

## THE VIABILITY OF YOGURT BACTERIA IN SELECTED PLANT BEVERAGES

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**Abstract.** The aim of this work was to investigate whether selected plant beverages can be a carrier of live cultures of lactic acid bacteria. Seven commercial dairy freeze-dried starters were analysed in the experiments. They were used to produce samples of fermented and non-fermented three market plant beverages (soy, rice, and coconut). The analysis of the plant beverage samples was carried out for three weeks of the refrigerated storage. The number of lactic acid bacteria cells (lactobacilli and streptococci) and pH were determined. In all starter cultures, a significantly higher share of streptococci than lactobacilli was observed. The viability of lactobacilli and streptococci was dependent on the type of plant beverage and the starter culture used, as well as on whether the beverage was fermented. Our experiments clearly show that the starter culture should be carefully selected for the specific type of plant beverage

**Key words:** fermented beverage, soy, rice, coconut, yogurt bacteria

### INTRODUCTION

Fermented milk beverages are very popular, and their health-promoting effect is scientifically documented [Donkor et al. 2005, Champagne et al. 2009, Rivera-Espinoza and Gallardo-Navarro 2010]. People consume them, not only because of their good taste, but mainly due to various health benefits. In addition, many people cannot consume cow's milk or other mammal products for nutritional (lactose intolerance or proteins contained in milk) or cultural (vegetarianism, religious precepts) reasons. Therefore, there have been attempts to formulate vegetable cow's milk substitutes. There are many different kinds of vegetable or plant beverages differing in qualitative and quantitative composition of

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sugars [Donkor et al. 2005, Champagne et al. 2009, Rivera-Espinoza and Gallardo-Navarro 2010]. For many years, attempts have been undertaken to obtain fermented plant beverages containing lactic acid bacteria present in fermented milk beverages [Chou and Hou 2000, Beasley et al. 2003, Farnworth et al. 2007, Champagne et al. 2009]. The high population level of microorganisms used in their production for the whole shelf life is one of the indicators of the quality of these products and their health-promoting properties. The minimum number of cells in such products should be at least  $7 \log \text{CFU} \cdot \text{cm}^{-3}$  or g for industrial bacteria, or at least  $6 \log \text{CFU} \cdot \text{cm}^{-3}$  or g<sup>-1</sup> for labelled additional microorganisms (including probiotic strains) [FAO/WHO 2001]. As it was previously described, the composition and acidity of a product has a significant impact on the viability of bacterial cultures as well as maintaining the required minimum populations of industrial bacteria [Beasley et al. 2003, Zaręba et al. 2008a].

The aim of this study was to investigate if selected plant beverages (non-fermented and fermented) can be a carrier of live lactic acid bacteria. Second target was to study if these beverages meet the criteria of the required minimum for introduced microbial populations throughout the period of refrigerated storage conditions.

## MATERIALS AND METHODS

Seven commercial freeze-dried dairy starter cultures of different microbiological composition, including yogurt starters containing probiotic strains, were used in the experiments: Yo-A, Yo-B, and Yo-S (containing *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, Mediterranea Biotechnologies, Italy); YC-X11; YC-X16 YO-Flex (containing *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, Chr. Hansen, Denmark), YC-180 YO-Flex (containing *Str. thermophilus* and *Lb. delbrueckii* subsp. *lactis* and *Lb. delbrueckii* subsp. *bulgaricus*, Chr. Hansen, Denmark); and ABY-3 (containing *Str. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* La-5, *Bif. animalis* subsp. *lactis* Bb-12, Chr. Hansen, Denmark), despite the fact that they were not differentiated in these studies and their population was not quantified (only the total number of bacteria was determined). The aim was to check a certain number of available starters for use in obtaining non-fermented and fermented plant beverages as a carrier of live lactic acid bacteria.

All beverages were produced from three natural flavoured plant beverages available on the market: soy, rice, and coconut beverages without sugars added (EcoMill; all purchased from local market). The samples of non-fermented plant beverages were transferred directly at 6°C immediately after the inoculation with yogurt cultures. The samples of fermented plant beverages were 37°C immediately before the inoculation with yogurt cultures. The fermentation of plant beverages was carried out at 37°C for 5 h after inoculation with 0,004% yogurt culture (according to the manufacturer's instructions). Each experiment was performed in three independent replicates. All samples of plant beverages were stored at 6°C for 21 days.

The analysis of plant beverage samples (the number of lactic acid bacteria cells and pH) was carried out at the time of inoculation, and in the 1st and 3rd weeks of refrigerated storage of beverages. The determination of lactic acid bacteria cells was performed using the classical plate method using agar media: M17 agar with a pH equal to  $7.2 \pm 0.2$

(Merck, Darmstadt, Germany, incubation at 37°C for 72 h under aerobic conditions), and MRS agar with a pH equal to 5.7 ± 0.2 (Merck, Darmstadt, Germany; incubation at 37°C for 72 h under anaerobic conditions). The final results were converted into colony-forming units in 1 cm<sup>3</sup> (CFU·cm<sup>-3</sup>) and calculated as the mean value of the logarithm of the number of bacterial cells (log CFU·cm<sup>-3</sup>) and the standard deviation of parallel determinations. The pH value was determined with a pH-meter CPO-505 (Elmetron, Poland).

These data were compared using Tukey HSD's ANOVA in Statgraphics Centurion XV (Statpoint Technologies, Inc., USA). Statistical significance was set at P < 0.05. To assess the possibility of sample classification based on the survival of bacteria in samples during storage, cluster analysis (PCA) was performed. The statistical analyses were conducted with Statistica v.12 software (StatSoft Inc., USA).

## RESULTS AND DISCUSSION

The fermented and non-fermented soy beverage samples differed in terms of both pH value and the viability of lactobacilli and streptococci. A reduction of pH value was observed in all of the soy beverages during refrigerated storage (Table 1). And the strongest changes were found in non-fermented beverage samples because the soy beverages contained sugars available for the cultures used. The major sugars present in soybean include glucose, fructose, sucrose, raffinose, and stachyose. The pH depended significantly not only on the storage time, or the fermentation process, but also on the starter culture used for beverage production. The lowest pH changes indicates a high stabilization of the present microbial activity. The lactobacilli and streptococci viability was determined by the fermentation process, the starter culture used, storage time, and the pH value. The intensity of pH value changes did not have a linear reference in changes in the population of lactobacilli (Table 1).

The acidity of rice beverages depended significantly on fermentation process, storage time and the type of starter culture used for production. The highest pH values were found in samples immediately after their production (Table 2). As in the case of soy beverages, the changes in the acidity of beverages, both fermented and non-fermented, were observed during the 21 days of storage, but the strongest changes were noted in non-fermented beverage samples. These changes in acidity can be explained by the presence of natural sugars in rice, because sucrose, glucose, fructose, maltose and raffinose are the major soluble sugars in rice. However, in the samples of fermented rice beverages, the changes in pH value were not so strong, that can be explained by the fact that the available sugars were already used by the starter bacteria, as well as, by the low pH value of these beverages what inhibited the further activity of present bacterial cells. The changes of starter culture population were observed with the changes in the acidity of the samples. These changes were statistically significant, but the correlation with the changes of pH value was not linear. Multivariate analysis of variance revealed that the population of lactobacilli and streptococci depended on the starter culture and the storage time (different superscripts in Table 2). On the 21st day of the experiments the number of bacterial cells was significantly lower compared to that on the first day. Refrigerated storage of

Table 1. Population of lactic acid bacteria (expressed as log CFU·cm<sup>-3</sup>) and pH value of fermented and non-fermented soy beverages (means and standard deviations)  
 Tabela 1. Populacja bakterii kwasu mlekovego (wyrażona jako log jfk·cm<sup>-3</sup>) i wartość pH fermentowanych i niefermentowanych napojów sojowych (wartości średnie i odchylenia standarodowe)

Starter culture Kultura	Storage time [days] Czas przechowywania [dni]	Fermented Soy Beverage Fermentowany napój sojowy						Non-Fermented Soy Beverage Niefermentowany napój sojowy					
		<i>Lactobacillus</i> spp.			<i>Streptococcus</i> sp.			pH			<i>Lactobacillus</i> spp.		
		średnia mean	SD średnia mean	SD średnia mean	SD średnia mean	SD średnia mean	SD średnia mean	średnia mean	SD średnia mean	SD średnia mean	średnia mean	SD średnia mean	SD średnia mean
ABY-3	1st day	4.77 a	±0.023	7.5 a	±0.05	8.8 a	±0.12	7.01 a	±0.013	7.0 a	±0.03	8.7 a	±0.13
	7th day	4.62 d	±0.010	6.6 a,b	±0.04	8.6 a	±0.11	6.11 b	±0.030	7.0 a	±0.04	8.3 a	±0.11
	21th day	4.48 e	±0.020	6.2 b	±0.13	8.2 a,b	±0.04	5.75 c	±0.024	6.7 b	±0.02	7.5 b	±0.14
YC 180	1st day	5.00 b,c	±0.023	8.1 a	±0.10	8.5 a	±0.07	7.02 a	±0.032	7.8 a	±0.04	8.5 a	±0.12
	7th day	5.00 b,c	±0.021	8.0 a	±0.05	8.3 a	±0.21	5.82 c	±0.023	7.8 a	±0.07	8.4 a	±0.06
	21th day	4.35 e	±0.020	7.4 a	±0.06	6.9 b,c	±0.34	5.01 f	±0.026	6.9 a,b	±0.35	7.5 b	±0.10
YC X11	1st day	4.80 a	±0.023	7.3 a	±0.12	8.7 a	±0.30	6.98 a	±0.031	7.7 a	±0.03	8.3 a	±0.14
	7th day	4.79 a	±0.021	6.8 a,b	±0.12	8.4 a	±0.26	5.64 d	±0.027	7.3 a	±0.11	8.0 a	±0.05
	21th day	4.16 f	±0.020	6.2 b	±0.09	7.4 b	±0.01	5.13 e	±0.024	6.0 b,c	±0.15	7.3 b	±0.21
YC X16	1st day	4.96 b	±0.021	7.3 a	±0.07	8.8 a	±0.10	7.03 a	±0.031	7.2 a	±0.05	8.7 a	±0.03
	7th day	4.90 b	±0.023	6.2 b	±0.21	8.5 a	±0.14	5.80 c	±0.025	6.1 b	±0.06	8.4 a	±0.06
	21th day	4.01 g	±0.020	5.2 c	±0.21	7.7 b	±0.03	5.15 e	±0.023	5.4 c	±0.03	7.5 b	±0.14
Yo A	1st day	4.64 d	±0.021	7.5 a	±0.14	8.9 a	±0.17	7.04 a	±0.030	7.5 a	±0.14	8.4 a	±0.17
	7th day	4.91 b	±0.024	7.0 a,b	±0.03	8.6 a	±0.05	6.50 b	±0.031	6.3 b	±0.00	8.4 a	±0.03
	21th day	4.25 f	±0.021	6.2 b	±0.06	8.4 a	±0.37	5.22 e	±0.024	5.6 c	±0.09	7.3 b	±0.07
Yo B	1st day	5.09 b,c	±0.023	7.6 a	±0.02	8.8 a	±0.02	7.07 a	±0.030	7.6 a	±0.04	8.6 a	±0.04
	7th day	5.06 b,c	±0.021	6.4 b	±0.26	8.5 a	±0.25	5.88 c	±0.026	6.4 b	±0.03	8.3 a	±0.07
	21th day	4.80 a	±0.022	6.2 b	±0.21	7.5 b	±0.14	5.16 e	±0.020	6.0 b,c	±0.07	7.6 b	±0.12
Yo S	1st day	4.70 a	±0.023	7.2 a	±0.10	9.0 a	±0.14	6.95 a	±0.027	7.0 a	±0.21	8.7 a	±0.13
	7th day	4.62 d	±0.021	5.9 b,c	±0.28	8.6 a	±0.04	6.02 b,c	±0.030	5.8 c	±0.21	8.7 a	±0.06
	21th day	3.86 g	±0.014	5.4 c	±0.06	7.9 b	±0.08	5.58 d	±0.026	5.4 c	±0.03	7.8 a,b	±0.21

a, b, c – different superscripts within a column show significant difference ( $P < 0.05$ )/różne indeksy w obrębie kolumny wskazują na statystycznie istotne różnice ( $P < 0.05$ ).

Table 2. Population of lactic acid bacteria (expressed as log CFU·cm<sup>-3</sup>) and pH value of fermented and non-fermented rice beverages (means and standard deviations)

Tabela 2. Populacja bakterii kwasu mlekovego (wyrażona jako log jfk·cm<sup>-3</sup>) i wartości pH fermentowanych i niefermentowanych napojów ryżowych (wartości średnie i odchylenia standardowe)

Starter culture Kultura	Storage time [days] Czas przechowywania [dni]	Fermented Rice Beverage										Non-Fermented Rice Beverage					
		Fermentowany napój ryżowy					Niefermentowany napój ryżowy					<i>Lactobacillus</i> spp.			<i>Streptococcus</i> sp.		
		pH mean średnia	<i>Lactobacillus</i> spp. mean średnia	<i>Streptococcus</i> sp. mean średnia	pH mean średnia	<i>Lactobacillus</i> spp. mean średnia	<i>Streptococcus</i> sp. mean średnia	pH mean średnia	<i>Lactobacillus</i> spp. mean średnia	<i>Streptococcus</i> sp. mean średnia	pH mean średnia	<i>Lactobacillus</i> spp. mean średnia	<i>Streptococcus</i> sp. mean średnia	pH mean średnia	<i>Lactobacillus</i> spp. mean średnia	<i>Streptococcus</i> sp. mean średnia	
ABY-3	1st day	4.02 a ±0.020	7.8 a ±0.03	8.6 a ±0.18	6.60 a ±0.033	7.8 a ±0.04	8.6 a ±0.08	7.8 a ±0.04	7.6 a ±0.05	8.5 a ±0.10	8.6 a ±0.08	7.7 a ±0.023	7.6 a ±0.05	8.5 a ±0.10	8.0 ab ±0.02	8.0 ab ±0.02	
	7th day	3.84 d ±0.019	7.7 a ±0.15	8.1 a ±0.12	4.79 d ±0.023	7.6 a ±0.07	8.5 a ±0.10	7.7 a ±0.04	7.7 a ±0.07	8.0 ab ±0.02	8.0 ab ±0.02	7.7 a ±0.024	7.7 a ±0.07	8.2 ab ±0.32	8.2 ab ±0.32		
	21th day	3.76 d ±0.019	7.2 a ±0.14	7.1 b ±0.28	4.37 f ±0.020	6.9 ab ±0.07	8.0 ab ±0.02	7.9 a ±0.11	7.9 a ±0.07	8.0 ab ±0.02	8.0 ab ±0.02	7.7 a ±0.021	7.7 a ±0.07	7.7 b ±0.07	7.7 b ±0.07		
YC 180	1st day	4.12 a ±0.021	8.1 a ±0.11	8.5 a ±0.14	6.54 a ±0.031	7.9 a ±0.11	8.6 a ±0.14	7.9 a ±0.11	8.6 a ±0.14	8.6 a ±0.14	8.6 a ±0.14	7.7 a ±0.024	7.7 a ±0.17	8.2 ab ±0.32	8.2 ab ±0.32		
	7th day	3.77 d ±0.019	7.9 a ±0.24	8.4 a ±0.26	5.35 c ±0.026	7.7 a ±0.17	8.5 a ±0.17	7.7 a ±0.17	7.7 a ±0.17	8.2 ab ±0.32	8.2 ab ±0.32	7.7 a ±0.024	7.7 a ±0.17	8.2 ab ±0.32	8.2 ab ±0.32		
	21th day	3.50 e ±0.018	7.4 a ±0.01	7.5 a,b ±0.18	4.81 d ±0.021	6.6 b ±0.04	7.7 b ±0.07	7.7 b ±0.07	7.7 b ±0.07	7.7 b ±0.07	7.7 b ±0.07	6.6 b ±0.021	6.6 b ±0.04	7.7 b ±0.07	7.7 b ±0.07		
YC X11	1st day	4.11 a ±0.021	7.6 a ±0.06	8.9 a ±0.04	6.55 a ±0.032	7.5 a ±0.07	8.8 a ±0.05	7.5 a ±0.07	8.8 a ±0.05	8.8 a ±0.05	8.8 a ±0.05	7.5 a ±0.021	7.5 a ±0.07	8.4 a ±0.10	8.4 a ±0.10	8.4 a ±0.10	
	7th day	3.93 a ±0.020	7.0 a,b ±0.16	8.5 a ±0.21	4.75 d,e ±0.021	6.8 ab ±0.021	8.8 a ±0.05	6.8 ab ±0.021	8.8 a ±0.05	8.8 a ±0.05	8.8 a ±0.05	6.4 b ±0.021	6.4 b ±0.03	7.3 b,c ±0.07	7.3 b,c ±0.07		
	21th day	3.69 d,e ±0.018	6.4 b ±0.00	7.5 a,b ±0.18	4.01 g ±0.01	6.4 b ±0.021	8.8 a ±0.05	6.4 b ±0.021	8.8 a ±0.05	8.8 a ±0.05	8.8 a ±0.05	6.4 b ±0.021	6.4 b ±0.03	7.3 b,c ±0.07	7.3 b,c ±0.07		
YC X16	1st day	4.25 b ±0.021	7.7 a ±0.24	8.9 a ±0.11	6.58 a ±0.031	7.5 a ±0.25	8.8 a ±0.10	7.5 a ±0.25	8.8 a ±0.10	8.8 a ±0.10	8.8 a ±0.10	7.3 a ±0.023	7.3 a ±0.04	8.7 a ±0.07	8.7 a ±0.07		
	7th day	4.13 a ±0.021	6.8 a,b ±0.12	8.6 a ±0.11	4.88 d ±0.021	6.8 ab ±0.021	8.8 a ±0.10	6.8 ab ±0.021	8.8 a ±0.10	8.8 a ±0.10	8.8 a ±0.10	6.8 ab ±0.021	6.8 ab ±0.04	8.7 a ±0.07	8.7 a ±0.07		
	21th day	3.78 d ±0.018	6.0 b ±0.16	8.3 a ±0.16	4.15 g ±0.021	6.8 ab ±0.021	8.8 a ±0.10	6.8 ab ±0.021	8.8 a ±0.10	8.8 a ±0.10	8.8 a ±0.10	7.3 a ±0.023	7.3 a ±0.04	8.7 a ±0.07	8.7 a ±0.07		
Yo A	1st day	4.15 a ±0.020	8.0 a ±0.16	8.2 a ±0.03	6.38 b ±0.030	8.0 a ±0.16	8.5 a ±0.05	8.0 a ±0.16	8.5 a ±0.05	8.5 a ±0.05	8.5 a ±0.05	7.3 a ±0.021	7.3 a ±0.03	8.1 ab ±0.14	8.1 ab ±0.14		
	7th day	4.12 a ±0.021	7.5 a ±0.09	7.2 b ±0.28	5.53 c ±0.021	7.8 a ±0.03	8.5 a ±0.05	7.8 a ±0.03	8.5 a ±0.05	8.5 a ±0.05	8.5 a ±0.05	7.3 a ±0.021	7.3 a ±0.04	8.1 ab ±0.14	8.1 ab ±0.14		
	21th day	3.84 d ±0.019	7.3 a ±0.11	6.9 b ±0.08	4.76 d ±0.021	7.2 a ±0.21	8.5 a ±0.05	7.2 a ±0.21	8.5 a ±0.05	8.5 a ±0.05	8.5 a ±0.05	7.2 a ±0.021	7.2 a ±0.04	8.1 ab ±0.12	8.1 ab ±0.12		
Yo B	1st day	4.21 a,b ±0.021	7.4 a ±0.11	8.7 a ±0.07	6.44 a,b ±0.031	7.3 a ±0.04	8.6 a ±0.04	7.3 a ±0.04	8.6 a ±0.04	8.6 a ±0.04	8.6 a ±0.04	7.3 a ±0.021	7.3 a ±0.04	8.3 ab ±0.21	8.3 ab ±0.21		
	7th day	3.71 d ±0.018	7.1 a ±0.34	8.5 a ±0.21	4.59 e ±0.021	7.3 a ±0.16	8.5 a ±0.05	7.3 a ±0.16	8.5 a ±0.05	8.5 a ±0.05	8.5 a ±0.05	7.3 a ±0.020	7.3 a ±0.04	7.7 b ±0.07	7.7 b ±0.07		
	21th day	3.16 f ±0.015	6.7 b ±0.03	7.7 a,b ±0.07	4.15 g ±0.020	6.6 b ±0.21	8.5 a ±0.05	6.6 b ±0.21	8.5 a ±0.05	8.5 a ±0.05	8.5 a ±0.05	7.2 a ±0.021	7.2 a ±0.04	8.1 ab ±0.12	8.1 ab ±0.12		
Yo S	1st day	5.04 c ±0.025	7.5 a ±0.14	8.8 a ±0.03	6.63 a ±0.032	7.1 ab ±0.17	8.3 ab ±0.16	7.1 ab ±0.17	8.3 ab ±0.16	8.3 ab ±0.16	8.3 ab ±0.16	7.1 ab ±0.031	7.1 ab ±0.05	8.3 ab ±0.16	8.3 ab ±0.16		
	7th day	4.73 c ±0.020	7.4 a ±0.06	8.5 a ±0.21	6.35 b ±0.031	6.9 ab ±0.09	8.1 ab ±0.12	6.9 ab ±0.09	8.1 ab ±0.12	8.1 ab ±0.12	8.1 ab ±0.12	6.9 ab ±0.026	6.9 ab ±0.03	7.5 b ±0.18	7.5 b ±0.18		
	21th day	4.19 a ±0.018	7.1 a ±0.26	8.0 a ±0.19	5.88 c ±0.026	6.4 b ±0.03	7.5 b ±0.18	6.4 b ±0.03	7.5 b ±0.18	7.5 b ±0.18	7.5 b ±0.18	6.4 b ±0.026	6.4 b ±0.03	7.5 b ±0.18	7.5 b ±0.18		

a, b, c – different superscripts within a column show significant difference ( $P < 0.05$ )/różne indeksy w obrębie kolumny wskazują na statystycznie istotne różnice ( $P < 0.05$ ).

both fermented and non-fermented rice beverages had a statistically significant effect on the viability of streptococci.

A reduction of pH value was observed also during the refrigerated storage of the coconut beverage samples and this was a statistically significant phenomenon which depended on the starter culture used and the fermentation process of coconut beverage (Table 3). Coconut beverage naturally contains very little carbohydrates of any kind, including fructose. The storage time and the type of starter culture also significantly determined the viability of lactobacilli and streptococci, but in a different range than in the case of soy and rice beverages (different superscripts in Table 3). In all samples of coconut beverage, the changes in the population of lactobacilli were observed. This means that the refrigerated storage conditions of non-fermented coconut beverages did not stop the present bacteria against the activity. In the case of the population of streptococci in coconut beverages the situation was different. The number of *Str. thermophilus* cells was significantly dependent on the storage time, and the type of starter culture used.

Excellent viability of the microbial starter culture is the first and the most important criterion to ensure a good and healthy quality of the product. This is because the careful selection of starter cultures as well as the storage parameters is a guarantee of the good organoleptic properties of the final product, which is determined by the metabolites of lactic acid bacteria produced during the fermentation process as well as during storage time [Zaręba et al. 2014]. In our studies, the number of lactobacilli and streptococci in most of the fermented beverages was at a similar level as in the non-fermented beverage samples. Farnworth et al. [2007] observed approximately 2 log increases in the number of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* in fermented soy beverage. This was also confirmed by Chou and Hou [2000] and Tang et al. [2007]. They indicated that the fermentation time is dependent on the bacterial strain used. Our research confirmed that the number of lactobacilli and streptococci cells increases during the fermentation of plant beverages, but this trend is reversed during refrigerated storage. The reduction in lactobacilli and streptococci population during refrigerated storage was depending on the culture and the type of plant beverage. Their viability depended on the fermentation process, the starter culture used, storage time, and the pH value. Shimakawa et al. [2003] obtained results similar to those demonstrated in our study. In contrast, some researchers have observed an increasing of bacterial population for a few first days of refrigerated storage, and later reduction in the population [Beasley et al. 2003]. Initially, the pH value of non-fermented plant beverage samples was significantly higher than that in fermented samples in our research. The changes in the pH value of the plant beverages during the fermentation process are evidence that the lactic acid bacteria found sugars in biochemical pathways useful for conversion into the energy required for the growth of the bacterial cells [Chou and Hou 2000, Champagne et al. 2009, Mousavi et al. 2011]. The increase in acidity (i.e., decrease in the pH value) due to the fermentation process was important and statistically significant and confirmed the fermentation ability of lactic acid bacteria cultures used. In all cases, a lowering of the pH value was observed during refrigerated storage of plant beverage samples. However, a drastic reduction of the pH value was detected only in non-fermented samples. More importantly, the differences in the reduction of pH values were higher after 3 weeks than at 1 week of storage, which was also confirmed by the principal component analysis (PCA). The PCA (Fig. 1) shows the behaviour of the survival of lactic acid bacteria in terms of three variables: the difference

Table 3. Population of lactic acid bacteria (expressed as log CFU·cm<sup>-3</sup>) and pH value of fermented and non-fermented coconut beverages (means and standard deviations)  
Tabela 3. Populacja bakterii kwasu mlekovego (wyrażona jako log jfk·cm<sup>-3</sup>) i wartości pH fermentowanych i niefermentowanych napojów kokosowych (wartości średnie i odchylenia standardowe)

Starter culture Kultura	Storage time [days] Czas przechowywania [dni]	Fermented Coconut Beverage Fermentowany napój kokosowy						Non-Fermented Coconut Beverage Niefermentowany napój kokosowy					
		<i>Lactobacillus</i> spp.			<i>Streptococcus</i> sp.			<i>Lactobacillus</i> spp.			<i>Streptococcus</i> sp.		
		pH mean średnia	SD mean średnia	SD mean średnia	SD mean średnia	SD mean średnia	SD mean średnia	pH mean średnia	SD mean średnia	SD mean średnia	pH mean średnia	SD mean średnia	SD mean średnia
ABY-3	1st day	4.40 c	±0.015	7.8 a	±0.42	9.0 a	±0.10	6.24 a	±0.030	7.2 a	±0.03	8.8 a	±0.12
	7th day	4.01 d	±0.020	7.2 a	±0.03	8.8 a	±0.05	5.15 e	±0.025	6.9 a,b	±0.03	8.5 a	±0.04
	21th day	3.75 e	±0.015	6.5 b	±0.15	8.3 a	±0.21	4.92 e	±0.021	6.3 b	±0.17	7.6 b	±0.07
YC 180	1st day	4.60 a	±0.020	7.6 a	±0.02	8.7 a	±0.11	6.45 b	±0.032	7.6 a	±0.05	8.5 a	±0.09
	7th day	4.61 a	±0.025	7.3 a	±0.21	8.4 a	±0.12	5.09 e	±0.020	7.4 a	±0.12	8.2 a	±0.21
	21th day	3.85 e	±0.018	6.5 b	±0.18	7.5 b	±0.09	4.58 f	±0.022	6.4 b	±0.17	7.3 b	±0.16
YC X11	1st day	4.47 b	±0.010	7.2 a	±0.10	8.7 a	±0.11	5.93 c	±0.023	7.1 a	±0.06	8.4 a	±0.30
	7th day	4.42 b	±0.019	6.1 b	±0.06	8.5 a	±0.10	5.28 d	±0.024	6.8 a,b	±0.21	8.0 a,b	±0.37
	21th day	3.97 d	±0.019	5.4 c	±0.03	7.7 b	±0.07	4.93 e	±0.024	5.1 c	±0.49	7.4 b	±0.26
YC X16	1st day	4.41 b,c	±0.020	7.2 a	±0.21	8.5 a	±0.25	5.92 c	±0.021	7.1 a	±0.01	8.1 a,b	±0.12
	7th day	4.40 c	±0.020	6.2 b	±0.21	8.3 a	±0.07	5.30 d	±0.024	6.9 a,b	±0.09	7.8 b	±0.21
	21th day	3.89 d,e	±0.019	5.2 c	±0.21	7.6 b	±0.04	4.66 f	±0.022	5.1 c	±0.37	7.5 b	±0.18
Yo A	1st day	4.57 a,b	±0.021	7.2 a	±0.26	8.6 a	±0.04	6.43 a	±0.031	7.0 a	±0.16	8.5 a	±0.05
	7th day	4.24 c	±0.020	6.6 b	±0.06	8.5 a	±0.01	5.76 c	±0.026	6.9 a,b	±0.18	8.2 a	±0.34
	21th day	4.03 d	±0.019	5.5 c	±0.10	8.1 a,b	±0.37	4.36 g	±0.023	5.6 c	±0.04	8.2 a	±0.09
Yo B	1st day	4.48 b	±0.021	7.0 a	±0.06	8.9 a	±0.21	6.33 a	±0.030	7.2 a	±0.14	8.5 a	±0.28
	7th day	4.15 c,d	±0.021	6.2 b	±0.05	8.8 a	±0.18	5.31 d	±0.024	6.1 b	±0.07	8.4 a	±0.09
	21th day	4.01 d	±0.020	5.3 c	±0.04	8.2 a	±0.09	4.92 e	±0.021	5.3 c	±0.04	8.4 a	±0.06
Yo S	1st day	4.75 a	±0.017	7.6 a	±0.06	8.2 a	±0.01	6.46 b	±0.032	7.4 a	±0.10	8.1 a,b	±0.09
	7th day	4.44 b	±0.022	6.2 b	±0.09	8.0 a,b	±0.39	5.33 d	±0.020	6.2 b	±0.05	8.0 a,b	±0.23
	21th day	3.89 d,e	±0.018	5.3 c	±0.0	7.4 b	±0.30	4.92 e	±0.020	5.4 c	±0.03	7.4 b	±0.26

a, b, c – different superscripts within a column show significant difference ( $P < 0.05$ )/różne indeksy w obrębie kolumny wskazujące istotyczne różnice ( $P < 0.05$ ).

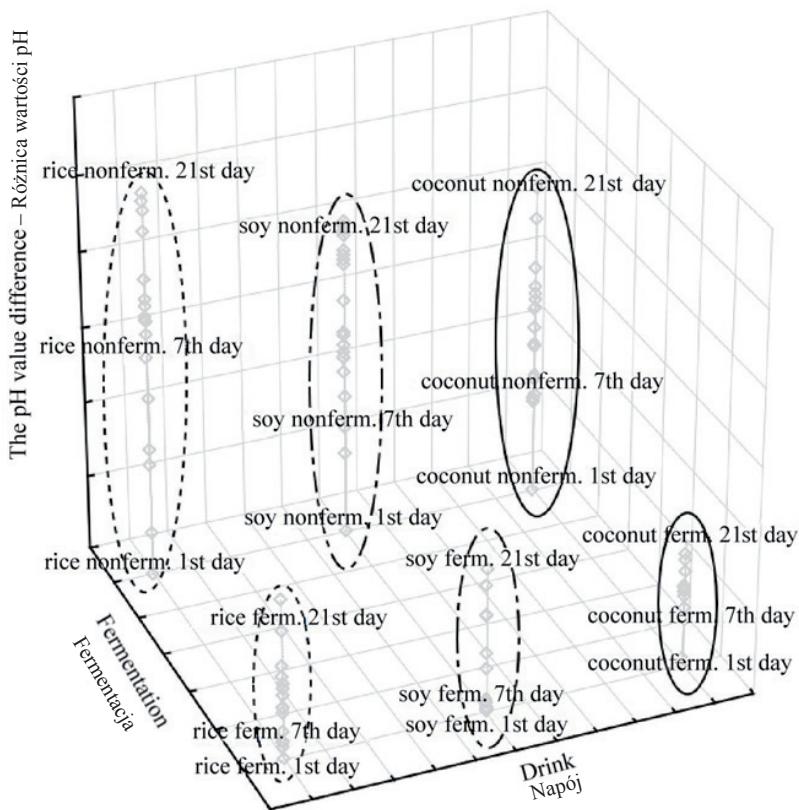


Fig. 1. Principal Component Analysis (PCA) of the survival rate of lactic acid bacteria in terms of three variables: the differences of pH value, the fermentation process, and the type of milk used in the experiments

Rys. 1. Analiza PCA (Principal Component Analysis) przeżywalności bakterii kwasu mleko-wego z uwzględnieniem trzech zmiennych: zmiany wartości pH, procesu fermentacji i rodzaju napoju roślinnego użytego w doświadczeniach

in the pH value, the fermentation process and the type of milk used in the test. The greater change of pH value was observed in the non-fermented samples during refrigerated storage compared to the fermented beverages, both in the 1st and 3rd weeks of storage. There were no evident significant differences between the different types of plant beverages in this regard. The available references relate only to the measurement of acidity in beverages fermented by single strains, but not commercial starter cultures. However, a similar trend for pH value reduction was noted by Beasley et al. [2003] and Zaręba et al. [2008a]. Acidity has a significant impact on the number of compounds affecting the taste and flavour of the final product. As is known, acidity also is a factor important to bacterial cell viability [Tang et al. 2007].

## CONCLUSIONS

Our experiments clearly show that not only fermented plant beverages may be carriers for viable cells of lactic acid bacteria cultures, but also non-fermented beverages inoculated with live starter cultures. Non-fermented plant beverages may also provide high numbers of microorganisms, initially inoculated with lactic acid bacteria and stored under refrigeration without fermentation process. The starter culture should be carefully selected for the specific type of plant beverage, as well as the storage temperature of the final product should also be adjusted.

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## PRZEŻYWALNOŚĆ BAKTERII JOGURTOWYCH W WYBRANYCH NAPOJACH ROŚLINNYCH

**Streszczenie:** Celem pracy było zbadanie, czy wybrane napoje roślinne mogą być nośnikiem żywych kultur bakterii kwasu mleковego. W doświadczeniach analizowano siedem komercyjnych liofilizowanych starterów mleczarskich. Były one użyte do otrzymania próbek fermentowanych i niefermentowanych trzech rynkowych napojów roślinnych (sojowego, ryżowego i kokosowego). Analizę próbek napojów prowadzono przez trzy tygodnie przechowywania w lodówce. Określono liczbę komórek bakterii kwasu mlekovego (pałeczek kwasu mlekovego i paciorkowców) i pH. W przypadku wszystkich starterów, zaobserwowano znacznie większy udział paciorkowców niż pałeczek kwasu mlekovego. Żywotność pałeczek kwasu mlekovego i paciorkowców zależała od rodzaju napoju roślinnego i zastosowanej kultury startowej, a także od tego, czy napój był fermentowany. Doświadczenia autorów dowiodły, że starter powinien być starannie dobrany do konkretnego rodzaju napoju roślinnego.

**Słowa kluczowe:** napój fermentowany, soja, ryż, kokos, bakterie jogurtowe