Potential use of *Thymus mastichina* extract as a natural agent against cheese spoilage microorganisms

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INTRODUCTION

- In cheese production, the development of some spoilage microorganisms during ripening can:
  - reduce cheese shelf-life and commercial value
    e.g. *Yarrowia lipolytica* (responsible by cheese browning)
  - endangers consumer’s health
    e.g. *Listeria monocytogenes*
To reduce the presence of these microorganisms, cheese producers can:

- do frequent washings and scrapings;
- apply substances such as natamycin;

• But this increases the production costs;

• On the other hand, there are natural substances with antiseptic properties;

• Thymus mastichina L. is a mediterranean plant with recognized antiseptic properties.
Question:

- Is the ethanolic extract of thyme effective in reducing microbial contamination of cheese surfaces?
OBJECTIVES

- to evaluate \textit{(in vitro)} the antimicrobial activity of \textit{Thymus mastichina} ethanolic extract on some spoilage microorganisms;

- to evaluate the behaviour of yeasts, moulds and enterobacteria when we use this extract directly on the cheese surface, comparing with natamicyn;

- to find out if there is some physical-chemical change when applying the extract;
MATERIAL AND METHODS

1. Ethanolic extract preparation:

50 g of dried and crushed leaf with 1 L of ethanol, shaken for 1 hr (giving an extract concentration of 2 mg/mL w/v);

✓ The extract was filtered using filter paper *

✓ For microbiological analyzes was filtered with sterile filters **

✓ The extract was stored refrigerated.

* Whatman, 150 mm diameter; ** Whatman, 0.2 μm porosity, 25 mm diameter
2. “in vitro” study:

- **Escherichia coli** (ISA)
  - 1x10^8 cel/mL
  - Transfer 0.1 mL after 3, 6, 12 and 24 h
  - Nutritive Broth

- **Staphylococcus aureus** (IPMA)
  - 1x10^8 cel/mL
  - Transfer 0.1 mL after 3, 6, 12 and 24 h
  - Nutritive Broth

- **Yarrowia lipolytica** (PYCC 4159)
  - 1x10^8 cel/mL
  - Transfer 0.1 mL after 3, 6, 12 and 24 h
  - Glucose Yeast Maltose Agar + tirosine; 25°C; 3 d
3. Cheese Production:

- Milk reception
- Filtration
- Pasteurization
- Addition of animal rennet
Then, cheeses are pressed

Whey separation

Ripening in the curing chamber 10 °C, 4 weeks
**Sampling:** 40 cheeses divided into 4 groups with different treatments

- **QEE** - Surface application of ethanolic extract (2 mg/mL)
- **QEA** - Surface application of aqueous extract (2 mg/mL)
- **QNT** - Surface application of natamycin (2,5 mg/mL)
- **QST** - Without application of any substance
4. Analysis carried out after manufacture and then once a week:

**Microbiological Analysis**
- Enterobacteria (ISO 21528-2: 2004)
- Molds and yeasts (ISO 6611: 2004)
- Total aerobic (ISO 4833: 2003)
- Y. lipolytica (Carreira et Loureiro, 1998).

**Physicochemical analysis**
- $a_w$
- pH (PHM61 Laboratory pH Meter, Denmark).
- Titratable acidity
- Color (colorimeter CIELAB system).
- Texture  (Texture Analyzer, 24 with 4mm Ø)
The amount of *E. coli*, *Sth. aureus* and *Y. lipolitica* was higher when no extract was applied.

The strains used are sensitive to this extract,
In cheese with extract is evident:

- a maintenance of the values of total aerobic, in the end of ripening;
In cheese with extract is evident:

- a decrease of enterobacteria

- more pronounced than in cheese with natamycin
In cheese with extract is evident:

- a decrease of molds and yeasts
- but less pronounced than in cheese with natamycin
There are no differences between the different groups of cheeses when compared within the same time of ripening.
Results of colour and texture analysis

About texture at the end of ripening, the hardness of cheeses with and without extrat present the same order of magnitude.
CONCLUSIONS

• The “in vitro” tests results evidenced the antimicrobial activity of *T.mastichina* extracts against the studied strains;

• The study performed directly on the cheese surface also exhibited an evident antimicrobial activity;

• The improvement of extraction methods and adjustment of the concentration of the extracts will contribute to the use of *T.mastichina* as a healthy alternative to natamycin;

• The physic and chemical evaluation showed that the cheeses treated with ethanolic extract did not present considerable differences when compared to cheeses without treatment or treated with natamycin.
FURTHER WORK

We have to accomplish:
✓ the quantification by the determination of MIC;
✓ antibiosis studies with other pathogenic microorganisms such as *Listeria monocitogenes*;

There are many challenges for the future
Thank you for your attention!

Obrigada!