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The Correlation between the Dynamic of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* CFU/mL and pH Value during the Shelf-Life of Yoghurt

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Abstract. The major objective of this study was following the dynamic of *Streptococcus thermophilus* CFU/mL and *Lactobacillus delbrueckii subsp. bulgaricus* CFU/mL and pH during the shelf-life of yoghurt.The initial count of *Streptococcus thermophilus* in yoghurt was different from one source to another, but it had a range between 10^8 and 10^9 CFU/mL yoghurt. After 22 days of refrigeration, a decreasing number of *Streptococcus thermophilus* with up to 1 log (over 10^7CFU/mL) was revealed for all investigated samples. Also, *Lactobacillus delbrueckii subsp. bulgaricus* count recorded a downward trend during the validity period of the product, corresponding concentrations drop by less a log.The pH value recorded small variations from a determination to another.

Key words: *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus*, yoghurt, pH.

INTRODUCTION

The starter culture for yoghurt production is a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Although they can grow independently, the rate of lactic acid production is much higher when used together than either of the two organisms grown individually. The yoghurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yoghurt mix to approximately 5.0 and the lactobacilli are responsible for a further decrease to pH 4.5. Thus, the question: "How does the pH value of yoghurt advance during the shelf-life?" is justifiable:

MATERIALS AND METHODS

Four classic yoghurt samples (2,8% fat) from different sources (T, D, P and C) were investigated in this work. All yoghurt samples were taken immediately after the production and were subjected to refrigeration during the previously established shelf life (4°C). *Streptococcus thermophilus* count, *Lactobacillus delbrueckii subsp. bulgaricus* count (CFU/mL yoghurt) and pH value was performed right after sampling and every 7 days throughout the investigation period. Decimal dilutions of samples in peptonated bacteriological water were prepared for each sample, followed by inoculation of 1 mL of each concentration in two Petri dishes. In each Petri plates, melted *Streptococcus thermophilus* agar and MRS agar, respectively, were poured after being cooled down to 45°C. After solidification, Petri plates were turned upside down. Those with *Streptococcus thermophilus*

agar were subjected to incubation at 37°C, for 48 hours, whereas those with MRS agar were incubated under anaerobic condition at 37°C, for 72 h. Enumeration was made using the pour plate technique. Plates containing 25 to 250 colonies were enumerated and recorded as colony forming units (CFU) per mL of the product.

RESULTS AND DISCUTIONS

The results are listed in table 1 and illustrated in figure 1.

Tab. 1

The dynamic of pH values and of count S*treptococcus thermophilus* CFU/mL and *Lactobacillus delbrueckii subsp. bulgaricus* CFU/mL from yoghurt in validity period

Sample	Investigated Parameters	Determination I (day 1)	Determination II (day 8)	Determination III (day 15)	Determination IV (day 22)
T	pH	4,23	4,23	4,25	4,23
	Strep. therm CFU/mL	$6,8x10^8$	$5,1\ x10^8$	$2,1x\ 10^8$	$1,5x\ 10^8$
	L.delbr.bulgar CFU/mL	$3,4\ x10^8$	$2,2\ x10^8$	$1,6\ x10^8$	$1,2\ x10^8$
D	pH	4,32	4,34	4,34	4,32
	Strep. therm CFU/mL	$8,9\ x10^8$	$7,7\ x10^8$	$5,3\ x10^8$	$2,5\ x10^8$
	L.delbr.bulgar CFU/mL	$2,8\ x10^8$	$2,1\ x10^8$	$1,7\ x10^8$	$1,3\ x10^8$
P	pH	4,24	4,24	4,24	4,23
	Strep. therm CFU/mL	$2,3\ x10^8$	$9,3\ x10^7$	$4,5\ x10^7$	$2,9\ x10^7$
	L.delbr.bulgar CFU/mL	$4,2\ x10^8$	$3,1\ x10^8$	$2,2\ x10^8$	$1,8\ x10^8$
C	pH	4,21	4,17	4,14	4,17
	Strep. therm CFU/mL	$3,5\ x10^9$	$2\ x10^9$	$1,7\ x10^9$	$1,1\ x10^9$
	L.delbr.bulgar CFU/mL	$3,3\ x10^9$	$2,1\ x10^9$	$1,9\ x10^9$	$1,2\ x10^9$

The initial *Streptococcus thermophilus* concentration in yoghurt was different from one sample to another, the count differences ranging from 10^8 to 10^9 CFU/mL yoghurt. A decreasing shift in viable germs with up to 1 log, was revealed for all investigated samples. After 22 day of refrigeration, the number of *Streptococcus thermophilus* was over 10^7CFU/mL (Fig. 1).

As it can be seen in Fig.2, the graphs of the dynamic of *Lactobacillus delbrueckii subsp. bulgaricus* count recorded a downward trend during the validity period of the product, corresponding concentrations dropped by less a log. In case of sample C, the initial count was the biggest ($3,3\ x10^9$ CFU/mL), and after 22 days of conservation, the count of this bacterium remained increased ($1,2\ x10^9$ CFU/mL).

For all of the samples the populations of streptococi and lactobacilli had been at the end of the validity period of the product over the minimum amount of 10^6 CFU/mL (proposed criterion of a “therapeutic minimum”), which is considered as a product probiotic develops its beneficial effects for the consumer body.

The dynamic of pH value was also followed and very low variations in the shelf-life of the products were observed (Fig. 3).

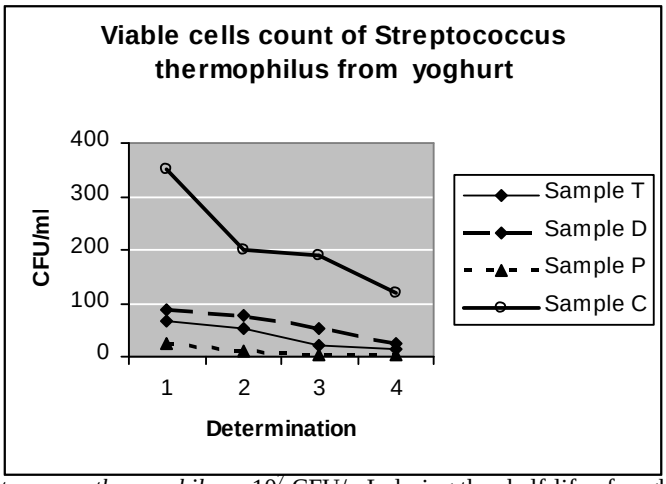

Fig. 1. *Streptococcus thermophilus* x 10^{7} CFU/mL during the shelf-life of yoghurt

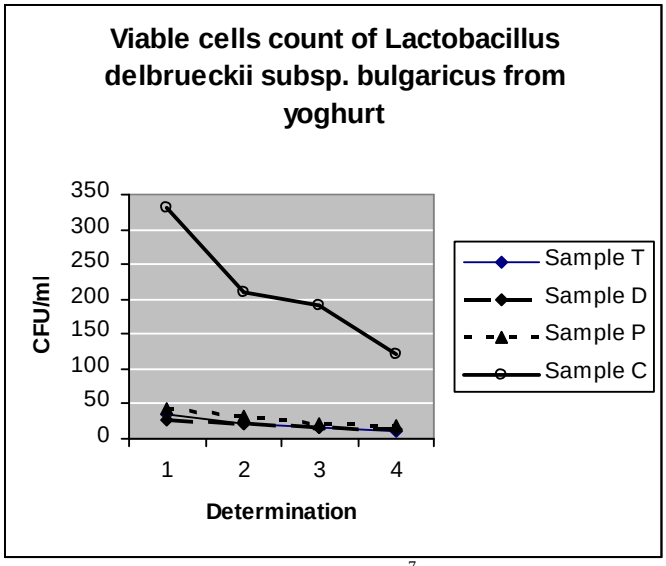

Fig. 2. *Lactobacillus delbrueckii subsp. bulgaricus* x10^{7} CFU/mL in shelf-life of yoghurt

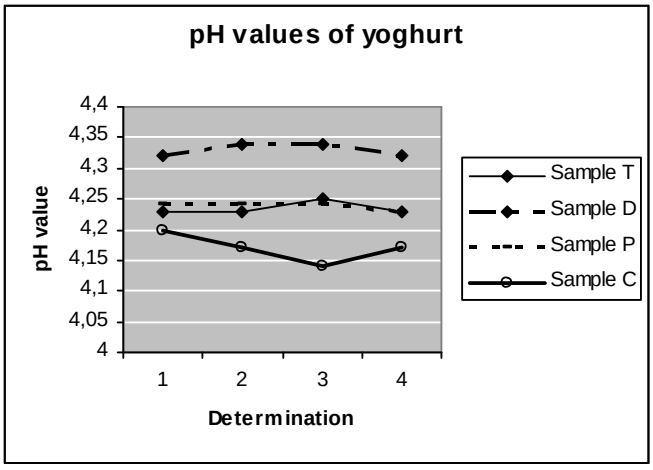

Fig. 3. The evolution of pH value during the shelf-life of yoghurt

Tab. 2

The correlation coefficient between pH and the yoghurt population of lactobacilli and streptococi

LAB	Day 1	Day 8	Day 15	Day 22
Streptococcus thermophilus	-0,21749	-0,19814	-0,32135	-0,28451
Lactobacillus delbrueckii subsp. bulgaricus	-0,63854	-0,75917	-0,8552	-0,75625

The results were statistically processed using SPSS program.

The value of correlation coefficient between viable cells counts of both bacterial species (CFU/mL) and pH value was calculated (Tab. 2). A negative correlation between the pH dynamic and the lactic acid bacteria (LAB) populations from yoghurt was observed. This was more intensely in case of *Lactobacillus delbrueckii subsp. bulgaricus* (-0,64.... -0,86) comparatively with *Streptococcus thermophilus* (-0,19... -0,32).

CONCLUSIONS

3.1. The present study revealed a decrease of viable *Streptococcus thermophilus* cells for all analyzed yoghurt samples, with approximately 1 log, during the product's shelf life (from 10^8 and 10^9 CFU/mL yoghurt initially, down to 10^7CFU/mL yoghurt, after 22 days of conservation).

3.2. The population of lactobacilli in all samples registered a steadily decline less than 1 log during storage.

3.3. The final level of lactic acid bacteria was approximately 1 log above the minimal admitted value of 10^6CFU/mL, considered for a product to have probiotic effect.

3.4. Very low variations of pH value in the shelf-life of the yoghurt were observed.

3.5. There is a more significant negative correlation between *Lactobacillus delbrueckii subsp. bulgaricus* CFUmL and the pH value, than *Streptococcus thermophilus* CFU/mL.

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