

ORIGINAL RESEARCH PAPER

THE PREBIOTIC AND PROTECTIVE EFFECTS OF BUCKWHEAT
FLOUR AND OAT BRAN ON *LACTOBACILLUS ACIDOPHILUS*

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During conservation, the probiotic bacteria currently used in food industry have been shown to have less viability in the matter of fermented products microbiota and also to present lower levels of colonization and survival *in vivo*. This study describes a new way of improving the behavior and functionality of *Lactobacillus acidophilus* - LA 5[®] commercial strain by using buckwheat flour (*Fagopyrum esculentum*) and oat bran (*Avena sativa*) as fermentation ingredients, in view of their high content of bioactive compounds that have a great impact both on fermentation microorganisms and consumers' health. The effect of these two raw vegetal products on LA 5[®] strain was studied by cultivation on MRS broth and on milk. The supplementation of the fermentation medium with 4.0% - 6.0% of buckwheat flour or oat bran, respectively, had a positive effect on metabolic activity and viability of lactic acid bacteria. Thus, the rapid decrease of the pH and the increase of the multiplication rate were observed after 6 hours of lactic acid fermentation. Furthermore, the presence of the vegetal substrates substantially improved the cells survival during the storage of the fermented products for 28 days at 4 °C, comparing to samples without supplementation used as control.

Keywords: *Lactobacillus acidophilus* (LA 5[®]), buckwheat flour (*Fagopyrum esculentum*), oat bran (*Avena sativa*), synbiotics

Introduction

The concept of prebiotics is relatively new (Gibson and Rastall, 2004), however they have been practically used since ancient times. Prebiotics are non-digestible food ingredients with effect upon the stimulation of growth and metabolic activity of probiotic bacteria, especially *in vivo* (Kelly, 2008). In order to have a positive effect on health and well-being, these food ingredients have to resist the gastric

acidity, to avoid hydrolysis by the mammalian enzymes and not to be absorbed in the upper gastrointestinal tract (Gibson and Rastall, 2004).

Dietary fibers (non-digestible carbohydrates) exhibit prebiotic activity, acting as an important player during the colonic fermentation, by causing several changes in the gut microbiota. Such dietary components can stimulate fermentation, thus leading to an increase in bacterial mass and consequently in the amount of fermented carbohydrates (Cummings and Macfarlane, 1991). The commercially available prebiotics are inulin-based, extracted from a food source (chicory root) or synthesized from sucrose. Usually, inulin stimulates the growth and activity of the beneficial bacteria (bifidobacteria and lactic acid bacteria), having a positive effect *in vitro* (Kaplan and Hutkins, 2000; Perrin *et al.*, 2001), and *in vivo* (Gibson *et al.*, 1995; Niness, 1999).

The market for prebiotic foods is rapidly developing due to the fact that these biological active components may target specific functions in the body. They are usually found in functional foods, such as fermented milk by maintaining the viability of probiotic bacteria. Their role as stimulators of the growth and development of bacteria has been previously described extensively (Coman *et al.*, 2013; Al-Sherajia *et al.*, 2013; Valcheva and Dieleman, 2016). This close relationship between lactic acid bacteria and prebiotics is based on the high nutritional demands of these specific microorganisms for some nutrients such as non-protein-nitrogen and B-group vitamins. This synbiotic concept is widely used for the production of functional products with many benefits on consumers' health (Rao, 2002). The main characteristic of the synbiotic products concept is represented by the synergistic relationship between the beneficial bacteria and their selective substrates, providing protective effect for the cells when passing through the gastrointestinal tract (Hammes and Hertel, 2002; Bielecka *et al.*, 2002).

Préstamo *et al.* (2003) showed that buckwheat is a nutritional product rich in essential bioactive compounds (aminoacids, fatty acids, B₁ and B₂ vitamins and minerals), that has beneficial effects on health, and can be also used as prebiotic for lactic acid fermentation (Coman *et al.*, 2013). In recent years, oats have been in the spotlight of many researchers, due to the fact that this vegetal by-product has a high content of soluble and insoluble fibers with positive effect on lactic acid bacteria metabolism (Gupta *et al.*, 2010; Nionelli, *et al.*, 2014).

The aim of this study was to investigate the qualitative and quantitative influence of the buckwheat flour and oat bran on the multiplication of commercial Christian Hansen starter culture, *Lactobacillus acidophilus* (LA 5[®]), during cultivation in MRS broth and milk. In addition, the cells survival during fermented products conservation in specific conditions for obtaining synbiotic products was also tested.

Materials and methods

Lactic acid bacteria strain, prebiotics and media

Lactobacillus acidophilus - LA 5[®] commercial starter was provided by Christian Hansen (Braşov, Romania) as freeze-dried culture (10¹¹ CFU/g). The preservation

of the stock culture was carried out in agreement with the recommendation of the manufacturer.

The buckwheat was purchased from a specialized market from Cahul, Republic of Moldova, and the oat bran was purchased from the Plafar market of Galati, Romania. The raw materials were first sterilized by UV radiation at the wavelength of 230 nm, and were then ground through Retsch GM 200 laboratory mill (Retsch GmGT, Germany).

The MRS medium was purchased from Scharlab SL Company (Spain) and used as MRS agar and broth containing (g/L): polypeptone 10.0, meat extract 10.0, yeast extract 5.0, glucose 20.0, Tween 80 1.0, dipotassium phosphate 2.0, sodium acetate 5.0, ammonium citrate 2.0, magnesium sulfate 0.2 and manganese sulfate 0.05. The pH was adjusted to 5.5 at 25°C.

The UHT milk produced by LaDorna (Romania), containing 3.5% fat, 4.5% carbohydrates and 2.9% proteins was used in the experiments.

The fermentation medium was obtained from MRS basal broth (without added glucose) and milk supplemented with different concentrations (2%, 4% and 6% w/v) of buckwheat flour or oat bran.

Inoculum preparation

The inoculum was freshly prepared for each cultivation step as follows: 1 g of stock freeze-dried culture (10^{11} CFU/g) was weighed in sterile Erlenmeyer and mixed for reactivation with 10 mL of MRS broth and then incubated for 15 minutes at 37°C.

Lactic acid fermentation

A volume of 10 mL inoculum was mixed with 90 mL of fermentation media, MRS basal broth and milk, being afterwards supplemented with buckwheat flour or oat bran. The control samples were prepared following a similar protocol without the addition of vegetal substrates. All samples were incubated at 37°C. The pH was monitored hourly using a pH-meter (Portamess®91, Switzerland). The fermentation stopped when the pH reached the value of 4.6. Lactic acid bacteria multiplication was assessed by indirect viable cell counts at every 2 hours, until the pH reached the value of 4.6.

After fermentation, all samples were stored at 4°C and the cells viability was also monitored, by plate counts, during the products storage, after 7, 14, 21 and 28 days.

Counting of lactic acid bacteria

The cells viability was quantified through the indirect counting method by performing serial dilutions in peptonized water (Merck) 0.1% (w/v), followed by cultivation in Petri dishes on MRS agar medium. The dishes were incubated in anaerobic jars (Merck) with Anaerocult® A kit (Merck), for 48 h at 37°C (BS ISO 20128:2006; ISO 8261 IDF122:2001). The results were expressed as log CFU/mL.

Statistical analysis

All the experiments were done in triplicate and the data presented here represents the mean of these replicates. The data was analyzed by using SPSS (IBM Corporation, version 10) statistical software.

Results and discussions

The cells multiplication, lactic acid fermentation dynamic and viability of LA 5[®] starter culture by cultivation on MRS broth and milk supplemented with 2%, 4% and 6% w/v buckwheat and oat bran, respectively, during 8 h of lactic acid fermentation, were analyzed.

Figure 1 and Figure 2 present the obtained results for the experiments when the cultivation took place on a glucose free MRS broth basal medium.

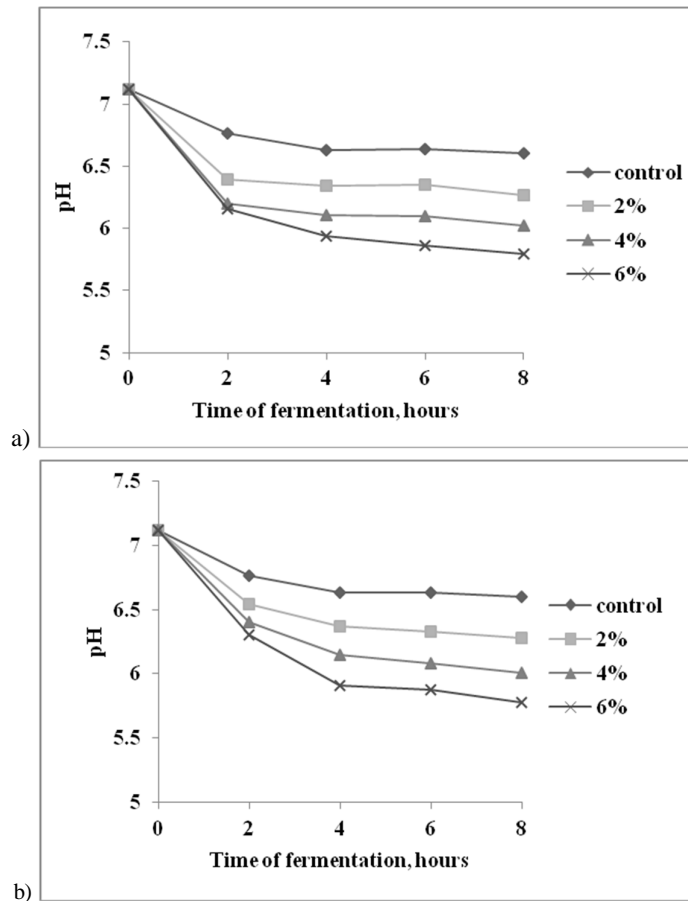


Figure 1. pH evolution during LA 5[®] cultivation on MRS basal broth, supplemented with different concentrations of buckwheat flour (a) and oat bran (b)

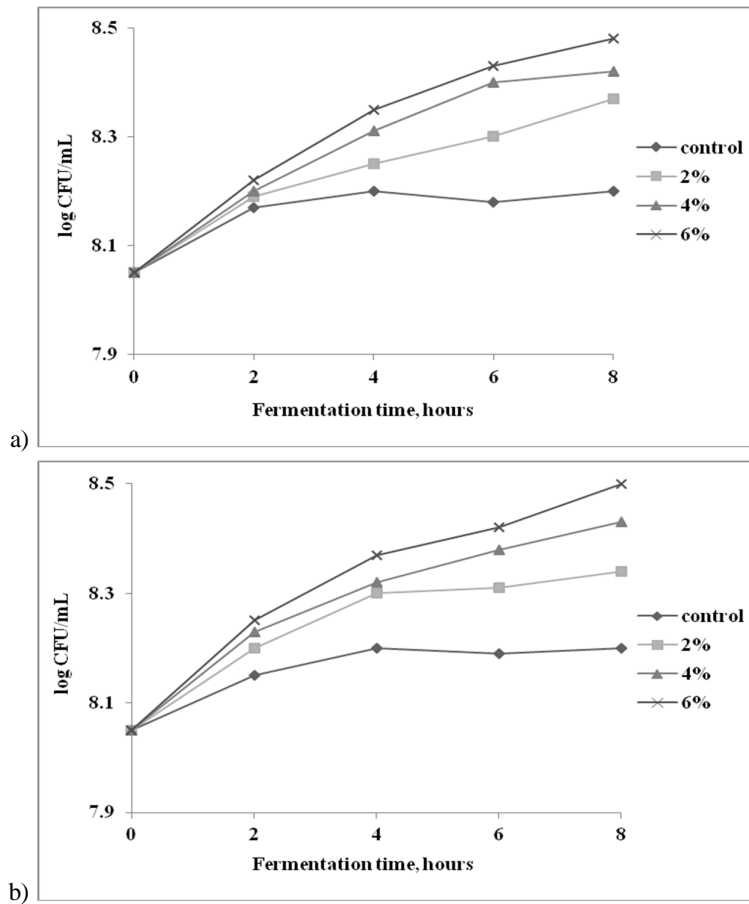


Figure 2. The quantitative influence of buckwheat flour (a) and oat bran (b) on the multiplication rate of LA 5[®] strain in MRS basal broth

The results showed that the supplementation of the fermentation medium with 2% vegetal substrates did not have a significant influence on the bacteria behavior, when compared to the control samples. On the other hand, by increasing the concentration of buckwheat flour or oat bran at 4% and 6%, the fermentation time required to obtain a final pH value of 4.6 was shorter (12h and 10h, respectively) comparing with the control samples (16h). After 8 hours of fermentation, an increase of the multiplication rate by 0.48 log CFU/mL and 0.5 log CFU/mL was observed, compared to control, when the basal MRS medium was supplemented with 6% buckwheat flour or oat bran, respectively.

It is well known that the probiotic bacteria have the ability to hydrolyze both the β -(2-1) linkage of oligosaccharides, due to the fact that these bacteria produce specific enzymes (Klein *et al.*, 1998; Semjonoves, *et al.*, 2004), and also β -glucosidase (Martinez-Villaluenga and Gomez, 2007) and β -fructofuranosidase

(Janer *et al.*, 2004). Kontula *et al.* (1998) studied the influence of oat bran oligosaccharides in regard to the carbohydrate utilization and the fermentation end-products using three lactic acid bacteria strains, *Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactococcus lactis*. They also demonstrated that oat bran oligosaccharides have an influence both on the bacteria multiplication and also on the quality and quantity of the fermentation end-products formed during the fermentation.

The results obtained in this study are in concordance with those reported by Angelov *et al.* (2006). They obtained a drink through lactic acid fermentation of the whole-grain oat substrate with lactic acid bacteria. The fermentation processes lasted for 8 h. At the end of the process, the viable cell number reached 7.5 log CFU/mL. Kedia *et al.* (2008) reported a high multiplication rate during *Lactobacillus plantarum* strain cultivation on an oat flour-based medium upon comparing with white flour or bran.

The best effect was observed for milk supplemented with 6% vegetal substrates. The pH of this sample reached the value of 4.6 after 6 hours of fermentation. Good results were also obtained when milk was supplemented with 4% buckwheat flour or oat bran. In this case the pH reached the value of 4.6 after 8 hours of fermentation, with 4 hours less compared to the control sample (Figure 3).

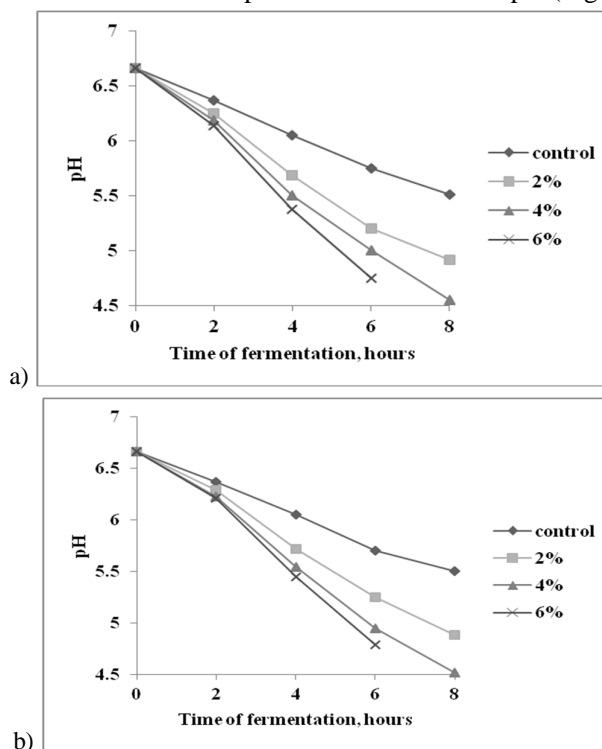


Figure 3. Lactic acid fermentation dynamic performed by LA 5[®] strain in milk supplemented with buckwheat flour (a) and oat bran (b).

Figure 4 presents the multiplication dynamics of LA 5[®] strain in milk supplemented with vegetal substrates. The most stimulating effect on cells multiplication was also obtained when milk was supplemented with 6% vegetal substrates. Buckwheat flour had a strong influence on LA 5[®] strain multiplication compared to oat bran at the same concentration. After 6 hours of lactic acid fermentation, the concentration of bacteria reached values of 1.2 log CFU/mL in the medium with buckwheat flour, and 0.9 log CFU/mL, respectively, in the medium with oat bran.

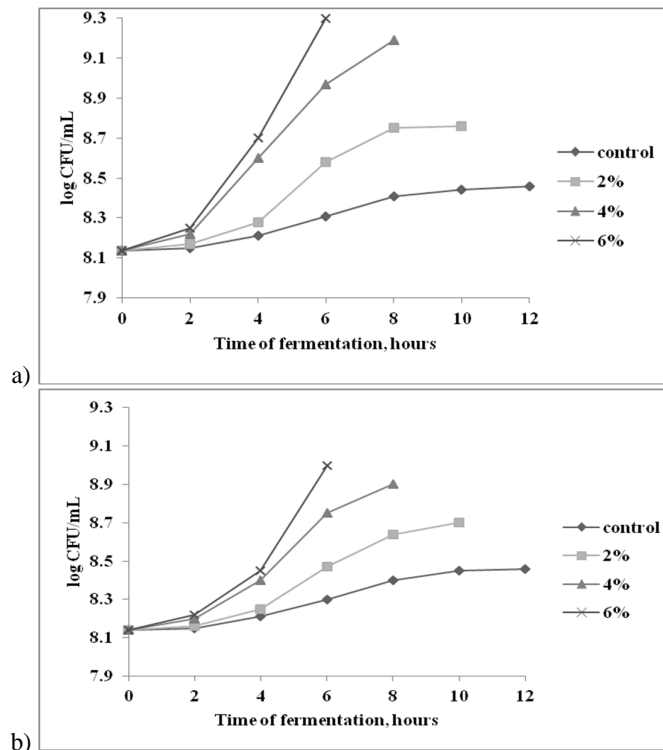


Figure 4. The qualitative and quantitative influence of buckwheat flour (a) and oat bran (b) on LA 5[®] strain multiplication during milk fermentation. The fermentations were stopped when reaching the pH of 4.6

The obtained results are in line with those obtained by Lazaridou et al. (2014). The scientists presented the positive impact of supplementation of skimmed milk (12% total solids) with 1.4% oat β -glucan with reference to the fermentation with a co-culture of *Lactobacillus paracasei* subsp. *paracasei* B117, *Lactobacillus delbrueckii* subsp. *bulgaricus* Y 6.15 and *Streptococcus thermophilus* Y 4.10, in terms of reducing the fermentation time and to increase the lactic acid bacteria efficiency with regard to the metabolic activity.

In order to have a probiotic effect, in fermented products the probiotic bacteria have to be present in a sufficiently high number. Thus, if the concentration is somewhere in the domain of 6 log CFU/mL to 7 log CFU/mL, the consumption of

100 mL of fermented product is necessary in order to reach the recommended daily dose of $8 \div 9$ log CFU. These results are in concordance with those obtained by Oliveira *et al.* (2011; 2012), which demonstrated the prebiotic role of inulin for lactic acid bacteria in skim milk lactic acid fermentation.

The survival rate of LA 5[®] in fermented milk supplemented with vegetal substrates was analyzed, during 28 days of storage, at 4°C (Figure 5). A storage period up to 28 days is the most recommended for fermented food preservation in order to ensure the shelf life of products (Desai *et al.*, 2004).

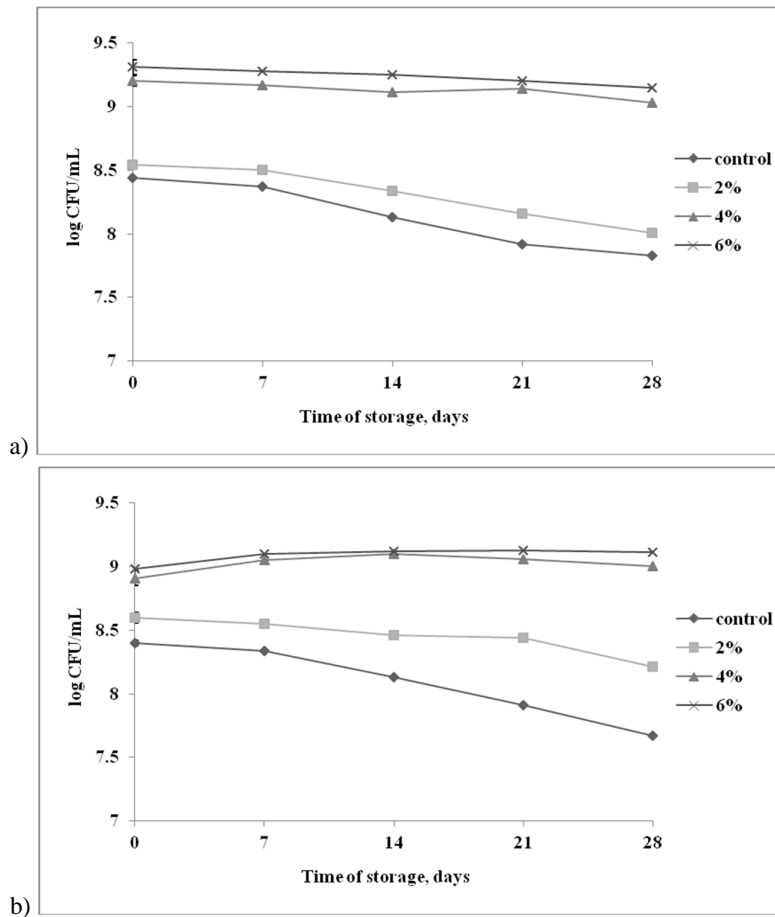


Figure 5. LA 5[®] viability in fermented milk supplemented with buckwheat flour (a) or oat bran (b), during fermented product storage at 4°C

The logarithmic reductions of cell viability of LA 5[®] at the end of the refrigeration period were similar for the fermented milk supplemented with buckwheat flour and oat bran, and significantly lower when compared to the control samples.

The addition of 4% and 6% oat bran and buckwheat flour had a positive effect on the growth and survival of LA 5[®] in dairy fermented products that are preserved for

long time in refrigeration conditions. Therefore it could be considered that these products play prebiotic and protective role on lactic acid bacteria cells and stimulate bacteria to well perform *in vivo* and *in vitro* (Shah, 2000a and 2000b; Mortazavian *et al.*, 2007).

The effect of buckwheat flour and oat bran as prebiotics, on the production of fiber-enriched fermented milks, was previously demonstrated by Coman *et al.* (2013) by investigating the kinetics of acidification using as starter a co-culture of *Lactobacillus rhamnosus* IMC 501[®] and *Lactobacillus paracasei* IMC 502[®] (ratio of 1:1; combination named SYN BIO[®]). These authors demonstrated that the supplementation of whole milk with the two vegetable substrates induced a significant decrease of the pH that reached the value of 4.6, in a significantly shorter fermentation time, and thus suggesting that the fermentation process was significantly influenced by buckwheat flour and oat bran. Also, an enhancement of the stability of *L. rhamnosus* IMC 501[®] and *L. paracasei* IMC 502[®] was recorded during storage at 4°C for 28 days (Coman *et al.*, 2013).

Gupta *et al.* (2010) showed that the concentrations of oats and sugar are the main factors that have the greatest influence on the growth and survival of *Lactobacillus plantarum*. Under refrigeration conditions, the shelf life of the synbiotic product based on oat bran was estimated to be 21 days, when a reduction of less than 1 log CFU/mL was observed. That explains the protective effect of the vegetal substrate.

Conclusions

When developing new probiotic foods, one should take into consideration not only the biotechnological properties of starter strains, but also the ability of the food matrices to provide good environmental conditions for bacteria, as well as to protect the cells for high survivability upon the gastrointestinal tract passage.

The results demonstrate that the buckwheat flour and oat bran can act as stimulating and protective additives for *Lactobacillus acidophilus* (LA 5[®]) strain, hence having a positive effect on bacteria physiology and also for the nutritional and functional quality of the fermented products.

This study gives new perspectives to obtain synbiotic products based on buckwheat flour or oat bran, through mixing prebiotic and probiotic ingredients in different food matrices, in order to provide *in vitro* and *in vivo* protective effect for beneficial microorganisms.

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