Bioactive-Rich Mushrooms for Food Reformulation (BIOMUSH-FOOD)

Giulia Bearzi (giulia.bearzi@unimi.it)

Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy

Tutor: Prof. Cristina Alamprese; Co-tutor: Prof. Manuela Rollini

This PhD research project is aimed at the development and optimisation of foods enriched with bioactive-rich mushrooms (BRMs) by applying DoE techniques and RSM. After the optimization of BRMs cultivation conditions, the obtained biomasses will be included in different food products. In particular, BRMs integration will be optimized for milk- and plant-based desserts as well as for traditional and gluten-free baked products. The enriched foods will be studied for quality characteristics, including sensory properties, and in vitro digestibility to evaluate the fate of bioactive compounds in digestate.

Funghi ricchi in composti bioattivi per la riformulazione di prodotti alimentari (BIOMUSH-FOOD)

Questo progetto di ricerca di dottorato è finalizzato allo sviluppo e all'ottimizzazione di alimenti arricchiti con funghi ricchi di composti bioattivi (BRMs) mediante l'applicazione di tecniche DoE e RSM. In seguito all'ottimizzazione delle condizioni di coltivazione dei BRMs, le biomasse ottenute saranno incluse in diversi prodotti alimentari. In particolare, l'integrazione con BRMs sarà ottimizzata per dessert a base latte e vegetale, nonché per prodotti da forno tradizionali e senza glutine. Gli alimenti arricchiti saranno valutati in termini di caratteristiche qualitative, incluse le proprietà sensoriali, e di digeribilità in vitro, al fine di indagare il destino dei composti bioattivi nel digestato.

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

Global concerns around the consumption of animal products and their adverse effects on health and environment have led to significant growth in the plant-based protein field. Plants, like other natural products including mushrooms, have seen an increase in their production also due to their content in bioactive and health-promoting substances.

Bioactive-rich mushrooms (BRMs) can be defined as macroscopic fungi, mostly higher *Basidiomycetes*, which are used in the form of extracts or powder for prevention, alleviation, or healing of diseases and/or for nutritional reasons (Lindequist *et al*., 2014). Presently, BRMs are mainly used as dietary supplements or functional foods, especially in Eastern countries. The most important BRMs species are *Ganoderma lucidum, Lentinula edodes, Agaricus brasiliensis, Pleurotus ostreatus, Cordyceps sinensis, Grifola frondose*,and some others.

From a nutritional point of view, mushrooms are valuable health foods since they have a significant amount of dietary fiber and are poor in calories and fat (Roncero-Ramos *et al*., 2017). Moreover, they have a good protein content (20–30% of dry matter), which includes most of the essential amino acids, a nutritionally significant content of vitamins, and trace minerals. They contain bioactive compounds of high medicinal value such as lectins, polysaccharides, phenolics, and volatile organic compounds, which are considered as relevant responsible agents for healthy activities including antitumor, antioxidant, antihypercholesterolemia, and antidiabetic effects.

Besides nutritional composition of mushrooms, it could be interesting to evaluate their *in vitro* digestibility, especially to study whether bioactive compounds are available during digestion, which can have benefits on health. Different *in vitro* methods have been developed to simulate digestion processes, from the oral to the small intestinal phases and, occasionally, large intestinal fermentation, taking into account the digestive enzymes, their concentration, pH, and digestion time (Minekus *et al*., 2014). In particular, an international network of multidisciplinary experts harmonized the digestion conditions by publishing the INFOGEST 2.0 method (Brodkorb *et al*., 2019).

Thanks to the valuable characteristics, BRMs could be used to reformulate foods to enrich the nutritional profile and provide consumers with good alternative proteins. In the literature there are still few food applications of mushroom biomasses, mainly focused on bakery products (Li *et al*., 2008; Prodhan *et al*., 2015), thus asking for new investigations and for the health benefits validation in the final products. Lu *et al*. (2020) reported that novel applications for mycelia- and fruiting body-based macrofungal foods are being explored for the improvement of food flavor and nutrition. However, in some cases an extraction phase is necessary, which may negatively influence the structure and biological activity of the bioactive compounds.

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

The overall objective of this PhD research project is to integrate BRMs, grown on agro-food wastes, in different food formulations to improve their nutritional profile. To fulfil the overall objective, the PhD project will be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Literature survey about BRMs.** The different characteristics of BRMs will be studied, focusing, in particular, on the nutritional aspects and the bioactive compounds with health implications. Moreover, a survey about cultivation methods and conditions will be performed, to study the relationship with biomass properties.

A2) **Optimization of mushroom biomass cultivation conditions.** Mushrooms with the best technological and nutritional characteristics will be chosen to study cultivation performances both in solid and liquid state. BRMs will be grown on different agrifood wastes, applying different environmental parameters (i.e., temperature, humidity, organic components) to study the effect on the biomass characteristics and to optimize cultivation conditions. A further in-depth analysis of the biomass will involve the evaluation whether to use it as such or to proceed with the extraction of proteins and bioactive components.

*Milestone (M) 1*: Optimized cultivation conditions to obtain valuable BRMs biomasses for food applications.

*Risk (R) 1*: Difficulties to obtain a constant quality biomass. *Mitigation action*: introducing new parameters to control the BRMs growing or changing mushroom species.

*R2*: Difficulties to obtain dry biomasses that maintain the nutritional properties. *Mitigation action*: considering an extraction phase and/or use a milder preservation technology that respects the biomass values.

A3) **Food development and optimization.** Design of Experiment techniques (DoE) and Response Surface Methodology (RSM) will be used to find the best integration level of BRMs in terms of final product quality and to optimize food formulations. In particular, BRMs integration will be optimized for milk- and plant-based ice creams and mousses as well as for traditional and gluten-free bread and cookies. For each product, a comparison will be made considering reference food formulations. Each product will be analysed for quality attributes, including sensory features, and for nutritional properties. In particular, *in vitro* digestibility will be studied according to the INFOGEST protocol to verify the digestion fate and the bioaccessibility of the bioactive compounds.

*M2*: Optimized formulations of BRMs enriched foods.

*R3*: Difficulties in the design of foods with the chosen mushroom biomasses. *Mitigation action*: revision of the formulation and/or choice of different food products.

A6) **Data elaboration.** The most suitable statistical tools will be applied to all the collected data.

A7) **Manuscript preparation.** During the three-year project, scientific papers and oral/poster communications will be prepared, thus assuring proper dissemination of the results.

***Table 1***Gantt diagram of the PhD thesis project

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|  | | ***Year 1*** | | | | | | | | | | | | ***Year 2*** | | | | | | | | | | | | ***Year 3*** | | | | | | | | | | | |
| Activity | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A1) | ***Literature survey*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Optimization of BRMs cultivation conditions*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Food development and optimization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Baked products* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Desserts* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Data elaboration*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A7) | ***Manuscript preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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