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THE IMPACT OF CARROT JUICES ON INTESTINAL AND PROBIOTIC BACTERIA

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ABSTRACT

There is a growing interest in non-dairy probiotic products. The main aim of the study was to evaluate the impact of juice prepared from 15 various cultivars of carrot on the growth of representatives of human intestinal microbiota (*Bifidobacterium catenulatum, Escherichia coli*) and probiotic strains (*Lactobacillus acidophilus* LA-5, *Lactobacillus casei* 01). Carrot juice was added to liquid medium at a final concentration of 5.0% and their impact on the bacteria number was assessed by measurement of the turbidity after 24 h of culture. The number of cells was expressed as % of positive control (medium without juice addition). Juices prepared from all tested cultivars of carrot inhibited the growth of *Bifidobacterium catenulatum*, and the strongest inhibitory effect was observed for juices obtained from the 'Kongo F1' cultivar (3.40 ±2.85% of positive control), 'Rumba F1'(4.17 ±2.27%) and 'Broker F1' (5.35 ±2.14%). The majority of tested juices also inhibited the growth of E. coli, but those prepared from the 'Niland F1', 'Napa F1', 'Afro F1'and 'Samba F1' cultivars stimulated the growth of this bacterium. The probiotic strains were less sensitive to carrot juice impact than intestinal species, however both stimulation and inhibition could be observed. Juices made from the cultivars 'Kongo F1' and 'Deep Purple F1' acted negatively on the growth of both probiotic strains, while juice from 'Bangor F1' cultivar inhibited *L. casei* 01 growth, but stimulated the growth of LA-5. The obtained results suggest that 'Kongo F1' and 'Deep Purple F1' cultivars are not suitable as an additive or raw material for the production of probiotic products, because of their inhibitory properties against probiotic strains. Concluding, carrots can be used as raw material for the production of probiotic beverages, however both the cultivar of carrot and the strains of probiotic bacteria used for the production should be selected carefully. The most suitable for production of probiotic beverages seems to be the 'Rumba F1', followed by the 'Polka F1' and 'Yellowstone F1' cultivars. They inhibited the growth of intestinal bacteria, which are undesirable in the final product, without negative effect on the probiotic species.

Keywords: carrot juice; antibacterial activity; intestinal microbiota; probiotic

INTRODUCTION

Generally, there are two basic groups of carrots: with orange roots (*Daucus carota* ssp. *Sativus* var. *Sativus*) and purple-violet (*Daucus carota* ssp. *Sativus* var. *Atroruben*); but varieties of yellow and white root are also known. Various cultivars differ in chemical composition, shape, and size of root, the length of the growing season, the storage stability and application. The most visible difference is associated with the root color and it is caused by the presence of high content of carotenoids in orange varieties, while in purple ones xanthophylls dominate **(Alasalvar et al., 2001; Gajewski et al., 2007; Majkowska-Gadomska and Wierzbicka 2010; Mech-Nowak et al., 2012)**.

The energy value of 100 g edible parts of carrots is only 27 kcal; moreover, a carrot is easy to digest, which makes it a valuable component of many diets, including for infants, convalescents, and people wanting to lose weight. Additionally, carrots are rich in antioxidants, both of a lipophilic fraction (carotenoids) as well as hydrophilic (phenolic compounds) **(Gajewski et al., 2007; Leja et al., 2010; Mech-Nowak et al., 2012)**.

Poland is the second European producer of carrots. In 2011, the domestic production has been estimated at about 900 thousands of tons. Carrot is a vegetable available almost all year round and can be eaten raw in the form of salads, cremogenes or juices. It is also suitable for cooking and freezing.

Owing to its taste, color and nutritional value is increasingly fashionable to use different cultivars of carrots for production of fresh unpasteurized carrot juices or as an ingredient in juice multi-vegetable juices. The more that the diversity of cultivars with different root color (from white through orange to the almost black) allows for a wide selection and enables to create interesting compositions. Some producers try also to introduce vegetable juices with added probiotic bacteria, into the market **(Nazzaro et al., 2008)**. The rich composition of carrots is certainly valuable to the consumer organism, but it can have negative effects on microorganisms added. It was proved that some polyphenols can inhibit the growth of intestinal bacteria **(Duda-Chodak, 2012)** and probiotics, such as *Lactobacillus casei* **(Duda-Chodak et al., 2008)**. The effect of carrot juice on the probiotic strains of bacteria or bacteria that reside in the human intestine has not been studied yet.

The aim of the study was to evaluate the impact of juices prepared from 15 cultivars of carrot on the growth of selected species of intestinal and probiotic bacteria.

MATERIAL AND METHODOLOGY

Material

Fifteen varieties of carrot (*Daucus carota* L.) with different colors of their edible root were selected for the study: 'Samba F1', 'Salsa F1', 'Polka F1', 'Rumba F1', 'Afro F1', 'Korund F1', 'Broker F1', 'Kongo F1', 'Niland F1', 'Nerac F1', 'Bangor F1', 'Napa F1' (all have orange root), 'Deep Purple F1' (purple root), 'Yellowstone F1' (yellow root), 'White Satine F1' (white root). All cultivars were obtained from the Experimental Station of the University of Agriculture in Krakow, located in Mydlniki, Poland.

Bacterial culture

Bifidobacterium catenulatum (DSM 16992) /BF/ and *Escherichia coli (*DSM 1116) /EC/ were used as the representatives of intestinal microbiota, and were purchased from Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ Germany). The probiotic strains *Lactobacillus acidophilus* LA-5 /LA5/ and *Lactobacillus casei* 01 /LC01/ were obtained from Christian Hansen (Denmark).

Bacteria were grown anaerobically at 37°C in media specially designed for particular microorganisms. The medium for *Bifidobacterium* contained (per 1 L): peptone from casein, pancreatic digest (10.00 g), yeast extract (5.00 g), beef extract (5.00 g), soy peptone (5.00 g), glucose (10.00 g), K_2HPO_4 (2.00 g), $MgSO_4 \times 7\ H_2O$ (0.20 g), $MnSO_4 \times H_2O$ (0.05 g), Tween 80 (1.00 mL), NaCl (5.00 g), cysteine-HCl x H_2O (0.50 g), resazurin (1 mg), and 40 mL salt solution. Composition of salt solution (per 1 L): 0.25 g $CaCl_2 \times 2\ H_2O$, 0.50 g $MgSO_4 \times 7\ H_2O$, 1.00 g K_2HPO_4, 1.00 g KH_2PO_4, 10.00 g $NaHCO_3$, 2.00 g NaCl. For lactic acid bacteria MRS Broth was used, while a Nutrient Broth was used for *E. coli* (both from Biocorp, Poland).

Preparation of carrot juice

The raw material for the preparation of the juice were thoroughly washed roots of appropriate carrot cultivar from which inedible parts were removed. The juice (ap. 1 L from each cultivar) was prepared with the use of a juicer. Then the juice was centrifuged at 14 000 rev./min. and the clear supernatant obtained after centrifugation was separated, frozen and stored (at -20°C) as such until the experiments.

The impact of carrot juice on the growth of bacteria

The impact of the carrot juices on bacteria was assessed in liquid medium inoculated with the tested bacteria (3×10^6/mL) with or without 5% of carrot juice. Biomass of bacterial cultures after 24 h of incubation at 37°C was assessed nephelometrically by measuring the turbidity in McFarland scale (densitometer DEN-1B, Biosan), which then enabled to calculate cells number.

As controls the number of bacteria in medium without carrot juice (positive control), and turbidity of empty medium without bacteria (double blank) was determined. As the addition of carrot juices (especially those from dark cultivars) to different media changed the medium color and turbidity, blank samples (medium with appropriate concentration of juice without bacteria) were also prepared. The value obtained for the blank was each time subtracted from the value obtained for the sample turbidity, taking into account the kind of medium.

The obtained results were expressed as % of positive control in order to facilitate the comparison between different bacteria species.

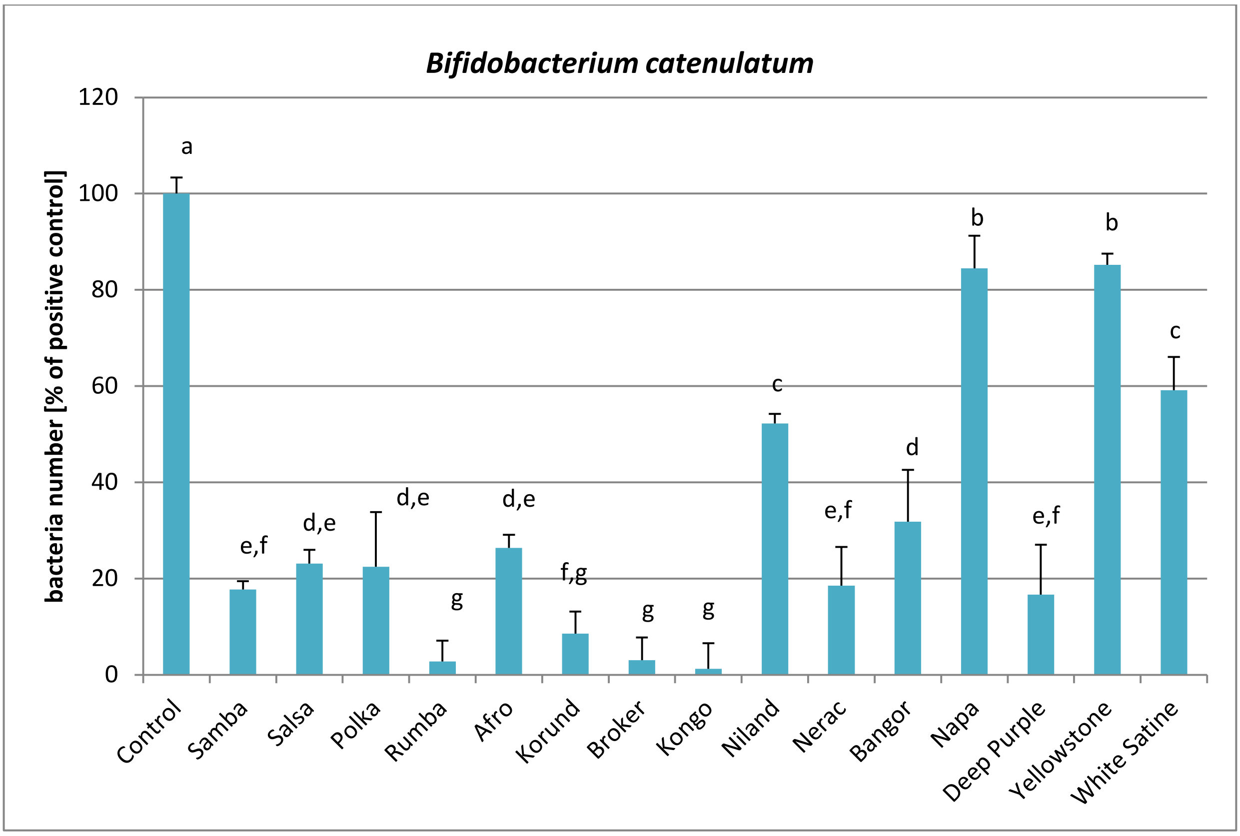
All determinations were performed at minimum three replications, and the results were presented as arithmetic mean ± SD. The ANOVA with a *post hoc* Tukey test was applied to perform statistical analysis (GraphPad InStat version 3.01 for Windows, GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

Analysis of the impact of carrot juice to *Bifidobacterium catenulatum* showed that all 15 cultivars inhibited the growth of the bacterium under conditions of the experiment (Fig. 1). The strongest inhibitory effect was observed for juices obtained from the 'Kongo F1' cultivar (3.40 ±2.85% of positive control), 'Rumba F1' (4.17 ±2.27%) and 'Broker F1' (5.35 ±2.14%).

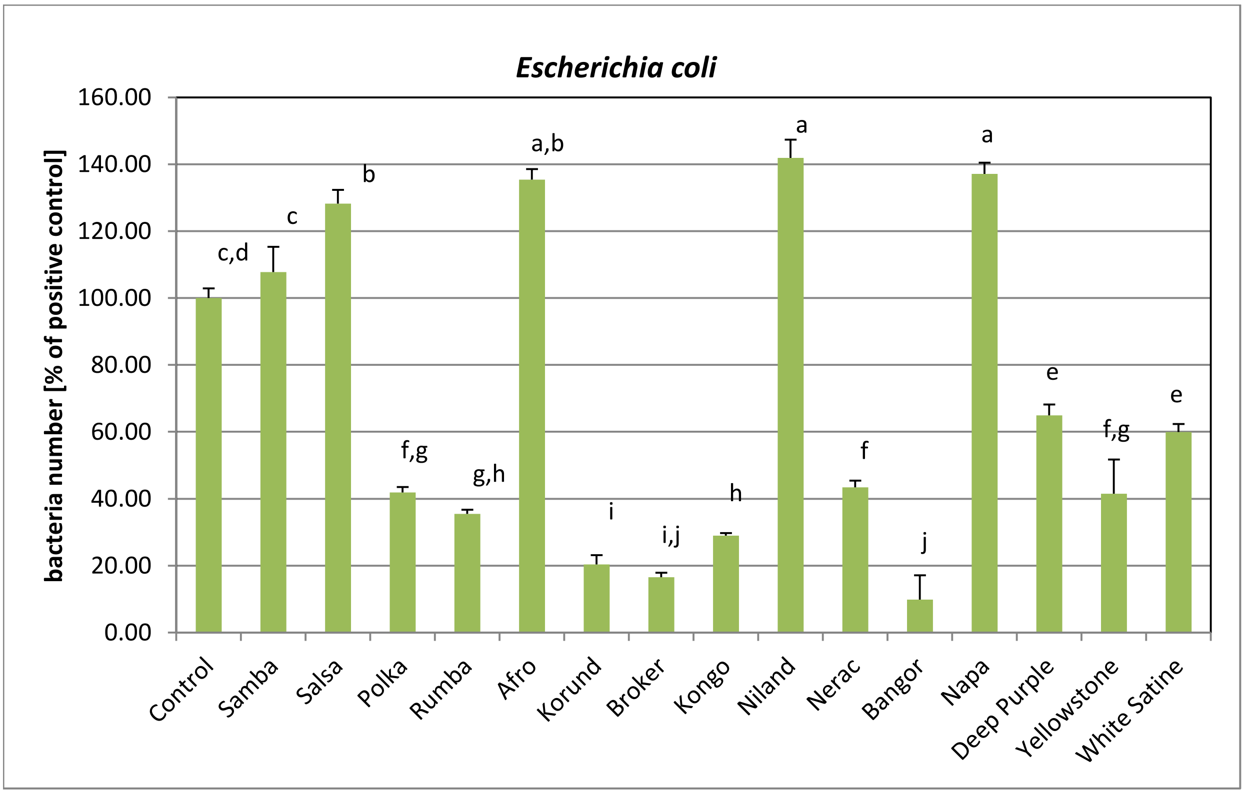
In the case of *E. coli* some cultivars of carrot positively influenced growth of the bacterium (Fig. 2). The highest stimulation of *E. coli* growth was observed in medium with the addition of 5% of juice prepared from the 'Niland F1' cultivar (141.91 ±5.45% of positive control), 'Napa F1' (137.09 ±3.39%), ' Afro F1' (135.43 ±3.14%), 'Salsa F1' (128.25 ±4.07%) and 'Samba F1' (107.78 ±7.51%). Juices made from other cultivars of carrots caused inhibition of the bacterium, and the stronger inhibition was characteristic of the 'Bangor F1' (14.67 ±1.49%) and 'Broker F1' (16.58 ±1.30%).

Generally, probiotics were less sensitive to the presence of carrot juices. The growth of *Lactobacillus acidophilus* LA-5 was inhibited by juices obtained from the cultivars 'Napa F1' (55.10 ±1.84% of positive control), 'Kongo F1' (58.30 ±2.08%), 'Deep Purple F1' (72.20 ±0,00%), and to a lesser extent by the 'Salsa F1', 'Broker F1', 'Niland F1' and 'Nerac F1' cultivars (Fig. 3). In contrary, the juices prepared from cultivars 'Bangor F1' and 'White Satine F1' slightly stimulated growth of this probiotic strain (105.29 ±2.06% and 104.83 ±2.62%, respectively). Juices from the 'Deep Purple F1' and 'Kongo F1' carrots inhibited also *Lactobacillus casei* 01 (52.25 ±28.62% and 62.51 ±3.71%, respectively), but the stronger inhibition of LC01 was observed for the 'Bangor F1' cultivar (37.71 ±3.06%) (Fig. 4).



a,b,c – different letters indicate that differences between the number of bacteria are statistically significant at $p < 0.05$

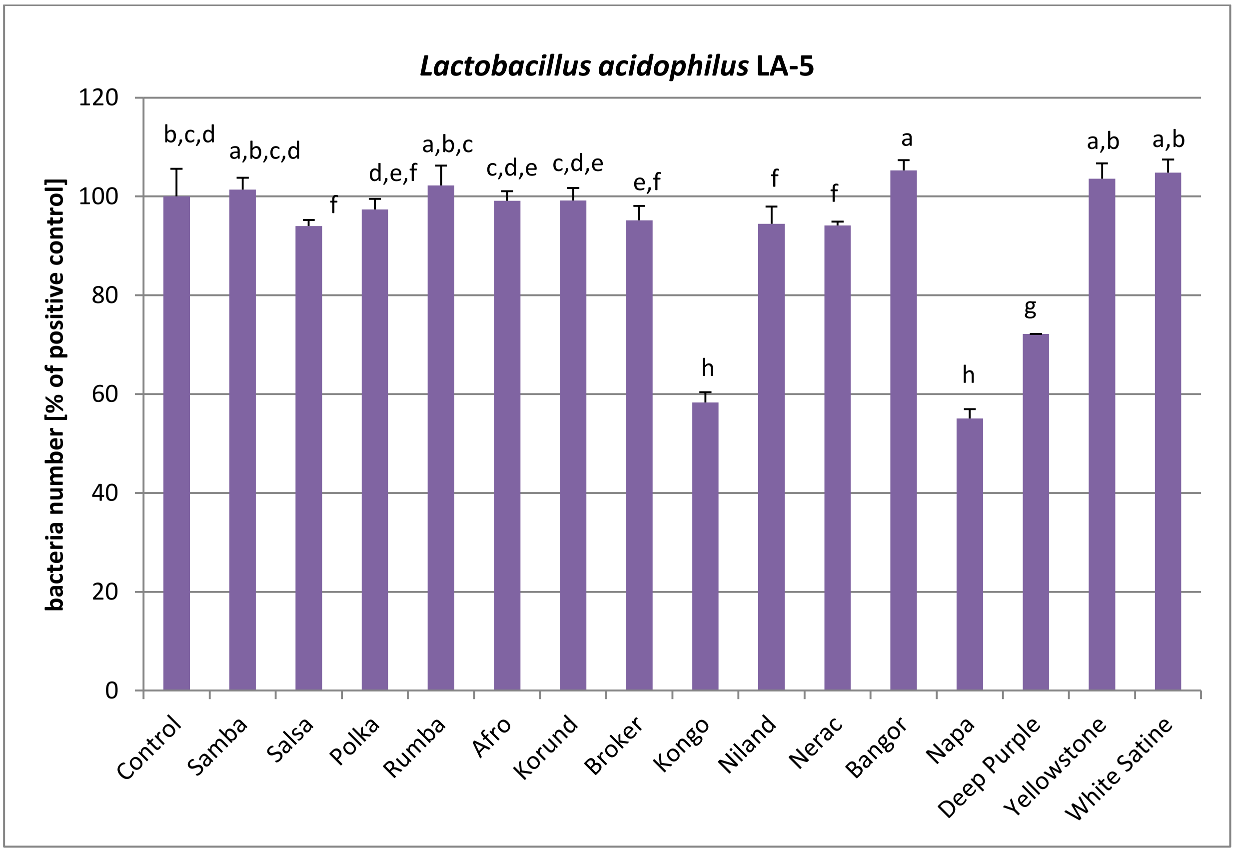
Figure 1 The impact of carrot juices on the growth of *Bifidobacterium catenulatum.*



a,b,c – different letters indicate that differences between the number of bacteria are statistically significant at $p < 0.05$

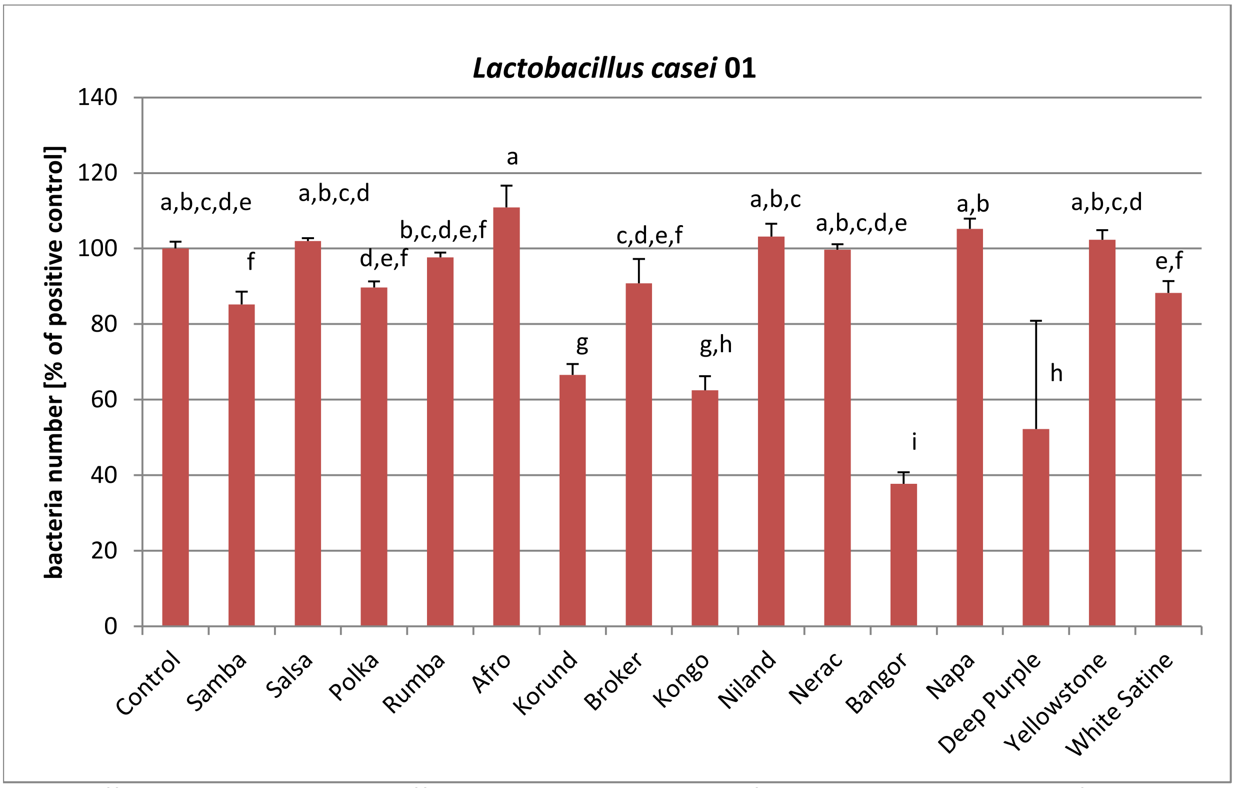
Figure 2 The impact of carrot juices on the growth of *Escherichia coli.*

There is a growing interest in non-dairy probiotic products because of the rising frequency of lactose intolerance, elevated blood cholesterol, as well as due to economic aspect, traditions, and dietary modifications



a,b,c – different letters indicate that differences between the number of bacteria are statistically significant at $p < 0.05$

Figure 3 The impact of carrot juices on the growth of *Lactobacillus acidophilus* LA-5.



a,b,c – different letters indicate that differences between the number of bacteria are statistically significant at $p < 0.05$

Figure 4 The impact of carrot juices on the growth of *Lactobacillus casei* 01.

caused by lifestyle (eg. a vegan diet) **(Prado et al., 2008)**. The obtained results suggest that some carrot cultivars are not suitable as an additive or raw material for the production of probiotic products. The cultivars 'Deep

Purple F1' as well as 'Kongo F1' inhibited growth not only of intestinal bacteria, which is a desirable effect, but also of probiotic strains. Likewise, the 'Korund F1', 'Bangor F1' and 'White Satine F1' cultivars should not be used for the production of probiotics products. Our results revealed that the most suitable cultivar for this purpose was 'Rumba F1'; it inhibited growth of intestinal bacteria but had no impact on probiotic strains. The 'Polka F1' and 'Yellowstone F1' cultivars also could be used. The 'Bangor F1' cultivar of carrot can be considered as additive for probiotic products, but the proper selection of probiotic strains should be made. Juice prepared from the 'Bangor F1' inhibited strongly the growth of intestinal bacteria but also of the *L. casei* 01, whereas the growth of LA5 was stimulated. The juice obtained from 'Afro F1' cultivar stimulated growth of probiotic strain LC01, but stimulated also *Escherichia coli* growth, so it should not rather be used for probiotic juices production.

CONCLUSION

Carrot juice can be used as raw material for the production of probiotic beverages, however both the cultivar of carrot and the strains of probiotic bacteria used for the production should be selected carefully. The most suitable for production of probiotic beverages seems to be the 'Rumba F1' cultivar, followed by 'Polka F1' and 'Yellowstone F1'. Their valuable property was the inhibition of the growth of intestinal bacteria without negative effect on the tested probiotic species.

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