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Production of probiotic Bulgarian yoghurts obtained from an ultrafiltered cow's milk

S. Kodinova[1], M. Dushkova[1†], M. Miteva-Petrova[2], V. Yanakieva[3], S. Petrov[2], Z. Denkova[3]

[1]Department of Process Engineering, Technical Faculty, University of Food Technologies, Plovdiv, Bulgaria
[2]Department of Organic Chemical Technologies, Technical Sciences Faculty, University "Prof. Asen Zlatarov", Burgas, Bulgaria
[3]Department of Microbiology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria

Abstract

Ultrafiltration of skim cow's milk with a UF10-PAN membrane at volume reduction ratios (VRRs) of 2 and 3 was performed. The ultrafiltration retentates obtained were used for production of probiotic yoghurts with three different starters. A control sample was prepared using skim cow's milk. All yoghurts were analysed according to the following parameters: titratable acidity, dry matter, organoleptic characteristics, number of specific microorganisms (Lactobacillus bulgaricus and Streptococcus thermophilus) and the total count of viable lactic acid bacteria for 28 d of storage. The results showed that the increase in the VRR during ultrafiltration increased the titratable acidity, as well as the dry matter of all yoghurts. Ultrafiltration concentration led to an increase in the count of viable lactic acid bacteria in all yoghurts which improved their functional properties. The highest values of the total number of viable lactic acid bacteria were determined in yoghurts obtained with starter 1CM, followed by starters MZ_2 and ZD for both VRRs. Probiotic yoghurts with the highest organoleptic evaluation were obtained from ultrafiltration retentates at VRR = 2 and starters 1CM and MZ_2.

Keywords

Cow's milk • probiotic yoghurt • ultrafiltration

Introduction

Ultrafiltration is widely used in the dairy industry for concentration, purification and fractionation of milk components as it has the following advantages in comparison with the traditional separation methods: environmental friendliness (Kumar *et al*., 2013; Tamime, 2013), lower energy consumption (Baldasso *et al*., 2011), increased yield (Macedo *et al*., 2012; Ong *et al*., 2013) and improved quality (Reschke da Cunha *et al*., 2006; Domagala and Wszolek, 2008; Heino *et al*., 2010; Domagala *et al*., 2012) of the final product, reduction in the production costs (Mehaia, 2005) and completion of the process at room temperature to treat heat-sensitive products and keep their natural properties (in comparison with thermal evaporation, for example; Baldasso *et al*., 2011).

Many fermented milk products are produced by ultrafiltration. When ultrafiltration was used for Greek yoghurt, it was established that yoghurts produced by ultrafiltration contained more lactic acid bacteria than those produced by traditional technology without ultrafiltration (Tamime *et al*., 2005). Sodini *et al*. (2005) used whey protein concentrates, obtained by ultrafiltration, to increase the protein content and enhance the development of lactic acid bacteria in the probiotic yoghurts produced.

Ymer is a national Danish soured milk product with an increased protein content, which can be obtained by traditional technology or by using membrane processes (Fonden *et al*., 2006). The ultrafiltration method for obtaining Ymer includes the following processing operations: heat treatment of skim milk at 85°C for 15 s, ultrafiltration at a volume reduction ratio (VRR) of 1.9 and a temperature of 50–55°C, standardisation of the retentate by the addition of cream, reheating to 85°C for 5 min, homogenising and cooling to 22°C. The coagulation is performed by mesophilic starter culture at 20–22°C for 14–16 h. The advantage of this technology is higher protein content, which leads to an increase in yield from 8% to 15%.

Özer (2006) developed a technology with ultrafiltration for the traditional sweet strained Indian yoghurt Shrikhand: skim cow's milk was subjected to pasteurisation at 85–90°C for 10–20 min; then, it was cooled at 21–22°C and coagulated with mesophilic lactic acid bacteria during 15–16 h. The fermented milk was reheated to 60°C for 5 min, cooled at 50°C and subjected to ultrafiltration to increase the dry matter to 16%. The product

† Corresponding author: M. Dushkova
E-mail: maria_douchkova@yahoo.fr

obtained had a better taste, texture, colour and appearance than that obtained by traditional technology.

Kumis is an ancient fermented milk drink, commonly consumed in Eastern Europe and Central Asia. Traditionally, it is made from mare's milk, and its healing and nutritional properties are well known but the quantity of mare's milk is limited and the price is quite high. Küçükçetin *et al*. (2003) investigated the possibility of using cow's milk for the production of Kumis: skim cow's milk was treated by ultrafiltration to obtain a protein-enriched concentrate. The casein and whey proteins in the ultrafiltration concentrate were separated by microfiltration, and the resulting retentates had a composition close to the mare's milk.

Labneh is a traditional fermented milk product, popular in different parts of the world, especially in the Balkans. It has a sour taste, milky white colour, smooth and creamy texture. In traditional Labneh technology, whole yoghurt is drained through filtering tissue to obtain dry matter from 22% to 26% (Otaibi and Demerdash, 2008). A comparative assessment of the chemical composition, rheological and organoleptic properties of Labneh obtained from cow's milk using traditional technology and using ultrafiltration retentate with or without added concentrated permeate was made (Shamsia and El-Ghannam, 2012). The authors found that the addition of 1% concentrated permeate containing 84% lactose, 11% mineral substances, 5% water and 1% glucono delta-lactone (GDL) resulted in a significant reduction in coagulation time and an increase in dry matter. The most significant reduction in coagulation time was observed when using a GDL. Compared with Labneh, obtained by traditional technology, when ultrafiltration was used the product was characterised by a higher content of total and soluble proteins, fats, minerals, acidity and pH. The addition of 1% concentrated permeate during the production of Labneh by ultrafiltration results in an improvement in taste, appearance and structure of the product.

Mehaia (2005) explored the possibility of the production of Labneh from goat's milk by traditional technology and by using ultrafiltration before and after coagulation with starter culture. The author found that Labneh, produced by membrane technology, had higher acidity, higher protein, fat, dry matter and lower pH. Ultrafiltration before and after coagulation led to an increase in yield of about 14.5%. The production time was significantly reduced by 75%, as well as the amount of starter used before (12.5%) and after (62.5%) ultrafiltration.

The aim of this research was to investigate the possibilities for the production of probiotic Bulgarian yoghurts obtained by ultrafiltration of skim cow's milk with a UF10-PAN membrane and assessment of their physicochemical, microbiological and organoleptic characteristics.

Materials and methods

Materials

Milk

The skim cow's milk was delivered by BCC Handel Ltd., Elena, Bulgaria. The milk was analysed for the following parameters: dry matter content (International Standardisation Organisation [ISO], Geneva, Switzerland 6731:2010); total protein content (Bulgarian State Standard [BSS] EN ISO 8961-1:2014); fat content (ISO 2446:2008); mineral substances (BSS 6154:1974). All these analyses were conducted with threefold repetition.

Starter cultures

Three probiotic starter cultures were used for the production of Bulgarian yoghurts: starter culture ZD consisting of a probiotic strain of *Lactobacillus bulgaricus* (National Bank for industrial microorganisms and cell cultures NBIMCC 3706) and *Streptococcus thermophilus* (3); starter culture MZ_2 consisting of a probiotic strain of *L. delbrueckii* subsp. *bulgaricus* (NBIMCC 3708) and *S. thermophilus* (TMZ_2 I); starter culture 1CM consisting of a probiotic strain of *L. delbrueckii* subsp. *bulgaricus* (NBIMCC 3708) and *S. thermophilus* (T3).

The ratio of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* was 1:2 in all starter cultures. The starter cultures were kindly provided by Prof. Zapryana Denkova from the Department of Microbiology at University of Food Technologies, Plovdiv, Bulgaria.

Media for development and maintenance of lactic acid bacteria: Sterile skim milk with a titratable acidity of 16–18°T – dried skim milk was provided by Scharlau, Barcelona, Spain, reconstituted to 9% dry matter content, autoclaved for 15 min at 118°C and cooled for storage at room temperature. Liquid medium (LAPTg10) for the development of lactic acid bacteria was prepared as follows: peptone – 15.0 kg/m^3 (Fluka, Bucharest, Romania); tryptone – 10.0 kg/m^3 (Fisher Scientific, Difco Laboratories, Hampton, USA); yeast extract – 10.0 kg/m^3 (Scharlau), glucose – 10.0 kg/m^3 (Sigma Aldrich, Merck, St. Louis, MO, USA); Tween 80 – 1.0 kg/m^3 (Sigma Aldrich). The pH of the liquid medium was 6.6–6.8 and the solid medium of LAPTg10 was 15.0 kg/m^3 agar (Sigma Aldrich).

Methods

Cultivation and storage of probiotic starter cultures for yoghurt

The starter cultures used (ZD, MZ_2, 1CM) were inoculated every 20 d in sterile skim milk with a titratable acidity of 16–18°T and stored at 4–6°C or as stock cultures at -20°C.

Figure 1. Scheme of laboratory equipment with a replaceable plate and frame membrane module. 1: valve; 2, 3, 4: manometers; 5: replaceable plate and frame membrane module; 6: pump; 7: tank for initial solution; 8: cylinder for permeate.

Ultrafiltration experiments

Ultrafiltration was carried out with polyacrylonitrile membrane UF10-PAN with 10 kDa molecular weight cut-off. Membrane was prepared by the dry–wet phase inversion method of polymer solutions with a solvent of dimethyl sulphoxide (Sigma Aldrich). Then, it was heat-treated in an aqueous medium for 10 min at 60°C. The membrane was prepared and kindly provided by the University Prof. Dr. Asen Zlatarov, Burgas, Bulgaria. Ultrafiltration experiments were carried out on laboratory equipment with a replaceable plate and frame membrane module (Figure 1). Ultrafiltration was undertaken at the following operating conditions: VRR = 2 and VRR = 3; working pressure, 0.5 MPa; temperature, 50°C; volumetric flow rate, 330 dm^3/h. The retentates obtained were then pasteurised at 65°C during 10–15 min and cooled at 42 ± 1°C. VRR was calculated by the following formula:

$$\mathrm{VRR} = \frac{V_0}{V_\mathrm{R}} \quad (1)$$

where V_0 is the volume of the feed solution and V_R is the volume of retentate.

Production of probiotic Bulgarian yoghurts

The coagulation of cow's milk and retentates was performed under aseptic conditions in sterile plastic containers of 100 cm^3 with 1.5% probiotic starter. The containers were placed in an incubator at 41–42°C for the coagulation of milk or retentates for 2.5–3 h. After coagulation, the yoghurts were cooled and stored at 2–6°C for 28 d.

Analysis of milk, retentates and yoghurts

The initial skim cow's milk and retentates obtained were analysed according to titratable and active acidity, total number of mesophilic anaerobic and facultative anaerobic microorganisms, as well as specific microorganisms, while the yoghurts were analysed according to dry matter, protein content, titratable acidity, specific microorganisms, number of viable lactic acid bacteria and organoleptic characteristics using the following methods.

Physicochemical methods

Dry matter was measured according to ISO 6731:2010; total protein content was investigated according to BSS EN ISO 8961-1:2014. The ability of lactic acid bacteria to form acids (titratable acidity, °T) was measured by the Turner method according to BSS 1111:1980. 1°T was equal to 1 cm^3 of 0.1 N NaOH (Sigma Aldrich), necessary for neutralisation of an equivalent quantity of organic acid in 100 cm^3 of culture medium. 10 cm^3 from every sample was taken, and 20-cm^3 distilled water was added. The titration was performed with 0.1 N NaOH using an indicator phenolphthalein until the appearance of light pink coloration, persistent for 1 min. To measure the active acidity (pH) a pen-type pH metre (PH-03 [I]; Hinotek, China) was used. All these analyses were conducted with threefold repetition.

Microbiological methods

The number of viable lactic acid bacteria was measured as appropriate serial dilutions of the yoghurts in saline solution NaCl (5 g/dm^3; Sigma Aldrich) were prepared and the spread plate method was applied. 0.1 cm^3 of the last three dilutions was used to inoculate in LAPTg10-agar for 3 d at 37°C until the appearance of countable single colonies. The total number of mesophilic anaerobic and facultative anaerobic microorganisms was measured according to BSS EN ISO 4833-1:2013. The count of *Escherichia coli* was established according to BSS EN ISO 16649-2:2014. The number of

Table 1. Organoleptic analyses of indices and hedonic scale for evaluation of probiotic Bulgarian yoghurts

Organoleptic indices for evaluation of probiotic Bulgarian yoghurts	
Indices	**Characteristics and norms**
1. Colour	White with different shades of creamy hue depending on the raw materials used
2. Appearance of coagulum	Dense, smooth, lateral tear is allowed depending on the type of milk
3. Structure at cutting	Smooth surface, with or without a grain-shaped structure, with or without a slight separation of the whey depending on the raw materials used
4. Consistency at shattering	Uniform, homogeneous, cream-like, light-grained or grained structure depending on the raw material used
5. Taste and aroma	A pleasant, lactic acid. No side taste and odour is allowed
Hedonic scale for evaluation of probiotic Bulgarian yoghurts	
Evaluation	**Points**
I dislike extremely	1
I dislike	2
I neither like nor dislike	3
I like	4
I like extremely	5

Staphylococcus aureus was identified according to BSS EN ISO 6888-1:2005+A_1:2005. The concentration of *Salmonella* was defined according to BSS EN ISO 6579:2003. To measure yeasts and moulds, BSS EN ISO 6611:2006 was used. All these analyses were conducted with threefold repetition.

Organoleptic analysis

Organoleptic analysis was performed using a 5-point hedonic scale for evaluation, and the basic organoleptic indices are presented in Table 1. A nine-member experienced panel drawn from the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, was used to evaluate the samples. The panellists rated the samples three times in a random order for colour, appearance of coagulum, structure at cutting, consistency at shattering, taste and aroma. Room temperature water and unsalted crackers were given to the panellists for mouth rinsing between samples to eliminate carry-over effects.

Statistical method

The least significant difference (LSD) method was used at the level of significance 0.05, using Microsoft Excel 2010, for comparison between the control and retentates VRR = 2 and VRR = 3, as well as between the three starter cultures.

Results

The main components, titratable acidity and pH of the initial skim milk and ultrafiltration retentates at VRR = 2 and VRR = 3 are presented in Table 2. It can be seen that the increase in VRR led to an increase in the dry matter, protein, fat contents and mineral substances. The experimental results of the titratable acidity and pH showed that the lowest values of the titratable acidity were observed for the control followed by the ultrafiltration retentates at VRR = 2 and 3. Titratable acidity increased from 23 ± 0.36°T (VRR = 2) to 31 ± 0.09°T (VRR = 3) in comparison with the control (16 ± 0.18°T). Table 2 also shows that the pH decreased when using the ultrafiltration process.

The results of the total number of mesophilic aerobic and facultative anaerobic microorganisms, specific microorganisms (*E. coli*, *S. aureus* and *Salmonella,* moulds and yeasts in the initial skim milk and ultrafiltration retentates show that the increase in VRR led to an increase in the total number of mesophilic aerobic and facultative anaerobic microorganisms ($P < 0.05$). The lowest values were found for the control ($1.8 \times 10^2 \pm 0.1 \times 10^2$ cfu/cm^3), followed by the ultrafiltration retentate at VRR = 2 ($2.5 \times 10^2 \pm 0.1 \times 10^2$ cfu/cm^3) and VRR = 3 ($3.8 \times 10^2 \pm 0.13 \times 10^2$ cfu/cm^3). The analysis for specific microorganisms in probiotic yoghurts obtained from the initial skim milk (control) and ultrafiltration retentates at VRRs of 2 and 3 showed that *E. coli* and *S. aureus* were less than 10 cfu/g, and *Salmonella* was not found in 25 g of the product. The count of moulds and yeasts was below 10 cfu/g in all tested probiotic yoghurts.

The results of the dry matter and protein content of the probiotic yoghurts obtained are presented in Table 3. The dry matter content of the controls was as follows: for ZD, (8.80 ± 0.14%); for MZ_2, (8.87 ± 0.16%); for 1CM, (8.90 ± 0.11%). The dry matter of the yoghurts obtained from ultrafiltration retentate at VRR = 2 was as follows: for ZD, (12.20 ± 0.10%); for MZ_2, (12.25 ± 0.12%); for 1CM, (12.40 ± 0.10%). The highest values were defined at VRR = 3: for ZD, (15.30 ± 0.16%); for MZ_2, (15.35 ± 0.12%); for 1CM, (15.38 ± 0.13%). The data show that the highest values of the protein content

Table 2. Main components and chemical properties of initial skim milk and ultrafiltration retentates at VRR = 2 and VRR = 3

Indices	Sample			Average values ± s.d.
	1	2	3	
Membrane UF10-PAN				
Skim milk				
Dry matter content, %	8.90	8.85	8.87	8.87 ± 0.03[a]
Total protein content, %	3.21	3.26	3.27	3.25 ± 0.03[a]
Fat content, %	0.1	0.1	0.05	0.08 ±0.04[a]
Mineral substances, %	0.71	0.72	0.72	0.72 ± 0.01[a]
Titratable acidity, °T	15.82	16.0	16.18	16.0 ±0.18[a]
pH	6.75	6.76	6.74	6.75 ± 0.01[a]
VRR = 2				
Dry matter content, %	12.23	12.24	12.25	12.24 ± 0.01[b]
Total protein content, %	5.59	5.59	5.74	5.64 ± 0.09[b]
Fat content, %	0.2	0.2	0.1	0.17 ± 0.06[a]
Mineral substances, %	0.96	0.96	0.97	0.96 ± 0.01[b]
Titratable acidity, °T	22.64	23	23.36	23 ± 0.36[b]
pH	6.60	6.65	6.63	6.62 ± 0.02[b]
VRR = 3				
Dry matter content, %	15.33	15.37	15.35	15.35 ± 0.02[c]
Total protein content, %	6.93	7.37	7.39	7.23 ± 0.26[c]
Fat content, %	0.3	0.3	0.15	0.25 ± 0.09[a]
Mineral substances, %	1.24	1.24	1.25	1.24 ± 0.01[c]
Titratable acidity, °T	30.91	31	31.09	31 ± 0.09[c]
pH	6.50	6.52	6.51	6.51 ± 0.01[c]

[a–c]To compare the composition of skim milk and retentates at VRR = 2 and VRR = 3.
VRR, volume reduction ratio.

Table 3. Dry matter and protein content of probiotic Bulgarian yoghurts from initial skim milk (control) and ultrafiltration retentates at VRR = 2 and VRR = 3

Probiotic yoghurts with different starters	Dry matter, %			Average values ± s.d.	Protein content, %			Average values ± s.d.
	1	2	3		1	2	3	
ZD (control)	8.66	8.80	8.94	8.80 ± 0.14[a]	3.22	3.24	3.28	3.25 ± 0.03[a]
ZD (VRR = 2)	12.10	12.20	12.30	12.20 ± 0.10[b]	5.60	5.70	5.63	5.64 ± 0.05[b]
ZD (VRR = 3)	15.14	15.30	15.46	15.30 ± 0.16[c]	7.28	7.20	7.22	7.23 ± 0.04[c]
MZ_2 (control)	8.71	8.87	9.03	8.87 ± 0.16[a]	3.26	3.30	3.31	3.29 ± 0.03[a]
MZ_2 (VRR = 2)	12.13	12.25	12.37	12.25 ± 0.12[b]	5.70	5.74	5.62	5.69 ± 0.06[b]
MZ_2 (VRR = 3)	15.23	15.35	15.47	15.35 ± 0.12[c]	7.25	7.31	7.35	7.30 ± 0.05[c]
1CM (control)	8.79	8.90	9.01	8.90 ± 0.11[a]	3.32	3.28	3.32	3.31 ± 0.02[a]
1CM (VRR = 2)	12.30	12.40	12.50	12.40 ± 0.10[b]	5.81	5.69	5.66	5.72 ± 0.08[b]
1CM (VRR = 3)	15.25	15.38	15.51	15.38 ± 0.13[c]	7.30	7.33	7.40	7.34 ± 0.05[c]

[a–c]To compare the dry matter and protein content of the obtained yoghurts with three probiotic starters (ZD, MZ_2, 1CM), and they indicate that mean values in the columns are significantly different ($P < 0.05$).
VRR, volume reduction ratio.

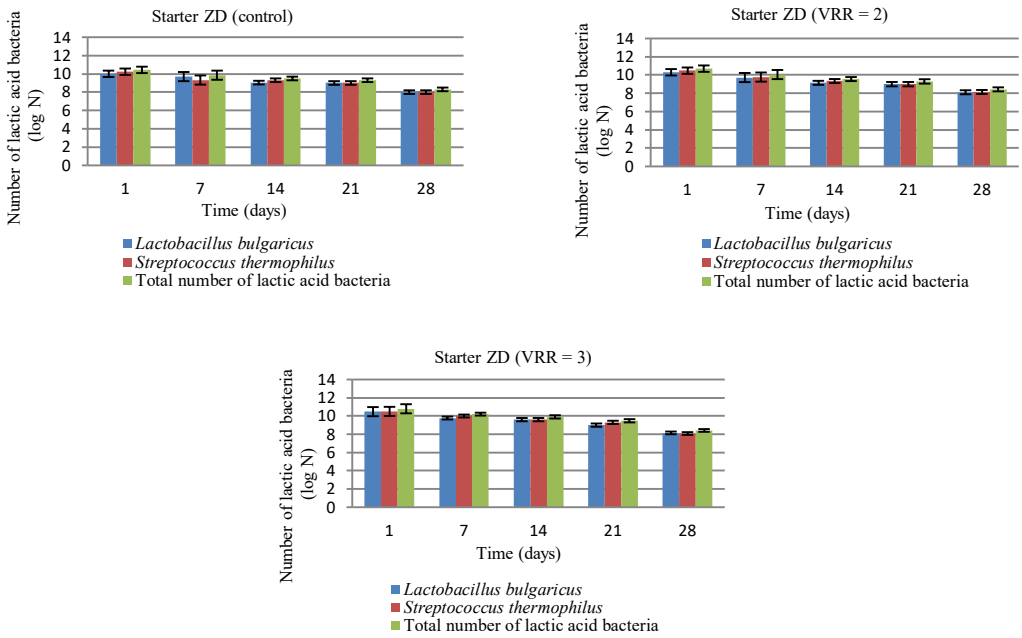

Figure 2. Microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter ZD. VRR, volume reduction ratio.

were defined for yoghurts obtained from retentate at VRR = 3: for ZD, (7.23 ± 0.04%); for MZ_2, (7.30 ± 0.05%); for 1CM, (7.34 ± 0.05%).

The changes in the number of *Lactobacillus bulgaricus*, *S. thermophilus*, as well as the total number of viable lactic acid bacteria for 28-day storage at a temperature of 2–6°C for all probiotic yoghurts were investigated. The results of experimental investigations are shown in Figures 2–4. The comparison of *L. bulgaricus* for each of the storage stages for the three types of yoghurt (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter ZD (Figure 2) showed that on the first day of the storage period the number of rod-shaped forms was higher ($P < 0.05$) at VRR = 2 (2 x 10^{10} ± 0.35 x 10^{10} cfu/g) and VRR = 3 (3 x 10^{10} ± 0.5 x 10^{10} cfu/g) in comparison with the control – 1 x 10^{10} ± 0.35 x 10^{10} cfu/g. Similar results were obtained for coccus-shaped forms – 3 x 10^{10} ± 0.35 x 10^{10} cfu/g at VRR = 2 and 3.2 x 10^{10} ± 0.5 x 10^{10} cfu/g at VRR = 3 in comparison with 1.7 x 10^{10} ± 0.35 x 10^{10} cfu/g in the control. The total count of viable lactic acid bacteria was highest in yoghurt obtained from ultrafiltration retentate at VRR = 3 (6.2 x 10^{10} ± 0.5 x 10^{10} cfu/g), followed by ultrafiltration retentate at VRR = 2 (5 x 10^{10} ± 0.35 x 10^{10} cfu/g) and control (2.7 x 10^{10} ± 0.35 x 10^{10} cfu/g). The concentration of viable cells of the probiotic strain *L. bulgaricus*, *S. thermophilus* and the total number of viable lactic acid bacteria remained high during the whole storage period, above – 2 x 10^8 cfu/g, as the strongest reduction was observed on the 28th day of the storage period.

The change in *L. bulgaricus* and *S. thermophilus*, as well as the total number of lactic acid bacteria of probiotic yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter MZ_2, is presented in Figure 3. The results indicate that on the first day of the storage period the number of rod-shaped forms was higher ($P < 0.05$) at VRR = 2 (2.8 x 10^{10}±0.34 x 10^{10} cfu/g) and VRR = 3 (3.8 x 10^{10} ± 0.20 x 10^{10} cfu/g) in comparison with the control – 2 x 10^{10} ± 0.32 x 10^{10} cfu/g. A similar trend was observed for the coccus-shaped forms: 1.1 x 10^{10} ± 0.32 x 10^{10} cfu/g in control in comparison with 1.5 x 10^{10} ± 0.34 x 10^{10} cfu/g at VRR = 2 and 4 x 10^{10} ± 0.20 x 10^{10} cfu/g at VRR = 3. The amount of viable cells was kept high during all storage periods, and on the 28th day it was above 8 x 10^8 cfu/g.

The dynamics of the change in *L. bulgaricus, S. thermophilus* and the total number of lactic acid bacteria of probiotic yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter 1CM is presented in Figure 4. The data show that their concentration is greatest in yoghurt from ultrafiltration retentate at VRR = 3, followed by the yoghurt from ultrafiltration retentate at VRR = 2 and the control. A reduction in the amount of viable lactic acid bacteria was observed during the studied storage period, and the biggest decrease was observed from the 21st to the 28th day.

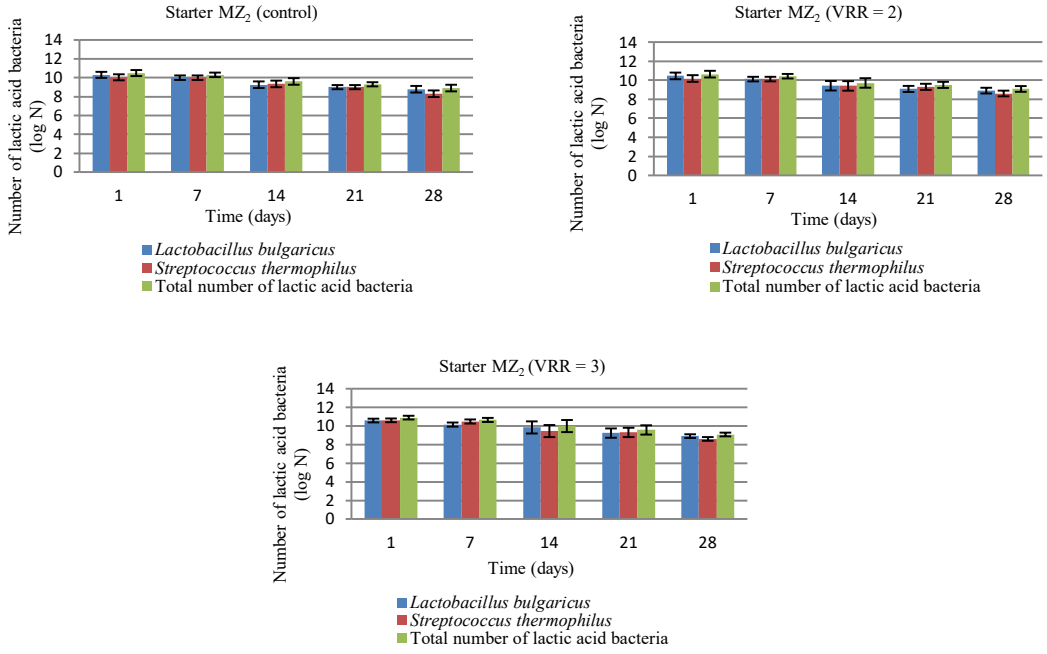

Figure 3. Microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter MZ_2. VRR, volume reduction ratio.

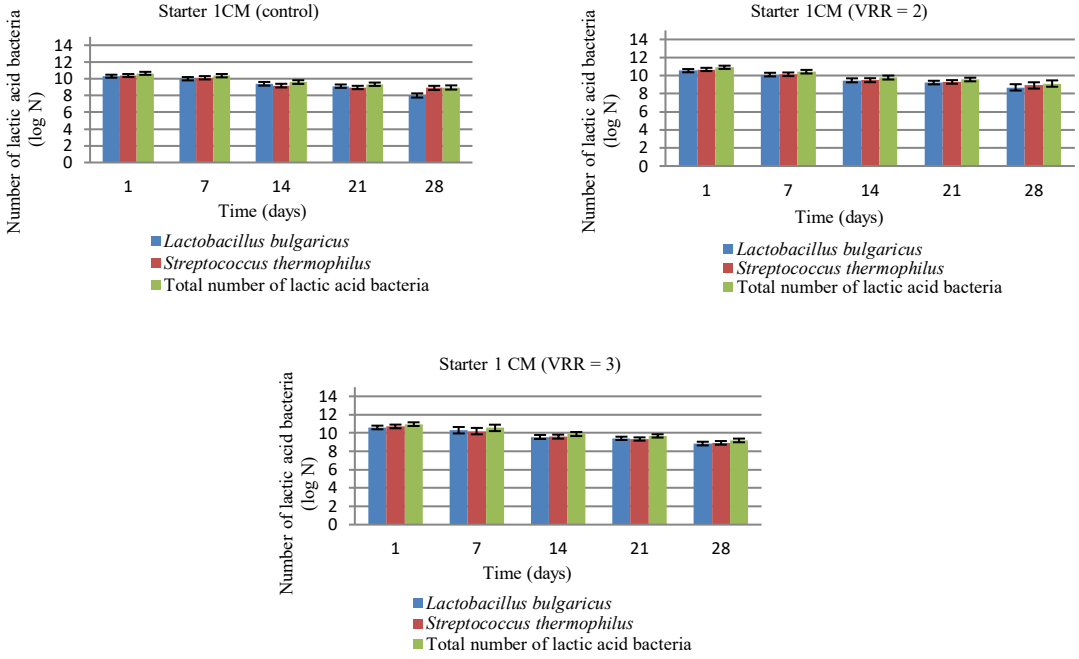

Figure 4. Microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter 1CM. VRR, volume reduction ratio.

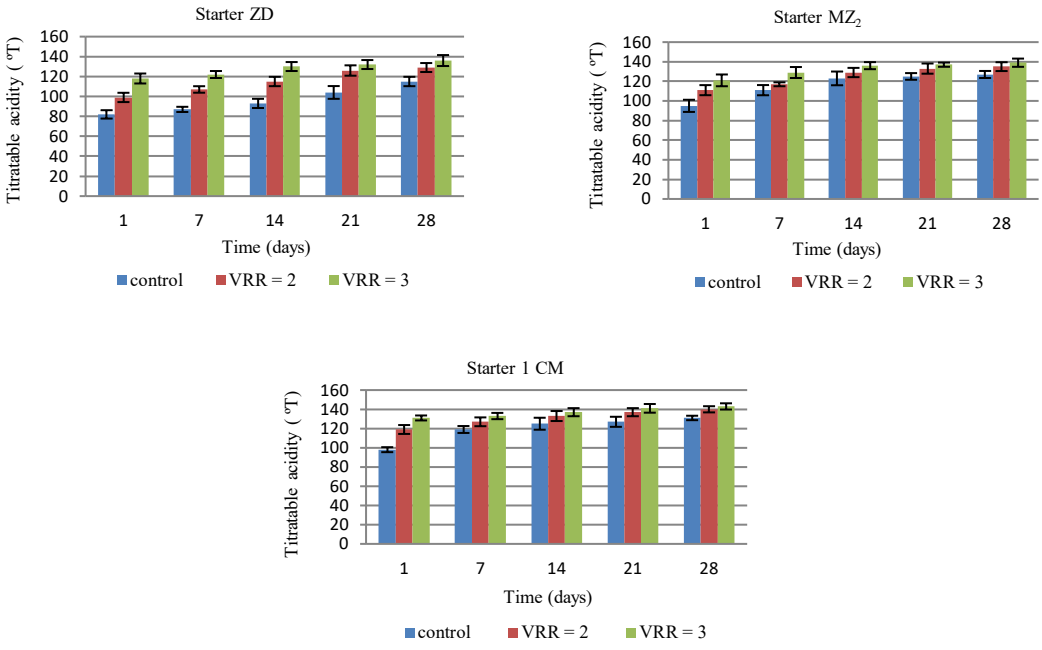

Figure 5. Kinetics of titratable acidity of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starters ZD, MZ_2 and 1CM. VRR, volume reduction ratio.

The titratable acidity of the yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with three types of probiotic starters was determined (Figure 5). The results show that the titratable acidity of all yoghurts increased ($P < 0.05$) with VRR of the milk used in manufacture and with storage time.

The results of organoleptic evaluation of the probiotic yoghurts are presented in Table 4. The data show that the yoghurts from retentate at VRR = 2 with all starter cultures had the highest number of points. Yoghurts, which had the higher total number of points, were these with starters MZ_2 and 1CM in comparison with starter ZD.

Discussion

Table 2 shows that the increase in VRR led to an increase in titratable acidity ($P < 0.05$). This could be explained by the higher protein content obtained with ultrafiltration. Moreno-Montoro *et al*. (2015) reported that the increased protein content leads to a higher buffering capacity which results in greater titratable acidity. The authors established significantly higher values of titratable acidity in ultrafiltered retentates from goat's milk in comparison with skim goat's milk and enhanced nutritional value because of the increase in dry matter, protein, fat contents and mineral substances. The increase in VRR resulted in a decrease in the active acidity (pH) of the investigated samples ($P < 0.05$).

Low levels of ultrafiltration concentration (VRR = 2 and VRR = 3) were used for the production of probiotic yoghurts because at higher levels the dry matter and protein content increase the density and the viscosity of the milk, which slows down the acid coagulation (Meletharayil *et al.,* 2015; Arango *et al*., 2018).

The increase in the total number of mesophilic aerobic and facultative anaerobic microorganisms in retentates could be explained by the decrease in the volume of the initial skim milk and higher concentration of microorganisms during ultrafiltration. It can be seen that in microbiological analysis, the initial skim milk and ultrafiltration retentates at VRR = 2 and 3 were in agreement with the admissible hygienic and epidemiological assessment norms according to the instruction from 31 July 2004 for the Maximum Allowable Quantities of Pollutants in Foods (Official Journal of Bulgarian Government, issue 88/8, 2004).

The statistical analysis of the data in Table 3 shows that there was no significant difference ($P > 0.05$) between the dry matter content of the yoghurts obtained with the three probiotic starters (ZD, MZ_2, 1CM) in all tested combinations. It can also be seen that the increase in VRR led to an increase in the dry matter content of the samples which could

Table 4. Organoleptic characteristics of probiotic Bulgarian yoghurts from skim milk (control and retentates at VRR = 2 and VRR = 3) with different starters

Indices	Type of probiotic yoghurt		
	Starter ZD		
	Control	**VRR = 2**	**VRR = 3**
Appearance of coagulum	Loose, smooth coagulum with slight lateral tearing during inclination of the package – 4 points	Dense, smooth coagulum – 4 points	Dense, grainy coagulum – 4 points
Consistency at shattering	Homogenous – 4 points	Homogenous – 5 points	Homogenous – 4 points
Colour	White with creamy hue – 5 points	White with creamy hue – 5 points	White with creamy hue – 5 points
Structure at cutting	Smooth surface, with abundant separation of whey – 3 points	Smooth surface, with slight separation of whey – 4 points	Smooth surface, with slight separation of whey – 4 points
Taste and aroma	Slight cream-like taste – 2 points	Slight cream-like taste – 4 points	Slight cream-like taste – 3 points
Total points	18 points	22 points	20 points
	Starter MZ_2		
	Control	**VRR = 2**	**VRR = 3**
Appearance of coagulum	Loose, smooth coagulum with slight lateral tearing during inclination of the package – 4 points	Dense, smooth coagulum – 5 points	Dense coagulum – 3 points
Consistency at shattering	Homogenous – 5 points	Homogenous – 5 points	Homogenous – 4 points
Colour	White with creamy hue – 5 points	White with creamy hue – 5 points	White with creamy hue – 5 points
Structure at cutting	Smooth surface, with abundant separation of whey – 3 points	Smooth surface, with slight separation of whey – 4 points	Smooth surface, with slight separation of whey – 4 points
Taste and aroma	Slight cream-like taste – 5 points	Pleasant cream-like taste – 4 points	Strong cream-like taste – 3 points
Total points	22 points	23 points	19 points
	Starter 1CM		
	Control	**VRR = 2**	**VRR = 3**
Appearance of coagulum	Loose, smooth coagulum with slight lateral tearing during inclination of the package – 3 points	Dense, smooth coagulum – 4 points	Dense, grainy coagulum – 4 points
Consistency at shattering	Homogenous – 4 points	Homogenous – 5 points	Homogenous – 3 points
Colour	White with creamy hue – 5 points	White with creamy hue – 5 points	White with creamy hue – 5 points
Structure at cutting	Smooth surface, with abundant separation of whey – 4 points	Smooth surface, with slight separation of whey – 5 points	Smooth surface, with slight separation of whey – 5 points
Taste and aroma	Slight cream-like taste – 4 points	Pleasant cream-like taste – 4 points	Strong cream-like taste – 3 points
Total points	20 points	23 points	20 points

VRR, volume reduction ratio.

be explained by the volume reduction during ultrafiltration concentration.

There was an increase in concentration due to an increase in the total number of lactic acid bacteria (Figure 2). Similar results were reported by Damianova *et al*. (2009) who demonstrated that the addition of plant proteins stimulated the development and viability of lactic acid bacteria and contributed to maintaining their higher amounts in the final lactic acid product. Marafon *et al*. (2011) found that the addition of whey protein concentrate and sodium caseinate resulted in an increase in the number of viable cells of *L. bulgaricus*, *S. thermophilus* and the probiotic strain of *Bifidobacterium animalis* in the yoghurts obtained.

Figure 3 shows that the lowest values of the total number of lactic acid bacteria were observed in the controls, followed by the ultrafiltration retentates at VRR = 2 and VRR = 3. This could be explained by the different dry matter content in the samples investigated. Mahdian and Tehrani (2007) reported that increased dry matter content keeps higher concentrations of *L. bulgaricus* and *S. thermophilus* in the yoghurt obtained.

There was research on the change in viable probiotic bacteria in the production of stirred-type yoghurt from goat's milk (Martìn-Diana *et al*., 2003). The authors found that the addition of 3% whey protein concentrate resulted in an increase in the amount of *S. thermophilus* ST-20Y, *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 – from 6.4 log units to 8.7 log units due to the increased protein content. Figure 3 also shows that a reduction was observed during the storage period and the biggest decrease was from the 21st to the 2th day. Similar results were also obtained from the experimental work of Oliveira *et al*. (2002) on the 28th day of storage in lactic acid beverages.

Comparing the total number of viable lactic acid bacteria of the three starter cultures used, it could be seen that the highest values were observed in the yoghurt with starter 1CM ($P < 0.05$), followed by the starter MZ_2 ($P < 0.05$) and starter ZD ($P < 0.05$). This dependence concerns both controls and yoghurts derived from ultrafiltration retentates at VRR = 2 and VRR = 3.

Figure 5 shows that the lowest values of titratable acidity were obtained in the controls, followed by yoghurts from ultrafiltration retentates at VRR = 2 and VRR = 3. This was probably due to the higher protein content in ultrafiltered retentates, which was favourable for the growth of lactic acid bacteria. This led to a higher concentration of lactic acid. The strongest increase in titratable acidity was observed in the period from 1 to 14 d, after which it remained practically unchanged. This could be explained by the inhibition in the growth of lactic acid bacteria from accumulated lactic acid during the storage period (Kondratenko and Simov, 2003). Figure 5 shows that yoghurt with the highest acidity was obtained with starter 1CM, followed by starters MZ_2 and starters ZD. This was probably due to the higher content of *S. thermophilus* in the yoghurts examined. The pH of the yoghurts was not measured as it is likely to have decreased with VRR owing to the increase in titratable acidity.

Conclusion

The results show that the increase in VRR led to an increase in the titratable acidity of initial milk, ultrafiltered retentates and yoghurts obtained. The level of ultrafiltration concentration led to an increase in the count of viable lactic acid bacteria in all yoghurts which improved their functional properties. The highest values of the total number of viable lactic acid bacteria were determined in Bulgarian yoghurts obtained with starter 1CM, followed by starters MZ_2 and ZD. Probiotic yoghurts with the highest organoleptic evaluation were obtained from ultrafiltration retentates at volume reduction ratio VRR = 2 and starters 1CM and MZ_2.

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