



# Article Microbiological, Physicochemical, Organoleptic, and Rheological Properties of Bulgarian Probiotic Yoghurts Produced by Ultrafiltered Goat's Milk

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**Abstract:** In this experimental work, the microbiological, physicochemical, organoleptic, and rheological properties of yoghurts produced by ultrafiltered goat's milk using two volume-reduction ratios and three probiotic starters were studied. It was established that the dry matter, fats, proteins, count of lactic acid bacteria, titratable acidity, and dynamic viscosity increased and the pH decreased with the rise of the volume-reduction ratio during ultrafiltration. All yoghurts exhibited Bingham plastic flow behaviour. We recommend using a volume-reduction ratio of 3 and MZ<sub>2</sub>f + *Bifidobacterium bifidum* BB-87 to produce probiotic Bulgarian yoghurts with the highest dry matter contents (23.02%), protein contents (10.20%), fat contents (9.80%), number of viable lactic acid cells (9.34 logN), viscosity (4.99 Pa·s at shear rate of  $1.22 \text{ s}^{-1}$ ), and organoleptic properties and the highest score (15) in the range of this experiment.

**Keywords:** probiotic starters; goat's milk; yoghurt; ultrafiltration; *Bifidobacterium bifidum; Lactobacillus acidophilus* 

# 1. Introduction

Milk and dairy products are some of the most indispensable natural foods due to their alimentary wholesome benefits. They are deservedly preferred by many people around the world [1–4]. The conductive effect of these products can be explained by their composition and the fact that they have an important place in the rational, prophylactic, and dietary nutrition of humans because they are vital for the maintenance of the human body [1,5,6].

Yoghurt is a food obtained by the bacterial fermentation of milk and is consumed all over the world [7]. The bacteria used for yoghurt production are known as yoghurt cultures or starter cultures. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the two microorganisms involved in the fermentation of yoghurt [8–10]. These bacteria provoke the fermentation of milk and produce lactic acid in order to change the yoghurt's texture and flavour. Different kinds of milk (cow's, sheep's, goat's, mare's, camel's) can be used for yoghurt production. There has been an increasing scientific and consumer interest in goat milk production in recent years [11]. Goat's milk is the most easily digestible dairy product and its amino acid composition is similar to that of human milk, which distinguishes it from other milks. It has high-quality proteins, fats, vitamins, and minerals [12,13]. This milk can be consumed by people who cannot consume cow's milk because it does not contain  $\alpha$ s1-casein, which causes various types of intolerance to milk [10]. Goat's milk can be successfully used for yoghurt production, individually or mixed with cow's, sheep's, and mare's milk [14].



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Microbiological, physicochemical, organoleptic, and rheological characteristics are the fundamental characteristics of yoghurts that influence the acceptance and choices of consumers [8]. One way to improve the texture quality of yoghurts is to increase the dry matter content, which, in the traditional method, can be achieved via the addition of milk concentrated by evaporation, powdered milk, or protein concentrates [15–17]. The conventional method for milk concentration is evaporation [18]. It is important to note that this process has many negative effects because it can change the characteristics of thermolabile whey proteins [19]. The application of an evaporation process can reduce the product quality due to the negative effect of thermal treatment on heat-sensitive milk components [20].

Nowadays, consumer interest is focused on natural foods produced transparently and with a minimal addition of additives. Membrane processes, such as nanofiltration, ultrafiltration (UF), microfiltration, and reverse osmosis, can allow producers to avoid the addition of milk powder and offer products with a more healthful profile [21,22]. The separation and concentration of milk substances can be conducted via UF [23–26]. This method is preferred due to its advantages—low energy costs and a novel nonthermal environmentally friendly technology that reduces the negative effects of temperature rises, such as phase changes, the denaturation of proteins, and changes in sensory properties [27,28]. In previous our work [29], we investigated the possibility of applying UF with an UF10-PAN membrane to improve the physical, chemical, and microbiological characteristics of the yoghurts obtained.

The aim of this experimental work was to study the fundamental properties (microbiological, physicochemical, organoleptic, and rheological) of goat yoghurts produced by UF with an UF25-PAN membrane at different concentration levels and starter cultures.

## 2. Materials and Methods

- 2.1. Materials
- 2.1.1. Milk

This work was performed with the pasteurized goat's milk "Olympus". It was purchased from the commercial market and had a fat content of 3.5%.

# 2.1.2. UF Membrane

An UF25-PAN membrane from polyacrylonitrile with a molecular weight cut-off of 25 kDa was used for the needs of the experiment.

#### 2.1.3. Starters for Yoghurt Production

One main probiotic starter culture named  $MZ_2f$  was used for the production of yoghurts. The microorganisms of this culture were a mixture of a probiotic *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) LB-51, and *Streptococcus thermophilus* (*S. thermophilus*) ZH in a 1:2 ratio. Yoghurts with acidophilic or bifidobacteria were also obtained by adding 0.5% of the active cells of *Lactobacillus acidophilus* (*Lb. acidophilus*) LAB-8 and *Bifidobacterium bifidum* (*B. bifidum*) BB-87 to the main starter.

#### 2.2. Methods

## 2.2.1. Growth of Bacteria

The maintenance and growth of lactic acid bacteria and bifidobacteria were achieved by using a sterile skim milk whose titratable acidity ranged from 16 °Th to 18 °Th (Thörner degrees). The production of the above-mentioned milk was performed by using a dried skim milk supplied by Scharlau (Barcelona, Spain), which was then reconstituted to a 10% dry matter level. After that, the milk was autoclaved for 15 min at a temperature of 118 °C and cooled to room temperature. The growth of lactic acid bacteria—MZ<sub>2</sub>f and *Lactobacillus acidophilus* LAB-8 was provided by a liquid medium LAPTg10—broth (CondaLab, Madrid, Spain) (BS 12:2010) with a pH range of 6.6–6.8. LAPTg10—agar medium was obtained with the addition of 15.0 g/dm<sup>3</sup> of agar (Sigma Aldrich, Hamburg, Germany) to the liquid medium of the same composition. For the growth of *B. bifidum* BB-87, transoligosaccharide propionate agar (Merck, Darmstadt, Germany) was used with an MUP selective supplement (Merck, Germany) [30]. The multiplication of microbial organisms in the starter culture ( $MZ_2f$ ), as well as the strains of *Lactobacillus acidophilus* LAB-8 and *B. bifidum* BB-87, was accomplished through an inoculation every 20 days in sterile skim milk (16 °Th to 18 °Th). The microorganisms were stored at a temperature ranging from 4 °C to 6 °C; otherwise, they were stored as stock cultures at a temperature of -20 °C.

#### 2.2.2. Ultrafiltration

The UF experiment was conducted with a replaceable plate and frame membrane module equipped with an UF25-PAN membrane. The scheme of the laboratory equipment is described in [31]. The UF was performed at two different volume-reduction ratios (VRR 2 and VRR 3), a transmembrane pressure of 0.5 MPa, a temperature of 20 °C, and a volumetric-flow rate of 330 dm<sup>3</sup>/h. Ultrafiltration was followed by a pasteurization process performed at 65 °C for 10 min and a cooling process performed at 42 °C  $\pm$  1 °C. The VRR was calculated as follows:

$$VRR = V_0 / V_R \tag{1}$$

where  $V_0$  is the feed liquid's volume, dm<sup>3</sup> and  $V_R$  are the retentate's volume, dm<sup>3</sup>.

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### 2.2.3. Yoghurt Preparation

Three different types of probiotic yoghurts have been developed:

- Sample A1—yoghurt without membrane concentration with MZ<sub>2</sub>f (control sample);
- Sample A2—yoghurt without membrane concentration with MZ<sub>2</sub>f + *Lactobacillus acidophilus* LAB-8 (control sample);
- Sample A3—yoghurt without membrane concentration with MZ<sub>2</sub>f + *B. bifidum* BB-87 (control sample);
- Sample B1—yoghurt from double-concentrated goat's milk with MZ<sub>2</sub>f;
- Sample B2—yoghurt from double-concentrated goat's milk with MZ<sub>2</sub>f + *Lactobacillus acidophilus* LAB-8;
- Sample B3—yoghurt from double-concentrated goat's milk with MZ<sub>2</sub>f + B. bifidum BB-87;
- Sample C1—yoghurt from triple-concentrated goat's milk with MZ<sub>2</sub>f;
- Sample C2—yoghurt from triple-concentrated goat's milk with MZ<sub>2</sub>f + *Lb. acidophilus* LAB-8;
- Sample C3—yoghurt from triple-concentrated goat's milk with MZ<sub>2</sub>f + *B. bifidum* BB-87.

All yoghurts were aseptically prepared in sterile polymeric vessels (100 cm<sup>3</sup>) using 1.5% of the active cells [32,33] of the main starter MZ<sub>2</sub>f or combined with 0.5% of the live cells of *Lb. acidophilus* LAB-8 or *B. bifidum* BB-87. The coagulation of the milk and retentates was performed at 41 °C  $\pm$  1 °C for 2.5 h to 3 h, followed by cooling and storage in refrigerated conditions (4 °C  $\pm$  1 °C) for 30 days.

## 2.2.4. Physicochemical Analyses

The following psychochemical parameters of the goat's milk, UF retentates, permeate, and yoghurts at the beginning of storage were determined:

- Total solid content: by drying at 105 °C [34];
- Total protein content: the determination of the nitrogen content and crude protein was made by using the Kjeldahl method [35];
- Milk fat content: Gerber method using Gerber centrifuge [36];
- Ash content: using a muffle furnace at 550 °C [37];
- Titratable acidity: determined by titration using the Thörner method [38];
- Level of pH: pen-type pH meter (PH-03 [I], Hinotek, Ningbo, China).

The yoghurts' active and titratable acidities were assessed every 10 days within a month.

#### 2.2.5. Microbiological Analyses

The following microbiological parameters were investigated at the beginning of storage:

- Total count of mesophilic aerobic and facultatively anaerobic microorganisms [39];
- Escherichia coli [40];
- Staphylococcus aureus [41];
- *Salmonella* spp. [42];
- Yeasts and fungi [43];
- Lactic acid bacteria: The spread-plate technique was used as a viable counting method for the enumeration of active lactic acid cells on the 1st, 10th, 20th, and 30th days of yoghurt storage. The usage of this method required dilutions of the sample using NaCl—5 g/dm<sup>3</sup> (Sigma Aldrich, Darmstadt, Germany). The plates were placed at 37 °C until the growth of the colonies (3 days);
- Bifidobacteria: The pour-plate method, using a transoligosaccharide propionate agar medium, was used for the determination of bifidobacteria. The methodology for the enumeration of the bifidobacteria was similar to that of the lactic acid bacteria.

#### 2.2.6. Sensory Analyses

A five-point hedonic scale of evaluation (1—dislike extremely; 2—dislike; 3—neither like nor dislike; 4—like; 5—like extremely) was used to evaluate the organoleptic characteristics of the yoghurts. The main organoleptic indices (colour, taste and aroma, appearance of coagulum, consistency at shattering, structure at cutting) and their norms are presented in [29]. This analysis was conducted at the beginning of the storage period by nine panellists—specialists from the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria. Acting as neutralizers between the degustations of each sample, room temperature water and unsalted crackers were used.

#### 2.2.7. Rheological Analysis

A Brookfield RV-DV II + Pro (Brookfield AMETEK, Middleborough, MA, USA) rotating viscometer with an accuracy of 1% was used for the examination of the rheological characteristics of the yoghurts.

The accurate implementation of the rheological study required covering the upper conical area of the spindle with the product; this was an obligatory condition. The cylinder was filled with 10.4 mL of each sample during the examination. The viscometer had a temperature probe (0 °C to 100 °C  $\pm$  0.1 °C) for monitoring the yoghurts' temperatures. An insulating cover was applied to the cylinder's neck before the measurements in order to determine the range of shear rates that would be used. The software Rheocalc 32 was used for presenting the results. Fifteen shear rates ranging from 1.22 s<sup>-1</sup> to 4.89 s<sup>-1</sup> were generated. After keeping the shear rate for one minute, the program notified us of the value of the apparent viscosity and the shear stress.

The results were approximated by the models of Bingham (Equation (2)), Casson (Equation (3)), Ostwald–De Waele (Equation (4)), and Herschel–Bulkley (Equation (5)), where the correlation coefficient  $R^2$  exceeds 95%:

$$\tau = \tau_0 + \eta_B . \gamma, \text{ Pa·s}$$
<sup>(2)</sup>

$$\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta_{ca} \cdot \gamma}, \text{ Pa·s}$$
 (3)

$$\tau = K.\gamma^{n}, Pa \cdot s \tag{4}$$

$$\tau = \tau_0 + K.\gamma^n, \text{ Pa·s}$$
(5)

#### 2.2.8. Statistical Analysis

All analyses were carried out in triplicate. Fisher's exact test was used in this experimental work to compare the obtained averages of the main characteristics of the yoghurts. The significance level used was 0.05.

## 3. Results

#### 3.1. Physicochemical Properties

The dry substance, protein, and fat contents increased during UF (p < 0.05); the highest increase was at VRR 3 (Table 1). It also can be seen that the concentrations of proteins and fats were approximately equal. The increase in ash was small—from 0.87% (goat's milk) to 1.32% (VRR 3). The UF concentration led to an increase (p < 0.05) in titratable acidity—from 17.4 °Th (goat's milk) to 33 °Th (retentate at VRR 3). An inverse relationship was observed concerning pH; it decreased during UF.

Table 1. Physicochemical properties.

Indice	Goat's Milk *	Retentate at VRR 2	Retentate at VRR 3	Permeate	
Dry matter, %	$12.30\pm0.26$ $^{\rm a}$	$16.48\pm0.90^{\text{ b}}$	$23.00\pm0.15^{\text{ c}}$	$5.19\pm0.08~^{\rm d}$	
Protein, %	$3.53\pm0.10~^{\rm a}$	$6.01\pm0.12$ <sup>b</sup>	$9.60\pm0.05$ <sup>c</sup>	$0.25\pm0.09$ <sup>d</sup>	
Fat, %	$3.50\pm0.10$ $^{\rm a}$	$6.00\pm0.10$ <sup>b</sup>	$9.50\pm0.20~^{\rm c}$	-	
Ash, %	$0.87\pm0.02$ <sup>a</sup>	$0.98\pm0.01$ <sup>b</sup>	$1.32\pm0.03$ <sup>c</sup>	$0.61\pm0.02$ d	
Titratable acidity, °Th	$17.40\pm0.90$ a	$25.20 \pm 1.50$ <sup>b</sup>	$33.00\pm1.20~^{\rm c}$	$8.30 \pm 1.00$ <sup>d</sup>	
pH	$6.76\pm0.07~^{a}$	$6.51\pm0.07^{\text{ b}}$	$6.20\pm0.04$ $^{\rm c}$	$6.36\pm0.11~^{\rm d}$	

Note: \* The results for the control samples are from our previous investigation [42]; <sup>a,b,c,d</sup> Means in a row for a specific sample with different letters were significantly different (p < 0.05).

#### 3.2. Specific Microorganisms

The availability of *E. coli*, *S. aureus*, *Salmonella* spp., fungi, and yeasts is important for the production of safe and quality products [44]. The total count of mesophilic aerobic and facultatively anaerobic microorganisms in all samples was less than 1 cfu/cm<sup>3</sup>; that of fungi, yeasts, *E. coli.*, and *S. aureus* - less than 10 cfu/cm<sup>3</sup> while *Salmonella* spp. was not detected in 25 cm<sup>3</sup>. Concerning the condition of the yoghurts during the storage period, the results were as follows: *E. coli*, *S. aureus*, fungi, and yeasts—less than 10 cfu/g, *Salmonella* spp. was not detected in 25 g, and the mesophilic aerobic and facultatively anaerobic microorganisms—less than 10 cfu/g. All specific microorganisms were according to the requirements described in the standards in Section 2.2.5., Microbiological Analyses.

## 3.3. Chemical Compositions of the Yoghurts

The chemical compositions of the yoghurts produced by UF are shown in Table 2. The yoghurts with different starters did not show a significant difference (p > 0.05). As expected, the dry substance, protein, and fat contents of the yoghurts increased when UF was used. The results are in accordance with those obtained in Table 1.

Sample	Dry Matter, %	Total Protein, %	Fat, %
A1 *	$12.27\pm0.16$ $^{\rm a}$	$3.59\pm0.10$ <sup>a</sup>	$3.50\pm0.10$ a
A2 *	$11.92\pm0.24$ a	$3.64\pm0.12$ a	$3.40\pm0.12$ a
A3 *	$12.08\pm0.27$ <sup>a</sup>	$3.50\pm0.08$ <sup>a</sup>	$3.50\pm0.14$ $^{\mathrm{a}}$
B1	$16.68\pm1.49~^{\mathrm{b}}$	$5.95\pm0.12$ $^{\mathrm{b}}$	$5.20\pm0.14$ <sup>b</sup>
B2	$16.53\pm0.49$ <sup>b</sup>	$6.20 \pm 0.10^{ m b}$	$5.40\pm0.12$ b
B3	$16.39\pm0.32$ <sup>b</sup>	$5.90 \pm 0.20$ <sup>b</sup>	$5.50 \pm 0.20$ <sup>b</sup>
C1	$23.12\pm0.13~^{\rm c}$	$10.00\pm0.14~^{ m c}$	$9.70\pm0.16$ <sup>c</sup>
C2	$23.02\pm0.14~^{\rm c}$	$10.00\pm0.12~^{\rm c}$	$9.80\pm0.18$ <sup>c</sup>
C3	$23.22\pm0.05~^{\rm c}$	$10.20\pm0.10$ $^{\rm c}$	$9.80\pm0.22$ <sup>c</sup>

Table 2. Main chemical compositions of the yoghurts.

Note: \* The results for the control samples are from our previous investigation [29]; a,b,c Means in a column for samples A, B, and C with different letters were significantly different (p < 0.05).

## 3.4. Microbiological Growth in Yoghurts during Storage

Figure 1 presents the microbiological growth that occurred in the yoghurts during storage. For the whole storage period, the rise in the volume-reduction ratio positively affected the total number of lactic acid bacteria (p < 0.05); this tendency was valid for all three starters studied. Despite the reduction of the total viable acid bacteria during the storage period, their count remained high—above  $6 \times 10^8$  cfu/g.



**Figure 1.** Microbiological growth in yoghurts during storage: (a) sample A1; (b) sample B1; (c) sample C1; (d) sample A2; (e) sample B2; (f) sample C2; (g) sample A3; (h) sample B3; (i) sample C3. Note: <sup>a,b,c</sup> Means for samples A, B, and C with different letters were significantly different (p < 0.05).

## 3.5. pH and Titratable Acidity during Storage

The UF concentration caused a rise in the titratable acidity of the yoghurts at p < 0.05 (Figure 2). During all storage periods, an increase in the titratable acidity and a decrease in the active acidity (pH) were noticed. The highest value of titratable acidity was observed for the yoghurt with starter MZ<sub>2</sub>f + *Lb. acidophilus* LAB-8, followed by that of the yoghurts with starter MZ<sub>2</sub>f + *B. bifidum* BB-87 and starter MZ<sub>2</sub>f. A comparison of pHs of the yoghurts with different starters showed that sample A was characterized with the highest values of pH, then samples B and C. The active acidities of the yoghurts from retentate at VRR 2 and VRR 3 were statistically equal (p > 0.05).



**Figure 2.** Titratable acidity and pH during the storage of yoghurts with starters: (a)  $MZ_2f$ ; (b)  $MZ_2f + Lb.$  *acidophilus* LAB-8; (c)  $MZ_2f + B.$  *bifidum* BB-87. Note: <sup>a,b,c</sup> Means for samples A, B, and C with different letters were significantly different (p < 0.05).

## 3.6. Organoleptic Characteristics of Yoghurts

The results for the organoleptic characteristics of the yoghurts are shown in Figure 3. Comparing the starters in the yoghurts, it could be seen that *Lb. acidophilus* LAB-8 and *B. bifidum* BB-87 had higher scores than  $MZ_2f$ . Yoghurts produced with *Lb. acidophilus* LAB-8 and *B. bifidum* BB-87 had relatively equal scores. Ultrafiltration had a positive effect on the organoleptic properties of the yoghurts with all three starters; the highest score was observed for sample C, then for sample B and sample A, as we established in our prevoius work [29] with another type of membrane.



**Figure 3.** Organoleptic properties of probiotic Bulgarian yoghurts with starters: (a)  $MZ_2f$ ; (b)  $MZ_2f + Lb$ . *acidophilus* LAB-8; (c)  $MZ_2f + B$ . *bifidum* BB-87.

## 3.7. Rheological Properties of Yoghurts

The rheological behaviours of the yoghurts with the three studied starters are shown in Figure 4. The rheograms showed that all yoghurts exhibited Bingham plastic flow behaviours. The flow curves were located as being the lowest for sample A, followed by samples B and C for all starters used. The shear stress increased with the rise in the level of the UF concentration.



**Figure 4.** Rheograms of probiotic Bulgarian probiotic yoghurts with starters: (a)  $MZ_2f$ ; (b)  $MZ_2f + Lb$ . *acidophilus* LAB-8; (c)  $MZ_2f + B$ . *bifidum* BB-87.

The parameters of the Herschel–Bulkley, Bingham, Casson, and Ostwald–de Waele models are shown in Table 3. The change of yield stress  $\tau_0$  is of great significance to the structure of the product. All models had  $R^2 > 95\%$ , which means that they were suitable for describing the rheological behaviours of the yoghurts.

Sample	Bingham		Casson		Ostwald-de-Waele		Herschel-Bulkley		
	τ <sub>0</sub> , Pa	η <sub>B</sub> , Pa∙s	τ <sub>0</sub> , Pa	η <sub>ca</sub> , Pa∙s	K, Pa∙s <sup>n</sup>	n	τ <sub>0</sub> , Pa	K, Pa∙s <sup>n</sup>	n
A1	0.67	0.031	0.28	0.019	0.229	0.58	0.07	0.199	0.61
B1	4.02	0.032	3.23	0.065	2.623	0.19	0.98	1.793	0.24
C1	2.04	0.886	0.95	0.483	2.672	0.53	1.49	1.33	0.81
A2	0.28	0.035	0.06	0.028	0.096	0.78	0.07	0.077	0.83
B2	3.98	0.036	3.16	0.078	2.562	0.21	3.89	0.526	0.92
C2	5.10	0.393	4.22	0.07	5.263	0.17	4.5	0.903	0.64
A3	0.54	0.033	0.19	0.022	0.177	0.64	0.04	0.162	0.66
B3	4.27	0.033	3.49	0.006	2.873	0.18	3.96	0.103	0.75
C3	5.98	0.377	5.10	0.058	6.111	0.15	3.29	2.861	0.28

Table 3. Rheological models for probiotic Bulgarian yoghurts.

The dependence of dynamic viscosity on the shear rates of the yoghurts with the three probiotic starters is presented in Figure 5. For all yoghurts, there was a rise in dynamic viscosity when the UF concentration level increased. The highest value of dynamic viscosity was obtained at VRR 3 for all starters studied.



**Figure 5.** Viscosity curves for probiotic Bulgarian yoghurts with starters: (**a**)  $MZ_2f$ ; (**b**)  $MZ_2f + Lb$ . *acidophilus* LAB-8; (**c**)  $MZ_2f + B$ . *bifidum* BB-87.

#### 4. Discussion

Table 1 shows the rise in the dry substances, fats, and proteins during UF. This could be explained by the passage of the water and low molecular weight substances (such as lactose, salts, and some vitamins) through the membrane [45]. Tamime et al., in 1991 [46], established that UF caused a slightly more pronounced increase in fats than in proteins. Ultrafiltration and constant-volume diafiltration were used for obtaining the milk protein concentrates of protein contents > 80% on a dry basis [47]. The ultrafiltration of skim milk was used to partially remove water, lactose, peptides, and other solutes. Gavazzi-April et al., in 2018 [48], proved that the molecular weight cut-off of the membrane affects the retention of the main components—an UF membrane of a molecular weight cut-off of 10 kDa showed better protein retention in comparison to that of a 50 kDa cut-off. Domagala, in 2012 [49], found a significant increase in the acidity of the retentates; however, the pH stayed mostly the same after a two-fold level of an UF concentration of goat's milk with a 30 kDa membrane. Higher acidity in retentates could be explained by the change in the buffering capacity, which depends on the milk composition, including protein content [50].

The values of specific microorganisms showed that the yoghurts obtained were safe for consumption. The availability of *E. coli*, *S. aureus*, *Salmonella* spp., fungi, and yeasts, is important for the production of safe and quality products because these microorganisms cause gastrointestinal diseases in human bodies [44].

As expected, the dry matter, fats, and proteins in the yoghurts increased with the rise in the concentration level during UF (Table 2). In the past few years, consumer interest in high-protein yoghurt has increased because of its better taste and texture. There is also more scientific data on the health benefits of dairy proteins. Different processing techniques, including membrane processes, affect the composition, rheology, structure, and sensory characteristics of yoghurts [51].

Upon comparing the microbiological properties of the yoghurts with different starters, it could be concluded that the probiotic yoghurts with the highest counts of viable cells

were those with  $MZ_2f + B$ . *bifidum* BB-87, followed by those with  $MZ_2f + Lb$ . *acidophilus* LAB-8 and those with  $MZ_2f$  alone (Figure 1). According to Ordonez et al., in 2007 [52], UF could be applied to increase the protein content, which led to higher concentrations of *Lb. acidophilus*, *B. bifidum*, *S. thermophilus*, and *Lb. bulgaricus*. According to Moineau-Jean et al., in 2019 [53], the application of a 50 kDa UF membrane to obtain a retentate with a 10.6% protein content resulted in a rise in the concentrations of *Lb. helveticus* R0052 and *S. thermophilus*. The application of UF increased the peptides and amino acids and, thus, improved the survival of probiotic strains [54]. Ultrafiltration causes a concentration of caseins that leads to a rise in milk's buffering capacity and a reduction in pH. This leads to a better growth of lactic acid bacteria in UF retentates during fermentation compared to goat's milk without UF.

The UF increased the protein content of yoghurts and, thus, favoured the growth of lactic acid bacteria. This provoked a rise in the titratable acidity of yoghurts obtained by UF [55,56]. The highest value of pH was established for MZ<sub>2</sub>f at the beginning of storage— 5.05. The lowest value was obtained for *Lb. acidophilus* LAB-8 at the end of the storage period (Figure 2)—4.08. This was probably due to the lactic and succinic acid produced by *Lb. acidophilus* LAB-8 [57]. Figure 2 indicates that UF caused a decrease in pH for all starters studied. The concentration of casein fractions during UF increased the enzymes' activity and, thus, improved the sugar metabolism of *Lb. bulgaricus and S. thermophilus*, as well as lactic acid production [58]. The concentration created by UF led to a rise in the number of viable lactic acid bacteria cells in comparison to the number found in plain yoghurt [59]. Therefore, the higher count of lactic acid bacteria in yoghurts obtained by ultrafiltration provokes higher acid production, leading to higher titratable acidity during storage [55].

Figure 3 shows that sample C had the highest score, followed by sample B and sample A, for all three starters. The last indicates that the use of UF improves the organoleptic properties of yoghurts. According to Jørgensen et al., in 2019 [51], membrane processes change yoghurt's composition, structure, and, thus, the sensory properties of its products. It could be seen that the UF concentration being at a low level (VRR 2 and 3) enhanced the organoleptic scores of yoghurts; however, a higher VRR would probably embarrass the coagulation of milk and make the final product have a high fat content.

Skriver et al., in 1993 [60], studied the effect of the dry matter content, temperature during fermentation, and composition of bacteria cultures on the rheological behaviour of stirred yoghurt. A pseudoplastic behaviour was valid for some yoghurts; however, some of the specimens displayed Bingham plastic behaviour. The profile of the flow curves depended on the applied manufacturing conditions. Our results showed that when comparing the three starters, it could be seen that  $MZ_2f + B$ . bifidum BB-87 yoghurts had the highest values of shear stress, followed by  $MZ_2f + Lb$ . acidophilus LAB-8 yoghurts and  $MZ_2$  f yoghurts (Figure 4). Prasanna et al., in 2013 [61], established that the use of starters in producing higher concentrations of exopolysaccharides leads to an improvement in the physicochemical and rheological properties of food products. Girard et al., in 2007 [62], found that using exopolysaccharide-producing strains of lactic acid bacteria results in a dense structure regarding the products because the exopolysaccharide structures cause linkages between the proteins in the milk and dairy products. This also led to an increase in the strength and viscosity of the resulting products. The highest shear stress was observed when an UF concentration at VRR 3 and B. bifidum BB-87 was used. According to Krzeminski et al., in 2011 [63], the shear stress of yoghurt was increased by fat and protein levels. Miocinovic et al., in 2016 [64], proved that the addition of milk proteins to goat's milk improved its textural, rheological, and sensory characteristics. Meena et al., in 2015 [45], found a positive correlation between firmness, shear stress, and protein content.

The rise in the UF concentration level increased the yield stress of yoghurts obtained with three probiotic starters (Table 3). According to Damin et al., in 2008 [65], the increase in the protein content increased the yield stress. It led to protein-chain extension and increased the elasticity and stability of the obtained products.

The dynamic viscosity of yoghurts increased when UF was applied (Figure 5). Shaker et al., in 2000 [66], investigated the influence of fats on the rheological characteristics of yoghurt during the coagulation process and found that an increase in the milk's fat content led to a significant increase in the viscosity. According to Delgado et al., in 2017 [67], the use of UF led to the higher dry matter content of milk, which improves the rheological and texture characteristics of dairy products. There was an increase in the viscosity, hardness, and cohesiveness of the coagulum; all of these factors affected the final quality of the product. The highest values of dynamic viscosity were established in milk with the starter  $MZ_2f + B. bifidum$  BB-87, followed by milk with  $MZ_2f + Lb.$  acidophilus LAB-8 and milk with  $MZ_2f$ .

#### 5. Conclusions

- The dry matter, fats, proteins, count of lactic acid bacteria, titratable acidity, and dynamic viscosity increased and the pH decreased with the rise of the volume-reduction ratio during ultrafiltration;
- The ultrafiltration concentration improved the microbiological, physicochemical, organoleptic, and rheological properties of yoghurts;
- All yoghurts exhibited Bingham plastic flow behaviour;
- Probiotic yoghurts with the highest dry matter contents, protein contents, fat contents, counts of viable lactic acid cells, viscosity, and score for organoleptic properties were established for MZ<sub>2</sub>f + *B. bifidum* BB-87 and VRR 3.
- Therefore, this volume reduction ratio and this starter can be used for the production
  of yoghurts using concentrated goat's milk via ultrafiltration to improve the quality
  and functionality of the final product.

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