

TITLE: DISTINCT MICROBIOME PATTERNS ARE PRESENT WITHIN CANASTRA CHEESE PRODUCING REGION.

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ABSTRACT:

Canastra cheese has great importance for the local culture and economy, being part of the Intangible Heritage of Brazil, and having received a protected Designation of Origin certification in 2012. It's made using raw milk, salt, rennet and an endogenous starter culture, popularly called "pingo". This study aimed to characterize the microbiota of Serra da Canastra's cheese and the pingo used in its production, using next generation sequencing to identify the bacteria and fungi present in 81 distinct producers. The bacterial community identified was compared with microbiota datasets from other Brazilian and world cheeses available in the literature.

Our study was able to separate the microbiota from Canastra cheeses and other cheeses in Europe and North America, and pH was a factor contributing to this segregation. A difference was also observed between the microbiota of Canastra and other Brazilian cheeses. In addition, we observed a relationship between the microbiota's global composition and the geographical distance between producers, as well as seasonality as a influencing factor on distinct microbial taxa in pingo and cheese.

We created novel scales to discriminate the level of good manufacturing practices and the producer's local infrastructure, and observed that specific bacterial and fungal taxa could discriminate between higher and lower ends of these scales. Low levels of good manufacturing practices (GMPs) were marked by bigger proportions of genera *Kocuria* in pingo and *Staphylococcus* in cheese, while higher levels of *Streptococcus* were related to cheeses from producers with higher levels of GMPs. Furthermore, we observed an inverse relationship between fungal genera *Debaryomyces* and *Trichosporon* within cheeses, which seems to be related to shifts in a climate transition from the dry season to the rainy season.

Using large-scale molecular sequencing techniques, we observe a connection between pingos and cheeses' microbiota. We observed distinct microbial patterns in different areas within the the Canastra region, with a possible effect of seasonality on fungal and bacterial compositions. Furthermore, we show that *Streptococcus*, *Staphylococcus* and *Kocuria* genera are correlated with good practices. These results may influence the development of tracking methods for specific Canastra subregions and assist producers to manufacture good quality cheeses, while maintaining the specific characteristics of their region.

Keywords: Cheese; Serra da Canastra; microbiota; 16S rDNA sequencing; ITS sequencing

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