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## Microbiological and Physicochemical Evaluation of Traditional Greek Kopanisti Cheese Produced by Different Starter Cultures

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Abstract: Kopanisti cheese is a Greek soft Protected Designation of Origin (PDO) cheese made of raw cow, sheep or goat milk, or mixtures of them, with similar organoleptic characteristics to that of Roquefort cheese. Traditional manufacturing of Kopanisti cheese is limited in small-scale dairies, without the addition of starter cultures. Instead, an amount of over-mature Kopanisti cheese, called Mana Kopanisti, is used to initiate ripening. Therefore, the selection of proper starter cultures and the understanding of the contribution of various microbial groups to its overall quality is crucial for the production of a high-quality final product with standardized organoleptic and physicochemical characteristics. Taking the above into account, the aim of the present study was the investigation of Kopanisti cheese microbiota and its role in cheese quality. For this purpose, four different types of Kopanisti were produced in triplicates, all with pasteurized cow milk, with the addition of (A) the typical mesophilic species Lactococcus lactis and Lactobacillus paracasei used as starters in the production of soft spread cheeses, (B) strains of Lactobacillus acidipiscis and Lactobacillus rennini previously isolated from Kopanisti and Mana Kopanisti, (C) all the species from (A) and (B) as inoculum, and finally (D) the species from (A) and Mana Kopanisti. Physicochemical and microbiological analysis was performed for milk and cheese samples during ripening. Enumeration was performed for major groups of lactic acid bacteria (LAB), total mesophilic bacteria, yeasts as well as hygiene indicator microorganisms. Bacterial isolates from all the different LAB groups, apart from enterococci, alongside yeasts isolates, were initially grouped using repetitive sequence-based polymerase chain reaction (rep-PCR) and then identified at the species level using 16S rRNA gene and internal transcribed spacer (ITS) DNA region sequencing, respectively. Sensory evaluation was also performed for final cheese samples at the end of the ripening period (35 days). Based on the results of the classical microbiological analysis, the average counts of the total mesophilic bacteria and LAB, apart from enterococci, ranged between 7 and 10 log colony forming unit (CFU) g<sup>-1</sup>, phychrotrophic bacteria, and yeast extract glucose chloramphenicol (YGC) isolates between 4 and 8 log CFU g<sup>-1</sup>, while coliforms and enterococci up to 2 log CFU g<sup>-1</sup> throughout ripening in cheese samples A, C and D. In contrast, in cheese sample B, the average counts of the total mesophilic bacteria and LAB, apart from enterococci, phychrotrophic bacteria, and YGC isolates ranged between 0 and 10 log CFU g<sup>-1</sup> and coliforms and enterococci up to 2 log CFU g<sup>-1</sup>. Although the microbial counts were not that different among samples, identification of the bacterial and yeasts isolates revealed the complex microbial community structure present in each cheese sample. Differences in the physicochemical characteristics among the cheese samples were also observed, with pH ranging from 4.3 to 5.3 and moisture from 49.6 to 58.0 % in the final cheese products. Interestingly, the sensory evaluation also revealed differences among samples, with cheese sample B ranking first based on the total score. Overall, the combination of these analyses highlighted the impact of different starter cultures on the Kopanisti microbiota as well as on the physicochemical and sensory characteristics of the final product.

**Keywords:** Kopanisti cheese, microbiota, classical microbiological analysis, physicochemical analysis **Conference Title:** ICADST 2021: International Conference on Advanced Dairy Science and Technology

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