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Application of fluorescence spectroscopy as a field method in the determination of varietal differences parsley (*Petroselinum crispum*) accessions after harvesting

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Abstract: The aim of this study is to establish the application of fluorescence spectroscopy as a field method for determining post-harvest varietal differences in parsley accessions (*Petroselinum crispum*). The proposed method includes studies of leaves from four standard varieties of parsley by means of fluorescence spectroscopy. Due to varietal differences of a given genotype, they will be compared in terms of determining the spectral distribution. Specimens were grown under uncontrolled field conditions. This will allow the approach to be practiced non-invasively in the quality control of parsley production in undefined premises and outdoors.The experimental studies were carried out locally on the farm. The spectral installation for generating emission fluorescence spectra is mobile. A system engineering approach based on the classical principles of modern optoelectronics was applied in its setup (optical setup). The results can be used to optimize the time for the analysis of the varietal difference of parsley genotypes after harvest, under uncontrolled conditions. The stability of the breeding variety and its common blacks with an established variety of the same species can be observed by monitoring the intensity of the signal. This will support the process of determining the belonging of a specific accession to a given variety (even for samples of unknown origin, when it is necessary to qualify the result of samples in a short time.

Keywords: Parsley accessions *(Petroselinum crispum)*; varietal differences; uncontrolled conditions; field method; fluorescence spectroscopy

INTRODUCTION

Parsley (*Petroselinum crispum*) is a biennial herb and spice widely distributed in the Middle East, Europe, North and South America (Sarwar et al., 2016). Curly parsley and Italian flat-leaf parsley are the two most popular types of parsley. The Italian variety is more aromatic and less bitter in taste than the curly one. There is another type of parsley known as root turnip (or the Hamburg variety) that is grown for its burdock-like roots. Parsley belongs to the umbel family. People began consuming parsley as a spice sometime during the Middle Ages in Europe (Punosevac et al., 2021; Quan et al., 2017).

In some countries, the curly variety is more popular. This stems from ancient preferences for this species, as people likened plantain parsley to a type of poisonous grass (Bouasla et al., 2022). The development of rapid and accurate methods such as optical diagnostics based on non-destructive analysis will help to overcome the barriers in studying and monitoring the processes related to the qualitative analysis of selected target genotypes in breeding programs.

Optical-electronic methods for assessing the quality of plants are non-contact, fast-acting, selective and do not violate the integrity of the examined sample. Based on them, it is possible to create non-invasive methods for post-harvest evaluation of parsley (*Petroselinum crispum*) specimens. So far, there is no data on their research using the proposed mobile method.

Fluorescence spectroscopy in the food industry is widely used for quantitative analysis. It is sensitive and specific enough to detect even small concentrations of compounds (Qin & Lu, 2008; Valeur et al., 2012). For example, changes in the structures of proteins, carbohydrates and lipids in oils can be detected through it. This is useful for verifying the authenticity of food products (Bachmann, 2006; Hof et al., 2005). Advances in fiber optic technology offer outstanding opportunities for the development of a wide range of highly sensitive fiber optic sensors in many new application areas. Fiber-optic components are successfully adapted to assemblies with micro-optics elements such as lenses, mirrors, prisms, gratings and others (Dakin & Brown, 2006; Mitchke, 2010). Fluorescence spectroscopy in agricultural sciences is applied to the analysis of tomatoes (Hoffmann et al., 2015) and cereals (Karoui & Blecker, 2011). Their characterization through this technique is done by grouping objects with similar characteristics to establish methods related to their classification.

In connection with the demands of consumers for high food quality, the conducted research can serve as a basis for the creation of mobile detecting devices, with which to carry out instant analysis of warehouse production of parsley in uncontrolled conditions, both in processing plants and in food retail outlets.

The present study aims to establish the function of fluorescence spectroscopy in the act of field method in the determination of varietal differences after parsley harvesting. They will be compared in terms of determining the spectral distribution due to the varietal differences of a particular genotype. The specimens were grown under uncontrolled field conditions. This will permit the technique to be applied non-invasively in the quality control of parsley production in unspecified rooms and outdoors.

MATERIAL AND METHODS

Material

Accessions from four standard parsley varieties, were investigated:

- Italian flat-leaf parsley – It is an early very high-yielding variety distinguished by extremely large and leafy rosettes that reach a height of 69-90 cm. The leaves are highly aromatic, broad, glossy and of a rich dark green color. The variety is very adaptable, does not form root crops and is preferred both for fresh consumption and as a dried spice. Vegetation period 75-85 days.

- Curly parsley - It is an early and productive variety. The leaves are strongly curled, tender and fragrant. Medium green. It is suitable for decorating salads and dishes. Grows best in full sun. Curly parsley is sown directly and you do not need to prepare seedlings. Spring sowing of the seeds takes place between February 20 and March 10. Do the pre-winter sowing of the seed in the period between December 1 and 15. The vegetation period lasts about 70-80 days. It is grown for its highly aromatic curly leaves, which are rich in essential oils and numerous vitamins.

- Einfache Schnitt - Smooth-leaved, very aromatic variety with a high yield. With late sowing, a harvest is possible in autumn until the following spring. Suitable for beds, containers and balcony boxes. Make sure the soil is loose and avoid waterlogging. Parsley loves a moist, semi-shady, humus-rich and nutritious location.

- Komon 2 - This variety is a standard selection for market and home gardens. It produces flat, smooth, deeply indented, dark glossy green, medium-sized leaves with a strong aroma. Parsley grows best in a mostly sunny location with relatively rich, moist and well-drained soil.

Parsley was grown using standard technology. The seeds of the target investigated varieties were sown at a depth of 1.6 cm at a distance of 0.6 cm. Then they were covered with light soil. Before germination, the soil layer is moist to a depth of 2 cm. They are watered once every 4 days.

Fluorescence spectroscopy

The mobile fiber-optical spectral (Figure 1) installation for the study of fluorescence signals is designed specifically for the rapid analysis of plant biological samples. The mobile experimental setup used by fluorescence spectroscopy includes the following components:

• Laser diode (LED) with an emission radiation of 245 nm with a supply voltage in the range of 3V. It is housed in a hermetically sealed TO39 metal housing. The emitter has a voltage drop from 1.9 to 2.4V and a current consumption of 0.02A. The minimum value of its reverse voltage is – 6 V.

• Rod lens of the achromatic doublet type. It is composed of two bonded lenses with different Schott and Corning dispersion coefficients with an anti-reflective coating. The radii of the two lenses are selected so that the chromatic aberration of one lens compensates for that of the other. The tolerance of the diameter of the forming optics is -0.005 mm

• The multimode optical fiber is FG200LEA. It has a core diameter of 200 μm and a step index refraction

• Quartz glass area 4 cm 2. Its optical properties are to be transparent to visible light and to ultraviolet and infrared rays. This allows it to be free of inhomogeneous that scatter light. Its optical and thermal properties exceed those of other types of glass due to its purity. Light absorption in quartz glasses is weak.

• CMOS detector with photosensitive area 1.9968×1.9968 mm. Its sensitivity ranges from 200 nm to 1100 nm. Its resolution is $\delta\lambda$= 5. The profile of the detector sensor projections along the X and Y axes is also designed for very small amounts of data, unlike widely used sensors.

The sample is irradiated by the . LED; after which it fluoresces. The emission signal is received at 45o by the rod lens, which transmits it through the optical fiber to the detector.

The three unique advantages of this scheme are:

• Inclusion of the Rod lens in the construction of the system, due to their increased light transmission efficiency by almost completely filling the air gaps between the individual lenses.

• Unique design of optical fiber coupling from a headquarters lens in a duralumin housing. In this way, the most optimal for compiling with optical fibers and forming images from laser diodes with low levels of intense losses is achieved.

• Reception of the emission signal at 45^{o}.

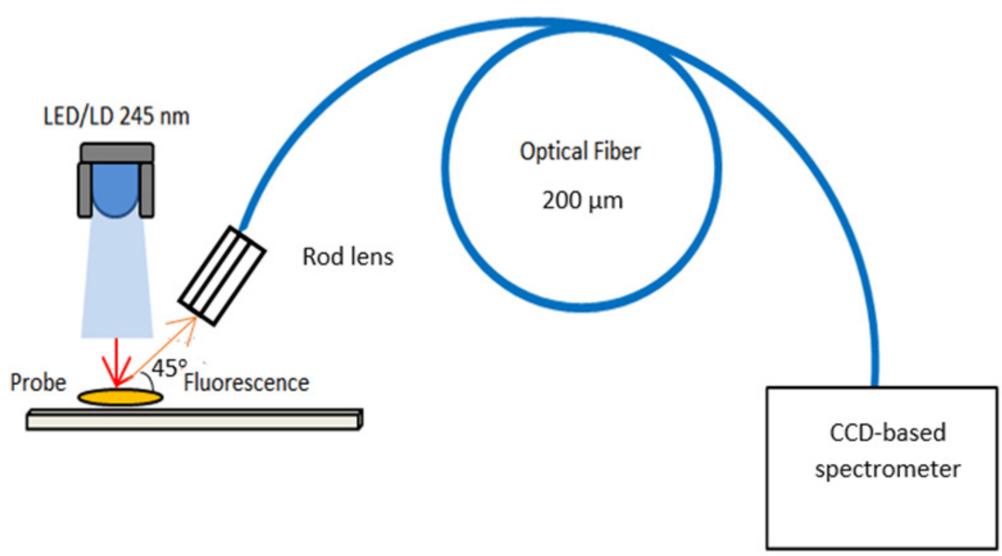

Figure 1. Mobile experimental installation used by fluorescence spectroscopy

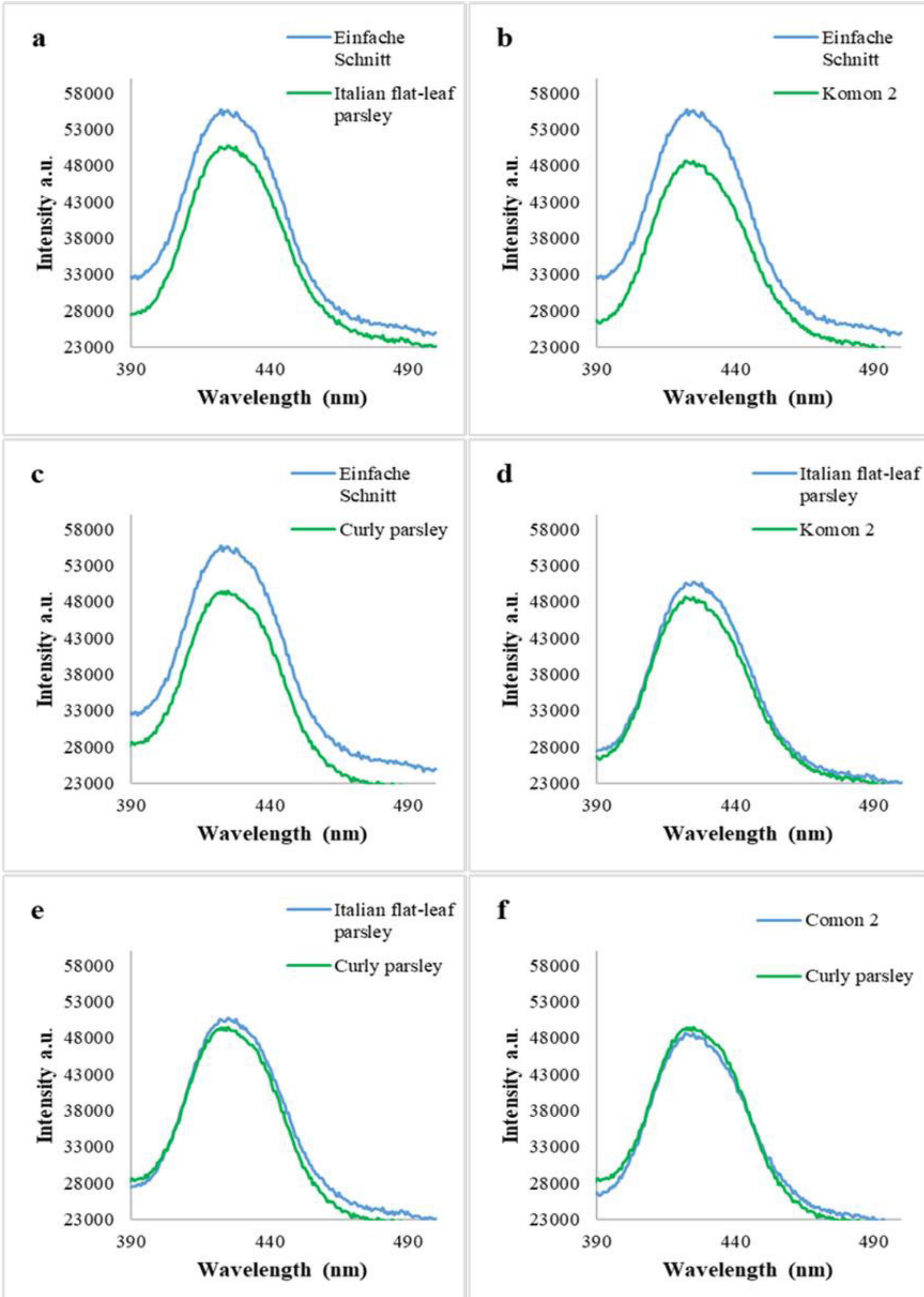

Figure 2. Difference in emission wavelength for Einfache Schnitt, Italian flat-leaf parsley, Komon 2 and Curly parsley

RESULTS AND DISCUSSION

The optical properties of parsley are determined by its energy structure, which includes both the occupied and free electronic energy levels, as well as the energy levels of the atomic vibrations of the molecules or the crystal lattice. The possible transitions between these energy levels, as a function of photon energy, are specific to parsley, resulting in spectra and optical properties unique to it. Parsley contains particles smaller than the wavelength of visible light. Particles in the turbid medium, such as parsley, act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce. Therefore, fluorescence spectroscopy finds application for analysis in this culture. The optical parameters and spectral properties also change as a function of temperature, pressure, external electric and magnetic fields, etc., which allows obtaining essential information about changes in the chemical and cellular morphological composition of parsley. This gives us reason to claim that, for the first time, fluorescence spectroscopy has been applied to the analysis of parsley samples regarding their varietal affiliation under uncontrolled conditions. A difference in the emission fluorescence signal of parsley accessions from different varieties is clearly observed.

The results give reason to conclude that fluorescence spectroscopy can be successfully applied as a rapid tool to establish the origin of unknown parsley leaves in the presence of a rich library of spectra. This will be an applied tool in breeding programs. By tracking signal intensity, one can monitor the stability of a variety and its general characteristics with other varieties. Emission fluorescence signals of Italian flat-leaf parsley, Komon 2 and Curly parsley (Figure 2 d, e and f are close in terms of wavelength localization and signal intensity level.

This is expected from the fact that the cultivars have a similar cell morphological composition when grown outdoors. However, the method of fluorescence spectroscopy can be applied to distinguish the leaves of these three cultivars, since the correlation in the spectral distribution is sufficiently distinct and distinguishable to determine practically qualitatively the belonging of the leaves to a given cultivar. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of a parsley leaf to a given variety.

CONCLUSON

The fluorescence spectroscopy method is fast-acting in application as a field method in the determination of varietal differences after parsley harvesting locally under uncontrolled conditions. (It has been proven that fluorescence spectroscopy will successfully apply as a rapid tool to establish the origin of unknown parsley accessions in the presence of a rich library of spectra). This will be an applied tool in breeding programs. By monitoring the signal intensity, the stability of a breeding line and its common blacks with an established variety of the same species can be monitored.

The differentiation of related varieties is a laborious and time-consuming task. For these reasons, the development of techniques that could assist in an early, quick and accurate differentiation of related varieties is of the utmost importance.

As conclusion, it has been established that the system engineering approach for adjustment (optical adjustment) of a specialized installation for applied research with fluorescence spectroscopy is applicable in the determination of varietal differences during parsley breeding.

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