THE IMPACT OF 'SPIRULINA' ON THE MICROBIOLOGICAL STABILITY OF UNPASTEURISED APPLE JUICE

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Abstract: The aim of this study was to assess the influence of the supplementation with 'Spirulina' (biomass of *Arthrospira platensis* cyanobacteria) on the microbiological stability of unpasteurised apple juice. Total number of mesophilic aerobic bacteria, yeasts and lactic acid bacteria were enumerated at different doses of 'Spirulina': 0.5, 1.0 and 1.5% (w/v). It was demonstrated that cell number of all tested microorganisms increased significantly at the third day of storage. Presence of cyanobacteria biomass at dose 1.5% in the juice caused a decrease in the number of total mesophilic bacteria, but enhanced growth of yeasts and lactic acid bacteria. It was concluded that 'Spirulina' might hold some potential for extension of shelf life of unpasteurised juices, but further studies are required.
Key words: Spirulina¸ unpasteurised apple juice, lactic acid bacteria, yeast, shelf life

INTRODUCTION

Due to the high demand on low processed food products unpasteurised juices seem to be a good option to provide some health promoting nutrients. However, their serious disadvantage, from the point of view of manufacturers and retailers, is their short shelf life, so there is an increasing demand to extend it and prevail their nutritional value at the same time (**Rupasinghe, Yu 2012**). Most of the studies focused on the supplementation of food products regarded the control of (potentially) pathogenic microorganisms (**Hetta, et al. 2014**), but there is very little known on how to inhibit growth of saprophytic microflora causing food spoilage using some materials of natural origin.

'Spirulina' is a dietary supplement which is manufactured from cyanobacteria *Arthrospira platensis* biomass known for their beneficial nutrient composition and health promoting properties like lowering blood cholesterol, scavenging free radicals, enhancing immune system and others (**Duda-Chodak, et al. 2010**).

The aim of this study was to assess if supplementation of unpasteurised apple juice with 'Spirulina' enhances its microbiological stability. To achieve this the number of total mesophilic aerobic bacteria (MAB), lactic acid bacteria (LAB) and yeasts (Y) were assessed throughout five days of juice storage at 4 °C.

MATERIALS AND METHODS

Microbiological media were obtained from BIOCORP (Gliwice, Poland) and 'Spirulina' (Ivarsson's Hawaian Spirulina) was manufactured by Ivarsson's Produkte für's Leben (Schriesheim, Germany). Apples of the cultivar Cox's Orange were obtained from the private orchard in Łososina (Poland). 'Spirulina' was mixed with ethanol (F.H.U. Dor-Chem, Krakow, Poland) at the ratio 1:1 (w/v) and homogenised (Ultra Turrax T-25-basic homogeniser, IKA®-Werke GmbH & Co. KG, Staufen, Germany) for 2 min at 16 000 rpm. Juice was prepared using home juicer MPM J-45 (MPM AGD S.A., Milanówek, Poland), filtered through cheese cloth and divided into 200 ml aliquots to capped glass bottles and pre-treated 'Spirulina' was added at three different doses: 0.5, 1.0 and 1.5 %. Then juice was stored in the fridge at 4 ˚C and samples were collected every 24 h. Microbiological analysis were carried out as described in Table 1. All experiments were conducted in triplicates and microbiological analysis were carried out in duplicates for each replicate and expressed as mean values ± standard deviation. Statistical analysis was carried out with GraphPad InStat program, version 3.01 for Windows (GraphPad Software, San Diego, California, USA). A

Kołmogorov–Smirnov test was applied to examine the normality of distribution. A single-factor Analysis of Variance test (ANOVA) with a post hoc Tukey test was applied to assess the differences among means.

Table 1. Description of microbiological analysis

Group of microorganisms	Method used	Temperature of incubation (C)	Time of incubation (h)
Total number of aerobic mesophilic bacteria	Spread plate method	30	72 ± 3
Lactic acid bacteria	Pour plate method	30	48 ± 2
Yeast	Spread plate method	28	72 ± 3

RESULTS AND DISCUSSION

The most of manufacturers of unpasteurised apple juices state that the shelf life of these products is no greater than 48 h which is quite short period of time considering logistics of delivering juices to retailers and shops straight after bottling them. ‘Spirulina’ is already used as an additive for fruit juices in doses not exceeding 1.5 % because of its benefits to human health (**FAO, 2008**), but according to our knowledge there were no studies assessing its influence on microbiological stability of these products. According to the current database, two group of microorganisms may potentially deteriorate the quality of those beverages and these are yeasts and lactic acid bacteria (**Raybaudi-Massilia, eta al 2009, Wareing, Davenport 2005**).

It was shown that ‘Spirulina’ at the dose of 1.5 % inhibited growth of MAB since the second day of storage (Table 2), however, statistically significant differences were noted on the day 5. Also, it might be concluded that juice quality deteriorates when the cell number exceeds $3.00 \cdot 10^4$ cfu per ml, but on the basis of the data presented in Table 3 it could be noticed that the time of spoiling juice is related to the significant increase in LAB population which was additionally induced by the presence of ‘Spirulina’. It was previously shown that the biomass of *Arthrospira platensis* enhances growth of probiotics (**Beheshtipour et al. 2012**), but it wasn’t yet investigated how it affects other representatives of lactic acid bacteria.

Table 2. The number of mesophilic aerobic bacteria ($\cdot 10^4$ cfu.ml^{-1}) in unpasteurised apple juice supplemented with different doses of ‘Spirulina’

Day	‘Spirulina’ dose (% w/v) 0	0.5	1.0	1.5
0	1.61 ± 0.62^a	1.67 ± 0.56^a	3.15 ± 2.27^a	2.25 ± 2.19^a
1	1.48 ± 0.43^a	2.43 ± 0.03^a	$>3.00^a$	1.76 ± 0.75^a
2	$>3.00^a$	$>3.00^a$	$>3.00^a$	1.73 ± 0.66^a
3	$>30.0^a$	$>30.0^a$	13.1 ± 4.90^a	6.18 ± 5.11^a
4	$>300^a$	$>300^a$	40.8 ± 27.0^a	45.0 ± 37.6^a
5	$>300^a$	282.5 ± 42.9^a	$>300^a$	157 ± 75.0^b

Means followed by the same superscripts in each row do not differ statistically at $p<0.05$. Results are expressed as arithmetic means ± SD obtained from six enumerations.

Table 3. The number of LAB (· 10^2 cfu.ml^{-1}) in unpasteurised apple juice supplemented with various doses of 'Spirulina'

Day	'Spirulina' dose (% w/v)			
	0	0.5	1.0	1.5
0	6.03 ± 0.68[a]	6.35 ± 0.30[a]	5.72 ± 0.51[a]	6.05 ± 0.58[a]
1	1.33 ± 0.06[a]	2.68 ± 0.85[a]	2.88 ± 1.05[a]	4.47 ± 0.77[a]
2	2.22 ± 0.58[a]	4.18 ± 0.18[a]	8.51 ± 0.72[a]	12.7 ± 5.40[a]
3	11.6 ± 2.20[a]	15.8 ± 3.80[a]	62.6 ± 1.90[a]	137 ± 83.8[a]
4	97.5 ± 49.2 [a]	167 ± 34.0 [a]	532 ± 63.0 [a]	940 ± 164 [b]
5	1400 ± 890 [a]	1450 ± 253.8 [a]	>3000 [b]	>3000 [b]

Means followed by the same superscripts in each row do not differ statistically at $p<0.05$. Results are expressed as arithmetic means ± SD obtained from six enumerations.

Table 4. The number of yeasts (· 10^3 cfu.ml^{-1}) in unpasteurised apple juice supplemented with various doses of 'Spirulina'

Day	'Spirulina' dose (% w/v)			
	0	0.5	1.0	1.5
0	3.95 ± 0.66 [a]	3.25 ± 0.64 [a]	4.75 ± 0.70 [a]	3.32 ± 0.89 [a]
1	4.38 ± 0.47 [a]	3.77 ± 0.92 [a]	5.63 ± 1.89 [a]	3.53 ± 0.78 [a]
2	7.17 ± 0.94 [a]	9.67 ± 0.96 [a]	10.7 ± 2.13 [a]	7.58 ± 0.95 [a]
3	35.2 ± 13.0 [a]	29.0 ± 8.20 [a]	25.7 ± 9.00 [a]	52.3 ± 35.8 [a]
4	45.2 ± 7.90 [a]	84.3 ± 11.7 [a]	178 ± 40.0 [a]	1630 ± 450 [b]
5	480 ± 205 [a]	252 ± 92 [a]	1000 ± 560 [b]	1630 ± 960 [b]

Means followed by the same superscripts in each row do not differ statistically at $p<0.05$. Results are expressed as arithmetic means ± SD obtained from six enumerations.

The number of yeasts in unpasteurised apple juice was not affected by the presence of 'Spirulina' for the first two days of storage (Table 4), however, it increased significantly on day 4 and 5. The explanation of this phenomenon could be that after initial digestion of nutrients which are introduced with cyanobacteria biomass by bacteria, yeasts could assimilate them.

CONCLUSION

According to the presented results it could be stated that 'Spirulina' has some potential for limiting the growth of mesophilic bacteria in unpasteurized beverages, however applying 'Spirulina' for the extension of their shelf life needs further studies.. More detailed experiments are necessary to understand the mechanism of changes in microbial population, especially LAB and yeasts, in the presence of biocompounds from cyanobacteria biomass.

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