Effect Of Inulin Suplementation Of Milk To Prepare Fermented Biomilks

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Actually, functional fermented milks have to exhibit multiple benefits for the health, associated to good organoleptic characteristics. These are strengthened by the addition of probiotics as well as certain soluble fibers, among which inulin. The aim of this work was the evaluation of the effect of inulin supplementation of milk basis on the production of probiotic fiber-enriched fermented milks. To this purpose, we investigated the kinetics of acidification of inulin supplementation of milk (0, 1, 2 and 4 g/100 g) throughout the fermentation, the probiotic survival, the post-acidification and the firmness of fermented fiber-enriched milks, stored at 4°C either for 24 h or after 7 days since their preparation. This work shows that the biomilk preparation is influenced either by the supplemented amount of inulin or by the co-culture composition. According to the selected co-culture, inulin addition to the milk influenced the kinetic parameters of acidification, the concentration of probiotics, the post-acidification and the firmness of fermented fiber-enriched milk.

1. Introduction

Nowadays, consumers are demanding for foods with increasingly properties, such as pleasant flavor, low-calorie value or low fat content, and benefic health effects. Within this context, food industry has been trying to offer products with improve flavor and appearance. In addition, functional dairy products offer requirements, benefits to health that are strengthened by the addition of probiotics as well as by certain types of soluble fibers known as prebiotic.

Prebiotics are non-digestible food components that beneficially act on the host because they selectively stimulate either the proliferation or the activity of bacterial populations that are desirable in the colon. Moreover, prebiotics may inhibit pathogen multiplication, ensuring additional benefits to host's health. Such components mostly

act on the large intestine, even though they can also impact the microorganisms inside the small one (Roberfroid 2000; Mattila-Sandholm et al. 2002).

Lactic-acid bacteria (LAB) isolated from humans' and animals' gastrointestinal tract is known as probiotics. These organisms, when used in large amounts in the preparation of foods and dairy products, should survive the passage through the upper digestive tract. Besides, probiotic microorganisms should be able to adhere to intestinal cells, providing beneficial effects in the intestinal tract (Gilliland 1989). When such organisms are largely consumed, they help in the intestinal balance (Ferreira 2003). In order to produce the desired benefits, probiotics bacteria should be present in the products in viable counts during their whole shelf-life.

To this purpose, we investigated throughout the fermentation by *Streptococcus thermophilus* in co-culture with *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus, Lactobacillus rhamnosus and Bifidobacterium animalis* subsp. *Lactis* the kinetics of acidification of inulin supplementation of milk (1, 2 and 4 g. 100 g^{-1}), the probiotic survival, the post-acidification and the firmness of fermented fiber-enriched milks, stored at 4°C either for 24 h or after 7 days since their preparation.

2. Material and Methods

2.1 Experimental Procedure

Five strains of pure commercial *starter* freeze-dried cultures (Danisco, Sassenage, France) were used: *Streptococcus thermophilus* TA040 (St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB340 (Lb) (yogurt microorganisms) and *Lactobacillus acidophilus* LAC4 (La), *Lactobacillus rhamnosus* LBA (Lr), and *Bifidobacterium animalis* subsp. *lactis* BL 04 (Bl) (probiotics microorganisms). Pre-cultures were prepared in which bacteria average counts in the different pre-cultures ranged from 6.1 to 6.5 LogCFU.mL^{-1}.

Milk prepared adding 13 g of skim powder milk (Molico, Nestlé, Araçatuba, Brazil) in 100 g of distilled water (M) was supplemented with inulin (Beneo TM, ORAFTI Active Food Ingredients, Oreye, Belgium), specifically 1 (SM1), 2 (SM2) and 4 (SM4) g.$100g^{-1}$, and them thermally treated at 90°C for 5 min in water bath (550 THE, Fisatom, São Paulo, Brazil). The heat treated milk was collected into 1.0 L sterile flasks, cooled in ice bath, distributed into 250 mL sterile Shott flasks inside laminar flow chamber, and stored at 4°C for 24 h, before using.

After inoculation, flask samples were transferred on water bath equipment assembled to a CINAC (*Cynetique d´acidification*, Ysebaert, Frépillon, France) system and, batch fermentations were performed at 42^{o}C up to pH 4.5 corresponding to the final fermentation time. Fermentations were carried out in duplicate and monitored using the CINAC system which allows to continuously measure and record of pH as well as to evaluate acidification rates throughout the run (Spinnler and Corrieu, 1989). From the collected data, the acidification rates (dpH/dt) were calculated as the time variation of pH and expressed as 10^{-3} pH units . min^{-1} (V_{max}). At the end of the incubation period, the following kinetic parameters were also calculated: (i) t_{max} (h): time in which the

maximum (V_{max}) acidification rates were reached and (ii) $t_{pH4.5}$(h): time to reach pH 4.5. Not supplemented milk (M) was used as control.

2.2 Milk Chemical Composition and Firmness Analyses

Physico-chemical analyses (fat, gross protein, lactose and total solids) were carried out in inulin-supplemented milk according to Schmidt-Hebbel (1956), A.O.A.C (1995) and INSTITUTO ADOLFO LUTZ (1985) methods, in triplicate. The results were expressed in g.100 g^{-1}. Fermented milk post-acidification was determined by pH measurement using a pHmeter model Q-400M1 (Quimis, São Paulo, Brazil). Acidity, expressed in percentage of lactic acid, was evaluated by titration using a 0.11N NaOH solution according to AOAC (1995) methodology No. 907.124. Fermented milk firmness was measured at 8ºC using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Godalming, United Kingdom). The operation conditions were: probe type acrylic P/25P (2.5 cm diameter), 10 mm penetration, and 10 mm s^{-1} speed). Firmness was expressed as the maximum penetration, in N.

2.3 Counts of Probiotic Bacteria

Bacterial enumerations were carried out either after 24h or after seven days since storage of fermented milks at 4ºC. One mL of sample was diluted with 9 mL of 1% sterile peptonated water. Afterwards, serial dilutions were done and bacteria were counted applying the in-depth platelet technique. All media were obtained from Merck (Darmstadt, Germany). St colonies were enumerated in M17 agar by aerobic incubation at 37 ºC for 48 h. Lb enumeration was carried out in MRS Agar medium with pH adjustment at 5.4 by acetic acid addition, and aerobic incubation at 37 ºC for 48 h. La and Lr were enumerated in the same medium with pH adjustment at 5.4 by acetic acid addition, and anaerobic incubation at 37 ºC for 72 h. Bl was enumerated as La and Lr without any pH adjustment (IDF 1996, 1997, 2003).

2.4 Statistical Analyses

Results were submitted to analyses of variance (ANOVA) using the Statistica Software 6.0. Mean values were compared using the Tukey test at $P<0.05$.

3. Results and Discussion

3.1 Milk Chemical composition

Fat, protein and solid contents ranged respectively from 0.03 to 0.06 g·100 g^{-1}, 4.00 to 4.10 g·100 g^{-1}, and 13.20 to 15.63 g·100 g^{-1}. No statistically significant difference was observed in fat and protein contents whereas those of total solids values were

statistically different. Lactose content ranged from 4.43 to 4.63 g·100 g^{-1}, with significant statistical differences when 4 g·100 g^{-1} of inulin was added.

3.2 Kinetics of Acidification

The fermentation times ($t_{pH4.5}$) ranged between 5.2 to 11.2 h. There was a statistically significant variation in this parameter, which was shorter in milks that received inulin as supplement mostly at 4 g.100 g^{-1}concentrations, whereas St-Lr cultures was strongly affected at such inulin level. However, for St-Lb (SM1), St-Lr (SM1 and SM2), and St-Bl (SM1) co-cultures, the fermentation times were close to those obtained at the other concentrations (Figure 1).

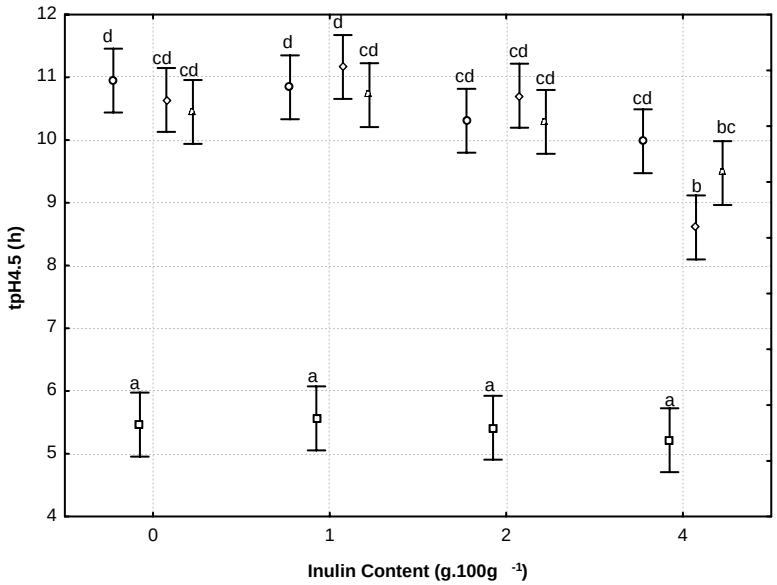

Fig. 1. *Effect of inulin addition to milk on the duration of fermentation ($tpH_{4.5}$) by Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, and Bifidobacterium lactis in co-culture with Streptococcus thermophilus. (o) St-La;(□) St-Lb; (◊) St-Lr; (Δ) St-Bl.*

Inulin influenced significantly the acidification kinetics of the milk for probiotics cultures. Maximum acidification rates (V_{max}) increased with inulin contents. Milk supplementation with 4 g.100 g^{-1} of inulin reduced the fermentation time of *S. thermophilus* and *L. rhamnosus* co-culture.

3.3 Post-Acidification and Acidity

pH values ranged from 4.32 (St-La/M) to 4.48 (St-Lr/M) after one day of fermentation (D1), and from 4.15 (St-La/SM4) and 4.37 (St-La/M) after seven days (D7). On

average, there was a 0.14 upH decrease after seven-day storage at 4°C. Using the St-Lb and St-La co-cultures, the post-acidification was stronger when using 4 g.100 g^{-1} of inulin, while there was a higher post-acidification of milk supplemented with 1 g.100 g^{-1} of inulin using St-Lr and St-Bl co-cultures. After one day of fermentation, the acidity ranged from 0.81 (St-Bl/M) to 1.03 g.100g^{-1} of lactic acid (St-La/SM2). Using the St-Lb co-culture the amount of lactic increased with inulin concentration in the formulation, but only the highest inulin concentration (4 g.100 g^{-1}) exhibited a statistically significant difference. Using the St-La co-culture the addition of 2 g.100 g^{-1} of inulin yielded the highest production of lactic acid compared to all tested co-cultures. Concerning St-Lr co-culture the highest production of lactic acid (0.94 g.100 g-1) was obtained using 1 g.100 g^{-1} of inulin. The lowest values of lactic acid were found using the St-Bl co-culture, and they ranged from 0.81 g.100 g-1 of lactic acid in Ml to 0.86 g.100 g-1 in SM1, with a statistically significant difference between them. After seven days of fermentation (D7), lactic acid production was always higher than after one day (D1), ranging from 0.90 g.100 g^{-1} (St-Lr/M) to 1.20 g.100 g^{-1} of lactic acid (St-La/SM2). Taken as an average, the increase in lactic acid production was 0.13 g.100 g^{-1}. The addition of inulin supported higher post-acidification for all investigated co-cultures.

3.4 Probiotic Bacteria Counts

The counts for *S. thermophilus* after 1 day of fermentation ranged from 8.67 (St-Bl) to 9.45 Log CFU.mL^{-1} (St-Lr) in SM1, without no statistically significant difference between the cultures. After 7 days of fermentation, counts ranged from 8.18 (St-Bl) to 9.42 Log CFU.mL^{-1} (St-Lr) in the same medium, with statistically significant differences. In average, the addition of 4g100 g^{-1} of inulin resulted in 0.40 log CFU.mL^{-1} decrease for La and 0.69 Log CFU.mL^{-1} for Lr.

After one day of fermentation, probiotic counts ranged from 7.37 (St-Lr/ M) to 9.13 Log CFU.mL^{-1} (St-Bl/SM4). There was a statistically higher growth of St-Lb in milks supplemented with inulin. Compared to the control the growth of La in milk supplemented with inulin was slightly higher, with a statistically significant difference. However, no appreciable variation between counts of Lr in milk supplemented or not with 1 g.100 g^{-1} of inulin was observed, whereas it became appreciable when 2 and 4 g.100 g^{-1} of inulin was added. Indeed, inulin showed a statistically significant *in vitro* bifidogenic effect, with higher counts in milks supplemented with inulin. After 7 days of fermentation he viability of *L. acidophilus* and *B. lactis*, probiotic bacteria, decreased by ~0.04 and ~0.15 Log CFU.mL^{-1}, respectively. However, counts remained stable for *L. rhamnosus* and *L. bulgaricus* cultures. Milk supplemented with inulin had a significant influence on the survival of these probiotic bacteria.

Probiotic counts were higher than 7.37 Log CFU.mL^{-1}. The inulin showed an *in vitro* bifidogenic effect, stimulating the growth of *B. lactis*; this effect was more pronounced using *B. lactis* than the other probiotic cultures. Counts remained almost stable after seven days of storage at 4°C.

3.5 Firmness

After one day of fermentation (D1), firmness ranged from 0.28 (St-Lb/M) to 0.47 N (St-Lr/SM4). At seven days (D7) it increased, on average, when compared to D1 by ~0.06 N, varying from 0.33 (St-Lb/M) to 0.54 N (St-Lr/SM4). Such results demonstrate that the supplementation of milk with inulin resulted in stronger firmness for all products and agree with those reported by MARTIN et al. (1999) and OLIVEIRA et al. (2001).

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