Fermented sausage microbiome: investigation, storage and exploitation

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The aim of this doctoral research project is to study the microbiome of spontaneously fermented sausages and to define protocols for its conservation and propagation for subsequent exploitation in different ecosystems, using a multi-omics approach.

Microbioma di salami fermentati: studio, conservazione ed utilizzo

L'obiettivo di questo progetto di dottorato è studiare il microbioma di salumi a fermentazione spontanea e definire protocolli per la conservazione e propagazione del microbioma per un successivo riutilizzo in diversi ecosistemi, utilizzando approcci multi-omici.

# 1.State-of-the-Art

The food fermentation process was an ancient form of food preservation technology. Nevertheless, the technology has evolved beyond food preservation to become a tool for creating desirable organoleptic, nutritional, and functional attributes in food products, and has become a cultural and traditional norm within communities. The particular characteristics of typical fermented foods from each region are linked to the use of local ingredients and specific production techniques. In particular, the qualitative characteristics of spontaneously fermented sausages depend on the quality of ingredients and raw materials, and on microbial composition involved in specific processing and ripening conditions (Aquilanti et al., 2007).

Meat fermentation is driven by a complex succession of microbial consortia as weel as by biochemical transformations able to produce a variety of metabolites responsible for flavors, odors, and texture (Srinivas et al., 2022). In modern sausage production, the use of starter cultures is increasing to guarantee safety and standardize the properties of the final product (Cocolin et al., 2001). However, commercial starters, mainly composed by lactic acid bacteria and coagulase-negative cocci, reduce the peculiar organoleptic characteristics of spontaneously fermented sausages and lead to losses the typicity (Daga et al., 2021, Franciosa 2022). Besides, according to the FAO report "The State of the World's Biodiversity for Food and Agriculture”, the biodiversity present in our ecosystems is in decline worldwide (FAO,199). Consequently, knowledge and conservation of the microbial biodiversity of spontaneously fermented sausages play an important role in protecting typical national products in order to maintaining biodiversity and sustainability.

Therefore, this PhD thesis project will be to study of the microbiome of Italian fermented sausages, through:

-characterization of community structure, diversity, activity and interactions in their natural environments;

-optimization of conditions and protocols for long-term preservation and propagation of microbiomes;

-identification of analytical methods to follow the structure of the microbiomes during the storage and the propagation.

# 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 display in Table 2:

A1) **Definition of Standard Operation Procedures (SOPs**) for the sampling (A1.1) and DNA extraction from fermented sausages (A1.2)

A2) **Study the microbiome of different typical spontaneously fermented sausages** using microbiological (A2.1), metabolomic using GC-MS instrument (A2.2) and metagenomic (Shotgun sequencing) analysis (A2.3).

A3) **Optimization of conditions and protocols** for long-term preservation and propagation of microbiomes using different preservation matrices (pellet, meat, first 10-fold serial dilution) (A3.1) and propagation matrices (Nutrient Broth, Brain heart infusion, meat juice) (A3.2)**.**

A4)**Identification of analytical methods** to follow the quality of microbiomes during storage and propagation using microbiological (A4.1), metabolomic (A4.2) and metataxonomic (16S sequencing) analysis (A4.3).

A5) **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) | ***SOPs*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1)SOPs definition |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) SOPs Validation |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***Study of the microbial ecology*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Microbial and sensory analysis |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Metabolomic analysis |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
|  | 3) Metagenomic analysis  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Protocols optimization*** |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Microbiome preservation protocols |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Microbiome propagation protocols |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Quality control of microbiomes***  |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 1) Microbial analysis |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 2) Metabolomic analysis |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 3) Metataxonomic analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Thesis and Paper Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# 3. Selected References

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