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The influence of the processing conditions on the salty cheese with *Propionibacterium shermanii* addition

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Abstract

In this study, the influence of the salting and ripening operations on the characteristics of the salty buffalo cheeses with *Propionibacterium shermanii* addition was evaluated. These bacteria, used in the Swiss cheese production, can synthesize amino acids and vitamins, especially vitamin B12, thus bringing a functional role to the final products. The research involved 4 types of salty cheese: cheese matured at 8 °C in 10% concentration brine for 7 days, cheese matured at 8 °C in 10% concentration brine for 14 days, cheese matured at 14 °C in 20% concentration brine for 7 days, cheese matured at 14 °C in 20% concentration brine for 14 days, in comparison with a commercial cheese. Considering the sensory, physical-chemical, and microbiological aspects of the final products, the cheese matured at 14 °C in 20% concentration brine for 7 days was the most appreciated and with the most appropriate characteristics.

Keywords: salty cheese, Propionibacterium shermanii, vitamin B12

1. Introduction

Buffalo milk is considered a good source for cheese production due to the higher yields, renneting properties and the faster coagulation process, related to a greater susceptibility of the buffalo kappa-casein to chymosin action and to the higher amounts of calcium and protein [11].

Recently, potential probiotic bacteria were isolated from dairy foods obtained from buffalo milk [4]. Probiotic products are the leading components in the "functional food" area, generating basic nutrition and health benefits [10].

Swiss-type cheeses are characterized by a hard or semi-hard texture, with a controlled propionic acid fermentation caused by propionic acid bacteria (especially *Propionibacterium freudenreichii* ssp. *freudenreichii* and *Propionibacterium freudenreichii* ssp. *shermanii*) [9]. *Propionibacterium freudenreichii* is a probiotic, nonmotile and Gram-positive bacterium. It has the ability for metabolite biosynthesis, especially propionic acid (a preservative of food and feed), acetic acid and vitamin B12 [7].

It is used in the Swiss-type cheeses production for its ability to produce the nutty and sweet flavours and the carbon dioxide by-product, responsible for the eyes formation [2]. The sweet flavour has also been associated with the water-soluble fraction containing amino acids (proline and valine), water-soluble fatty acids, mainly propionic acid, salt, magnesium ions, calcium ions, tri, and tetra peptides [3]. During the cheese ripening period, these types of bacteria can grow and generate this large amount of flavour compounds in warm but also in cold storage conditions [6]. Cheese ripening is the technological process whensoever biochemical and microbiological changes take place, resulting in the development of a specific flavour and consistency of the matured product [5]. Cheese flavour highly impacts the consumer enjoyment and preferences [1].

Thus, the possibility of *Propionibacterium freudenreichii* ssp. *shermanii* development in salty cheeses was evaluated in this study. Also, the way in which the salting and maturing operations influence the characteristics of the salty cheeses' assortments were investigated.

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2. Materials and Method

2.1. Materials

The raw buffalo milk was purchased from the local market. The rennet and the calcium chloride were bought from a local veterinary pharmacy. *Propionibacterium shermanii* starter culture was isolated from an Emmental cheese and then inoculated in the raw milk (Figure 1).

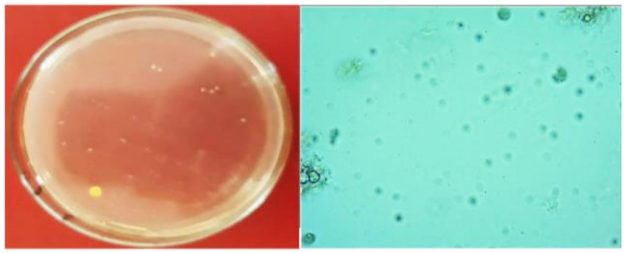


Figure 1. *Propionibacterium shermanii* isolated from the Emmental cheese

2.2. Salty cheeses production

The research involved 4 types of salty cheese: cheese matured at 8 °C in 10% concentration brine for 7 days (B.M.C.10%), cheese matured at 8 °C in 10% concentration brine for 14 days (B.M.S.10%), cheese matured at 14 °C in 20% concentration brine for 7 days (B.M.C.20%), cheese matured at 14 °C in 20% concentration brine for 14 days (B.M.S.20%), in comparison with a control cheese bought from the market (B.C.). The four different types of cheeses were produced by the conventional method using buffalo milk, which was processed through the main operations of pasteurization, cooling, inoculation with calcium chloride, rennet and *Propionibacterium shermanii* starter culture, coagulation, curd processing, salting and ripening, respecting different specific conditions (Figures 2-3).

Figure 2. Aspects during curd processing

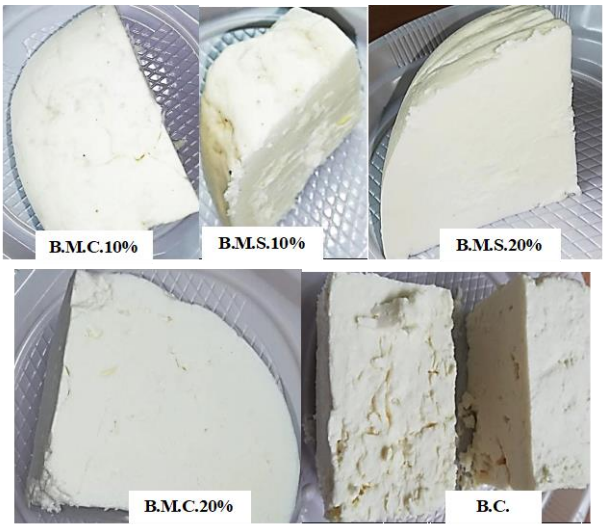


Figure 3. Sections through the four types of salty cheeses compared with the control cheese

2.3. Sensory, physical-chemical, and microbiological analysis of the cheeses

The four types of cheeses were analyzed considering: *the sensory characteristics* based on a scoring assessment scale, *the physical-chemical indicators* which involved the following determinations: dry substance by drying in an oven at 102 ± 2°C, acidity by the Thörner method, fat content by butyrometric method, salt content by argentometry titration method and *the microbiological parameters*, represented by the total number of germs (determined by Plate Count Agar according to SR ISO 4833-1:12013) and yeasts and molds (according to a the standardized method from STAS ISO 7954-2001 using Sabouraud Agar with Chloramphenicol as culture medium), and also, *the ability of Propionibacterium shermanii to generate vitamin B12* based on the thin layer chromatography method.

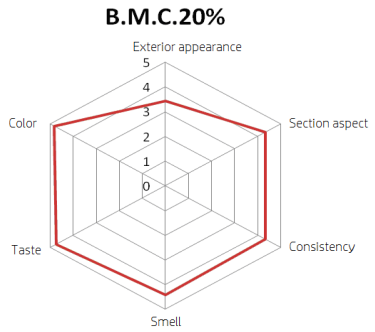
The highlighting of vitamin B12 in cheese was done by adapting the method proposed by Ponder et al [8]. Vitamin B12 was extracted from the cheese samples in a mixture of methanol: 1: 3 water (25% V). Thus, for this extraction, 5 g of cheese were weighed and mixed with 50 mL of the mixture of methanol and water. The extract was separated by centrifugation for 15 minutes at 5000 rpm. Then it was dried on the plate and redissolved in 10 mL of the 1: 3 methanol: water mixture. Vitamin B12 was detected in the cheese samples by thin layer chromatography on GF60 254 silica gel plates impregnated with fluorescent substance using as mobile phase methanol, chloroform, acetic acid, ammonia, and water in a ratio of 7: 4: 5: 1: 0 ,5. 3

µL of cheese extracts and 3 µL of standard vitamin B12 solutions in a concentration of 50 µg / mL and 25 µg / mL were spotted on the chromatographic plate.

3. Results and Discussion

3.1. The results of the cheeses sensory evaluation

Considering the sensory analysis of the final products, the cheese matured at 14°C in 20% concentration brine for 7 days (B.M.C.20%) was the most appreciated and with the most appropriate characteristics (Figure 4). It had a more uniform appearance, with slight traces of gauze on the surface and a pleasant white color both on the outside and in its section. In the section, the cheese can be described like a creamy porcelain paste with no traces of impurities or foreign bodies. Also, this type of cheese had a uniform consistency, both on the surface and in the section, being a compact, well-connected mass that breaks easily without shattering. As well, it had a pleasant smell and a sweet taste with slight nuances of nuts, which indicates the development of *Propionibacterium shermanii*.



3.2. Cheeses physical chemical evaluation

The most appreciated type of cheese, B.M.C.20%, was characterized by the following values of the physical-chemical parameters (table 1):

Characteristic	Value
Dry substance, %	46.241
Acidity, °T	89.2
Fat content, relative to the dry matter %	80.4881
Salt content, %	3.6572

Vitamin B12 highlighting

Examining the silica gel plate with the samples of cheeses extracts and the standard solutions of vitamin B12 in UV light at 254 nm, it was observed the spots presence at the same Rf value (0.54) or at close values which shows that in all 4 samples of cheeses the vitamin B_{12} was present. The shape of the spots in the cheeses samples is different from that of the standard because of the more complex matrix of the cheese's extracts (Figure 5).

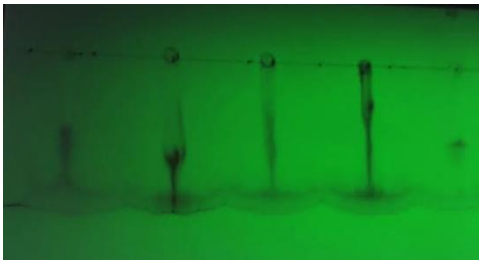


Figure 5. The chromatogram obtained for cheeses extracts compared to the vitamin B12 standard (last spot in the figure)

3.3. Cheeses microbiological evaluation

When analyzing the total number of germs, higher values were obtained for the 4 types of cheese, compared to the commercial cheese due to the development of *Propionibacterium shermanii*, inoculated in the studied cheeses (Figure 6).

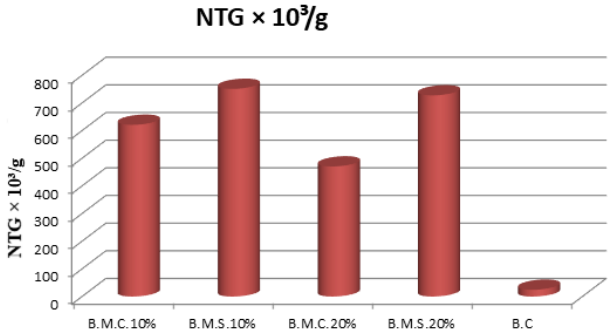


Figure 6. TNG values for the analyzed cheeses

After the yeasts and molds evaluation, the commercial cheese recorded the highest value, most probably due to unsatisfactory pasteurization and storage conditions, while the B.M.C.20% type of cheese was characterized by the lowest value, 2.75 x10³/g (Figure 7).

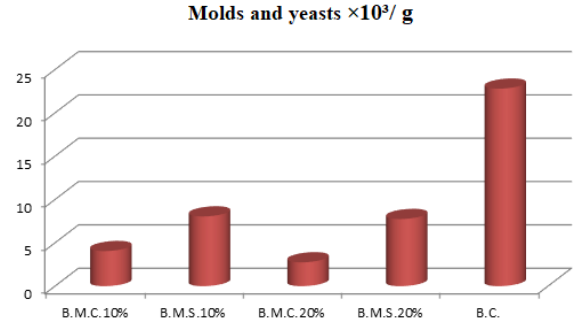


Figure 7. Molds and yeasts values for the analyzed cheeses

4.Conclusion

After the application of different salting and ripening conditions, it was found that the use of a brine with a concentration of 20%, followed by 7 days of maturation at 14°C generated a cheese with optimal sensory, physical-chemical and microbiological characteristics, which also allowed the development of bacteria of the genus *Propionibacterium shermanii* which led to the formation of vitamin B12, giving functional properties to the product.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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