From agri-food waste to high-value compounds via green technology approaches

Agostina Colacicco (agostina.colacicco@unimi.it)

Dept. of Food, Environmental and Nutritional Sciences, University of Milan, Italy

Tutor: Dr. Martina Letizia Contente

The aim of this PhD project regards the use of green technologies such as biocatalysis, enzyme immobilization, and “in continuous” processes to transform molecules or extracts derived from agri-food waste in natural valuable compounds/enriched extracts with attractive biological properties (circular economy approach). The combination of the above cited technologies demonstrated to enhance the sustainability, selectivity and productivity, of chemical processes.

Dai rifiuti agoalimetari ai composti ad alto valore attraverso approcci di green technologies

L'obiettivo di questo progetto di dottorato riguarda l'uso di tecnologie green come biocatalisi, immobilizzazione enzimatica e processi “in continuo” per trasformare molecole o estratti derivati da scarti agroalimentari in composti naturali di pregio/estratti arricchiti con interessanti proprietà biologiche (approccio di economia circolare). È stato dimostrato che la combinazione delle tecnologie sopra citate migliora la sostenibilità, la selettività e la produttività dei processi chimici.

# 1. State-of-the-Art

With the population growth approaching now 8 billion people as well as the modification of the life-style and the eating habits, a large increase of the agri-food companies involved in the production and processing of food-related compounds was observed, so much that agri-food-industrial waste became a significant environmental and economic problem (Sagar *et al*., 2018). In fact, tones of agri-food residues generally made up of seeds, peels, leaves, skins, branches, trunks, roots are typically unexploited and need to be managed and disposed. Moreover, most of these residues have demonstrated to contain natural valuable compounds with attractive biological properties. In this context, waste minimization together with the possibility of bioactive recovery and their modification through green methodologies can represent an appealing business opportunity for the agri-food, nutraceutical, cosmetic and pharmaceutical companies, among others (Tonini *et al*., 2018).

Biocatalysis, the branch of biotechnologies aiming at using living systems or their parts (enzymes) to catalyze chemical reactions has been recognized as a valuable tool for chemists if stable and robust biocatalysts can be employed (Contente *et al*., 2021). In this context, “extremozymes” proteins derived from microorganisms adapted to live in drastic environments (e.g., high/low temperature solvent and pH) demonstrated to be more tolerant to industrial processes with respect to the mesophilic counterparts.

To further increase enzyme stability and allow their recover and reuse, immobilization techniques on solid supports can be exploited, also facilitating catalyst incorporation in flow chemistry reactors (Bommarius *et al*., 2013).

Among the advantages of “in continuous” systems high local concentration of the biocatalyst, superior mass and heat transfer, impacting on reaction times as well as the addition of in-line work-up and purification steps enhancing the system automation, are noteworthy. Moreover by adding more bioreactors in series it is possible to set up multi-enzymatic cascade reactions (Benítez-Mateos *et al*., 2021).

In this project we decided to focus our attention on natural glycosides and their transformation into the corresponding aglycones characterized by superior bioavailability and/or bioactivity. To do this extremophilic β-glycosidases were selected as biocatalysts together with four different agri-food residues as glycosides natural sources.

Soybean cultivation produces one of the largest agri-waste worldwide. The residues contain three types of isoflavones (daidzein, genistein, and glycitein), which can be found mainly as glycosides. Recently, commercial preparations of isoflavones have come to the public attention due to their positive effects on cognitive function. However, when the biological activities of these compounds are considered, the bioavailability of the aglycones has been suggested to be higher than that of the glycosides; but they represent only a minor constituent of soy-residues. B-glycosidases can be used to hydrolyze isoflavone glycosides to their aglycones, thus obtaining high-value products. Among natural fragrances, vanilla is the most employed in the perfume, cosmetic and food industry, due to its aromatic characteristics. Being its plant extraction quite expensive, several synthetic strategies have been developed for vanillin preparation. As an important fraction of vanillin is still present as glucovanillin in the plant residues as well as in the wastewater distillation, β-glycosidases can be used to hydrolyze this glycoside to its aglycone.

Enzymes can also be associated in cascade reactions. For example for the preparation of hydroxytyrosol one of the most powerful antioxidants starting from oleuropein, the major component of olive leaves and branches as well as for the obtainment of the aglycones from citrus rutinosides (e.g., rutin and hesperetin) mainly present in the inedible parts of citrus fruits, especially peels and leaves.

According to FDA, EMA and EFSA regulation, processing natural molecules through biocatalytic approaches allows for the final compounds to be claimed as natural, thus increasing their market value.

# 2. PhD Thesis Objectives and Milestones

**Table 1***Gantt diagram for this PhD thesis project.*

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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 | ***Glycoside green extraction, purification, and analysis*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2 | ***Batch reaction optimization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3 | ***Enzyme immobilization*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4 | ***Flow processing*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5 | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Glycoside green extraction, purification, and analysis.** Green methodologies involving enzyme- ultrasound- and microwave-assisted extractions will be employed. Green solvents, ionic liquids (IL), deep eutectic solvents (DES), or non-polar GRAS solvents such as 2-methyl tetrahydrofuran (2-MeTHF), D-limonene, ethyl acetate will be investigated in order to obtain safer, eco-friendly and more efficient extractions (Gullon *et al*., 2020; Chemat *et al*., 2019). Identification and quantification of the desired glycosides will be carried out via HPLC/GC analysis. This part will be performed in collaboration with partners with strong expertise in the field of green extraction and analysis.

A2) **Batch reaction optimization.** Single-step and one-pot, multi-step reactions will be firstly optimized in batch mode. The obtained results will be fundamental to understand the catalyst compatibility and to select the best immobilization strategy.

A3) **Enzyme immobilization.** Catalyst stability will be improved via enzymatic immobilization techniques. Particular attention will be paid on a covalent bond between the protein and methacrylate or agarose microbeads demonstrated to be the most suitable carriers for “in continuous” operations. The best-performing enzymes will be immobilized and assayed for operational stability and reusability.

A4) **Flow processing.** In-continuous processes will be developed to increase the productivity and sustainability of the process. To solve solubility problems, water-miscible co-solvents, multi-phase enzymatic reactions, or pure organic solvents will be studied. In-line extractions and purification protocols will be carried out, thus avoiding any manual handling and increasing the safety and automation of the system.

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

# 3. Selected References

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