Original paper

THE USE OF THE YEAST KLUYVEROMYCES FRAGILIS B0399 IN THE PRODUCTION OF PROBIOTIC YOGURT

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Abstract

Rising interest for probiotics in the recent years was caused by the possibility of their use in prevention and cure of different types of human and animal intestinal disorders. During 20th century many research studies were concentrated on finding new types of probiotic cultures. In this work, for the production of probiotic yogurt, we used commercially available, new generation probiotic lactic yeast "Turval B0399", produced by Italian company Turval Laboratories. Turval B0399 is the culture of yeast species Kluyveromyces marxianus fragilis B0399. This yeast is characterized by the unique ability of fermenting with the enzyme β-galactosidase and by production of lactic acid, a fundamental substance in cell metabolic reactions. This probiotic yeast, naturally resistant to antibiotics, mitigate negative effects of antibiotics - by competitive colonisation of intestine it regulates intestinal dismicrobism by preventing the growth of pathogens, such as *Candida albicans*, while increasing the number of residential bifidobacterias. It keeps intestinal homeostasis, improves immunity (in *in vitro* studies it was shown to decrease the production of proinflammatory cytokines, while in studies on patients with atopic dermatitis it decreased the IgE level). It improves the general metabolism and is very successful in prevention and treatment of different intestinal disorders (Crohn's disease and Irritable Bowel syndrome).

In this work we studied the growth of the yeast *Kluyveromyces fragilis* B0399 and its influence on the growth of the probiotic bacteria *Lactobacillus acidofilus* LA5 and *Bifidobacterium lactis* BB12 with the final aim of achieving the maximal number of live cells during the production of probiotic yogurt ($>10^6$ cfu/g). The experimental production of the probiotic yogurt with Turval B0399 was done in the Dairy Laboratory of the Department of Animal Science, Faculty of Agriculture, University of Novi Sad, while all microbiological analyses were done in JPS Dairy Institute, Novi Beograd. During the production of probiotic yogurt we followed the activity of the yeast *Kluyveromyces fragilis* B0399 in different concentrations – 0.5%; 1%; and 3% and under different fermentation temperatures - 39◦C; 23,5◦C and 4◦C (in the cooled probiotic yogurt).

Among all studied conditions we managed to obtain the sufficient number of live yeast cells in the final product when adding 1% of Turval product during the fermentation phase on 23,5◦C, when the number of live cells is $3.5x10^7$cfu/g probiotic bacteria and $3.6x10^5$ cfu/g *Kluyveromyces fragilis* B0399. Clinical studies have shown that in order to exhibit its probiotic functions the daily uptake of the yeast *Kluyveromyces fragilis* has to be ≥10 millions of live cells (certified by the Italian Ministry of Health).

Sensor properties of this probiotic yogurt, odour, taste and colour, are preserved up to expiry date of 30 days. Final product is safe for use and has beneficial properties for good intestinal performance and general health of its consumers.

Key words: *Kluyveromyces marxianus*, *probiotic culture, Turval-B0399, yogurt*

Introduction

Food industry companies have rather high expectations in food products that meet the consumers' demand for a healthy life style. This especially addresses foods that are not intended only to satisfy hunger and provide humans with necessary nutrients, but also to prevent nutrition-related diseases and increase physical and mental wellbeing of consumers, so called "functional food" (Granato et al., 2010; Jankovic et al., 2010; Kaplan et al., 2014). One of the most promising areas for the development of functional foods lies in the modification of gastrointestinal tract activity by the utilization of beneficial microbes (probiotics) in diary products or other dietary supplements, which, when administrated in adequate amounts are aimed at promoting human health (Food and Agriculture Organization/World Health Organization (FAO/WHO), 2001; Jankovic et al., 2010). According to a new market report published by Transparency Market Research, "Probiotics Market: Global Industry Analysis, Market Size, Share, Trends, Analysis, Growth and Forecast", the global probiotic market has been in constant growth, of 7.6% over the previous 5-year period, and is expected to reach 22 billion euros by 2015 (Saxelin, 2008; Pedretti, 2012).

Probiotics have a documented therapeutic role in reducing certain human illnesses, particularly gastrointestinal diseases, caused by deficient or compromised gut microflora (Allen et al., 2004; Canani et al., 2007; Enck et al., 2011); anti-cholesterol activity and anti-high blood pressure effect (Lye et al., 2009; Jahreis, 2002); alleviation of lactose intolerance symptoms by active lactose hydrolysis (Yoshida et al., 2010), promotion of beneficial immune responses (Leyer GJ et al., 2009); beneficial skin effects and reduction of allergies' symptoms (Krutmann, 2009); antimicrobial, anticarcinogenic and anti-mutagenic activities (Orrhage et al., 1994; Rea et al., 2007). There are several proposed molecular mechanisms underlying these functions, including short-chain fatty acid (SCFA) production, the enhancement of the barrier function of the intestinal epithelium, the suppression of the growth and binding of pathogenic bacteria, and alterations of the immune activity of the host (Tuomola et al., 1999; Ventura et al., 2009; Aragon et al., 2010). Furthermore, probiotics can alter colonic fermentation and stabilize symbiotic microbiota (Spiller, 2008), improving the dynamic interplay between the resident bacterial community and the host.

Increasing evidence about the therapeutic potential of probiotics is substantiating constant research and selection of novel microbial species and strains with greater probiotic potential and better properties according to selection criteria (Havenaar et al., 1992), such as: total safety for the host, resistance to gastric acidity and pancreatic secretions, adhesion to epithelial cells, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, evaluation of resistance to antibiotics, tolerance to food additives and stability in the food matrix. The probiotics used today have not been selected on the basis of all these criteria, but the most commonly used probiotics are specific strains of lactic acid bacteria (LAB), belonging mainly to the genus *Bifidobacterium* or *Lactobacillus*. Less frequently used organisms are strains of *Propionibacterium freudenreichii*, bacilli, or yeasts (Wassenaar and Klein, 2008). *Kluyveromyces marxianus fragilis* (found also as *Kluyveromyces fragilis* or *Kluyveromyces marxianus*) is lactic yeast isolated from different dairy products, mainly

kefir (Zhou et al., 2009; Bolla et al., 2011). The importance of this species in food development and fermentation is well documented, while its probiotic activities have been recently well recognized for one strain, named *K. marxianus* B0399, isolated from whey and curds of cow's milk and deposited at the Belgian Coordinated Collection of Microorganisms (BCCM) (accession number MUCL 41579) by Turval Laboratories Italy. Well characterized, health-promoting, probiotic properties of this strain include: strong adhesion to mucosal surfaces and persistence in the intestine; capacity to finely tune the immune response by decreasing the production of the proinflammatory cytokines TNF-α, IFN-γ, IL-6 and the chemokines IP-10 and IL-8 (known to play a crucial role in host defense mechanisms) in PBMCs and Caco-2 cells in the presence of inflammatory stimuli such as LPS, IL-1β, or enteropathogenic bacteria (Maccaferri et al., 2012a and 2012b). Furthermore, two studies have independently proved that this yeast can improve the growth and survival of bifidobacteria in complex food matrices and proximal and transverse colon (Rada, 1997; Maccaferri et al., 2012). It is capable of survival during gastric transit, maintaining its vitality and fermentation capacity (Mustacchi et al., 2010). These scientific evidences are supporting the valuable therapeutic effects of this strain, seen in multiple clinical studies such as: Irritable Bowel Syndrome (Andreoli and Lovrovich 2009; Roda and Cornia 2009), Irritable Colon (Andreoli, 2006), candidiasis (Cettolo and Riul, 2006; Mustacchi et al., 2009), antibiotic-associated diarrhea (Vaughan, 2002) etc. For all these reasons, *K. marxianus* B0399 is the active ingredient in different functional foods currently marketed in several countries worldwide and it is included in the European Food Safety Authority (European Food and Safety Authority (EFSA), 2010) list of qualified presumption of safety (QPS) biological agents added to food and feed (EFSA, 2010). As such, this strain is of particular interest for the global functional food industry.

Since the highest consumption of probiotic products in Europe is associated with probiotic yogurt (Wassenaar and Klein, 2008), our aim in this work was to optimize the production conditions of the new generation, yogurt-like, probiotic dairy beverage, containing mixed probiotic population: yeast *Kluyveromyces fragilis* B0399 and probiotic bacteria *Lactobacillus acidofilus* LA5 and *Bifidobacterium lactis* BB12. We studied the *Kluyveromyces* growth and its influence on the growth of the probiotic bacteria. The final aim was to achieve the maximal number of live cells upon the production of probiotic yogurt ($>10^6$ cfu/g) and after a desired, 30 day, storage period. Production process was further optimized to reach both the 'therapeutic minimum' of live *Kluyveromyces* B0399 cells in the final product ($> 8x10^4$ cfu/g of 125g yogurt package or $> 1x10^7$ cfu/diem, indicated by the Italian Ministry of Health (Bottona et al., 2008)) as well as the lower cost of the production process, acceptable for the Serbian market.

Materials and methods

Milk

Pasteurized and homogenized milk with 1.6% of milk fat was used for the production of fermented dairy beverages. Milk was taken from "Mlekara Dana", Vrbas, and the quality of milk was in accordance with "The regulations on the quality of raw milk" (the Official Gazette of the RS 21/2009) and "The regulations on the quality of dairy products and starter cultures" (the Official Gazette of the RS, 33/2010 and 69/2010).

Starter cultures

Two different types of starter cultures were used for the production of the fermented, probiotic, dairy beverages. A classic probiotic yoghurt (commercial name) was produced

with mixed, ABY-6 starter culture (Chemibiotech s.r.l, Italy), which contains classical yogurt strains: containing *Lactobacillus delbreukii* subsp.*bulgaricus* and *Streptococcus salivarius* subsp.*thermophiles,* as well as two probiotic strains, *Lactobacillus acidofilus* LA5 and *Bifidobacterium lactis* BB12. In addition, the new generation probiotic product was produced using bacterial ABY-6 starter together with a non-conventional, semi-processed, liquid yeast starter, TURVAL B0399® (Turval Laboratories, Udine, Italy). Turval B0399 starter contains probiotic lactic yeast strain *K. marxianus* B0399 in the concentration $\geq 1{,}4x10^7$ yeast cells/ml.

Production of fermented dairy beverages

Milk used for the production of probiotic beverages is low fat milk, standardized to 1.6% milk fat, homogenized and pasteurized initially at 72° for 20s, subjected to bio-chemical analysis of the milk quality, then additionally pasteurized at 92-96°C for 10 and cooled to the inoculation temperature. During the production of the new generation probiotic yogurt, yeast *K. marxianus* B0399 was inoculated in three different concentrations – 0.5%, 1% and 3%, and under different fermentation temperatures – 39°C, 23.5°C and 4°C (in the cooled fermented product). Precisely, in 1000 ml of milk, cooled to a certain inoculation temperature, bacterial and yeast inoculum were added in three different ways as follows: A) alongside, both cultures were added to heated milk, at a temperature of 39°C; or B) alongside, both cultures were added to milk at room temperature (23.5°C); and C) separately, bacterial inoculum was added to heated milk, at a temperature of 39°C while yeast inoculum was added at the end of the fermentation process, in the cooled product (4°C), before mixer homogenization.

In all cases fermentation was interrupted when the fermented product reached 4.75-4.65 pH level and 32-36°SH, which means 4h for 39°C fermentation and about 24h for 23.5°C fermentation.

Fruit containing, yogurt-like fermented milk products with probiotic properties were made in two ways: a) a classical 'fruit containing probiotic yogurt' was made by inoculating ABY-6 probiotic starter culture and 39°C while the new generation 'fruit containing probiotic yogurt' was made as previously described for B). In all cases, according to the manufacturer's suggestion, 10% of fruit paste was added to the cold product (4°C) before mixer homogenization. Apart from 52% of blended strawberries, fruit paste contained small amount of sucrose, water, citric acid, colors and aromas.

All previously mentioned products, containing *K. marxianus* B0399, were further optimized in order to achieve both the 'therapeutic minimum' of live yeast cells in the final product ($\geq 8x10^4$ cfu/g for 125g yogurt package) and the lowest production cost. This optimization was done by varying the size of yeast inoculum form 3% to 0.5%.

ABY-6 probiotic starter culture was added to achieve a concentration of 0.005% in manufacturing yogurt samples.

Products were stored for 30 days in a fridge, at the temperature of 4-8ºC.

Analysis of milk and fermented dairy products

Chemical composition of milk was analyzed right before the yogurt production:

- dry matter was measured with direct method at the temperature of 102±1 ºC;
- milk fat was measured by the Gerber method; - total proteins by Kjeldahl method.

Organoleptic (taste, smell, colour, consistency) and physicochemical analysis of all products were performed on the day 1 after production, and on the day 30 after production.

For this purpose standard methods of analysis were used: active acidity was measured with pH meter and titratable acidity was measured by Soxhlet-Henkel method.

Microbiological analysis (probiotic bacteria and yeast count) was also performed at two previously mentioned time points (day 1 and day 30 after production). Sample suspensions and serial dilutions were prepared in accordance with the standards defined by ISO 6887-5:2010 (Microbiology of food and animal feeding stuffs -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 5: Specific rules for the preparation of milk and milk products). The number of live *K. marxianus* B0399 cells was determined by the colony count method according to the standards defined by ISO 21527-1:2008 (Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0.95). Similarly, the number of probiotic bacteria, *L. acidofilus* LA5 and *B. lactis* BB12, was determined by colony-count technique described in ISO 20128:2006 and ISO 29981:2010 standards respectively (Milk products -- Enumeration of presumptive Lactobacillus acidophilus on a selective medium/ Enumeration of presumptive bifidobacteria -- Colony-count technique at 37 degrees C).

Results and discussion

Production of the innovative, plane, dairy probiotic beverage containing *K. marxianus* B0399

Table 1 shows the 'chemical composition of milk used for the production of probiotic beverages'.

Table 1. *Chemical composition of milk used for the production of probiotic beverages*

Milk fat (g/100g)	Total proteins (g/100g)	Total lactose (g/100g)	Dry matter without fats (g/100g)	Dry matter (g/100g)	Somatic Cells (*1000/ml)
1.59	3.32	4.53	8.61	10.185	149

Probiotic species in a specific probiotic food carrier should be alive to an adequate number in order to exert their positive effects on the health of the host. This attribute is known as 'viability' or 'therapeutic minimum' in literature, defined by the adequate number of live probiotic cells in a food product at the time of consumption (Korbekandi, 2011). Various factors have been recognized to affect the viability of probiotic species during storage of fermented dairy products such as interaction between species present, culture, fermentation time and temperature, level of inoculation, carbohydrate source in the fermentation medium, final acidity, dissolved oxygen, and storage time and temperature (Korbekandi et al., 2011; Mohammadi and Mortazavian, 2011). While synergic growth-promoting effects between *L.acidophilus* and *Bifidobacterium* species are well documented (Kneifel et al., 1993) there is no data in the literature about the co-inoculation effect of kefir-derived yeast *K. marxianus* on the survival of these two probiotic bacteria. We tested this effect by comparing the number of probiotic bacteria between two products: i) fermented product made by co-inoculation of 'ABY6' bacterial culture and 'Turval' yeast culture and ii) yogurt fermented with ABY6 only, where yeast culture was added at the end of the production process, in the cooled coagulum. While in the first product the milk is used as

the growth medium for both probiotic bacteria and yeast, in the later product it is the classical 'probiotic yogurt' that is used as a carrier for the innovative probiotic lactic yeast.

Data in Table 2A ('Maximising number of probiotic species by varying fermentation temperature') show that the presence of the yeast strain *K. marxianus* B0399, when added post-fermentation, (in the cooled coagulum) does not significantly influence the final count of probiotic bacteria. The results in Table 2A also suggest that it is rather a long fermentation time (24h) at low temperature (23°C±1°C), which is beneficial for both bacterial, and yeast growth during the production process. This result is supported by the literature data that lower incubation temperature favours the growth of bifidobacteria (Kneifel et al., 1993), and it also shows that the room T was better choice for *K. marxianus* growth, while typical fermentation T of 39°C seemed to even negatively effect the number of *K. marxianus* cells in the final product. This result is in agreement with the multi year fermentation practice of kefir products containing *K. marxianus* strain (Nambou et al., 2014).

Table 2A. *Maximising number of probiotic species by varying fermentation temperature*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temperature	Fermentation Time	Probiotic culture added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/ml)
ABY-6 culture	**39°C**	**7h**	/	**8.1×10^6**	/
ABY-6 culture + **3%** Turval	**39°C**	**7h**	/	**7×10^6**	**2×10^5**
ABY-6 culture	**39°C**	**7h**	3% Turval	**7.5×10^6**	**3.75×10^5**
ABY-6 culture + 3% Turval	**23.5°C**	**24h**	/	**9.4×10^6**	**9.5×10^5**

Table 2B. *Maximising number of yeast cells by varying inoculum size of Turval B0399*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temp.	Fermentation Time	Probiotic culture added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/ml)
ABY-6 culture + **1%** Turval	**39°C**	**7h**	/	**7.5×10^6**	**4.8×10^4**
ABY-6 culture	**39°C**	**7h**	1% Turval	**7.1×10^6**	**9×10^4**
ABY-6 culture + 1% Turval	**23.5°C**	**24h**	/	**3.5×10^7**	**3.6×10^5**
ABY-6 culture + 0.5% Turval	**23.5°C**	**24h**	/	**1.9×10^7**	**2.8×10^5**

After we identified the ideal fermentation conditions for our mixed microbial population we tested whether it was possible to decrease the yeast inoculum size with respect to the initial 3% concentration and thus decrease the production cost of the new probiotic product. The 3% inoculum size is the Turval concentration that should be applied when the product is made by adding Turval in the cooled coagulum (4°C) in order to satisfy the 'therapeutic minimum' at the time of the consumption ($\geq 1 \times 10^7$ live yeast cells/diem) (Bottona et al., 2008). We hypothesized that by co-inoculating 1% or even 0.5% of yeast culture under favourable fermentation conditions (24h at room temperature) we could still

obtain the *Kluyveromyces* 'therapeutic minimum' in the final product because of the yeast cell proliferation under these conditions. The results presented in Table 2B ('Maximising number of yeast cells by varying inoculum size of Turval B0399') confirm this hypothesis. Even though 0.5% of inoculum gave the satisfactory number of yeast cells in the final product ($2.8x10^5$ /ml) we recommend 1% yeast inoculum as the best compromise between the high cell number and acceptable production cost (to compensate for the possible decline in the concentration of the probiotic organisms during storage of a probiotic). To question whether the number of probiotic bacteria declines below the proposed 'therapeutic minimum' during refrigerated storage, we investigated the viability of probiotic species (yeast and bacteria) in yogurts stored in cold, 30 days after production.

Table 3. *Microbiological analysis of the new generation fruit probiotic dairy beverage*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temp.	Fermentation Time	Fruit paste added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/m)
ABY-6 culture	**39°C**	**7h**	**10%**	**$8.1x10^6$**	**-**
ABY-6 culture + 1% Turval	**23.5°C**	**24h**	**10%**	**$2.9x10^7$**	**$3.3x10^5$**

Table 4. *Stability of physico-chemical characteristics of the new generation probiotic dairy beverages during long term (30 days), refrigerated storage*

Sample type: Probiotic cultures	Fermentation Temp, Time	pH value	Titratable acidity (°SH)	pH value	Titratable acidity (°SH)
		1st day after production		**30th day after production**	
ABY-6 culture	39°C, 7h	4.77	32.0	4.58	35.0
ABY-6 culture + **1% Turval**	23.5°C, 24h	4.72	33.0	4.60	35.0
ABY-6 culture + **0.5% Turval**	23.5°C, 24h	4.73	32.0	4.60	35.0
ABY-6 culture + **10% fruit paste**	23.5°C, 24h	4.79	32.0	4.18	39.0
ABY-6 culture + **1% Turval + 10% fruit paste**	23.5°C, 24h	4.68	34.0	4.20	39.0

The results are shown in Table 5 ('Microbiological analysis of the new generation probiotic dairy beverages after 30 days of refrigerated storage'), showing 1 log decrease in the number of live probiotic bacteria after long-term storage and a lesser increase in the yeast count. Likewise, the viability of probiotic bacteria in products over a long shelf life at refrigeration temperature has been reported before to be unsatisfactory (Rybka and Kailasapathy, 1995, Lourens-Hattingh and Viljoen, 2001b). This is mainly due to a certain level of the 'over-acidification' during storage, which if it reaches pH values under 4.6 may be harmful for probiotic species, particularly for bifidobacteria (Kailasapathy and Chin, 2000, Lourens-Hattingh and Viljoen, 2001b). As shown in Table 4 ('Stability of physico-chemical characteristics of the new generation probiotic dairy beverages during long term (30 days), refrigerated storage') the acidity of both classical probiotic yogurt

(ABY-6 species) and innovative, lactic yeast-based probiotic product (ABY-6 culture + Turval) decreased to pH 4.6 over the storage period. This could explain the slight decrease in the viability of probiotic bacteria, shown in Table 5, although the final count remained acceptable (above the therapeutic minimum). On the other hand, the ability to hydrolyse the residual lactose, utilisation of glucose and galactose produced by LAB and resistance to the low pH of *Kluyveromyces* cells are all plausible explanations for a minor increase in the yeast cell number over a long storage period (Table 5). However, this did not interfere with the sensorial characteristics of the product, which remained the same during the whole storage period.

Table 5. *Microbiological analysis of the new generation probiotic dairy beverages after 30 days of refrigerated storage*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temp.	Fermentation Time	Probiotic culture or Fruit paste added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/ml)
ABY-6	**39°C**	**7h**	/	$1.3x10^6$	/
ABY-6 + 1% Turval	**23.5°C**	**24h**	/	**$1.3x10^6$**	**$6 x10^5$**
ABY-6	**39°C**	**7h**	**10% fruit paste**	$2.7x10^6$	/
ABY-6 + 1% Turval	**23.5°C**	**24h**	**10% fruit paste**	**$1.5x10^6$**	**$2.5x10^6$**

Production of the innovative fruit dairy probiotic beverage containing *K.marxianus* B0399

The 1% Turval B0399 inoculum was further used to produce an alternative, fruit-containing, diary probiotic product by its co-inoculation with ABY-6 culture under optimal fermentation conditions (RT, 24h). As shown in Table 3 ('Microbiological analysis of the new generation fruit probiotic dairy beverage') the number of yeast cells in the product was satisfactory and similar to its concentration in the plane yogurt (>$3x10^5$/ml). Sensorial characteristics of this product were similar to the classical 'fruit probiotic yogurt' (ABY-6 culture, 39°C fermentation temp.), while the number of probiotic bacteria in the Turval-based product is slightly bigger than in the classical one, similarly to the plane probiotic products described previously (probably the result of the preferable fermentation conditions). In addition, we investigated the effect of commercial strawberry preparations on the viability of probiotic bacteria and probiotic yeast during storage (30 days) at refrigerated temperature. Again, we could observe the correlation between the post-storage over-acidification of fruit beverages (Table 4) and slightly decreased viability of probiotic bacteria (Table 5). While the number of probiotic bacteria in the fruit products slightly decreased over time but still remained satisfactory until the expiry date (30 days), the yeast cell viability showed exactly the opposite, increasing trend (Table 5). This 1 log increase in the yeast count is mainly due to the presence of proportions of sucrose and fructose derived from the fruit (Kailasapathy et al., 2008). Still, this did not interfere with the sensorial characteristics of the product, which remained the same in terms of taste and odour while a fairly recognisable decrease in acidity could be noticed, just like for the classical 'fruit probiotic yogurt' without lactic yeast.

Conclusion

The results obtained in this study demonstrated that fermented dairy products are great choice as food-carriers for the new generation probiotic yeast strain, *K. marxianus* B0399.

The sensory properties such as: appearance, texture, flavour and overall quality of probiotic dairy beverages containing Turval B0399 probiotic lactic yeast were comparable with standard 1.6% fat probiotic yogurt and they remained stable during the 30 day storage period. The pH values in all samples decreased during storage, where a bit higher dynamics could be observed for the fruit-containing yogurt and was not attributable to the presence of yeast. These innovative products are lacking yeasty flavour typical for dairy products containing Saccharomyces species (Lourens-Hattingh and Viljoen, 2001a), which may be explained by a distinct diapason of aroma compounds produced by *K.marxianus* strain (Fonseca, 2008). Even though it was not directly measured in this study, it is important to mention that *K. marxianus* B0399 produces very small amount of CO2 and ethanol at the end of 35-day shelf life of the commercially available, Italian branded, fruit based bio-yogurt EUFYR (Coop Italia, personal communication). This can be explained by strictly anaerobic metabolism of ethanol production of this yeast strain (Fonseca et al., 2008), eliminating what was known to be the major constraints for incorporating another probiotic yeast strain, *Saccharomyces boulardii* into bio-yogurt (Lourens-Hattingh and Viljoen, 2001a).

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