AgriTomActive

"Valorization of agricultural wastes of unripe (green) tomatoes through the cascade extraction of bioactive molecules with eco-friendly and sustainable technologies for applications in the agri- food, packaging and non-food sectors" Acronym: AgriTomActive

The "*Agritomactive*" project, financed by the AGRITECH *National Research Centre for Agricultural Technologies (Innovation at the service of the circular economy: Agritech Center's Spoke 8 PNRR: https://agritechcenter.it/it/presentazione-del-bando-a-cascata-spoke-8-new-models-of-circular-economy-in-agriculture-through-waste-valorization-and-recycling),* directed by *Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA) Research Foundation*, in collaboration with a processing tomato Company - *La Torrente S.r.l.*, scientifically addresses the valorization of the agro-industrial by-product unripe green tomatoes, with the aim to develop a sustainable and easily scalable methods for the extraction of high-value added (bioactive molecules) naturally present in this secondary material.

This project falls under a broader plan to foster the move toward a circular economy that ultimately encourages the reduction of waste and recycling of by-products from production into new secondary fresh matter for food, cosmetic, nutraceutical, and packaging industries. Green unripe tomatoes, indeed, contain a rich group of bioactive molecules with functional and biological properties to be valorized.

Among the agri-food productions, tomatoes (*Solanum lycopersicum L.*) are certainty one of the most cultivated and consumed vegetables worldwide. World produces around 190 million metric tons of tomatoes per year[1]. The Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) reported tomato global production exceeding 180 million metric tons in the three-year period of 2020–2022. Italy is the 3rd largest producer of industrial tomatoes in the world[2]. As a consequence of this massive demand, the agri-food industries of tomatoes produce about 15 million tons of waste pre- and post-processing. The pre-processing waste amounts to about 1–6% of the total tomato production[3]. After harvesting tomato fruits, huge amounts of biomass residues are left on the field (about 24,000 kg/ha, depending on cultivation conditions), namely tomato harvest stalks which include plant residues, plant wastes, damaged tomatoes and green tomatoes. The percentage of green tomatoes left in the field after harvest can be estimated included among 0.5-3%[4]. During the processing tomato mechanical harvesting operations, green unripe tomatoes are separated by electronic color-sorting device that equipped the mechanical harvesters and usually are directly discarded in the fields.

[1] https://www.worldostats.com/post/tomato-production-by-country-2023
[2] https://www.tomatonews.com/en/background_47.html
[3] Garcia-Marti, M. and J. Simal-Gandara. 2023. Chapter 5: Chemical and Biological Valorization of Tomato Waste. *In Agri-Food Waste Valorisation*; Royal Society of Chemistry: London, UK, Volume 78. pp. 326.
[4] Leoni, C. 1997. "Scarti" in the tomato processing industry: a contribution to disentanglement among culls, rejected tomatoes, production reject and processing wastes. Industria Conserve. 73: 278-290.

In addition, also industrial tomato processing generates this type of waste (green unripe tomatoes) during the first operation of quality evaluation and selection, inspection and washing carried out in the industrial plants. The green unripe tomatoes therefore represent an important collateral residue both in the agricultural field with great impact on the environment and in the industrial field for all tomato processors.

The overall objective of *AGRITOMACTIVE* project is to valorize this particular waste, the green unripe tomatoes, left primary in the field and then discarded also in the industry as waste, through the set up and development of cascade extraction processes, which used innovative and ecological technologies and which allow to recover bioactive molecules to be used in different application sectors. These bioactive molecules of added values (Glycoalkaloids, Polyphenols and Cutin) recovered from green unripe tomato waste will be exploited as food ingredients and/or for the formulation of bio-phytopharmaceuticals to be reused in agricultural operation as natural antibacterial extract for the nurse and transplanting uses, for the pharmaceutical and cosmetic potentiality and as new coatings for packaging materials meeting the concept of the circular economy. The extraction of these molecules has been carried out applying a cascade approach to maximize the potential of the unripe green tomatoes. In order to increase the valorization of this waste, the final residues of the extraction processes will be additionally exploited in anaerobic digester for the production of biogas. The project will result in achievable processes to draw a new process prototype.

The recovery of bioactive molecules extracted in this project from unripe green tomato waste with novel, green and environmentally sustainable technologies meet the upcycling principles. The core target of upcycle is to promote the value of the waste and turn it to be wealth, enhancing its added value. In fact, the extracted molecules will be valorized and from discarded waste they will become ingredients of added value to be used in agriculture, nutraceutical and packaging sectors, so thanks to the new extraction processes it's possible to give an additional value to waste, recycling them and regenerating new products/prototypes.
The extraction processes will be developed and set up from a circular economy view not only because they will process the waste, but also because the exhausted solids produced by these processes will be allocated to the biogas production as further valorization, with a view to creating cascade extraction processes that are as circular as possible and with waste close to zero. In this sense the circular economy helps to guarantee and increase the sustainability of agri-food systems, as it provides solutions that enable integrated and persistent problems to be addressed, such as the transformation of waste into bioproducts[5].
According to Velasco-Munoz et al.[6] with respect to the environment, implementing the circular economy can contribute to combatting climate change, as it is estimated that it is able to reduce emissions by 5.6 billion tons of CO_2 equivalent by 2050. In this respect, the transition from a linear economic model to a circular economy model represents a challenge that requires the development and application of new knowledge that will enable the creation of innovative, technological and sustainable processes, products and services.
The target is to divert significant amounts of this selected waste from current low value use such as animal feed and low value compost, into applications with a 2-3 times greater value by applying a cascade approach.

To realize this project, over a 12-month work plan, the following specific objectives linked to their respective performance indicators (KPI) later used as milestones, will be fulfilled:

- **Objective 1: Map the availability of unripe green tomatoes**.

- **Objective 2: Definition of the cascade extraction protocol of Glycoalkaloids and Polyphenols complex (GPC) and Cutin from unripe green tomato.**

[5] Stillitano, T., E. Spada, N. Iofrida, G. Falcone and A.I. De Luca. 2021. Sustainable Agri-Food Processes and Circular Economy Pathways in a Life Cycle Perspective: State of the Art of Applicative Research. *Sustainability*. 13: 2472. https://doi.org/ 10.3390/su13052472

[6] Velasco-Muñoz, J.F., A. Jose, A. Sánchez, B. López-Felices and I.M. Román-Sánchez. 2022. Circular economy in agriculture. An analysis of the state of research based on the life cycle. *Sustainable Production and Consumption.* 34: 257-270. ISSN 2352-5509.

- **Objective 3: Flow chart drawing, extraction, concentration of novel bio-Phyto-pharmaceutical formula extracted from Green Tomatoes; Glycoalkaloids (Alpha Tomatine, Tomatidine, etc.) and Polyphenols complex (GPC Formula)**

- **Objective 4: Verification of GPC (Glycoalkaloid and Polyphenols Complex) formula prototype stability over time.**

- **Objective 5: Development of Cutin based coatings with improved barrier properties for food packaging application.**

- **Objective 6: Valorization of extraction by-products and return to the agricultural field in a circular economy perspective.**

Summarizing, this project concerns the extraction of bioactive molecules (Glycoalkaloids, Polyphenols and Cutin) from the agricultural waste of unripe green tomatoes with innovative and sustainable extraction techniques (Ultrasounds, ASE, Microwaves, NADES) and the use of the extracted molecules for the creation of natural bio-Phyto-pharmaceuticals, for food ingredients and for the formulation of bio-based packaging, thus creating a circular economy model in agriculture and increasing sustainable agri-food production.

The major tomato Glycoalkaloids complex (Tomatine and Tomatidines) are the first type of natural molecules to be considered. Steroid glycoalkaloids (SGAs) are a class of cholesterol-derived metabolites commonly found in the *Solanaceae* plants. α-Tomatine, a well-known bitter-tasting compound, is the major SGA in tomato, accumulating extensively in all plant tissues, particularly in the leaves and immature green fruits that accumulates significantly during early fruit development when tomatoes are green (as a and reduces in subsequent stages of ripening. The compound, which is part of the plant's own defense system, has been of interest to science because of its potent antimicrobial and antifungal activities. The ripening-related transformation of α-tomatine is dependent on the ripening inducer plant hormone ethylene. When fruit is not ripe, the immature fruit is rich in α-tomatine, which is beneficial to resisting diseases and pests and ensuring the survival of the plant. When the fruit is ripe, α-tomatine is degraded into non-bitter and non-toxic Esculeoside A, which makes the fruit delicious and conducive to feeding and seed spread. This mechanism might be regarded as a survival strategy for tomatoes.

Besides glycoalkaloids, green tomatoes also contain high level of Polyphenols, a class of antioxidant compounds with various health impacts, and Cutin, a biopolymer found in tomato skin, which can be utilized as a precursor for the production of biodegradable materials, e.g., protective coatings and natural packaging materials.

The valorization of fruit and vegetable waste not only minimizes the environmental impact associated with waste disposal but also generates added value, strengthening the economic and environmental sustainability of agri-food businesses. From a circular economy perspective, obtaining active biomolecules from agribusiness waste is attracting considerable interest in the scientific community. In particular, the tomato industry, which is one of the most important sectors of the global food industry, may have enormous potential, as it produces huge amount of waste, which includes green unripe tomatoes. These residues have a negative impact on the sustainability of the food industry, as their disposal is a major problem in terms of environmental and economic impact. However, they represent an economic and renewable biomass that,

under the biorefining model, could be exploited for energy production and chemical extraction, thus contributing to the sustainability of this production chain [7].

Extraction of bioactive compounds in green unripe tomatoes involves procedures for obtaining such compounds using modern methods that are able to retain their functionality. Despite such promising potential, current use of green unripe tomatoes is still limited to pilot studies and experimental systems. The upscaling from laboratory-scale extraction to industrial scale is accompanied by a set of challenges such as process optimization, cost-effective extraction processes, and food safety legislations. The seasonality of tomato production further makes it challenging to create a stable supply chain for green tomato residues. The gap between potential mapped for biomass feedstock and existing levels of green tomato valorization indicates that many opportunities lie untapped. Such imbalance requires that more research and development must be aimed at improving the technologies employed in extraction as well as integration into existing agro-industry systems [8].

Typically, target molecules exist in a trapped state in plant cells, which presents a physical mass transfer barrier hindering their extraction; usually conventional extraction methods are used that include maceration, Soxhlet extraction, and hydro-distillation, are largely reliant on the use of organic solvents, elevated temperatures, and extended processing times. All these methods often lead to high-energy consumption, solvent wastage, and potential thermal degradation of thermolabile compounds. Besides, toxic solvents pose health and environmental hazards, making classical extraction techniques economically unviable in the long term.

Recently, innovative technologies are being investigated in order to improve the yield and sustainability of bioactive compounds extraction. As the research progresses, it is expected that these technologies will not only improve the output but also contribute to eco-friendlier practices of waste valorization in food industries. Consequently, to obtain these molecules, our studies have aimed at green extraction technologies based on Green Physical and Chemistry principles. Special emphasis has been placed on methods that maintain the target compounds using moderate temperatures, non-toxic solvents, and physical treatments.

From our previous research experience, the main physical tool used was ultrasound technology, which increases extraction efficiency through the disruption of tomato cells, hence minimizing extraction time, cost, and solvent utilization. In fact, tests conducted on tomato processing waste consisting of skins and seeds have indicated that the use of ultrasonic extraction to separate polyphenols from tomato skins and seeds increases the concentration of polyphenols in the obtained extracts by about 50%. Although ultrasound extraction is superior for hydrophilic compounds, cell wall disruption also facilitated the solubilization of carotenoids.

SSICA based the development of the AGRITOMACTIVE extraction protocols on two studies concerning the Tomatine extraction and one for the extraction of both Tomatine and Polyphenols, but paying particular attention on the solvent to use in order to set up a more sustainable and greener protocol.

Besides empirical experimentation, the project encompassed computational analysis to predict the chemical response of target molecules to various extraction conditions. The strategy enabled the determination of ideal extraction parameters in line with the principles of Green Chemistry and the goals of a circular economy. Considering the Cutin, its extraction can be effectively predicted and modeled using methods developed for

[7] Boccia, F.; Di Donato, P.; Covino, D.; Poli, A., "Food waste and bio-economy: A scenario for the Italian tomato market", Journal of Cleaner Production, 2019, 227

[8] Martinez, F.; Garcia, M.; Lopez, A., "Valorization of green unripe tomatoes: Extraction of antioxidants and bioactive compounds for functional foods", Food Research International, 2020, 132, 109–118.

lipid and fatty acid extraction, as both share similar structural components. Research in lipid and fatty acid extraction often utilizes organic solvents such as ethanol and hexane, which are also suitable for dissolving Cutin, since they can disrupt the plant cell wall and membrane, facilitating the release of Cutin from the epidermis. Furthermore, the extraction of fatty acids from plant tissues often involves hydrolysis or saponification, processes that are also relevant for Cutin extraction.

The residual biomasses deriving from extraction procedures will be valorized through anaerobic digestion processes for the production of biogas and digestate. Digestate will be characterized and its Phyto stimulant or phytotoxic effect will be evaluated.

The developed technologies have been testing at SSICA pilot scale plants, in order to assess their feasibility in industrial environments. In addition, an operational workflow has been developing to enable smooth integration into existing processing lines, thus ensuring efficiency as well as sustainability.

The studies are nowadays in progress and as soon as possible will be published in Peer Review Scientific Journals and in usual tomato stakeholder's divulgation channels.

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Figures:

Fig. 1 Green unripe tomatoes: manual and optical sorted at La Torrente Industrial Tomato Plant

Fig. 2 - SSICA – Technological Tomato Pilot Plant – Angri (SA)

Fig. 3 Ultra Sound Pilot Plant Application in SSICA Tomato Plant – Angri (SA)

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Acknowledgements AGRITECH

The AgriTomActive project - Valorization of agricultural wastes from unripe green tomatoes through the cascade extraction of bioactive molecules with eco-friendly and sustainable technologies for applications in the agricultural, food, packaging and other non-food sectors - CUP: G43C22001330005, was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.