Chapter 078

Enhancement of waste from the agri-food chain as innovative ingredients for the formulation of functional foods and their impact in chronic kidney disease

**Abstract.** The largest Italian agri-food chains are represented by the production of extra virgin olive (EVO) oil and wine. Their production leads to a great amount of agri-food wastes that can represent an innovative and sustainable source of secondary raw materials that can be used as ingredients for functional foods. In this study, production chain wastes from EVO oil, wine, such as olive leaves, free oil olive pulp, grape skin, and grape seeds, were selected and characterized for the content in antioxidant compounds by HPLC-DAD-MS analysis. Using innovative technologies, micronized powders have been obtained from waste rich in antioxidant bioactive compounds, used as functional ingredients for the formulation of two bars based on EVO oil, rich in polyphenols, fruit and vegetables. The bars were analyzed to evaluate their total antioxidant capacity and the antiradical activity. The formulated bars were tested for their functional action in a preliminary *in vivo* study on renal patients who took bars and performed adapted physical activity. The preliminary results show that the association of adapted physical activity with the consumption of functional bars, rich in antioxidant and bioactive compounds, lead to an improvement in body composition, other clinical parameters.

**Keywords.** Antioxidant compounds, Functional food, Waste recovery, *Olea europaea* L., *Vitis vinifera* L.

# Introduction

Recent studies have shown that the waste from the agri-food chain is rich in bioactive molecules (Romani et al., 2020). In Italy the production of extra virgin olive oil and wine lead to large quantities of waste. The vegetable matrices *Olea europaea* L. and *Vitis vinifera* L. are chemically characterized by the presence of molecules with high active and biological value such as polyphenols. As regards the active molecules of *Olea europaea* L. we find: hydroxytyrosol, tyrosol, oleocanthal, oleacin, oleuropein, verbascoside and ligstroside, these have in common that they have a high antioxidant and antiradical power (Romani et al., 2019). We find these molecules in EVO oil and are directly linked to the health claim of EFSA 432/2012 relating to the prevention of oxidation of blood lipids and consequent cardiovascular diseases. Some of the same molecules have also been identified in the by-products of the oil supply chain, including olive leaves (oleuropein) and free oil olive pulp (hydroxytyrosol) (Romani et al., 2016). *Vitis vinifera* L. is also a matrix characterized by the presence of bioactive molecules belonging to the class of anthocyanins and procyanidins. All these molecules are linked to important functional properties, mainly antioxidant and antiradical which allows these waste products to have an important value and potential to be used as innovative secondary raw materials in several product sectors. Some possible applications of these products are in food sector both as a functional antioxidant ingredient and as a natural preservative in place of chemicals. Further uses can be in the cosmetic, nutraceutical, agronomic, feed and phytotherapeutic sectors. Recent studies have shown how the action of active molecules of the Olea europaea L. matrix such as hydroxytyrosol, oleacin and oleocanthal, in synergy, can assist in the therapy of patients suffering from chronic kidney disease (CDK) (Noce et al., 2021). Considering the antioxidant molecules that characterize the matrices selected in this work, olea and vitis, it is possible to hypothesize that these innovative ingredients can are used as the basis of functional food formulations for sport and well-being, but also as an integration in the diet for CDK patients.

# Material and methods

### HPLC-DAD-MS analysis

The analyses for the qualitative and quantitative evaluation of bioactive compounds of micronized products and EVO oil extract were obtained using an HP-1260 liquid chromatograph equipped with a DAD detector (Agilent-Technologies, Palo Alto, USA). The HPLC system is interfaced with an Agilent MS system equipped with an ESI source (Agilent Corp, Santa Clara, CA, USA). Analyses were acquired in full-scan mode and the mass range was set to m/z 100–1500 in negative and positive modes. Analytical columns and chromatographic methods are described in Romani et al., 2020. Polyphenols found in the extracts were identified by comparing retention times and UV/Vis spectra with those of the authentic standards. Each compound was quantified at the selected wavelength (240, 280, 330, 350, 520 nm) using a five- point regression curve and applying the correction of the molecular weights (Romani et al., 2020).

### Composition of innovative bars

Two functional bars were designed starting from innovative ingredients coming from the recovery and enhancement of waste matrices from agri-food chains. In particular, two vegan and organic bars have been formulated based on fruit, vegetables and secondary raw materials from *Olea europaea* L. and Vitis vinifera L., both bars are characterized by EVO oil as the main lipid source, with a content in compounds active polar minors (MPC) equal to 775.98 mg / L (HPLC-DAD-MS analysis). The two bars designed are green and blue, the green is characterized by the presence of a functional ingredient from Olea, the micronized olive leaves and micronized free oil olive pulp, the blue bar is characterized by the presence of innovative products coming from the recovery of wine-making waste, such as micronized grape film and grape seeds. The two bars have a weight of 32g and a caloric content of 122kcal / bar. Table 1 shows the compositions of the two innovative bars formulated.

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| Table 1. Composition of innovative bars. BB: blue bar; GB: green bar | |
| **Name** | **Ingredients** |
| BB | dates, thompson grapes, cashews, almonds, plums, acerola powder, cabbage powder, beetroot powder, açai powder, blueberry powder, rhubarb powder, kiwi powder, carob flour, micronized grape film, micronized grape seeds, EVO oil. |
| GB | dates, cashews, thompson grapes, figs, apple, fennel powder, cabbage powder, celery powder, spinach powder, barley grass powder, carob flour, kiwi powder, grape skin micronized, grape seeds micronized, micronized olive leaves, micronized free oil olive pulp, EVO oil. |

### Total antioxidant capacity (Folin- Ciocalteu assay)

The total antioxidant capacity on two bars was evaluated using the Folin-Ciocalteu method adapted to the samples. 500 µl of water and 125 µl of Folin reagent were added to 125 µl of suitably diluted extract and it was reacted for 6 minutes in the dark. Subsequently, 1.25 ml of Na2CO3 at 20% in H2O were added, then the solution is brought to the volume of 3 ml with water. The solution is left in the dark for 85 minutes then centrifuged for 5 minutes at 5000 rpm. The spectrophotometric reading is carried out at 725 nm using a Lambda 25 spectrophotometer (PerkinElmer, Waltham, MA, USA) and using the extraction solvent (70:30 hydroalcoholic solution pH 3.2) as blank. The results are expressed in mg of gallic acid (GAE) per g of bar.

### Antiradical activity (DPPH assay)

A further investigation carried out on the samples under study was the evaluation of the antiradical properties. The method chosen is a spectrophotometric method based on the use of the stable radical DPPH • (radical 1,1-diphenyl-2-picrilidrazil).

Through this test it is possible to obtain information on efficiency by evaluating the percentage antiradical activity after 20 minutes of contact with the DPPH radical. 1 mL of aqueous solution of the various samples, suitably diluted, was added to 1 mL of an ethanolic solution of DPPH • (obtained by solubilizing 4.0 mg of DPPH • in 100 ml of ethanol). Absorption was measured at 517 nm immediately and after 20 min using a Lambda 25 spectrophotometer (PerkinElmer, Waltham, MA, USA) versus an ethanol: H2O 50:50 solution as a blank.

The reducing activity A.R.% was calculated by applying the following formula:



# Results and discussion

### HPLC-DAD-MS characterization of by-products from Olea europaea L.

The by-products of the oil supply chain were analyzed by HPLC-DAD-MS, including micronized olive leaves and de-oiled olive pulp. The main molecules of these two products are represented by oleuropein for the olive leaves and hydroxytyrosol for the free oil olive pulp, many studies have shown the functional value of these two molecules reacting to their antioxidant and antiradical power (Romani et al. 2019). The tables below show the qualitative and quantitative characterizations of the analyzed samples. (Table 2, 3)

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| Table 2. Quali-quantitative HPLC-DAD-MS analysis of polyphenols in the micronized powder of olive leaves, data expressed in mg/kg | |
| **Compounds** | **mg/kg** |
| Hydroxytyrosol | 0,60 |
| Verbascoside | 2,71 |
| luteolin 7-O-glucoside | 5,11 |
| Oleuropein | 29,55 |
| Oleuropein aglycone | 5,32 |
| **Total polyphenols** | **43,30** |

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| Table 3. Quali-quantitative HPLC-DAD-MS analysis of polyphenols in the micronized powder of free oil olive pulp, data expressed in mg/kg | |
| **Compounds** | **mg/kg** |
| Hydroxytyrosol Glycol | 340,00 |
| Hydroxytyrosol | 1845,50 |
| Tyrosol | 450,36 |
| Oleoside | 316,17 |
| Verbascoside | 1269,68 |
| Verbascoside Derivatives | 728,93 |
| Oleuropein | 4142,81 |
| Luteolin Glucoside | 815,00 |
| Luteolin Derivatives | 174,00 |
| **Total polyphenols** | **10082,45** |

### HPLC-DAD-MS characterization of by-products from Vitis vinifera L.

The by-products of the wine chain including the micronized grape skin and grape seeds were analyzed by HPLC-DAD-MS. The main molecules of these two products are represented by procyanidins and anthocyanins, many studies have shown the functional value of these two molecules reacting to their antioxidant and antiradical power (Romani et al., 2020). The tables below show the qualitative and quantitative characterizations of the analyzed samples (Table 4)

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| Table 4. Quali-quantitative HPLC-DAD-MS analysis of polyphenols in the micronized powders, data expressed in mg/g compounds in each of the analyzed powders. GSE: micronized greape seeds ; GSK: micronized greape skin | | |
|  | **GSE** | **GSK** |
| Gallic acid | 0,072 | 0,052 |
| Other procyanidins | 0,772 | 0,08 |
| Catechin dimer B3 | 1,446 | 0,416 |
| Epicatechin | 0,281 | 0,012 |
| Catechin trimer | 2,082 | 0,435 |
| Epicatechin gallate dimer | 0,552 | 0,192 |
| Catechin tetramers | 24,694 | 15,875 |
| Epigallocatechin dimer | 1,03 | 7,6 |
| Catechin/epicatechin trimers digallated | 27,307 | 28,368 |
| Delphinidin-3-glucoside | 0,009 | 0,017 |
| Cyanidin-3-glucoside | 0 | 0,002 |
| Petunidin-3-glucoside | 0,014 | 0,024 |
| Peonidin-3-glucoside | 0 | 0 |
| Malvidin-3-glucoside | 0,048 | 0,09 |
| Delphinidin-3-acetylglucoside | 0,003 | 0,002 |
| Cyanidin-3-acetylglucoside | 0,005 | 0,009 |
| Petunidin-3-acetylglucoside | 0,004 | 0,005 |
| Malvidin-3-acetylglucoside | 0,004 | 0,009 |
| Malvinidin-3-caffeoylglucoside | 0,014 | 0,036 |
| **Total polyphenols** | **58,335** | **53,224** |

### Total antioxidant capacity (Folin-Ciocalteu) and antiradical activity (AAR%)

### The two bars formulated based on micronized Olea europaea L. and Vitis vinifera L. from circular agriculture standardized in active compounds and traced quality EVO oil were analyzed to evaluate their functionality. The total antioxidant capacity (Folin - Ciocalteu) and the percentage antiradical activity (AAR%) were evaluated using the DPPH radical. As shown in table 5, both bars have optimal values, proclaiming a marked functional antioxidant and anti-radical activity, both exceeding 80% AAR. These values allow us to affirm that the products from the recovery of agrifood chains standardized in active compounds in association with EVO oil with a high content of antioxidant compounds can be considered innovative ingredients to be used for the food, nutraceutical, but also cosmetic sector, agronomic and feed.

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| Table 5. Total antioxidant capacity (TAC) mg GAE / 32g and antiradical activity (AAR%). BB: blue bar; GB: green bar | | |
|  | **TAC** | **AAR%** |
| BB | 250,12 | 84,49 |
| GB | 207,47 | 80,53 |

# Conclusions and future perspectives

The study represents an example of a circular model for the recovery of waste from the agri-food chain and the development of innovative functional ingredients starting from secondary raw materials. The recovery and controlled transformation of the by-products of wine and EVO oil production have made it possible to apply the principles of circular economy and to obtain new products to be used in various product sectors, including food, nutraceutical, agronomic, feed, and cosmetic. The selected sector is functional sports-wellness nutrition. Two bars based on fruit and vegetables were formulated and added with standardized micronized in antioxidant compounds of olive leaves, free oil olive pulp, grape skin and grape seeds. The association of these innovative ingredients with the EVO oil with a high CMP content has made it possible to obtain two functional antioxidant products thanks to the synergy created between the molecules present in the formulation. The antioxidant and antiradical functionality has been tested *in vitro* with the Folin-Ciocalteu and DPPH test, but these bars have also been selected for an *in vivo* study entitled "Evaluation of the possible energetic and beneficial action induced by the association of a supplement food with sports activities for the treatment of uremic sarcopenia "in collaboration with the Tor Vergata University of Rome and the University of the Foro Italico in Rome. Preliminary results show that the association of physical activity and the intake of functional bars rich in antioxidant active compounds lead to an improvement in body composition and parameters, such as blood pressure and lipid metabolism, improving the health of the affected patient. from chronic kidney disease and subsequent uremic sarcopenia. A study is underway which includes a larger patient population to demonstrate the hypothesis proposed in the pilot study Grazioli et al., 2022.

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