Chapter 28
The Cultivation of *Iris pallida* as an Opportunity for the Enhancement of Tuscan Agro-Biodiversity and a Resource for the Local Economy

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Abstract *Iris pallida* is produced in Tuscany (Italy) in two limited hilly areas. The cultivation of Iris has undergone a progressive contraction, mainly due to the high labour requirements of the crop and to the reduction in the price of dry rhizomes on the international market of the perfume industry. To improve the value of the Iris crop as a resource for the local economy, a key aspect is to improve the quality of Iris in the Tuscan territory. Innovative biocidal and repellent products from arbutus and olive trees containing antimicrobial active ingredients have been developed to improve the critical points that compromise the quality and microbiological safety of Iris rhizomes. Gas chromatography–mass spectrometry (GC-MS) and innovative GCxGC-time-of-flight (TOF) and GC-vacuum ultraviolet (VUV) analyses have been optimized for the exhaustive characterization of irones and volatile compounds in flours with different aging degrees and in the absence or presence of cuticles to obtain an identity card of the aromatic profile of Iris, to monitor the quality of the product and to enhance the biodiversity of Tuscany. High performance liquid chromatography–photodiode-array detection–mass spectrometry (HPLC-DAD-MS) was also used to characterize the nonaromatic component with antioxidant activity to enhance its possible innovative use in the cosmetic and liqueur sectors of both flour and byproducts of the supply chain.

Keywords Irones · Isoflavones · Innovative product · Byproduct · Quality · Safety

Annalisa Romani died before publication of this work was completed.

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28.1 Introduction

Among more than 300 species of the genus Iris (from the family Iridaceae), only *Iris pallida* and *Iris germanica* are currently used to produce natural irones, which are widely used in the perfume industry because of their attractive violet-like and powdery fragrance (Roger et al. 2010). Four main irones have been described (trans-α, cis-α, trans-γ, and β-irones), and their isomeric distribution and "sensory" properties depend on the iris species (Firmin et al. 1998). At present, *Iris pallida* is produced in Italy only in Tuscany in two limited hilly areas: in Chianti (near Florence) and in Pratomagno (near Arezzo), and it is usually complementary to olive oil and wine production. *Iris pallida* is a minor crop with high added value, and its rhizome is used by the international fragrance industry. In Italy, despite the wide globalization that has developed in the agri-food system, some traditional agricultural crops may play an important role in the environmental preservation and social and economic development of some marginal rural areas. The interest in the *Iris pallida* came from the high added value of the production, the expected potential of the existing network of local producers, and the sensitivity of the local stakeholders to the value of the traditional product not only for direct marketing reasons but also for indirect benefits coming from its high aesthetic value for the landscape and therefore for tourism and for its role in the environmental preservation of the area (Belletti et al. 2013). Once important in Western herbal medicine, *Iris pallida* rhizomes are now mainly designated to three markets: the French perfumery industry, the Italian alcoholic drinks industry, and the German children dummy industry.

Iris pallida is a rustic, vigorous, and hardy species capable of successfully adapting to difficult cultivation environments, especially in terms of physical and chemical soil characteristics (Pignatti et al. 2000). Plants are frequently attacked by slugs, snails, and porcupines. Diseases affecting Iris include bacterial soft rot and leaf spot. One of the aims of this work is to improve the critical points that compromise the quality of the Iris rhizome through the design and creation of new biocidal and repellent products, which can be used for the microbiological safety of the bulb during the refinement and aging phase. Iris cultivation may have a biennial or triennial production cycle. In the first stages after cropping, the rhizomes are cleaned of roots and washed. After this stage, the producer can follow two different paths. To obtain black-type orris, the washed rhizomes were sliced and dried outside. To obtain white-type orris, rhizomes are peeled by hand and undergo a long process of 2–3 years of drying and aging. The specific hydrodistillation of dried and crushed iris rhizomes leads to an essential oil called Iris butter. One ton of Iris rhizome produces 2 kg of essential oil, making it a highly priced substance. As this essential oil almost exclusively contains irones and fatty acids with no smell (Garnero and Joulain 1981), its commercial value is directly determined by its iron concentration. Similarly, the value of Iris rhizomes is closely related to their iron content. Depending on the distillation process used, different orris fractions are obtained, used as base notes in fragrances by perfumers but most importantly as natural fixatives to enhance other aromas and as stabilizers in cosmetics (DeBaggio and Tucker 2009).

Orris root powder is also used for making gins and cordials as a very potent natural fixative for the other components (Tonutti and Liddle 2010) and in the baking, printing, and textile industries. Gas chromatography–mass spectrometry (GC-MS) analytical methods and innovative GCxGC-time-of-flight (TOF) and GC-vacuum ultraviolet (VUV) methods have been optimized for the analysis of aromatic metabolites in Iris flour and used to obtain an identity card of the aromatic profile of the Iris of Tuscan territory to monitor the quality of the product and enhance the biodiversity of Tuscany.

The roots and rhizomes of many Iris species are well known as precious sources of isoflavones. They have been used in traditional medicine to treat respiratory disorders and decrease smooth muscle activity. Recent studies have revealed potent anticholinesterase, antineoplastic, and anti-plasmodial activities of iris constituents (Kukula-Koch et al. 2015). Except for isoflavones, the main chemical components of Iris are xanthones, quinones, flavones, flavone C-glycosides, terpenes, and simple phenolics, with multiple biological activities. Numerous findings regarding their broad pharmacological implementation shed new light on the potential use of the rhizome and other Iris constituents; therefore, the aim of this work is also to characterize the non-aromatic component by high performance liquid chromatography–photodiode-array detection–mass spectrometry (HPLC-DAD-MS) analytical methods to enhance a possible innovative use in the cosmetic and liqueur sectors of both the flour and byproducts of Iris.

28.2 Material and Methods

28.2.1 Analysis of Volatile Compounds

28.2.1.1 SPME Conditions

The volatile compound (VOC) profile was determined by solid-phase microextraction (SPME). Five hundred milligrams of pulverized bulb were placed into a 20-mL screw cap vial fitted with polytetrafluoroethylene (PTFE)/silicone septa. VOCs were absorbed by exposing a 2-cm divinylbenzene/carboxen/polydimethylsiloxane SPME fibre (DVB/CAR/PDMS by Supelco) at 60 °C for 15 min into the vial headspace and then immediately desorbed at 280 °C in a gas chromatograph injection port.

28.2.1.2 GC-MS Analysis

VOCs were analysed by a 7890a GC system operating in splitless mode, separated by a DB InnoWAX column (0.4 μm dfx0.2 mm i.d., 50 m) and detected by a quadrupole mass spectrometer 5975c MSD (Agilent Technologies, Palo Alto, CA, USA) operating in EI mode at 70 eV. The initial oven temperature was set at 40 °C, held for 0.5 min, raised to 260 °C at 6 °C/min, and held at 260 °C for 1 min. For each chromatogram, the peaks were integrated using the total ion current (TIC) areas. The percentage distribution of VOCs was obtained by normalizing the area of the peaks to the total area. In the case of irons, a quantitative analysis was carried out using 6-point calibration curves constructed using methyl-α-ionone (α-IRONE) (range 10–100 ppm, 0.99 R^2).

28.2.1.3 GCxGC-TOF Analysis

GCxGC was performed by a flow modulation system consisting of an Agilent 7890B GC (Agilent Technologies, Palo Alto, CA, USA) with a capillary flow modulator device for 2D separation coupled with a time-of-flight mass spectrometer (TOF-DS; Markes International Ltd., Llantrisant, UK) as reported in Ieri et al. (2019).

28.2.1.4 GC-VUV Analysis

A GC SRA-Agilent 7890B connected to a VUV 101 detector (VUV Analytics) equipped with a 60 m HP-Innowax 0.25 mm i.d. × 0.5 μm d.f column was used. Conditions were reported in Ieri et al. 2019.

28.2.2 HPLC-DAD-MS Analysis

The analyses for the qualitative and quantitative evaluation of non-volatile compounds were obtained using an HP-1260 liquid chromatograph equipped with a DAD detector (Agilent-Technologies, Palo Alto, USA). The HPLC system was 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 both positive and negative modes. Different columns and different analytical conditions were used to descend the treated matrix (arbutus, olive leaves, iris, and liqueurs).

28.3 Results and Discussions

To enhance the crop of Iris in the Tuscan territory as a resource for the local economy, a key aspect is to improve the critical points that compromise the quality of the Iris rhizome. Innovative natural products have been developed that can be used in the growth phase of the bulb for the reduction of microbial attacks or as repellents from porcupines, greedy eaters of iris bulbs. Solutions containing antioxidant and antimicrobial active ingredients, applicable on the bulb or by immersion or by direct nebulization during the drying phase, have been optimized. Standardized solutions containing active ingredients with high repellent activities are obtained by applying aqueous or steam extraction of plant species such as arbutus and olive leaves. The solutions were characterized by the optimization of a GC/MS method for the volatile compounds and by the optimization of HPLC/DAD/MS analytical methods for the nonaromatic component with antiradical and antioxidant activity. The aqueous extract of arbutus contained procyanidins, gallic acid, monogalloyl glucose, gallotannins (polygalloylated glucose), gallagic tannins, and flavonols. The olive leaf extract was obtained by aqueous extraction of cv leccino, frantoio, rossellino, and moraiolo leaves in a rapid pressure extractor. The product obtained was enriched in hydroxytyrosol (50% of the total content). An innovative drying technology customized for the iris bulb was also used to optimize the drying phase and to preserve the molecules of interest and the organoleptic characteristics.

The value of Iris rhizomes is closely related to their irone content; therefore, various analytical methods have been optimized to obtain an identity card of the aromatic profile of the Iris of the Tuscan territory and to monitor the quality of the product. In particular, the VOC profile was determined by chromatography coupled to mass spectrometry (GC-MS) with sampling from the headspace using the solid-phase microextraction (SPME) technique. An innovative analytical method that uses a new GC detector based on vacuum ultraviolet spectroscopy (VUV), which measures the full absorption of the scan in the range 125–240 nm, has also been optimized. The VUV detector showed the ability to discriminate between different isomers of iron and quantify them in this high-value matrix. GC-VUV completes MS spectrometry by overcoming its limitations and providing a secondary confirmation method (Ieri et al. 2019). For the optimization of GC-MS and innovative GCxGC-TOF and GC-VUV analytical methods, flours of rhizomes at different degrees of aging and/or drying and with or without cuticle were analysed. Flours with and without cuticles have different relative percentages of irones; in particular, in the extracts obtained from white flour, the main iron is cis-α-Irone, and in those obtained from brown flour, the main iron is cis-γ-Irone. These results were confirmed by all GC methods (Fig. 28.1). These results suggest that irones are present in the cuticle, particularly cis-γ-Irone. These findings were confirmed by GC-MS analysis and valorization of this waste from rhizome processing.

The characterization of the nonaromatic component with antiradical and antioxidant activity was performed by HPLC-DAD-MS analysis, not only in flours of rhizomes at different degrees of aging and/or drying and with or without cuticle but also in the byproduct cuticle. The analyses confirmed the presence of compounds belonging to various polyphenolic classes, in particular isoflavones, xanthones, including mangiferin and neomangiferin and hydroxycinnamic derivatives, with a total polyphenol composition of 14.1 ± 0.05 mg/g in the cuticle waste. The phases of maceration in alcoholic and hydroalcoholic solutions of the medium-sized bulbs and of the flour have been optimized for the prototyping of macerates (0.4–0.6 mg GAE/mL) that can be used in the food sector as bases for spirits or alcoholic

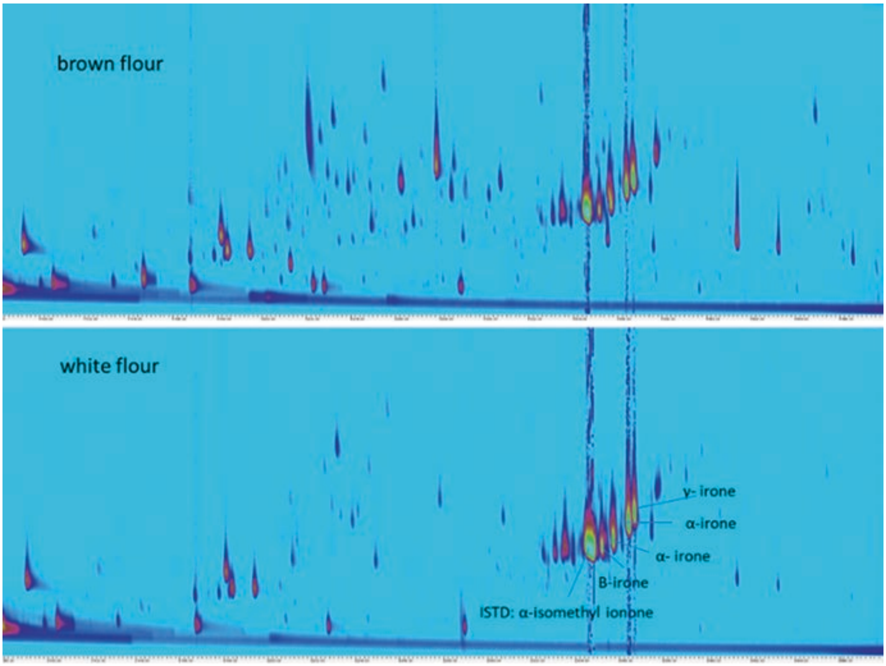

Fig. 28.1 GCxGC analysis of flours with and without cuticle

semifinished products, for food use in the bakery products sector (biscuits and cakes also from traditional recipes) or as a filling for creams and icings. The bulbs and iris flour were also extracted in a modulated steam current under vacuum to obtain suitably characterized fractions to be used for the formulation of cosmetic references. Through the innovative use in the cosmetic and food sector of iris flour and cuticle extracts, the aim is to valorize Iris with a line of food, cosmetic, and artisan products.

28.4 Conclusions and Future Perspectives

The increasing value of the traditional production of *Iris pallida* influences the territory not only for direct marketing reasons but also for the indirect benefits coming from its high aesthetic value for the landscape and therefore for tourism and for its role in the environmental preservation of the area. The creation of a line of food, cosmetic, and high added value products increases profitability and gives life to a model of territorial development. The goal is to create a territorial brand around Iris supported by a discipline of behaviours and good practices that are not only agricultural but also sociotouristic and environmentally friendly.

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