

Short Communication: Detection of *Hafnia alvei* in Brazilian's Serrano Cheese

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Abstract:- *Hafnia alvei* effects on cheese compounds have been studied recently. It can modulate the microbiota and favors the growth of other species involved on the flavor's compounds production. The Serrano cheese has a strong flavor but, until recently, the substances responsible for them were not fully identified, as well as their microbiota. This communication aims to inform the first identification of this microorganism in Serrano cheese. The study analyzed cultivable microbiota of nine samples of Serrano cheese. There were identified 59 microorganisms from 13 different species. One of them was *H.alvei*, detected in two different samples of Serrano cheese.

Keywords:- Serrano Cheese, *Hafnia Alvei*, Flavor.

I. INTRODUCTION

Hafnia alvei is an enterobacterium normally associated with poor hygiene in cheese production. However, its effect on the modulation of microbiota during maturation, as well as the production of compounds responsible for the desired sensory characteristics in some cheeses, has been reported in recent studies [1; 2].

Microbiota changes during cheese ripening. It happens because the compounds produced during this event are substrates for other microbial types, with a succession of populations over time. Therefore, the microorganisms present initially have primary importance in defining the sensory characteristics of the final product, as they create the environmental characteristics for the development of the others [1; 2; 3].

The compounds produced by the microbiota during ripening give the cheeses unique characteristics, so much that likely to receive the designation of origin because the peculiarities of their flavors. Serrano cheese, produced in the regions of the Santa Catarina mountains and in the region of the upper fields of the mountains in Rio Grande do Sul, obtained the designation of origin in 2020. It is produced from raw milk and matures for up to 60 days, been considered semi-hard, greasy and with a thin, light yellow rind, in addition to a strong flavor [4; 5; 6].

The detection of *H. alvei* in Serrano cheese produced in the fields on top of the mountain range, in Rio Grande do Sul,

in the midst of a study of the cultivable microbiota, may be the first step towards identifying the compounds responsible for the flavor of this cheese. Therefore, this communication aims to inform the first identification of this microorganism in Serrano cheese produced in Rio Grande do Sul.

Nine samples of Serrano cheese, each one obtained from different cheese factory, were collected, stored in sterile containers and sent to Lab of Inspection and Technology of

Dairy, Eggs and Honey from Federal University of Rio Grande do Sul for analysis. From each sample, 25g were ground and homogenized in 225mL of 0.1% peptone water with the aid of the stomacher. Decimal dilutions were inoculated in Plate Count Agar (PCA) and kept at 37°C for 48h to count mesophilic microorganisms, while psychrotrophic counting was performed after storage for 10 days at 7°C [7]. Based on the different colony morphologies, the microorganisms were isolated and kept in pure culture for further identification, which was performed by mass spectrometry with laser desorption ionization and time-of-flight analysis (MALDI TOF MS, AutoflexSpeed®, programs FlexControl® and Biotyper®-Bruker, Germany), using formic acid with ethanol extraction [8; 9].

The samples were analyzed for their cultivable bacterial microbiota. Until now, 59 bacteria from 12 different species were identified in the nine cheese samples. The identified microorganisms are present in table I. Among these species, *H.alvei* was identified in two of the collected samples. These bacteria are associated with the production of substances that give cheeses flavors that please the taste of consumers. However, it does not produce these substances itself, but interacts with other microorganisms that end up having stimulated growth with their presence. The microbiota containing *H. alvei* produces a largest quantity of aroma compounds, primarily corresponding to sulfur compounds [1; 2].

In a research carried out with controlled microbiota cheeses, the presence of *H. alvei* influenced the expression of genes associated to assimilation of sulfur compounds from *Brevibacterium aurantiacum*, which suggests a lower demand for sulfur amino acids by this microorganism [2]. This allows the catabolism of methionine and cysteine and, as a result of this reaction, the production of volatile sulfur compounds.

Methionine is more numerous in caseins so, the sulfur compounds in cheese mainly originate from it. The volatile sulfur compounds include a diverse class of molecules responsible for the characteristic aromatic notes such as “cheesy” or “garlic” in cheeses [1; 2; 10; 11; 12].

Another influence of *H. alvei* on cheese compounds is linked to lipolytic catabolism, although the presence of coding genes in its genetic code was not detected. This is due to the use of glycerol released from cheese triglycerides as a nutrient, which favors its growth. The cheese’s fatty acid profile interest researchers because some lipids are notably important to human nutrition. Isomers of conjugated linoleic acid (CLA) are one of the most interesting of them, being rumenic acid the highlight. Since CLA is naturally present in milk, cheese represents a good source of this fatty acid [2; 13].

TABLE I Partial number of isolated/identified microorganisms from Serrano cheese

Identified microorganism	Sample	Number of isolates
<i>Lactococcus lactis</i>	2; 3; 4; 7; 8	21
<i>Enterococcus faecalis</i>	1; 4; 5; 6; 7	18
<i>Hafnia alvei</i>	2; 3	4
<i>Raoutella ornithinolytica</i>	4; 7; 8	3
<i>Citrobacter freundii</i>	1; 5	3
<i>Escherichia coli</i>	8; 9	3
<i>Enterococcus durans</i>	5; 9	2
<i>Enterococcus faecium</i>	9	1
<i>Enterococcus italicus</i>	8	1
<i>Lactococcus garvinae</i>	5	1
<i>Lactococcus paracasei</i>	6	1
<i>Pichia cactophila</i>	4	1
	TOTAL	59

Although the importance of *H.alvei* in the modulation of microbiota, which influence in cheese’s aromatic compounds related in other cheese worldwide, in Serrano cheese it is the first report. Other studies related microbiota and its role on compounds of the artisanal cheeses in Brazil, principally on Minas artisanal cheese that is well studied. But in Serrano cheese it hasn’t been fully described. Normally studies had focus on the safety of the products, analyzing the pathogenic microorganisms present on them [4; 14; 15; 16].

As a conclusion, this current study should increase knowledge about of Serrano cheese’s microbiota and its role on production of compounds that give this cheese its unique flavor. The *H. alvei* finding could give us a clue of its importance on modulating microbiota and handle other researches to formulate yeasts containing microorganisms that are necessary to flavors constitution.

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