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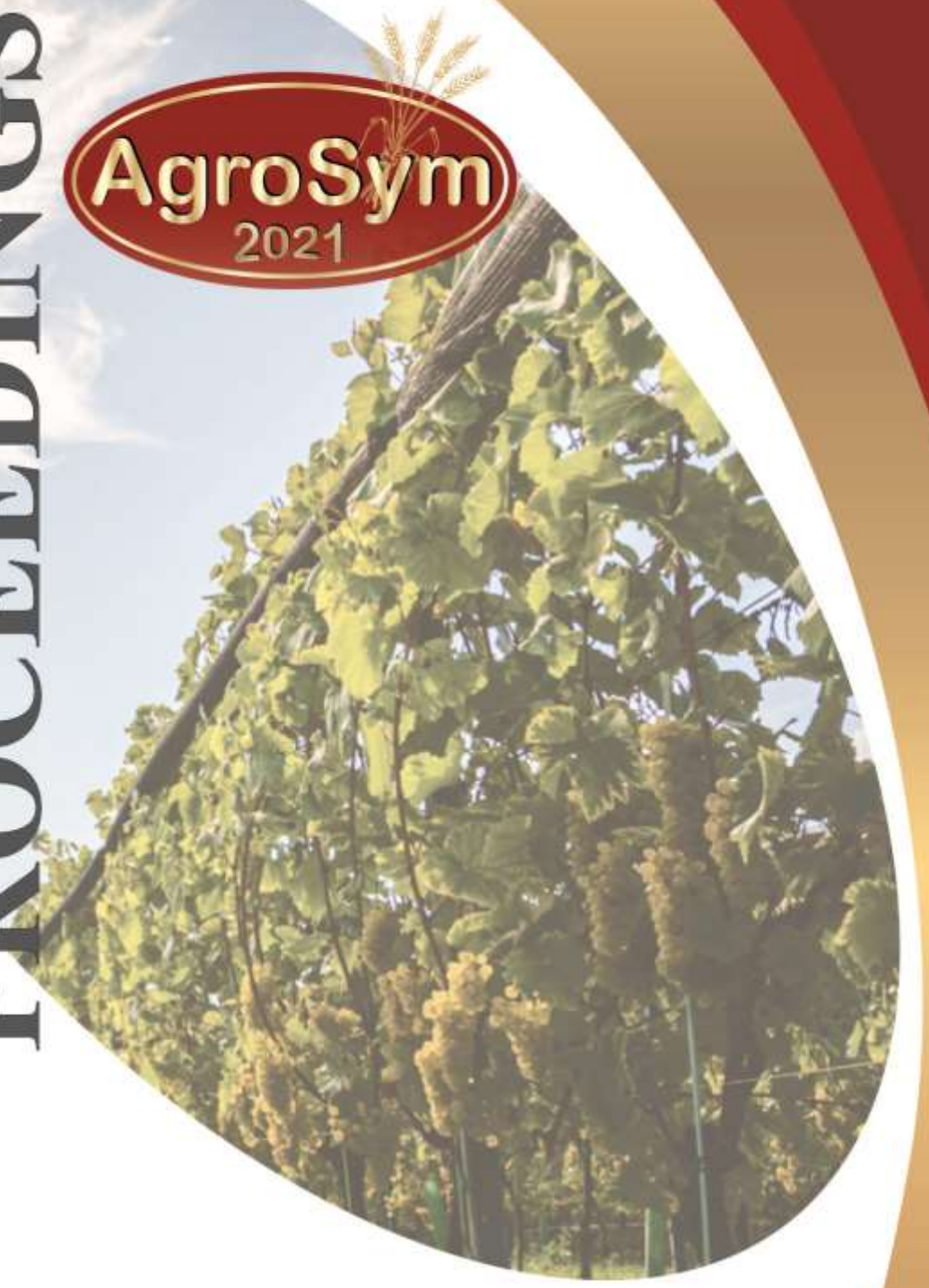

XII International Scientific Agriculture Symposium "AGROSYM 2021" October 7-10, 2021

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CHARACTERIZATION OF COLORED MAIZE SEED FRACTIONS USING FLUORESCENCE SPECTROSCOPY AND MULTIVARIATE ANALYSIS

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Abstract

Application of fluorescence spectroscopy combined with chemometrics algorithms provides rapid and non-destructive screening method in seed quality estimation, widely used in the agricultural industry and crop breeding. Fluorescence spectroscopy is a technique capable of detecting differs fluorophores among various colored maize seed cultivars and through different seed fractions. In the present study, we used the Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm to analyse the excitation-emission matrices (EEMs) of various cultivars of colored maize (*Zea mays* L.) seeds and its fractions. The EEMs were recorded as a set, with the excitation ranging from 280 nm to 330 nm and the emission spectra ranging from 300 nm to 550 nm. The MCR-ALS analysis yielded two major fluorescence components for all of the analysed samples. Both position and shape of component 1 (C1) varied among the samples. On the other hand, the position and shape were similar for component 2 (C2). C1 could be used as a marker for the discrimination of colored seeds and their fractions. The observed variations in C1 between the analysed seeds may be due to the presence of their individual fluorophores, assigned to anthocyanins, proteins, and phenolics. In conclusion, the MCR-ALS analysis of the seed emission spectra has a great potential for the rapid and non-expensive characterization of various cultivars of colored seeds.

Keywords: *maize seed, fluorescence, Multivariate Curve Resolution-Alternating Least Squares.*

Introduction

Maize (*Zea mays* L.) is considered one of the major food sources worldwide. Health benefits correlated to consumption of the whole grain are related not only to nutrients like carbohydrates, proteins, dietary fiber, vitamins, and minerals but also to the presence of various phytochemicals (Siyuan, Tong, and Liu 2018). Properties of these phytochemicals contribute to the high antioxidant activities of the maize seeds (Del Pozo-Insfran et al. 2006). It is well known that phenolic compounds, mainly phenolic acids, flavonoids, and tannins are major phytochemicals abundant in seeds, with different composition and distribution within the seed fractions (Ndolo and Beta 2014). Polyphenolics like ferulic and p-coumaric acid found in white maize as well as their derivatives, have antioxidant and anticarcinogenic effects according to reported studies. Red colored maize seeds on the other hand have higher anthocyanins content which also expresses antioxidant activity (Del Pozo-Insfran et al. 2006).

Fluorescence spectroscopy is a sensitive, non-destructive and rapid technique, which doesn't require complex sample pretreatment and preparation. It has been used in analysis of various kinds of food, such as cereals food, dairy products, wine, honey and other samples (Sádecká and Tóthová 2007). Fluorescence spectra of cereals are dependent on the species and the cultivar (Zandomeneghi 1999). Cereal food contains a large number of fluorescent molecules

(fluorophores) such as proteins, phenolics and others. The fluorescence spectrum of a food sample is complex, composed of the signals of the contributing fluorophores. In combination with suitable statistical analysis, fluorescence spectra are useful tools for various applications (Sádecká and Tóthová 2007). Applications of the fluorescence spectroscopy combined with Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) for food analyses have been reported previously in many studies (Bartolić et al. 2018; Stanković et al. 2019, 2021).
We developed methods for the measurement and analysis of emission spectra with MCR-ALS of macromolecules composed of different kinds of monomers such as proteins and polyphenols for characterization of different cultivars of pigmented maize seeds (white and red).

Material and Methods

The pigmented (white and red) maize seeds were purchased from the local market in Belgrade, Serbia. The two fractions, inner and outer, were separated using the corresponding laboratory sieves after homogenisation in a mill and subsequently with liquid nitrogen in a mortar with a pestle. Obtained powder samples were used without any further processing before the fluorescence measurements.
The front-face fluorescence measurements of the red and white maize fractions were recorded by an Fl3-221 P spectrofluorimeter (JobinYvob, Horiba, France), equipped with a 450 W Xe lamp and a photomultiplier tube. The ranges of the excitation spectra were 280 - 330 nm, while the range for the recorded fluorescence emission spectra was 300 - 550 nm. A spectral bandwidth of 2 nm was used for excitation and emission slits.
The Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm was applied to analyse the excitation-emission matrices (EEMs) of studied samples. The MCR–ALS has been used to decompose the overlapping mixture of spectral signals into individual components (Stanković et al. 2019).

Results and Discussion

The representative excitation-emission matrices (EEMs) for the inner and outer seed fractions of the various cultivars of pigmented *Zea mays* L. seeds, are presented in Figure 1 (A-D). The differences were observed in their EEMs, which could be explained by the presence of their individual fluorophores, assigned to anthocyanins, proteins, and phenolics (Sádecká and Tóthová 2007). The EEMs of white seeds (Figure 1 A and B), showed considerably weaker emission signal in the range 330 - 360 nm for the excitation 280 - 295 nm, compared to red seeds.

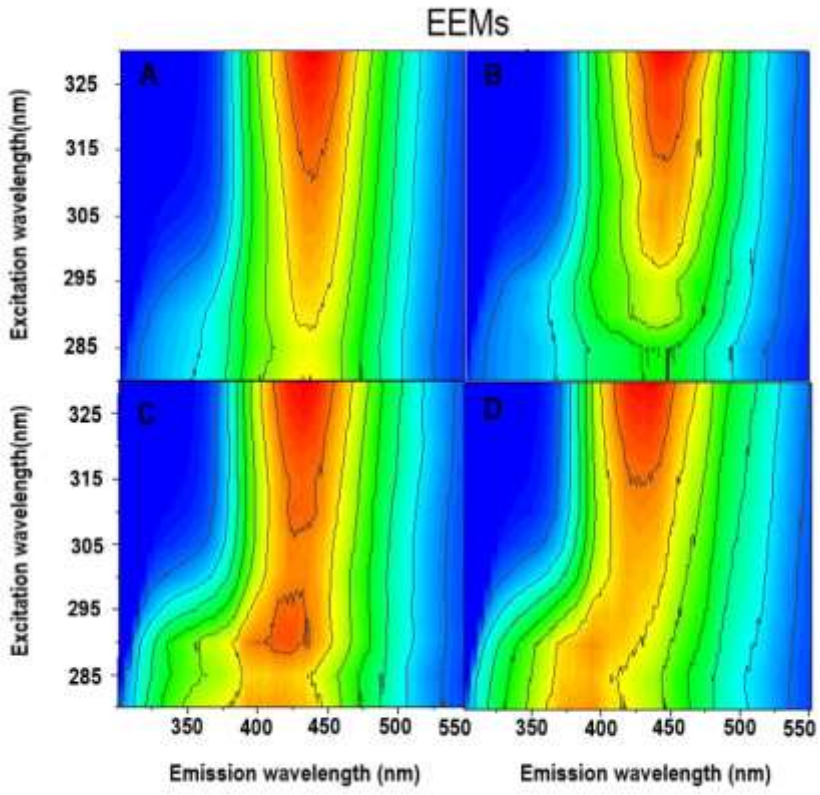

Figure 1. EEMs of the *Zea mays* L. seeds' fractions: inner (A-White and C-red) and outer (B-white and D-red).

Results of MCR-ALS analysis yielded two major fluorescence components. The position and shape of components C1 and C2 varied among the analysed samples.
As shown in Figure 2, the peak position of component 1 (C1) and component 2 (C2) were found around 360 nm and 450 nm, respectively. According to literature data, the fluorescence of the first component (C1) with the emission maximum at 360 nm originates from the aromatic amino acids present in cereal proteins (Zandomeneghi, 1999). Among the analysed samples, the C1 component of the red seeds fraction exhibited the highest relative intensity. Position of the emission maximum of C2 component, assigned to phenolic compounds, varied among the samples.

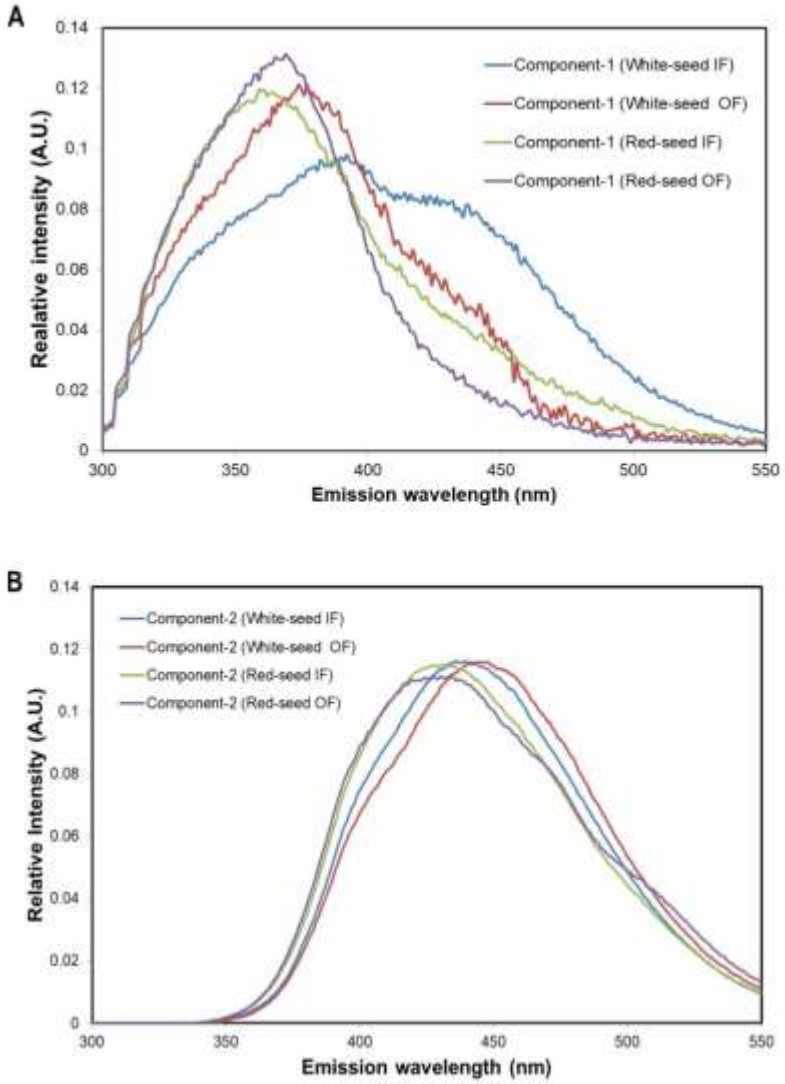

Figure 2. Emission profiles of the spectral components A) component 1 (C1) and B) component 2 (C2) obtained using the MCR-ALS method.

Conclusions

Our results imply that fluorescence spectroscopy combined with the MCR-ALS method could be applied to the rapid, simple, non-expensive characterization of various cultivars of colored seeds and their fractions. As the seed quality depends on different conditions, such as processing, storage, and others, MCR-ALS-derived components may be useful indicators for the screening of cereal seeds' health effects.

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