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Research Paper

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Stability of *Lactobacillus Plantarum* from Functional Beverage Based Sprouted Buckwheat in the Conditions Simulating in the Upper Gastrointestinal Tract

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ABSTRACT

Buckwheat (Fagopyrum esculentum Moench) is very rich in antioxidants, especially rutin. Buckwheat extract enriched with prebiotics and fermented with probiotic culture, namely Lactobacillus plantarum, becomes a functional drink with multiple beneficial effects on health, such as dyslipidemia, cardiovascular disease, obesity. This study follows the evolution of the number of viable cells of Lactobacillus plantarum in the fermented drink based on buckwheat extract. The evolution is followed under in-vitro simulated gastrointestinal conditions. The results of this study show a decrease by 0.32 logCFU/mL after the passage through the stomach, at pH 2. Under the action of pancreatic juice and bile salts, the number of cells of Lactobacillus plantarum will drop by 0.66 log CFU/mL. This relatively small drop may be due to either the prebiotics (buckwheat honey, inulin), or to the increased viscosity of the drink after inulin was added. These results demonstrate that due to the fermented drink composition, the number of viable cells of Lactobacillus plantarum remains at a high level even after having navigated the gastric and duodenal route.

KEYWORDS: Sprouted Buckwheat, Lactobacillus plantarum, in-vitro simulation, simulated gastric & intestinal condition

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is a pseudocereal rich in protein, carbohydrates, fibre, vitamins, minerals and polyphenols. What distinguishes it from other cereals is the fact that its composition includes an antioxidant, rutin (quercetin-3-rutinosid), which has the role of reducing capillary permeability and the risk of atherosclerosis (Vojtiskova, 2012). By sprouting, the content of phenolic compounds multiplies by 20 or even 25.

According to the FAO/WHO (2002), probiotics are defined as microorganisms which, when administered in adequate amounts, bring benefits to the host-organism. These probiotics are lactobacilli, bifidobacteria and non-pathogenic yeasts.

Research has shown that supplementation with probiotics such as *Lactobacillus* or *Bifidobacteria* gives significant body benefits: modulation of the immune system, prevention of gastrointestinal diseases and production of bacteriocins, vitamin synthesis and an increase in the bioavailability of minerals, as well as an ability to maintain intestinal homeostasis. In the probiotic choice, there are some prerequisites: survival of lactobacilli when passing through the gastrointestinal tract and their adhesion to the intestinal epithelium cells in order to prevent their elimination due to peristalsis. At the same time, it has to be decided whether they can achieve colonization.

Most international organisations (Codex Alimentarius, International Federation of Yoghurt, and The Standard Code of Food in Australia) recommend a minimum standard of 10^7 UFC/mL of live probiotic cells at the time of ingestion. The Association of Manufacturers of Drinks and Dairy Products in Japan recommend a concentration of living cells no lower than 10^8 ufc/mL, to ensure that it can offset reductions which were due to passage through gastrointestinal tract.

Lactobacillus plantarum is an optional heterofermentative species, with a relatively large genome, which indicates its ability to adapt to many different conditions (Kleerebezem, 2003). It includes two enzymes: PAD (phenolic acid decarboxylase) transforms the phenolic acids into their derived compounds: vinyl and phenylpropionic acids and tannase which it has able to metabolise phenolic acids. In recent years, research has demonstrated that *Lactobacillus plantarum* has the ability to reduce the size of adipocytes along with lowering the concentration of cholesterol and leptin and increases the concentration of adiponectin (Takemura, 2010), also, it reduces gastrointestinal symptoms during treatment with antibiotic (Lonnemrk, 2000).

This study follows the evolution of the number of viable cells of *Lactobacillus plantarum* in a fermented beverage based on sprouts buckwheat extract under in-vitro simulated gastrointestinal conditions.

2. Materials and methods

2.1. In order to obtain the functional beverage, the following are needed:

- Extract from buckwheat, germinated for 7 days;
- Starter Culture of *Lactobacillus plantarum*, 60 Vege Start by Christian Hansen, Denmark;
- Inulin, from Enzymes and Derivatives Company, Costica, Neamt, Romania
- Buckwheat honey, bought from a company in the USA.

The fermentation was achieved at a temperature of 30 °C, while the pH value did not go beyond 4.6.

2.2 Methods

2.2.1. Viable cell counts

Viable cell counts were determined by the standard plate method with MRS medium. Dilution of 1 ml broth was carried out in 9 ml NaCl 0.9% to plate the suitable dilution. The plates were incubated at 37^0 C for 48 h for cell enumeration.

2.2.2. The viability of *Lactobacillus plantarum* during simulated gastric and intestinal conditions

Simulated gastric and intestinal juices were prepared using the method Kos (2000). Simulated gastric juice was obtained by creating a suspension of pepsin (3 g/L) in a sterile solution of NaCl 0.5%. The pH was scaled to 2 with the help of HCl concentrate.

Simulated intestinal juice was obtained by suspending pancreatin (1 g/L) and bile salts (0.45 g/L oxigall) in a sterile solution of NaCl 0.5%. The pH was adjusted to 8 using 0.1 M NaOH solution.

The functional beverage based on germinated buckwheat (0.2 mL) was mixed with gastric and intestinal juice (1mL) and with 0.3 mL of 0.5% NaCl solution. It was then incubated at 37^0 C, 50 rpm (Kos, 2000). Changes in viability of *Lactobacillus plantarum* were monitored during gastric and intestinal treatment.

Because gastric chymus is released, at the rate of 2-3 kcal/min (Brener, 1983), it is considered that 100 ml of beverage (caloric value 41,65 kcal/100 ml) will reach the duodenum within 30 minutes and the intestinal passage will take 90 minutes.

How cell viability was calculated:

$V (\%) = (N_t / N_0) * 100$

V(%)=Cell viability

N_0= number of viable cells upon exposure

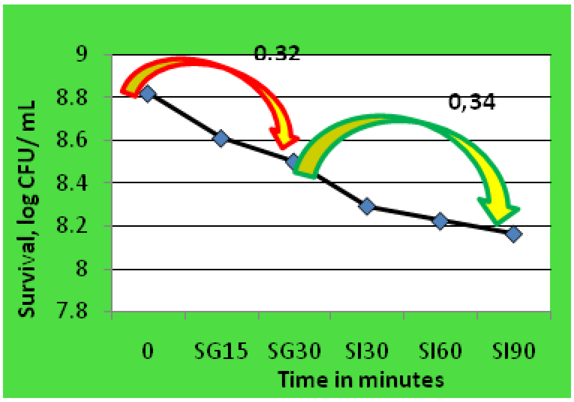
N_t = number of viable cells at moment t

3. Results and discussion

Table 1. Correspondence between the transit and the number of viable cells (log UFC/mL)

Zone	Time, min	Log UFC/mL
	0	8.82
Gastric (SG)	15	8.65
	30	8.50
Intestinal (SI)	30	8.29
	60	8.22
	90	8.16

Fig1. Correspondence between the transit and the number of viable cells (log UFC/mL)



Viability in the gastric area is 96,4%, in the duodenal area it is 96%, so that the overall viability is 92%. It is noted also that after passage through the stomach, only 16 log CFU/mL is left, which means the beverage keeps its probiotic properties.

Table 2.
Comparative study with other functional beverages

Beverage, culter starter	SG	SI	Author
Sprouted buckwheat based beverage, *Lb plantarum*	(30 min) 96.4%	(90 min) 96%	Brajdes et al,2013
Soy-based beverage, *Lb plantarum*	(30min) 94.5-99.3 %	(240min) 72.92-94.77 %	Lapsiri et al, 2012
Sesame based drinks, *Lb plantarum*	(30min) 92.3-99.19 %	(240min) 77.42-99.3 %	Lapsiri et al, 2012
Soy-based beverage, *Lb paracasei, Bifidobacterium longum*	(90 min) 98 %	98 (min) 98%	Cho,J., Y., 2012
Soy-based beverage and syrup of buckthorn, 5-10%, *Bifidobacterium bifidus*	86.85-80.85 (30 min)	81.31-85.22 (240 min)	Maftei et al, 2012

The factors which influence the viability of *Lactobacillus plantarum* cells under in-vitro simulated gastrointestinal conditions are represented by starter cultures (*Lactobacillus plantarum*), the extract from germinated buckwheat, buckwheat honey, inulin and the existing antioxidants.

The ability of *Lactobacillus plantarum* cells to withstand gastrointestinal conditions is due to an intrinsic resistance (Kashket, 1987), which is, in turn, accounted for by its large genome.

The sprouted buckwheat, the content of sugars and free nitrogen will be the energy sources for cells of *Lactobacillus plantarum* in its transit. Michida (2006) showed in his studies that malt extract shows better efficiency on the viability of *Lactobacillus plantarum* cells in vitro simulation system of gastrointestinal conditions than cereal extract.

In their studies, Nazzaro (2012) and Buriti (2010) showed that inulin induces better stress resistance to the cells of *Lactobacillus plantarum*, as compared to glucose. The adhesion of probiotics to the surface of enterocytes and of mucosal cells through self-assembly and co-aggregation is ten times higher in the presence of inulin than in the presence of glucose (Nazzaro, 2012) or honey (Saran et al, 2012). Moreover, autolysis of *Lactobacillus plantarum* cells is lower in the presence of inulin than in the presence of honey (Saran et al, 2012). Note that the combination of inulin with honey creates a synergistic effect in protecting the viability of *Lactobacillus plantarum* cells.

Lactobacillus plantarum, because of the two enzymes, tannase and decarboxylase, can decompose the tannic acid into gallic acid and glucose, and gallic acid into pyrogallol, which is used in the production of energy (Rodríguez, 2008). Pyrogallol is an antioxidant substance that can induce apoptosis in several types of cells mediated by superoxide anion (Han, 2009). Not only do phenol compounds influence positively the metabolism of bacteria, but they can also increase the consumption of nutrients, such as sugars (García-Ruiz, 2008). Hernandez (2011) suggests that polyphenols may exert prebiotic action, because the polyphenols are associated with dietary fibres.

3. Conclusions

Owing to its composition, the functional beverage based on sprouted buckwheat maintains its probiotic properties along the entire gastrointestinal route and may produce beneficial effects on health.

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