

Effects of different stages of lactation on the raw milk for Fontina cheese manufacturing: chemical composition and microbiota analysis by 454 –pyrosequencing

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In order to investigate the effect of lactation stage on the microbial quality of milk for fontina Fontina cheese production, nine cheese-making days were followed in two cheese factories over a four months period: three in the first 40 days after partum (early lactation stage S1), three in the following 40 days in the middle lactation (stage S2) and three in the last 40 days when cows were pregnant (stage S3). After plating, and basic chemical analysis, total microbial DNA was isolated from milk and used as template in Polymerase Chain Reaction (PCR) to study the hypervariable V1, V2 and V3 regions of the bacterial 16S rRNA gene and analysed by 454-pyrosequencing. A total of 683,128 sequence reads were generated by the pyrosequencing of 18 milk samples. An average of 498 OTUs were identified. The use of classical microbial approach and high-throughput sequencing allowed not only the description of the bacterial community but also to find difference for lactation stages. The milk samples collected at first lactation stage were characterised by higher counts of coliforms and enterococci and higher amounts of *Enterococcus* genus while the milk samples collected at later stage of lactation were characterised by higher amount of protein. Some recurrent species could be found at all times of sampling: *Staphylococcus*, *Lactococcus* and *Streptococcus* across *Firmicutes* and *Mesorhizobium* and *Ralstonia* among *Proteobacteria* phylum. This is the first study where the lactation stage is clearly linked to milk microbiota.