METABOLIC AND BIOCHEMICAL CHARACTERISTICS OF PROBIOTIC CULTURE IN MILK SUPPLEMENTED WITH RYE FLAKES AND MALT EXTRACT

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Abstract
Rye flakes and malt extract were added to milk in order to stimulate growth and fermentative activity 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 have a stimulating effect more pronounced than malt extract on acidification capacity of the probiotic culture, and to achieve an increase of the milk acidity of 7g lactic acid /dm^3 (in 6h at 39^{o}C) the two ingredients must be added in concentration of 2% and, respectively, 0.2%..
The probiotic culture reach the greatest proteolytic activity when rye flakes are added in the proportion of 3% and malt extract in the proportion of 0.1% and the amino acids released rate was 764.6 μg%. The lactose bioconversion rate was greater in the milk supplemented with rye flakes 3% and malt extract 0.1% and residual lactose was 3.84%.

Keywords: Probiotic culture, rye flakes, malt extract

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 [2, 9]. 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"[11].
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 [1, 7, 8, 14]. 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 [1, 10, 12].
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 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 [4].

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 [6].

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 [6].

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).

Malt extract is a source of nutrition, energy and of vitamin B and is sometimes used as a "tonic". Malt extract is an extremely attractive substitute for refined sugar because in addition to sweetness and energy, it delivers naturally derived nutrition in a particularly suitable form for human consumption. Nutrient content of malt extract (per 100g): carbohydrates 78 g (glucose 9 g, fructose 1 g, maltose 42 g, sucrose 1.2 g, 1-2 g of unidentified metabolites, probably phytates and β-glucan-derived oligosaccharides); minerals (potassium, 0.5 g; sodium, 0.01 g; calcium, 0.03 g; magnesium, 0.08 g; phosphorus, 0.38 g, iron, 0.0008 g; zinc, 0.0003 g; manganese, 0.00002 g; copper, 0.0007 g), amino acids (alanine, 0.39 g; arginine, 0.29 g; aspartic acid, 0.55 g; cysteine, 0.09 g; glutamic acid, 1.48 g; glycine, 0.36 g histidine, 0.17 g; isoleucine, 0.25 g; leucine, 0.5 g; lysine, 0.23 g; methionine, 0.18 g; phenylalanine, 0.34 g; proline, 0.90 g; serine, 0.36 g; threonine, 0.10 g; tyrosine, 0.16 g; valine, 0.38 g; nonidentified, 0.35 g) [5].

In this study was followed the influence of rye flakes and malt extract added in milk on metabolic and biochemical caracteristics of probiotic bacteria during incubation at 39°C. Also, the study followed the investigation of the impact of the proportion of rye flakes and malt extract on sensory properties.

2. MATERIALS AND METHODS

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 MBi (milk reconstituted with 12.0% nonfat dry milk) for hydratation and standardization of the inoculated cells. Then, this medium MBi was used like inoculum.

2.2. Preparation of fermented milk samples

In this study were prepared 8 variants of milk reconstituted from milk powder (12% nonfat dry milk) supplemented with rye flakes and malt extract (table 1). Milk with no added ingredients was used as control.

The milk samples were pasteurized at 90-95°C/ 2 minutes. Then, the milk samples cooled to 40°C were inoculated in the proportion of 2% with probiotic culture and incubated 6h at 39°C.

2.3. Physicochemical and biochemical methods

Proteolytic activity of probiotic culture was assessed by determining the amino acid content of milk samples during incubation at 39°C (at 0h, 4h and 6h). To assess the amino acid content of the milk samples was used the CD-ninhydrin reactive method described by Folkertsma and Fox [13] with some modifications. For this was prepared a standard curve using leucine and concentrations of 0.125 to 2.0 mM.

Table1. Variants of milk supplemented with rye flakes and malt extract

Milk variants	Milk powder (g%)	Extra milk powder (g%)	Rye flakes (g%)	Malt extract (g%)
MB	12,5	-	-	-
MS	12,5	-	1	-
SM I	12,5	-	1	0,1
SM II	12,5	-	2	0,2
SM III	12,5	-	3	0,3
SM IV	12,5	2	2	0,2
SM V	12,5	-	1	0,3
SM VI	12,5	-	3	0,1

The preparation of the milk samples for the analysis was obtained through a precipitation with 5% $ZnSO_4$ and then through a centrifugation of 15 minutes at 6000rot/min and the supernatant was filtrated through a quantitative filter paper.

Then, 50µl of filtrate were added to 950µl of water to obtain the total volume to 1ml. Moreover, 2ml of CD-ninhydrin reactive reagent (1g of $CdCl_2$ dissolved in 1ml of water, 0.8g of ninhydrin, 80ml of 90% ethanol and 10ml of glacial acetic acid) were added to the filtrate and then the composition was heated at 84°C and maintained for 5 minutes.

The absorbance was read to a spectrophotometer UV-VIS-NIR (Jasco V570) at 507nm wavelength and the amino acids were expressed in mg leucine/100 cm^3 milk (or fermented milk).

Lactose bioconversion capacity of probiotic culture was evaluated by determination of lactose content of milk samples at different time intervals (0h, 4h, and 6h). The lactose content of milk samples was assessed by the 3.5-Dinitrosalicilic acid method (3.5-DNS method), according to STAS 10902-89 of milk and dairy products. To establish the standard curve were prepared lactose solutions with concentrations of 0.5-5 mg/cm^3. To determine lactose, milk variants were precipitated with 5% ZnSO4 solution and filtering through quantitative filter paper (filtrate A). Then, 3.5-acid DNS reagent was mixed with the filtrate A and the mixture was kept 5 minutes on a boiling water bath.

The absorbance was read to a spectrophotometer UV-VIS-NIR (Jasco V570) at 530nm wavelength and lactose was expressed in g lactose/100 cm^3 milk (or fermented milk).

The acidity of milk samples, expressed in gram acid lactic/dm^3, was determined by titration with NaOH 0,1N in the presence of phenolphthalein, used as indicator. For the measure of pH was used a pH-meter Denver, and the calibration was made with standard solutions with pH=4 and pH=7.

3. RESULTS AND DISCUSSIONS

3.1. Effect of rye flakes and malt extract on the acidification rate of the probiotic culture

In the first 4 h of incubation, the acidity of milk samples supplemented with rye flakes and malt extract was higher than in control samples MB and MS, and its higher values were recorded in samples supplemented with 0.3% extract malt (figure 1). SM5 sample reached the highest acidity (6.39 g lactic/dm^3 acid) and it was 1.6 times higher than in MB and 2.05 times higher than that in MS.

These results show that malt extract should be used in a concentration of 0.2%, since at higher concentrations was observed that it has slightly inhibitory effect on the acidification capacity of the probiotic culture, probably due to the additional reducing sugars (mainly maltose) in milk.

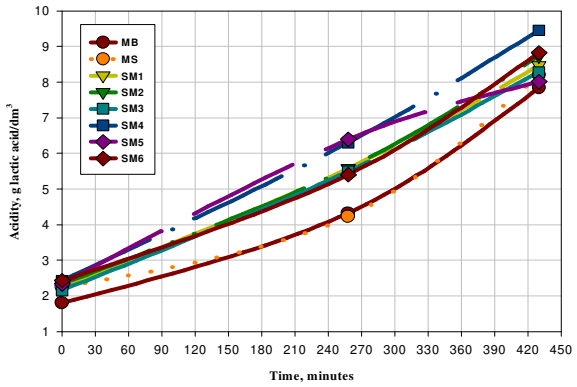

Figure1. The evolution of the titrable acidity in the milk variants during the incubation at 39°C

MB = reconstituted milk (12% skimmed milk powder); MS = MB + 1% rye flakes; SM1 = MB + 1% rye flakes + 0,1% malt extract; SM2 = MB+2% rye flakes + 0.2% malt extract; SM3 = MB + 3% rye flakes+ 0.3% malt extract; M4 = MB +2% milk powder + 2% rye flakes + 0,2% malt extract; SM5 = MB + 1% rye flakes+ 0,3% malt extract; SM6= MB +3% rye flakes + 0,1% malt extract;

After 6 h of incubation was observed that the highest rate of acidification was obtained in SM4, and the acidity was with 1.62 g lactic acid /dm^3 higher than in MB (milk without ingredients) and with 1.26 g lactic acid/dm^3 higher than in MS, which was supplemented only with rye flakes.

Acidification rate in MS was about two times higher in the last 2 h in MB than in the first 4h at 39^{o}C, which leads us to suppose that the lactic bacteria need a longer period of adaptation in milk supplemented only with rye flakes.

After 4 h of fermentation, the pH of all fermented milks ranged between 4.8 and 4.89.

In conclusion, for stimulation of fermentative activity of probiotic culture is recommended to use rye flakes in a rate of 2% and malt extract at a rate of 0.2%.

3.2. Effect of rye flakes and malt extract on the bioconversion rate of lactose by probiotic culture

The highest bioconversion rate of lactose (expressed as lactose consumed by probiotic culture/hour), after the first 4 h of incubation at 39°C, was reached in SM6 (milk with 3% rye flakes and 0.1% malt extract) and was of 241.12 mg lactose/ h (figure 2).

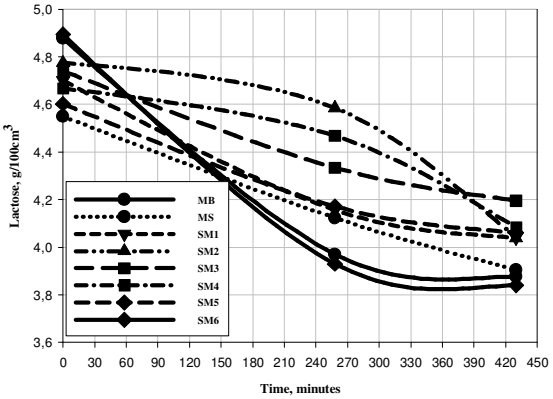

Figure 2. The evolution of the lactose content in the milk variants during the incubation at 39°C

MB = reconstituted milk (12% skimmed milk powder); MS = MB + 1% rye flakes; SM1 = MB + 1% rye flakes + 0,1% malt extract; SM2 = MB+2% rye flakes + 0.2% malt extract; SM3 = MB + 3% rye flakes+ 0.3% malt extract; M4 = MB +2% milk powder + 2% rye flakes + 0,2% malt extract; SM5 = MB + 1% rye flakes+ 0,3% malt extract; SM6= MB +3% rye flakes + 0,1% malt extract;

Figure 2 shows that the reduction of lactose content, after 6 h of incubation, was highest in SM6, and the amount of metabolized lactose by probiotic culture was higher than in MS sample (milk supplemented only with rye flakes) with 409 mg lactose. In other variants of milk, lactose bioconversion rate was lower than in controls (MB) and was clearly influenced by the proportion of malt extract added to the milk. Thus, the lowest lactose bioconversion rate of probiotic culture occurred in SM3 and SM5 samples (supplemented with 0.3% malt extract) and was 1.94 times lower than in SM6, which contains only 0.1% malt extract. Therefore, the lactose bioconversion rate was positively influenced by the proportion of rye flakes, being highest in the sample SM6 (with rye flakes 3%), in which the residual lactose content was 3.84g/100g and this result is comparable to that obtained by De Noni [3]. Compared with SM6, reducing lactose content in variant SM2, which contains rye flakes 2%, was lower with 318 mg lactose.

Rye flakes (3%) and malt extract (0.1%) provides an improvement in the bioconversion rate of lactose by probiotic culture.

3.3. The effect of the two sources of nutrients and growth factors on proteolytic activity of probiotic culture

Regarding the proteolytic activity of probiotic culture, after 4 hours of incubation, in the milk variants was observed a decrease in the amino acids proportion (expressed as mg leucină/cm^3 milk) and it was higher in milk samples with higher proportions of malt and smaller proportions of rye (SM5) (figure 3).

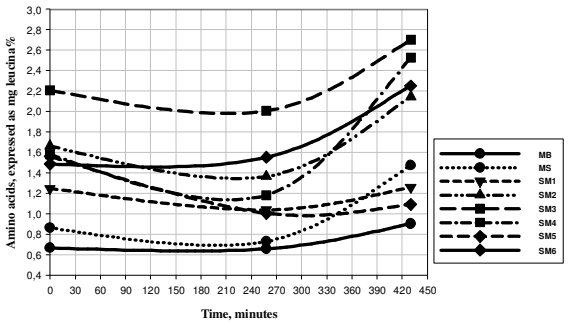

Figure 3. The evolution of the amino acids content, in the milk variants, during the incubation at 39°C

MB = reconstituted milk (12% skimmed milk powder); MS = MB + 1% rye flakes; SM1 = MB + 1% rye flakes + 0,1% malt extract; SM2 = MB+2% rye flakes + 0.2% malt extract; SM3 = MB + 3% rye flakes+ 0.3% malt extract; M4 = MB +2% milk powder + 2% rye flakes + 0,2% malt extract; SM5 = MB + 1% rye flakes+ 0,3% malt extract; SM6= MB +3% rye flakes + 0,1% malt extract;

Between 4-6h of incubation, proteolytic activity of probiotic culture is observed by increasing the proportion of amino acids in milk variants studied, and the highest values were recorded in variants of milk supplemented with 2% rye flakes and 0.2% malt extract (SM2 and SM4).

At the end of incubation, the highest proteolytic activity was observed in SM4 that was 5.51 times higher than in control MB, which does not contain those two ingredients. An important increase in amino acid rate (764.6 µg %) was observed in SM6, which contains 3% and 0.1% rye malt extract. The increased amino acid rate of this sample was 3.24 times higher than in MB (milk without those two ingredients).

In conclusion, to stimulate proteolytic activity of probiotic culture is recommended to use rye flakes in a rate of 2% and malt extract at a rate of 0.2% when the reconstituted milk contain 14% skimmed powder milk. In the case of the reconstituted milk, containing 12% skimmed powder milk, for obtained an elevated amino acids rate those two ingredients will be used in proportion of 3% rye flakes and 0.1% malt extract.

4. CONCLUSIONS

Rye flakes and malt extract represent a solution for the stimulation of fermentation activity of probiotic bacteria in milk with the 12% nonfat dry milk. In agreement with results obtained following observations were made:

- To improve the acidification capacity of probiotic culture used (bifidobacteria, Lactobacillus acidophilus, Lactobacillus lactis and Streptococcus thermophilus), it is recommended to use rye flakes at a rate of 2% and malt extract at a rate of 0.2%.
- To increase the lactose bioconversion rate of this probiotic culture is recommended to use rye flakes at a rate of 3% and malt extract at a rate of 0.1%. Under these conditions we obtain a lactose bioconversion rate of 175.5 mg / h;
- To stimulate proteolytic activity of this probiotic culture is recommended to use rye flakes at a rate of 3% and malt extract at a rate of 0.1%. In this case probiotic culture releases a higher proportion of amino acids (764.6 µg %) during fermentation.

In addition, using rye flakes and malt extract was obtained a probiotic fermented milk with special sensory attributes (pleasant aroma, sweet-sour taste, color like coffee milk and consistency like cream).

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