DETERMINATION OF GROWTH PARAMETERS OF PROBIOTICS IN MILK SUPPLEMENTED WITH OAT FLAKES

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Abstract
The aim of this study was to evaluate the addition of oat flakes on the growth rate of probiotics in milk, in order to obtain a probiotic product with special sensory attributes. Oat flakes were added to milk before pasteurization. Milk without oat flakes was used as a reference sample. Probiotic culture used in this study contains bifidobacteria, Lb. acidophilus, Lactobacilus lactis and Streptococcus thermophilus. Growth characterization of probiotic culture in milk supplemented with oat flakes was done by determining the number of lactic acid bacteria at different time intervals.
Based on the results obtained was observed that oat flakes stimulated growth of bifidobacteria in milk, their generation time is with 55 minute shorter than those of reference sample. The growth rate of bifidobacteria was 15 times higher than that of Lactobacilus acidophilus, when oat flakes were added to a concentration of 2%.
Lactobacillus species (Lb. acidophilus, Lactobacilus lactis) of milk variants with oat flakes were present in small numbers, because the ingredient used has a slightly inhibitory effect on them. Regarding the evolution of Streptococcus thermophilus was observed that the presence of oat flakes in the proportion of 2% this bacterium reaches a growth rate of 0.835h^{-1}.

Keywords: probiotic, oat flakes, fermented milks, growth rate, bifidobacteria, Lactobacillus acidophilus

1. INTRODUCTION

The nutritional and potentially therapeutic value of food is a key characteristic in the development of new value-added products that are manufactured for health-conscious consumers [1]. Many researchers have recognized the beneficial effects of consuming fermented milk products [2-6]. Dietary fiber may play a major role in the prevention of some illnesses, and a daily intake of 25 to 30 g of dietary fiber has been recommended for adults [1].
Dairy products appear to be good vehicles for the delivery of probiotics to humans *B. bifidum*, *B. longum* subsp. *infantis, B. longum* and *B. animalis* subsp. *lactis* are commonly used for the production of fermented milks. Dairy products containing bifidobacteria are made with pure cultures, alone or in combination with other lactic acid bacteria such as *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* group or *L. casei* group. Dairy-related bifidobacteria are already used in a wide variety of probiotic dairy products including milk, bifidus milk, cheese, frozen yoghurt-like product and ice cream. Yoghurt-like products are made using a single species of bifidobacteria in combination with lactic acid bacteria. Bifidobacteria can be incorporated to yoghurt like products before or after fermentation [7].
Oats are a complex carbohydrate food with potentially important metabolic effects in humans. There is convincing evidence that both whole oats and oat bran can reduce serum cholesterol levels in normal or hypercholesterolemic subjects, and they also have a relatively low glycemic effect. These effects have been attributed to the presence of a high proportion of viscous β-glucan gum in the nonstarch polysaccharide fraction, which can delay absorption in the small intestine and act as a substrate for fermentation in the colon. However, maldigestion of oat starch could also reduce the glycemic response to test meals and provide a separate source of fermentable material in the colon [8].

The mechanism by which a soluble fibre, such as β-glucan, exerts hypercholesterolemic and hypoglycemic effects are still debated but the most common hypothesis is based on increased lumen viscosity. It has been suggested that cereal β-glucan decrease the absorption and reabsorption of cholesterol, bile acids and their metabolism by increasing the viscosity of gastro-intestinal tract contents as well as delaying gastric emptying and the intestinal absorption of nutrient, such as digestible carbohydrates and thereby reducing post-prandial hyperglicemya and insulin secretion [9, 10].

Oat is an excellent source of dietary fiber (10.2–12.1% total dietary fiber and 4% β-glucan). Soluble fiber accounted for 40% of the total dietary fiber. Much of the healthful effects of dietary fiber have been associated with soluble fiber [11].

Oat is the only cereal containing a globulin or legume-like protein, avenalin, as the major (80%) storage protein. Globulins are characterized by water solubility; because of this property, oats may be turned into milk but not into bread. The more typical cereal proteins such as gluten and zein are prolamines (prolamins). The minor protein of oat is a prolamine: avenin. Oat protein is nearly equivalent in quality to soy protein, which has been shown by the World Health Organization to be equal to meat, milk, and egg protein. The protein content of the hull-less oat kernel (groat) ranges from 12–24%, the highest among cereals.

The objective of this study was to evaluate the potential for production of fiber fortified probiotic fermented milk with attractive sensorial attributes. Also, the study followed the influence of oat flakes added in milk on growth rate of probiotic bacteria, during incubation at 38°C.

2. Materials and Method

2.1. Characterization of probiotic culture

In this study was used a probiotic culture (MSK mix ABD V1-54, Danisco Cultor, Germany) containing the bifidobacteria, *Lactobacillus acidophilus*, *Lactobacillus lactis* and *Streptococcus thermophilus*. This lyophilized culture is recommended by the manufacturer to obtain fermented milk with moderate acidity and high viscosity. In addition, the culture is characterized by moderate flavoring capacity. Before inoculation, lyophilized probiotic culture was suspended in basic medium M0 (milk reconstituted with 12.0% nonfat dry milk) for hydratation and standardizes inoculated cells.

2.2. Preparation of fermented milk samples

In this study were made 2 variations of milk reconstituted from milk powder (12% nonfat dry milk), which were supplemented with oat flakes up to 2% (O1) and 3% (O2). Also, was prepared a milk sample without oat flakes to be used as a reference (M). The milk samples were pasteurized at 90-95°C/ 2 minutes. The milk samples cooled to 40°C were inoculated in the proportion of 2% with probiotic culture. Then, the milk samples were incubated at 38°C for 4h.

2.3. Methods physicochemical and microbiological

Evolution of probiotic bacteria populations (*Lactobacillus acidophilus* and bifidobacteria) and other lactic acid bacteria (Lactobacillus lactis and Streptococcus thermophilus) in milk variants was assayed by determining the number of lactic acid bacteria at different time intervals (0, 2 and 4 hours). Counting of lactic acid bacteria in the milk samples was performed using direct counting by Breed method and indirect counting by Koch method (MRS was used as culture medium). The numbering of lactic acid bacteria was made in colored smear (with methylene blue) obtained from the milk samples diluted in decimal system.

The characteristic parameters of the growth of bifidobacteria were calculated with the following relations [12]:

- the number division (n) was calculated with relation

$$n = \frac{(\lg N - \lg N_0)}{\lg 2} \quad (1)$$

- the growth rate or the number of divisions that are produced in an hour expressed in h^{-1} was calculated

$$\mu = \frac{(\lg N - \lg N_0)}{\lg 2(t - t_0)} \quad (2)$$

where:
N_0-the initial number of cells from milk, in colony forming units/cm^3
N-the number of the cells obtained by their multiplication during the cultivation, in cfu/cm^3
t_0- the zero time of the determination, in hours
t-the interval of time studied, in hours

- the generation time represents the interval of time for the duplication by division of a single cell

$$tg = \frac{t}{n} = \frac{1}{\mu} \quad (3)$$

where:
t- the interval of time studied, in hours
n-the number of generations produced in the time t.

Fermentation activity of probiotic culture was monitored by determining titrable acidity and pH at the same time. Titrable acidity was determined by titration with 0.1 N NaOH in the presence of phenolphthalein as indicator, and pH was measured with HACH pH meter.

The lactose content was determined by the iodometrical method (the Luff-Schoorl variant-STAS 10902-89 of milk and dairy products). An alkaline solution of cupric-salt is reduced by the lactose from the milk samples at a heat temperature and the cuprous oxide obtained from the reaction is indirectly titrated with sodium thiosulphate.

In order to evaluate quantitative the sensorial attributes of the milk variants supplemented with oat flakes and fermented with probiotic culture, was used a scoring method [13]. It was used an appreciate system of the sensory attributes with 5 points and for each characteristic (taste, smell, aspect and consistence) were establishes 6 appreciation steps (from 0 to 5). The appreciation steps within the 5 points system were: 5-very good (exceptional and ideal qualities); 4-good (qualities corresponsive to the norms); 3-satisfying (with easily defects); 2-insatisfying (with obvious defects); 1- bad (with acute defects); 0-alterated (with sever characteristic modifications). For each sensory characteristics evaluated with the scale score by 5 points was determined average score of the group of panelist. Then, using the ponderation factors was crossing the scale of 5 points to 20 points scale (according to STAS 12656-88 milk and dairy products- sensorial analysis- methods with scales points).

3. RESULTS AND DISCUSSION

3.1. The effect of oat flakes on the growth rate of probiotic bacteria (bifidobacteria and *Lactobacillus acidophilus*) of culture used

In the milk samples supplemented with oat flakes was observed that the bifidobacteria grew well in milk variant O1 (supplemented with 2% milk oat flakes and inoculated at a rate of 4% with probiotic culture), in which the growth rate of bifidobacteria was 0.7634 h^{-1}, and the bacterial population reached 4.23x10^7 cfu/cm^3 (fig.1).

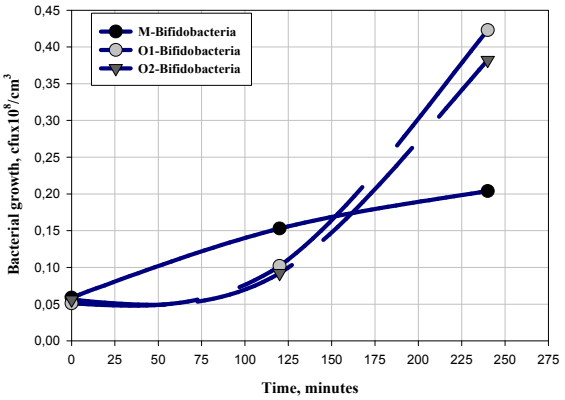

Figure 1. The evolution of bacteria in milk supplemented with oatmeal during incubation

The population of bifidobacteria in milk variant O1, inoculated with probiotic culture at a rate of 4%, was 2 times higher than in simple milk M (milk without oat flakes) and 11% higher

than in milk sample O2, supplemented with 3% oat flakes (table 1).

The generation time of bifidobacteria in O1 was with 8 minutes less than in O2 and 54 minutes shorter than in sample M.

Table1. Growth parameters of bifidobacteria in milk suplemented with oat flakes

Milk samples	Populations of bifidobacteria, log cfu/cm^3			Growth parameters of bifidobacteria at 4h		
	0 h	2 h	4 h	n	μ[h^{-1}]	tg [h]
M	6,768	7,184	7,309	1,7985	0,4496	2,2240
O1	6,707	7,008	7,626	3,0534	0,7634	1,3100
O2	6,749	6,962	7,582	2,7697	0,6924	1,4442

In conclusion, oat flakes have a stimulating effect on the growth rate of bifidobacteria and populations of bifidobacteria in fermented milks with oat flakes are within the limits imposed for probitotice (10^6-10^7 ufc/cm^3).

The growth rate of *Lb. acidophilus* in the presence of oat flakes was lower than of the bifidobacteria.

The populations of Lactobacillus acidophilus in milk supplemented with oat flakes were less numerous than in control milk (without oat flakes) as can be seen in figure 2.

The growth rate of Lb. acidophilus in simple milk M (without oat flakes) was 10 times higher than in variant O1 (with 2% oat flakes), which means that the generation time of the bacteria in the control was 16 times lower than in variant O1.

In the sample O2 (milk supplemented with 3% oat flakes), the population of Lb. acidophilus was smaller than in the O1 sample and the growth rate of bacteria was 4.65 times lower than in simple milk (M).

Based on the results obtained we concluded that oat flakes have a slightly inhibitory effect on Lb. acidophilus strain from probiotic culture used.

Lactobacillus lactis grew better in simple milk than in milk supplemented with oat flakes (Fig. 3) and recorded a growth rate of 0.0651 h^{-1} and generation time of 8 hours and 20 minutes. Growth rate of Lactobacillus lactis in sample M was 4.34 times than in sample O1 (milk supplemented with 2% oat flakes) and 2.94 times higher than in sample O2 (milk supplemented with 3% oat flakes).

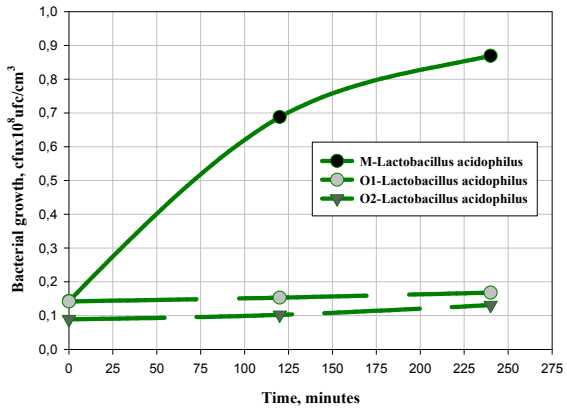

Figure 2. Evolution of Lactobacillus acidophilus in milk supplemented with oat flakes during incubation

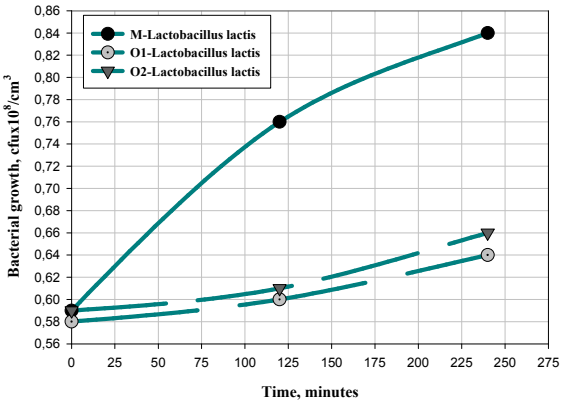

Figure 3. Evolution of Lactobacillus lactis in milk supplemented with oat flakes during incubation

It was also noted that the population of Lactobacillus acidophilus in O1 sample (milk supplemented with 2% oat flakes) was 2.5 times smaller than the population of bifidobacteria.

In addition, the results showed that oat flakes were more pronounced inhibitory effect on Lactobacillus acidophilus than on Lactobacillus lactis.

As regards the Streptococcus thermophilus strain (fig. 4), it was observed that the presence of oat flakes, add to milk at a rate of 2% (variant O1), the bacteria reach a growth rate of 0.835 h^{-1}, which means a generation time of 1h and 11 minutes. The population of Streptococcus thermophilus in the sample O1 was 1.27 times higher than in M sample and the generation time of lactic acid streptococcus in O1 sample was 11 minutes shorter than in the sample M.

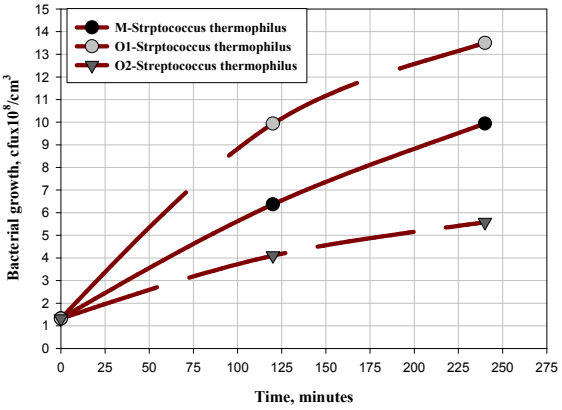

Figure 4. Evolution of Streptococcus thermophilus in milk supplemented with oat flakes during incubation

The growth rate of dairy streptococci O1 sample was slightly higher than that of Sour, and generation time was 8 minutes less. Because the growth of Str. thermophilus was better in O1 than in M, we assume that oat flakes have a stimulating effect on the growth rate of the bacteria in milk

When we use oat flakes at a rate of 3% (O2), Streptococcus thermophilus achieve a growth rate of less than 1.4 times than in simple milk (M) and 1.6 times lower than in O1, probably due to short incubation time.

At the end of incubation was observed that the acidity of milk sample O1 (milk with 2% oat flakes) was equal to that of sample M, but increase of the acidity in O1 was 0.538 g acid lactic/dm^3 greater than in M. Therefore, oat flakes stimulated fermentative activity of probiotic culture.

In the evolution of pH in the milk samples supplemented with oat flakes, during incubation, it was observed that it decreases with 2.41 units in O1 sample and with 2.39 units in O2.

Based on results obtained in the lactose determination, was observed that the decrease of lactose was 4.83 times higher in sample O1 than in sample O2.

3.2. The sensorial characterization of the fermented milks supplemented with oat flakes

The probiotic fermented milk supplemented with oat flakes was characterized through the following sensorial attributes:

- Aspect, color and consistence: coagulum homogenous with fine consistence like cream, and with smaller oat flakes fragments uniform distributed in the coagulum mass, without whey elimination; color white to yellowish uniform in the whole product mass, imprinted by oat flakes;
- smell: specific aroma of probiotic culture, pleasant aroma of lactic fermentation;
- taste: pleasant, acidulous, flavor specific of fermented milk.

On the basis of the quantitative evaluation of the sensorial attributes and taking into account the coefficient of ponderation, the fermented milks reached the following score: O1 (fermented milk supplemented with 2% oat flakes)- 20 points and O2 (fermented milk supplemented with 3% oat flakes)-18.5 points.

The sensorial profile of milk supplemented with 2% oat flakes is presented in fig. 5.

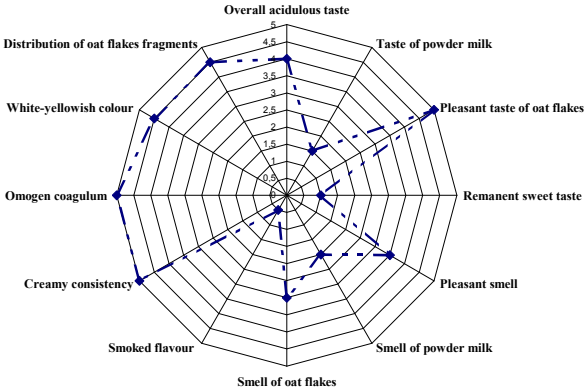

Figure 5. Sensorial profile of milk suplemeneted wih 2% oat flakes

4. Conclusions

Oat flakes represent a solution for the growth stimulation of probiotic bacteria in milk with the 12% nonfat dry milk because of the following considerations:

- by using oat flakes, bifidobacteria reached a high growth rate (0.763 h^{-1}), that was 1.7 times higher than in simple milk;
- the population of bifidobacteria in milk supplemented with oat flakes was larger than in milk. We recommend supplementation with 2% oat flakes in order to obtain a numerous population of bifidobacteria ($4x10^7$cfu/cm^3), in short time.
- oat flakes assures a great number of bifidobacteria in a short time (4h), so this ingredient contribute to reducing the incubation period of probiotic fermented milks with bifidobacteria.
- the oat flakes improve both the flavor and consistency of milk products with probitics.

On the basis of what it was discussed above, it was recommended utilization the oat flakes (in a proportion of 2%) in order to obtained probiotic fermented milks with numerous population of bifidobacteria and with attractive taste, creamy consistency and pleasant aroma

.5. REFERENCES

[1] Garcia E. F., McGregor J.U. and Traylor S., The Addition of Oat Fiber and Natural Alternative Sweeteners in the Manufacture of Plain Yogurt, *Journal of Dairy Science*,1998, 81:655–663

[2] Baron, M., Roy, D., Vuillemard, J.C.: Biochemical caracteristics of fermented milk produced by mixed-cultures of lactic acid starters and bifidobacteria, *Lait*, 2000, 80, 465-478

[3] Bollongue, J. ş.a., Action sur la flore intestinale de laits fermentes au Bifidobacterium, Lait, 1993, 73, 249-256

[4] Brady, L.J. ş.a. The role of probiotic cultures in the prevention of the colon cancer, *Journal of Nutrition*, 2000, 2S, 410S-414S

[5] Dave, R.I. and Shah, N.P. Evaluation of media for selectiv enumeration of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus and bifidobacteria, *Journal of Dairy Science*, 1996, 79, 1529-1536

[6] Roy Denis, Technological aspects related to the use of bifidobacteria in dairy products, Lait, 2005, 85, 39–56

[8] Lund, K. E. and Johnson, I.T., Fermentable Carbohydrate Reaching the Colon after Ingestion of Oats in Humans, *Journal of Nutrition*, 1991, 121: 311-317

[9] Lazaridou, A., Biliaderis, C.G., Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects; *Journal of Cereal Science,* 2007, Volume 46, Issue 2, Pages 101-118

[10] Dikeman, C.L., Murphy, M.R. and Fahey Jr., GC, Dietary Fibers Affect Viscosity of Solutions and Simulated Human Gastric and Small Intestinal Digesta, Journal of Nutrition, 2006, 136: 913–919

[11] Manthey F.A., Hareland G.A. and Huseby D.J., Soluble and Insoluble Dietary Fiber Content and Composition in Oat, Cereal Chemistry, 1999, 76(3): 417–420

[12] Bahrim, G. Microbiologie tehnica, Editura Evrika, Braila, 1999

[13] Segal, B. et al.. *Tehnologia produselor alimentare de protecţie*, Editura Ceres, Bucureşti, 1991, pp. 111-116