MP Biomedicals

Case study - *Cheese*

Food

Extraction of RNA from Cheese without Prior Separation of Microbial Cells.

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Overview

- **Keywords:** Cheese, Microbial flora, L.Lactis, RNA analysis, RT-PCR
- **Aim of the study:** Understanding the cheese microbial flora without separating the cells from the cheese matrix.
- **Application:** Quantification of L.Lactis rRNA and mRNA by real-time PCR
- **Sample name:** Cheese
- **Material:** FastPrep® Homogenizer, 2 ml Lysing Matrix tubes containing 0.1 mm silica/ zirconium beads
- **Buffer:** TRIzol reagent

Protocol and Parameters

1. Approximately 125 mg of cheese was placed into a 2 ml lysing matrix tubes.
2. 1.25 ml of TRIzol reagent was added immediately.
3. The tubes were vigorously shaken in a bead beater (FastPrep-24™ system) by using three 60 s mixing sequences at a speed of 6.5 m/s. The tubes were cooled on ice for 5 min before each mixing sequence.
4. After centrifugation for 10 min at 12,000 x g and 4 °C, each supernatant (approximately 1,100 µl) was transferred into a 2-ml tube containing 300 µl of a gel that improved separation of the aqueous and organic phases (Phase Lock Gel Heavy; Eppendorf, Hamburg, Germany).

Conclusion

- In the present work, RNA was successfully extracted from cheeses manufactured with L. lactis, and rRNA and mRNA transcripts were quantified by real-time PCR.
- The FastPrep® extraction method could be used or adapted for cheeses in which other microbial species are present.

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