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# PROBIOTIC YOGHURTS FROM ULTRAFILTERED CONCENTRATED MILK

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**Abstract**: In this paper, yoghurts from ultrafiltered concentrated whole milk with a volume reduction ratio 0, 2 and 3 with three different probiotic yoghurt starters were obtained. Their physiological, microbiological and rheological properties were examined. The concentrated probiotic yoghurts had high concentration of viable cells of the probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* (over $10^{10}$cfu/cm$^3$). For the preparation of concentrated probiotic yoghurts the most appropriate volume reduction ratio was 2. The probiotic yoghurts with starters $MZ_2$ and 1CM had the best structure. The concentrated probiotic yoghurts with all starters are functional foods.

**Keywords**: ultrafiltration, concentrated probiotic yoghurt, rheology of probiotic yoghurts, probiotic starters

## INTRODUCTION

Membranes have been major tools in the purification, fractionation and concentration of food products for more than 25 years. These processes are widely used in food industry (Daufin *et al*., 2001; Shi *et al*., 2014; Wang *et al*., 2008).

Ultrafiltration (UF) is a pressure-driven process using a semi-permeable membrane to separate macromolecules or colloids from liquids and is based

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on a simple sieving mechanism. Solute dimensions in relation to the membranes pore size distribution determine whether or not a solute molecule can pass through the membrane (De Bruijn *et al*., 2005; Van Reis *et al*., 2007). Membrane processes are used in the dairy industry to produce concentrated milk (skim or whole), which is added for normalization of milk to produce yoghurts, yoghurt beverages and soft cheese. These products have better texture, viscosity, taste and viability of cells compared to the traditional method for their production. Concentration by membrane filtration instead of thermal evaporation does not employ severe heating and preserves the natural taste of food products and the nutritional value of heat-sensitive components. The ultrafiltration of milk has little denaturing effect on proteins, vitamins and other biologically active components in milk (Le *et al*., 2014).

For the manufacture of concentrated yoghurt can be employed different ultrafiltration systems. In the production of concentrated yoghurt from ultrafiltration retentate, the milk was concentrated by ultrafiltration up to the desired TS level in the final product (~23 – 25 $g.100\ g^{-1}$) (Veinoglou *et al*., 1978; El-Samragy & Zall, 1988; Hofi, 1988; Tamime *et al*., 1989a, b; Ozer *et al*., 1997a, b, 1998a, b, 1999 a, b, c). The ultrafiltration module was operated at 0.7 – 0.8MPa inlet pressure and at 35 - 40°C. The protein and fat levels of the concentrated yoghurt were adjusted to 8.5 $g.100g^{-1}$ and 9.5 $g.100g^{-1}$, respectively, followed by heat treatment at 65°C for 10 minutes. The heat-treated retentate was further processed in a similar manner to the production of natural set yoghurt (Tamime *et al*., 1989b, 1991a, b, c, 2001; Ozer *et al*., 1997a, 1998a, 1999a; Le *et al*., 2014).

The purpose of the present work was to obtain probiotic yoghurts from ultrafiltered concentrated whole milk with different viscosity and to study their rheological properties.

## MATERIALS AND METHODS

Three probiotic yoghurt starters $MZ_2$ (probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* NBIMCC 3708), ZD (probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* NBIMCC 3706), 1CM (probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* NBIMCC 3708), provided by the Department of "Microbiology" at the University of Food Technologies, Bulgaria, were used for the production of the yoghurts.

The membrane filtration experiments were carried out on laboratory equipment with a replaceable plate and frame membrane module fitted with a UF25-PAN polyacrylnitrilic ultrafiltration membrane ("Ekofilter" Ltd, Bulgaria) with 25 kDa molecular weight cut-off (Fig. 1). This equipment was fitted with a plate and frame membrane module with membrane surface area of 1,250 $cm^2$; a threeplunger high-pressure pump (max 15 MPa) with a capacity of 330 $dm^3/h$; a pipeline system with two manometers (0-15 MPa) for measuring the inlet and outlet pressure; and a special working pressure regulating valve (Dushkova and Dinkov, 2009).

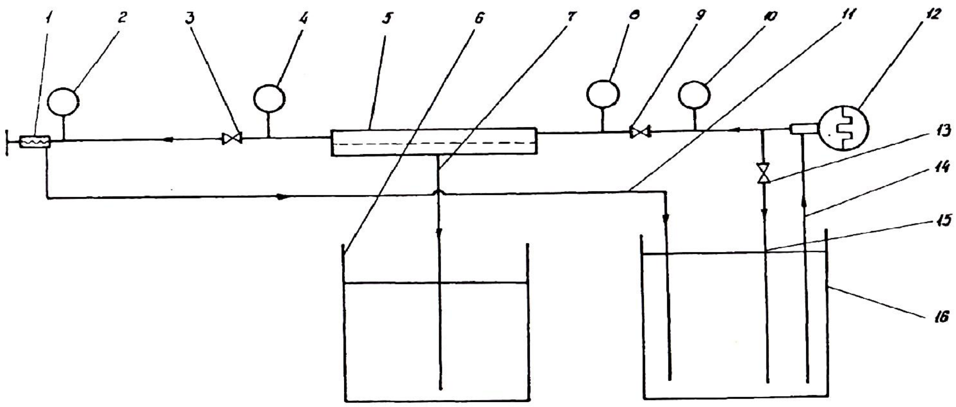


Figure 1. Scheme of laboratory equipment with a replaceable plate and frame membrane module
1 - valve; 2 - manometer (0–5 MPa); 3 - valve; 4 - manometer (0–0.6 MPa); 5 - replaceable plate and frame membrane module; 6 - tank; 7 - pipeline; 8 - manometer (0–0.8 MPa); 9 - valve; 10 - manometer (0–15 MPa); 11 - pipeline; 12 - pump; 13 - valve; 14 - pipeline; 15 - pipeline; 16 - tank.

The experiments were carried out under the following operating conditions: transmembrane pressure 0,5 MPa, volume reduction ratio (VRR) – 2 and 3. All experiments were carried out at a temperature of 50°C and input volumetric flow rate of 330 $dm^3/h$.

*Rheological examination*
The rheological tests were carried out with a rotary viscometer Rheotest-2 with a cylinder S1 and sample volume 25 ml. Samples were homogenized before measuring. The parameters of the rheological model were calculated using software “Curve Expert Professional” (Paskov *et al.*, 2010).

*Determination of the concentration of viable cells*
Appropriate serial dilutions in saline solution of the obtained yogurts were prepared and the spread plate method was applied. 0.1 $cm^3$ of the last three dilutions was used to inoculate LAPTg10-agar (for the enumeration of lactobacilli and streptococci) or the respective elective solid medium (for the enumeration of the specific microorganisms). The inoculated Petri dishes were incubated for 3 days at optimum temperature for the growth of the

respective microorganisms until the appearance of countable single colonies. The count of the colonies was then used to estimate the number of bacteria in the original sample.

*Determination of the titratable acidity*

Ten $cm^3$ of each sample were mixed with 20 $cm^3$ of distilled water. The titratable acidity was determined by titration of each sample with 0.1 N NaOH using phenolphthalein as an indicator until the appearance of a pale pink colour persisting over 1 min. One Torner degree (°T) corresponds to 1 $cm^3$ 0.1 N NaOH, needed for the neutralisation of an equivalent amount of organic acid, contained in 100 $cm^3$ of the cultural medium (Denkova, 2005).

*Dry matter content*

In all experiments samples of the initial milk prior to ultrafiltration and of the retentates and permeate after concentration to VRR = 2 and VRR = 3 were taken for analysis to determine the dry matter content according to ISO 6731:2010.

*Preparation of yoghurts*

In the production of ultrafiltered concentrated yoghurt, the natural warm whole cow‘s milk was ultrafiltered until the TS level reaches 23 – 25 g/100g. The ultrafiltered concentrated whole milk was pasteurized at 65 °C for 10 min and cooled to ~43 °C. The milk was then inoculated with yoghurt starters and fermented at 41±1 °C for 2,5 – 3 hours.

## RESULTS AND DISCUSSIONS

Yoghurts from double- and triple-concentrated whole pasteurized cow’s milk were obtained using three probiotic yoghurt starters - $MZ_2$, ZD, 1CM. Yoghurts from homogenized cow's milk with the same starters were prepared as blanks. The microbiological status of the whole milk before and after two- and three-fold concentration was determined. The results of these studies are shown in Table 1.

A comparative study on the concentration of viable cells, titratable acidity, the ratio of *Lactobacillus delbrueckii* ssp. *bulgaricus* to *Streptococcus thermophilus* cells and the dry matter content of the yoghurts from two- or three-fold ultrafiltered concentrated whole milk with the three probiotic starters was conducted. Data from these experimental studies are shown in Table 2 for the double-concentrated milk and Table 3 for the triple-concentrated milk.

Table 1. Microbiological analysis of the whole and concentrated milk.

| Sample | TBA, cfu/cm$^3$ | Specific microorganisms, cfu/cm$^3$ | | | Molds and yeasts, cfu/cm$^3$ |
|---|---|---|---|---|---|
| | | *E.coli* | *St.aureus* | *Salmonella* sp. | |
| **Blank$_{M32}$** | < 10 | < 10 | Not found | Not found | < 10 |
| **MZ$_2$ – 2UF** | < 10 | < 10 | Not found | Not found | < 10 |
| **MZ$_2$ – 3UF** | < 10 | < 10 | Not found | Not found | < 10 |

Table 2. Comparison of probiotic yogurts obtained from two-fold ultrafiltered concentrated milk

| Sample | DMC, % | TK, °T | N, cfu/cm$^3$ | Ratio *L.d.*ssp.*bulgaricus* : *Str. thermophilus* |
|---|---|---|---|---|
| **Starter ZD** | 20,82 | 77 | 1,4x$10^{11}$ | 1 : 6 |
| **Starter ZD – Blank** | 13,67 | 62 | 5x$10^{10}$ | 4 : 1 |
| **Starter MZ$_2$** | 20,15 | 89 | 3,2x$10^{12}$ | 1 : 1 |
| **Starter MZ$_2$- Blank** | 13,64 | 77 | 2,1x$10^{11}$ | 1 : 10 |
| **Starter 1CM** | 20,90 | 70 | 5,3x$10^{12}$ | 1 : 1 |
| **Starter 1CM – Blank** | 14,07 | 67 | 6,0x$10^{11}$ | 1 : 1,2 |

The results showed that probiotic yoghurts, prepared from ultrafiltered concentrated whole milk with concentration factor 2 and 3 were characterized not only by high dry matter content, but also by higher titratable acidity (Table 2 and Table 3).

Table 3. Comparison of probiotic yogurts obtained from three-fold ultrafiltered concentrated milk

| Sample | DM, % | TK, °T | N, cfu/cm$^3$ | Ratio *L.d.*ssp.*bulgaricus* : *Str. thermophilus* |
|---|---|---|---|---|
| **Starter ZD** | 26,97 | 82,0 | 1,3x$10^{12}$ | 1 : 1,5 |
| **Starter ZD – Blank** | 13,67 | 62,0 | 5x$10^{10}$ | 4 : 1 |
| **Starter MZ$_2$** | 27,20 | 82,0 | 4,5x$10^{12}$ | 1 : 1 |
| **Starter MZ$_2$- Blank** | 13,64 | 77,0 | 4x$10^{11}$ | 1 : 1 |
| **Starter 1CM** | 26,91 | 70,0 | 6,0x$10^{11}$ | 1 : 5 |
| **Starter 1CM – Blank** | 14,07 | 67,0 | 6,0x$10^{11}$ | 1 : 1,2 |

It is noteworthy that the concentrated milks stimulated slightly the growth of *Streptococcus thermophilus* from the starter. All yoghurts, regardless of the milk they had been made of, had significant amounts of viable cells (over $10^{10}$cfu/g). The studies showed that yogurts from triple concentrated milk were too dense, had an almost cheesy character with enhanced oil nuance and reduced yoghurt flavour. Better quality probiotic yoghurts were prepared from double concentrated milk.

The rheology of the probiotic yoghurts produced from concentrated ultrafiltered whole cow's milk with volume reduction ratio 2 and 3 was examined. The resulting data is shown on Fig. 2, Fig. 3 and Fig. 4.

The curves were curvilinear and had interface tension of displacement. Thus the examined probiotic yoghurts were non-ideal plastic bodies. Therefore to determine the rheological behavior the equation of Herschel - Bulkley for non-ideal plastic bodies was applied:

$$\tau = \tau_0 + K.\gamma^n \qquad (1)$$

where: $\gamma$ – shear rate, $s^{-1}$; $\tau$ - shear stress, Pa; $\tau_0$ - yield stress, Pa; K - consistency index, $Pa.s^n$ (shows the texture of the product); n – flow index indicating the degree of deviation from Newton's law.

The dynamic viscosity of the three probiotic yoghurts with the three starters was determined by the following equation:

$$\eta = \frac{\tau_0}{\gamma} + K.\gamma^{n-1} \qquad (2)$$

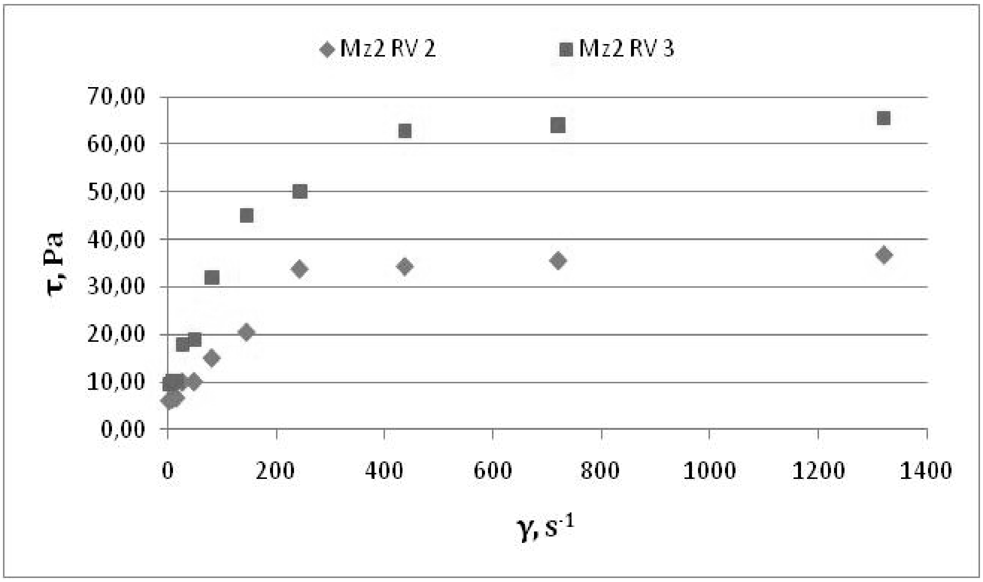


Figure 2. Flow curves of probiotic yoghurts from ultrafiltered concentrated whole cow's milk with Starter $MZ_2$

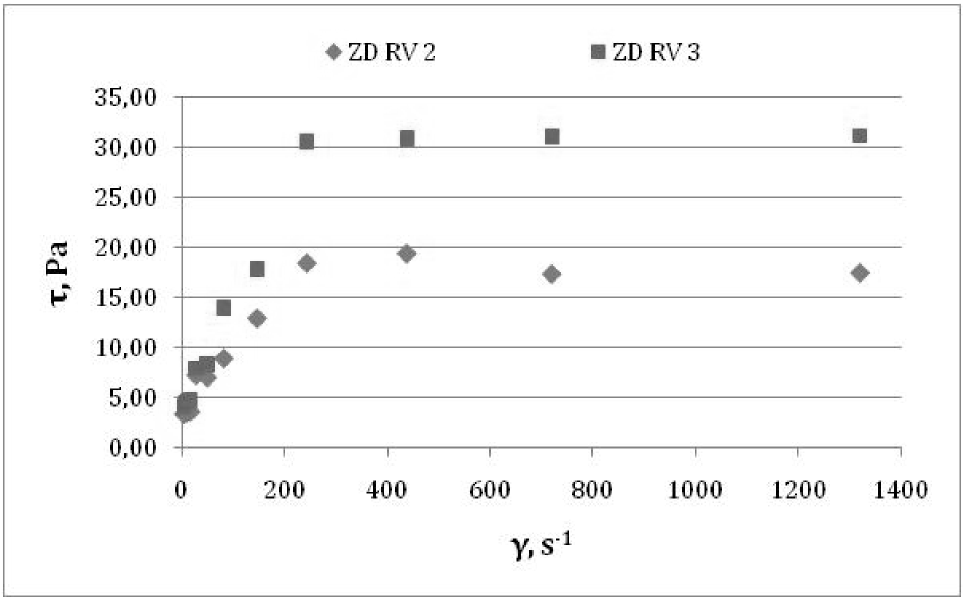


Figure 3. Flow curves of probiotic yoghurts from ultrafiltered concentrated whole cow's milk with Starter ZD

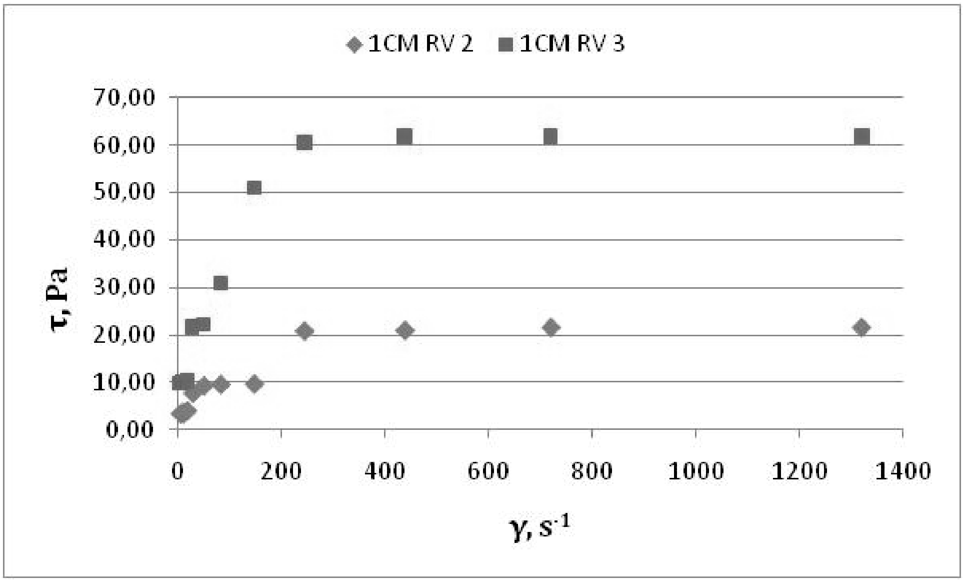


Figure 4. Flow curves of probiotic yoghurts from ultrafiltered concentrated whole cow's milk with Starter 1CM

The parameters of the model of Herschel - Bulkley for the yoghurts with probiotic starters are presented in Table 4.

Table 4. Parameters of the model of Herschel - Bulkley for the yoghurts with probiotic starters $MZ_2$, 1CM and ZD

| Starter | $\tau_0$ | K | N | $R^2$ |
|---|---|---|---|---|
| **$MZ_2$ 2UF** | 5,96 | 2,94 | 0,3788 | 0,9345 |
| **$MZ_2$ 3UF** | 9,53 | 4,64 | 0,4025 | 0,9380 |
| **ZD 2UF** | 3,50 | 1,94 | 0,4227 | 0,9108 |
| **ZD 3UF** | 4,16 | 2,10 | 0,3493 | 0,9430 |
| **1CM 2UF** | 6,50 | 1,88 | 0,3718 | 0,9238 |
| **1CM 3UF** | 9,87 | 5,05 | 0,3956 | 0,9094 |

The obtained results showed that the flow index ***n*** was not affected by the degree of concentration. The value of ***n*** was nearly equal for the probiotic yoghurts obtained from two- or three-fold concentrated milk with the probiotic starter 1CM. The consistency constant K of the probiotic yoghurts with the three starters increased with the increase in the dry matter content, the degree of concentration increased as well. The change in the static interface tension of flow which characterizes the texture of the product increased with the increase in the degree of concentration for all three starters. This showed improvement of the texture of the obtained probiotic yoghurts and the highest value of the static interface tension of flow was observed in yoghurt obtained from triple-ultrafiltered concentrated milk with probiotic Starters $MZ_2$ and 1CM (5.92 and 9.87, respectively).

Table 5. Equations for calculation of dynamic viscosity of probiotic yoghurts from ultrafiltered concentrated milk

| Starter / ultrafiltered concentrated milk | Dynamic viscosity |
|---|---|
| **$MZ_2$ 2UF** | $\eta = \frac{5,96}{\gamma} + 2,42\gamma^{-0,6366}$ |
| **$MZ_2$ 3UF** | $\eta = \frac{9,53}{\gamma} + 4,50\gamma^{-0,6086}$ |
| **ZD 2UF** | $\eta = \frac{3,5}{\gamma} + 1,90\gamma^{-0,6662}$ |
| **ZD 3UF** | $\eta = \frac{4,16}{\gamma} + 1,95\gamma^{-0,580}$ |
| **1CM 2UF** | $\eta = \frac{3,44}{\gamma} + 1,88\gamma^{0,6202}$ |
| **1CM 3UF** | $\eta = \frac{8,3}{\gamma} + 4,94\gamma^{-0,6223}$ |

Probiotic yoghurts obtained with Starter ZD had lower value of the static interface tension of displacement, which indicated low degree of texturing in comparison with the yoghurts with the other starters. The equations for calculating the dynamic viscosity of the yoghurts is given in Table 5. The results of these studies, shown on Fig. 5, Fig. 6 and Fig. 7 showed that with the increase in the dry matter content there was an increase in the viscosity as well. Fig. 5 and Fig. 7 show that the highest viscosity was observed in yoghurt obtained with ultrafiltered triple concentrated milk with the starters $MZ_2$ and 1CM. The dynamic viscosity of the yoghurts with starters 1CM and ZD from two-fold concentrated milk were comparable as seen from Figure 6 and Fig. 7. The same tendency was observed in yoghurts from three-fold concentrated milk prepared with starters $MZ_2$ and 1CM. The highest value for the dynamic viscosity was determined in yoghurt from double-ultrafiltered concentrated milk with starter $MZ_2$. Therefore, the most appropriate starters for the preparation of yoghurts from ultrafiltered concentrated milk with volume reduction ratio 2 were probiotic starters $MZ_2$ and 1CM, which had the highest values of the consistency constant (2,94 Pa.s and 2,10 Pa.s) and of the dynamic viscosity. This was confirmed by the ratio of *Lactobacillus delbrueckii* ssp. *bulgaricus* to *Streptococcus thermophilus* cells in the probiotic yoghurts obtained with ultrafiltered concentrated milk with volume reduction ratio 2, given in Table 2.

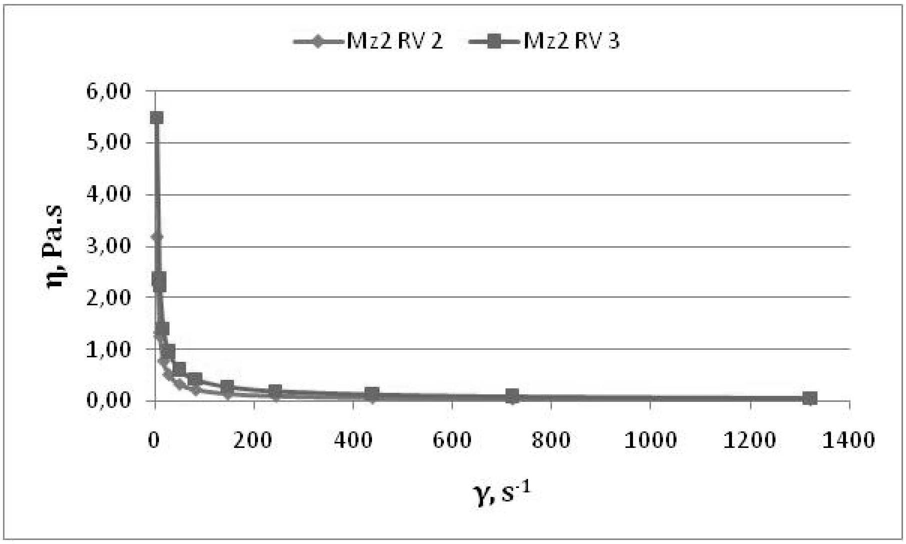


Figure 5. Relation between the dynamic viscosity and the velocity gradient in yoghurts from double and triple ultrafiltered concentrated milk with probiotic starter $MZ_2$

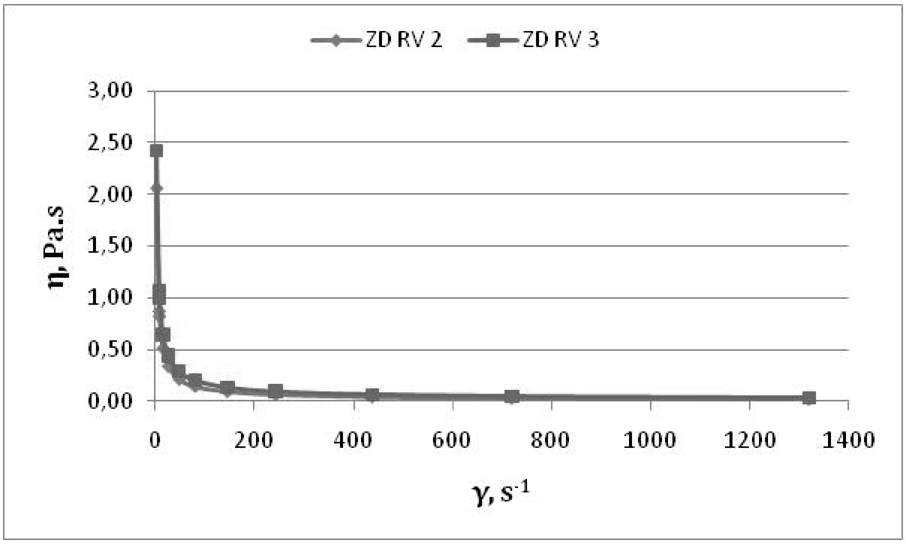

Figure 6. Relation between the dynamic viscosity and the velocity gradient in yoghurts from double and triple ultrafiltered concentrated milk with probiotic starter ZD

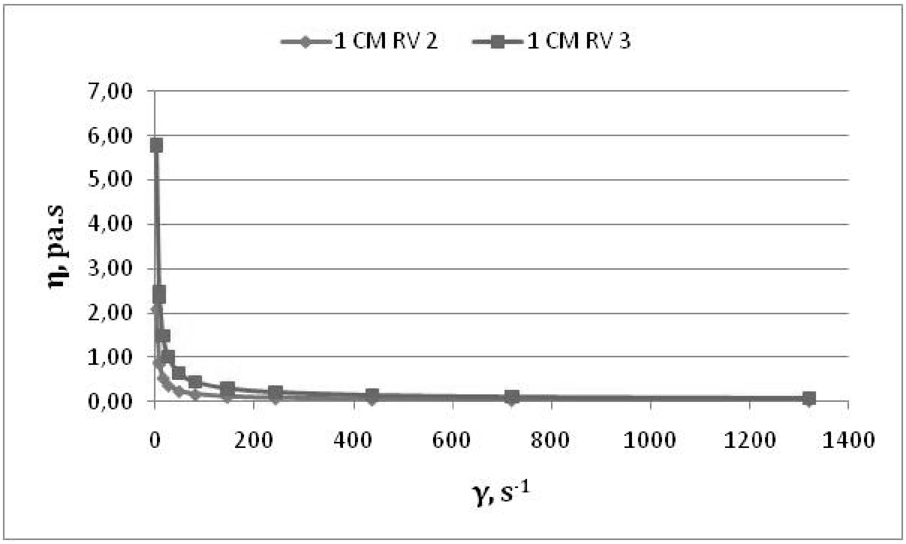

Figure 7. Relation between the dynamic viscosity and the velocity gradient in yoghurts from double and triple ultrafiltered concentrated milk with probiotic starter 1CM

## CONCLUSIONS

The conducted studies on the preparation of probiotic yoghurts from ultrafiltered concentrated milk demonstrated that the whole cow's milk subjected to ultrafiltration concentration with volume reduction ratio 2 was suitable for the preparation of fermented probiotic milk products with higher density and better rheological parameters, i.e. products with better texture. The type of the starter did not influence the rheological parameters significantly. The higher density favoured the growth of *Streptococcus thermophilus*. All three concentrated yoghurts with starters $MZ_2$, 1CM and ZD had significant amount of the respective probiotic strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* (over $10^9$cfu/$cm^3$), which makes them a suitable food for all age groups.

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