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The influence of fiber addition and various probiotic strains on bacterial viability, macroelements bioavailability, and the quality of sheep milk ice cream

Summary:

Sheep milk-based products are at the forefront of functional foods due to their nutritional and anti-allergenic properties and could be a suitable carrier of probiotic bacteria and prebiotics. Probiotic bacteria of the genus *Lactobacillus* and *Bifidobacterium* are most commonly used in food production. Adding prebiotics to dairy products, such as ice cream made from sheep's milk, is an excellent alternative to fiber supplementation in recommended doses, increasing their nutritional value and improving product quality parameters.

The purpose of this study was to determine the possibility of using five various probiotic strains (*Bifidobacterium animalis* ssp. *lactis* BB-12, *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus paracasei* L-26, *Lacticaseibacillus casei* 431, *Lactobacillus acidophilus* LA-5) in the production of ice cream from sheep's milk with the addition of inulin and apple fiber. The physicochemical properties of ice cream mixtures directly after conditioning and ice cream after 7 and 21 days of frozen storage were also determined. Additionally, the research aimed to assess the bioavailability of macroelements (calcium, magnesium, potassium, and phosphorus) and the survival rate of probiotic strains under simulated *in vitro* digestion conditions.

Replacing 1.5% apple fiber with inulin reduced the pH value of the ice cream mixtures even prior to fermentation, compared to samples containing 4% inulin. Following fermentation, the ice cream mixtures with apple fiber exhibited a significantly lower pH value, increased darkening, and a higher proportion of red color compared to mixtures containing only inulin. This study highlights a crucial issue in selecting probiotic bacteria for fermenting sheep's milk mixtures, as the partial replacement of inulin with apple fiber alters the fermentation conditions. Furthermore, the mixture with the addition of 1.5% apple fiber demonstrated a lower count of *L. casei* cells after fermentation. Conversely, adding apple fiber significantly increased the number of L. acidophilus cells in the mixture after fermentation. However, adding 1.5% apple fiber did not significantly affect the growth of *B. animalis*, *L. paracasei*, and *L. rhamnosus*. The freezing process reduced the live cell population of probiotic bacteria in all ice cream groups, except in the ice cream containing *L. rhamnosus*, where no significant reduction in bacterial population was observed immediately after freezing. The probiotic strain used for fermentation affected the ice cream's pH value, lactic acid content, color components, overrun, and organoleptic characteristics. There were no significant differences in the lactic acid content between ice cream with inulin and ice cream with apple fiber, regardless of storage duration. Extending the storage time from 7 to 21 days increased the brightness (L* value) across all ice cream groups. Adding fiber enhanced the ice cream's overrun compared to those made with inulin. In all groups of ice cream, an increase in storage time

led to a significant reduction in the first drop melting time. The total melting time also decreased with more extended storage periods. After 7 days of frozen storage at -22°C, the number of viable probiotic bacterial cells in all ice cream groups decreased compared to the cell count immediately after freezing. Extending the storage time from 7 to 21 days did not significantly impact the number of probiotic cells in any ice cream group. The *Lacticaseibacillus paracasei* strain exhibited the highest survival rate during storage, suggesting its suitability to produce probiotic ice cream from sheep's milk. The addition of apple fiber had noticeable effects on the visual characteristics of sheep's milk ice cream. Ice cream containing fiber exhibited a darker hue, a more apparent red coloration, and a less smooth texture with increased sandiness compared to ice cream solely containing inulin. Evaluation of organoleptic attributes revealed no significant influence of storage duration on these parameters. Analysis of the sheep's milk ice cream indicated a highly favorable calcium-to-phosphorus ratio ranging from 1.28:1 to 1.32:1, surpassing the recommended ratio of 1:1 or 1.5:1, suggesting its potential as an excellent calcium source. The highest calcium bioavailability, contingent on the strains of probiotic bacteria utilized, was observed in ice cream fermented by *Lacticaseibacillus paracasei*. Conversely, ice cream fermented with *Bifidobacterium animalis* ssp. lactis exhibited the highest phosphorus bioavailability. Additionally, ice cream fermented with *Lacticaseibacillus casei* demonstrated the highest magnesium bioavailability. However, the bacterial strain used in ice cream production did not influence the bioavailability of potassium. The addition of fiber limited the bioavailability of calcium and magnesium in sheep's milk ice cream. The reduction in the bioavailability of these macronutrients depended on the type of fiber, with the addition of 4% apple fiber reducing bioavailability the most by 6-12%. Adding 4% inulin reduced the bioavailability of calcium by about 3-5%, and adding 2.5% inulin with 1.5% apple fiber reduced the bioavailability of calcium by 4-8%. In contrast, the addition of 4% apple fiber did not significantly affect the bioavailability of phosphorus in ice cream. In contrast, the bioavailability of potassium was higher in ice cream without additives than in ice cream with fiber. A study of the effect of the strain used on the survival rate of probiotics under in vitro digestion conditions compared to the number of cells before digestion indicated that *Lactobacillus acidophilus* had the highest survival rate (>80%) in ice cream without added fiber. The lowest survival rate (<50%) was shown for the *Lacticaseibacillus rhamnosus* strain. The survival rate of *Lacticaseibacillus paracasei, Lacticaseibacillus casei, Bifidobacterium animalis* ssp. *lactis* strains exceeded 50%, compared to the number of bacterial cells in the ice cream before digestion. Using inulin and apple fiber in ice cream production influenced the survival rate of probiotic strains in the simulated gastrointestinal tract. The addition of 4% inulin increased the survival rate of *Lacticaseibacillus rhamnosus* by about 10% and the *Bifidobacterium animalis* ssp. *lactis* strain by about 22% compared to the survival rate in ice cream without the additive. In contrast, adding only apple fiber improved the survival of *Bifidobacterium animalis* ssp. *lactis* under in vitro digestion conditions by about 6%. In the case of *Lacticaseibacillus casei, Lacticaseibacillus paracasei* and *Lactobacillus acidophilus*, adding 4% apple fiber increased survival under gastrointestinal passage conditions by 5.7-11.2%.

It is challenging to identify a single strain whose use in ice cream production would be most beneficial for reasons of both technological and macronutrient bioavailability and survival in the

gastrointestinal tract. Considering the technological process (fermentation, freezing, frozen storage), the most preferred strain would be *Lacticaseibacillus rhamnosus*. However, the simulated digestion studies indicate that the *Lacticaseibacillus acidophilus* strain provides a therapeutic effect due to its very good survival rate in the gastrointestinal tract. The research results can be used to design new functional dairy products that combine probiotic benefits with increased prebiotic content. Studies of synbiotic ice cream from sheep's milk can be a good source for supplementing macronutrient deficiencies in the daily human diet.