RESEARCH ARTICLE

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Microbiological, physicochemical and organoleptic characteristics of probiotic Bulgarian yoghurts obtained by an ultrafiltered whole cow's milk

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ABSTRACT
Ultrafiltration of whole cow's milk with ultrafiltration polyacrilonitrile membrane with 10 kDa molecular weight cut-off at volume reduction ratios 2 and 3 was performed. The obtained ultrafiltration retentates were used for the production of Bulgarian yoghurts with three different probiotic starters. A control sample was prepared using a whole cow's milk with the same starters. All yoghurts were analyzed according to the following parameters: titratable acidity, dry matter, organoleptic characteristics, concentration of specific microorganisms, probiotic *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, as well as the total count of viable lactic acid bacteria for 28 days of storage. The results showed that the increase in the volume reduction ratio during ultrafiltration increased the titratable acidity, as well as the dry matter of all examined yoghurts. The increase in the level of ultrafiltration concentration resulted in a higher concentration of viable lactic acid bacteria in all tested yoghurts which improves their functional properties.

Key words: probiotic Bulgarian yoghurt, ultrafiltration, whole cow's milk

Introduction

In dairy production, the texture quality can be improved by increasing of total solids through the addition of evaporated milk, powdered milk and protein concentrates (Stack et al., 2010; Tratnik et al., 2006) and enzymatic crosslinking (Lorenzen et al., 2002; Park & Guo, 2006). The traditional concentration methods, for example, evaporation can change the characteristics of whey proteins because they are thermolabile.

Ultrafiltration is a very attractive alternative method to concentrate milk for production of dairy products because it has the following advantages in comparison with the traditional separation methods: environmental friendliness (Kumar et al., 2013), lower power consumption (Baldasso et al., 2011), increased yield (Ong et al., 2013) and quality of the final product (Domagala et al., 2012), reduction of the production costs (Mehaia, 2005), the realization of the process at room temperature in order to treat heat-sensitive substances and keep their natural properties (Baldasso et al., 2011; Ding et al., 2002; Gésan-Guiziou, 2013). Therefore, in the last several years, the application of ultrafiltration process to concentrate milk constituents and to increase the total solid content was investigated (Bergillos-Meca et al., 2015). It could be an important processing step in cheese and other dairy products manufacturing (Gésan-Guiziou, 2013).

The probiotic yoghurts prepared from goat's milk concentrated by ultrafiltration had better sensory properties and good texture (Domagala et al., 2012; Domagala & Wszolek, 2008). Bergillos-Meca et al. (2015) also investigated the application of the ultrafiltration process as an alternative to the addition of powdered milk in the production of fermented goat's milk. They concluded that ultrafiltration increased Ca, Zn and P concentrations and these products may constitute a better source of minerals compared to other products existing already on the market.

Ainaz and Ehsani (2008) investigated the chemical composition and the growth of *Lactobacillus acidophilus* and *Bifidobacterium lactis* as probiotic bacteria in yoghurt made from ultrafiltered milk and compared with the control yoghurt with 2 % (w/v) skim milk powder. The results showed that during ultrafiltration concentration the protein and total solid content increased which resulted in a higher buffering capacity, acidity and better growth of probiotic bacteria.

Narayana and Gupta (2015) studied the effect of the ultrafiltration process on the quality of cow's milk plain set yoghurt. Direct ultrafiltration of skim cow's milk at two different ultrafiltration concentration levels – 1.5 and 2 fold and highly concentrated skim cow's milk concentrate

obtained by ultrafiltration were used to adjust the total solids of milk. The results showed that the protein percentage increased and the lactose content decreased significantly ($p < 0.05$) in yoghurt with the increase in ultrafiltration concentration/ultrafiltration concentrate addition with similar total solids in milk. None of the quality parameters tested showed significant difference with the ultrafiltration process, so that both procedures can be recommended at 1.5 fold to produce yoghurt with good quality and enhanced protein content without the addition of stabilizers.

The aim of this research was to investigate the microbiological, physicochemical and organoleptic characteristics of probiotic Bulgarian yoghurts obtained by an ultrafiltered whole cow's milk.

Materials and Methods

Materials

Milk

The whole cow's milk was delivered by BCC Handel Ltd., Elena, Bulgaria. The milk was analyzed according to the following parameters: dry matter content (ISO 6731:2010) -; total protein content (ISO 8961-1:2014); fat content (ISO 2446:2008); mineral substances (BDS 6154:1974).

Starter cultures

Three probiotic starter cultures were used for the production of Bulgarian yoghurts: Starter culture ZD consisting of a probiotic strain of *Lactobacillus delbrueckii* subsp. *bulgaricus* (National Bank for industrial microorganisms and cell cultures NBIMCC 3706) and *Streptococcus thermophilus* (T3); 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 the University of Food Technologies, Plovdiv, Bulgaria.

Media for development and maintenance of lactic acid bacteria

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

Cultivation and storage of probiotic starter cultures for yoghurt

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

Membranes

Ultrafiltration was carried out with a polyacrylonitrile membrane UF10-PAN with 10 kDa molecular weight cut-off. The membrane was prepared by the dry-wet phase-inversion method of polymer solutions with a solvent of dimethyl sulfoxide (Sigma Aldrich Merck, St. Louis, MO, USA). Then it was heat-treated in an aqueous medium for 10 min at 60 °C.

Ultrafiltration experiments

Ultrafiltration experiments were carried out on laboratory equipment with a replaceable plate and frame membrane module fitted with a UF10-PAN polyacrilonitrilic ultrafiltration membrane with 10 kDa molecular weight cut-off (Figure 1). Ultrafiltration was realized at the following operating conditions: volume reduction ratio VRR = 2 and VRR = 3, working pressure of 0.5 MPa, the temperature of 50 °C and feed flow rate of 330 $dm^3.h^{-1}$. The retentates were then pasteurized at 65 °C during 10 min to 15 min after ultrafiltration and cooled at 42 °C ± 1 °C.

Figure 1. *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.

Production of probiotic Bulgarian yoghurts

For the production of probiotic yoghurts ultrafiltration retentates at volume reduction ratios 2 and 3 were used. Control samples from whole cow's milk without ultrafiltration with the same starters were also prepared. The coagulation was performed in sterile plastic containers of 100 cm^3 with a 1.5 % probiotic starter (ZD, MZ_2, 1CM). Three repetitions of every sample were made. The containers were placed in a thermostat at a temperature of 41 °C to 42 °C for coagulation of milk for 2.5 h to 3 h. After coagulation, yoghurts were cooled and then stored at refrigerator conditions at 2 °C to 6 °C during 28 days.

Methods for analysis of the milk products

The initial whole cow's milk and the retentates were analyzed according to titratable and active acidity, the total number of mesophilic aerobic and facultatively anaerobic microorganisms, as well as specific microorganisms, while the obtained yoghurts were analyzed according to dry matter content, titratable acidity, amount of specific microorganisms, number of viable lactic acid bacteria and organoleptic characteristics using the following methods:

Physicochemical methods

Dry matter content was measured according to ISO 6731:2010. The ability of lactic acid bacteria to form acids (titratable acidity; °T) was measured by the Toerner method according to BDS 1111:1980. To measure active acidity (pH) a pen-type pH meter (PH-03[I]; Hinotek, China) was used.

Microbiological methods

The number of viable lactic acid bacteria – appropriate serial dilutions in saline solution NaCl – 5 $g.dm^{-3}$ (Sigma Aldrich – Merc, St. Louis, MO, USA) of the obtained yoghurts 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 days at 37 ºC until the appearance of countable single colonies, used to estimate the number of bacteria in the original sample. The total numbers of mesophilic aerobic and facultatively anaerobic microorganisms were analyzed according to (BDS EN ISO 4833-1:2013); *Escherichia coli* - according to BDS EN ISO 16649-2:2014; *Staphylococcus aureus* - according to BDS EN ISO 6888-1:2005+A_1:2005; *Salmonella* - according to BDS EN ISO 6579:2003; Yeasts and moulds – according to BDS EN ISO 6611:2006.

Organoleptic analysis

The organoleptic analysis was performed using a 5-point hedonic scale for evaluation and the basic organoleptic indices were presented on 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 panelists rated the samples three times in a random order for color, the appearance of coagulum, the structure at cutting, consistency at shattering, taste and aroma. Room temperature water and unsalted crackers were given to the panelists for mouth rinsing between samples to eliminate carry-over effects. The basic organoleptic indices and their characteristic were presented on Table 2.

Table 1. *Hedonic scale for evaluation of organoleptic characteristics 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

Table 2. *Organoleptic indices for evaluation of probiotic Bulgarian yoghurts.*

Indices	Characteristic and norm
1. Color	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 odor is allowed

Statistical method

Fisher's least significant difference test at a significance level of 0.05 was used for comparison of the mean values of the physicochemical and the microbiological characteristics between the whole cow's milk and the retentates at volume reduction ratios VRR = 2 and VRR = 3, as well as between different yoghurts.

Results

The initial whole cow's milk was analyzed according to the dry matter, total protein, fat contents and mineral substances. The results were as follows: 12.48 % (w/v), 3.14 % (w/v), 3.60 % (v/v) and 0.74 % (w/v) respectively.

The experimental results for the titratable acidity and the pH of whole cow's milk and the obtained ultrafiltration retentates from this milk at volume reduction ratios 2 and 3 were presented on Table 3. The data showed that the increase in the volume reduction ratio led to an increase in the titratable acidity from 22 ºT ± 1.00 ºT (VRR = 2) to 29 ºT ± 0.58 ºT (VRR = 3), respectively ($p < 0.05$). The ultrafiltration concentration provoked a decrease in the active acidity from 6.71 ± 0.01 for retentate at VRR = 2 to 6.61 ± 0.04 for retentate at a volume reduction ratio 3 ($p < 0.05$), whereas no statistically significant difference ($p > 0.05$) was observed between the whole milk and ultrafiltration retentate at a volume reduction ratio 2.

Microbiological analysis of the initial whole milk and ultrafiltration retentates at volume reduction ratios 2 and 3 was presented on Table 4. The total number of mesophilic aerobic and facultatively anaerobic microorganisms, specific microorganisms - *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, as well as the presence of moulds and yeasts was defined. The data showed that the increase in the volume reduction ratio led to an increase in the total number of mesophilic aerobic and facultatively anaerobic microorganisms ($p < 0.05$). The lowest values of the mesophilic aerobic and facultatively anaerobic microorganisms were found for the initial milk ($1.6x10^2$ cfu.cm^{-3} ± $0.12x10^2$ cfu.cm^{-3}), followed by the ultrafiltration retentate at VRR = 2 ($3.5x10^2$ cfu.cm^{-3} ± $0.12x10^2$ cfu.cm^{-3}) and VRR = 3 ($4.5x10^2$ cfu.cm^{-3} ± $0.10x10^2$ cfu.cm^{-3}), respectively.

The results for the dry matter of the obtained probiotic yoghurts (control and ultrafiltration retentates) with the three probiotic starters (ZD, MZ_2, 1CM) were presented on Table 5. The statistical analysis of the data showed that there was no significant difference ($p > 0.05$) between the dry matter of the yoghurts obtained with three probiotic starters. The dry matter content of the controls had an average value of 12.58 % (w/v), followed by the ultrafiltration retentate at VRR = 2 (19.69 %) and the ultrafiltration retentate at VRR = 3 (26.56 %).

The microbiological status of the investigated probiotic yoghurts was presented in Table 6. The analysis for specific microorganisms show that *E. coli* and *St. aureus* were less than 10 cfu.g^{-1}, and *Salmonella* was not found in 25 g of the product. The count of moulds and yeasts was under 10 cfu.g^{-1} in all tested probiotic yoghurts.

The change in the concentration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, as well as their total number in the investigated yoghurts with starter ZD was presented in Figure 2.

The results indicated that on the first day of the storage period the concentration of *Lactobacillus bulgaricus* was highest in yoghurt from ultrafiltration retentate at VRR = 3 (10.60 logN ± 0.20 logN), followed by ultrafiltration retentate at VRR=2 (10.48 logN ± 0.32 logN) and control (10.30 logN ± 0.24 logN).

A similar trend was observed for the coccus-shaped forms: 10.60 logN ± 0.20 logN, 10.51 logN ± 0.32 logN and 10.38 logN ± 0.24 logN, respectively.

All probiotic yoghurts were characterized by a high content of viable lactic acid cells - above $2x10^8$ cfu.g^{-1}.

Table 3. *Titratable acidity and pH of initial whole milk and ultrafiltration retentates*

Sample	Titratable acidity, °T			Average values ± SD	pH			Average values ± SD
	1	2	3		1	2	3	
Whole milk	17.00	19.00	19.00	18.00 ± 1.15^{a}	6.73	6.76	6.78	6.76 ± 0.03^{a}
VRR = 2	21.00	22.00	23.00	22.00 ± 1.00^{b}	6.70	6.70	6.72	6.71 ± 0.01^{a}
VRR = 3	29.00	29.00	30.00	29.00 ± 0.58^{c}	6.58	6.60	6.65	6.61 ± 0.04^{b}

Note: Small letters (a, b, c) were used to compare the initial whole milk and ultrafiltration retentates and they indicated that mean values in the columns were significantly different ($p < 0.05$).

Table 4. *Microbiological analysis of initial milk and ultrafiltration retentates.*

Sample	TBA, cfu.cm^{-3}	Specific microorganisms			Moulds and yeasts, cfu.cm^{-3}
		E. coli, cfu. cm^{-3}	*St. aureus*, cfu.g^{-1}	*Salmonella* sp. in 25 cm^3	
Whole milk	$1.6x10^2$ ± $0.12x10^{2a}$	< 1	< 10	Not found	< 1
VRR = 2	$3.5x10^2$ ± $0.12x10^{2b}$	< 1	< 10	Not found	< 1
VRR = 3	$4.5x10^2$ ± $0.10x10^{2c}$	< 1	< 10	Not found	< 1

Note: Small letters (a, b, c) were used to compare the initial whole milk and ultrafiltration retentates and they indicated that mean values in the columns were significantly different ($p < 0.05$).

Table 5. *Dry matter of probiotic yoghurts (control and ultrafiltration retentates).*

Type of probiotic yoghurt with different starter	Dry matter, %			Average values ± SD
	1	2	3	
ZD (control)	12.36	12.48	12.60	12.48 ± 0.12^{aA}
ZD (VRR = 2)	19.24	19.56	19.88	19.56 ± 0.32^{bB}
ZD (VRR = 3)	25.92	26.41	26.90	26.41 ± 0.49^{cC}
MZ_2 (control)	12.48	12.60	12.72	12.60 ± 0.12^{aA}
MZ_2 (VRR = 2)	19.50	19.69	19.75	19.65 ± 0.13^{bB}
MZ_2 (VRR = 3)	26.49	26.61	26.73	26.61 ± 0.12^{cC}
1CM (control)	12.53	12.67	12.81	12.67 ± 0.14^{aA}
1CM (VRR = 2)	19.75	19.85	19.95	19.85 ± 0.10^{bB}
1CM (VRR = 3)	26.46	26.67	26.84	26.66 ± 0.19^{cC}

Note: Small letters (a, b, c) were used to compare the dry matter of the obtained yoghurts with three probiotic starters, and they indicated that mean values in the columns were significantly different ($p < 0.05$), capital letters (A, B, C) were used to compare dry matter of the obtained yoghurts (control, VRR = 2 and VRR = 3).

Table 6. *Microbiological analysis of probiotic yoghurts*

Type of probiotic yoghurt with different starter	Specific microorganisms			Moulds and yeasts, cfu.g^{-1}
	E. coli, cfu.g^{-1}	*St. aureus*, cfu.g^{-1}	*Salmonella* sp. in 25 g	
ZD (control)	< 10	< 10	NF	< 10
ZD (VRR = 2)	< 10	< 10	NF	< 10
ZD (VRR = 3)	< 10	< 10	NF	< 10
MZ_2 (control)	< 10	< 10	NF	< 10
MZ_2 (VRR = 2)	< 10	< 10	NF	< 10
MZ_2 (VRR = 3)	< 10	< 10	NF	< 10
1CM (control)	< 10	< 10	NF	< 10
1CM (VRR = 2)	< 10	< 10	NF	< 10
1CM (VRR = 3)	< 10	< 10	NF	< 10

Note: NF means not found

The growth of the lactic acid bacteria in the investigated yoghurts with starter MZ_2 was presented in Figure 3. The increase in the volume reduction ratio increased the concentration of the rod-shaped forms in yoghurts from ultrafiltration retentates in comparison with the control ($p < 0.05$). The amount of rod-shaped forms was highest in the yoghurt from ultrafiltration retentate at VRR = 3 (10.66 logN ± 0.18 logN), followed by VRR = 2 (10.54 logN ± 0.20 logN) and the control (10.48 logN ± 0.30 logN). The concentration of the coccus-shaped forms for the control was 10.60 logN ± 0.32 logN, while these amounts in the yoghurts from ultrafiltration retentates were higher - 10.65 logN ± 0.24 logN (VRR = 2) and 10.70 logN ± 0.20 logN (VRR = 3), respectively.

The change in the concentration of the rod-shaped and the coccus-shaped forms, as well as their total number in the yoghurts with starter 1CM was presented in Figure 4. The data showed that the concentration of *Lactobacillus bulgaricus* on the 1st day of storage period was highest in the yoghurt from ultrafiltration retentate at VRR = 3 (10.72 logN ± 0.26 logN), followed by ultrafiltration retentate at VRR = 2 (10.70 logN ± 0.35 logN) and the control - (10.48 logN ± 0.20 logN). The count of *Streptococcus thermophilus* was highest in the yoghurt from ultrafiltration retentate at VRR = 3 (10.78 logN ± 0.28 logN), followed by ultrafiltration retentate at VRR = 2 (10.68 logN ± 0.33 logN) and the control - (10.51 logN ± 0.22 logN). The total number of the lactic acid bacteria was lowest in the control (10.79 logN ± 0.24 logN), followed by the ultrafiltration retentates at VRR = 2 (10.99 logN ± 0.36 logN) and VRR = 3 (11.05 logN ± 0.24 logN). The figure showed that the total number of lactic acid bacteria is the lowest at the 28th day.

The experimental results for the titratable acidity of the yoghurts with three probiotic starters were presented in Figure 5. The data showed that the increase in the volume reduction ratio led to an increase in the titratable acidity throughout the storage period for all tested samples comparing the 1st day and the 28th day ($p < 0.05$). The titratable acidity increased most significantly between 1st day to 7th day, excluding starter ZD at VRR = 2. Then from 21st day to 28th day it remained practically unchanged, excluding starter ZD and control 1CM. This could be explained by the inhibition growth of the lactic acid bacteria from the accumulated lactic acid during the storage period (Kondratenko & Simov, 2003). The starter with the strongest acid-forming ability was 1CM, followed by the starters MZ_2 and ZD.

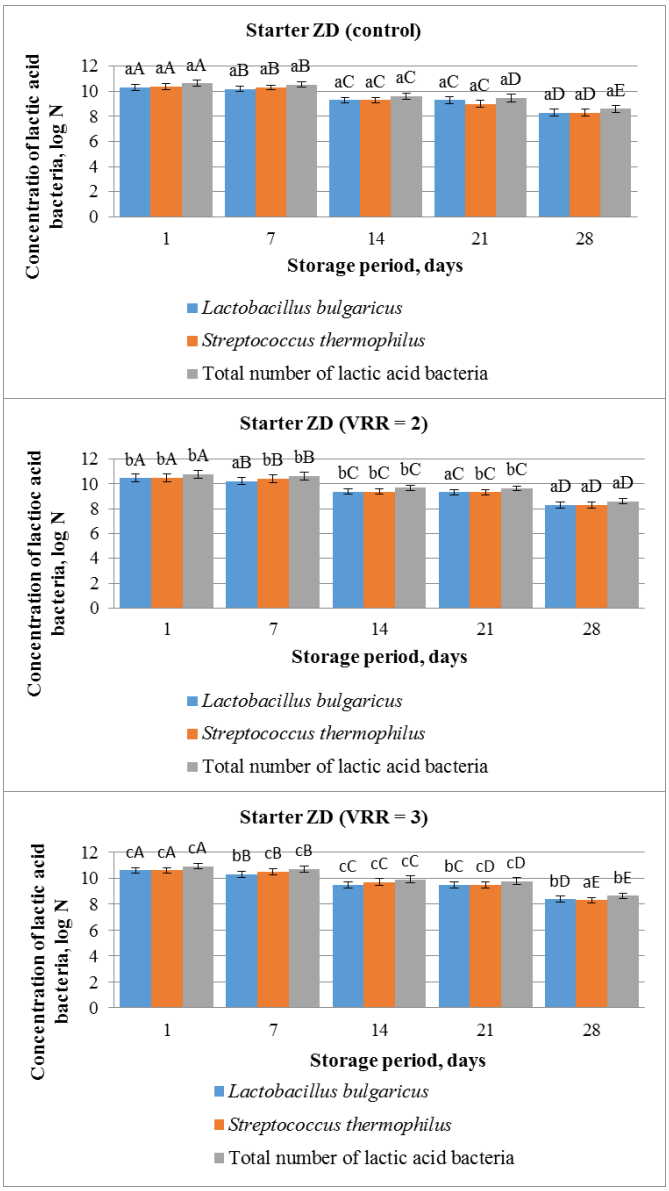

Figure 2. *Microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter ZD*

Note: Small letters (a, b, c) were used to compare the concentration of lactic acid bacteria in the obtained yoghurts (control, VRR = 2 and VRR = 3), capital letters (A, B, C, D, E) were used to compare the lactic acid bacteria on the 1 – 7 – 14 – 21 – 28 day.

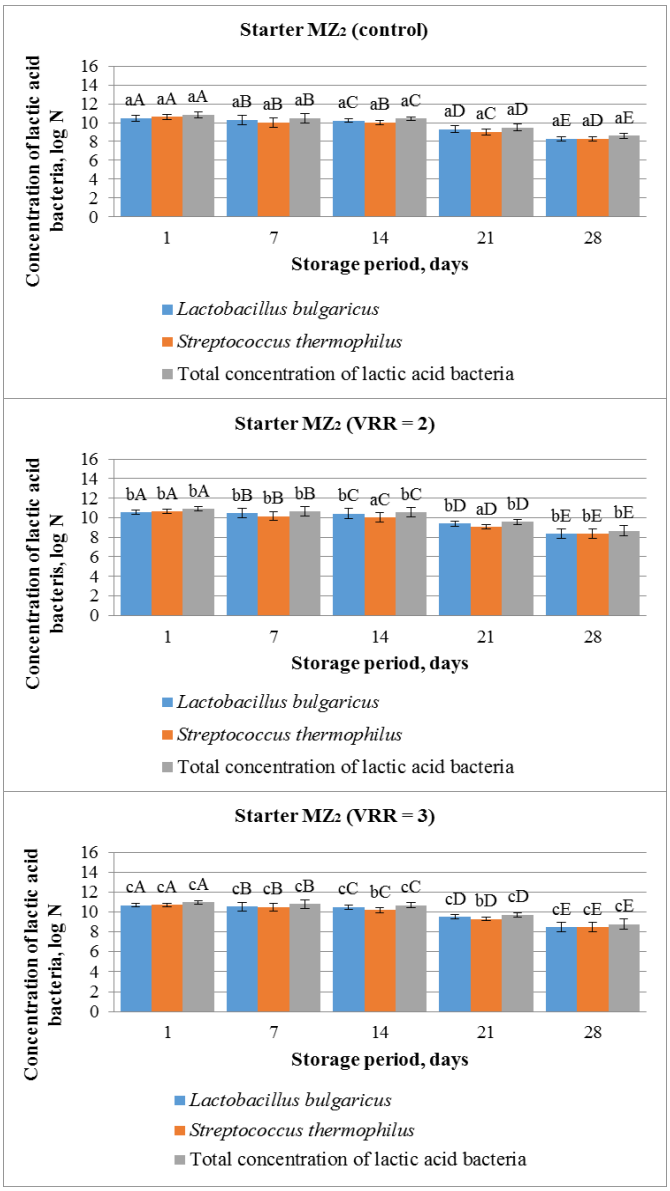

Figure 3. *Microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter MZ_2*

Note: Small letters (a, b, c) were used to compare the concentration of lactic acid bacteria in the obtained yoghurts (control, VRR = 2 and VRR = 3), capital letters (A, B, C, D, E) were used to compare the lactic acid bacteria on the 1 – 7 – 14 – 21 – 28 day.

The organoleptic evaluation of the probiotic yoghurts (controls and ultrafiltration retentates at VRR = 2 and VRR = 3) was made, as follows: the appearance of coagulum, consistency at shattering, color, the structure at cutting, taste and aroma (Table 7, Table 8, Table 9). The highest number of points was obtained for the yoghurts from ultrafiltration retentate at VRR = 2 with the three probiotic starters. The lowest number of points was observed for the controls. Yoghurt with the highest organoleptic evaluation was produced with starter MZ_2, followed by starter ZD and starter 1CM.

Discussion

Table 3 showed that ultrafiltration concentration provoked an increase in the titratable acidity and a decrease in the pH. The lowest value of the titratable acidity was observed for the initial milk.

Shamsia and El-Ghannam (2012) found higher values of titratable acidity for yoghurts produced by ultrafiltration compared to these produced by traditional technology without ultrafiltration.

This could be explained by the higher protein content in the retentates which was due to the retention and concentration of all proteins in milk (casein and whey proteins) with a molecular weight above the retention capacity of the membrane.

The higher concentration of mesophilic aerobic and facultatively anaerobic microorganisms (Table 4) in the samples could be explained by the fact that the volume of the retentates decreased.

The data showed that the initial milk and the obtained ultrafiltration retentates were in agreement with the requirements for obtaining a high quality and safety final product (Instruction from 31 July 2004 for the Maximum Allowable Quantities of Pollutants in Foods (Official journal of Bulgarian Government, issue 88/8, 2004).

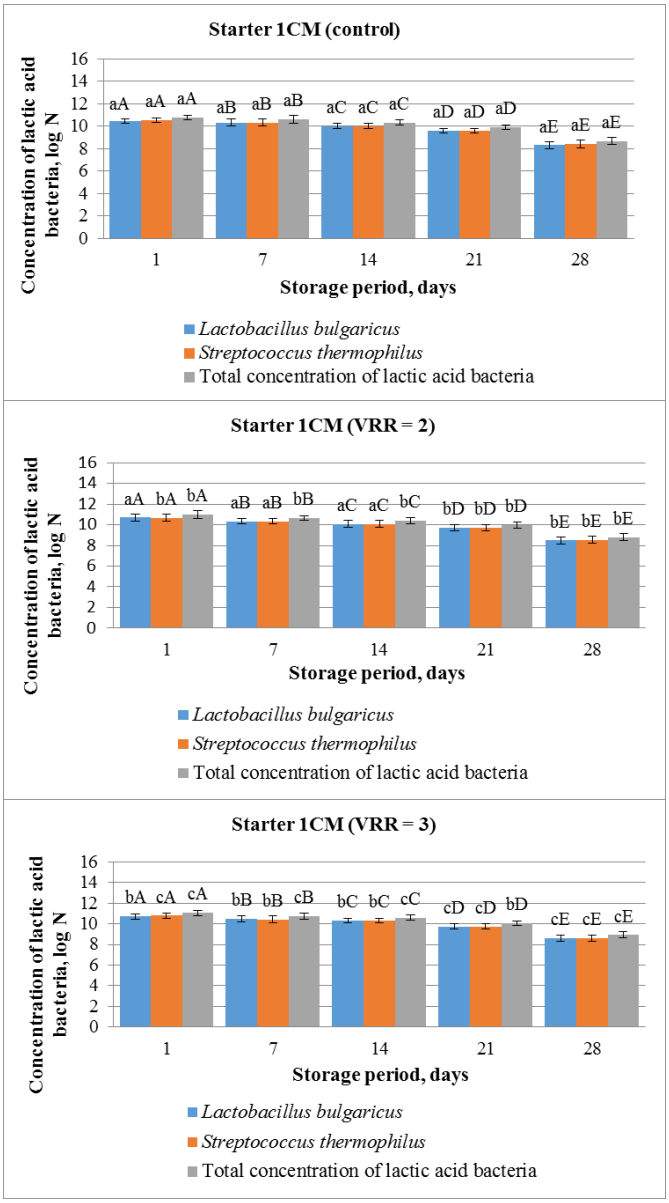

Figure 4. *Microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter 1CM*

Note: Small letters (a, b, c) were used to compare the concentration of lactic acid bacteria in the obtained yoghurts (control, VRR = 2 and VRR = 3), capital letters (A, B, C, D, E) were used to compare the lactic acid bacteria on the 1 – 7 – 14 – 21 – 28 day.

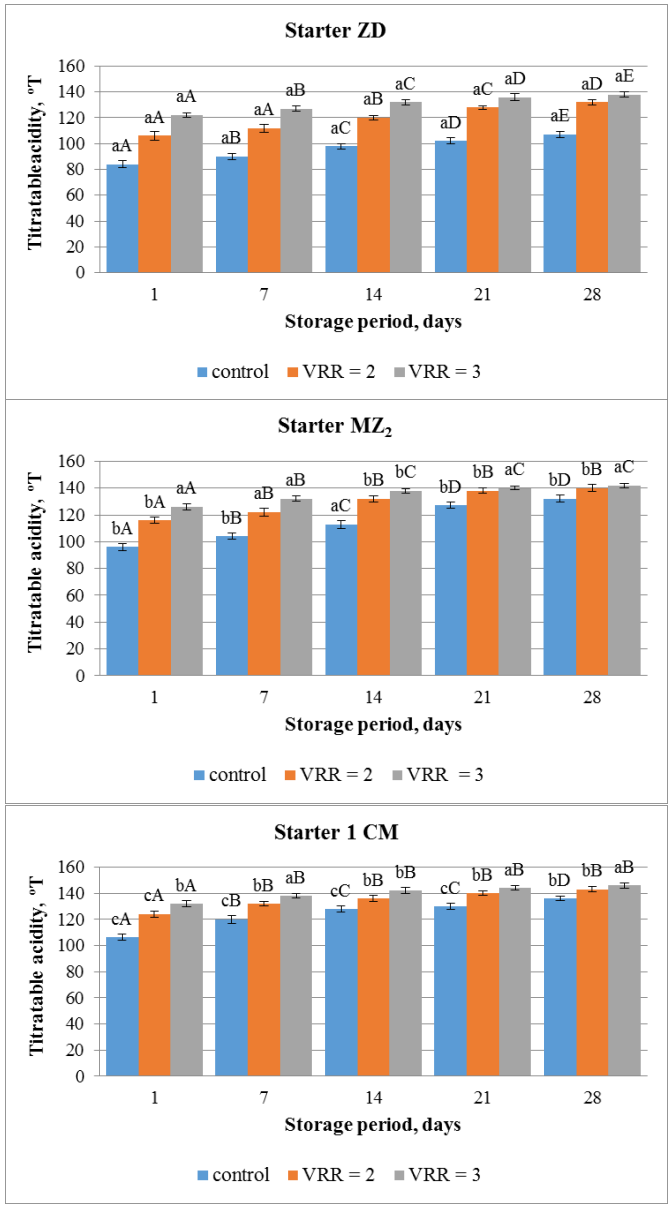

Figure 5. *Titratable acidity of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starters ZD, MZ_2 and 1CM*

Note: Small letters (a, b, c) were used to compare the titratable acidity of the obtained yoghurts (control, VRR = 2 and VRR = 3), capital letters (A, B, C, D, E) were used to compare the titratable acidity on the 1 – 7 – 14 – 21 – 28 day.

Table 7. *Organoleptic evaluation of probiotic yoghurts with starter ZD.*

Indices	**Type of probiotic yoghurt**		
	Starter ZD		
	control	**VRR = 2**	**VRR = 3**
Appearance of coagulum	smooth coagulum without lateral tear at the inclination of the package 4 points	smooth, dense coagulum without lateral tear at the inclination of the package 5 points	dense coagulum without lateral tear at the inclination of the package 4 points
Consistency at shattering	homogeneous 5 points	homogeneous 5 points	homogeneous 5 points
Color	white with creamy hue 5 points	white with creamy hue 5 points	white with creamy hue 5 points
Structure at cutting	smooth surface with strong separation of the whey 4 points	smooth surface with a slight separation of the whey 5 points	smooth surface without separation of the whey 5 points
Taste and aroma	a pleasant lactic acid taste 3 points	a pleasant lactic acid taste 4 points	a weak lactic acid taste 2 points
Total points	21 points	24 points	21 points

Table 8. *Organoleptic evaluation of probiotic yoghurts with starter MZ_2*

Indices	**Type of probiotic yoghurt**		
	Starter MZ_2		
	control	**VRR = 2**	**VRR = 3**
Appearance of coagulum	smooth, dense coagulum without lateral tear at the inclination of the package 4 points	smooth, dense coagulum without lateral tear at the inclination of the package 5 points	smooth, dense coagulum without lateral tear at the inclination of the package 5 points
Consistency at shattering	homogeneous 5 points	homogeneous 5 points	homogeneous 5 points
Color	white with creamy hue 5 points	white with creamy hue 5 points	white with creamy hue 5 points
Structure at cutting	smooth surface with a slight separation of the whey 5 points	smooth surface 5 points	smooth surface 5 points
Taste and aroma	a pronounced lactic acid taste 4 points	a pleasant lactic acid taste 5 points	sweet weak lactic acid taste 4 points
Total points	23 points	25 points	24 points

The results in Table 5 showed that the use of ultrafiltration concentration increased the dry matter of the yoghurts investigated. The comparison of the dry matter content of the yoghurts showed that the increase was approximately 1.57 times for VRR = 2 and 2.11 times for VRR = 3 for the three starter cultures. This could be explained by the fact that during the ultrafiltration process the water and low molecular substances like lactose and minerals pass through the membrane whereas the high molecular substances (fat and proteins) are retained on the membrane surface and subjected to further concentration.

Figure 2 for the change in concentration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, as well as their total number in the investigated yoghurts with starter ZD showed that the during storage period the concentration of lactic acid bacteria decreased, comparing the 1st and the 28th day ($p < 0.05$). It also could be seen that ultrafiltration had a positive effect on the concentration of lactic acid bacteria ($p < 0.05$). This was due to the fact that the use of the ultrafiltration process resulted in an increase in the dry matter content of the retentates in comparison with the control, which favored the growth of lactic acid bacteria. These results were in agreement with the experimental work of Marafon et al. (2011) which found that the use of whey protein concentrate in the production of yoghurts resulted in

Table 9. *Organoleptic evaluation of probiotic yoghurts with starter 1CM.*

Indices	**Type of probiotic yoghurt**		
	Starter 1CM		
	control	**VRR = 2**	**VRR = 3**
Appearance of coagulum	smooth, dense coagulum without lateral tear at the inclination of the package 3 points	smooth, dense coagulum without lateral tear at the inclination of the package 5 points	smooth, dense coagulum without lateral tear at the inclination of the package 4 points
Consistency at shattering	homogeneous 5 points	homogeneous 5 points	homogeneous 5 points
Color	white with creamy hue 5 points	white with creamy hue 5 points	white with creamy hue 5 points
Structure at cutting	smooth surface with mean separation of the whey 3 points	smooth surface without a separation of the whey 4 points	smooth surface withouth a separation of the whey 4 points
Taste and aroma	stronger lactic acid taste 4 точки	a pleasant lactic acid taste 4 points	sweet lactic acid taste 3 points
Total points	20 points	23 points	21 points

The microbiological status of probiotic Bulgarian yoghurts (control and ultrafiltration retentates at VRR = 2 and VRR = 3) with starter MZ_2 and starter 1CM, presented in Figures 3 and 4, confirmed the positive effect of ultrafiltration concentration on the growth of lactic acid bacteria ($p < 0.05$), like the starter ZD (Figure 2). The experimental results indicated that the concentration of lactic acid bacteria decreased slightly during all storage periods ($p < 0.05$). Despite this, the amount of viable bacteria cells was above $2x10^8$ cfu/g and that made these yoghurts suitable for all human age groups due to their functional properties.

The comparison of Figures 2, 3 and 4 showed that probiotic yoghurt with the highest amount of viable cells was obtained with starter 1CM, followed by starters MZ_2 and ZD, respectively. This dependence was valuable for controls and yoghurts from ultrafiltration retentates at VRR = 2 and VRR = 3.

The organoleptic evaluation of the yoghurts (Tables 7, 8, 9) showed that ultrafiltration improved the body, the texture and the overall acceptability of the final product due to the dry matter and the protein increase (Meena et al., 2015). Our experimental results indicated that ultrafiltration concentration at low levels (VRR = 2) was suitable for the production of yoghurts, but these obtained from retentates at VRR = 3 had a creamy structure and were more convenient for cheese production.

Conclusion

This research showed the possibilities for the application of ultrafiltration for the production of probiotic Bulgarian yoghurts. The results showed that the ultrafiltration concentration increased the dry matter content and the count of viable lactic acid bacteria in all examined probiotic yoghurts. 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, respectively. Probiotic yoghurts with the highest organoleptic evaluation were obtained from ultrafiltration retentates at volume reduction ratio VRR = 2 and starters MZ_2 and ZD.

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