

LACTOCOCCUS LACTIS

Study of *Lactococcus lactis* during advanced ripening stages of model cheeses characterized by GC-MS.

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INTRODUCTION

Lactococcus lactis, known as dairy starter, has recently been detected, as metabolically active cells, in advanced ripening stages of some cheeses, by culture-independent methods. To better understand the role of the *L. lactis* in ripening processes our goals were to confirm the vitality of the microorganism up to 180 days of ripening of model cheeses, and to evaluate the potential correlation between the metabolism of this and volatile aromatic profiles of the cheeses.

RESULTS

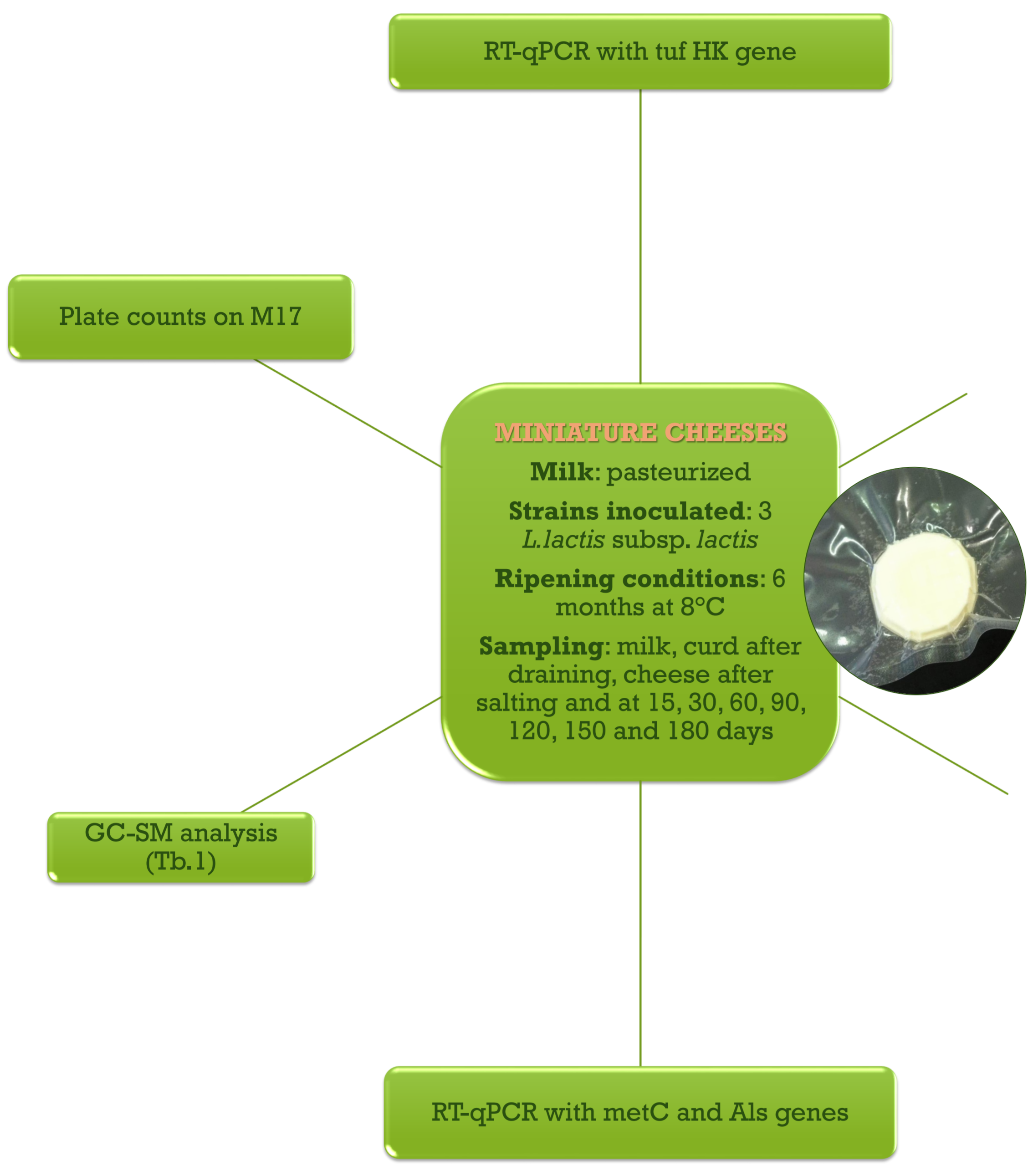


Table 1: Analysis conditions to determine the volatile compounds in the miniature cheeses by solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS).

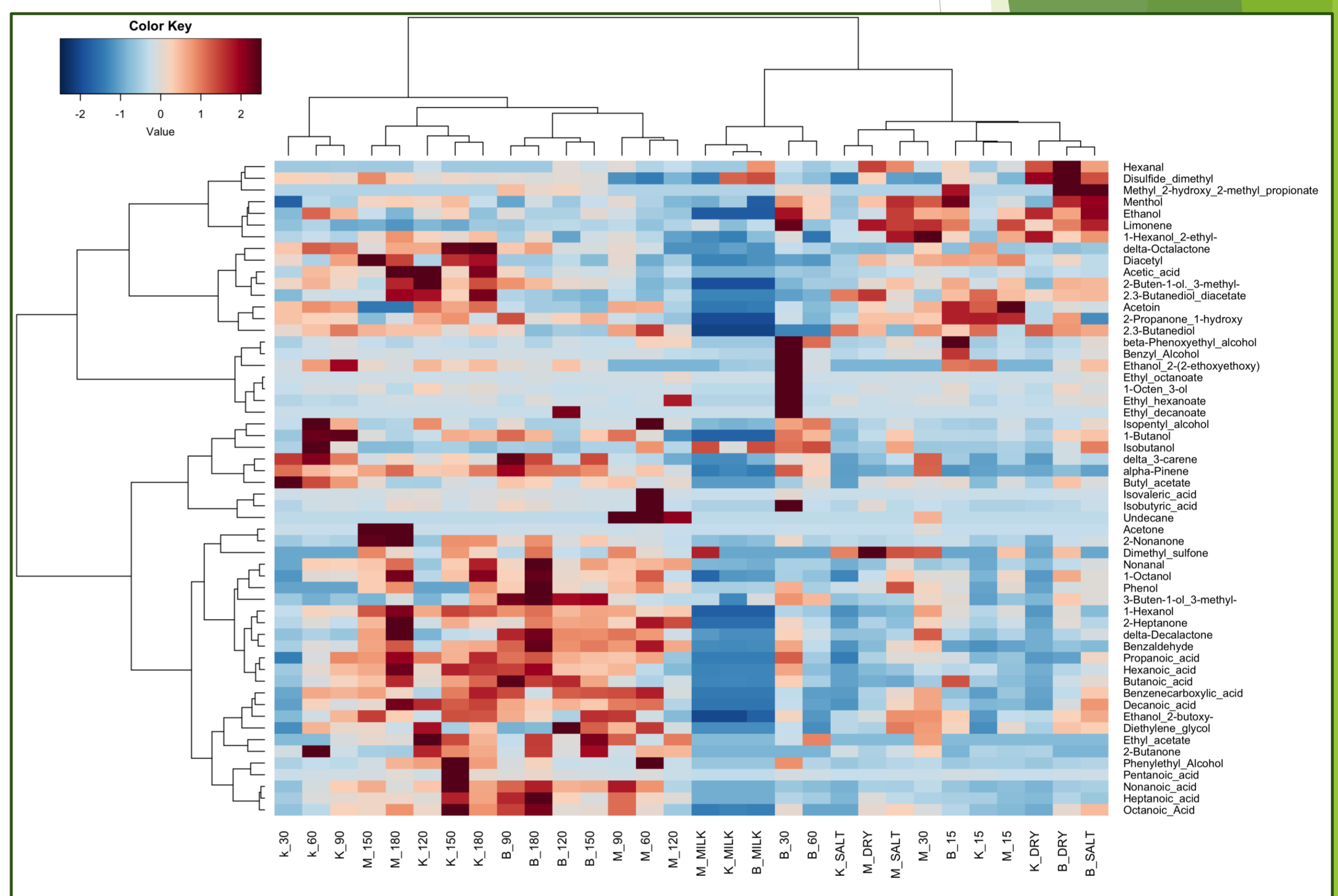
| GC | MS |
|--|--|
| Column DB-WAXETR (30 m x 0.25 mm, 0.25 µm) | Ionization energy 70 eV |
| Carrier Gas Helio | Capture speed 0,30 s ⁻¹ |
| Flow rate 1 mL·min ⁻¹ | Modo Full Scan 33-300 |
| Injection port temperature 260 °C | Range m/z: |
| Method HS-SPME | Modo TIC One Ion quantificator m/z Two ions qualifier m/z |
| Temperature program: 40 °C for 5 min, increased at a rate of 10 °C min ⁻¹ to 80°C, then at rate 5°C min ⁻¹ to 240°C per 3 min | Software: GCMSolution |
| Total time for analysis 44 min | Peak identification of each volatile metabolite was performed by comparison of the retention time and mass spectra of eluting compound to those of the pure standard. |
| | The amount of each compound was performed as area by the peak area of a selected quantifier ion of the volatile metabolite. |

Table 2: Detection of alive cells of *L. lactis* throughout manufacturing and ripening of miniature cheeses: comparison of the results obtained by culture-independent and -dependent approaches

| Strain | Step | K | | | B | | | M | | |
|----------|------|--------------|--------|-------------|-------------|------|-------------|-------------|------|-------------|
| | | RNA (CFU/g)* | BULK** | M17 (CFU/g) | RNA (CFU/g) | BULK | M17 (CFU/g) | RNA (CFU/g) | BULK | M17 (CFU/g) |
| Man.ing | milk | 1,71 | + | 8,15 | 2,78 | + | 7,74 | 2,37 | + | 7,34 |
| | dry | 6,91 | + | 9,23 | 8,01 | + | 9,90 | 8,11 | + | 9,74 |
| | salt | 7,01 | + | 9,45 | 8,32 | + | 8,69 | 8,34 | + | 9,91 |
| Ripening | 15d | 7,18 | + | 8,31 | 6,52 | + | 7,75 | 7,50 | + | 8,46 |
| | 30d | 7,15 | + | 6,78 | 6,86 | + | 6,72 | 7,12 | + | 7,15 |
| | 60d | 7,00 | + | 5,98 | 6,44 | + | 3,85 | 6,52 | + | 3,85 |
| | 90d | 6,66 | NC | NC | 6,34 | + | 4,20 | 6,52 | + | 4,71 |
| | 120d | 6,75 | NC | NC | 6,12 | NC | NC | 7,41 | NC | NC |
| | 150d | 4,21 | NC | NC | 6,02 | NC | NC | 4,32 | NC | NC |
| 180d | 3,21 | NC | NC | 4,15 | NC | NC | 3,43 | NC | NC | |

*The values, expressed as microbial loads, referred to standard curve described in a previous work (Ruggirello et al. 2013)
 ** "+": *L. lactis* was detected; "NC": *L. lactis* was not detected.

Figure : Heatplot of volatile compounds in miniature cheeses from manufacturing to 180 days of ripening. The colour scale represents the scaled abundance of each variable, denoted as Z-score, with red indicating high abundance and blue indicating low abundance.



The comparison of culture-dependent and -independent analysis highlighted, as just reported in our previous work (Ruggirello et al., 2016), a discordance between the results. The three starters of *L. lactis* were found alive until the 180 days of ripening with loads of 10³ and 10⁴ CFU/g by RT-qPCR although the microorganism did not grow on plate after 90, 120 and 120 days respectively for K, B and M strains. The absence of other microorganisms on M17 medium, in accordance with the negative bulk for *L. lactis*, underlined that *L. lactis* was the only microorganism in the cheese.

The aroma profiles of the cheeses disclosed the presence of important contents of volatile compounds related to the metabolism of carbohydrates, amino acids and lipids. Focusing the attention on two of the typical aroma compounds found in ripened cheeses, we followed, by RT-qPCR, the expression of two genes related at the formation of DMDS (metC) and Acetoin (Als). Preliminary results showed an expression of these genes both in early stages of ripening and at 180 days; data were corroborated by the presence of these aromas along the whole process of manufacturing and ripening of the cheeses. We could suppose a correlation between the metabolism of *L. lactis* and the formation of these aromas. Further investigations will allow a better understanding of the role of this microorganism and its metabolic activity in the aging steps of cheese.

*Metatranscriptomic analysis of the samples at 15 days was carried out (data not shown) showing the expression, in the cheeses, of all genes involved in the pathways related at the formation of DMDS, Acetoin, Diacetyl, 2,3 Butanediol, Acetic Acids, all peculiar aroma compounds of ripened cheese.

DISCUSSIONS

| Sample | Als | metC |
|--------|-----|------|
| K SALT | + | |
| K 15 | + | |
| K 30 | + | |
| K 180 | + | |
| M SALT | + | |
| M 15 | + | |
| M 30 | + | + |
| M 180 | + | + |
| B SALT | + | + |
| B15 | + | + |
| B 30 | + | + |
| B 180 | + | + |

Table 3: RT-qPCR for the metC and Als genes, involved respectively in the formation of DMDS and Acetoin compounds. The analysis was so far carried out in cheeses at salt step and 15, 30 and 180 days of ripening, showing a detection signal in all samples (+). The Ct values have to be normalised and for this reason they are not reported in the table.

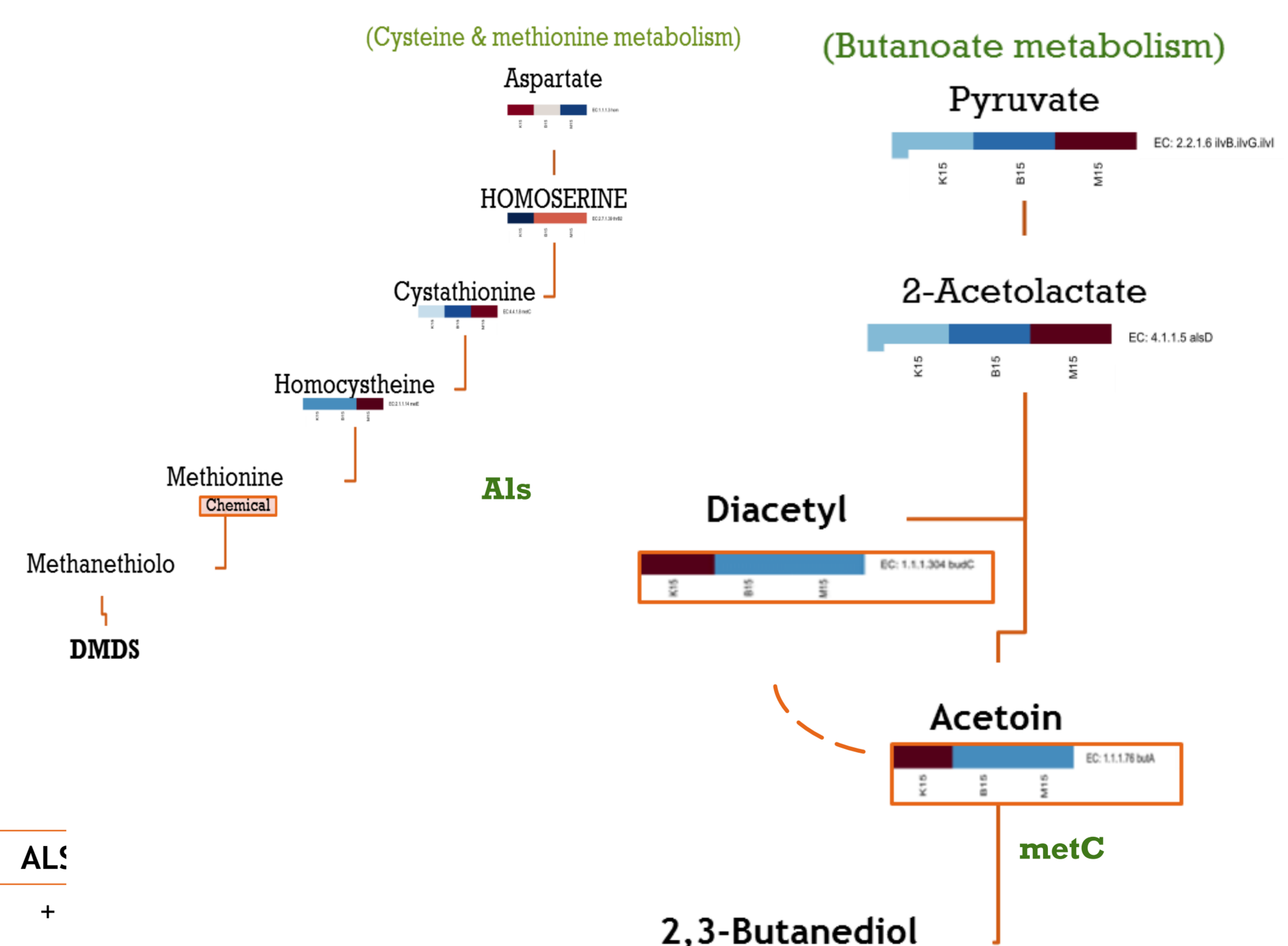


Fig. 2: Pathways of formation of DMDS, Acetoin, Dyacetyl and 2,3-Butanediol volatile compounds, with related expression data of cheeses at 15 days of ripening.