JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2013, 19(4), 474-479

*Journal of Agroalimentary Processes and Technologies*

# Influence of *Spirulina platensis* biomass over some starter culture of lactic bacteria

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**Received: 23 November 2013; Accepted: 21 December 2013**

***Abstract***

The aim of this research was to investigate the effect of a cyanobacterial (*Spirulina platensis*) biomass on the microflora of a probiotic fermented dairy product during incubation and storage time. *Spirulina*-enriched and control fermented milks were produced using pasteurized milk, powder milk and two starter culture BB12 and LA-5.

During incubation and storage time there were followed parameters as: the titratable acidity, the *p*H, the syneresis, the water holding capacity, the dynamic viscosity and the lactic bacteria number. The final product was stored at 5 ± 1°C for 15 days. The results showed that the *Spirulina platensis* biomass had a beneficial effect on the survival of the BB12 and LA-5 starter bacteria during the entire storage period. The abundance of bioactive substances in *Spirulina platensis* have great importance from a nutritional point of view because the cyanobacterial biomass provides a new opportunity for the production of functional dairy foods.

***Keywords***: *Spirulina platensis*, *Bifidobacterium animalis ssp. lactis*, *Lactobacillus acidophilus*, flowing proprieties

## 1. Introduction

The probiotic bacteria are defined as "*living microorganisms which have benefits over the health of the host organism if they are prescribed at the proper moment*" [1,2].

To observe a positive health effect of their consumption, a minimum level of live microorganisms is required. This level, depending upon the strains used and the required health effect, it is usually between $10^8$ and $10^{11}$ cfu/g [3].

Yogurt, a nutrient-dense food, is one of the most popular fermented milk products worldwide [4]. Yogurt is obtained by fermenting fresh milk or reconstituted milk with lactic acid bacteria, and preferred by the customers because of its effects of improving the intestinal environment and enhancing the body immunity [5]. Yoghurt and other fermented milk contribute to health with natural nutrients and enrich the intestinal flora with lactic acid bacteria (LAB). Therefore, assuming a daily consumption of fermented dairy products of 100g, they should

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contain between $10^6$ cfu/g to $10^9$ cfu/g of these live bacteria at the time of consumption.

Some strains produce certain health promoting metabolites including proteins and fattyacids which are desirable from a nutritional and/or physiological perspective. However it should be emphasized that the ingestion of probiotic organisms opens up the possibility that these health promoting metabolites may also be produced in vivo [6]. The general consumption of dairy products and, particularly, of probiotic dairy products reached a new dimension during the last years due to the favorable effects over the health attested by the records of nutritionists and doctors [7].

Food products which contain probiotics can be categorized as functional aliments and together with the prebiotics they represent the largest segment of the functional food market in Europe, Japan and Australia [2]. The lactic bacteria, mainly lactobacillus and bifidobacteria, are the primary agents of the probiotics in the functional food industry [8].

*Spirulina platensis*, a cyanobacterium is a photoautotrophic microorganism, widely distributed in nature and is consumed as human food supplement for centuries because of its best known nutritional value. It contains 78% proteins [9], vitamins [10,11,12], 4-7% lipids [13,14], minerals [15], carbohydrates [16] and some natural pigments [17].

Due to the presence of these phytonutrients, it has corrective properties against several diseases like cancer, hypertension, hypercholesterolemia, diabetes, anaemia etc. Recently [18] reported the growth promotion effect of LAB by *Spirulina platensis*. The purpose of our study was to evaluate the effect of *Spirulina platensis* biomass on the growth of LAB.

## 2. Materials and methods

### *2.1. Materials*

Fresh, cow milk (≈ 20 L) for yogurt production was purchased from a dairy factory from Galati, Romania. *Spirulina platensis* biomass was obtained from the S.C. Hofigal Export Import S.A., (Bucharest, Romania). The *Bifidobacterium animalis ssp. lactis* (BB-12) and *Lactobacillus acidophilus* (La-5) (Chr. Hansen, Denmark) were used as a starter culture. Powder milk was from Euro Food Prod S.R.L. (Bucharest, Romania). MRS agar was from Amyl Media (Danenong, Vic, Australia).

### *2.2. Methods*

Before yogurt preparation, raw milk was pasteurised (95 ºC, 5 min) and cooled to 44 ºC. Control yogurt and the four *Spirulina platensis* yogurts (Table 1) were prepared on the same day. *Spirulina platensis* biomass (0.5% and 1%) and powder milk (5%) were added into pasteurised and cooled milk followed by the addition of 1% BB12/La-5 culture. Inoculated mixes were then poured into 200 mL sterile polypropylene cups with lids and incubated at 42 ºC to achieve a pH of 4.6–4.8 (6 h, the same time for all yogurts). The finished yogurts were immediately cooled in an ice bath and then stored at 5 ± 1°C for 15 days.

***Table 1.*** Variants of the new probiotic product with *Spirulina platensis* biomass

| Code | DVS culture | Products |
|---|---|---|
| Control ($C_{BB}$) | BB12 | Milk + BB12 + powder milk |
| Sample 1 ($S_{1BB}$) | | Milk + BB12+ powder milk + 0.5% *Spirulina platensis* biomass |
| Sample 2 ($S_{2BB}$) | | Milk + BB12 + powder milk + 1% *Spirulina platensis* biomass |
| Control ($C_{La}$) | La-5 | Milk + La-5 + powder milk |
| Sample 1 ($S_{1La}$) | | Milk + La-5 + powder milk + 0.5% *Spirulina platensis* biomass |
| Sample 2 ($S_{2La}$) | | Milk + La-5 + powder milk + 1% *Spirulina platensis* biomass |

*2.2.1. Physicochemical analyses*

The pH of the yogurts was monitored using a digital pH meter (Eutech, Cyberscan 1000, Singapore). Titratable acidity, expressed as g of lactic acid per mL of the yogurt, was evaluated by titration method. The water holding capacity (WHC) of yogurt was measured by centrifugation of a five gram yogurt sample at 2500 rpm for 10 min at 20 ºC. The WHC was calculated as follows:

$$WHC\ (\%) = (1 - W_1/W_2) \times 100$$

where: $W_1$ = Weight of whey after centrifugation, $W_2$ = Yoghurt weight [19].

*2.2.2. Microbiological analysis*

Total population of viable microorganisms was counted on regular MRS medium (*p*H = 5.5). All plates were incubated anaerobically at 42°C for 48 h. The lactic bacteria number was established, through indirect counting using an automatic colony counter ACOLYTE. All the experiments was in duplicate and the results were expressed as cfu/mL.

*2.2.3. Rheological measurements*

The dynamic viscosity and the torque, of the probiotic dairy product with *Spirulina platensis* biomass, were measured at 9 ºC using a rotary viscosimeter BROOKFIELD DV – E, equipped with a LV 2 spindle [20].

*2.2.4. Statistical analysis*

Data analyses were performed using a statistical software (Statistica 7.0). One way ANOVA was used to analyse data on physico-chemical properties. A p value < 0.05 was considered statistically significant for all analyses.

## 3. Results and discussion

*3.1. Physico-chemical characterization*

Figure 1 presents the titratable acidity of the yogurts and Figure 2 shows the pH changes in the yogurts during the storage period. The titratable acidity is a definitive parameter of the fermented dairy products. During the storage period, significant diferences were found between the control and other yogurt samples for titratable acidity values.

During storage period titratable acidity values of the control and other yogurts tended to increase. Higher values are registered for the samples obtained with La-5 culture (0.069 g lactic acid/mL product for $S_{1La}$ sample and 0.693 g lactic acid/mL product for $S_{2La}$ sample). At the end of the 15$^{th}$ days of storage, higher values of this parameter are registered for sample $S_{2BB}$ (0.99 g lactic acid/mL product) and for $S_{2La}$ sample (0.936 g lactic acid/mL product).

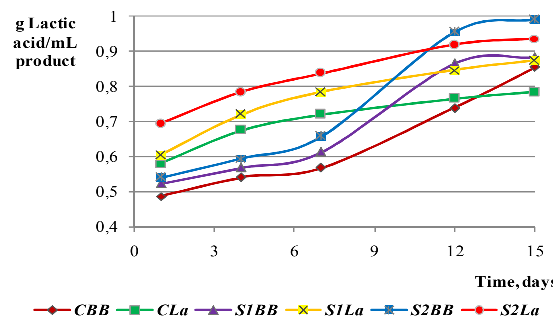


***Figure 1.*** Titratable acidity variation during the storage period of 15 d at 5 ± 1°C

According to Figure 2 the increase in the *Spirulina platensis* powder content caused slight decrease in the *p*H values of the experimental yogurt samples (p<0.05). *p*H values of the six yogurt types were decreased to approximately 4.10 – 4.50 during the storage period. This was probably caused from the addition of powdered *Spirulina platensis* which promoted the growth of lactic acid bacteria.

Similar findings related to this decrease in yogurts caused by *Spirulina platensis* powder, were also notified by [14] in the *Spirulina* added yogurts [14].

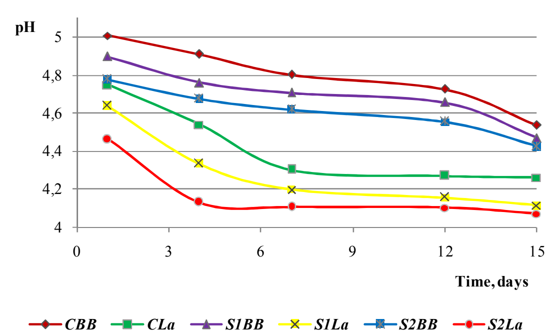


***Figure 2.*** pH variation in yogurt during the storage period of 15 d at 5 ± 1°C

WHC is one of the most important physical properties, i.e., the higher the value, the better the curd stability. The WHC of samples produced with *Spirulina platensis* biomass was higher compared to the samples produced without *Spirulina platensis* biomass (Figure 3). The sample with 1% *Spirulina platensis* biomass had the highest WHC values.

The highest WHC on the first day of cold storage was obtained for sample $S_{2La}$ (96.08%). On the 15$^{th}$ days of the storage period higher values of this parameter are registered for sample $S_{2BB}$ (78%).

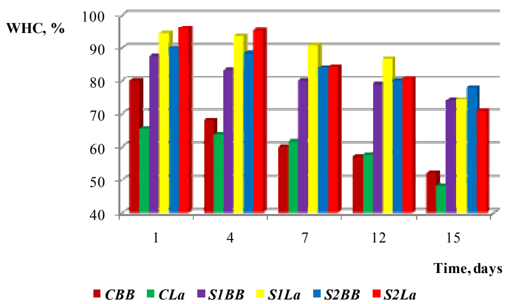


***Figure 3.*** Water holding capacity of the new probiotic product with *Spirulina platensis* biomass

*3.2. Microbiological analysis*

Both probiotic bacteria need nutrients to grow and survive. *Spirulina platensis* powder may represent a unique source of nutrients for these bacteria since it contains significant concentrations of amino acids, precursors of nucleic acids, vitamins, mineral and etc., among them also derivates of vitamin B which is a well known promoter for the probiotic bacteria [21]. From the survival curves (Figure 4), it can be seen that *Spirulina platensis* powder addition into the all yogurt types resulted in better growth of all added bacteria. It was probably caused by the nutritive properties of *Spirulina platensis* designated by [22].

In general, the viability of the bacteria in all yogurts increased when *Spirulina platensis* powder was added during the storage period. However, the difference among higher (1 %) and lower (0.5 %) addition was not seen always observed. After 1 % of *Spirulina platensis* powder addition, the viable counts were 6.8 and 7.5 log cfu·mL$^{-1}$ at the end of the storage period. The difference in the viable counts of the samples with *Spirulina platensis* powder between initial and last day of storage was maximum 1.0 log cfu·mL$^{-1}$. Similar effects were also reported by [22] in yoghurt and [14] in fermented milk.

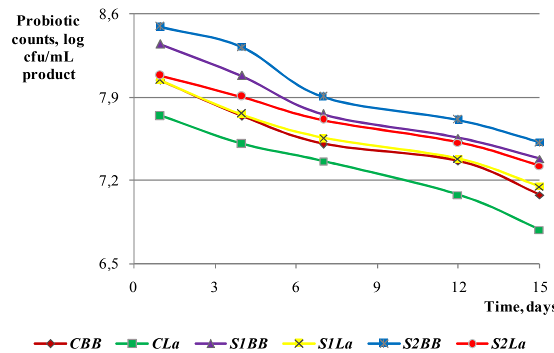


***Figure 4.*** Viable counts variation during storage period

The viability of *Bifidobacterium animalis ssp. lactis* was higher than of *Lactobacillus acidophilus* at the end of the storage period.

*3.3. Rheological analysis*

The rheological behavior of the probiotic dairy products with *Spirulina platensis* biomass is presented in Figure 5 (the shearing stress variation according to the shearing rate) and Figure 6 (the dynamic viscosity variation according to the shearing stress).

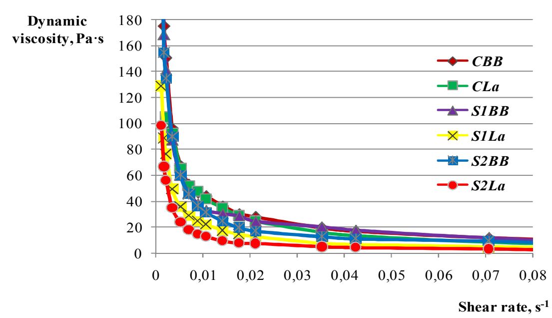


***Figure 5.*** Dynamic viscosity variation depending on the shear rate

There was determined that samples have a rheological behavior similar with the one of the nonNewtonian fluids, time independent, therefore a pseudoplastic behavior. Specific for a fluid with this type of behavior is the flow resistance decrease as a result of the fluid shearing rate increase.

For all samples, it was noted that for low values of shear rate, tangential shear stress variation depending on shear rate was increasing (regression coefficient $R^2$ values varies from 0.843 for sample $S_{2BB}$ and 0.985 for sample $S_{1La}$).

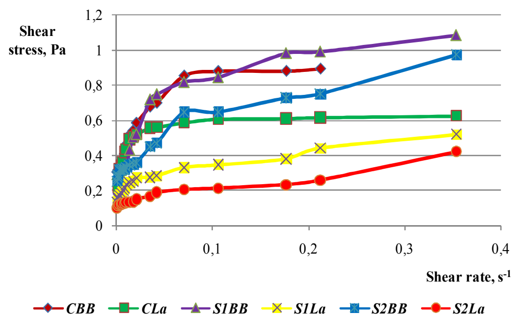


***Figure 6.*** Shear stress variation depending on the shear rate

Shear stress variation depending on the shear rate had shown a development, especially at higher shear rate values 0.05 $s^{-1}$. The dynamic viscosity of samples containing *Spirulina platensis* biomass at lowest values of shear rate then 0.05 $s^{-1}$ varied from 24 Pa·s for sample $S_{2La}$ at 67.8 Pa·s for sample $C_{BB}$. At values of shear rate of 0.35 $s^{-1}$ the dynamic viscosity varied between 1.195 Pa·s for sample $S_{1La}$ to 4.215 Pa·s for sample $C_{BB}$.

## 4. Conclusions

Some functional foods, which are therapeutically efficient for the human body, can be obtained by combining the milk and the *Spirulina platensis* biomass. As a result of the lactose fermentation, the titratable acidity is growing fast during the incubation period.

The highest titratable acidity value was obtained for the $S_{2BB}$ (0.99 g lactic acid/mL product) and for $S_{2La}$ sample (0.936 g lactic acid/mL product) and the lowest for the $C_{La}$ sample (0.783 g lactic acid/mL product) at the end of the storage period. The pH for all products has decreased during incubation, being stabilized at storage.

For the sample with *Spirulina platensis* biomass, water holding capacity after 15th days of storage is reduced with 13.39%.

The results of this research demonstrated that the *Spirulina platensis* biomass has stimulatory effect on the growth of coccus shaped starter bacteria. At the end of the storage period, the highest number of probiotic bacteria was encountered at the sample $S_{2BB}$ sample ($3.3\cdot10^7$ cfu/mL product)

The rheological analysis showed that the addition of *Spirulina platensis* biomass does not modify significantly the flowing proprieties of the probiotic dairy products.

**Compliance with Ethics Requirements:**

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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