Obtaining chocolate masses with probiotic lactobacilli strains

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Obtaining chocolate masses with probiotic lactobacilli strains. *Milk chocolate variants with probiotic lactobacilli were obtained. They were stored at room temperature of 20 - 22°C and low relative humidity for the duration of two months. Decrease in the concentration of viable cells of probiotic bacteria in the course of storage of the chocolate bars was observed, but there remained enough amount of beneficial microflora (10^5 - 10^8cfu/g) to carry out its inherent preventive role in the consumption of this type of functional food. It was shown that chocolate mass is a suitable matrix for the inclusion of probiotic bacteria.*

Key words: *Lactobacillus*, probiotic, chocolate, pathogen

INTRODUCTION

The development of new functional foods is an opportunity to improve the quality of food offered to consumers in order to positively affect their health. Food products with added value are subjected to increasing industrial and social interest in the contemporary society.

The selection of strains of lactobacilli, bifidobacteria and propionic acid bacteria and yeasts for the development of starters for a wide range of food products - dairy, meat, cereals, etc. is especially prospective for production and development of functional foods. Thus the currently used preservatives in food production can be reduced or replaced.

According to FuFoSE, in a coordination with ILSI, Europe a food product can be considered functional if together with its basic nutritional effects it has beneficial effects on one or more functions of the human body, thereby improving the overall and the physiological condition, or reducing the risk of developing a disease.

Lactic acid bacteria play a key role in food fermentations, contributing to the development of desired organoleptic properties of the final product and the microbiological safety [3, 8].

For many years, chocolate was consumed entirely for pleasure, but in the last 20 years, several studies have shown that dark chocolate and cocoa may have beneficial effects on human health due to the high content of polyphenols. Many studies have shown that polyphenols and/or polyphenol-rich foods play an important role for the health of the consumer due to their antioxidant properties [2, 4, 6]. These properties allow them to act as anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral and antiallergic compounds [1, 7, 9]. In addition to reducing blood pressure levels, cocoa polyphenols probably influence the control of cholesterol. Chocolate and cocoa contain not only polyphenols but also methylxanthines, which could further contribute to the impact of these foods on health [5].

The purpose of the present study was to investigate the possibilities for applying probiotic *Lactobacillus* strains in the manufacture of milk chocolate.

MATERIALS AND METHODS

Microorganisms

Two lyophilisates of two probiotic strains of the genus *Lactobacillus* - *Lactobacillus plantarum* X2 and *Lactobacillus paracasei* RN5 were used in the present study.

Media

LAPTg10 - broth. Composition (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6 - 6.8, and Tween 80 is added - 1cm^3/dm^3. Sterilization - 20 minutes at 121ºC.

LAPTg10 - agar. Composition (g/dm^3): medium LAPTg10-broth agar - 20. Sterilization - 20 minutes at 121ºC.

LBG - agar. Composition (g/dm^3): tryptone – 10; yeast extract – 5; NaCl – 10; glucose – 10; agar - 15. pH is adjusted to 7.5. Sterilization - 20 minutes at 121°C.

Saline solution. Composition (g/dm^3): NaCl - 5. Sterilization - 20 minutes at 121ºC.

Obtaining milk chocolate variants with probiotic lactobacilli

For the preparation of milk chocolate variants, enriched with probiotic lactobacilli strains chocolate raw material was melted in a water bath, then cooled to 45°C and 1% of the lyophilizate of the corresponding probiotic strain was added. The mixture was homogenized, tapped into chocolate bar trays and cooled to obtain solidified chocolate bars. The obtained chocolates were stored at room temperature (20-22°C) and low relative humidity for 2 months. The changes in the values of the physico-chemical (dry matter, titratable acidity and pH) and microbiological (concentration of viable cells of probiotic lactobacilli, contaminant microflora) were monitored in the course of storage of the milk chocolate variants. To determine the number of viable microorganisms appropriate tenfold dilutions were prepared, followed by spread plating. The inoculated plates were cultured for 3 days at optimum temperature until the appearance of countable single colonies.

Microbiology

Microbiological status of the obtained milk chocolate variants Indicators:

- lactic acid bacteria, cfu/g;
- total bacterial abundance (TBA) - cfu/g;
- *Escherichia coli* in 0.1 g of the product (ISO 16649-2:2001);
- *Salmonella* sp. (BS EN ISO 6579:2003) in 25.0 g of product;
- *Staphylococcus aureus* in 1.0 g of product (BS EN ISO 6888-1:2005 + A1: 2005);
- molds and yeasts in 1.0 g of product, cfu/g (BS ISO 21527-1:2011 and BS ISO 21572-2:2011).

RESULTS AND DISCUSSION

Milk chocolate variants were prepared with the inclusion of 1% of the lyophilisates of two *Lactobacillus* strains with probiotic properties. The resulting variants were stored at room temperature and low relative humidity for 2 months.

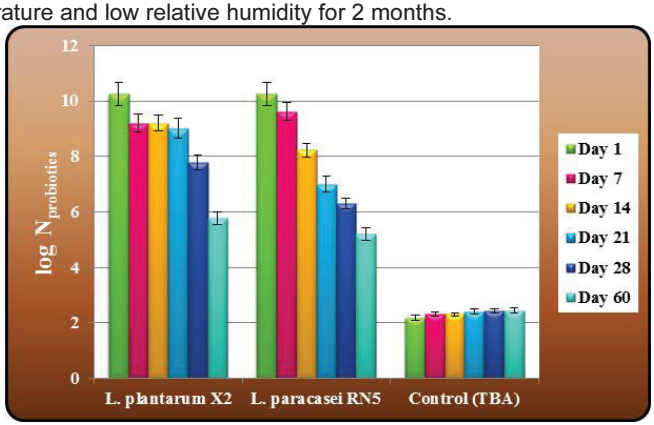

Fig. 1. Changes in the concentration of probiotic lactobacilli in milk chocolate variants and TBA of the control during storage for 2 months

The changes in the physico-chemical (dry matter content, pH and titratable acidity) and the microbiological (viable cell count of probiotic lactobacilli, contaminant microflora) parameters in the process of storage were monitored (Fig. 1, Table 1 - Table 5).

Chocolate proved to be a suitable matrix for the inclusion of the cells of the probiotic lactobacilli. Immediately after the preparation of the milk chocolate variants, the concentration of viable cells of probiotic bacteria exceeded 10^9cfu/g. This provided the required amount of beneficial microflora.

For 60 days of storage the amount of active probiotic cells decreased with 10^4-10^5 cfu/g, depending on the species, but still maintaining high levels of beneficial microorganisms in both milk chocolate variants.

The changes in the microflora of the chocolate mass (control) showed that the contaminant microflora also retained active in the course of storage at room temperature (Fig. 1). The change in pH values of the chocolate control was probably due to the activity of acidifying microorganisms (Table 3).

Table 1. Change in the dry matter content (in %) of the variants of milk chocolate during storage for 2 months

Additive	*Day 1*	*Day 7*	*Day 14*	*Day 21*	*Day 28*	*Day 60*
L.plantarum X2 lyophilisate	99	99	99	99	99	99
L.paracasei RN5 lyophilisate	96	96	96	96	96	96
Control	98	98	98	98	98	98

The dry matter content of the chocolate masses varied in the range between 96-99% for the different variants, and the values did not change during storage for 2 months (Table 1). The low water activity of the milk chocolate variants restricted the growth of microorganisms, providing their microbiological purity (Table 4).

Table 2. Changes in the titratable acidity (in °T) of the variants of milk chocolate during storage for 2 months

Additive	*Day 1*	*Day 7*	*Day 14*	*Day 21*	*Day 28*	*Day 60*
L.plantarum X2 lyophilisate	14	14,2	14,8	18,7	18,4	18,6
L.paracasei RN5 lyophilisate	17	17,5	17,9	16,5	17,2	18,0
Control	13	13,3	15,8	18,6	18,7	18,9

Slight increase in the values of the titratable acidity of the chocolate masses was observed, suggesting little change in their acidity (Table 2). At the same time the pH of the medium decreased (Table 3).It was compensated by the increased sweet taste of the milk chocolate variants with probiotic bacteria. More significant changes in the pH values of the chocolate mass occurred after the 14^{th} day, which was the result of the metabolic processes of the probiotic strains, which were activated during storage at room temperature of 20 - 22°C (Table 3). The obtained milk chocolate variants met the standard criteria for microbiological purity of food for all the tested microbiological indicators (Tab 4).

Table 3. Change in the pH of the milk chocolate variants during storage for 2 months

Additive	*Day 1*	*Day 7*	*Day 14*	*Day 21*	*Day 28*	*Day 60*
L.plantarum X2 lyophilisate	6,42	6,34	6,29	5,26	4,50	4,27
L.paracasei RN5 lyophilisate	6,30	6,29	6,25	5,70	5,64	4,24
Control	6,52	6,47	5,62	5,15	4,75	4,19

Table 4. Microbiological indicators in the variants of milk chocolate with probiotic bacteria

Additive	*TBA, cfu/g*	*Molds and yeasts, cfu/g*	*Salmonella sp., cfu/g*	*E.coli, cfu/g*	*Staph. aureus, cfu/g*
L.plantarum X2 lyophilisate	< 10	< 10	Not found	< 100	< 100
L.paracasei RN5 lyophilisate	< 10	< 10	Not found	< 100	< 100
Control	$1,5*10^2$	< 10	Not found	< 100	< 100

CONCLUSION

It has been found that chocolate masses are suitable matrices for the inclusion of probiotic bacteria. Variants of milk chocolate with two lyophilisates of the probiotic strains *Lactobacillus plantarum* X2 and *Lactobacillus paracasei* RN5 were obtained. The concentration of viable cells of probiotic bacteria decreased in the course of storage of the chocolate bars, but there remained enough amount of beneficial microflora (10^5 - 10^6cfu/g), to carry out its inherent preventive role in the consumption of this type of functional food.

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This paper has been reviewed