VINE LEAVES HARVESTING AND UTILIZATION FOR NUTRACEUTICAL PURPOSES - VINE LEAF FOR LIFE PROJECT

Giulia Santunione¹, Filippo Ottani², Nicolò Morselli², Marco Puglia², Giuseppe Nigro³, Matteo Mora⁴, Giulio Allesina^{2,5}, Simone Pedrazzi^{2,5}

Department of Life Science (Agro-Food Science Area), BIOGEST – SITEIA Interdepartmental Centre, University of Modena and Reggio Emilia - Piazzale Europa 1A, Reggio Emilia, Italy.

Department of Engineering "Enzo Ferrari" - University of Modena and Reggio Emilia – Via Vivarelli 10/1, 41125, Modena, Italy.

RI.NOVA Soc. Coop. - Via Dell'Arrigoni120, Cesena (FC), Italy.

⁴ CANTINE RIUNITE & CIV - Via Brodolini 24, Campegine (RE), Italy.

⁵INTERMECH - Inter-departmental Center, University of Modena and Reggio Emilia, Via Vivarelli 2 – 41125 Modena, Italy.

ABSTRACT: VineLeaffor Live is a Emilia Romagna regional project focused on the valorization of the vine leaves of two regional grapes variety with high levels of nutraceutical compounds in the leaves: "Grasparossa" and "Salamino". The project is started on 28th September 2022 and during the first 8 months of activities a complete chemical characterization of 10 leaves samples from representative vineyards has been done. Furthermore, a first raw prototype of mechanical harvester has been developed and tested. Next activities will be analyze the presence of residual active compounds in the leaves and improve the mechanical harvesting doing and testinga second version of the machine optimized in terms of harvesting efficiency and reliability.

Keywords: agricultural residues, biobased products, green chemistry, food, food additives.

1 INTRODUCTION

Vitis vinifera L. is one of the most important and cultivated species in the world, includes numerous cultivars with different phenological and qualitative characteristics. Although, wine and grapes represent the best known and most valuable products, various other products also derive from the vine plant, among the most abundant of which are vine leaves, a rich source of vitamins, minerals, crude fiber and phenolic compounds [1].

These are considered a real treasure chest of health and delicacy in many Mediterranean countries, such as Turkey, Greece and Bulgaria where specific varieties are grown for the consumption of fresh and preserved leaves.

Recently, the growing search for healthier diets and new health products has placed vine leaves in the spotlight in Italy as well. In fact, they contain numerous bioactive molecules, in particular phenolic substances capable of protecting and delaying oxidative processes. Among the various beneficial properties for human health, these secondary metabolites of the leaf show a protective effect against the development and progression of pathological conditions such as cancer, ageing, cardiovascular problems and diabetes [2].

Due to the growing interest in the consumption of vine leaves, some studies have also focused on their culinary use and on the processes necessary to preserve their health properties during the food processing phases. Furthermore, their use in healthy infusions is spreading more and more. This scientific and applicative approach is in line with the constant regulatory evolution that is affecting products intended for food integration and the use of natural substances in food preparation [3].

For companies in the nutraceutical, food, cosmetic and pharmaceutical sectors, the possibility of having access to new sources of biomolecules of dietary healthy interest, from vine leaves, therefore represents a significant boost and a strategically important tool for providing the market with answers able to satisfy the current growing demand. However, in Italy, at present, these companies source red vine leaves almost exclusively from foreign countries (e.g. Spain, France, etc.) [4].

In this context, considering the vast regional ampelographic base, the Emilia-Romagna winery is offered an important opportunity for product diversification and, therefore, a new source of income, especially if it is part of a cooperative group that acts synergistically in a certain area, on the basis of specific supply chain agreements.

Once the local vine varieties of interest have been identified, it is necessary to proceed with an objective characterization of the leaves that allows us to understand their most valuable intended use. Furthermore, for the producer who intends to diversify his income with the transformation and sale of the harvested leaves, it will be necessary to adopt strategies to defend the vineyard which allow the use of the leaves for dietetic - health purposes (treatments with products with low residuals), for which adequate technical-agronomic support is required.

Finally, it is essential to implement innovative methods of mechanized harvesting of the leaves, which guarantee their integrity and preserve their properties, as well as respecting the physiology of the vine.

2 MATERIAL AND METHODS

2.1 Project goals and objectives

Vine LeafforLifw is a on-going research project funded by the Emilia-Romagna region focused to the development of an integrated approach that will allow the beneficiary company CantineRiunite& CIV to open up to its members a privileged sales channel for highly specialized products, improving supply chain relationships. The project has the duration of 18 months, it was started on 28th September 2022 and it will finish on the 28th of March 2024. The general objective of the VINE LEAF FOR LIFE project consists in objectively characterizing vine leaves of local varieties, harvested mechanically with an innovative machine, to be used for the extraction of nutraceutical compounds, for the development of new dietetic-health products capable of increasing profit for the winery industries. In particular, the working will focus on the following specific objectives:

- Characterization and use of grape variety leaves for the extraction of active ingredients for nutraceutical use;
- Design and implementation of an innovative mechanized system for the recovery of vine leaves to be used for nutraceutical purposes;
- Evaluation of the corporate defense strategy and of the residual nature of the active ingredients used on the leaves;
- Market analysis and new methods of marketing vine leaves and verification of the qualitative perception by the consumer.

3 RESULTS AND DISCUSSION

3.1 Chemical characterization of grapevine leaves

10 samples of "Salamino" and "Grasparossa" leaves from different vineyards were analyzed.

Table I resumes the chemical composition of the samples evaluated through a CHNS-O elemental analyzer [5], a 550 °C furnace (for the ash content) [6] and a 105 °C drying stove for the moisture [7].

Leaves have similar CHNS-O composition, however sensible differences are present in the ash content and moisture content. Ash contents depends on the soil structure of the vineyards.The"GREGORINI SALAMINO 2022" samples has the higher ash content value (11.36 %wt. dry conditions), instead the "SABBATINI DANILO SALAMINO 2022" samples has the lower value (5.09 %wt. dry conditions).

About moisture content, this value depends on the radiation and geographical position of the vineyards. "I BOSCHI GRASPAROSSA 2022" sample has the higher content (67.74 %wt.) and "SABBATINI DANILO

SALAMINO 2022" has the lower one (57.31 % wt.).

3.2 Heavy metal content of the vine leaves samples Cd, Hg Cu and Pb contents in the leaves are estimated through leaves mineralization and ICP-MS analysis [8]. The value obtainer are compared to the limits of theRegulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs [9].

Figure 1 shows Hg and Cd levels, the EU Regulation reports a limits of 50000 ng/kg (dry condition) only for the Cd, then all the samples have a value of Cd much lower than the limits. Figure 2 shows the Cu values that are lower the limits of 10000ng/kg (dry conditions) and Figure 3 reports the Pb values that are lower than the limits of 100 μ g/kg (dry conditions).







Figure 2: Cu contents of vine leaves ($\mu g/kg$ of dry weight)

Table I:	Vine	leaves	chemical	composition
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Leaves sample	N [%wt] dry	C [%wt] dry	H[%wt] dry	S [%wt] dry	ASH [%wt] dry	M [%wt]ar
SELOGNA SALAMINO 2022	1.41	47.69	5.99		7.97	60.96
PIRONDI GRASPAROSSA 2022	1.24	46.27	5.67		10.52	67.51
I FRUTTI DI GAIA GRASPAROSSA 2022	1.56	48.49	6.11		9.80	67.33
LA FOLA GRASPAROSSA 2022	1.51	48.5	6.04		7.71	61.93
FRUTTI DI GAIA SALAMINO 2022	1.44	46.62	5.88		10.21	59.00
SALSI GRASPAROSSA 2022	1.25	46.68	5.84		11.26	65.58
I BOSCHI GRASPAROSSA 2022	1.84	50.35	6.54		8.74	67.74
BASSOLI SALAMINO 2022	1.17	45.48	5.92		10.45	63.26
GREGORINI SALAMINO 2022	0.98	46.25	5.57		11.36	66.69
SABBATINI DANILO SALAMINO 2022	1.5	46.51	6		5.09	57.31



Figure 3: Pb contents of vine leaves (ng/kg of dry weight)

3.3 Nutraceutical compound extraction from vine leaves

Figure 4 resumes the nutraceutical ingredients (Polyphenols, Flavonoids and Anthocyanins) contents in the vine leaves sample.



Figure 4:Nutraceutical compounds contents in the leaves Polyphenols are evaluated through Folin-Cicalteu method and spectrum-photometric analysis at 760 nm [10]. Flavonoids are estimated through methanol – HCl extractionand spectrum-photometric analysis at 280 nm [11]. Anthocyanins are estimated through methanol – HCl extraction and spectrum-photometric analysis at 540 nm [11]. The "SALSI GRASPAROSSA 2022" sample shows the higher value of active compounds followed by the "PIRONDI GRASPAROSSA 2022" sample.

3.4 Mechanical harvesting of the vine leaves

One of the goals of the project is to implement an optimize a prototype for the mechanized harvesting of grapevine leaves to be used for the extraction of nutraceutical compounds. A first raw prototype depicted in Figures 5 and 6 has been implemented and tested. Howeverseveral issues need to be fixed, first of all the leaves create agglomerated in the tubes that stops the harvesting. Furthermore, the big-bags charge and discharge is complex and not time optimized. In order to overcome these issues a 3D model of a new harvesting apparatus has been developed (Figure 7) and under construction right now.



Figure 5:1st version of the vine leaves harvesting apparatus



Figure 6: 1st version of the vine leaves harvesting apparatus during operation



Figure 7: CAD 3D model of the 2st version of the vine leaves harvesting apparatus

4 CONCLUSIONS

In the first 8 months of the Emilia Romagna regional project VINE LEAF FOR LIFE, an extensive characterization of the leaves of two grapes variety of the Emilia Romagna ("Grasparossa" and "Salamino") has been done. Chemical analysis shows that leaves compositions are similar for different vineyards of the same variety and the presence of heavy metals is under the limits for food applications following the Regulation (EC) No 1881/2006. Specific chemical analysis assessed the valuable content of high value nutraceutical compounds in the leaves. Finally an initial prototype for vine leaves harvesting has been implemented and tested.

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7 LOGO SPACE

