

Short Communication

Influence of fructooligosaccharides on the fermentation profile and viable counts in a symbiotic low fat milk

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Abstract

This study evaluated the effects of prebiotics on fermentation profile and growth of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, and *Bifidobacterium lactis* in co-cultures with *Streptococcus thermophilus*. Acidification rate and viability were positively influenced by the co-culture with *B. lactis* and by both inulin or oligofructose in low fat milk.

Key words: *Lactobacillus*, *Bifidobacterium*, Fructooligosaccharide, fermented milk.

In recent years, consumers are increasingly demanding foods with pleasant flavor, low-calorie value or low fat content, and beneficial health effects (Vasiljevic and Shah, 2008; Paseephol and Sherkat, 2009). Within this context, the food industry has been trying to offer products with improved flavor and appearance. In addition, functional dairy products offer requirements and benefits to the health that are strengthened by the addition of probiotics, as well as of certain types of soluble fibers, such as oligofructose, inulin and lactulose, known as prebiotics.

Inulin and oligofructose are common forms of prebiotics (Gibson and Roberfroid, 1995; Angus *et al.*, 2005). According to Gibson and Roberfroid (1995), prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health. Regarding technological applications, inulin is mostly applied to obtain products with low fat content, while oligofructoses are employed in low-calorie fruit preparations such as yoghurts, as well as to balance the sweetness and mask the high-intensity sweetener's residual flavor used in food preparations (Niness, 1999). In addition, Oliveira *et al.* (2011) reported that inulin has the additional advantage of improving the firmness of functional dairy fermented products.

On the basis of this background, oligofructose and inulin appear as important food ingredients that should be additionally explored for the production of new functional dairy products. Therefore, the aim of the present study was to evaluate the effects of these prebiotics on the fermentation profiles and viable counts of co-cultures of *Lactobacillus acidophilus*, *L. bulgaricus*, *L. rhamnosus*, and *Bifidobacterium lactis* with *Streptococcus thermophilus*.

Streptococcus thermophilus TA040 (St), *Lactobacillus delbrueckii* subsp. *bulgaricus* LB340, *Lactobacillus acidophilus* LAC4 (La), *Lactobacillus rhamnosus* LBA (Lr), and *Bifidobacterium animalis* subsp. *lactis* BL 04 were used as commercial starter cultures (Danisco, Sassenage, France) in this study. All strains were inoculated in sufficient amounts to get initial counts of 10⁶ cfu/mL. Figure 1 represents a schematic flow chart of milk preparation and fermentation process. The skim milk (Castroni, Reggio Emilia, Italy) was supplemented with the prebiotics oligofructose (Orafti®P95) and inulin (Orafti®GR) (Orafti Active Food Ingredients, Oreye, Belgium). The maximum acidification rate (V_{max}) was calculated as the time variation of pH (dpH/dt) and expressed as 10⁻³ pH units/min. The time to reach pH 4.5 (T_f) was assumed as the time to complete the fermentation. Viable cell counts were made in triplicates after fermentation, as previously de-

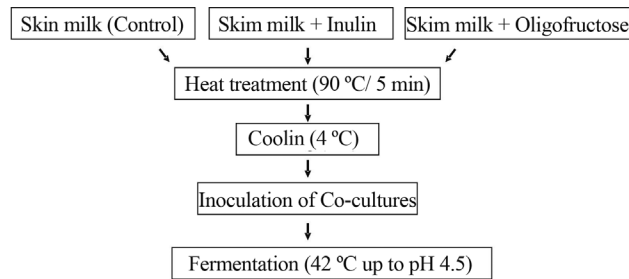


Figure 1 - Schematic flow chart of milk preparation and fermentation process.

scribed by Oliveira *et al.* (2009a). Samples (1.0 mL) were added to 9.0 mL of 0.1% sterile peptone water; then, appropriate dilutions were made, and each bacterium was counted in the three most appropriate dilutions, applying the pour plate technique. Subsequently, *S. thermophilus* was plated into M17 agar (Oxoid, Basingstoke, UK) and then submitted to aerobic incubation at 37 °C for 48 h. Enumeration of *L. bulgaricus* was carried out in MRS agar (Oxoid), acidified to 5.4 with acetic acid, followed by aerobic incubation at 37 °C for 48 h. *L. acidophilus* and *L. rhamnosus* were grown anaerobically at 37 °C for 72 h in MRS agar (Oxoid). *B. lactis* was counted in MRS agar containing 50 g/L cysteine, after jar anaerobic incubation at 37 °C for 72 h. Anaerobic conditions were ensured by the use of AnaeroGen (Oxoid). Variations with respect to the mean values were presented as standard deviations. Mean values of these parameters were submitted to analysis of variance (ANOVA), using the Statistica Software 6.0

(StatSoft, Tulsa, USA). They were compared using the Tukey test at significance level $p < 0.05$.

Table 1 shows the results obtained for fermentations of low fat milk as such (control) or supplemented with 40 mg/g of inulin or oligofructose by strains *L. bulgaricus* St-Lb, *L. acidophilus* St-La, *L. rhamnosus* St-Lr or *B. lactis* St-BI in binary co-cultures with *S. thermophilus*.

V_{\max} ranged from $17.06 \cdot 10^{-3}$ upH/min (St-Lr in the control) to $23.17 \cdot 10^{-3}$ upH/min (St-La in milk supplemented with oligofructose). In the presence of oligofructose and inulin, the average V_{\max} values obtained with all the co-cultures were 12.2% and 6.4% higher than in the control, respectively, and approximately 15% higher than those obtained in previous study (Oliveira *et al.*, 2009a) both in the presence and in the absence of inulin, probably due to the presence of 0.5% fat in the milk used in this study. Recent studies reported that fat components present in milk, like conjugated linoleic acid, provided functional characteristics (Oliveira *et al.*, 2009b; Rodrigues *et al.*, 2011). Probably, this component may have positively influenced the fermentation profile and growth of probiotic bacteria.

It should be noted that the time to complete fermentation (T_f) ranged from 4.2 h (St-Lb in the presence of oligofructose) to 11.3 h (St-Lr in the control), which means that the fermentation profile depended, not only on the interactions between St and the others microorganisms, but also on the type of prebiotic supplemented. In the presence of oligofructose, the average time to complete fermentation by all the binary co-cultures was in fact approximately 21%

Table 1 - Maximum acidification rate (V_{\max}), time to reach pH 4.5 (T_f), and viable cell counts in fermentations of low fat milk (M), supplemented with 40 mg/g inulin (M+I) or oligofructose (M+O), by *S. thermophilus* in co-culture with *L. bulgaricus* (St-Lb), *L. acidophilus* (St-La), *L. rhamnosus* (St-Lr) or *B. lactis* (St-BI)

Milk	Co-culture	V_{\max} (10^{-3} pH units/min)	T_f (h)	Viable cell counts (log cfu/mL*)
M	St-Lb	20.880.25 ^a	4.39 ± 0.12 ^c	7.41 ± 0.21 ^c
M	St-La	19.47 ± 0.31 ^b	10.33 ± 0.21 ^k	7.35 ± 0.06 ^b
M	St-Lr	17.06 ± 0.25 ^b	11.31 ± 0.08 ^l	7.03 ± 0.24 ^a
M	St-BI	18.91 ± 0.19 ^c	8.99 ± 0.18 ⁱ	7.67 ± 0.13 ^h
M+I	St-Lb	20.55 ± 0.25 ^h	4.26 ± 0.20 ^b	7.55 ± 0.12 ^c
M+I	St-La	21.35 ± 0.24 ^j	8.55 ± 0.14 ^h	7.78 ± 0.07 ^g
M+I	St-Lr	19.52 ± 0.31 ^c	9.03 ± 0.24 ^j	7.88 ± 0.11 ^d
M+I	St-BI	19.77 ± 0.18 ^f	8.41 ± 0.15 ^g	8.55 ± 0.19 ^k
M+O	St-Lb	21.22 ± 0.19 ⁱ	4.19 ± 0.20 ^a	7.45 ± 0.12 ^f
M+O	St-La	23.17 ± 0.22 ^k	7.44 ± 0.08 ^d	7.66 ± 0.07 ⁱ
M+O	St-Lr	20.36 ± 0.31 ^g	8.13 ± 0.11 ^c	7.44 ± 0.11 ^j
M+O	St-BI	20.88 ± 0.22 ^a	7.99 ± 0.11 ^f	8.11 ± 0.19 ^j

Means ($n = 3$) ± standard deviation with different letters in the same column are significantly different ($p < 0.05$).

*Counts of *L. bulgaricus* (St-Lb), *L. acidophilus* (St-La), *L. rhamnosus* (St-Lr) or *B. lactis* (St-BI), according to the microorganism employed along with *S. thermophilus*.

and 8% shorter than in the control and in the presence of inulin, respectively (Table 1). Such an effect demonstrates that both oligofructose and inulin stimulated the metabolism of the binary co-cultures. Moreover, it is noteworthy that the use of these prebiotics decreased, in general, the fermentation time of all the binary co-cultures, mainly of St-Lr, for which the addition of inulin and oligofructose decreased T_f by no less than 20% and 28%, respectively, when compared to the control. This last result confirms that the combination of St-Lr and prebiotics could be satisfactorily exploited in the dairy industry (Tannock *et al.*, 2000; Capela *et al.*, 2006; Rodríguez-Alcalá *et al.*, 2011).

Of the two prebiotics, oligofructose accelerated the acidification more than inulin did, likely because its polymerization degree is much lower than that of inulin (Angus *et al.*, 2005), which made its partial hydrolysis quicker.

Table 1 shows the counts after 1 day-storage at 4 °C since the end of fermentations of co-cultures of *S. thermophilus* with either Lb or the selected probiotics, both in the presence and in the absence of oligofructose or inulin. Viable St counts (results not shown) were not significantly influenced by the type of prebiotic or probiotic used, varying only between 8.9 and 9.1 log cfu/mL. On the other hand, significant differences were observed for viable counts of the probiotic bacteria, which were strongly influenced by the presence of FOS as well ($p < 0.05$). As expected by the above considerations, the mean probiotics viable counts were 7.8% and 4.1% higher than in the control, respectively, in the presence of oligofructose and inulin. The influence of FOS on probiotic survival is consistent with the observations of several authors, who observed a clear beneficial action of these prebiotics on the viability of *L. rhamnosus*, *L. acidophilus*, and *Bifidobacterium* spp. in fermented milk and other dairy products (Dave and Shah, 1996; Oliveira *et al.*, 2009a; Paseephol and Sherkat, 2009).

Finally, St-BI was the binary co-culture most stimulated by the presence of inulin and oligofructose; it did, in fact, show average viable counts 6% and 12% higher, respectively, than in the control. These results demonstrate that the interaction between pre- and probiotics was positive, according to the definition proposed by Gibson and Roberfroid (1995).

According to Angus *et al.* (2005), both inulin and oligofructose promote the growth of bifidobacteria in absolute terms. Briefly, fructose released from their partial hydrolysis catalyzed by inulinase may be metabolized as an additional carbon and energy source (Mayo *et al.*, 2010). In the presence of external oligosaccharides and polysaccharides like inulin or oligofructose, bifidobacteria are, in fact, able to uptake the monomers from their hydrolysis through the fructose-6-phosphate shunt (McKellar and Modler, 1989; Tharmaraj and Shah, 2003; van der Meulen *et al.*, 2006).

In general, these results as a whole not only confirm St-Lb as the best co-culture for yoghurt preparation, but

also suggest that the alternative probiotics employed in this study (La, Lr, and BI) would be able to ensure satisfactory results in terms of fermentation profile. Moreover, oligofructose and to some extent also inulin were shown to significantly accelerate either acidification or fermentation, thus proving to be interesting prebiotics to improve the production of functional dairy products.

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