

## Monitoring shelf life of a whey-based beverage: proteolytic activity by *Bifidobacterium animalis* subsp. *lactis* BB-12<sup>R</sup>

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### RESUMEN

**Introducción.** Uno de los productos lácteos más consumidos alrededor del mundo es el queso, y en su proceso de elaboración se obtiene un residuo líquido por precipitación denominado suero, destacando a este producto por su mayor aporte nutricional y funcional, que el de la misma leche. La leche y sus derivados son considerados alimentos funcionales por sus aportes nutricionales, en especial los productos lácteos fermentados, ya que con este proceso se incrementan los beneficios a la salud que aportan los nutrientes naturales de la leche. Es por ello, que, al fermentar suero de leche con probióticos, se obtienen productos con alto valor funcional. **Materiales y Métodos.** En el presente trabajo se realizó una comparación del efecto proteolítico de *Bifidobacterium animalis* subsp. *lactis* BB-12<sup>R</sup>, durante su fermentación en suero de leche, y adicionalmente se monitoreó su acidez titulable y su pH, durante su vida en anaquel (21 días). **Resultados y discusión.** Con los resultados obtenidos del presente estudio, se demuestra que las bebidas a base mantuvieron los probióticos vivos, ya que se incrementó constantemente la acidez durante la vida en anaquel de las bebidas, adicionalmente, se observó que la actividad proteolítica fue variable dependiendo de las condiciones de fermentación.

**Palabras clave:** fermentación, proteólisis, queso.

### ABSTRACT

**Introduction.** One of the most consumed dairy products around the world is cheese, and in its production process a liquid residue is obtained by precipitation called whey, highlighting this product for its greater nutritional and functional contribution, than that of milk itself. Milk and its derivatives are considered functional foods due to their nutritional contributions, especially fermented dairy products, since this process increases the health benefits provided by the natural nutrients in milk. That is why, when fermenting whey with probiotics, products with high functional value are obtained. **Materials and methods.** In the present work, a comparison of the proteolytic effect of *Bifidobacterium animalis* subsp. *lactis* BB-12<sup>R</sup>, during its fermentation in whey, and additionally its titratable acidity and pH were monitored during its shelf life (21 days). **Results and Discussion.** With the results obtained from the present study, it is shown that the based drinks kept the probiotics alive, since the acidity was constantly increased during the shelf life of the drinks, additionally, it was observed that the proteolytic activity was variable depending on the conditions fermentation.

**Keywords:** cheese, fermentation, proteolysis.

**Área:** Lácteos.

## INTRODUCTION

Milk and milk products are a source of vitamins, minerals, lipids, and proteins of high biological value. For decades, fermented dairy products are considered beneficial foods for the health of human beings; this is due to a large part of the microorganisms involved in the fermentation and to the products released during this process (Alvarez-Rosales et al., 2019). The probiotics bacteria, such as *Bifidobacterium* spp. are very important in functional foods, due to their ability to acidify the food as well as to the positively influence in the sensory properties of dairy products, by means of the fermentation mainly the derivation of the hydrolysis of the proteins. That is why the proteolytic activity of the probiotics is very important for the quality of the dairy products, since it has favorable with the production of desirable volatile compounds (by enhancing the sensory properties), and additionally, with nutritional effects, (González et al., 2011), such as increasing the digestibility of milk proteins and increasing the production of bioactive peptides, well it is know that, he proteolytic activity of probiotics like *Bifidobacterium* spp., in the milk fermentation process produces bioactive peptides that provide additional benefits in consumer health (Alvarez-Rosales et al., 2019). For all the above, that when fermenting whey with probiotics such as *Bifidobacterium* spp., products with high functional value are obtained. The objective of the present experiment was to evaluate the proteolytic effect to whey fermentation by *Bifidobacterium animalis* subsp. *lactis* BB-12<sup>R</sup>, during self-life (21 days).

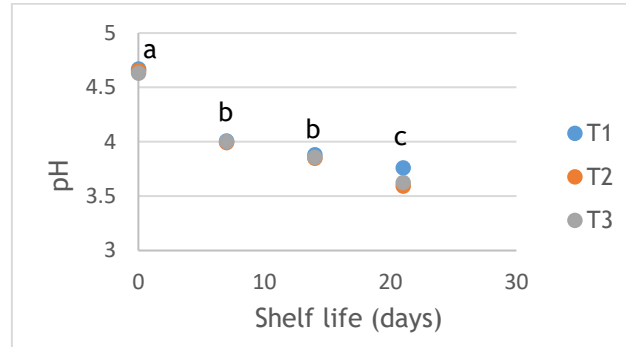
## MATERIALS Y METHODS

The treatments were whey-based beverages were evaluated during the self-life. The whey was obtained from the own elaboration of fresh cheese. For this, the milk was pasteurized at 75°C for 15 minutes, later, the temperature was lowered to 45°C, and added the CaCl<sub>2</sub> (1g/L), after that the renin was added (force 1:15000 L), and after 30 minutes of rest, the desuered was done. The whey obtained was fermented at 37°C, with by *Bifidobacterium animalis* subsp. *lactis* BB-12<sup>R</sup>, (CHRS- HANSESN), three treatments were evaluated: one with the BB-12<sup>R</sup> and with prebiotic carboximetilcelulose (CMC); other with BB-12 and without CMC; and the control without BB-12<sup>R</sup> and without CMC. The beverages were carried out at 0, 7, 14 and 21 days to determine pH, titratable acidity (NOM-155-SCFI-2012) and proteolytic activity (Church et al.,1983). For this one, were obtained filtrates according to Donkor et al. (2007) and were kept at -20°C until analysis. The proteolytic activity was determined in triplicate based on the reaction of the free primary amines (NH<sub>3</sub>) with O-phtaldialdehyde (OPA) and b-mercaptoethanol. The statistical analysis will be carried out using the SAS statistical package, in which an analysis of variance was carried out with the GLM procedure (treatments were used as qualifying variables and as variables of response proteolysis, pH and titratable acidity).

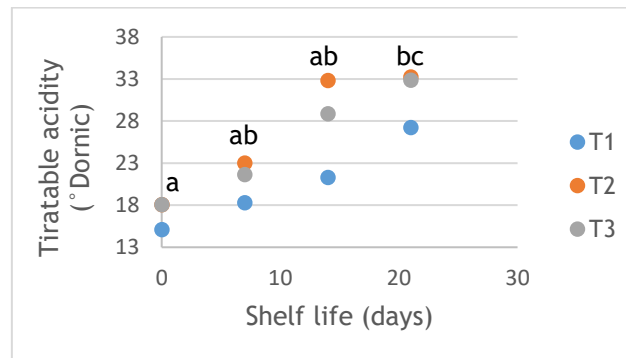
## RESULTS AND DISCUSSION

Significant differences (P<0.01) were observed in all variables evaluated (pH, titratable acidity, and proteolytic activity) due to effect to shelf life. Additionally, only in the proteolytic activity, significant differences (P<0.01) were observed in the treatments evaluated in this study was, of which, the T1 - the control without *Bifidobacterium animalis* subsp. *lactis* (BB-12<sup>R</sup>) and without CMC, T2 - with BB-12<sup>R</sup> and without CMC. T3-with the BB-12<sup>R</sup> and with prebiotic carboximetilcelulose (CMC). Graph 1 shows the pH of the whey-based beverages, which significant differences (P<0.01) were observed due to the effect to shelf life and no significant differences (P>0.01) were observed due to the effect to treatments. Graph 2 shows the titratable acidity of the whey-based beverages and significant differences (P<0.01) were observed due to the effect to shelf life and no significant differences (P>0.01) were observed due to the effect to treatments. Graph 3, indicate the percentages of proteolytic activity of each type of fermented whey-based beverages, and significant differences (P<0.01) were observed due to the effect to shelf life and due to the effect to treatments. Similar results found Álvarez-Rosales et al., (2019), when seeing a constant change in the proteolysis during the shelf life of the fermented beverages with *Lactobacillus acidophilus*, due to the effect of the fermentation conditions (treatments). Additionally,

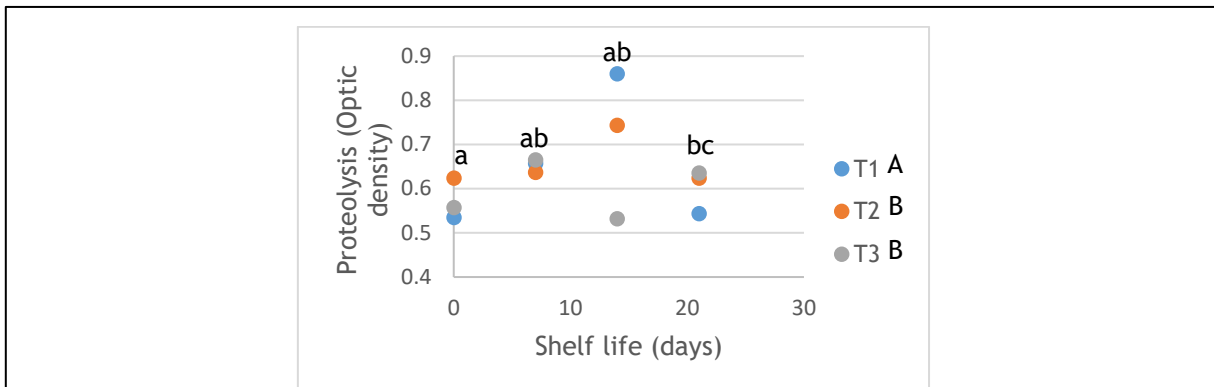
they mention that the production of fermented beverages is a process that involves many physical and chemical changes during its production and shelf life. One of these changes is proteolysis, which consists in the progressive hydrolysis of milk caseins to smaller polypeptides, peptides, and amino acids by intracellular peptidases.



**Graph 1** pH of the whey-based beverages. a,b,c Significant differences ( $P < 0.01$ ) were observed due to the effect to shelf life. No Significant differences ( $P > 0.01$ ) were observed due to the effect to treatments. Treatments: T1 - the control without *Bifidobacterium animalis* subsp. *lactis* (BB-12<sup>R</sup>) and without CMC, T2 - with BB-12<sup>R</sup> and without CMC. T3-with the BB-12<sup>R</sup> and with prebiotic carboximetilcelulose (CMC).



**Graph 2** Titratable acidity of the whey-based beverages. a,b,c Significant differences ( $P < 0.01$ ) were observed due to the effect to shelf life. No Significant differences ( $P > 0.01$ ) were observed due to the effect to treatments. Treatments: T1 - the control without *Bifidobacterium animalis* subsp. *lactis* (BB-12<sup>R</sup>) and without CMC, T2 - with BB-12<sup>R</sup> and without CMC. T3-with the BB-12<sup>R</sup> and with prebiotic carboximetilcelulose (CMC).



**Graph 3.** Proteolysis of the whey-based beverages. Significant differences ( $P < 0.01$ ) were observed due to the effect to shelf life (lowercase) and due to the effect to treatments (Capital letters). Treatments: T1 - the control without *Bifidobacterium animalis* subsp. *lactis* (BB-12<sup>R</sup>) and without CMC, T2 - with BB-12<sup>R</sup> and without CMC. T3-with the BB-12<sup>R</sup> and with prebiotic carboximetilcelulose (CMC).

## BIBLIOGRAPHY

- Álvarez-Rosales, J.D., Ozuna, C. Salcedo-Hernández S. y Rodríguez-Hernández G. 2019. Comparision of Antioxidant Activity of Cow and Goat Milk During Fermentation with *Lactobacillus acidophilus* LA-5. In Prebiotics and Probiotics – Potential Benefits in Nutrition and Health. Intech Open. Swiss.
- Church, F. C., Swaisgood, H. E., D. H. Porter y G. L. Catignani. 1983. Department of food science spectrophotometric assay using o-phthalaldehy de for determination of proteolysis in milk and isolated milk proteins J. Dairy Sci. 66: 1219-1227.
- Donkor, O. N., A. Henriksson, T. K. Singh, T. Vasiljevic y N. P. Shah. 2007. ACE-inhibitory activity of probiotic yoghurt. Int. Dairy J. 17:1321–1331
- González-González, C. R., K. M. Tuohy y P. Jauregi. 2011. Production of angiotensin - I converting enzyme (ACE) inhibitory activity in milk fermented with pH and peptides on the ACE-inhibitory activity. Int. Dairy J. 21: 615-622
- Norma Oficial Mexicana NOM-155-SCFI-2012, Leche-Denominaciones, especificaciones fisicoquímicas, información comercial y métodos de prueba.