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Researches on maintaining functionality in the storage of probiotic dairy product – Afinolact

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Abstract

Probiotic bacteria are the most important microorganism group which it comes to improve consumers' health. Since the dawn of time, and to nowadays, people have been successfully using medicinal herbs to prevent and treat diseases. The probiotic dairy product AFINOLACT seeks to combine the beneficial effects of probiotic bacteria with the therapeutic virtues of medicinal herbs. In this paper we have studied the probiotic dairy product AFINOLACT storage behavior (manufactured from cow`s milk, liquorice extract and blueberry extract), from a physico-chemical and microbiological point of view. It has been discovered that AFINOLACT does not lose its functional properties during storage (1•108 – 1•109 CFU/ml probiotic bacteria).

Keywords: probiotic bacteria, functional product, blueberry extract, liquorice extract

1. Introduction

In recent years an important part of food researches has been primarily focused on the new probiotic products. Fermented dairy products are very popular all over the world because of their pleasant sensory characteristics and the potential they have in maintaining and even improving the health of consumers.

The consumption of dairy products in general and fermented dairy products in particular has reached a new dimension in recent years due to their beneficial effects on health, effects proven during years of nutritional and medical research [1, 3].

Food containing probiotics can be included in the functional foods category which, together with prebiotics, represents the largest segment of the functional food market in Europe, Japan and Australia. The market for these products is growing simultaneously with the consumers' awareness of their beneficial effects on health [9].

Scientific studies have stimulated interest in the use of beneficial bacteria in dairy products. Lactic bacteria, especially *Lactobacillus* and *Bifidobacterium* spp., are the probiotics main in functional food industry [8].

Use of medicinal herbs to cure disease dates from the very ancient times, prehistoric, when man living in nature and fighting through various means to ensure his own existence, he realized that some plants were good for food, others for healing of diseases, others were toxic. World Health Organization recently announced that 75 – 80 % of the world's population is treated with natural remedies [10].

Blueberry is the most important source of ascorbic acid (12-20 mg), axerophthol (3-5 mg), nicotinic acid (0,2 mg), aneurin (0,02 mg), lactoflavin (0,02 mg), tocopherol and minerals: potassium (50 mg), calcium (10 mg), phosphorus (8 mg), sulfur (8 mg), magnesium (6 mg), chlorine (5 mg), manganese (3 mg), iron (1 mg) [5, 7].

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Liquorice is rich in free amino acids (aspartic acid, serine, proline, threonine, glycine, valine, alanine, isoleucine); sugar represented by glucose (0,6-4,1%), fructose (0,3 -1%), saccharose (7,5-20,3%), maltose (0,1—0,6%); vitamins from B group and minerals: calcium, natrium, phosphorus, iron, manganese, zinc, copper, molybdenum [6].

Due to the importance of fermented dairy products, probiotics, blueberry extract and liquorice extract, this paper studies physical-chemical and microbiological point of view, a new type of fermented dairy product – AFINOLACT.

2. Materials and Method

- Cow milk purchased from a collecting center in the Galati district, featuring the following properties, determinate with Milk Lab equipment: minerals: 0,72 %; nonfat dry substance: 9,08 %; lactose: 4,32 %; protein: 3,52 % fat: 1,5 % and titration acidity: 20 ºT;
- Blueberry extract and liquorice extract thus obtained: a known quantity of material plant has been subjected to an aqueous extraction, at room temperature for 2 hours. Aqueous extracts obtained were filtered through filter paper and then concentrated in a Buchi Rotavapor at 50°C and a pressure of 200 mbar, stored of 4°C until use;
- freeze-dried culture of lactic bacteria ABY 3, made by Chr. Hansen containing *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium* ssp.

Were made 3 types of new product AFINOLACT encoded as follows:
A: milk + 5 % inoculum;
B: milk + 5 % inoculum + 6 % blueberry extract;
C: milk + 5 % inoculum + 6 % blueberry extract + 6 % liquorice extract.

The physical-chemical analyses were performed according to the effectual standards and aimed the determination of the following parameters:

- dry substance – has been made with the classical drying method in oven (Memmert drying oven) at 102-105°C, according to AOAC 925.23 Ch. 33.2.09;
- fats by the butirometric method;
- titration acidity – the volume of sodium hydroxide 0,1 N necessary to neutralize acidity in 100 ml milk, expressed in ^{0}T, acc. to STAS 9535/1987;
- lactose content – was determined by 3,5-dinytrisalicylic acid (DNS) method reading absorbance at 540 nm in spectrophotometer UV / Vis. 6505 Jenway, using a calibration curve;
- yogurt water-holding capacity – was determined using a procedure by Guzman Gonzalez, Morais, Ramos and Amigo. 5 g of yoghurt was centrifuged for 10 minute at 2500×g and 20 °C;
- pH values have been obtained with a pH–meter IQ-SCIENTIFIC after calibration with reference of pH 4, 7 and 9.

Microbiological analyses were performed at the moment of start and then every two hours during the incubation time, who was lasting for 5 hours and was focused on the evolution of the lactic bacteria number during this time, and also for the first 8 days of storage. In microbiological examination, numerical evaluation of these microorganisms was performed by indirect method, using an automatic colony counter, Acolyte.

3. Results and Discussion

Physical-chemical analysis. For fermented dairy product AFINOLACT was determined the dry substance and fat, values recorded in Table 1.

Table 1. Samples content in dry substance and fat

Sample	*A*	*B*	*C*
Dry substance %	86,7	88,9	94,7
Fat, %	1,5	0,6	0,4

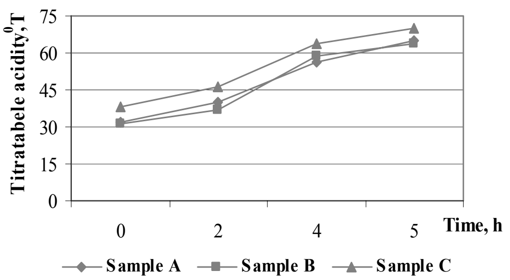

Figure 1. Titration acidity variation during incubation time

A characteristic parameter for the fermented dairy products is the titration acidity, which was measured during incubation (Figure 1) and storage time (Figure 2).

Titration acidity grows rapidly during incubation time, higher values of this parameter are recorded for sample C (70 °T), and then acidity increases slower, as a result of lactose fermentation. During storage time higher values of titration acidity have samples A (82 °T) and C (83 °T).

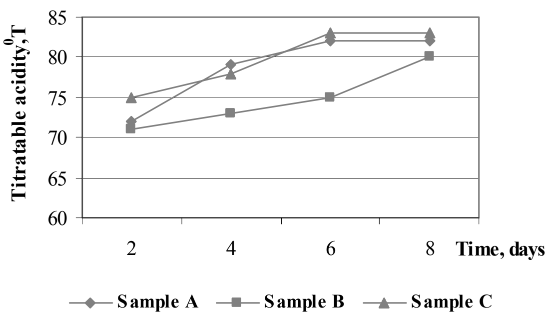

Figure 2. Titration acidity variation during storage time

Regarding the pH variation (from 6,473 to 4,584 for sample A and from 6,07 to 4,976 for sample C) there is a slight decrease during incubation time (figure 3), later during storage time (figure 4) values are mentained at a constant level (from 4,559 to 4,538 for sample A and from 4,817 to 4,707 for sample C). pH evolution is correlated with the intensity of lactose fermentation, but is influenced by buffer substances which are formed in yogurt.

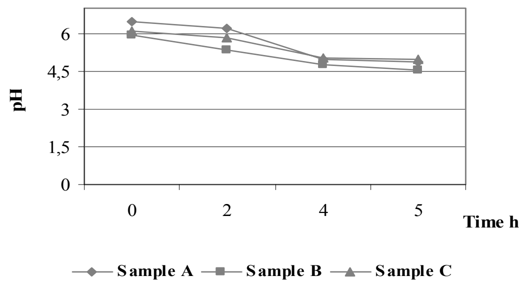

Figure 3. pH variation during incubation time

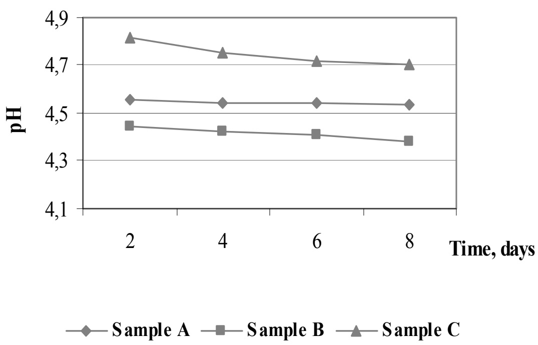

Figure 4. pH variation during storage time

The conversion of lactose is shown by reducing the pH values and, by increasing titration acidity.

Lactose degradation starts immediately after adding the DVS culture and continues during incubation time and storage time. During incubation time (figure 5) sample B has the lowest the level of lactose (3,83 %), and sample A have the highest level of lactose (4,27 %).

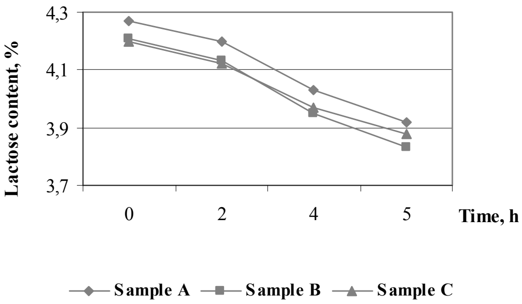

Figure 5. Variation of lactose content during incubation time

During storage time lactose content decrease with 0,5 units for sample A and 0,39 units for sample C is mainly due to the fermentation by probiotic culture. Lactose concentration until the 8 storage days decreased to 3,63 % for sample B and to 3,57 % for sample C.

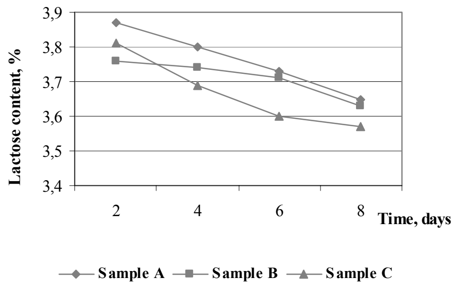

Figure 6. Variation of content lactose during storage time

The water-holding capacity was lower for sample C (milk + 5 % inoculum + 6 % blueberry extract + 6 % liquorice extract), 47,6 % after 8 days of storage compared with the samples A (66,8 %) and B (66 %) stored the same time (Table 2).

Table 2. Water-holding capacity of yogurt during storage time

Time, days / *Sample*	*2*	*4*	*6*	*8*
A	63,6	64	65,6	66,8
B	64	64,4	65,2	66
C	54,5	54	51,6	47,6

Microbiological analyses

For each sample was followed the evolution of the microorganisms number during incubation and storage time. Analyzing the graphical representation in Figure 7 it can be seen that the number of lactic bacteria has not been significant increased in first hour during incubation time, number of lactic bacteria began to grow after the second hour of incubation, the highest amount of lactic bacteria are reported for the sample B ($4 \cdot 10^7$ CFU/ml).

From Figure 8 it can be seen that lactic bacteria are growing during the 2nd day of storage because the sample temperature is still high, 37 °C, when they are placed in cold.

At the end of the 8th days of storage time the number of lactic bacteria is still high, the highest values recorded for the sample C and sample B.

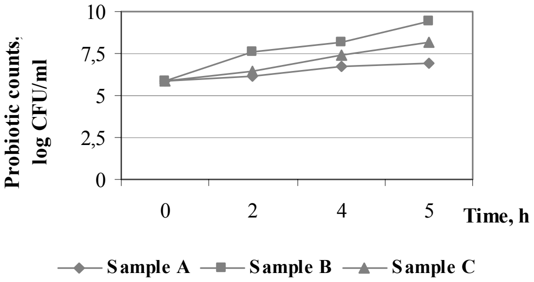

Figure 7. Viability of probiotic organisms during incubation time

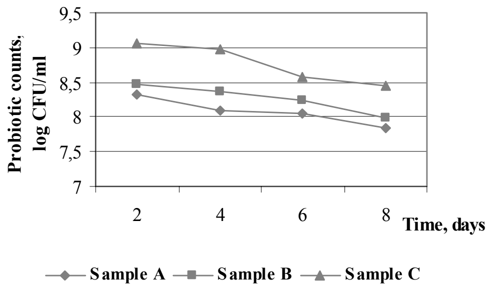

Figure 8. Viability of probiotic organisms during storage time

4. Conclusion

Creating new products based on milk and medicinal herbs extract may lead to the achievement of efficient therapeutic functional foods and less harmful to the human body.

As a result of lactose fermentation, the acidity is growing rapidly. The pH of the samples AFINOLACT decreases during incubation time to further stabilization during storage time.

The number of lactic bacteria is maximum after 2nd day of storage (for sample C this is $1{,}12 \cdot 10^9$ CFU/ml). During storage time, the biggest number of lactic bacteria is registered for sample C ($2{,}8 \cdot 10^8$ CFU/ml) and B ($0{,}97 \cdot 10^8$ CFU/ml).

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