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Application of fluorescence spectroscopy in the determination of varietal differences after potato harvesting

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Abstract

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The present study aims to establish the application of fluorescence spectroscopy as a field method in the determination of varietal differences after potato harvesting.

The experimental studies were conducted on site at the Institute of Vegetable Crops "Maritsa" after potato harvesting in 2021. The fluorescence analysis was carried out with a source with an emission wavelength of 285 nm and a portable fiber-optical spectrometer model, AvaSpec-ULS2048CL-EVO.

The subject of this research are tubers from Tresor (early variety standard), Sante (medium early variety standard), Agria (medium late variety standard), C 617 (early breeding line), D 486 (early breeding line), C 619 (medium early breeding line), B 782 (medium early breeding line), B 783 (medium-late breeding line), C 716 (medium late breeding line).

The correlation between their emission signals of the samples was established. This fact allows fluorescence spectroscopy to be successfully applied as a rapid tool to establish the origin of unknown tubers in the presence of a rich library of spectra as an applied tool in breeding programs.

The results of the experiment can be used to optimize the time for the analysis of the varietal difference of the potato genotypes after harvest.

Fluorescence spectroscopy in a fiber-optical configuration will support the process of determining the belonging of a specific variety to a given variety (even for samples of unknown origin, when it is necessary to qualify the result of accessions in a short time.

Keywords: potato tubers; fluorescence spectroscopy; breeding line; variety; emission wavelength

Introduction

Potato (*S. tuberosum* L.) is the most important non-cereal crop and ranks fourth in global production after maize, wheat and rice (Londhe, 2016). Their wide distribution and versatile use as food, fodder and raw material for industry (Tunio et al., 2020) is determined by the high taste and nutritional qualities of the tubers, whose chemical composition is characterized by an optimal ratio of organic and mineral substances.

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 have been successfully adapted to assemblies with micro-optics elements such as lenses, mirrors, prisms, gratings, and others (Dakin & Brown, 2006; Mitchke, 2010).

Fluorescence spectroscopy is an important research tool in many analytical areas of science. It is currently the dominant methodology and is widely used in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, agriculture and genetic analysis, as well as in many other application areas. Methods using this light phenomenon are highly sensitive, time-consuming, and do not require the expense and difficulty of using radioactive tracers (Becker et al., 2003; Albani, 2007).

Various techniques have been investigated for non-invasive spectrospectrometric analysis of potatoes. To verify the quality and safety of raw potato, and sweet potato tubers have been successfully applied spectroscopic methods. Conventional quality assessment (internal and external) is applied to these, followed by a description of spectroscopy as a non-destructive technique and a summary of recent successful implementations of these tools in relation to quality assessment (Sanchez et al., 2020).

Both NIR-excited fluorescence spectra and pure Raman spectra can be used to identify three potato cultivars (Dai et al., 2014).

The effects of light are used applicable to spectral analysis such as fluorescence, transmission and diffuse reflectance for quality control of vegetable crops, incl. in potatoes. Also, they can serve as a method in the determination of varietal differences after potato harvesting, since fluorescence emission is visualized in the visible spectral range and from ultraviolet rays.

The spectral distribution of the emission signal in potato tubers consists mainly of two maxima in the visible range (Leo et al., 2007).

The ability of near-infrared spectroscopy (NIRs) to predict the acrylamide content of French fries has been successfully applied. NIRS can be used as a screening tool in potato breeding and potato processing research to reduce acrylamide in the food supply (Adedipe et al., 2016).

Visible (VIS) and near-infrared (NIR) spectroscopic systems have been successfully applied as a tool to measure the diffuse spectroscopy of three different types of potatoes. As a result, the model created with their layout can quickly and accurately identify potato varieties without any destruction, which provides a new way to detect potato quality and variety identification (Chen et al., 2016).

Quantification of reducing sugar content in sweet potatoes by near-infrared (NIR) reflectance mining has been successfully applied. Thus, reducing sugar content in sweet potatoes was successfully detected by NIR spectra in a rapid manner (Hong et al., 2022).

The established application of fluorescence spectroscopy as a determination of varietal differences after potato harvesting is the aim of the present study.

Materials and Methods

Materials

Tubers were produced at "Maritsa" Vegetable Crops Research Institute using a block method with 9 variants, 4 repetitions, 20 plants in a repetition, with an inter-row distance of 70/25 cm and an experimental area size of 3.5 m^2. Planting was done in the first half of March with planting material obtained from our mountain base in the village of Pavelsko. The agrotechnical measures were carried out in the optimal timings for sowing.

The accessions that are the subject of the study are:

Three variety standards:

- Tresor: early variety standard characterized by large (180 g), oval tubers with shallow eyes, yellow skin and light yellow flesh;
- Sante : medium early variety standard, characterized by large (180 g), oval tubers with shallow eyes, yellow skin and light yellow flesh;
- Agria: medium late variety standard characterized by large to very large (190 g), oblong-oval tubers with shallow eyes, yellow skin and dark yellow flesh.

Six breeding line created at "Maritsa" Vegetable Crops Research Institute:

- C 617: early breeding line characterized by medium-large (100 g), round-oval tubers with shallow eyes, yellow skin and light yellow flesh;
- D 486: early breeding line characterized by with medium to large (140 g), oval tubers with very shallow eyes, yellow skin and flesh;
- C 619: medium early breeding line characterized by large (190 g), round-oval tubers with medium deep eyes, yellow skin and flesh;
- B 782: medium early breeding line characterized by medium large (110 g), round-oval tubers with shallow eyes, yellow skin and flesh;
- B 783: medium-late breeding line characterized by medium-large (120 g), round-oval tubers with very shallow eyes, lta skin and flesh;
- C 716: medium late breeding line characterized by very large (200 g), round tubers with shallow eyes, yellow skin and flesh.

Fluorescence spectroscopy

This study was conducted with a fiber optic spectrometer, which allows the generation of emission fluorescence signals from 200 nm to 1200 nm in connection with the implementation of the activities on the set task. The device is used for fluorescence spectroscopy of solid samples with a photosensitive area of 1.9968×1.9968 mm. The experimental setup includes a laser diode (emission wavelength 285 nm, optical power 16 mW, DC), a portable spectrometer model AvaSpec-ULS-2048CL-EVO. The sample is placed on a duralumin stand, which allows reception of an emission signal from it under 180° from a U-shaped optical fiber. This reduces aberrations and allows the generation of a better quality emission fluorescence signal (Figure1). The resolution of the spectrometer can be in the range of 0.06 – 20 nm, with that of the setup used for our experiment being 0.09 nm. Since the fluorescence is often very weak and also omnidirectional, in order not to saturate the receiver, the useful fluorescence signal is measured in a direction that is less than 180° to the excitation radiation. It is preferable to use a laser diode (LED) as a source in the scheme, since its spectral width is very small. LED used in the experiment has a relatively wide emission spectral width of about 30-40 nm and the angular distribution of its emission is in a large angular range of +/-30°. The sensitivity of the spectrometer is in the range from 200 nm to 1200 nm. Its resolution is $\delta\lambda$= 5 nm. The spectral setup based on fluorescence signals allows recording both the emission spectrum and the spectrum of the excitation source.

Fig. 1. The experimental setup used for the fluorescence spectroscopy. Measurements

For the particular circuit, the photodetector is of the CMOS model S9132 type. Its sensitivity is in the range of 200 nm to 1200 nm. Its resolution is $\delta\lambda$= 5 nm. S9132 was chosen because it can detect very high intensity colubene emission radiation.

The laser radiation is deflected from the source and irradiates the accession. After the sample fluoresces, the emission signal is incident on a U-shaped optical fiber with a core diameter of 200 µm with a step index of refraction and a numerical aperture of 0.22.

It takes him to the detector. In the spectrometer, the light signal is converted to electrical-digital using a USB 2.0 wire, downloaded to a computer with AvaSoft8 software and exported to Excel. This allows analysis, processing and visualization of the results of the conducted research.

Study Indicators

Spectral analysis – a spectral analysis of 10 tubers of each variety and each breeding line was carried out. The emission spectrum represents the wavelength distribution of an emission measured for a constant excitation wavelength. The excitation spectrum represents the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength. This spectrum is represented as a function of the wavelength of the light intensity incident on the photodetector in the spectrometer.

Spectral analysis indicators are:

- Exciting wavelength;
- Emission wavelength;
- Intensity strength.

Results and Discussion

The optical properties of potato tubers are determined by their 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 are tuberous-specific as a function of photon energy. As a result, the spectra and optical properties are only characteristics of it. Tuberous contains particles with sizes smaller than the wavelength of visible light. Particles in turbid media such as tuberous act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce. Therefore, fluorescence spectroscopy finds application for analysis in this vegetable crop. 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 the tuberous.

The averaged graphs from the research conducted as regulations are graphically presented as follows: Figure 2 – Early potatoes, Figure 3 – Average early potatoes, Figure 4 – Average late potatoes, Figure 5 – Spectral distribution of the three varieties: Tresor, Agria and Sante. In the early potatoes, the emission fluorescence signal of the breeding lines was of much lower intensity than that of the confirmed variety. In mid-early potatoes, the fluorescent emission signal of the breeding lines is closer in intensity to that of the confirmed variety. In medium-late potatoes, the fluorescent emission signal of the breeding lines is of a fairly close intensity to that of the confirmed variety. In the case of the three confirmed varieties, a clear correlation between their emission signals is visible.

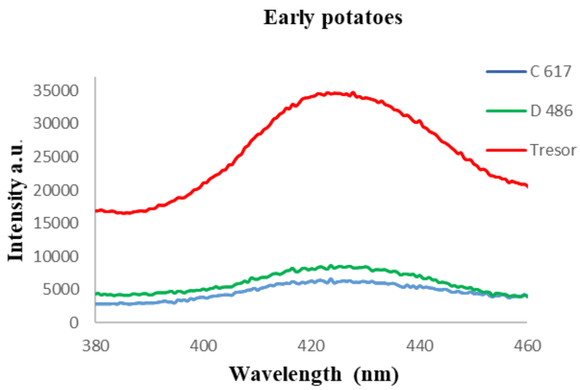

Fig. 2. Emission wavelengths of early potatoes

A literature survey was conducted to conduct similar research. It turned out that until now the described experimental approach for the field method in the determination of varietal differences after potato harvesting has not been applied nationally and internationally. This gives us reason to claim that for the first time, fluorescence spectroscopy was applied to the application of fluorescence spectroscopy as a field method in the determination of varietal differences after potato harvesting under uncontrolled conditions.

The method is successfully applied to distinguish tuberous from different varieties. Fluorescence spectroscopy can be applied to analyze tuberous of unknown cultivars and establish their origin with a sufficiently well-structured data library. Because it can be applied topically to test specimens.

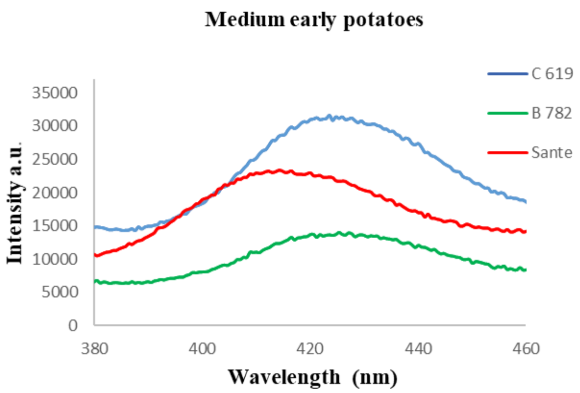

Fig. 3. Emission wavelengths of medium early potatoes

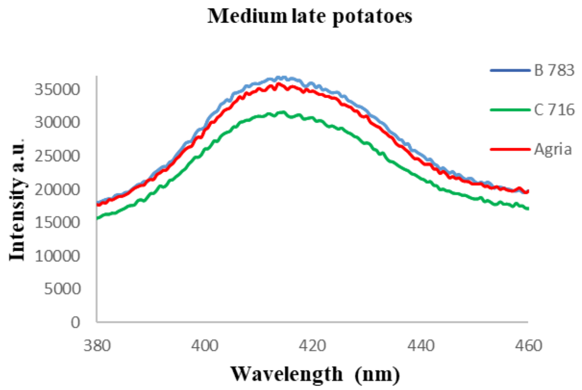

Fig. 4. Emission wavelengths of medium late potatoes

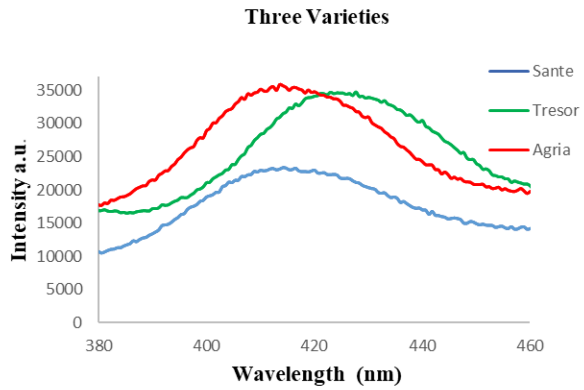

Fig. 5. Emission wavelengths of three varieties

It eliminates sample damage during transport and provides a highly sensitive assay.

Conclusion

It was established the method of fluorescence spectroscopy is fast-acting in the determination of varietal differences after potato harvesting.

A systems engineering approach to the setup (optical setup) of a dedicated fluorescence spectroscopy applied research facility was found to be applicable in determining varietal differences in potato breeding.

It has been shown that fluorescence spectroscopy will help to be successfully applied as a rapid tool to establish the origin of unknown tubers in the presence of a rich library of spectra.

It was established this will be an applied tool in breeding programs. By monitoring the signal intensity, the stability of the breeding line and its common blacks with an established variety of the same species can be observed.

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