

A MASTER'S DEGREE RESEARCH: PROTEOMIC APPROACH ON EWE'S CHEESE

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ABSTRACT

A master's programme structure is usually flexible and involves a comparably dissertation that is based on a research or a practice-led research project. It involves a scientific exploration that helps students obtaining investigation skills and acquiring some transversal competences. **The main challenge of this academic process is to build an idea, identify the problematic, organize the hypothesis and stablishing the best methodology to get answers according to the objectives.** This master's degree research was developed within a multidisciplinary project, and the main objective was to investigate which proteomic methodology was better to investigate the degradation of cheese nitrogen fractions, with the resources available at University of Évora.

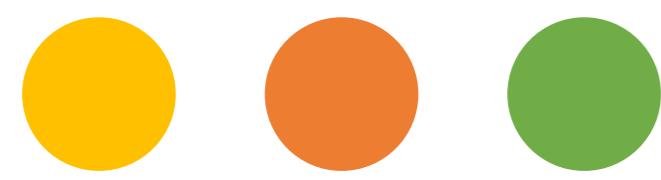
Ewe's cheeses have great tradition in Portugal, and have a high intrinsic value, arising from their very appreciated unique sensory characteristics, coupled with long-recognized social and economic impacts. Therefore, it's mandatory to understand the metabolic pathways in cheese ripening, and specifically, proteolysis. Three electrophoretic techniques have been used: urea polyacrylamide gel electrophoresis (Urea-PAGE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for the insoluble fraction of cheese and two-dimensional gel electrophoresis (2-DE) for the soluble fraction.

Results showed that urea-PAGE was the best method for cheese insoluble fraction analysis, mainly because it separates proteins not only by molecular mass, but also having charge into account, and since caseins have similar molecular masses SDS-PAGE is not able to separate them (1–3). This research investigation also concluded that both urea-PAGE and 2-DE methods are complementary in the study of proteolysis of ewe's cheese. Urea-PAGE results showed a degradation of the insoluble fraction, the caseins, during maturation, and the 2-DE showed an increase of the soluble fraction, that according to other results (3) we believe that are peptides resulting from the degradation of caseins.

Keywords: ewe's cheese; proteolysis; electrophoresis; caseins.

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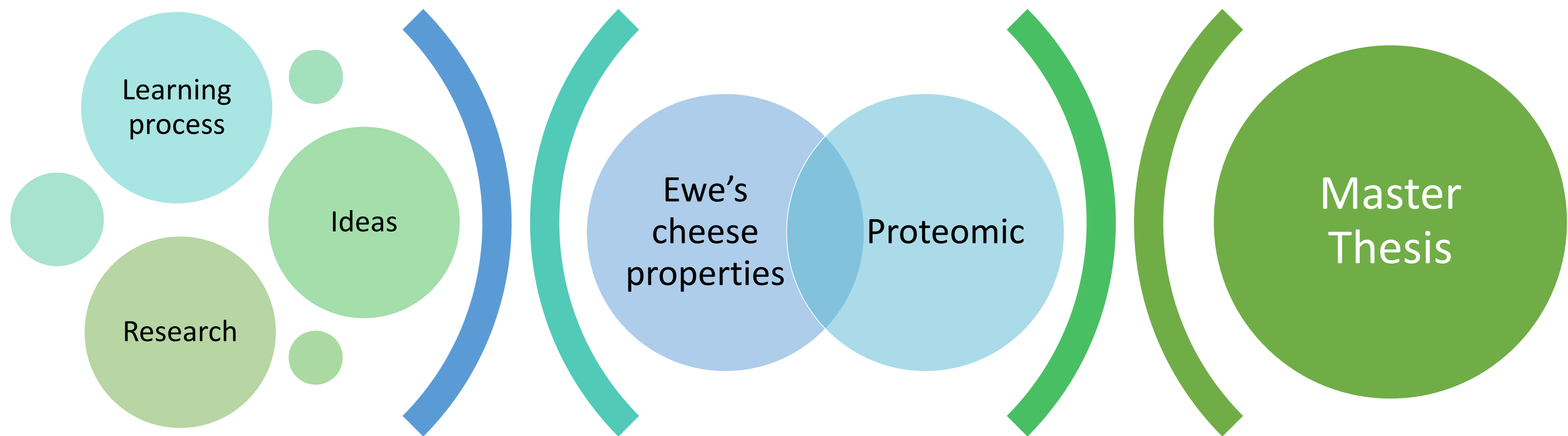


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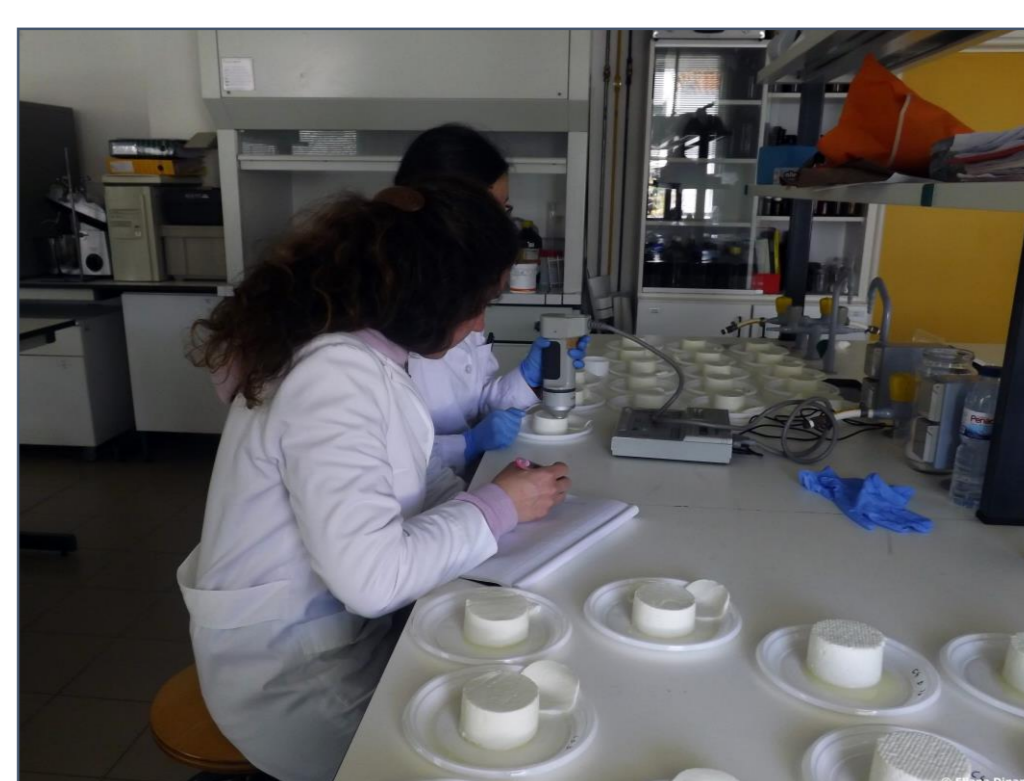
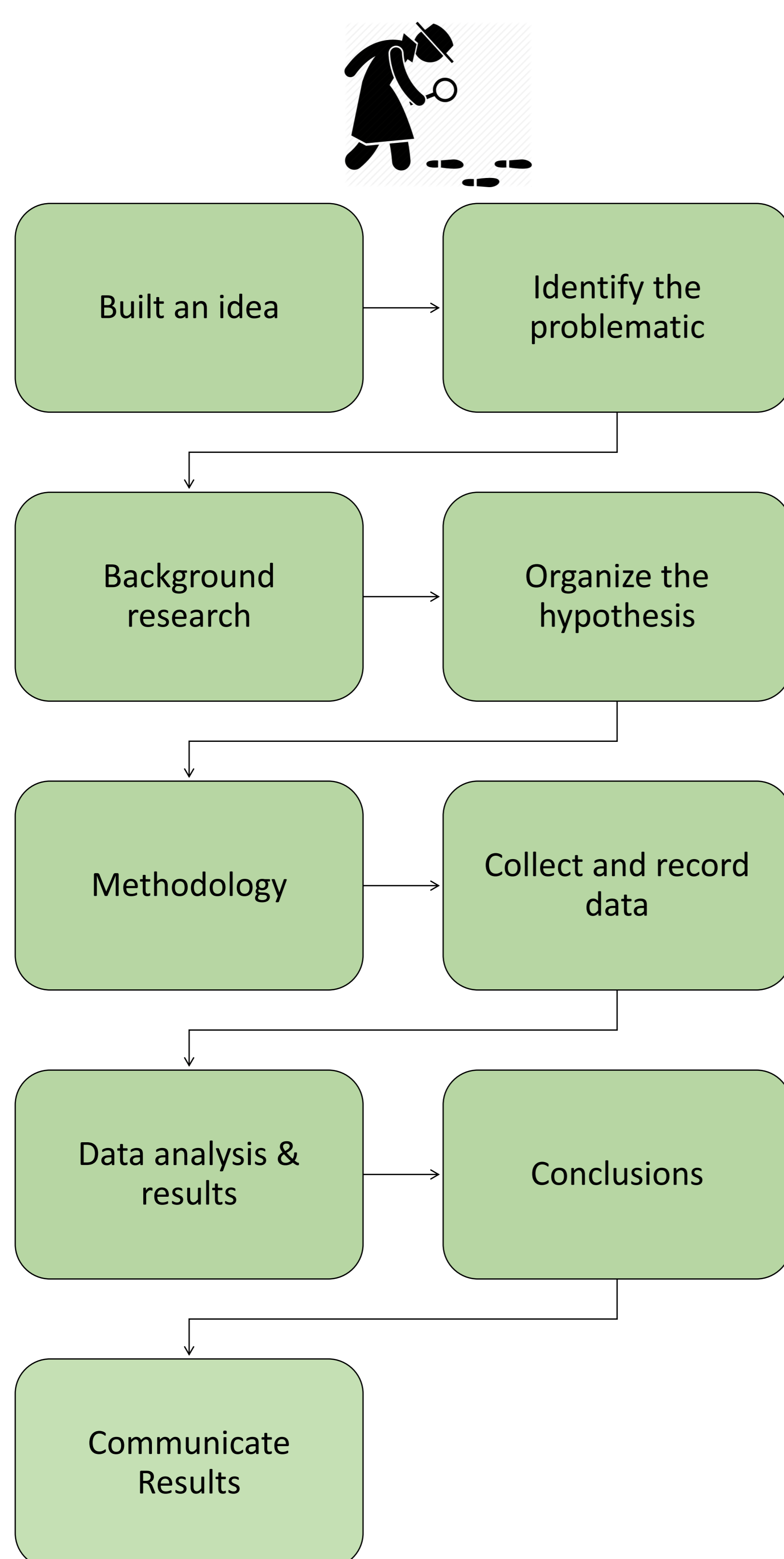


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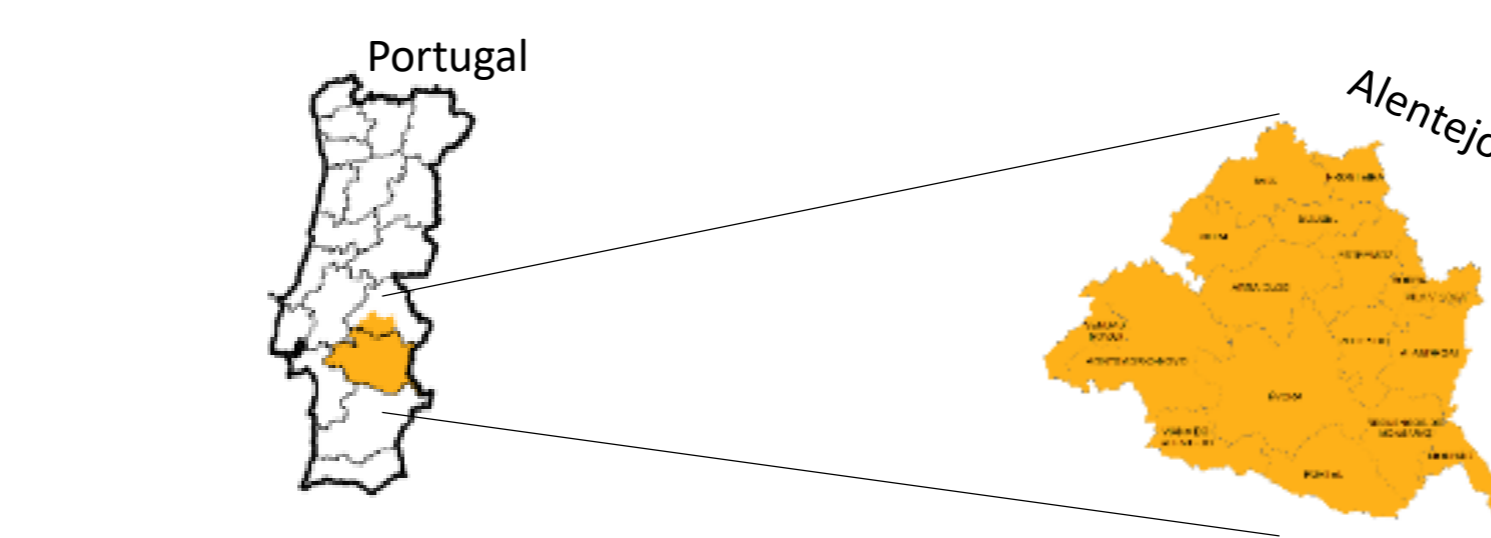
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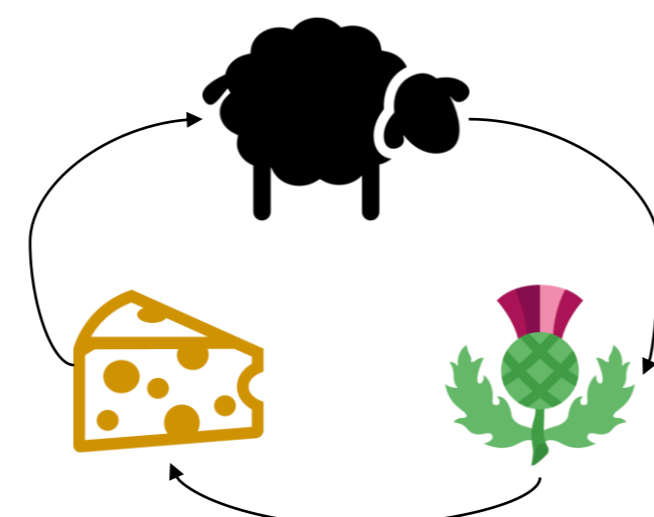
1. Following the steps of a Master's research



2. Background

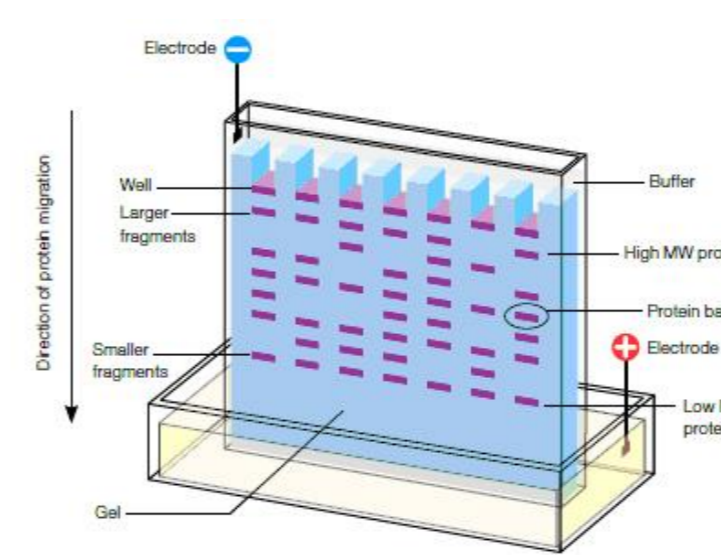


Ewe's cheeses have great tradition in Portugal, specially in Alentejo region, and have a high intrinsic value



Very appreciated unique sensory characteristics, coupled with long-recognized social and economic impacts

It's mandatory to understand the metabolic pathways in cheese ripening, and specifically what happens during **cheese proteolysis**



Schematic of electrophoretic protein separation in a polyacrylamide gel (Source: Bio-Rad Laboratories)

Objective

To determine the best **proteomic method** to study cheese proteolysis of soluble and insoluble fractions of cheese



3. Analysis and conclusions

Three electrophoretic techniques have been analysed

For the insoluble fraction of cheese

Urea polyacrylamide gel electrophoresis (Urea-PAGE)

11 bands were identified

According to Marcos *et al.* (1979) are translated in 4 casein (CN) areas:

γ-CN
β-CN
α₂-CN
pre-α₁-CN

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Difficult to separate caseins since they have **similar molecular masses**

For the soluble fraction of cheese

Two-dimensional gel electrophoresis (2-DE)

It's possible to observe a various number of spots, which are translated into proteins

In order to know more about this, there is a need of an identification of the differentially expressed proteins

Conclusions

- 1 **Urea-PAGE** → Best method for cheese insoluble fraction analysis
- 2 2-DE showed an increase of the soluble fraction during maturation, that according to other results we believe that are peptides resulting from the degradation of caseins, showed by the urea-PAGE results.
- 3 **Urea-PAGE & 2-DE** → Complementary in the study of proteolysis of ewe's cheese.