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Research Article

Investigation of the antimicrobial activity of polyphenol-enriched extracts against probiotic lactic acid bacteria

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Abstract

The antimicrobial activity of polyphenol-enriched extracts from industrial plant by-products (strawberry and bilberry press residues and distilled rose petals) against probiotic lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus* S10 and S19; *Lactobacillus rhamnosus* YW and S25; *Lactobacillus gasseri* S20; *Streptococcus thermophilus* S13 and S32) was investigated. The minimum inhibitory concentration (MIC) in most strains tested was found to be relatively high (from 6.25 mg.mL⁻¹ to 12.50 mg.mL⁻¹). The maximum concentration of polyphenols without any inhibitory effect (MCWI) ranges from 0.390 mg.mL⁻¹ to 0.781 mg.mL⁻¹. The results from the present study showed that among the tested lactic acid bacteria, *Lactobacillus delbrueckii subsp. bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13 had the best growth characteristics in a polyphenol-enriched culture medium. These strains had the highest MIC and MCWI values and could thus be used as starter cultures for polyphenol-fortified fermented milk. Practical applications: Polyphenol-enriched extracts from industrial plant by-products (waste) – distilled rose petals (by-products of rose oil production) and strawberry and bilberry press residues (by-products of fruit juice production) can help improve economic outcomes and solve environmental problems in the food industry. Consequently, the development of functional fermented milks with a combination of probiotic starter cultures and polyphenol extracts represents a positive research direction for the food industry.

Keywords: antimicrobial activity, polyphenol-enriched extracts, probiotics

Abbreviations:

MIC - Minimum inhibitory concentration

MCWI - Maximum concentration of polyphenols without inhibitory effect

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Introduction

Phenolic compounds are plant nutraceuticals representing a huge structural diversity, including chlorogenic acids, hydrolysable tannins, and flavonoids (flavonols, flavanones, flavan-3-ols, anthocyanidins, isoflavones, and flavones). Most of these compounds occur as glycosylated derivatives in plants and foods (Marín et al. 2014). Food polyphenols have been widely studied and confer many important bioactivity benefits such as prevention of cardiovascular diseases, many types of cancer and age-related illnesses (Velderrain-Rodríguez et al. 2014). Epidemiological studies have shown that these health effects are attributed to the antioxidant capacity of phenolic compounds (Scalbert et al. 2005). The biological benefits of polyphenols depend on their bioavailability. For example, a significant portion of dietary polyphenols is not absorbed in the small intestine and can thus interact with colonic microbiota (Manach et al. 2004). These compounds reach the colon, where they are deglycosylated and metabolized by microbial enzymes (Velderrain-Rodríguez et al. 2014). Their metabolites are also described as modulators of human gut microbiota (Cardona et al. 2013). Polyphenols may act as promoting factors of growth, proliferation or survival for beneficial gut bacteria - mainly *Lactobacillus* strains - and thus exert prebiotic actions that inhibit the proliferation of some pathogenic bacteria such as *Salmonella* and *Helicobacter pylori* species (Hervet-Hernández and Goñi 2011). Probiotic bacteria and polyphenols have already demonstrated health-promoting properties (Maukonen and Saarela 2014). The use of these two bio-active ingredients in popular consumer foods such as fermented milk indicates an approach to create more functional foods. Moreover, the antimicrobial activity of polyphenols has been extensively investigated against a wide range of microorganisms. For example, extensive studies have been done on the antimicrobial property of polyphenols on pathogenic bacteria, but few studies have been carried out on the effect of polyphenols on probiotic lactic acid bacteria (Gyawali and Ibrahim 2012; 2014). Therefore, the objective of this study was to investigate the anti-

microbial activity of polyphenol-enriched extracts from industrial plant by-products (strawberry and bilberry press residues and distilled rose petals) against selected probiotic lactic acid bacteria.

Materials and Methods

Plant materials. The plant by-products of strawberry and bilberry were supplied by Santulita Ltd. (Sofia, Bulgaria). The press residues were stored frozen at -18°C until used to produce the polyphenol extracts. Rose (*Rosa damascena* Mill.) petals were supplied by Ecomaat Ltd. (Mirkovo, Bulgaria). The petals were dried in a thin layer at room temperature (25÷27°C) for one week before final hot air drying (50°C, 1h). Dried rose petals were stored in a desiccator in dark until used.

Bacterial strains and storage conditions. The cultures of lactic acid bacteria used in the present study (*Lactobacillus delbrueckii* subsp. *bulgaricus* S10 and S19; *Lactobacillus rhamnosus* YW and S25; *Lactobacillus gasseri* S20; *Streptococcus thermophilus* S13 and S32) belong to the laboratory collection of the Department of Microbiology at UFT – Plovdiv. The cultures were stored frozen at -20°C until used.

Plant extracts preparation. Frozen by-products were thawed, hot air-dried (60°C, 8h) and milled until a particle size < 0.4 mm was obtained. Strawberry and bilberry pomace (approximately 300 g) were extracted with 70 % aqueous ethanol acidified with HCl (1 %, v/v) at a liquid to solid ratio of 20:1 (v/w). After 1 h of stirring at ambient temperature, the extraction mixtures were filtered through a paper filter (Machery-Nagel, Düren, Germany) on a Büchner funnel, and organic solvent was evaporated under vacuum (40°C). To remove sugars, salts and amino acids from extracts samples were purified using a column (465×30 mm) filled with Amberlite XAD 16 HP. Prior to sample application the resin was conditioned and equilibrated by rinsing with 500 mL of ethanol and 1000 mL of water, acidified with trifluoroacetic acid (TFA, pH 2). Subsequently, 250 mL of the extracts were applied and the column rinsed with 1000 mL

of acidified water (TFA, pH 2). For elution of the pigments at least 500 mL of a mixture of ethanol and acidified water (TFA, pH 2) (95:5 v/v) was applied until the column was colorless. The organic solvent of the eluate was evaporated under vacuum (40°C). After that, the residue was lyophilized for 48h. Rose petal polyphenols were extracted with 30 % aqueous ethanol using approximately 350 g of finely milled pomace (particle size < 4 mm) at a liquid to solid ratio of 20:1 (v/w). After 1h of stirring at ambient temperature, the extraction mixture was filtered using a paper filter and the organic solvent was evaporated under vacuum (40°C). The extract obtained was also purified on a column (465×30 mm) filled with Amberlite XAD 16 HP. Prior to sample application the resin was conditioned and equilibrated as described above. Then 250 mL of the extract was applied and the column subsequently rinsed with 1000 mL of acidified water (TFA, pH 2). For the elution of the rose petals polyphenols 500 mL of a mixture of ethanol and acidified water (pH 2) (95:5, v/v) was applied to the column. After evaporation and concentration under vacuum (30°C), the polyphenols were lyophilized for 48h.

Determination of minimum inhibitory concentration (MIC) and maximum concentration without inhibitory effect (MCWI)

The minimum inhibitory concentration of the polyphenol extracts against probiotic lactic acid bacteria was determined by measuring the changes in optical density of culture medium at an absorbance of 600 nm. A solution of the polyphenol extract was first pre-prepared by adding lyophilized polyphenol extract to 10 mL of liquid culture medium (MRS-broth for lactobacilli and M17-broth for streptococci, MERCK, Germany) to obtain a final concentration of polyphenols in the medium 50 mg.mL⁻¹. Serial dilutions were prepared from the primary dilution as follows: Eleven numbered screw tubes (16 mm x 100 mm) were taken with 5 mL of liquid culture medium (MRS-broth for lactobacilli and M17-broth for streptococci, MERCK, Germany). For the first tubes of the series, 5 mL of solution of the poly-phenol extract (50 mg.mL⁻¹) were added. Tube 1 was stirred and 5 mL were withdrawn and transferred to tube 2. This serial

transference was repeated until tube 10. All tubes were inoculated with 0.2 mL inoculum of the tested strain of lactic acid bacteria. The tubes were incubated at optimal temperature (37°C) for 36h and the optical density was measured. The tube 11 (liquid culture medium + inoculum) was used as control. The maximum concentration of polyphenols without inhibitory effect (MCWI) was determined as the highest concentration of polyphenols in which the growth of the tested strains of lactic acid bacteria was not inhibited.

Results and Discussion

The results obtained for the growth of the tested strains of lactic acid bacteria in culture medium containing different polyphenol extracts are shown in Figs. 1 – 4.

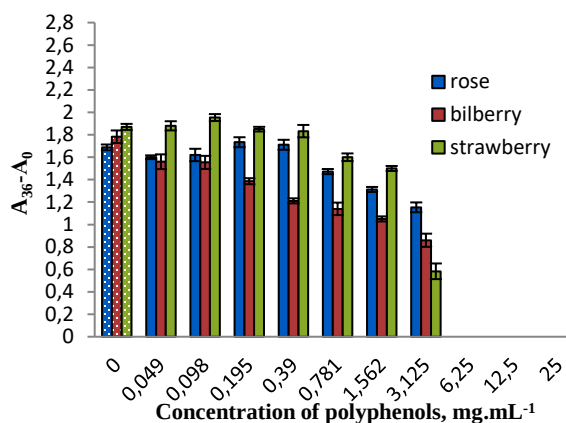


Figure 1. Growth of *Lactobacillus gasseri* S20 in culture media with different concentrations of polyphenol extracts from strawberry, bilberry and distilled rose petals.

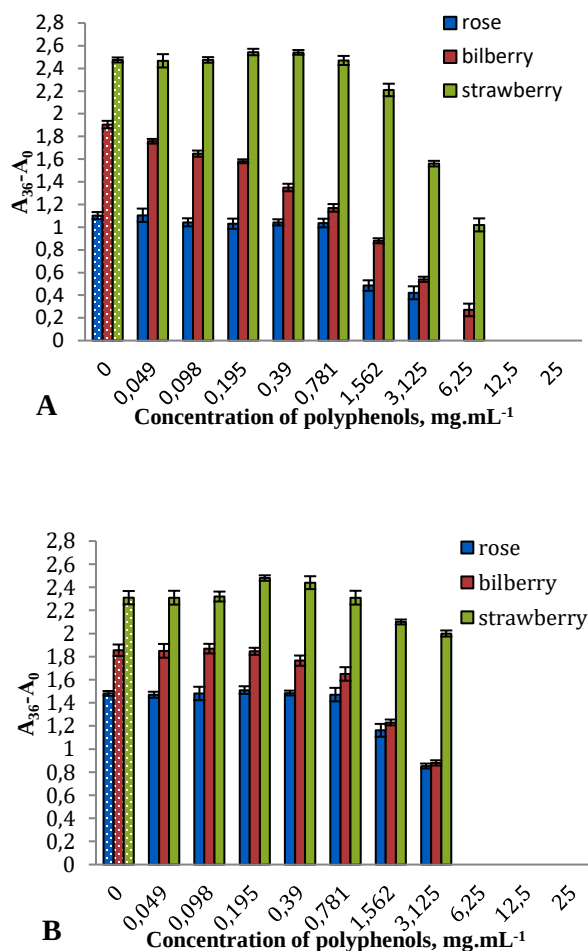


Figure 2. Growth of *Lactobacillus bulgaricus* S10 (A) and S19 (B) in culture media with different concentrations of polyphenol extracts from strawberry, bilberry and distilled rose petals.

It is evident that the growth of lactic acid bacteria was influenced by the type of extract added. Bacterial growth in a medium containing polyphenol extract from bilberry was significantly slower in comparison with the media with polyphenol extracts from strawberry and distilled rose petals. This due to the different composition of the polyphenols in these extracts used suggesting a specific effect on the lactic acid microflora. It is known that the effect of phenolic compounds on bacterial growth depends on the microbial strain, the structure of polyphenols and their concentration in the medium (Almajano et al. 2008; Daglia 2012).

Pacheco-Ordaz et al. (2017) have evaluated the effect of phenolic compounds on the growth of selected probiotic bacteria. The authors found that the polyphenol compounds had selective effect on the growth of probiotic lactobacilli. Among the tested strains of lactic acid bacteria in the present study *Lactobacillus rhamnosus* YW had significantly higher growth in the culture media with polyphenol extracts. In strawberry extract enriched medium the growth of this strain was not inhibited at polyphenol concentrations up to 1.562 mg.mL⁻¹

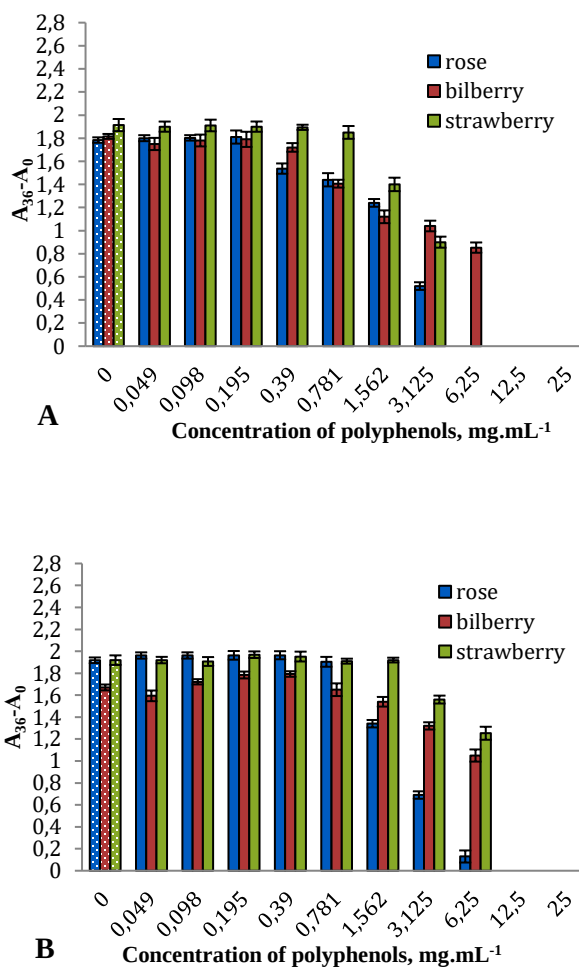


Figure 3. Growth of *Lactobacillus rhamnosus* S25 (A) and YW (B) in culture media with different concentrations of polyphenol extracts from strawberry, bilberry and distilled rose petals.

The tested extracts had an inhibitory effect on the growth of lactic acid bacteria strains. This effect increased at the higher concentrations of polyphenol extracts in the medium.

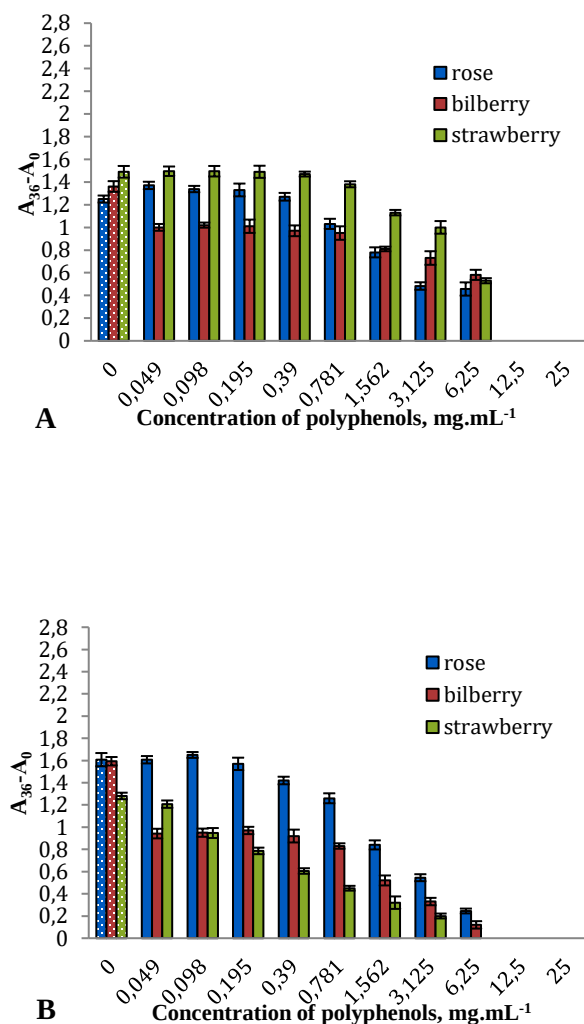


Figure 4. Growth of *Streptococcus thermophilus* S13 (A) and S32 (B) in culture media with different concentrations of polyphenol extracts from strawberry, bilberry and distilled rose petals.

In a similar study, a grape seed extract supplemented with catechin (25 mg.mL⁻¹) and gallic acid (5.5 mg.mL⁻¹) had an inhibitory effect on different species of *Lactobacillus* at a higher concentration (Tabasco et al. 2011).

The results obtained for the minimum inhibitory concentration (MIC) and the maximum concentration of polyphenols without inhibitory effect (MCWI) of the tested strains of lactic acid bacteria are shown in Table 1. It was found that the MIC values in most strains were relatively high and ranged from 6.25 mg.mL⁻¹ to 12.50 mg.mL⁻¹. *Str. thermophilus* (S13 and S32) had a minimum inhibitory concentration higher than *Lactobacillus*. An exception was the *Lactobacillus rhamnosus* YW, which also had high MIC values (12.50 mg.mL⁻¹) in all tested polyphenol extracts. This showed that the *Str. thermophilus* S13 and S32 and *Lactobacillus rhamnosus* YW had lower sensitivity to poly-phenols in the extracts used in comparison with the other test lactic acid bacteria.

For most strains the maximum concentration of polyphenols in the medium in which there is no inhibitory effect on the tested lactic acid bacteria ranges from 0.390 mg.mL⁻¹ to 0.781 mg.mL⁻¹. These data are in agreement with the results obtained from other authors (Cueva et al. 2010), who reported that concentrations of polyphenols in the medium above 1 mg.mL⁻¹ decreased in vivo viability of investigated *Lactobacillus* species.

In the present study the lowest MCWI values were found for the growth of the tested strains in a medium with polyphenol extract from bilberry press residues. The polyphenol extract from bilberry press residues had the highest inhibitory effect towards tested lactic acid bacteria strains. These results indicated that the addition of polyphenol extract from bilberry to milk will have a negative impact on the growth of lactic acid microflora and will retard the process of lactic acid fermentation and coagulation. Therefore, polyphenol extracts from strawberry press residues and distilled rose petals could be recommended for the production of polyphenol-fortified fermented milks.

Table 1. Minimum inhibitory concentration (MIC) and maximum concentration of polyphenols without inhibitory effect (MCWI).

<i>Bacterial strains</i>	<i>MIC mg.mL⁻¹</i>			<i>MCWI mg.mL⁻¹</i>		
	Extract from rose	Extract from bilberry	Extract from strawberry	Extract from rose	Extract from bilberry	Extract from strawberry
<i>Lactobacillus gasseri</i> S20	6.25	6.25	6.25	0.390	-	0.390
<i>Lactobacillus bulgaricus</i> S10	6.25	12.50	12.50	0.781	-	0.781
<i>Lactobacillus bulgaricus</i> S19	6.25	6.25	6.25	0.781	0.195	0.781
<i>Lactobacillus rhamnosus</i> S25	6.25	12.50	6.25	0.195	-	0.390
<i>Lactobacillus rhamnosus</i> YW	12.50	12.50	12.50	0.781	0.781	1.562
<i>Streptococcus thermophilus</i> S13	12.50	12.50	12.50	0.390	-	0.390
<i>Streptococcus thermophilus</i> S32	12.50	12.50	6.25	0.195	-	-

Conclusions

The results obtained in this study showed that among the tested lactic acid bacteria, *Lactobacillus delbrueckii subsp. bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13 had the best growth characteristics in a polyphenol-enriched culture medium. These strains had the highest MIC and MCWI values and could thus be used as starter cultures for polyphenol-fortified fermented milk. Based on these results the industries can use combinations of probiotic lactic acid bacteria and polyphenol-enriched extracts from plant by-products to develop new functional fermented milks. However, the influence of polyphenol-enriched extracts to growth and activity of probiotic lactic acid bacteria must be further studied in situ to better define future recommendations.

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References

- Almajano M.P., Carbó R., Jiménez J., Gordon M. Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, 2008, 108(1): 55-63. <https://doi.org/10.1016/j.foodchem.2007.10.040>
- Cardona F., Andrés-Lacueva C., Tulipani S., Tinahones F.J., Queipo-Ortuño M.I. Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry*, 2013, 24(8): 1415-1422. <https://doi.org/10.1016/j.jnutbio.2013.05.001>
- Cueva C., Moreno-Arribas M., Martín-Álvarez P.J., Bills G., Vicente M.F., Basilio A., Rivas C.L., Requena T., Rodríguez J.M., Bartolomé B. Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Research in Microbiology*, 2010, 161(5): 372-382. <https://doi.org/10.1016/j.resmic.2010.04.006>
- Daglia M. Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 2012, 23(2): 174-181. <https://doi.org/10.1016/j.copbio.2011.08.007>
- Gyawali R., Ibrahim S.A. Impact of plant derivatives on the growth of foodborne pathogens and the functionality of probiotics. *Applied Microbiology and Biotechnology*, 2012, 95(1): 29-45. <https://doi.org/10.1007/s00253-012-4117-x>
- Gyawali R., Ibrahim S.A. Natural products as antimicrobial agents. *Food Control*, 2014, 46(1): 412-429. <https://doi.org/10.1016/j.foodcont.2014.05.047>
- Hervet-Hernández D., Goñi I. Dietary Polyphenols and Human Gut Microbiota: a Review. *Food Reviews International*, 2011, 27(2): 154-169. <https://doi.org/10.1080/87559129.2010.535233>
- Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L. Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, 2004, 79(5): 727-747. <https://doi.org/10.1093/ajcn/79.5.727>

- Marín L., Miguélez E.M., Villar C.J., Lombó F. Bioavailability of Dietary Polyphenols and Gut Microbiota Metabolism: Antimicrobial Properties. *BioMed Research International*, 2015, 2015: 18 pages. <http://dx.doi.org/10.1155/2015/905215>
- Maukonen J., Saarela M. Human gut microbiota: does diet matter? *Proceedings of the Nutrition Society*, 2015, 74(1): 23-26. <https://doi.org/10.1017/S0029665114000688>
- Pacheco-Ordaz R., Wall-Medrano A., Goñil M.G., Ramos-Clamont-Montfort G., Ayala-Zavala J.F., González-Aguilar G.A. Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. *Applied Microbiology*, 2018, 66(1): 25-31. <https://doi.org/10.1111/lam.12814>
- Scalbert A., Manach C., Morand C., Rémésy C., Jiménez L. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 2005, 45(4): 287-306. <https://doi.org/10.1080/1040869059096>
- Tabasco R., Sánchez-Patán F., Monagas M., Bartolomé B., Moreno-Arribas M.V., Peláez C., Requena T. Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. *Food Microbiology*, 2011, 28(7): 1345-1352. <https://doi.org/10.1016/j.fm.2011.06.005>
- Velderrain-Rodríguez G.R., Palafox-Carlos H., Wall-Medrano A., Ayala-Zavala J.F., Chen C.O., Robles-Sánchez M., Astiazaran-García H., Alvarez-Parrilla E., González-Aguilar G.A. Phenolic compounds: their journey after intake. *Food Function*, 2014, 5(2): 189-197. <https://doi.org/10.1039/C3FO60361J>