PhD DISSERTATION PROJECTS

Investigation of fermentation process to improve the sensory and nutritional attributes of cheeses

Matteo Roattino (Matteo.Roattino@student.unibz.it)

Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy

Tutor: Prof. Marco Gobbetti

Co-supervisor: Dr.ssa Olga Nikoloudaki

This PhD thesis research project is aimed to deal with the new challenges in dairy science. Novel plant based (PB) sources have been investigated to find a combination of plant-based ingredients (flours and vegetable oil source) to reproduce the sensory and nutritional characteristics of a traditional animal based (AB) cheese. Furthermore, considering that the market request of biologically produced cheeses is on the rise, we aim to study the variations and evolution in the microbiota of a semi-hard South-Tyrolean AB cheese, produced on a bio farm from cows both grazing and enclosed.

Studio degli effetti della fermentazione nel miglioramento del profilo sensoriale e nutrizionale dei formaggi

Questo progetto di tesi di dottorato affronta le nuove sfide del settore lattiero-caseario. Nuovi ingredienti vegani sono studiati per valorizzare la combinazione (fonti di farine e oli vegetali) capace di riprodurre il profilo sensoriale e nutrizionale di un formaggio tradizionale nei formaggi vegani. Inoltre, grazie all’aumento della domanda di formaggi biologici, ci proponiamo ad investigare la variazione e l’evoluzione del microbiota di uno specifico formaggio tradizionale Altoatesino prodotto da un’azienda a conduzione biologica con latte proveniente da vacche al pascolo e in stalla.

# **1. State-of-the-Art**

There is an amazing microbiome behind every great cheese: a dynamic ecology made of starter and non-starter bacteria that evolve throughout fermentation and ripening, depending on the unique cheese type and manufacturing conditions (Ercolini, 2020). The cheese microbiome must be understood since the bacteria and their primarily biochemical processes will have a significant impact on the end product (Yeluri Jonnala et al., 2018). Traditionally, the research of artisanal dairy products has been based on culture dependent approaches, but the complexity of the cheese microbiota can have a negative impact on their efficiency (Biolcati et al., 2021). Hence, cultural independent approaches (such as metagenomics) have become more popular to better understand the microbial ecology, providing a surprisingly frequent identification of genera and species not previously associated with cheese ecosystems (Cotter & Beresford, 2017; Ercolini, 2020).

Organic production in the US has increased rapidly over the past decade with a consumer market for organic milk increasing from 1.9 to 5.0% of total milk sales (Snider et al., 2022). In order to satisfy physiological, epidemiological and developmental requirements for animals such as those which have free access to outdoor areas, organic animal farming management ensures better conditions than the conventional one. The regulation further establishes that at least 60% of feedstuffs are produced on the farm itself and includes specific guidelines for veterinary treatment. Moreover, organic farming is also providing benefits for the environment, such as a reduction in chemical inputs, soil conservation and reducing aquifer pollution (Scozzafava et al., 2020). The cheese microbiota comes from three sources: the indigenous milk microbiota, the inoculated starter cultures, and the cheese production environment (Anastasiou et al., 2022). There is a lack of data on the extent to which milk microbiota play an essential role in defining the product specificity of mountain dairy products. However, it has been hypothesized that the summer transhumance to mountain pastures would alter the microbial population of the milk, and that milk microbiota could affect the cheese-making process (Secchi et al., 2023). Furthermore, the most significant contributor to the environmental impact of cheese production is the raw milk production phase (Rencricca et al., 2023). Tzamaloukas et al. (2021) investigated the effect of the different farming management on Cyprus´s cheese biochemical profile. Accordingly, the ruminants' milk fat content was not influenced by any of their farming practices. However, under organic farming practices, milk and cheese contained increased values of total mono-unsaturated FA (MUFA) and poly-unsaturated FA (PUFA). Nevertheless, there was an increase in total mono-unsaturated FA (MUFA) and poly-unsaturated FA (PUFA) in milk and dairy products and a decrease in total saturated FA (SFA) as a result of organic farming practices.

The potential development of plant protein ingredients as a substitute for milk-based cheese are currently being investigated. It is, however, difficult to develop plant products that are composed and functionally compatible with the properties of traditionally made cheese. At this time, commercially available alternatives to cheese with plant proteins have relied significantly on not protein components such as starch and coconut oil for delivery of functions that result in low levels of protein and high fat content when a finished product is made (Grasso et al., 2023). Grossmann & McClements (2021) define plant-based cheese as follows: “Plant-based cheese is an edible material prepared from plant ingredients that is designed to have a similar appearance, texture, and flavour as animal-based cheeses”. In line with that definition, the design principle behind plant-based cheese production is to match the physicochemical and sensory attributes of a specific traditional cheese, a result which can be achieved by using a diversity of different ingredients and structuring methods determine by the cheese features required (Grossmann & McClements, 2021).

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **PB cheese - Studying the best combination of different ingredients and microorganisms** to find out the best quantities of different ingredients that can simulate the feature of a milk based Crescenza soft fresh cheese and evaluate the effect of the fermentation process.

A2) **AB cheese - Culture dependent approach** to roughly identify the cultivable microbial components of cheese.

A3) **AB cheese - Culture independent approach** to better understand the overall microbial ecology of the cheese over time. Consequently, the data obtained by the previous method will be compared with this one.

A4) **PB cheese - Evaluation of the texture and biochemical attributes** to explore the biochemical changes of the cheese during the fermentation process and to know its emulsion stability.

A5) **AB cheese - Physiochemical and biochemical analyses** to investigate the chemical components and their interaction in the cheese over time.

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***PB cheese - Studying the***  ***best combination of different***  ***ingredients and microorganisms*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Formulation of cheese alterative samples |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Selection of starter cultures |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***AB cheese – Culture dependent approach*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) microbial counts, isolations and DNA Extractions |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Rep-PCR and Identifications |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***AB cheese - Culture independent approach*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Metagenomics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***PB cheese - Evaluation of***  ***the texture and biochemical attributes*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Confocal Laser Scanner Microscopy |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2)Texture and Rheological analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Colloidal stability |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 4) Sensory Analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***AB cheese - Physiochemical and biochemical analyses*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Urea-polyacrylamide gel electrophoresis (PAGE) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Reversed-Face-Liquid Chromatography (FPLC) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Volatile Free Fatty Acids (VFAA) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 4) Volatile Organic Component (VOC) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

Anastasiou, R., Kazou, M., Georgalaki, M., Aktypis, A., Zoumpopoulou, G., & Tsakalidou, E. (2022). Omics Approaches to Assess Flavor Development in Cheese. In *Foods* (Vol. 11, Issue 2). MDPI.

Biolcati, F., Ferrocino, I., Bottero, M. T., & Dalmasso, A. (2021). Mycobiota composition of robiola di roccaverano cheese along the production chain. *Foods*, *10*(8).

Cotter, P. D., & Beresford, T. P. (2017). Microbiome Changes During Ripening. In *Cheese: Chemistry, Physics and Microbiology: Fourth Edition* (Vol. 1, pp. 389–409). Elsevier Inc.

Ercolini, D. (2020). Secrets of the cheese microbiome. In *Nature Food* (Vol. 1, Issue 8, pp. 466–467). Springer Nature.

Grasso, N., Bot, F., Roos, Y. H., Crowley, S. V., Arendt, E. K., & O’Mahony, J. A. (2023). Plant-Based Alternatives to Cheese Formulated Using Blends of Zein and Chickpea Protein Ingredients. *Foods*, *12*(7).

Grossmann, L., & McClements, D. J. (2021). The science of plant-based foods: Approaches to create nutritious and sustainable plant-based cheese analogs. In *Trends in Food Science and Technology* (Vol. 118, pp. 207–229). Elsevier Ltd.

Rencricca, G., Froldi, F., Moschini, M., Trevisan, M., Ghnimi, S., & Lamastra, L. (2023). The environmental impact of permanent meadows-based farms: A comparison among different dairy farm management systems of an Italian cheese. *Sustainable Production and Consumption*, *37*, 53–64.

Scozzafava, G., Gerini, F., Boncinelli, F., Contini, C., Marone, E., & Casini, L. (2020). Organic milk preference: is it a matter of information? *Appetite*, *144*(February 2019).

Secchi, G., Amalfitano, N., Carafa, I., Franciosi, E., Gallo, L., Schiavon, S., Sturaro, E., Tagliapietra, F., & Bittante, G. (2023). Milk metagenomics and cheese-making properties as affected by indoor farming and summer highland grazing. *Journal of Dairy Science*, *106*(1), 96–116.

Snider, M. A., Ziegler, S. E., Darby, H. M., Soder, K. J., Brito, A. F., Beidler, B., Flack, S., Greenwood, S. L., & Niles, M. T. (2022). An overview of organic, grassfed dairy farm management and factors related to higher milk production. *Renewable Agriculture and Food Systems*, *37*(6), 624–632.

Tzamaloukas, O.; Neofytou, M.C.; Simitzis, P.E.; Miltiadou, D. (2021). Effect of Farming System (Organic vs. Conventional) and Season on Composition and Fatty Acid Profile of Bovine, Caprine and Ovine Milk and Retail Halloumi Cheese Produced in Cyprus. *Foods 2021, 10, 1016.*

Yeluri Jonnala, B. R., McSweeney, P. L. H., Sheehan, J. J., & Cotter, P. D. (2018). Sequencing of the cheese microbiome and its relevance to industry. In *Frontiers in Microbiology* (Vol. 9, Issue MAY). Frontiers Media S.A.