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The use of hyperspectral imaging to predict the distribution of internal constituents and to classify edible fennel heads based on the harvest time

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ABSTRACT

The objective of this study was to use hyperspectral imaging to predict the internal concentration of soluble solids (SSC), individual sugars and organic acids, phenols, and antioxidant activity of fennel heads in relation to different sheath layers and harvest times. Thirty-five fennel heads were collected during 7 different harvest times over a period of 3 weeks. For each fennel VisNIR (400–1000 nm) and NIR (900–1700 nm) images of the perpendicular section were acquired. From the external to the internal part of the fennel chemical analysis of each leaf was done summing up to 160 samples. Similarly, for hyperspectral images three regions of interest (ROI) were extracted and averaged for each corresponding leaf. A calibration set of 105 samples and a validation set of 31 samples was used to develop the PLSR models, after removing 20 samples without correct reference values and 4 outlier spectra. Among the predicted parameters only SSC, DPPH and phenols could be predicted with satisfactory accuracy. Particularly, for SSC, mean centering gave an R^2 of 0.87, 0.81, 0.77 for calibration, cross validation, and prediction, respectively (RMSEP of 0.515 over a range of values from 4 to 9%). First derivative combined with SNV applied for DPPH gave the same accuracy with R^2 of 0.81, 0.76, 0.78 (RMSEP of 2.460 over a range of 20–250 mg kg^{-1}). The best preprocessing technique for phenols was MSC (mean) yielding RMSEP of 3.042 (over a range from 50 to 350 mg kg^{-1}). In addition it was possible to map the constituent concentrations on the hyperspectral images showing the increase of soluble solids, phenolics and antioxidant activity from the external to the internal leaves. As for classification of fennels according to harvest time using PLS-DA, all the classes were distinguished with a non-error rate of 89.29% in calibration 75.71% in cross validation and 88.57% in prediction. Except for some samples of class 5 in calibration and 2, 4 and 5 in case of cross validation, all others were nearly correctly classified. In conclusion results of this work showed the potentiality of hyperspectral imaging in the Vis-NIR spectral range to predict internal constituents and to classify fennel heads according to the harvest time.

1. Introduction

Fennel (*Foeniculum vulgare* Mill.) belongs to the family Apiaceae (formerly the Umbelliferae) and it is native to southern Europe and the Mediterranean region (Azeez, 2008). It is cultivated as a vegetable crop in Mediterranean countries: the edible part is the swollen basal part, also called "grumolo", but more often referred to as "head", which results in overlapped leaf sheathes that appear as concentric rings in transverse section, also embracing the stem in the middle. Consumers mainly appreciate this crop for its organoleptic properties such as its sweet-taste, the aroma and aniseed-flavor but also for its crunchiness. It is consumed raw in salads or cooked. Generally, the most used instrumental techniques to measure quality attributes of fruits and vegetables are destructive and involve a considerable amount of manual work, primarily due to sample preparation. In addition, most of these analytical techniques are time consuming, and sometimes, may require sophisticated equipments (i.e. the analyses of total phenolic compounds and antioxidant activity by spectrophotometer, sugars, organic acids, and vitamin C and phenolic composition by HPLC). Moreover, destructive analyses can be performed only on a limited number of samples and, thus, their statistical relevance may be limited. In recent years, researches have been focused on the development of non-destructive techniques suitable to increase the number of specimens that can be analysed, to repeat more times the same analysis on the same sample at a given time

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or during its physiological evolution, and in order to achieve real-time information (Costa et al., 2009). Hyperspectral imaging, among non destructive spectral techniques, has gained importance in the past few decades due to its potentiality for accurate, robust and non-destructive prediction of food quality of different types, including fruits and vegetables.

Many scientific studies have been carried out to determine the essential oil content and composition of fennel seeds and plant extracts for pharmaceutical applications by using different spectroscopic techniques. Schulz et al. (2000) measured the essential oil content in fennels using both dispersive spectrometry and FT-IR technique and obtained good results for the two harvests considered. Strehle et al. (2005) investigated the cross sections of fennel seeds using Raman spectroscopy for mapping the essential oil and the results obtained were satisfactory. Furthermore, in the same study the chemical composition of the fennel oil was studied by taking the Raman spectra of the oil only to avoid interference of the tissue signals. In another study Gudi et al. (2014) used the IR and Raman spectroscopy for the classification of different fennel chemotypes based on their individual profile of volatile substances. It was concluded by the study that ATR-FTIR and NIR both can effectively discriminate intact fennel heads of different chemotypes. NIR FT Raman microspectroscopy has also been used by Baranska et al. (2004) to study the fennel head to obtain detailed information regarding its chemical composition and microstructure. The results of microscopic Raman mapping demonstrated the presence of main essential oil component named anethole in the whole mericarp with the highest concentration on the top. On the other side there is a lack in the literature of studies aimed to predict fennel head composition in terms of maturity-related attributes, also to better assess the harvest time and the internal organoleptic and nutritional properties. This has been assessed for many fruits and vegetables; many studies demonstrated the capability of NIR and Vis-NIR to predict the soluble solids content (SSC) and titratable acidity of apples (Iyo and Kawano, 2001; Zude et al., 2006), kiwifruits (Ying et al., 2005; Moghimi et al., 2010), mushrooms (Roy et al., 1993) citrus fruit (McGlone et al., 2003; Guthrie et al., 2005; Gómez et al., 2006), strawberries (ElMasry et al., 2007), tomatoes (Flores et al., 2009), mangoes (Marques et al., 2016). Moreover at the moment current applications include the estimation of nutritional compounds (Givens et al., 1997; Liu et al., 2015), the single organic acid (Ignat et al., 2012) and sugars detection (Rady et al., 2015) in different species.

All these successes in the prediction of various analytes using Vis-NIR and NIR provided motivation to test the capability of these techniques for the prediction of similar analytes in fennels. Particularly, soluble solids, individual sugars and organic acids, phenols, and antioxidant activity were monitored also in relation to different sheath layers of fennel heads in order to have a spatial distribution of these constituents. A second objective of this work was to test the possibility of using hyperspectral imaging to discriminate among fennel heads from different harvest times.

2. Materials and methods

2.1. Experimental design and spectral acquisition

Thirty-five fennel heads were collected during 7 different harvests over a period of 3 weeks, in order to enlarge the span of the variation interval of each individual constituent. For each fennel 2 images of the perpendicular section (cut in the middle of the head) were acquired using a Hyperspectral scanner (version 1.4, DV srl, Padova, Italy) with 2 spectrographs, one in the Vis-NIR range (from 400 to 1000 nm) and the second in the range of 900–1700 nm (spectral resolution of 5 nm and spatial resolution). For each fennel section 5 leaves or sheathes were individuated from the external to the internal part (including the stem).

From each corresponding sheath layer of a single fennel, three regions of interest (ROI) were acquired separately from the images of the Vis-NIR range and the NIR range, using the image cropping tool in the PLS toolbox, as shown in Fig. 5. For prediction purpose, the mean spectra of the ROI were averaged, obtaining one spectra per each fennel layer. Over 160 samples analysed, 20 samples were eliminated for some problems in the recording of the reference values of one or more quality parameters, having a total of 140 spectra, as the corresponding number of samples and reference measures.

2.2. Chemical analysis

The same 5 leaves for each fennel section, used for NIR and Vis-NIR acquisitions, were separately analysed for the following quality parameters.

Total soluble solid (SSC) content and titratable acidity: Twenty grams of fennel tissues were transferred in a falcon tube, homogenized in an Ultra-Turrax (IKA T18 basic, Wilmington, NC, USA) and filtered with two layers of cheesecloth. Few drops of the fennel juice obtained were used to measure SSC content with a digital refractometer (Atago PR32-Palette, Tokyo, Japan). Another fraction of 1 g of fennel juice was used to measure the titratable acidity (reported as mEq NaOH 100 g^{-1} of fresh weight), with an automatic titrator (Titrator T50, Mettler Toledo) titrating with NaOH 0.1 N until final pH of 8.1.

Total phenolic content and antioxidant activity: The same extraction was carried out for analyses of total phenolic content and antioxidant activity, following the procedure described by Amodio et al. (2014) with slight modifications. Five grams of fresh fennel tissue were homogenized in 2 mM sodium fluoride (NaF) methanol: water solution (80:20) for 1 min, using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA). The homogenate was filtered through two layers of cheesecloth and then centrifuged (PK 121R, Thermo Electron Corporation, France) at 10,000 rpm for 10 min at 4 °C. The pellet was discarded and the supernatant was retained and used as the extract. The total phenolic content was determined according to the method reported by Singleton and Rossi (1965). Each extract (100 μL), appropriately diluted, was mixed with 1.58 mL distilled water, 100 μL of Folin-Ciocalteau reagent and 300 μL of a sodium carbonate solution (Na_2CO_3 200 g L^{-1}). After 2 h of incubation at room temperature in the dark, the absorbance was read at 725 nm against a blank using a spectrophotometer (Shimadzu UV-1700, Jiangsu, China). The total phenolic content was calculated based on the calibration curves of gallic acid (0–500 μg/mL) and expressed as mg of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} fw). Antioxidant activity was performed following the procedure described by Brand-Williams et al. (1995) with minor modifications. Each extract (50 μL), appropriately diluted, was mixed with 950 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution to initiate the reaction. The absorbance was read at 515 nm after overnight incubation at room temperature in the dark. Antioxidant activity (abbreviated as DPPH) was calculated as mg of Trolox equivalents per 100 g of fresh weight (mg TEAC 100 g^{-1} fw) using a Trolox standard curve (0–625 μM).

Simultaneous analysis of organic acids and sugars: Organic acid and sugars were extracted homogenizing 10 g of fresh fennel tissue with 20 mL of ultrapure water using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) at 14,000 rpm for 1 min. The homogenate was centrifuged at 9000 rpm for 10 min at 5 °C. The supernatant was filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and then through a 0.2 μm cellulose acetate filter (INCOFAR, Modena, Italy). All extracts were performed in