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Lactic acid beverage based on wort and mint (*Menta piperita* L.)

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A new type of wort-based beverages with addition of mint in concentrations of 0.5 – 1.5 g/dm^3, fermented by probiotic lactic acid bacteria strains *Lactobacillus casei* ssp. *paracasei* PX3 and *Lactobacillus casei* ssp. *rhamnosus* LBRC11 was developed. The influence of the mint dose on the phenolic content, the antioxidant capacity and the fermentation dynamics was studied. Both lactobacilli strains grew well in wort with mint at each experimental mint dose and accumulated a sufficient number of viable cells necessary to achieve the desired probiotic effect. Mint addition led to an increase in the biological value of the beverages because the total phenolic content rose up to 22 % and the antioxidant capacity rose up to 53 %. The increase in the mint dose by 0.5 g/dm^3 was not sufficient to achieve a significant increase in the phenolics content, but it was enough to cause an increase in the antioxidant capacity. It can be hypothesized that the antioxidant capacity of the developed beverages was not determined only by the phenolic compounds. The maximum phenolic content and antioxidant capacity were observed at the maximum mint amount (1.5 g/dm^3).

Keywords: wort, mint, lactic acid fermentation, antioxidant capacity, phenolic content

INTRODUCTION

Functional foods contain not only the necessary nutrients for the body but also ingredients that contribute to improving the individual's health. One of the approaches to the development of functional foods involves the addition of components that are not present in most foods and which are not nutrients, but have proven a positive effect on the body, such as plant extracts, probiotic microorganisms, etc. [1].

In the last decade, articles related to the effect of fermentation on phenolic compounds and antioxidant activity in plant-based foods, the interaction between food phenolics and lactic acid bacteria, the antioxidant activity of lactic acid bacteria, cereal-based functional fermented food and drinks have appeared [2-5].

Fermentation of cereals such as oats, corn and wheat under the action of probiotic microorganisms is useful because it provides better digestibility of food. [6]. There is evidence in the literature that the survival of lactic acid bacteria is significantly improved by the use of barley malt as a basis for beverage production [7, 8].

Peppermint (*Menta piperita* L.) is a very widespread plant, which has a pleasant aroma and contains pharmacologically active ingredients. There are many articles related to the antioxidant and antimicrobial activities, the phenolic content and the essential oil of peppermint [9-11].

Wort from barley malt has a high nutritional value and is a suitable medium not only for alcoholic fermentation. However, scarce information is still available on the lactic acid fermentation of wort in the presence of herbs [2].

The two strains used in the present research have proven probiotic properties - antimicrobial activity against pathogenic and saprophytic microorganisms, antibiotic resistance and resistance to different concentrations of bile salts and different pH values, possibility for conduction of industrial processes (fermentation, freeze-drying) with accumulation and maintaining of a high concentration of viable cells, thus being very suitable for the development and production of functional beverages (unpublished data). Some members of the current scientific team behind the present research have worked on the examination of some probiotic properties of other representatives of the species *Lactobacillus casei*, proving their

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probiotic potential like antibiotic resistance and resistance to different concentrations of bile salts and different pH values [12], possibility for conduction of industrial processes (fermentation, freeze-drying) with accumulation and maintaining of a high concentration of viable cells [13].

The aim of the present study was to investigate the influence of mint (*Menta piperita* L.) addition on the fermentation dynamics, the antioxidant activity and the phenolic content of lactic acid beverages based on wort. The aim of the study will be achieved by studying the fermentation process of wort-based beverages with mint fermented by two *Lactobacillus casei* strains (*Lactobacillus casei* ssp. *rhamnosus* LBRC11 and *Lactobacillus casei* ssp. *paracasei* PX3).

EXPERIMENTAL

Materials

Pilsner malt from WEYERMANN, Germany was used. The malt was ground on a Corona hand mill. Dried milled peppermint (*Menta piperita* L.), bought from a local market was used. The used probiotic lactic acid bacteria (LAB) strains were *Lactobacillus casei* ssp. *rhamnosus* LBRC11 and *Lactobacillus casei* ssp. *paracasei* PX3. They are part of the culture collection at the department of Microbiology at the University of Food Technologies, Plovdiv.

Folin-Ciocalteu's phenol reagent and gallic acid were supplied by Merck - Germany. Trolox, DPPH radical and TPTZ were purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals used were of analytical grade and were obtained from local producers.

Experimental design

Wort was obtained from barley malt by an infusion method using a brewery equipment Braumaister 20L of Speidel Germany. The wort was boiled for 10 min after mashing and lautering. The wort extract was 11.4°P. Immediately after boiling, peppermint was added to the wort at three different concentrations - 0.5 g/dm^3; 1.0 g/dm^3 and 1.5 g/dm^3. The mint was left to macerate for 30 min. After that the wort was filtered through cloth filter, cooled and inoculated with LAB. The wort without mint was inoculated too and served as a control. The quantity of the LAB suspension was 5 % of the wort volume. The initial concentration of viable LAB cells in all beverage variants was 10^7 CFU/cm^3. The fermentation was carried out at 15°C for 7 days.

Analyses

Sample preparation. Before determination of the total phenolic content and the antioxidant capacity, the samples were frozen and stored in a freezer until the day of analysis. After the samples were defrosted, they were treated with methanol, filtered to remove formed precipitates and diluted properly with methanol.

Wort extract and pH. Wort extract was determined using a pycnometer according to Analytica – EBC, Method 8.3 (2005). The pH value was determined using Sartorius PB-11 pH meter according to Analytica – EBC, Method 8.17 (2005) [14].

DPPH radical scavenging activity. The ability of the samples to scavenge DPPH radicals was determined by the modified method of Brand-Williams *et al.* (1995) [15]. Freshly prepared 6×10^{-5} M solution of DPPH was mixed with the diluted sample at a ratio of 9:1 (v/v). After 30 min incubation at room temperature, the absorption was measured at 517 nm. The DPPH radical scavenging activity was defined as a function of the Trolox concentration having equivalent antioxidant activity and was expressed as μmol TE per dm³ sample.

Ferric-reducing antioxidant power assay (FRAP). The FRAP assay was carried out according to the procedure of Benzie and Strain (1999) [16]. The suitably diluted sample (150 μl) was mixed with 2850 μl of FRAP reagent, allowed to react for 4 min at room temperature and the absorbance was measured at 593 nm. The results were expressed as μmol TE per dm³sample.

*Total phenolic compounds (TPC). T*he FC reagent according to ISO 14502-1 [17] and a version of the Glories method modified by Mazza *et al.* (1999) were used [18]. The diluted sample was mixed with 0.1% HCl solution in 96% ethanol and 2% solution of HCl at a ratio of 1:1:18.2 (v/v/v). After incubation for 15 min at room temperature the absorbance at 280 nm was measured. The calibration curves were prepared using gallic acid and the total phenolic content was expressed as g gallic acid equivalent (GAE) per dm³.

Determination of the concentration of viable lactobacilli cells. Appropriate ten-fold dilutions of the samples taken from the relevant fermentation stage were prepared. Amounts of 0.1 cm^3 from the last three dilutions were used for spread-plating on LAPTg10-agar. The inoculated Petri dishes were incubated at 37±1°C for 48-72 hours until the appearance of countable single colonies. The number of single colonies was used to estimate the

concentration of viable lactobacilli cells in the test sample.

The concentration of viable cells and the pH value were determined during the fermentation process – immediately after inoculation, on the first, fourth and seventh day of fermentation.

Statistical analysis. The obtained data were analyzed statistically using MS-Excel 2010 software. The analyses were performed in triplicate. The results were expressed as mean value ± standard deviation. One-way ANOVA and Scheffe's multiple range test at $p<0.05$ as described by Donchev *et al.* [19] were used.

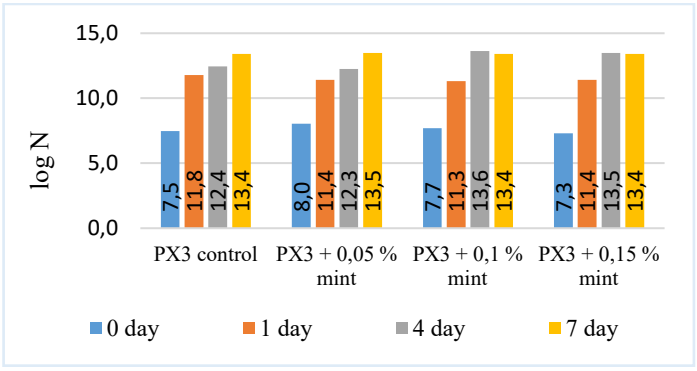
RESULTS AND DISCUSSION

In an initial series of experiments, both strains of lactic acid bacteria grew well on wort. Beverages with very good sweet/sour balance, with pronounced freshness and harmony were obtained as a result of fermentation. They were evaluated by an organoleptic panel as the most preferred among all beverages obtained by fermentation with different strains of different species of the genus *Lactobacillus* (unpublished data).

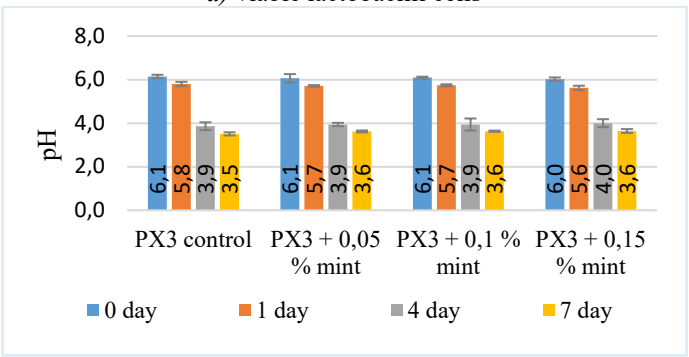
Development of a new type of wort-based beverages with mint addition fermented by L. paracasei PX3 or L. rhamnosus LBRC11

During the first day, an exponential phase in the growth of *L. paracasei* PX3 in all variants was observed, as the concentration of added mint did not significantly affect the reproduction and growth of the strain (Fig. 1). After the first day, the concentration of viable cells increased significantly and reached 10^{11}CFU/cm^3, at an initial concentration after inoculation of 10^7 CFU/cm^3 (the change was 4logN). Then their concentration continued to increase, and at the end of the process it reached 10^{13} CFU/cm^3. At higher mint doses (1.0 g/dm^3 and 1.5 g/dm^3), the entry of the cells into the stationary phase was after the fourth day of fermentation.

The pH of the medium in all beverage variants with *L. paracasei* PX3 gradually decreased from the beginning of the fermentation process (Fig. 1).



a) viable lactobacilli cells

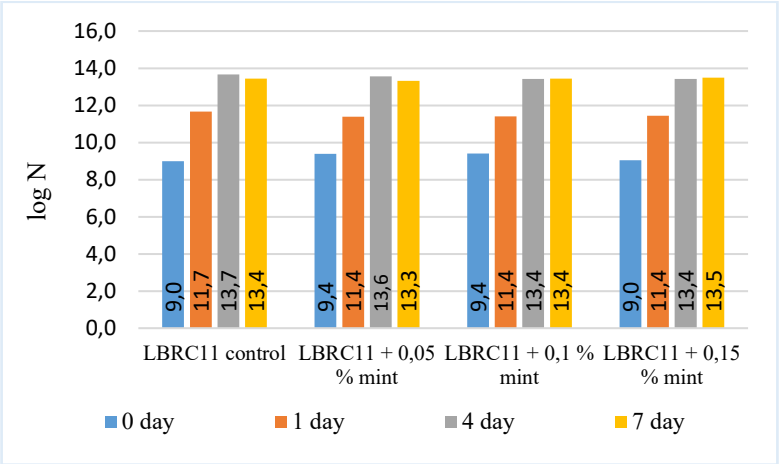


	control	with 0,05% mint	with 0,10% mint	with 0,15% mint
0 day	a	a	a	a
1 day	b	b,c	b,c	c
4 day	d	d	d	d
7 day	e	e	e	e

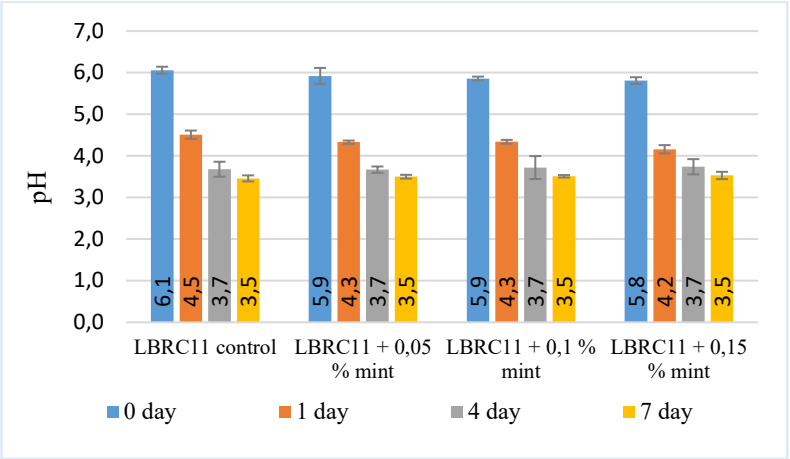
The same letter for a given sample means no significant differences (95% confidence level)

b) pH value

Fig. 1. Changes in the concentration of viable lactobacilli cells and pH value during lactic acid fermentation with *Lactobacillus casei* ssp. *paracasei* PX3



a) viable lactobacilli cells



	control	with 0,05% mint	with 0,10% mint	with 0,15% mint
0 day	a	a	a	a
1 day	b	b, c	b, c	c
4 day	d	d	d	d
7 day	d	d	d	d

The same letter for a given sample means no significant differences (95% confidence level)

b) pH value

Fig. 2. Changes in the concentration of viable lactobacilli cells and pH value during lactic acid fermentation with *Lactobacillus casei* ssp. *rhamnosus* LBRC11

Due to the buffering nature of wort on the first day, the change in pH was within 0.3-0.4 units, then by the end of the fermentation process the total decrease in pH was 1.6-1.9 units. As a result of the fermentation process, the pH decreased from 6.0-6.1 to 3.5-3.6 for the different wort/mint combinations.

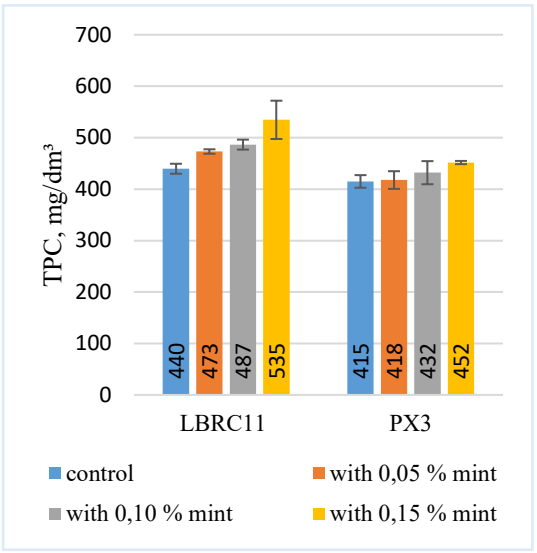
Within 24 hours of the start of the fermentation by *L. rhamnosus* LBRC11 the concentration of viable cells increased by two logarithmic units, and in the next 96 hours by two more logarithmic units (Fig. 2). After the fourth day, there was an entry into the stationary phase in all experimental variants. *L. rhamnosus* LBRC11 accumulated a high concentration of viable cells, regardless of the experimental combination of wort and mint.

Regardless of the specific percentage of added mint, the variants fermented by *L. rhamnosus* LBRC11 retained lower pH values (Fig. 2) compared to the beverages obtained with *L. paracasei* PX3. The pH value of the beverages with *L. rhamnosus* LBRC11 on the day of inoculation ranged from 5.8 to 6.1. Within 24 hours, the pH dropped sharply in all experimental variants. The pH values on the fourth and the seventh day of fermentation were comparable. In all variants of beverages fermented by *L. rhamnosus* LBRC11 a pH = 3.5 was reached at the end of the fermentation process. Both strains grew well in a medium of wort with mint added in amounts between 0.5 g/dm^3 and 1.5 g/dm^3 accumulating a sufficient amount of viable cells necessary to achieve the

desired probiotic effect upon consumption of the beverages. In the first 24 hours, both strains accumulated a significant amount of viable cells. In the fermentation with the strain *L. rhamnosus* Oly a slightly lower concentration of biomass was reported during the process [2], but in the end the biomass amount was equalized with that in the fermentation with the strains *L. rhamnosus* LBRC11 and *L. paracasei* PX3. In *L. rhamnosus* LBRC11 and *L. paracasei* PX3 the stationary phase was reached after the fourth day, while in the strain *L. rhamnosus* Oly biomass accumulation continued until the end of the process [2]. The fermentation process proceeded normally, organic acids, mainly lactic acid, accumulated and the pH of the medium decreased. *L. rhamnosus* Oly accumulated the least amount of acids and lowered the acidity of the medium slightly [2], while the greatest amount of acids accumulated in the fermentation with *L. rhamnosus* LBRC11.

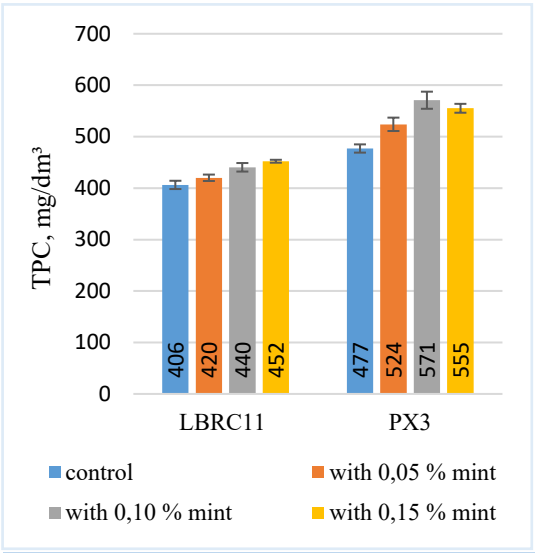
Phenolic content and total antioxidant capacity of beverages based on wort and mint, fermented by L. paracasei PX3 and L. rhamnosus LBRC11.

The total phenolic content determined by the direct spectral method (Glories method) in the beverages with mint and fermented by *L. paracasei* PX3 (Fig. 3) was between 10% and 20% higher than that of the beverage without mint. The increasing of the mint dose between 0.5 g/dm^3 and 1.0 g/dm^3 by a step of 0.5 g/dm^3 led to a significant increase in the phenolic content. The beverages with mint added in amounts of 1.0 g/dm^3 and 1.5 g/dm^3 had almost equal phenolic content. No significant change in the results obtained with the FC reagent (Fig. 3) when mint was added at doses up to 1.0 g/dm^3 was observed. Only the beverage with 1.5 g/dm^3 mint had 9% higher phenolic content compared to the control.



	control	with 0,05% mint	with 0,10% mint	with 0,15% mint
LBRC 11	a	a, b	b	
PX3	c	c	c, d	d

a) colorimetric method with Folin-Ciocalteu reagent



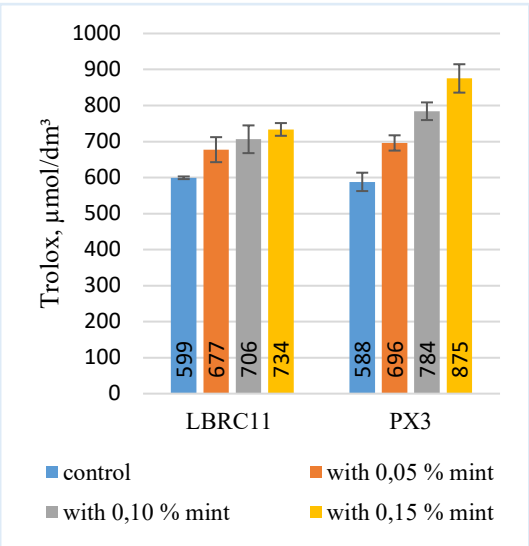
	control	with 0,05% mint	with 0,10% mint	with 0,15% mint
LBRC 11		a	a, b	b
PX3			c	c

b) Glories method

The same letter for a given sample means no significant differences (95% confidence level)

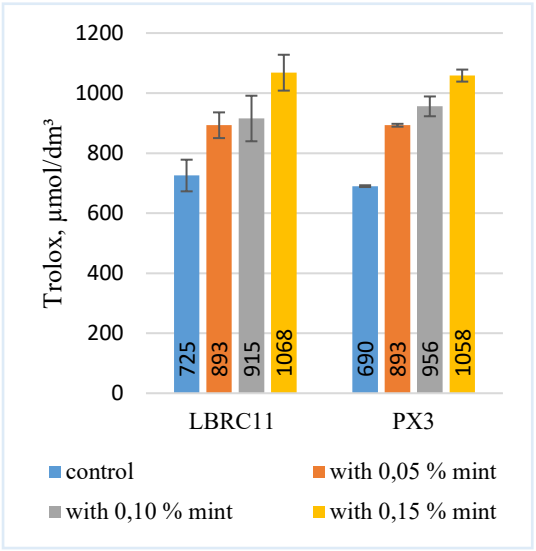
Fig. 3. Changes in the total phenolic content (TPC) during fermentation by *Lactobacillus casei* ssp. *rhamnosus* LBRC11 and *Lactobacillus casei* ssp. *paracasei* PX3

The mint addition and the increase in the mint dose in the beverages fermented by *L. paracasei* PX3, resulted in an increase of the beverage ferric-reducing antioxidant power (FRAP) between 29% and 53% compared to the control sample (Fig. 4). A similar trend was observed in the DPPH radical scavenging activity of the beverages (Fig. 4). It was by 18 – 49 % higher compared to the control and it increased with the increase in the amount of the mint.



	control	with 0,05% mint	with 0,10% mint	with 0,15% mint
LBRC 11		a	a,b	b
PX3				

a) DPPH assay



	control	with 0,05% mint	with 0,10% mint	with 0,15% mint
LBRC 11		a	a	
PX3				

b) FRAP assay

The same letter for a given sample means no significant differences (95% confidence level)

Fig. 4. Changes in the total antioxidant capacity during fermentation by *Lactobacillus casei* ssp. *rhamnosus* LBRC11 and *Lactobacillus casei* ssp. *paracasei* PX3

The mint addition to the beverages fermented with *L. rhamnosus* LBRC11 was accompanied by an increase in the total phenolic content determined by the Glories method (Fig. 3). Compared to the control, it was by 8 - 11% higher. It turned out that the increase in the mint amount by 0.5 g/dm^3 did not lead to significant changes in the total phenolic content, but the increase in the mint dose by 1.0 g/dm^3 was sufficient for an increase in the total phenolics. A similar trend was observed in the results for the total phenolic content determined by the FC reagent (Fig. 3). The phenolic concentration was by 8 - 22% higher compared to the control sample and it was necessary to increase the mint dose by 1 g/dm^3 to have a significant increase in the total phenolic content.

The DPPH radical scavenging activity of the beverages with mint (Fig. 4) was by 13 – 22% greater than that of the control. More significant increase in the radical scavenging activity was observed when the mint dose rose from 0.5 g/dm^3 to 1.5 g/dm^3. A similar trend was established in the results from the FRAP assay (Fig. 4). The ferric-reducing antioxidant power of the beverages with mint was by 23 – 47 % higher compared to the control. The beverages with 0.5 g/dm^3 and 1.0 g/dm^3 mint did not show any significant difference in the FRAP value.

The obtained results for the total phenolic content determined by both methods showed that the addition of mint to wort led to an improvement in the biological value of the lactic acid beverages due to the higher content of phenolic compounds.

The increase in the total phenolic content was not proportional to the increasing values of the mint dose. In most cases, the increase in the mint amount by 0.5 g/dm^3 did not lead to a significant difference in the total phenolic content. A significant increase was achieved by increasing the mint dose by 1.0 g/dm^3. The observed trend was probably due to the different processes that occur when adding mint to wort and because of the influence of the lactic acid fermentation. On one side, various substances were extracted from mint, including phenolic compounds. On the other side, extracted compounds fall into a protein-rich environment, which is a prerequisite for the formation of insoluble protein-phenolic complexes [20]. In addition, it was possible that the cell wall of *L. rhamnosus* LBRC11 and *L. paracasei* PX3 adsorbed some of the phenolic compounds [21]. Therefore, in some cases, the increase in the mint dose by 0.5 g/dm^3 was not sufficient to cause an increase in the total phenolic content of the beverages despite the higher mint amount for extraction.

The differences in the total phenolic content determined by FC and Glories methods can be explained by the features of both methods. On one side, the Glories method determines the amount of oxidized and non-oxidized phenolics, unlike the FC method which determines the amount of only non-oxidized phenolics. On the other side, the FC reagent is not specific only to the phenolic compounds. It can react with many other substances such as ascorbic acid, monosaccharides, aromatic amines, etc. [22].

It is well known that phenolic compounds possess DPPH radical scavenging activity and ferric-reducing antioxidant power. However, they are not the only compounds in wort and mint that have such abilities. Wort is rich in peptides, which also have antioxidant properties [23]. Peppermint essential oil has iron-reducing and DPPH scavenging activities as well [10, 11]. The addition of mint to the boiling wort was a prerequisite for extracting some of the mint essential oil into the resulting beverages. The presence of compounds with antioxidant activity other than phenolics can be explained by the observed differences between the changes in the total phenolic content on one side and the values of the FRAP and the DPPH assays on the other side. The use of different strains to carry out lactic acid fermentation had certain impact as well. The increase in the mint dose by 0.5 g/dm^3 led to a significant growth in the antioxidant capacity of the beverages fermented by *L. paracasei* PX3. The same trend was observed in the fermentation by *L. rhamnosus* Oly [2], but the antioxidant capacity of the beverages fermented by *L. rhamnosus* LBRC11, especially the value determined by the DPPH assay, was not affected significantly when the mint dose rose by 0.5 g/dm^3.

CONCLUSION

Wort-based beverages with mint added in concentrations between 0.05 g/dm^3 and 0.15 g/dm^3, fermented under the action of two probiotic strains of the species *Lactobacilus casei* - *Lactobacillus casei* ssp. *rhamnosus* LBRC11 and *Lactobacillus casei* ssp. *paracasei* PX3 were prepared. It was found that both strains of lactic acid bacteria grew well in wort medium with added mint in all experimental concentrations, accumulating a sufficient number of viable probiotic lactobacilli cells necessary for the manifestation of the probiotic effect upon consumption. Their growth was strain-specific. Fermentation with both strains proceeded normally with accumulation of organic acids and lowering of the pH of the medium from 6.1 to 3.5.

The addition of mint (*Menta piperita* L.) and the increase in its dosage between 0.5 g/dm^3 and 1.5 g/dm^3 led to higher antioxidant capacity and total phenolic content in the obtained lactic acid beverages based on wort. Thus, the biological value of the finished beverages rose. Probably, the degree of the antioxidant capacity and the phenolic content changes were affected by different factors like extraction processes, formation of water-insoluble complexes, variety of the lactobacilli strains and adsorption of some phenolics onto the lactobacilli cell walls. Additional comprehensive investigations are needed to clarify the role of multiple mechanisms and the interactive influence of different factors on the observed changes in the phenolic content and the antioxidant capacity.

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