**Pilot and industrial scale design, optimization and development of innovative plant-based functional food and supplements**

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This PhD thesis research project is aimed at developing biotechnological protocols for obtaining new functional foods and beverages. The research will involve the set-up of fermentation protocols, including the selection of *i*) ingredients, *ii*) starters, and *iii*) process parameters. The optimized formulation will undergo a more in-depth characterization of the compounds responsible for the functional activities as well as their effect on human health, followed by the industrial scale-up of the experimental prototypes.

Strategia sperimentale per la progettazione ottimale di alimenti e bevande funzionali

Questo progetto di dottorato è finalizzato alla messa a punto di protocolli biotecnologici per lo sviluppo di alimenti e bevande funzionali, attraverso l’uso di batteri lattici selezionati e la selezione di ingredienti vegetali. La formulazione ottimizzata subirà una caratterizzazione più approfondita dei composti responsabili delle attività funzionali e del loro effetto sulla salute umana, seguita dallo scale-up industriale dei prototipi sperimentali.

1. **State-of-the-art**

The concerns about environmental impact and sustainability of animal-based diets, as well as human health issues thereof related, have fuelled consumer demand for dairy alternatives, paving the way to plant-based yogurt-like (YL). The term YL refers to products similar to conventional yogurt in terms of structure, sensory properties and ability to keep lactic acid bacteria alive and viable for a long time, however, obtained from matrices other than milk. Cereals, pseudocereals and legumes are widely used as main ingredients in YL formulations given the wide availability and moderate cost and since they still represent the main source of macro and micronutrients worldwide. These grains represent alternative protein sources to animal-derived ingredients being rich in proteins of high biological value, fibres, and bioactive compounds (Gobbetti et al., 2020). Nevertheless, the nutritional and functional value of plant matrices can be compromised by the presence of anti-nutritional factors (ANF), which can adversely affect their nutritional and sensory profile. The most common ANF in plants are condensed tannins, saponins, phytic acid, α-galactosides and trypsin inhibitors. Fermentation, in addition to having a positive impact on the nutritional value, sensory and technological properties of vegetables products, has been thoroughly investigated as a biotechnological process capable of reducing the impact of ANF (Gobbetti et al., 2020).

Being characterized by proteins of different nature compared to milk once, plant protein rarely precipitate due to acidification and this is one of the major problems related to the production of YL from vegetable matrices. YL are mainly obtained after the fermentation of aqueous extracts or suspensions in water of cereal flours, pseudocereals, legumes, homogenized fruits and over the years there have been different attempts to obtain a structure similar to that of traditional yogurt. Moreover, often YL production process is long and expensive, because compared to animal sources plant matrices have lower protein content with different coagulation modes thus requiring structuring agents and emulsifiers. The acidification caused by fermentation also leads to a destabilization of plant protein causing, during storage, a loss of viscosity with consequent separation of the aqueous phase (Bernat et al., 2014), hence, different solutions can be adopted. Although not responding to the growing demand for clean-label products, additives can be used. Alternatively, lactic acid bacteria strains able to produce exopolysaccharides (EPS) which improve YL structural properties can be employed as starters or starch gelatinization can be carried out before fermentation. Starch gelatinization can also prevent phase separation while reducing contamination by endogenous microorganisms before inoculation of selected starters (Pontonio et al., 2020). Based on the above considerations the aim of this research project is to set-up biotechnological protocols for the development of functional YL which comply with the concept of clean label.

1. **PhD Thesis Objectives and Milestones**

This PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Exploiting the possibilities of functionalization (enrichment in bioactive compounds) of a rice-based matrix** intended as main base for YL production, will be achieved through the following operational phases:

Screening of i) different plant-based ingredients to be used for the YL fortification (A1.1); ii) lactic acid bacteria selected for pro-technological and functional characteristics (e.g. GABA and EPS synthesis) (A1.2). The analytical plan for the full set of the YL prototypes will include the determination of the nutritional label, kinetics of acidification, total titratable acidity; concentration of organic acids (lactic and acetic acids), free amino acids (including the functional GABA), and peptides. Microbiological analysis will also be performed during the different bioprocessing stages and during storage in refrigerated shelf-life. Aiming at defining the technological properties and EPS synthesis by the starter, viscosity will be also determined. The recipes will be then optimized, based on the results obtained from the nutritional and functional characterization of the YL (A1.3).

A2) **The experimental prototypes will be evaluated through a sensory characterization** of the product (A3.1) and the most interesting theses, will be object of industrial scale-up of the production process (this activity will be carried out during the 6-months periods in Celery company (A2.2).

A3) **A nutritional-functional characterization of the selected YL prototypes** will also include the evaluation of antioxidant activity by *in vitro* and *ex-vivo* (on human or animal cell cultures) assays; the characterization of the molecules responsible for the antioxidant activity (this activity will be performed during the 6-months period at University of Granada, Spain); the evaluation of the *in vitro*-protein digestibility and *in vitro* glycaemic index (A3.2).

A4) **Evaluation of YL effects on microbiota.** Once the development of the selected prototypes has been completed, collaborative investigations will be carried out aiming at determining the effect of the new YL products on the gut microbiota and on the diet (A4).

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) | ***Possibilities of functionalization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Screening of ingredients |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Screening of LAB |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) YL optimization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Sensory characterization and scale-up*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Sensory characterization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Industrial scale-up |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Nutritional-functional characterization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Evaluation of antioxidant activity |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Evaluation of protein digestibility and glycaemic index |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Evaluation of YL effects on microbiota*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Writing and editing*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

1. **Selected References**

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