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TECHNOLOGICAL ASPECTS RELATED TO PREBIOTICS INCLUSION IN THE MACEDONIAN DAIRY INDUSTRY

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Abstract - Probiotic dairy products have been defined as "food containing live micro-organisms believed to actively enhance human health by improving the intestinal microbial balance". This study was carried out to evaluate the growth and viability of probiotic bacteria Bifidobacterium bifidus in natural set-yogurt during the period of 28 days (0, 1, 7, 14, 21, 28 days). Three series of fermented cow's milk have been taken, prepared in three variants as follows: the first variant (B) was prepared by the addition of the prebiotic Fibregum B, the second variant (P) with the addition of a prebiotic Fibregum P and the third variant of the fermented milk without prebiotic, served as the control variant (K). The dynamic of probiotic's bacteria growth and development was determined using a colony reader (pbi international F4). The count of probiotic bacteria Bifidobacterium bifidus was higher in natural set yogurt with prebiotic inclusion compared to the control variant without prebiotics. It has been concluded that the development of bacteria depends on the conditions and stage of production and the type of prebiotic.

Keywords - prebiotic, Bifidobacterium bifidus, natural - set yogurt, dynamic.

I. INTRODUCTION

Probiotics and Prebiotics still have a significant role in the field of preventive nutrition and their further development is therefore required [1]. Fermented dairy products have been and still are knowingly and unknowingly an important part of the human diet. Enrichment of the probiotic dairy products with prebiotics, has a sole purpose for multiplicating representative probiotics from the Bifidobacterium genera [2]. Prebiotics have primarily been characterized as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that provide benefits" [3]. Hence, in some instances, a prebiotic is known to have a beneficial outcome on probiotics assisting in the growth and viability of probiotic bacteria [4].

The viability of the probiotic bacteria "in vitro" and "in vivo" through the digestive tract can be enhanced by including prebiotics compounds such as Fructooligosaccharides, Inulin, etc., which act symbiotically [6,11]. As in previous studies characterizing a range of probiotic species and strain, a very high dose such as a minimum of 108 CFU/ day, mostly in the range of 1010–1011 CFU/day - was required for the respective health benefits [7].

The Fibregum is a natural, purified exudate of the tree Acacia, it contains more than 80% vegetable soluble fibres. It is composed of cellulose, hemicelluloses, oligosaccharides, pectin, waxes and lignin [8]. Inclusion of prebiotics Fibregum, during the technological process for production of natural-set yogurt, presents the following features: High stability in heat treatment, high stability in storage, high solubility at room temperature, improved water retention capacity (lower syneresis), low viscosity, pH and yeast fermentation, low hygroscopic traits, neutral taste, odor and color [9,10].

The aim of this study is to establish the length of survival of the probiotic bacteria during storage period (day 0 to day 28). The research is to confirm that prebiotics inclusion into a probiotic fermented dairy product is crucial from the technological aspect.

II. DETAILS EXPERIMENTAL

2.1. Technological operations of probiotic natural set - yogurt with prebiotic Fibregum inclusion

The experiment consists of a number of interrelated phases. First the bulk tank milk was taken and distributed to the dairy in Pelagonia region in Macedonia. The collected cow's milk was standardized on fat content of 1 ± 0, 1 % and was homogenized at temperatures of 65°C.

A quantity of 1% of milk powder was added into the homogenized milk with continuous mixing. In the next phase the milk was pasteurized with a heat treatment of 92°C and inoculated with a starter culture F-DVS ABT 10, (Probio-Tec-Chr. Hansen, Copenhagen, Denmark) composed of Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium bifidus at a temperature of 43°C. After, in two variants of milk B and P, prebiotics Fibregum B and Fibregum P (C.N.I. - Rovencedex.- France) were added at a rate of 1.5% (w/v) of each of

the prebiotic in order to increase the growth and viability of the above mentioned cultures. As a control variant (K) a sample of the natural - set yogurt without prebiotics was used. Further on the enriched milk samples with prebiotics were packed in plastic tins for sales and placed in a thermostatic chamber (Instrumentaria Sutjeska, Serbia) at 43 ± 1° C until terminal coagulation.

The coagulated samples were placed in the refrigerator at 4-6°C after 4 hours. All of the variants B, P and K, were kept stored for 28 days and during this period the number of viable cells in experimental variants has been measured.

2.2. Assessment of pH values and titrable acidity (TA) ^{0}SH

The assessment of the pH values has been conducted with pH-meter (Mettler Toledo Seven Eazy). The calibration of the pH-meter with buffer solutions of pH=7 and pH=4 has been done previously. The pH value was monitored for probiotic acid milk up to 28 day of storage. Titrable acidity was expressed in ^{0}SH which was determined by the titration according to Sokslet –Henkel.

The titration was conducted with 0.1 mol NaOH and indicator Phenolphthalein up to the change of color of the milk from white to pale pink. Titrable acidity was monitored after inoculation of the starter culture for the storage time as well.

2.3. Microbiological analysis

2.3.1. Monitoring the growth of the total number of bacteria in probiotic natural set -yogurt with the addition of prebiotics

The growth of probiotic bacteria B.bifidus has been done in anaerobic conditions using specific nutritional medium. Previously a serial diluting was conducted, and then the diluted solution was seeded in a Petri plate with the nutritional medium MRS – IM. In this medium in anaerobic conditions an intensive growth of B.bifidus was observed, but low amounts of St.thermophillus was present.

The nutritional medium MRS –IM was prepared by adding three types of solutions: Solution A: was prepared by diluting of Dichloxacillin in distillated water and filtering through the sterile filter 0,22µm. Solution B: was prepared by diluting of lithium chloride in distillated water and filtering through the sterile filter 0,22µm. Solution C: Cysteine hydrochloride was diluted in distillated water and was sterilized in an autoclave for 15 minutes at 120°C. The prepared Petri plates with the MRS-IM agar and added the solution from probiotic natural set-yogurts was placed in anaerobic conditions at 37°C for 3 days.

III. RESULTS AND DISCUSSION

In this research the assessment of the growth of the bacteria B.bifidus was conducted on two variants ("B" and "P") of a probiotic natural set- yogurts with prebiotics, as well as to the control variant "K".

Probiotic bacteria B. bifidus in diary-fermented products has been growing intensively up to the day 7 of storage and its development was further on decreasing. However, on day 28 it was still present in sufficient amount. The above mentioned can be seen in Table 1 and Figure no.1. The growth intensity of this bacteria is stronger with the variant P (Fibergum P included).

Table 1. Growth dynamics of B. bifidus during storage period

Dynamics of Bifidobacterium Bifidus ($\bar{x}$)						
Variants	Storage period					
	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28
	$x10^6$	$x10^{10}$	$x10^{10}$	$x10^6$	$x10^6$	$x10^6$
K	28.00^{ab}	46.00	72.33	64.00	49.33	28.33^{ab}
B	56.00^{a}	65.33	84.67	67.33	60.33	46.33^{a}
P	62.00^{b}	79.33	102.67	83.67	69.00	54.33^{b}

^{ab,}Values about dynamics of B.bifidus at days 0,14 and 28, followed by the same superscripts within 1 column are significantly different ($p < 0.05$).
Starting from the moment of the inoculation with the starter culture (F-DVS ABT 10- Probio-Tec.) marked as day zero, the growth of the bacteria B.bifidus was constantly increasing up to day 7. B.bifidus was present even on day twenty eight of storage.

During the first week in storage the average number of living cells of B.bifidus was higher in the probiotic natural- set yogurt with prebiotic P inclusion, (102.67 x 10^{10}cfu/ml) compared with the variant B (84.67 x 10^{10}cfu/ml) as well as with the control variant K (72.33 x 10^{10}cfu/ml). Thus, the influence of the prebiotic on the bacterial growth has been proved. Such obtained results were also found in better agreement with the other workers in dairy science [3,5,6,9,12].

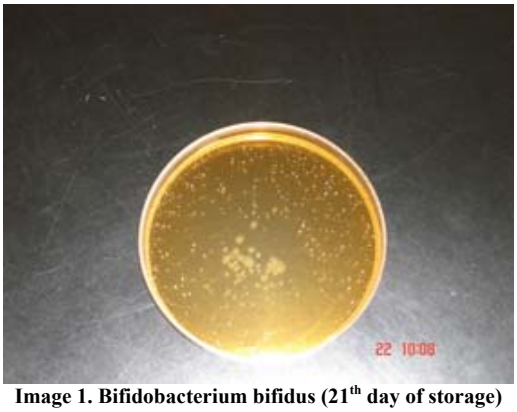

Image 1. Bifidobacterium bifidus (21th day of storage)

Table 2. TA dynamics of three variants probiotic natural set-yogurt during storage period

Dynamics of titrable acidity (TA) ^{0}SH (x̄)					
Variants	Storage time				
	Day 1	Day 7	Day 14	Day 21	Day 28
K	28.23	29.53	30.87	36.17	37.33^{ab}
B	29.33	31.50	32.70	33.03	34.53^{a}
P	30.43	32.30	33.87	34.27	34.90^{b}

^{ab,}Values about dynamics of ^{0}SH at days 1, 14 and 28, followed by the same superscripts within 1 column are significantly different ($p < 0.05$).

In Table 2 and Figure 2 the results about the change of titrable acidity of three variants probiotic natural set-yogurt in a period of 28 days has been shown. The results gained indicate that the titrable acidity was constantly increased in all variants. The value of titrable acidity at two variants with prebiotic inclusion has been found almost

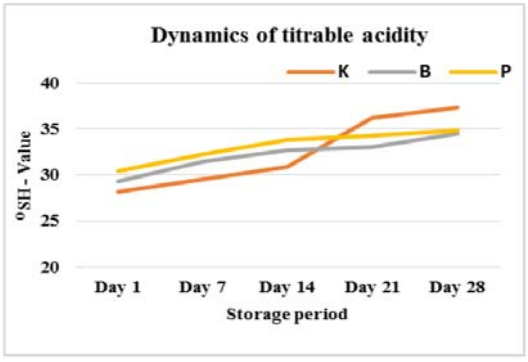

Figure 2. Dynamics of titrable acidity of three variants probiotic natural set-yogurt during storage period

Table 3. pH dynamics of three variants probiotic natural set-yogurt during storage period

Dynamics of pH (x̄)					
Variants	Storage time				
	Day 1	Day 7	Day 14	Day 21	Day 28
K	4.34	4.25	4.17^{a}	4.11	4.07
B	4.37	4.26	4.24^{a}	4.20	4.15
P	4.36	4.22	4.15	4.14	4.13

^{a,}Values about dynamics of pH at days 1, 14 and 28, followed by the same superscripts within 1 column are significantly different ($p < 0.05$).

In Table 3 and Figure 3 are presented the results about the pH value of three variants probiotic natural set-yogurt during storage period. It can be seen that the pH value was constantly decreased in all variants. We noticed an insignificant change in pH value of two variants with prebiotic inclusion, (4.15 and 4.13), but in such medium the probiotic bacteria can survive up to day 28 of storage. Our results were also found in better agreement with the other authors [9,11,12].

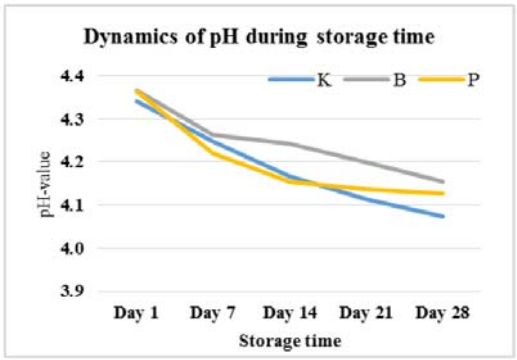

Figure 3. Dynamics of pH value of three variants probiotic natural set-yogurt during storage period

CONCLUSIONS

The obtained results of this study indicate that Fibregum prebiotics can enhance the growth of this representative probiotic strains in vitro. This indicated that the B.bifidus was growing intensively up to the day 7 of storage and its development was further on decreasing. However, on day 28 it was still present in sufficient amount. It has been concluded that the development of bacteria depends on the conditions and stage of production as well as the type of prebiotic.

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