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Growth characteristics of probiotic in milk supplemented with rye flakes

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Abstract

Rye flakes were added to milk in order to stimulate growth of probiotic bacteria and to obtain a probiotic product with pleasant sensory attributes. Probiotic culture used in this study contains bifidobacteria, *Lb. acidophilus*, *Lactobacilus lactis* and *Streptococcus thermophilus*.

Rye flakes had a pronounced stimulating effect on growth of bifidobacteria and the population of beneficial bacteria recorded after only 4 hours of incubation was $4x10^8$ufc/cm^3. *Lactobacillus acidophilus* population obtained in the presence of rye flakes was 2 times higher than in simple milk, which means that the ingredient had good stimulating effect on these bacteria. The growth rate reached by *Streptococcus thermophilus* (0.813h^{-1}) in milk with rye flakes was slightly than in simple milk, so the stimulating effect of the rye flakes on this lactic acid streptococcus was weak.

Keywords: bifidobacteria, probiotic dairy products, rye flakes, growth rate, probiotic population

1. Introduction

During the last three decades, attempts have been made to improve the health status of human by modulating the intestinal microbiota using live microbial adjuncts called probiotics [8]. So a probiotic is "a preparation or a product containing viable, defined microorganisms in sufficient numbers, witch alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host"[10].

The consumption of fermented milks with probiotic bacteria (bifidobacteria and Lactobacillus acidophilus) may affect the composition of indigenous microflora and may have several beneficial effects on human health such as the maintenance of a balanced flora, alleviation of lactose intolerance symptoms, resistance to enteric pathogens, immune system modulation, an antihypertensive effect as well as certain anti-carcinogenic effects [2, 6, 7, 11]. The property of probiotic bacteria to modulate the intestinal microbiota is very important in prevention of some intestinal disorders such as: antibiotic-associated diarrhoea, rotavirus gastroenteritis, traveler's diarrhoea and radiation-induced diarrhoea. In order to obtain the desired therapeutic effects, the fermented milks must contain a minimum of viable probiotic bacteria at the precise moment when it is consumed. Therefore, the viable probiotic bacteria should be present in fermented milks to a minimum level of 10^6cfu/g product and the daily intake recommended is of a10^8cfu (meaning the product in a proportion of 100g with 10^6cfu/g) and it was established in order to compensate for the possible bacteria reductions which take place during their passage through the stomach and the intestine [2, 9]. Unfortunately, this level of probiotic bacteria isn't always retrieved in the probiotic products because of the following causes: the probiotic bacteria grow slowly in milk, are sensitive to various medium factors (pH, rh), their viability in fermented milk like yoghurt is

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reduced. Regarding the probiotic bacteria growth, especially of bifidobacteria, the cow's milk doesn't satisfy their nutritional requirements, being poorly in growth factors (especially in amino acids and peptides with a lower molecular mass), and these ones don't have a proteolytic activity and can't assure their nitrogen sources easily assimilable.

Rye is a good source of dietary fiber (the part of plant foods that is not digested and absorbed in the upper gastrointestinal tract in humans). The main dietary fiber component in rye is the partly soluble arabinoxylan. Arabinoxylan from rye may have potential as a prebiotic substrate for the proliferation of Bifidobacterium longum. In plus, rye grain contained 4.6-6.6 g of fructan/100g, depending on the growth conditions [5].

Rye is a rich source of manganese, a mineral that acts a co-factor for more than 300 emzymes, including enzymes involved in the body's use of glucose and insulin secretion. In addition, rye is an especially good source of several mineral, e.g. iron, copper, zinc, selenium, magnesium and fluoride [5].

The potential health effects of diets high in rye are: rye fiber increases faecal volume and reduces the intestinal transit time (this promotes proper bowel function and prevent constipation); intake of rye fiber increases the excretion of energy (this may help to prevent the development of obesity).

In this study was followed the influence of rye flakes added in milk on growth rate of probiotic bacteria during incubation at 38°C. Also, the study followed the investigation of the impact of the proportion of rye flakes on sensory properties. Sensory analysis results were compared with growth parameters of probiotic bacteria as support for our observations and conclusions.

2. Materials and Method

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.

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 rye flakes up to 2% (S1) and 3% (S2). Also, was prepared a milk sample without rye flakes to be used as a reference (M0). 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.

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 [1]:

- ⇨ the number division (n) was calculated with relation

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

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

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

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

$$tg = \frac{t}{n} = \frac{1}{\mu}$$

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 rye flakes and fermented with probiotic culture, was used a scoring method (Segal et al., 1985). 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

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

In the first two hours of incubation was observed that the growth rate of probiotic bacteria (bifidobacteria and Lactobacillus acidophilus) was higher in the milk samples supplemented with rye flakes then in milk without ingredient (table 1), which means that this source of dietary fiber stimulates the growth of probiotic bacteria.

Table 1. The populations of probiotic bacteria and parameters of bacterial growth

Milk samples	Populations of bifidobacteria (log cfu/cm^3)			Parameters of bifidobacterial growth at 2h			Populations of Lb. acidophilus (log cfu/cm^3)			Parameters of Lb. acidophilus growth at 2h		
	0h	2h	4h	n	μ [h^{-1}]	tg [h]	0 h	2 h	4 h	n	μ [h^{-1}]	tg [h]
M	6,768	7,184	7,821	1,38	0,69	1,44	7,154	7,485	7,838	1,09	0,54	1,81
S1	6,804	7,527	8,406	2,40	1,20	0,83	7,122	7,520	7,883	1,32	0,66	1,51
S2	6,70	7,911	8,582	4,00	2,00	0,50	7,184	7,876	8,140	2,29	1,14	0,87

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

In the firs 2 hours, the most numerous population of bifidobacteria was registered in S2 sample (fig.1), supplemented with 3% rye flakes, and it was 5.3 times higher than in simple milk (M) and 2.4 times higher than in S1 sample, with 2% rye flakes. Consequently, bifidobacteria had the highest growth rate in S2 (2.0 h^{-1}), which means that the generation time of bifidobacteria in milk supplemented with 3% rye flakes was only 30 minutes.

In sample S1, which has a quantity of rye flakes with 1% less than S2, bifidobacteria had a generation time of 50 minutes, which means that the growth rate was 1.66 times lower than in S2.

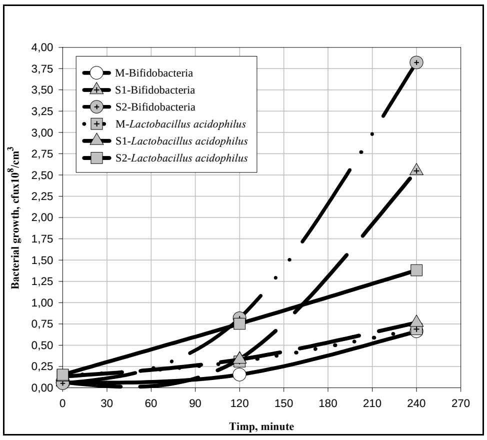

Figure 1. The dynamic of growth of probiotic bacteria in milk variants with or without rye flakes during the incubation at 38°C

Lactobacillus acidophilus has recorded the highest growth rate in the S2 sample with 3% rye flakes, but it was 1.74 times lower than bifidobacteria. The generation time of *Lactobacillus acidophilus* in S2 was with 22 minutes higher than the bifidobacteria and was almost equal to the bifidobacteria in S1 sample.

The population of *Lb. acidophilus* in sample S2 was 1.72 times higher than in M sample (milk without rye flakes), which means that the rye flakes stimulated the growth of this probiotic bacteria.

The results obtained after 4 hours of incubation at 38°C showed that the population of bifidobacteria continued to increase numerically, though the growth rates of bifidobacteria were lower than the ones noted at 2 hours of incubation. Thus, after the first 4 hours of incubation the most numerous population of bifidobacteria was registered in the S2 milk variant, supplemented with 3% rye flakes, though the rate growth of bifidobacteria was 1.29 times lower than the one from the first 2 hours of incubation. Referring to the S1 (milk with rye flakes in a proportion of 2%) was observed that the growth rate from S2 was higher with 16.54% and the generation time was with 7 minutes lower. The population of bifidobacteria from S2 was 6 times higher than in the M (milk without rye flakes) and with 50% higher than in the S1 milk variant, supplemented with 2% rye flakes.

After 4 hours of incubation, the greatest population of *Lactobacillus acidophilus* was observed in S2 sample (fig.1), supplemented with 3% rye flakes, and it was 2 times higher than in simple milk (M) and 1.8 times higher than in S1 sample, with 2% rye flakes. Consequently, Lactobacillus acidophilus had the highest growth rate in S2 (0.793 h^{-1}), though this growth rate was 1.44 times lower than the one from the first 2 hours of incubation. The generation time of Lactobacillus acidophilus in milk supplemented with 3% rye flakes was 1hours and 15 minutes, meaning the 23.4 minute higher than that recorded after 2h of incubation.

Growth parameters obtained for Lactobacillus acidophilus in the M sample (milk without rye flakes) are close of those obtained by Brizuela (2001) for the *Lactobacillus acidophilus* LB-12 strain.

In conclusion, based on observations recorded during incubation, rye flakes stimulates growth of probiotic bacteria and ensure obtaining a population of bifidobacteria of $4x10^8$ufc/cm^3 and a population of *Lactobacillus acidophilus* of $1.38x10^8$ufc/cm^3 in only 4h.

The effect of rye flakes on the growth rate of other lactic acid bacteria (*Lactobacillus lactis* and *Streptococcus thermophilus*) of culture used

During incubation (4 hours) was observed that the growth rate of *Lactobacillus lactis* was higher in the samples milk supplemented with rye flakes than in milk without ingredient, being maximum ($0.353h^{-1}$) in the sample S2 supplemented with 3% rye flakes (fig. 2). The population of *Lactobacillus lactis* in the sample S2 was $4x10^7$cfu/cm^3, being 3 times higher than in the sample S1 (milk supplemented with 2% rye flakes) and 4.96 times higher than in the sample M (milk without rye flakes) .

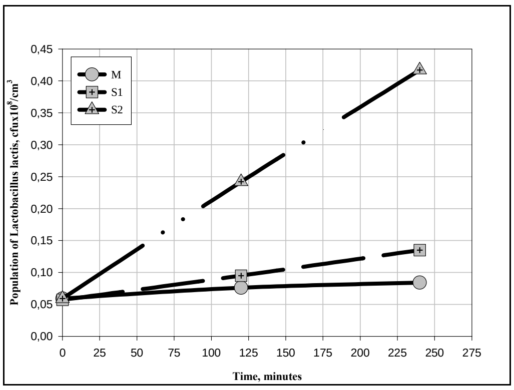

Figure 2. The dynamic of growth of Lactobacillus lactis in milk samples with or without rye flakes during the incubation at 38°C

In comparison with the sample S2, the generation time of Lactobacillus lactis in the sample S1 was 199 minute, which means with 1 hour and 55 minute higher than in S2 and with 4 hours and 21 minute lower than in the sample M (milk without rye flakes). These results show that the supplementation of milk with rye flakes had a lower stimulating effect on *Lactobacillus lactis* than on bifidobacteria.

Regarding the evolution of *Streptococcus thermophilus*, rye flakes had a slightly stimulating effect on this bacterium, as we found by comparing the results of milk samples studied. After incubation (4 hours), the largest population of Streptococcus thermophilus was obtained in the sample S2 and was $1.2x10^9$cfu/cm^3.

Fermentation activity of starter culture in milk supplemented with rye flakes was more intense than in milk without the ingredient, and the acidity developed during incubation was about 5g acid lactic/dm^3. The highest increase of acidity was registered in the sample S2 (pH=4.5), where the acidity was with 5.19 g lactic acid/dm^3 higher than the initial one, and with 0.89 g acid lactic/dm^3 higher than in M (milk without rye flakes). Because residual lactose content of sample S2 is equal to the sample M, high acidity of S2 is probably explained by the partial consumption of rye flakes carbohydrates by lactic acid bacteria.

The sensorial characterization of the fermented milks supplemented with rye flakes

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

- aspect, color and consistence: coagulum homogenous with fine consistence like cream, and with smaller rye flakes fragments uniform distributed in the coagulum mass, without whey elimination; color yellowish brown (like milk with coffee) uniform in the whole product mass, imprinted by rye flakes;

- smell: specific aroma of probiotic culture, pleasant aroma of lactic fermentation;
- taste: pleasant, acidulous, flavor specific 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: S1(fermented milk supplemented with 2% rye flakes)- 20 points and S2 (fermented milk supplemented with 3% rye flakes)-18.5 points.

4. Conclusions

Rye 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 rye flakes, bifidobacteria reached a high growth rate (2.00 h^{-1}), that was 2.9 times higher than in simple milk;
- the population of probiotic bacteria (bifidobacteria and *Lactobacillus acidophilus*) in milk supplemented with rye flakes were larger than in milk. We recommend supplementation with 3% rye flakes in order to obtain a numerous population of bifidobacteria ($4x10^8$cfu/cm^3) and *Lactobacillus acidophilus* ($1.38x10^8$cfu/cm^3), in short time.;
- rye flakes contributes to improvement of the cultivation conditions of bifidobacteria because they are rich in vitamins of the B complex which are necessary for the growth of these bacteria. Moreover, rye contains prebiotics (meaning a non-digestible food ingredients that stimulate the bifidobacteria in colon) like fructooligosaccharides;
- rye flakes assures a great number of bifidobacteria and *Lactobacillus acidophilus* in a short time (4h), so this ingredient contribute to reducing the incubation period of probiotic fermented milks with bifidobacteria and *Lactobacillus acidophilus*;
- the rye flakes improved both the flavor of the fermented milk with probiotic culture and its color;
- On the basis of what it was discussed above, it was recommended utilization the rye flakes (in a proportion of 3%) in order to obtained probiotic fermented milks with numerous population of bifidobacteria and with taste and aroma pleasant.

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