Application of innovative technologies for the functionalisation of alternative proteins and the associated functional and rheological characterisation

Federico Drudi (federico.drudi3@unibo.it)

Dept. Food Science and Technology, University of Bologna, Cesena, Italy

Tutor: Dr. Urszula Tylewicz Co-tutor: Dr. Silvia Tappi

The growing market for plant-based products necessitates specific ingredients to address new technological challenges. Non-thermal technologies, including cold plasma (CP), pulsed electric field (PEF), ultrasound (US), and high-pressure processing (HPP), offer sustainable and efficient approaches for protein functionalization. This PhD project aims to evaluate the use of these innovative treatments on legume flours, studying their effects on functional properties. The modified flours and their potential applications in bakery, snack, or dairy replacement products will be assessed. The project also investigates the environmental sustainability of the functionalization process. The research contributes to understanding protein modification techniques for developing improved plant-based alternatives.

Applicazione di tecnologie innovative per la funzionalizzazione di proteine alternative e relativa caratterizzazione funzionale e reologica

Il crescente mercato dei prodotti a base vegetale richiede ingredienti specifici per affrontare specifiche sfide tecnologiche. Le tecnologie non termiche, come CP, PEF, US, e HPP, offrono approcci alternativi per la funzionalizzazione delle proteine. Questo progetto mira a valutare l'utilizzo di tali trattamenti su farine di legumi, studiandone gli effetti sulle proprietà funzionali, per poi esplorare le potenziali applicazioni in prodotti da forno, snack o sostituti dei latticini. Il progetto si prefigge di analizzare anche la sostenibilità ambientale del processo di funzionalizzazione. Questo progetto mira a contribuire alla comprensione delle tecniche di modifica applicabili su farine proteiche.

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

Following recent trends in the food sector, an increasing market share of plant-based products can be observed, ranging from dairy substitutes to alternative meats (GFI Europe, 2023), trend that is expected to increase further, also thanks to a shift in consumer preferences towards more environmentally friendly and cruelty-free products. Finally, especially in the richest countries, the development of protein foods from vegetable matrices has experienced tremendous growth (Fasolin *et al*., 2019; Aschemann-Witzel *et al*., 2021). As a result of this situation, more and more companies are launching such products, creating a need for specific ingredients. As it is well known, many plant-based products are characterised by a long list of ingredients used to overcome technological problems and meet specific requirements in terms of sensory properties and stability, to mimic the animal counterpart (Akharume *et al*., 2021). Unfortunately, proteins derived from plant sources differ not only in terms of nutritional value but also in terms of technological properties. Solubility and gelling properties are generally lower compared to those of animal origin (especially at pH close to neutrality), making their use in formulations more complex (Akharume *et al*., 2021).

One response to these needs can be the modification of plant proteins to obtain products with specific properties. Protein functionalisation has been used in the food sector for several years (Panyam and Kilara, 1996; Messens *et al.,* 1997). Recently, however, the interest of scientific research has shifted from the traditional chemical-enzymatic modifications (e.g. glycosylation, acetylation, hydrolysis and cross-linking) to physical modifications obtained by applying non-thermal technologies such as cold plasma (CP), pulsed electric field (PEF), ultrasound (US), high-pressure processing (HPP) and extrusion, which make the whole functionalisation process more sustainable and efficient (Mirmoghtadaie *et al*., 2016; Sun-Waterhouse *et al*., 2017; O'sullivan *et al*., 2022).

Although the four technologies mentioned above are all considered non-thermal, they are based on different functional mechanisms. CP is able to favour the rearrangement of the protein structure thanks to the main action of the reactive oxygen and nitrogen species (RONS) formed (Basak and Annapure, 2022), while in HPP, the denaturing effect on the proteins is achieved by the compression that causes the collapse of the structures with empty spaces (such as the β-sheets) (Wang *et al*., 2022). One of the most studied technologies is the application of US, where the short and localised pressure and temperature shocks (thanks to the cavitation phenomenon) can act both at the macroscopic level on the size of the particles and at the microscopic level, denaturing the proteins and exposing the most lipophilic areas (O'sullivan *et al*., 2022). Finally, the least used technology for this purpose is PEF, as its efficacy and mechanism of action on proteins is still controversial (Han *et al*., 2018).

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

The aim of the project is to evaluate the possibility of using innovative non-thermal treatments to modify the functional properties of legume flours and subsequently use these optimised ingredients in product formulation to meet specific needs. The project can be divided into the following tasks, which are also time-framed in Table 1.

A1) Literature review of previous studies on the application of non-thermal technologies for flour modification and protein denaturation and research on the specific needs in the formulation of new plant-based products.

A2) Evaluation of the properties of different legume flours with the aim of identifying one or two specific legumes to work on.

A3) Application of different treatments to the selected flours, with the aim of studying the effects on functional properties. The effect of PEF, US, HPP and CP will be evaluated and optimisation of parameters for the best treatment will also be studied.

A4) Evaluation of the functional and rheological properties of the modified flours, with the aim of identifying some key aspects in which the flour has been modified and try to find a suitable use in a final product.

A5) Development of a bakery, snack or dairy substitute product using the ingredients obtained to understand if the functionalisation process can improve the performances of the final product.

A6) Assessment of the sustainability level of the overall functionalisation process. An environmental and economic assessment will be done for each newly developed ingredient, taking into account other, more conventional functionalisation systems.

A7) Writing and editing of the final dissertation, scientific papers and attending conferences with oral and/or poster presentations.

*Table 1:* Gant chart with the expected duration of different research activities.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activity Months** | **2** | **4** | **6** | **8** | **10** | **12** | **14** | **16** | **18** | **20** | **22** | **24** | **26** | **28** | **30** | **32** | **34** | **36** |
| **A1** | Literature review and research |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **A2** | Flour characterization and identification of target characteristics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **A3** | Treatments application and optimization for flour functionalization (PEF, US, HPP, CP) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **A4** | Quality evaluation and identification of possible usages for the product |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **A5** | Application of the created flours for new final products |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **A6** | LCA and LCC of developed processes |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **A7** | Writing dissertation, scientific papers |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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