Chapter N (please do not write anything in this line. Editors will annotate the chapter number)

Study of the volatile component and the flavonoid content of edible flowers

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**Abstract.** In recent years, a growing interest among farmers and chefs has been oriented toward edible flowers, which are now popular in modern cuisine and appreciated by consumers. Their expansion is not limited to the aesthetic value, but also to flavor and nutritional aspects that supply to food and beverages. The aim of our research is to characterize edible flowers in terms of aromatic profile and the nutritional component to provide an objective approach able to discriminate one product from another on the market considering the peculiarities of each variety available. In the first step of the study, reported in this paper, an HS-SPME/GC-MS and an ASE/HPLC-UV methods were developed to assess the volatile fingerprint and the flavonoid content in edible flowers. The resulting chromatographic profiles of the analysis of a wide set of samples will then be processed by multivariate statistical analysis to build classification models (next step of research, in progress).

**Keywords.** Edible flowers, flavor, flavonoids, HS-SPME/GC-MS, ASE-HPLC/UV

**N.1 Introduction**

According to scientific studies, edible flowers are nontoxic and innocuous flowers, and their consumption can contribute to several health benefits due to their content of bioactive compounds (Lu et al., 2016). In recent years, the evolution in food habits towards healthier and correct lifestyles and the research on innovative dishes by professional chefs resulted in a significantly increased demand for these products. The use of flowers as a food ingredient recently gained new popularity although it is an ancient tradition dating to 140 B.C. (Falconnier, 2006). Today’s edible flower availability includes several tens of species differing in form, color, and flavor, typically used to improve the appearance, color, and nutritional value of proposed dishes. Edible flowers are regarded as unique and imaginative ingredients that can be used both as a garnish and as a component in salads, soups, appetizers, desserts, and beverages. They are widely consumed fresh, but can also be dried, powdered, crystallized, or used as foams (molecular gastronomy). The current demand for more attractive and tasty food combined with new information concerning their nutritional value sees the edible flower global market in a phase of accelerated expansion (Rop et al., 2012). However, for most of them, limited information on the production on a global scale has been found, while are only available producers’ annual reports and sales data of the agricultural companies (Fernandes et al., 2020). In 2017, Europe, the Middle East, and Africa had the largest market share (Technavio, 2018). There are still no official lists by international organizations such as FAO, WHO, and EFSA of the several flower varieties; however, Lu *et al.* have listed 97 families, 100 genera, and 180 species, highlighting that the number of edible flowers varies from country to country (Lu et al., 2016). Today, a wide variety of these flowers with different appearances and organoleptic characteristics such as size, shape, color, aroma, and taste are available on the domestic market. The sensory and nutritional features assessment represents for the food industry an important criterion to gain this market segment, and for small producers to support local agriculture. Also on the consumer’s side, these features have a significant impact on choices of food consumption focused on the research of a pleasing sensory experience and a healthy lifestyle (Kelly, et al., 2001). Although sensory qualities and flower variety significantly influence their acceptability in the human diet, several studies report that many other factors, such as packaging, price, education, gender, and socioeconomic class of consumers, can also impact (Rodrigues et al., 2017). In this context, the volatile and nutritional component evaluation of edible flowers is crucial to valorize food ingredients able to provide beneficial properties and aesthetic value. The volatile profile is defined by a combination of plenty of aromatic compounds, such as alcohols, alkanes, carbonyls, esters, and terpenes, that determine its flavor (Deng et al., 2004; Movafeghi et al., 2010); some of them, are also beneficial for health. For example, caryophyllene is regarded to be the principal anti-inflammatory agent in carnations (Lyra et al., 2008). The solid phase microextraction (SPME) method is the most widely used procedure to analyze volatile compounds in fresh flowers because it avoids the development of newly formed compounds potentially resulting from liquid or enzymatic extraction procedures (Deng et al., 2004; Fernando and Grun, 2001; Movafeghi et al., 2010; Rout et al., 2012; Ye, 2013). As regards the edible flowers’ bioactive component, several studies have shown that flavonoids are the substances present in greater quantities (Yan et al., 2012). Epidemiological research also confirmed the inverse relationship between the intake of flavonoids and the risk of chronic diseases, in addition to their high capacity as an antioxidant component (Oliveira et al. 2014, Fernandes et al. 2019, Negro et al., 2021). This paper reports the preliminary results of our research, still in progress, focused on the characterization based on flavor profiles and the nutritional component of different edible flower typologies, to expand the set of objective approaches available to consumers and chefs to make informed choices. The first project step involved the development of an HS-SPME/GC-MS method to assess the volatile fingerprint, and an HPLC-UV method to determine the flavonoid content. The next step of the research will involve the analysis of a sample set of different edible flowers available on the market to characterize by statistical classification tools.

**N.2 Materials and methods**

Trays containing edible flowers of mixed colors and varieties were purchased on Amazon. The flowers were bio-certified and produced by an Italian agricultural company. The samples were kept in the refrigerator on their original tray and analyzed within 3 days of arrival in the lab. Before analysis flowers were separated by typology (Antirrhinum, Dianthus and Violet). 1.5 g of fresh flower sample was placed in a 20 mL glass vial for autosampler to assess the volatile profile by HS-SPME/GC-MS (Triplus Autosampler/Trace 1300 Gas Chromatograph-ISQ 7000 Single Quadrupole, Thermo Fisher Scientific). A 50/30 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stable Flex fibre (2 cm) was selected. The sample vial was preheated to 40 °C for 5 minutes in the autosampler. The fibre was then exposed in the headspace for 10 minutes at the same temperature. The splitless mode was chosen as the injection modality maintaining the valve closed for 5 minutes. The injector temperature was set to 220 °C with a 50 mL/min split flow. A VF-WAXms capillary column (30 m x 0.25 mm ID, 0.25 mm) was used for the chromatographic separation employing helium as carrier gas (flow rate of 1 mL/min). A temperature gradient was programmed at 60 °C for 1 min, ramped at 3 °C/min to 230 °C for 5 min. The detection was carried out using a single quadrupole in full-scan acquisition mode in a 45–450 amu range. Both ion source and transfer line were kept at 250 °C.

An accelerated solvent extraction (ASE) followed by an HPLC/UV method was optimized to determine the flavonoid content (Dionex ASE 150, Dionex Ultimate 3000 HPLC, Thermo Fisher Scientific). Before ASE extraction, flower samples were dried in a lab oven at 40 °C for 8 hours, then ground for 10 seconds using an IKA A10 basic mill, and subsequently analysed. In the ASE system, 0.5 g of dried flowers were extracted under the following conditions: a single static cycle of 15 min, a temperature of 120 °C, ethanol as extraction solvent. The extraction pressure was kept constant at 1000 psi with a flush volume of 40%. A rotary evaporation system kept at 40 °C was employed for extract evaporation. Before HPLC analysis, the extracts were dissolved in 6 mL of methanol. A Kinetex® C18 LC column (5 μm 250 x 4.6 mm ID) maintained at 40 °C was used for separation. The mobile phase was a binary solvent system consisting of water (A) and acetonitrile (B), both containing 0.1 % formic acid. The elution gradient was programmed as follows: 5 min at 15% B, 5-13 min at 25% B, 13-19 min at 40% B, and 20-30 min system was restored to initial conditions. The flow rate was 0.5 mL/min, and the wavelength was set at 280 nm. Data were collected by Chromeleon 7 software both for GC and HPLC analysis.

**N.3 Results and discussion**

The initial phase of this study focused on the development of an HS-SPME/GC-MS method for flavor investigation and an ASE/HPLC-UV method for flavonoid content determination in order to characterize different typologies of flowers. The data collected from the chromatographic analyses of a wide sample set will be then processed using multivariate statistical analysis in a second phase of the research still ongoing. The goal will be to build models able to classify the various types of flowers in terms of their flavor and antioxidant component and be able to place unknown edible flowers in the appropriate class. At present, only a few fresh edible flower samples belonging to three different classes (5 for each class) were analyzed. Color and taste distinguish the investigated categories: Antirrhinum with pastel mixed colors produces bitter sensations; Dianthus with red, pink, and white hues produces sweet and spicy sensations; and Violet with mixed colors produces delicate and sweet tastes. By comparing the chromatographic profiles of both the volatile and the bioactive components of the analyzed samples, preliminary qualitative results were obtained. Figure 1 shows the flavor fingerprint of the Antirrhinum and Dianthus samples. Caryophyllene, ethylene glycol, eugenol, isoledene, linalool, and longipinene were detected in Dianthus, but there were not found in the other groups (except for caryophyllene, which is also a component of Violets). Acetanisole, bergamotene, eremofilene, isocitronelle were found only in Antirrhinum. Violet samples were instead characterized by allocimene, farnesene, humulene, limonene, menthatriene, myrcene, and ocimene. The identification of compounds was performed by comparing their mass spectra to mass spectra available in the NIST library, considering satisfactory the compounds with R-match higher than 900. At this stage of the research, the qualitative analysis without the use of reference standards should be reported as a “putative compounds identification” (Sumner et al., 2007). Figure 2 compares instead the flavonoid profiles of Antirrhinum and Dianthus, showing also in this case significant differences between the two groups.

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*Fig. 1. HS-SPME/GC-MS flavor profiles of Antirrhinum (left) and Dianthus (right). The chromatograms are reported in the same scale of the x- and y-axis.*



*Fig. 2. Flavonoid components obtained by HPLC-UV analysis of Antirrhinum (left) and Dianthus (right). The chromatograms are reported in the same scale of the x- and y-axis.*

**N.4 Conclusions and future perspectives**

In this study, an HS-SPME/GC-MS and an ASE/HPLC-UV methods were developed, and then applied to a limited set of edible flowers. The preliminary qualitative investigation showed significant differences among the investigated varieties with the identification of specific compounds that characterize one class over the another. This first step of the research will allow the future edible flower characterization of different typologies from both the standpoint of flavor and the bioactive component. Multivariate analysis and the obtained classification models could represent an objective approach to enable the choice of one product compared to another available on the market and identify aromatic and flavonoid compounds that could be used as product markers.

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