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Antibacterial activity against gastrointestinal pathogens of novel powdered beetroot juices

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INTRODUCTION & AIM

Lactic acid fermentation is an innovative approach in the food industry that has emerged as a promising technique, known to improve food preservation, physico-chemical properties, and microbiological safety. Beetroot juice rich in bioactive compounds (e.g. saponin, tannins, alkaloids, flavonoids, cardiac glycosides), can be fermented with selected probiotic bacteria, to increase their antioxidant activity, improve flavor profile and potential health benefits.

The aim of this study was to evaluate the antibacterial activity of unfermented (NF-PBJ) and *Lactobacillus plantarum* 299v-fermented (F-PBJ) beetroot juices against a broad spectrum of bacterial strains that can be considered as food poisoning or gastrointestinal pathogens. Prior to analysis, the beetroot juices were subjected to powdering processes applying spray-drying (SD) and freeze-drying (FD), with 20% oligofructose added as a carrier and prebiotic agent.

METHODS

- The production of powdered juices (NF-PBJs and F-PBJs) followed the method described by Brzezowska et al. (2023)[1].
- The reference microbes (*Micrococcus luteus* ATCC 10240*, Staphylococcus epidermidis* ATCC 12228*, Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC BAA-1707 methicillin-resistant, MRSA*) , Bacillus subtilis* ATCC 6633*, Bacillus cereus* ATCC 10876*, Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853*, Klebsiella pneumoniae* ATCC 13883*, Proteus mirabilis* ATCC 12453*, Acinetobacter baumannii* ATCC 19606*,* and *Salmonella* Typhimurium ATCC 14028*),* obtained from the American Type Culture Collection (ATCC), being a part of collection of the Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland.
- The antibacterial potential of the obtained products was analyzed (in a concentration range of **15.625–32000 µg/mL**) by using the broth microdilution method in Mueller-Hinton broth medium, according to EUCAST guidelines (2024)[2]. The method entailed the preparation of microbial suspensions at a standardized density (0.5 on the McFarland scale, corresponding to 1.5×10^8 colony-forming units per mililiter CFU/mL) .
- The antibacterial activity was presented by the following parameters: the lowest concentration of a tested compound that will inhibit the visible growth of a microorganism after overnight incubation (MIC), and minimum bactericidal concentrations (MBC) as the lowest concentration of PBJs that will prevent the growth of an organism after subculture on to compound-free media and MBC/MIC ratio (≤4 was considered to have a bactericidal effect, >4 as a bacteriostatic effect).

RESULTS & DISCUSSION

According to previous results[1], BPJs with oligofructose have shown beneficial functional properties, including high total phenolic content, strong antioxidant capacity or chemoprotective and antiproliferative activity against various cancer cells (e.g. cervical cancer cells, prostate cancer cells, leukemia cell lines).

The results of this study also showed additive antimicrobial properties of PBJs with 20% oligofructose. The results were species dependent. For all probiotic beetroot juices (PBJs), the MICs ranged from 2000 to 8000 µg/mL, while the MBCs ranged from 4000 to >32000 µg/mL. The lowest activity was observed against *B. cereus* ATCC 10876 (a food-borne toxin-producing pathogen, a major cause of gastrointestinal illness), with an MIC in the range of 4000-8000 µg/mL. Based on the MBC/MIC ratio, the tested compounds exhibited bacteriostatic activity against the majority of bacterial strains, except for the unfermented freeze-dried beetroot juice, which displayed bactericidal activity at MIC=16000 µg/mL. The lowest MIC value of 2000 µg/mL was reported for the fermented freeze-dried beetroot juice powder with 20% oligofructose and the unfermented spray-dried beetroot juice powder with 20% oligofructose against Gram-negative rods, including *E. coli* ATCC 25922 and *A. baumannii* ATCC 19606. Additionally, fermented spray-dried beetroot juice powder with 20% oligofructose also exhibited this MIC value against *A. baumannii* ATCC 19606. In all cases, the activity was bactericidal.

According to others[3-4], the juice extract of the beetroot was more effective against *Staphylococcus* sp., *Bacillus* spp., including *B. cereus*, and *E. coli* but showed no effect on *Salmonella* spp. and *Klebsiella* spp. Unfortunately, most of the works were based on non-referential, screening and low-precision methods for determining antimicrobial activity, i.e. the disc diffusion method, so their results cannot be compared with ours. Salamatullah et al.[5] showed that oven-dried beetroot (pulp and peel) inhibited the growth of *S. aureus*, *Listeria monocytogenes*, *E. coli* and *Pseudomonas aeruginosa* at MIC=4-8 mg/mL.

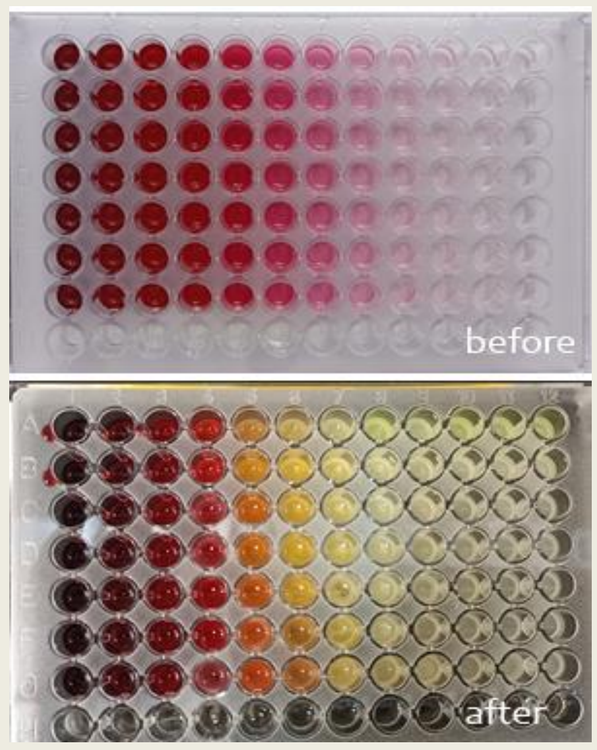

Figure 1. Filled 96-well plate before and after 24-hour incubation

Table 1. Activity of the fermented and unfermented beetroot juice powdered with 20% oligofructose against Gram-positive and Gram-negative bacteria

Type of beetroot juice powdered with 20% oligofructose	Type of drying	MIC [mg/L] MBC [mg/L] bactericidal (+) / bacteriostatic (-)											
		Gram-positive						Gram-negative					
		Ml ATCC 10240	Se ATCC 12228	Sa ATCC 6538	Sa ATCC 1707	Bs ATCC 6633	Bc ATCC 10876	Ec ATCC 25922	Pa ATCC 27853	Kp ATCC 13883	Pm ATCC 12453	Acb ATCC 19606	ST ATCC 14028
F-PBJ	FD	8 16 (+)	8 16 (+)	8 16 (+)	8 32 (+)	8 >32 (-)	8 >32 (-)	2 4 (+)	8 16 (+)	8 16 (+)	8 16 (+)	2 4 (+)	8 16 (+)
	SD	4 32 (-)	8 16 (+)	8 32 (+)	8 32 (+)	8 8 (+)	4 >32 (-)	4 4 (+)	4 8 (+)	8 16 (+)	8 16 (+)	2 4 (+)	8 16 (+)
NF-PBJ	FD	32 >32 (-)	16 16 (+)	16 16 (+)	8 32 (+)	8 32 (+)	16 32 (+)	8 8 (+)	8 8 (+)	8 8 (+)	16 32 (+)	8 16 (+)	4 32 (-)
	SD	8 32 (+)	8 16 (+)	8 16 (+)	8 32 (+)	8 16 (+)	8 >32 (-)	2 2 (+)	8 16 (+)	8 16 (+)	8 16 (+)	2 4 (+)	8 16 (+)
CONTROL*		16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	16 >32 (-)	8 >32 (-)
Amoxicillin (5 mg/mL; MIC/MBC results expressed in µg/mL)		0.98 0.98 (+)	0.98 0.98 (+)	0.98 0.98 (+)	31.25 31.25 (+)	125 125 (+)	125 125 (+)	3.91 7.81 (+)	500 >1000 (-)	500 >1000 (-)	0.49 0.49 (+)	125 125 (+)	0.98 0.98 (+)

Figure 2. Microtitre plate with antibacterial activity of the fermented freeze-dried beetroot juice powdered with 20% oligofructose against Gram-positive and Gram-negative bacteria

Abbreviations: MIC - minimal inhibitory concentration [µg/mL]; MBC - minimal bactericidal concentration [µg/mL]; (+) means bactericidal activity based on MBC/MIC ≤ 4; (-) - bacteriostatic activity based on MBC/MIC > 4; * CONTROL - fresh beetroot juice dissolved in DMSO (100 mg/mL); FD - freeze drying; SD - spray drying; Se ATCC 12228 - *Staphylococcus epidermidis* ATCC 12228; Sa ATCC 6538 - *Staphylococcus aureus* ATCC 6538; Sa ATCC 1707 - *S. aureus* MRSA ATCC 1707; Bs ATCC 6633 - *Bacillus subtilis* ATCC 6633; Bc ATCC 10876 - *Bacillus cereus* ATCC 10876; Ml ATCC 10240 - *Micrococcus luteus* ATCC 10240; Ec ATCC 25922 - *Escherichia coli* ATCC 25922; ST ATCC 14028 - *Salmonella* Typhimurium ATCC 14028; Pa ATCC 27853 - *Pseudomonas aeruginosa* ATCC 27853; Kp ATCC 13883 - *Klebsiella pneumoniae* ATCC 13883; Pm ATCC 12453 - *Proteus mirabilis* ATCC 12453; Acb ATCC 19606 - *Acinetobacter baumannii* ATCC 19606

CONCLUSIONS

The results highlight the antimicrobial potential of probiotic beetroot juices (PBJs), demonstrating effective activity against selected gastrointestinal or foodborne pathogens such as *E. coli, Salmonella* spp. (typically associated with foodborne illness) or *A. baumannii (*rarely associated with diarrheal illness). They are among the most common Gram-negative pathogens that poses major threat to human life. Thus the novel foodstuffs obtained might be a more convenient food formulations with significant technological and human health protection potential.

The results indicate also a wide applicability of PBJs in the development of innovative functional foods, pharmaceuticals, cosmetics, and dietary supplements. This area of research definitely needs to be further explored, in particular to investigate other prebiotic carriers (e.g. inulin, Nutriose®, maltodextrin) for their biological properties, including antibacterial or antifungal activities, either on reference strains or clinical isolates. It could also be interesting to check whether the drying technique of beetroot juice powder has some influence on the availability of active compounds and beneficial antimicrobial properties.

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