiotic strains. World Journal of Microbiology&Biotechnology 21, 1401-1408.

[9]. Gobbetti M., Di Cagno R., De Angelis M. (2010). Functional microorganisms for functional food quality. Critical Reviews in Food Science and Nutrition 50 (8), 716-727.

[10]. Hyun C., Shin H. (1998). Utilization of bovine plasma obtained from a slaughterhouse for economic production of probiotics. Journal of Fermentation and Bioenginering, 86, 34-37.

[11]. Khosravi-Darani K., Taheri P., Ahmadi N. (2015). Effect of Process Variables on the Probiotic and Starter Culture Growth in Sinbiotic Yogurt Beet. Research and Reviews: Journal of Food and Dairy Technology. Volume 3, Issue 2, pp. 13-24.

[12]. Lourens-Hattingh A., Viljoen B.C. (2001). Growth and survival of a probiotic yeast in dairy products. Food Research International, 34, 791-796.

[13]. Mazahreh A.S., Ershidat M.O. (2009). The benefits of lactic acid bacteria in yoghurt on the gastrointestinal function and health. Pakistan Journal of Nutrition 8.9: 1404-1410.

[14]. Parvez S., Malik K.A., Kang A., Kim H.Y. (2006). Probiotics and their fermented food products are benefitial for health. Journal of Applied Microbiology 100 (6), 1171-1185.

[15]. Saarela M., Mogensen G., Fonden R., Matto J., Mattila-Sandholm T. (2000). Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology, 84, 197-215.

[16]. Samaržija D. (2015). Fermentirana mlijeka. Hrvatska mljekarska udruga. Zagreb.

[17]. Soccol C.R., Vandenberghe L.P.S., Spier M.R., Medeiros A.B.P., Yamaguishi C.T., Lindner J.D.D., Pandey A., Soccol V.T. (2010). The Potential of Probiotics: A Review. Food Technol. Biotechnol. 48 (4), 413-434.

[18]. Sodini I., Lukas A., Oliveira M.N., Remeuf F., Corrieu G. (2002). Effect of milk base and starter culture on acidification, texture and probiotic cell counts in fermented milk processing. J. Dairy Science 85 (10), 2479-2488.

[19]. Shah N.P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science 83.4:894-907.

[20]. Stijepić M., Milanović S., Glušac J., Vukić V., Kanurić K., Đurđević-Milošević D., Ranogajec M. (2011). Effects of selected factors on rheological and textural properties of probiotic yoghurt. Mljekarstvo 61 (1), 92-101.

[21]. Tamime A.Y., Saarela M., Søndergaard A.K., Mistry V.V., Shah N.P. (2005). In: Tamime A.Y. (Ed.), Production and maintenance of viability of probiotic microorganisms in dairy products. Probiotic Dairy Products. Blackwell Publishing Ltd. Oxford, UK. pp.39-72.

[22]. Tammime A.Y., Robinson R.K. (2007). Yoghurt: science and technology. Woodhead Publishing.

[23]. Vinderola C.G., Bailo N., Reinheimer J.A. (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. Food Res. Int. 33, 97-102.

[24]. Vinko I., Božanić R., Golem Ž., Kesner-Koren I., Mahnet S. (2011). Change of lactose content after milk fermentation using various microbial cultures. Mljekarstvo 61 (2), 161-167.

[25]. Yilmaz-Ersan L., Kurdal E. (2014). The Production of Set-Type-Bio-Yoghurt with Commercial Probiotic Culture. International Journal of Chemical Engineering and Applications, Vol. 5, No. 5, October 2014 pp. 402-408.