

FELLOWSHIP SUMMARY REPORT

Name: Mattia Di Nunzio

Title: Innovative eco-friendly Bio-basEd processes to recover BioactivE compoundS from agri-food wasTe (BE BEST)

CRP fellowship theme: Theme 3 - Transformation Technologies and Innovation

Host institution: Dep. of preventive medicine and public health, food sciences, toxicology and forensic medicine, University of Valencia, Spain

Host collaborator: Prof. Maria Jose Esteve Mas

Date: 11th September – 17th December 2023

1. What were the objectives of the research project? Why is the research project important?

The main objective of the project was to investigate the possible and synergic application of combined hydrolytic enzymes (pectinase, cellulase, and protease) alone or in combination, at two different concentrations followed by extraction with different six different NaDES formulation (and two different conventional extraction as controls) for polyphenols recovery from orange fruit byproducts (peel).

The importance of the project is related to the fact that food processing generates high waste and byproducts, causing a negative environmental impact and significant disposal expenses. Many of these biomaterials, including citrus wastes, are a source of valuable compounds that could be used in various commercial sectors, including food industries. Several techniques have been used to recover polyphenols from food byproducts, but most are expensive and unsafe for human health and the environment. In recent times, several "green" novel extraction techniques have been used to optimize the extraction of bioactive compounds. Natural deep eutectic solvents (NaDES) have become an exciting candidate. Considering the extraction recovery of bioactive compounds, the type of solvent and the chemical form in which food bioactives are bound to the food matrix must be considered. The overall results obtained in this project will enhance the value of agri-food waste, fitting the challenge of the theme identified by the CRP research fellowship.

2. Were the objectives of the fellowship achieved?

The main objectives of the project were to define and fine-tune the extraction process and evaluate the best combinations between enzymatic treatments and NADES solvents used for the recovery of phenolic compounds from orange peel. The experimental conditions considered were:

- No enzyme treatment
- Enzyme treatment with pectinase at two different concentrations
- Enzymatic treatment with cellulase at two different concentrations
- Enzyme treatment with protease at two different concentrations
- Combination of all enzymes used individually at two different concentrations
- For each condition reported, extraction was performed with 6 different NADES (ChCl:LP:MA, LP:MA, Be:MA, LA:Glu, MA:Glu, and ChCl:LA) and 2 convection extractions with water and a 50% hydroalcoholic mixture. The total number of experimental conditions were 64, each performed in duplicate.

The main outcomes considered to evaluate the objectives were:

- Determination of total flavonoid content by colorimetric assay
- Determination of polyphenol content and profile by HPLC
- Evaluation of antioxidant capacity by DPPH assay and FRAP assay

At the end of the overseas sojourn, the extraction process setup (completed at the end of week 2) was carried out and the extraction processes for all experimental combinations considered. In addition, the best combination of various enzymatic treatments and NADES solvents for polyphenol recovery was determined by colorimetric assay of flavonoids. Also, at the end of the sojourn period, total antioxidant activity was evaluated, and samples were prepared and injected into HPLC. Chromatogram analysis for qualitative-quantitative determination of the phenolic fraction is currently underway.

3. What were the major achievements of the fellowship? (up to three)

As indicated above, the main achievements of the fellowship were:

- Set up of the extraction conditions.
- Determination of flavonoid content by colorimetric method and evaluation of total antioxidant capacity by DPPH and FRAP assay.
- Qualitative-quantitative evaluation of the phenolic profile by HPLC (chromatogram analysis currently in progress).

4. Will there be any follow-up work?

Yes, there will be.

Specifically, upon completion of further determinations that are scheduled between January and February at the University of Milan designed to more thoroughly characterize the chemical and physical characteristics of the extracts obtained during the stay at the University of Valencia, several conference abstracts and a paper are planned to be submitted by 2024 to an international conference and to a peer-reviewed journal, respectively. CRP fellowship will be included in the acknowledgement. In addition, the CRP-funded period abroad allowed for the consolidation of relations between the two universities through the implementation of a project that was submitted for a Spanish national competitive grant and the formalization of an application for an integration of stay abroad by a postdoctoral researcher to spend two years at the University of Milan to conduct scientific research.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Actors in the food supply chains are under increasing pressure to meet the demand for fruits and vegetables as the world's population is predicted to increase by 3 billion people over the course of the next 30 years. However, with the world's population growing and the demand for enough nutritious food growing, food loss and waste represent serious and growing problems. Greenhouse gas emissions from food waste decomposition in landfills and unnecessary emissions from cultivating, transporting, and selling food waste are also linked to food waste. Through a combined approach of bio-based methodologies and environmentally friendly extraction solvents with minimal environmental impact, BE BEST will enable the extraction of bioactive compounds from citrus processing byproducts with greater efficiency and to

maximize the value of waste from the agri-food chain. Additionally, we think that enhancing the recovery of bioactive chemicals will enable the agri-food chain's outputs to be better utilized and enhanced, raising their market value and enabling reuse—thus realizing the circular economy's goal.

6. How was this research relevant to:

BE BEST's goals align perfectly with the principles of the Circular Economy, which has been hailed as a solution to address our current unsustainable resource use and help businesses better understand the natural inputs that support them. These principles include waste valorization, cost reduction, and increased resource efficiency, all of which present substantial opportunities to reduce food waste. Additionally, we think that enhancing the recovery of bioactive chemicals will enable the agri-food chain's outputs to be better utilized and enhanced, raising their market value and enabling reuse—thus realizing the circular economy's goal. Furthermore, the CRP research fellowship recognized that the BE BEST project's goals align with the challenge of the topic "transformational technologies and innovation" under the title "novel waste reduction technologies."

7. Satisfaction:

I can only confirm that the CRP fellowship has undoubtedly confirmed my highest expectations and, as a senior assistant professor tenure track, has increased my opportunities to advance to associate professor and therefore my career possibilities. I did not encounter any problems or critical issues during the preparation and submission phase of the project. In addition, correspondence with the CRP offices via email was always fast and efficient.

8. Advertising the Co-operative Research Programme:

I learned about the CRP fellowship from some departmental colleagues. I have no particular suggestions for increasing visibility.