SENSORY AND CHEMICAL ASPECTS OF FROZEN SOY YOGURT FERMENTED WITH Enterococcus faecium AND Lactobacillus jugurti

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ABSTRACT: As recent studies have demonstrated the beneficial effects to health of soy yogurt fermented with E. faecium and L. jugurti, aim to offer new options of consumption or this product, the goal of this work was to present the processing of frozen soy yogurt and the evaluation of its sensory characteristics. Then compare it with the other ice creams regularly processed with cow and soymilk fermented with a mixed culture of L. delbrueckii ssp. bulgaricus and S. thermophilus and also with the non-fermented ice creams acidified with the addition of lactic acid. The effect of the lactic acid bacteria in the oxidation of the product was also evaluated. The results demonstrated that it is possible to have a frozen soy yogurt fermented with E. faecium and L. jugurti with good sensory characteristics up to a period of 180 days. The lactic bacteria were not able to stop the development of the oxidation process for a long period of storage but it did not alter the sensory characteristics of the product.

KEYWORDS: Frozen soy yogurt; Enterococcus faecium; sensory analysis; chemical analysis.

Introduction

The presence of frozen yogurt in the market is increasing those days not only because it is good for health due to its processing with probiotic bacteria but also because it presents the opportunity of substituting the regular high fat content ice cream12.

There are no international identity patterns for frozen yogurt although some countries have already defined some conditions for the product, that is, a minimum of tritatable acidity5.

Most of the manufacturers of frozen yogurt say that it must always have a very important element: the viable lactic acid bacteria2,3,20. However, not all the lactic bacteria found in the fermented products are probiotic. Fuller6 said that probiotics are products that have microorganisms that brings benefits to the user as they improve the balance and the properties of the native flora, are not destroyed by the gastrointestinal tract and remain in the intestines. Based on this concept, yogurts are not considered probiotic although they have demonstrated a great number of auxiliary properties to prevent and fight against some health disorders.

The relatively high costs of the milk-fermented products have restrained significantly their consumption/use and consequently the benefits they may bring to the population. Soymilk, as a powerful source of proteins, has been seen as an important substitute for cow milk in the processing of fermented products.

Recent studies related to soy fermentation have searched for new bacteria cultures, especially probiotic, in order to improve the product with especial characteristics. Enterococcus faecium, then, has been studied and tested mainly because of its capacity of reducing the seric cholesterol levels14,16 and of stimulating the immunological system13.

Trying to show the market a new option for the regular soy yogurt, this work presents the processing of a probiotic type of frozen soy yogurt fermented with Enterococcus faecium and Lactobacillus jugurti and evaluates its sensory characteristics in relation to other frozen yogurts processed with fermented and non fermented regular cow milk and soy milk with mixed cultures of L.delbrueckii ssp bulgaricus and S. thermophilus. It was also important to evaluate the effect of the lactic bacteria in the oxidation process of the product during the storage period once some studies have demonstrated that some lactic acid bacteria are able to inhibit, up to a certain point, the oxidation of the frozen yogurt8,21.

Material and methods

Material

Samples of frozen soy yogurt fermented with Enterococcus faecium e Lactobacillus jugurti.

Experimental protocol

The frozen soy yogurt fermented with E. faecium
and *L. jugurtii* was processed once and compared to the following products:
- frozen yogurt fermented with *L. delbrueckii* ssp *bulgaricus* and *S. thermophilus* (standard product)
- frozen soy yogurt fermented with *L. delbrueckii* ssp *bulgaricus* + *S. thermophilus*
- non fermented frozen soy yogurt.
- non fermented frozen yogurt

The sensory evaluations were done soon after the processing of the products and after 90 and 180 days of storage. The degree of oxidation was determined at the beginning of the process and at every 30 days up to the last period of storage (180 days).

**Methods**

**Processing of the non fermented and fermented products**

Soy yogurt with the regular culture (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) was processed as described by Rossi et al. The traditional yogurt (milk with *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) was processed following Tamine & Robinson. For the processing of the non fermented products, all the contents of the yogurt were added to the soy and cow milk and the final mixture was acidified with lactic acid bacteria up to pH 4.3 (pH identical to the fermented products).

**The processing of the samples (ice creams)**

The solid ingredients were added to both the non fermented and fermented products, i.e., 5.5% of skim powder milk, 15.5% of sugar and 1% of stabilizer. Those mixtures were homogenized in a mechanical mixer (Metalurgica Visa-L025) for 5 minutes. After adding 7% of glucose, 6% of hydrogenated vegetal fat and 1% of emulsifier previously melted in water bath, they were taken to the freezer for one hour for the maturation development and finally taken to an ice-cream machine (R.Camargo) type PHD 80 – 100, without overrun control. The ice creams were put in a 2 L thermo package and stored at the temperature of 23°C up to the moment of the analyses.

**Counting of the viable cells**

The product cells were evaluate as for the number of lactic bacteria viable cells, every 30 days, using the M17 agar medium (Difco) for the growing of the coccus and MRS agar (Difco) for the growing of the bacillus.

**Sensory analysis**

A panel of 30 persons evaluated the flavor, appearance and overall perception of the products soon after the processing, in 90 and 180 days of storage. They were based on an unstructured 9cm hedonic scale, where extremes were the words “disliked very much” and “liked very much.” The results were submitted to a variance analysis (ANOVA) and to the Turkey media tests by means of the SAS statistical program (Statistical analysis system – V.6.12).

**Determination of the oxidation degree**

The oxidation was evaluated by the TBA test (thiobarbituric acid) according to Rosmin et al. with the following modifications: A mixture of 2g of sample, 5ml of TBA (0.02M) and 10ml of 10% TBA was centrifuged for 5 minutes at 3500 rpm, the supernatant was filtered in no.1 Whatman paper and an aliquot of 8ml of the filtrated was melted in boiling water bath for 35 minutes. The absorbance was measured in 532 nm after the color development.

**Results and discussion**

Gonzáles and Mattila said that a probiotic product must have, at least, $10^9$ UFC/g of the inoculate probiotic microorganism. As the objective of this work was to produce a probiotic frozen yogurt, the counting of the number of viable cells was done in the resulting ice creams from fermented products during all the storage period.

Figures 1, 2, and 3 show the results of the cell counting. During the storage period (180 days), a diminishing of 2 logarithms cycles occurred in relation to the first numbers of microorganisms and it indicates that the freezing has a certain injuring effect on the bacteria cell. In relation to the product 3 in particular, the object of study, the final countings were about $10^6$ UFC/g, for coccus as well as for bacillus, and their probiotic characteristics were preserved.

Davidson et al., while studying the viability of the probiotic cultures in samples of frozen yogurt, concluded that this product presents itself as an appropriate vehicle for the incorporation of probiotic bacteria once they have little or none negative effect during the storage period.

FIGURE 1 - Viability coccus and bacillus in product 1 (frozen yogurt fermented with *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*).
The sensory evaluations results of the product, realized in 3 times along the storage period, are presented in Table 1.

The first time, soon after the processing, it is possible to notice that the frozen soy yogurt non fermented (product 4) is significantly different from the others for the 3 attributes in questions, i.e., it is the one which presents the lowest evaluation. It is interesting to point out that product 3, the object of study, was from the sensory point of view so good as product 1 considered the reference pattern.

At 90 and 180 days of storage all the products had no significant differences concerning flavor, appearance and overall impression, which is the same as to say that frozen soy yogurt fermented with *E. faecium* and *L. jugurti* (product 3) is similar to the pattern product (product 1) in sensory terms.

When the analysis of the results of the 3 evaluations during the storage period is put together, it is possible to notice that not only the overall impression, the most relevant parameter, but also flavor and appearance are different only in product 4 in the initial evaluation and not in the other times.

As Davidson et al. have defined acidity is the most important attribute for taste of frozen yogurts, this fact can explain the behavior of all the samples, except number 4 at the first time, once the products have the same pH value (4.3). According to these authors, the informations about the effect of the probiotic cultures in the sensory quality of frozen yogurts are still very limited.

![Figure 2](image1.png)

**FIGURE 2** – Viability of coccus and bacillus in product 2 (frozen soy yogurt fermented with *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*).

![Figure 3](image2.png)

**FIGURE 3** – Viability of coccus and bacillus in product 3 (frozen soy yogurt fermented with *E. faecium* and *L. jugurti*).

<table>
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<th>Storage period (days)</th>
<th>Product</th>
<th>Appearance</th>
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<th>Overall Impres.</th>
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The average in equal letters in the same column are not significantly different between themselves (<p> 0.05), by the Turkey test.

1: frozen yogurt fermented with *L. delbrueckii* ssp. *bulgaricus* + *S. thermophilus*; 2: frozen soy yogurt fermented with *L. delbrueckii* ssp. *bulgaricus* + *S. thermophilus*; 3: frozen soy yogurt fermented with *E. faecium* + *L. jugurti*; 4: frozen soy yogurt with all the ingredients of the yogurt, acidified with the addition of lactic acid; 5: frozen yogurt with all the ingredients of the yogurt, acidified with the addition of lactic acid.

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As far as the sensory evaluation, it is interesting to notice the behavior of each of the products, during the storage period. None alteration was observed during the storage period in none of the products.

Some authors say that the activity of the lactic acid bacteria during the storage period generally cause chemical alterations, i.e., an increase of acidity that affects the sensory characteristics of the products. This situation did not happen in this present study, probably because the bacteria cultures presented, in the storage temperature of (-23C), a metabolism low enough not to promote the increase in acidity during the 180 days of storage.

Ashaye et al. said that freezing temperatures are more satisfactory than the others in the conservation of the sensory attributes of soy yogurt, such as flavor, taste and overall impression. In a way, it also applies to the fact that the samples did not suffer sensory alterations during the storage period.

An interesting aspect must be highlighted; flavor is one of the main factors that affect the ice creams quality and, consequently, the market sales. From this point of view, the acceptance of the resulting products, particularly number 3, can still be emphasized by means of the addition of a flavor once the samples were presented to the panel in natura in order not to disguise some eventual defects that could appear.

As far as oxidation is concerned, Figure 4 shows that more or less all the products presented higher concentrations of malondialdehyde in the storage period, stating an increase in oxidation. Such situation is not in accordance with the literature data that show that some lactic bacteria strains are able to inhibit the oxidation but the mechanism is not completely known.

Figure 4 also shows that the frozen soy yogurt fermented with L.delbreuckii ssp. bulgaricus and S. thermophilus (product 2) suffered less oxidation than the frozen yogurt fermented with the same microorganisms (product 1), what implies that soy is less sensitive to oxidation than cow milk. In conclusion, the culture of L.delbreucki ssp. bulgaricus and S. thermophilus was effective to inhibit the oxidation process of the frozen soy yogurt and not with the cow milk products. On the other hand, the culture with E. faecium and L. jugurti was less effective than the conventional yogurt culture to prevent the oxidation of the stored products under conditions of this study.

Comparing the results of the sensory analysis (Table 1) and those of the oxidation process (Figure 4), it possible to see that the increase in the concentration of malondialdehyde in the different products, through the storage period, was not sufficient enough to make significant alterations in the sensory characteristics evaluated so as to endanger their acceptance.

**Conclusion**

The results allow the conclusion that it is possible the making and processing of a probiotic frozen soy yogurt fermented with E. faecium and L. jugurti, with similar sensory characteristics of the reference product, i.e., the one processed with frozen yogurt fermented with a mix culture of L.delbreucki ssp. bulgaricus and S. thermophilus. As for the sensory aspects, all the products remained unaltered during the storage period, even though there was an increase in the concentrations malondialdehyde.

sorvete de “iogurte” de soja fermentado com *E. faecium* e *L. jugurti* com boas características tecnológicas e sensoriais, mantidas por um período de 180 dias. Foi também observado que as bactérias láticas não foram capazes de impedir o desenvolvimento do processo oxidativo ao longo do período de estocagem, embora tal fato não tenha sido causa de alterações nas características sensoriais dos mesmos.

PALAVRAS-CHAVE: Sorvete de iogurte de soja; análises sensoriais; análises físico-químicas.

References
