RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2014, **4**, 55503

Quality attributes of a fermented whey-based beverage enriched with milk and a probiotic strain

Maja Lj. Bulatović,*[a] Tanja Ž. Krunić,[b] Maja S. Vukašinović-Sekulić,[a] Danica B. Zarić[c] and Marica B. Rakin[a]

Received 19th August 2014
Accepted 22nd October 2014

DOI: 10.1039/c4ra08905g

www.rsc.org/advances

Beverages produced by fermentation of whey have significantly lower viscosity, milder flavour and less viability of probiotic microorganisms compared to those obtained by fermentation of milk. Therefore, it is necessary to choose an adequate combination of cultures and supplements that can enhance these characteristics of the final product. The main objectives of this paper were to study the influence of milk and additional probiotic strain *Lactobacillus rhamnosus* ATCC 7469 on the quality attributes of a fermented whey-based beverage containing commercial ABY-6 starter culture. To formulate a beverage that meets the required criteria for probiotics, supplementation of whey with 30% milk and its fermentation by ABY-6 co-cultured with *L. rhamnosus* is advisable. The obtained whey-based beverage has desirable texture and sensory quality attributes similar to traditional products and meets consumers' demands. The beverage contains 7.49 log(CFU mL^{-1}) probiotic bacteria, expresses antioxidant activity of 45.1%, satisfactory sensory characteristics and has a shelf life of at least 20 days.

1. Introduction

Compared to various types of yoghurt and other fermented dairy products, whey is one of the least frequently consumed dairy products around the world. Its poor sensory attributes have a large negative effect on consumer acceptability. Consequently, whey is commonly used as a supplement, in the form of whey powder or whey protein concentrate.[1] Expensive processing procedures lead to the fact that Serbia exploits only 12% of whey, in contrast to developed countries that exploit 95% of this by-product.[2] The fermentation of whey by commercial starter cultures, designed for yoghurt production, could be an alternative to increase the sensory quality of whey. On the other hand, well known health benefits[3] of raw whey can be significantly improved by its fermentation. There is a large number of scientific reports that provide evidence of the health benefits of microorganisms including their production of antioxidants.[4] Thus, application of starters that produce exopolysaccharides, antioxidants or posses probiotic properties, can significantly improve whey quality due to their positive influence on the immune system as well as on gastrointestinal health. These benefits could be the key point for increasing whey exploitation by its integration into human nutrition.

Due to the low level of total solid content (approximately 6%, by weight), relatively high lactose-glucose ratio and high acidity, consumers perceive whey-based beverages as watery, sweet–sour liquid with the poor mouthfeel.[5] Likewise, beverages produced by fermentation of whey have significantly lower viscosity, milder flavour and less viability of probiotic microorganisms compared to those obtained by fermentation of milk. Therefore, it is necessary to choose an adequate culture or supplements that can enhance these characteristics of the final product. One of the possible ways could be the use of dairy starters in combination with high exopolysaccharide-producing strains or use of hydrocolloids.[5] On the other hand, in order to avoid hydrocolloids and preserve the completely natural composition of beverage, milk addition could be a good alternative. The aggregation of casein and whey proteins, during fermentation, leads to the formation of the gel that constructs the beverage structure, protects probiotic strains and improves overall quality of product.[6]

Cultures that are most frequently used as dairy starters are AB (*L. acidophilus* and *Bifidobacterium* spp.), ABC (*L. acidophilus*, *Bifidobacterium* spp. and *L. casei*), ABT (*L. acidophilus*, *Bifidobacterium* spp. and *S. thermophilus*) and ABY (*L. acidophilus*, *Bifidobacterium* spp., *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*).[7–10] Combination of these commercial cultures with strains marked as a good exopolysaccharide producers could improve quality of beverages in several ways. The presence of exopolysaccharides leads to the improvement of textural attributes (such as firmness and mouthfeel) of many food products. Many of them can form gels that will constitute food structure and enhance viscosity of solutions owing to their high

[a]*Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia. E-mail: mbulatovic@tmf.bg.ac.rs; Fax: +381 113370387; Tel: +381 113303775*

[b]*Innovation center Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia*

[c]*IHIS Techno Experts d.o.o. Research Development Center, Batajnicki drum 23, 11000 Belgrade, Serbia*

molecular weight.[11] Exopolysaccharides may act as prebiotics, selectively metabolised by beneficial bacteria, enhancing their growth, activity and viability in food products as well as in the gastrointestinal tract.[12–15] In the addition to the improvement in probiotic character, the exopolysaccharides also improves aroma of the final product, by stimulating the growth of microorganisms that produces aromatic compounds. This is very important since many studies have shown that the flavour is the first elimination parameter in the selection of food, followed by consideration of the health aspects.[16,17] Probiotic beverages with disagreeable sensory characteristics are not attractive to costumers even if its consuming has multiple benefits to their health.

There are a small number of literature reports about the use of commercial ABY cultures for fermented whey beverage formulation. There is practically no data on the characterisation of beverage obtained by fermentation of whey using commercial ABY-6 culture. In addition, there is no data concerning the possibility that additional *L. rhamnosus* ATCC 7469 strain can improve general quality of produced whey beverage. Therefore, the aim of our study was to evaluate the influence of milk and additional probiotic strain *L. rhamnosus* ATCC 7469 on the quality of whey-based beverage that contains commercial ABY-6 starter culture.

2. Materials and methods

2.1. Culture and media

Commercial lyophilized dairy starter culture that is known as 'Lactoferm ABY 6' used in this study was supplied by Biochem s.r.l. (Monterotondo, Roma, Italy). Starter culture is mixture of *Streptococcus salivarius* ssp. *thermophilus* (80%), *Lactobacillus acidophilus (*13%), *Bifidobacterium bifidum* (6%), *Lactobacillus delbrueckii* ssp. *bulgaricus* (1%). The culture that consists of 10 g lyophilised starter powder is the one currently used in dairy industry. The culture was maintained according to the manufacturer's instructions at −18 °C until use (no longer than 20 mounts). For each experiment, 1% (w/v) of starter culture was gently dissolved in sterilised skim milk (0.5% fat) and activated 30 min at 42 °C. Concentration of viable probiotic cells (*L. acidophilus and B. bifidum*) in activated culture was 5.58 ± 0.06 log(CFU mL^{-1}).

The strain *Lactobacillus rhamnosus* ATCC 7469, used in this study, was supplied by American Type Culture Collection (ATCC, Rockville, USA). Stock culture was stored at −18 °C in 3 mL vials containing De Man Rogosa Sharpe (MRS) broth (Fluka, USA) and 50% (v/v) glycerol as a cryoprotective agent. To prepare the laboratory culture, a drop of stock culture was transferred to 3 mL of MRS broth and incubated for 18 h under anaerobic conditions at optimal growth temperature (37 °C). The working culture was pre-cultured twice in MRS broth prior to experimental use. Concentration of viable cells in activated culture was 7.78 ± 0.165 log(CFU mL^{-1}).

After the activation, desired inoculum level of each culture was added into the fermentation medium, in accordance with the requirements of the experimental procedure (see Section 2.2).

Whey remained after cheese production and sterile skim milk with 0.5% fat were obtained from domestic dairy plant Imlek a.d. (Belgrade, Serbia). After collection, the whey was stored at −18 °C until use (no longer than one week). The chemical composition of whey was: total solids 9.8 ± 0.03% (w/v); protein 2.6 ± 0.012% (w/v); fat 1.05 ± 0.08% (w/v) and lactose 5.6 ± 0.114% (w/v).

2.2. Experimental procedure

Based on preliminary experiments (data not shown) 30% milk was used for beverage formulation as concentration that appropriate for appreciably sensory quality improvement. Whey (0% milk, v/v) and whey-milk mixture (30% milk, v/v) were pasteurized at 60 °C for 60 min, cooled at fermentation temperature (42 °C) and inoculated with following level of activated cultures. Four different beverages were formulated: AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v).

The flasks containing 300 mL of formulated beverage were prepared for each point of analyses. Prepared samples were incubated at 42 °C in a water bath. During the incubation, samples (2 ml) were taken every 1 h for determination of pH value. The fermentations were carried out for 4 h until pH = 4.6 ± 0.2 was attained. After 4 h fermentations were stopped by quick cooling. The fermented beverages were stored at 4 °C for 28 days. Analysis of the titratable acidity (TA, °SH), pH value, viable cell count (log(CFU mL^{-1})), syneresis (%), viscosity (cP), antioxidant activity (%) and overall acceptability was carried out during fermentation and 28 days of storage.

2.3. Chemical analysis

The titratable acidity was determined by the Soxhlet-Henkel method,[18] and the pH value was measured using a pH meter (Inolab, WTW 82362, Wellheim, Germany).

2.4. Microbiological analysis

One milliliter of fermented sample was diluted with 9 mL of sodium chloride (0.85%, w/v), and mixed uniformly. Subsequent serial dilutions were prepared and viable cell count was determined using pour plate technique. MRS-maltose (MRSM) agar and anaerobic incubation at 37 °C for 48 h were used for the enumeration of viable cell count of probiotic bacteria (*L. acidophilus* and *B. bifidum* in AW and AM beverages; *Lb. acidophilus*, *B. bifidum* and *L. rhamnosus* in RW and RM beverages).[19]

2.5. Texture analysis

2.5.1. Viscosity. The apparent viscosities were determined at 8 °C according to modified method.[20] A Brookfield DV II+ Pro viscometer (Brookfield Engineering Lab Inc, Stoughton, MA) was used. A spindle N°61 was set to 10 rpm. The viscosity measurements were continuous over 30 s required to collect 70 data points. Data points were averaged per sample per replication. The apparent viscosity was determined on three cups of

sample per replication. Three replications were conducted and values are expressed in cP.

2.5.2. Syneresis. Syneresis of fermented samples was determined according to the method.[21] The fermented samples (20.0 mL) were centrifuged at 1000 rpm for 10 min at 4 ± 1 °C. Collected supernatant was drained, weighed and the following equation was used for syneresis calculation:

$$\text{Syneresis}(\%) = \frac{\text{Weight of supernatant (g)}}{\text{Weight of fermented sample (g)}} \times 100\% \quad (1)$$

2.6. Sensory analysis

Sensory analysis of fermented beverage samples was conducted after 1, 7, 14, 21 and 28 days of storage according to the modified method.[22] Fifty-five untrained panellists (35 being women and 20 men, age between 25 and 55) from the faculty, including teachers, students and staff were randomly selected and invited to participate in the sensory evaluation of fermented whey-based beverages on the basis overall acceptability. The participants were asked to assess the overall acceptability of the four different fermented beverages: AW, AM, RW and RM. Each questionnaire consists of four questions: name, age, sex and overall acceptability for four consumed products.

The samples were presented monadically at 4 ± 1 °C, in individual plastic cups coded with 3-digit numbers, serving 20 mL samples to each panellist. The participants were given four samples at a time at storage temperature (4 ± 1 °C), a pencil, a questionnaire and a glass of cold water to rinse their mouths between samples. They have been asked to mark an value on the questionnaire scale which best represents how much they liked or disliked each of four samples with respect to overall acceptance, using a 9-point hybrid hedonic scale where 1 = disliked extremely; 5 = neither liked nor disliked and 9 = liked extremely. The sensory analysis was consisted of 275 questionnaires distributed into 5 sessions (5 times of storage). Prior to serving all samples were subjected to counts of yeasts, molds and coliforms to evaluate the hygienic and sanitary conditions of the products.

2.7. Antioxidant activity

Antioxidant activity of fermented whey-based beverages was determined by its ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, which was measured according to the modified method.[23] A stock solution of 0.1 mM DPPH (Sigma-Aldrich, Australia) was prepared by dissolving in methanol. After 4 h fermentation samples were macerated with methanol and centrifuged at 8000 rpm for 20 min at 4 °C. Methanol (1.5 mL) and DPPH (1.0 mL) were added to the supernatant (0.5 mL). Control sample was prepared by mixing methanol (1.5 mL) and DPPH (1.5 mL), while methanol was used as blank sample. Mixtures were allowed to stand 30 min in dark, at room temperature. The antioxidant activity was analyzed by reading the absorbance at 517 nm. Scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity } (\%) = [(\text{Ac} - \text{Aa})/\text{Ac}] \times 100 \quad (2)$$

where Aa and Ac represent absorbance of sample and control, respectively.

2.8. Statistical analysis

The experiments were performed in triplicate. All values are expressed as mean ± standard deviation. Mean values were analysed using two-way ANOVA. The Tukey post hoc test was performed for means comparison (Origin Pro 8 (1991–2007), Origin Lab Co., Northampton, USA). Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Chemical analysis

Fermentation of whey by commercial cultures designed for yoghurt production could be an interesting way of including whey in human consumption. Changes in pH and titratable acidity (°SH) during fermentation and storage period are specific for every product and depend on the microorganisms used for formulation as well as of the substrate composition.

A gradual decrease of pH was observed in all samples during 4 h fermentation as well as during 28 days of storage (Fig. 1). Values of pH were ranged from 4.34 to 4.51 in all samples after fermentation. Statistically significant difference ($P < 0.05$) in pH was recorded in samples AW (4.51) and RW (4.37). Observed difference, means that *L. rhamnosus* leads to significant drop of pH in sample formulated without milk. In samples supplemented with 30% milk (AM and RM) applied culture does not have statistically significant ($P > 0.05$) influence on pH. Comparing samples fermented by ABY-6 (AW and AM) and samples fermented by ABY-6 co-cultured with *L. rhamnosus* (RW and RM) regarding the milk content, it was observed that milk supplementation does not significantly ($P > 0.05$) affects pH value after fermentation.

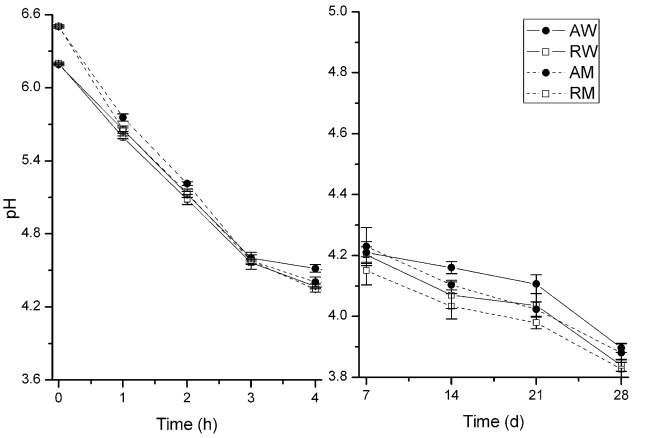

Fig. 1 Effect of milk and culture composition on pH value of whey-based beverages during 4 h fermentation and 28 days of storage. AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v). Vertical bars represent the standard deviation ($n = 3$) for each data point.

Compared to the pH values obtained after fermentation, pH values at the end of storage period were considerably lower, ranged from 3.82 to 3.89 in all samples (Fig. 1). A possible explanation of this behaviour could be that the used strains are capable to save their productivity during storage. It can be also assumed that buffering capacity of milk[24] is enough to suppress significant pH decreasing after fermentation but not after 28 days of storage. *L. rhamnosus* has statistically significant effect ($P < 0.05$) on pH in sample RW, as well as in sample RM.

On the other hand, milk supplementation does not significantly affect the pH after 28 days of storage. The obtained results are different to those reported by others[25,26] who reported pH of about 4.20 after 35 days of storage, when the initial pH value was about 4.60. Therefore, we could say that *L. rhamnosus*, as a strain with high lactic acid productivity,[27] significantly affects pH of whey-based beverage during 28 days of storage regardless of milk addition.

Based on the results, pH decreases faster in samples with *L. rhamnosus* during the fermentation as well as during storage period. As reported in the literature,[28] this strain is characterised by excellent proteolytic activity with high amount of free amino acids (FAA) produced during process of cheese production. Due to this specific ability, it provides amino acids to the strains present in ABY-6 culture and probably increases their metabolic activity. In addition, the increased strains activity leads to the production the higher amount of lactic acid and faster decrease of pH in these samples during fermentation as well as during storage period.

Titratable acidity of samples ranged from 16.1 to 24.2 °SH after fermentation, and from 23.2 to 35.4 °SH after 28 days of storage. As shown in Fig. 2, the highest titratable acidities of 24.2 °SH and 35.4 °SH were observed in sample RM after fermentation and after 28 days of storage, respectively. Based on the observed results we could observe that the presence of milk and *L. rhamnosus* increases titratable acidity of whey-based beverages. It is interesting to note that milk significantly ($P < 0.05$) affects titratable acidity of samples AM and RM, in contrast to the non-significant ($P > 0.05$) effect of milk on pH of above samples. The possible explanation could be the fact that the productivity of both cultures in the presence of milk proteins was enhanced, but produced lactic acid cannot be recorded by measuring pH.

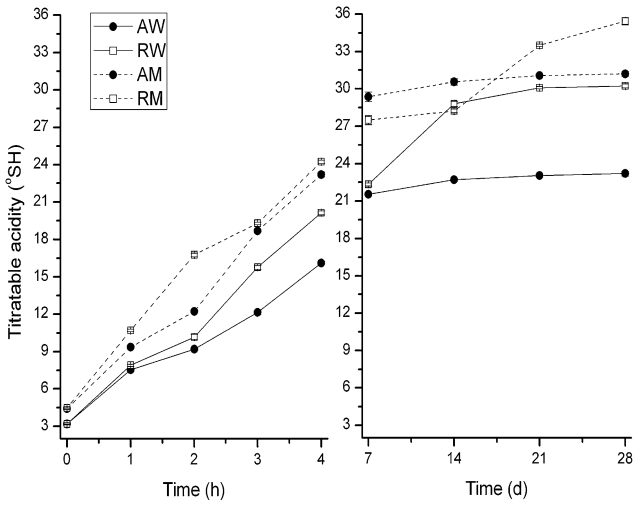

Fig. 2 Effect of milk and culture composition on titratable acidity of whey-based beverages during 4 h fermentation and 28 days of storage. AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v). Vertical bars represent the standard deviation ($n = 3$) for each data point.

Lactic acid has significant impact on the flavour of fermented milk products. A beverage is considered to have a good quality if it has a titratable acidity of approximately 44 °SH. In our study, due to very short fermentation time (4 h) and whey as poor substrate, strains present in ABY-6 culture are not able to produce satisfactory amount of lactic acid. The addition of highly productive strain and milk enhances amount of lactic acid present in produced beverage (Fig. 2). However, titratable acidities of the fermented whey-based beverages in this study were below value 53.0 °SH at which unpleasant acid taste could be detected.[29,30]

3.2. Microbiological analysis

The preferred option for whey fermentation is the use of culture containing probiotic strains. Probiotics in form of fermented dairy products are metabolically active products, which pass through some modifications during their shelf life, such as loss of culture viability and overall sensory quality. Whey does not contain an abundance of nutrients, but its enrichment can create the conditions present in the gastrointestinal tract, which is the natural habitat of probiotic bacteria and thus lead to improvements of their growth and viability. The changes in viable cell count of probiotic bacteria in beverages formulated with whey and whey-milk mixture, fermented by ABY-6 and ABY-6 co-cultured with *L. rhamnosus* for 4 h and stored for 28 days are shown in Fig. 3.

As indicated in Fig. 3 viable cell count of probiotic bacteria (*L. acidophilus* and *B. bifidum*) ranged from 4.88 to 5.19 log(CFU mL^{-1}) in samples AW and AM, respectively, after 4 h of fermentation. It suggests that milk have significant ($P < 0.05$) influence on growth of ABY-6 culture. Regardless of the positive effect of milk, both samples fermented by ABY-6 starter culture (AW and AM) did not meet the requirement (>6.0 log(CFU mL^{-1}) to be considered as probiotics.[31] Same statistically significant ($P < 0.05$) positive influence of milk observed in samples fermented by ABY-6 co-cultured with *L. rhamnosus* where the viable cell count of probiotic bacteria was ranged from 6.69 log(CFU mL^{-1}) in sample RW to 7.51 log(CFU mL^{-1}) in sample RM (Fig. 3). That confirms that milk has significant influence on the growth of these probiotic strains. According to earlier reports,[32] the remarkable effect of milk on the growth of microorganisms was recorded and it is caused mainly by presence of milk protein during the fermentation of whey-milk base. We could say that milk proteins protect probiotic strains, enhance their growth and probably viability.

Co-culturing of ABY-6 with probiotic strain *L. rhamnosus* significantly ($P < 0.05$) increases viable cell count of probiotic

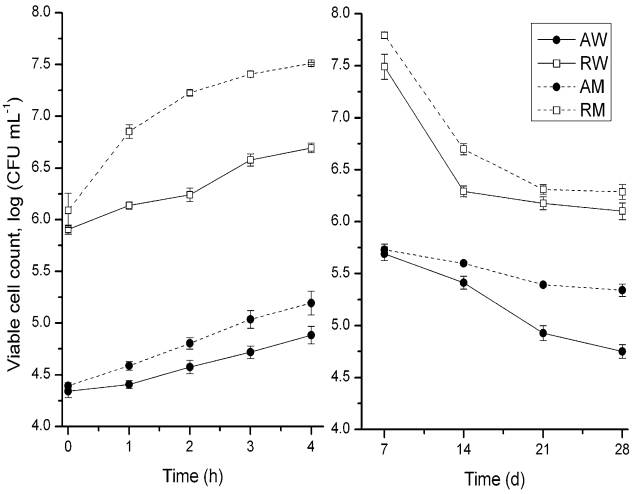

Fig. 3 Effect of milk and culture composition on viable cell count of probiotic bacteria in whey-based beverages during 4 h fermentation and 28 days of storage. AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v). Vertical bars represent the standard deviation ($n = 3$) for each data point.

bacteria regardless of the presence of milk. The reached count of viable probiotic bacteria was for about 1.5–2.3 log units higher in samples RW and RM than in samples AW and AM that contained only *L. acidophilus* and *B. bifidum* as probiotics (Fig. 3). Based on these results, addition of milk and highly productive probiotic strain *L. rhamnosus*, with excellent growth capability, improves the probiotic character of produced whey-based beverage. Maximal viable cell count of probiotic bacteria (7.51 log(CFU mL^{-1})) was reached in sample RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v) after 4 h of fermentation.

The rate of population reduction was significantly ($P < 0.05$) slower in samples supplemented with milk during 28 days of storage, regardless of culture. The observed results suggest that milk slows the probiotic viable cell count reduction.

As shown in Fig. 3, samples RW and RM have significantly ($P < 0.05$) higher probiotic viable cell count than samples fermented AW and AM during the whole storage period. Sample RM had significantly ($P < 0.05$) higher probiotic viable cell count (6.30 log(CFU mL^{-1})) than sample RW (6.10 log(CFU mL^{-1})) at the end of storage period. Both samples RW and RM meet the requirement (>6.0 log(CFU mL^{-1})) to be considered as probiotics. The obtained results are consistent to those reported in our previous research,[33] which suggests that synergistic effect of proteins and polysaccharides can positively affect growth and viability of probiotic bacteria. Sample RM supplemented with 30% milk and fermented by ABY-6 co-cultured with *L. rhamnosus*, achieved the maximal probiotic cell count of 7.51 log(CFU mL^{-1}) after 4 h fermentation and held that count of viable probiotic bacteria during 28 days of storage.

3.3. Texture analysis

The knowledge of rheological behaviour of whey-based beverages is a valuable tool in design of processing technologies and predicting the product stability during storage. The basic parameter, obtained during rheological study of liquid foods, is viscosity, used to characterize the fluid texture.[34–36] The changes in syneresis and viscosity of the beverages formulated with whey and whey-milk mixture, fermented by ABY-6 and ABY-6 co-cultured with *L. rhamnosus* and stored for 28 days are shown in Fig. 4 and Table 1.

As indicated in Fig. 4, the viscosity of samples fermented by ABY-6 increases and reaches values 1.6662 cP (AW) and 2.8350 cP (AM) during the first two weeks of storage. After 14 days, the viscosity of sample AW starts to declines reaching the value 1.5518 cP after 28 days of storage. On the other hand, in the sample formulated with whey-milk mixture (AM) viscosity increases during whole storage period reaching the value of 2.9529 cP after 28 days of storage. We can observe, that the viscosity of fermented whey-based beverages is significantly ($P < 0.05$) related to the presence of milk in formulation. Strong influence of milk on texture of whey-based beverage is in accordance with the results reported by others[5] who found that casein content had high influence on the texture of fermented milk products. Produced lactic acid reduces the pH of milk to the isoelectric point (pH = 4.6) of casein and leads to the formation of protein gel. This observation is also supported by previous studies[37,38] that pointed out that an additional amount of milk can cause a stronger texture due to stronger network of protein gel.

In the samples fermented by ABY-6 co-cultured with *L. rhamnosus* viscosity increases in both samples (RW and RM) reaching values 1.6281 cP and 2.7732 cP, respectively, after two

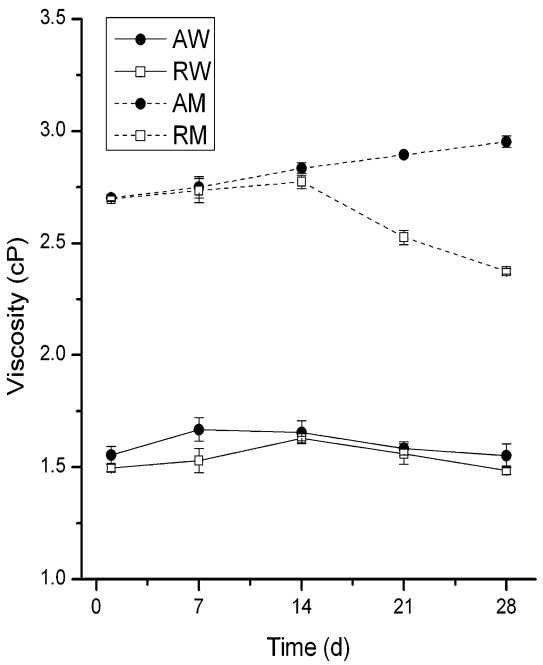

Fig. 4 Effect of milk and culture composition on viscosity of whey-based beverages during 28 days of storage. AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v). Vertical bars represent the standard deviation ($n = 3$) for each data point.

Table 1 Effect of milk and culture composition on syneresis of whey-based beverages during 28 days of storage. AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v)

	Syneresis (%)[a]			
	Sample			
Time (days)	AW	RW	AM	RM
1	88.3 ± 0.91	78.5 ± 1.05	67.5 ± 0.70	50.6 ± 0.60
7	90.1 ± 0.61	82.5 ± 1.05	72.3 ± 0.97	64.9 ± 0.85
14	91.2 ± 0.75	84.3 ± 1.15	75.1 ± 0.75	70.0 ± 0.68
21	87.9 ± 0.68	81.2 ± 0.95	76.1 ± 1.10	67.9 ± 0.80
28	85.0 ± 0.83	80.2 ± 0.58	78.3 ± 0.76	65.9 ± 0.86

[a] Data are the mean ± standard deviation calculated from three independent experiments ($n = 3$).

weeks of storage. In these samples viscosity values of 1.4852 cP for RW and 2.3755 cP for RM were observed at the end of storage period.

Different behaviour of samples fermented by ABY-6 and ABY-6 co-cultured with *L. rhamnosus* (AM and RM) could be explained by presence of highly productive strain *L. rhamnosus* both in lactic acid and exopolysaccharide as well. Lower pH values in sample, that contains *L. rhamnosus*, contribute to lowering stability of protein gel formed during fermentation. It is also interesting to note, that after 14 days of storage presence of *L. rhamnosus* leads to a considerable increase in the content of lactic acid. Protein gels are pH-sensitive and presence of lactic acid affects a polypeptide chain interaction, which leads to the uptaking of water inside the gel. Uptaking of water inside the gel weakens its structure and leads to the decrease in viscosity of these samples.[6]

Changes in syneresis during the storage were observed in all samples (Table 1). It appeared that syneresis increases during the 14 days of storage for samples inoculated with ABY-6 co-cultured with *L. rhamnosus* (RW and RM). After 14 days of storage syneresis of RW and RM samples were 84.3% and 70.0%, respectively. Further, syneresis starts to decline and values 80.2% for sample RW and 65.9% for sample RM were reached after 28 days.

In the samples inoculated with ABY-6 increase in syneresis was observed during 14 days of storage. After 14th day, syneresis in sample AW decreases, in contrast to the sample AM where increase in syneresis was observed to the end of storage period. Syneresis of AW and AM samples was 85.0% and 78.3%, respectively, after 28 days. During the whole storage period, syneresis values of samples were significantly different ($P < 0.05$) in favour of the sample supplemented with 30% milk. The observed results were correlated with the above results obtained for viscosity. An increase in viscosity correlates to the stronger protein gel that loses the ability to hold the whey. Whey drains from the protein matrix and appears on the surface of fermented milk.[39]

Comparing beverages formulated with whey-milk mixture it was observed that sample RM had significantly ($P < 0.05$) lower syneresis than sample AM. This result is in accordance to those reported by others[40,41] who observed lower level of syneresis in yoghurt gels made by EPS producing starters compared to those made by EPS non-producing starters.

We could say, that addition of probiotic *L. rhamnosus* strain, beside the slight reduction of viscosity, leads to the decrease of syneresis as the first eliminating parameter for beverage selection by consumers. Exopolysaccharide produced by *L. rhamnosus*[42]can form weak polysaccharide-protein interactions instead of more stable protein–protein ones.[43,38] This contributes to the formation of weak gel structure[44] that easily hydrates and thus reduces the syneresis of these beverages compared to the beverages fermented by ABY-6.

3.4. Sensory analysis

From 55 randomly panellists taking part in the overall acceptability test, 36.3% were male and 63.6% were female. Approximately 67.5% were between 25–45 years old. The analysis of whey-based beverages was conducted after 1, 7, 14, 21 and 28 days of refrigerated storage at 4 °C. The changes in acceptability values of fermented whey-based beverages are presented in Table 2.

The results indicated that supplementation of whey by 30% milk significantly ($P < 0.05$) affects sensory acceptance of whey-based beverages (Table 2). Samples AM and RM showed high acceptability values during the storage period, with mean values between 7.80 and 8.38. These results suggest that milk addition helps to avoid the poor sensory characteristics perceptible to consumers. Nonetheless, the acceptability values were significantly ($P < 0.05$) higher for sample AM, compare to the sample RM. Co-culturing of ABY-6 with *L. rhamnosus* leads to the decreases of acceptability values of whey-beverage during whole storage period. Based on our previous research[45] this problem can be solved by fortification of the whey-based beverage with various fruit bases that can enhance its sensory characteristics. Taking into consideration the fact that the count of viable probiotic bacteria is significantly higher in beverage that

Table 2 Effect of milk and culture composition on acceptability values of whey-based beverages during 28 days of storage. AW (0% milk, ABY-6 6%, v/v), AM (30% milk, ABY-6 6%, v/v), RW (0% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v)

	Acceptability values[a]			
	Sample			
Time (days)	AW	RW	AM	RM
1	6.20 ± 1.22	6.92 ± 1.11	8.52 ± 1.01	8.20 ± 0.76
7	6.08 ± 1.08	6.80 ± 1.19	8.52 ± 1.00	8.12 ± 1.01
14	5.96 ± 1.17	6.60 ± 1.15	8.40 ± 1.15	8.00 ± 1.04
21	5.80 ± 1.19	5.52 ± 1.29	8.32 ± 1.14	7.48 ± 1.08
28	5.52 ± 1.16	5.16 ± 1.11	8.12 ± 1.13	7.20 ± 1.04
Mean	5.91 ± 0.26	6.20 ± 0.80	8.38 ± 0.17	7.80 ± 0.44

[a] Data are the mean ± standard deviation calculated from three independent experiments ($n = 3$).

contain *L. rhamnosus*, we can observe, that the benefit of the strain addition is much greater than its relatively negative impact on the sensory profile of beverage. It is also necessary to emphasise the positive effect of *L. rhamnosus* on reduction of syneresis as a characteristic that largely determines the acceptability of the whey-based beverage by consumers.

3.5. Antioxidant activity analysis

Based on the aforementioned findings milk significantly affects the quality of the beverage. Thus, the beverage formulated with 30% milk was selected as acceptable. In addition, it was necessary to explore the effect of the EPS producing strain on antioxidant activity of fermented beverage formulated by 30% milk. The influence of *L. rhamnosus* on antioxidant activity of whey-based beverage formulated by 30% milk is shown in Fig. 5.

The antioxidant activity was significantly higher ($P < 0.05$) in sample RM during fermentation as well as during the whole storage period. Additional exopolysaccharide produced by *L. rhamnosus*, probably stimulate ABY-6 strains to produce metabolic products such as bioactive peptides that contribute to the higher antioxidant activity of beverage. The obtained results are in accordance to the results reported by other researchers,[46] who found that the metabolic products of LAB obtained by utilisation of oligosaccharides contribute to the higher antioxidant activity of yogurt prepared by *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus* and *L. plantarum*. On the other hand, *L. rhamnosus*, as a strain with high proteolytic activity[28] significantly contributes to the production of antioxidant peptides.

In addition, it was found that in both samples (AM, RM) antioxidant activity decrease from an initial value 46.0 and 51.2%, respectively, at the end of fermentation, to 38.1 and 44.1% by 14 days of storage (Fig. 5). After two week of storage, antioxidant activity of samples (AM, RM) starts to increases and reaches value 39.2 and 45.1%, respectively, at 21st day of storage. The obtained results are in agreement with earlier studies.[47] Increase in antioxidant activity after 14th day of storage could be explained by release of intracellular microbial enzymes by cell lysis that contribute to the antioxidant activity. This observation is in accordance with literature reports[28] about increased peptidase activities occurred during ripening of cheese produced by *L. rhamnosus* ATCC 7469. Therefore, it could be assumed that proteolysis[48] and lactic acid production[49] as the results of microbial activity during fermentation and refrigerated storage could be additional sources of antioxidant activity.

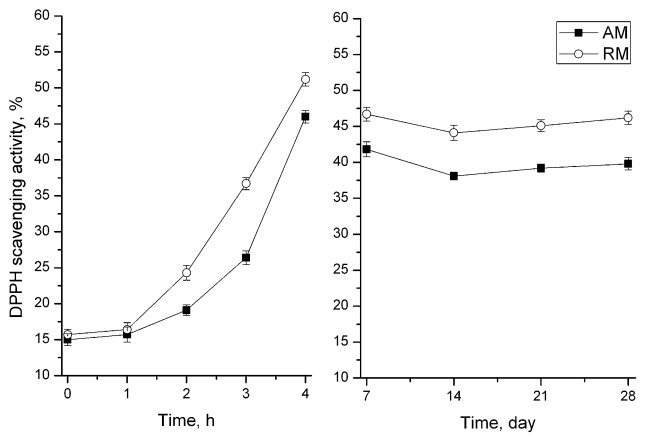

Fig. 5 Effect of culture composition on DPPH scavenging activity of whey-based beverages during 4 h fermentation and 28 days of storage. AM (30% milk, ABY-6 6%, v/v), RM (30% milk, ABY-6 4%, *L. rhamnosus* 2%, v/v). Vertical bars represent the standard deviation ($n = 3$) for each data point.

3.6. Conclusions

The present study is the first report on use of commercial ABY-6 culture in whey fermentation. Probiotic whey beverage was successfully formulated using milk and commercial ABY-6 culture co-cultured with *L. rhamnosus*. Co-culturing of commercial starter culture ABY-6 with probiotic *L. rhamnosus* strain increases viable cell count for about 2.60 log units compared to the beverages obtained in fermentations performed by ABY-6 culture. Milk helps to avoid the poor sensory characteristics perceptible to consumers and in synergy with exopolysaccharides greatly improves the viscosity and syneresis of beverage.

To formulate beverage that meets required criteria for probiotics (viable cell count $>10^6$ CFU mL^{-1}) supplementation of whey with 30% milk as well as co-culturing of ABY-6 and *L. rhamnosus* is advisable. The obtained beverage contains 7.49 log(CFU mL^{-1}) probiotic bacteria, expresses antioxidant activity of 45.1%, satisfactory sensory characteristics, has a shelf life of at least 20 days, and it can be introduced in the market.

Acknowledgements

This work was funded by the Serbian Ministry of Education, Science and Technological development (TR 31017).

References

1 B. Matijević, R. Božanić and Lj. Tratnik, *Mljekarstvo*, 2010, **60**, 175–182.
2 M. R. Silva, C. L. L. F. Ferreira, N. M. B. Costa and J. Magalhães, *Anais do XVIII Congresso Nacional de Laticínios*, Juiz de Fora, 2001.
3 E. Ha and M. B. Zemel, *J. Nutr. Biochem.*, 2003, **14**, 251–258.
4 L. Tapsell, I. Hemphill, L. Cobiac, C. Patch, D. Sullivan and M. Fenech, *Med. J. Aust.*, 2006, **185**, S4–S24.
5 V. Legarová and L. Kouřimská, *Mljekarstvo*, 2010, **60**, 280–287.
6 R. N. Zúñiga and E. Troncoso, in *Scientific, Health and Social Aspects of the Food Industry*, ed. B. Valdez, InTechOpen, Rijeka, 2012, ch. 15, pp. 295–320.
7 T. Saito, *Anim. Sci. J.*, 2004, **75**, 1–13.
8 G. Maiocchi, *Ind. Latte*, 2001, **XXXVII**, 94–98.
9 A. B. Martín-Diana, C. Janer, C. Peláez and T. Requena, *Int. Dairy J.*, 2003, **13**, 827–833.

10 E. B. Minelli, A. Benini, M. Marzotto, A. Sbarbati, O. Ruzzenete, R. Ferrario, H. Hendriks and F. Dellaglio, *Int. Dairy J.*, 2004, **14**, 723–736.
11 J. M. Aguilera and D. W. Stanley, in *Microstructural Principles of Food Processing and Engineering*, ed. J. M. Aguilera, and D. W. Stanley, Aspen Publishers Inc, Gaithersburg, 2nd edn, 1999, pp. 155–183.
12 G. R. Gibson, B. Rabiu, C. E. Rycroft and R. A. Rastall, in *Handbook of Functional Dairy Products*, ed. C. Shortt, and J. O. Brien, CRC Press LLC, Boca Raton, 2004, pp. 91–109.
13 T. Mizota, Functional and nutritional foods containing bifidogenic factors, *Bull. Int. Dairy Fed.*, 1996, **313**, 31–35.
14 R. Mohammadi, A. M. Mortazavian, R. Khosrokhavar and A. G. Cruz, *Ann. Microbiol.*, 2011, **61**, 411–424.
15 C. E. Rycroft, M. R. Jones, G. R. Gibson and R. A. Rastall, *J. Appl. Microbiol.*, 2001, **91**, 878–887.
16 R. Mohammadi and A. M. Mortazavian, *Food Rev. Int.*, 2011, **27**, 192–212.
17 H. Tuorila and A. V. Cardello, *Food Qual. Prefer.*, 2002, **13**, 561–569.
18 L. Varga, *Int. J. Food Microbiol.*, 2006, **108**, 272–275.
19 R. I. Dave and N. P. Shah, *J. Dairy Sci.*, 1996, **79**, 1529–1536.
20 K. J. Aryana and P. McGrewa, *LWT - Food Sci. Technol.*, 2007, **40**, 1808–1814.
21 M. K. Keogh and B. T. O'Kennedy, *J. Food Sci.*, 1998, **63**, 108–112.
22 J. Hemsworth, S. Hekmat and G. Reid, *Innov. Food Sci. Emerg.*, 2011, **12**, 79–84.
23 G. Balakrishnan and R. Agrawal, *J. Food Sci. Technol.*, 2012, DOI: 10.1007/s13197-012-0891-9.
24 S. Fadiloğlu, O. Erkmen and G. Şekeroğlu, *Food Technol. Biotechnol.*, 2004, **42**, 27–32.
25 R. I. Dave and N. P. Shah, *Int. Dairy J.*, 1997, **7**, 31–41.
26 S. E. Gilliland, S. S. Reilly, G. B. Kim and H. S. Kim, *J. Food Sci.*, 2002, **67**, 3091–3095.
27 M. Lj. Bulatović, M. Rakin, Lj. Mojović, S. Nikolić, M. Vukašinović-Sekulić and A. Đukić-Vuković, *J. Food Sci. Eng.*, 2012, **2**, 705–711.
28 R. D. Cagnoa, M. Quintob, A. Corsettic, F. Minervinia and M. Gobbettia, *Int. Dairy J.*, 2006, **16**, 119–130.
29 R. Pinthong, R. Macrae and J. Rothwell, *Int. J. Food Sci. Technol.*, 1980, **15**, 647–652.
30 C. H. Kehagias and T. N. Dalles, *J. Food Prot.*, 1984, **47**, 760–761.
31 A. Y. Tamime, V. M. E. Marshall and R. K. Robinson, *J. Dairy Res.*, 1995, **62**, 151–187.
32 K. Almeida, A. Y. Tamime and M. N. Oliveira, *LWT - Food Sci. Technol.*, 2009, **42**, 672–678.
33 M. Lj. Bulatović, M. Rakin, M. Vukašinović-Sekulić, Lj. Mojović and T. Krunić, *Int. Dairy J.*, 2014, **34**, 109–115.
34 M. A. Rao, *J. Texture Stud.*, 1997, **8**, 135–168.
35 M. L. Alonso, E. Garzon, B. Melkon and J. Zapico, *Alimentaria*, 1990, **27**, 53–57.
36 M. A. Rao and J. H. Cooley, *J. Texture Stud.*, 1992, **23**, 415–425.
37 M. Guven, K. Yasar, O. B. Karaca and A. A. Hayaloglu, *Int. J. Dairy Technol.*, 2005, **58**, 180–184.
38 L. Ramchandran and N. P. Shah, *J. Dairy Sci.*, 2009, **92**, 895–906.
39 M. F. Bezerra, D. F. S. Souza and R. T. P Correia, *Int. J. Dairy Technol.*, 2012, **65**, 1–7.
40 A. N. Hassan, J. F. Frank, K. A. Schmid and S. I. Shalabi, *J. Dairy Sci.*, 1996, **79**, 2098–2103.
41 D. Jaros, H. Rohm, A. Haque and W. Kneifel, *Milchwissenschaft*, 2002, **57**, 325–326.
42 G. Lorca, M. I. Torino, G. Font de Valdez and A. Ljungh, *FEMS Microbiol. Lett.*, 2002, **206**, 31–37.
43 D. M. Folkenberg, P. Dejmek, A. Skriver, H. S. Guldager and R. Ipsen, *Int. Dairy J.*, 2006, **16**, 111–118.
44 E. Fernández-García and J. U. McGregor, *Eur. Food Res. Technol.*, 1997, **204**, 433–437.
45 M. Rakin, M. Vukasinovic, S. Siler-Marinkovic and M. Maksimovic, *Food Chem.*, 2010, **100**, 599–602.
46 A. N. Madhu, N. Amrutha and S. G. Prapulla, *Probiotics Antimicrob. Proteins*, 2012, **4**, 90–97.
47 V. V. Illupapalayam, S. C. Smith and S. Gamlath, *LWT - Food Sci. Technol*, 2014, **55**, 255–262.
48 A. Lourens-Hattingh and B. C. Viljoen, *Int. Dairy J.*, 2001, **11**, 1–17.
49 I. Correia, A. Nunes, I. F. Duarte, A. Barros and I. Delgadillo, *Food Chem.*, 2001, **90**, 853–859.

