BOOK OF PROCEEDINGS

XIV International Scientific Agriculture Symposium

"Agrosym 2023"

Jahorina, October 05-08, 2023

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CHARACTERISATION OF MUNG BEAN (*VIGNA RADIATA* L.) SEEDS USING FLUORESCENCE SPECTROSCOPY AND MULTIVARIATE ANALYIS

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Abstract

Mung bean (*Vigna radiata* L.) is a leguminous plant cultivated mainly in south-east Asia and used as an ingredient in local cuisine. Its principal nutritional value is contained in its constituents such as starch, proteins, (poly)phenols, and natural antioxidants. Fluorescence spectroscopy is increasingly used as a method of choice for food analysis; due to the presence of different fluorophores originating from aromatic amino acids and secondary metabolites, it is useful for proteins and phenolics detection. In this study, the total protein and phenolic contents of mung bean seed extracts were determined using the Bradford method and Folin–Ciocalteu (FC) reagent, respectively. Antioxidant activity was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Fluorescence spectra were recorded for a series of excitation-emission wavelengths. Further, we used the multivariate analysis on the recorded excitation-emission fluorescence matrix of the studied samples. The results showed the presence of three different fluorescence components, with the position of the emission maximum corresponding to the fluorophore of proteins (component 1 with excitation/emission peak maxima at Ex 290/Em 345 nm) and phenolics (component 2 - Ex 295/Em 395 nm and component 3 - Ex 350/Em 450 nm). This fluorescence-based method could be a useful approach for estimating the nutrient properties of leguminous food.

Keywords: *Mung bean, fluorescence spectroscopy, MCR-ALS, TPC, DPPH.*

Introduction

Mung bean (*Vigna radiata* L.) belongs to Fabaceae (Leguminosae) family that possesses high nutritional value (Shi *et al*. 2016). Legume seeds are known as a functional food source due to their high content of proteins and essential amino acids, vitamins, and minerals but also contain bioactive components and (poly)phenols that contribute to a high antioxidant capacity (Singh *et al*., 2017; Amarowicz and Pegg, 2008). Secondary metabolites take part in the protection of the seed from infection during and after germination but are also beneficial during later growth stages of the crop (Yusnawan *et al*., 2019; Ganesan and Xu, 2018). The majority of the phenolic content of the mung bean seed include caffeic acid, syringic acid, chlorogenic acid, ferulic acid, and p-coumaric acid (Singh *et al*., 2017).

Fluorescence spectroscopy is a sensitive, fast, and noninvasive analytical method, capable of detecting low amounts of fluorescent compounds in a sample that contains fluorophores (Sádecká and Tóthová, 2017). A series of emission spectra with different excitation wavelengths are recorded to obtain excitation-emission matrices (EEMs). Fluorescence characteristics of specific groups of compounds are analyzed from the obtained EEMs using advanced statistical methods. Multivariate Curve Resolution-Alternating Least Square (MCR-

ALS) analysis was used to extract the position and shape of specific spectral components (Stanković *et al*., 2021).
The aim of this study was to estimate the nutritional value of the analyzed mung bean seeds using fluorescence spectroscopy combined with multivariate analysis.

Material and Methods

Plant samples and their preparation

Seeds of mung bean (*Vigna radiata* L.) were purchased from the Local Organic market in Maastricht, Netherlands. Whole seeds were grinded in a mill and further homogenized to obtain a fine powder with liquid nitrogen in a mortar with a pestle.

Chemicals

Pyrogallol, 2,2-diphenyl-1-picrylhydrazyl, gallic acid (GA), Coomassie Brilliant blue G-250 (CBB G-250), methanol, ethylenediaminetetraacetic acid (EDTA), polyvinylpyrrolidone (PVP) and phosphoric acid (H_3PO_4) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Tris and bovine serum albumin (BSA) were obtained from SERVA Electrophoresis GmbH (Heidelberg, Germany). Folin–Ciocalteu's phenol reagent and sodium carbonate anhydrous (Na_2CO_3) were obtained from Fluka Analytical (Buchs, Switzerland). Acetone and ethanol were obtained from Zorka Pharma (Šabac, Serbia).

Determination of total protein content

Protein concentration was determined using the Bradford method. Proteins were extracted from the powdered seeds with extraction buffer 0.1 M Tris-HCl pH 7.6, containing 1 mM dithiothreitol, 1 mM EDTA, and 2% PVP. The homogenates were incubated and stirred for 30 minutes at 4°C and centrifuged for 10 minutes at 10 000 rpm. Bradford reagent was prepared by dissolving 10 mg of CBB G-250 in 5 ml of 95% ethanol, containing 10 ml of H3PO4 filled with deionized water up to the final volume of 20 ml. Bradford reagent was diluted 5 times with deionized water before use. Sample aliquots of 5 µl were placed in microplates and mixed with 200 µl of diluted Bradford reagent. Absorbance at 595 nm after 5 minutes of incubation was detected using a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). Protein concentration was determined using a calibration curve in the range of 0.1-1.0 mg/ml BSA solution.

Determination of total phenolic content

Total phenolic content (TPC) was determined using Folin–Ciocalteu (FC) reagent. Phenol extraction was carried out by adding 80% methanol solution to 100 mg of seed powder in a 1/10 (w/V) ratio. Samples were incubated on a shaker for 60 minutes at 25°C, followed by centrifugation for 5 minutes at 10 000 rpm. From collected supernatants, aliquots of 50 µl were taken and mixed with 475 µl of 0.2 M FC reagent. After 3 minutes, 475 µl of 0.25 M Na2CO3 was added to each sample and incubated for 60 minutes. Absorbance at 724 nm was measured using a UV-VIS microplate reader. The calibration curve was prepared with gallic acid in 80% methanol in the range of 0.05-2.00 mM and used for the determination of TPC content. Obtained results were expressed in µmol equivalent of gallic acid per gram of dry weight (gDW).

Determination of DPPH radical scavenging activity

The antioxidant activity (AA%) of mung bean seeds was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. Powdered seed samples were added to the reaction mixture containing 0.1 mM DPPH in 70 % ethanol solution. After 30 minutes of incubation in the dark, 200 µl aliquots were taken and placed in microplate wells. Absorbance at 517 nm was measured in a UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). For blank and control, pure deionized water and 0.1 mM DPPH solution was used respectively. Antioxidant activity was defined as a percentage of DPPH-reagent consumed during the reaction.

Fluorescence spectroscopy and multivariate analysis

The fluorescence measurements of the mung bean seeds were recorded using an Fl3-221 P spectrofluorimeter (Jobin Yvob, Horiba, French Republic), equipped with a 450 W Xe lamp and a photomultiplier. The front-face (FF) configuration was used for the fluorescence measurements. The fluorescence emission spectra of the analysed seed samples were measured in the range of 270 to 515 nm with excitation wavelength ranging from 260 to 380 nm in 5 nm steps. The integration time was set at 0.1 s, while the spectral bandwidth of 1 nm was set for the excitation and emission slits. The multivariate analysis (Bartolić *et al*. 2018, Stanković *et al*. 2019) has been used to decompose and extract an optimal number of emission components (corresponding to the fluorophores) from the excitation-emission matrix (EEM) of the analysed sample. The analysis was performed using the Unscrambler X 10.4 (Camo Analytics, Oslo, Norway) software.

Results and Discussion

Results of total protein and phenolic content of the studied extract of the mung bean seed samples as well as antioxidant activity with their respective standard errors are presented in Table 1. Obtained protein content was calculated from the regression equation (R = 0,999, y = 0.478×x + 0.793), from the calibration curve and expressed in mg of protein per gram of dry weight (mg/gDW). Obtained results of total phenolic contents (TPC), calculated from the calibration curve using the regression equation (R=0.999, y =0.482×x + 0.033) are expressed in µmol equivalents of gallic acid per gram of dry weight of the samples (µmol eq.GA/gDW). The resulting value equals 56.91 µmol eq.GA/gDW and corresponds to the previously published results (Singh *et al*., 2017; Orak *et al*., 2018).

Table 1. Total phenolic content, total protein content, antioxidant activity of the whole mung bean seed with their corresponding standard errors out of 4 replicates.

Total protein content (mg/gDW) [a]	Total phenolic content (µmol eq.GA/gDW) [b]	Antioxidant activity (%)
56.91 ± 2.43	0.353 ± 0.025	54.52 ± 1.77

[a] DW – dry weight, [b] eq.GA – equvalent of Gallic acid.

In this study, DPPH radical scavenging activity of the whole mung bean seed extracts was 54.52% ± 1.77. The antioxidants were determined by a stable, purple-coloured organic free radical DPPH. Its ability of reduction was followed by accepting an electron and loss of its absorption spectral band at 517 nm, and a visually noticeable change into the yellow-coloured DPPH radical.

Further, fluorescence spectroscopy was used for the characterization of the nutritional composition of organic mung bean seeds. Figures 1a and 1b show the EMMs for the mung bean seeds. Two distinct spectral maxima on the contour map are displayed, one at about

340–360 nm corresponds to fluorescent spectra of proteins (Stanković et al., 2019) and the other at about 430–450 nm to phenolic compounds. MCR-ALS was applied to the EMM to distinguish an optimal number of spectral components which are displayed in Figures 1c and 1d.

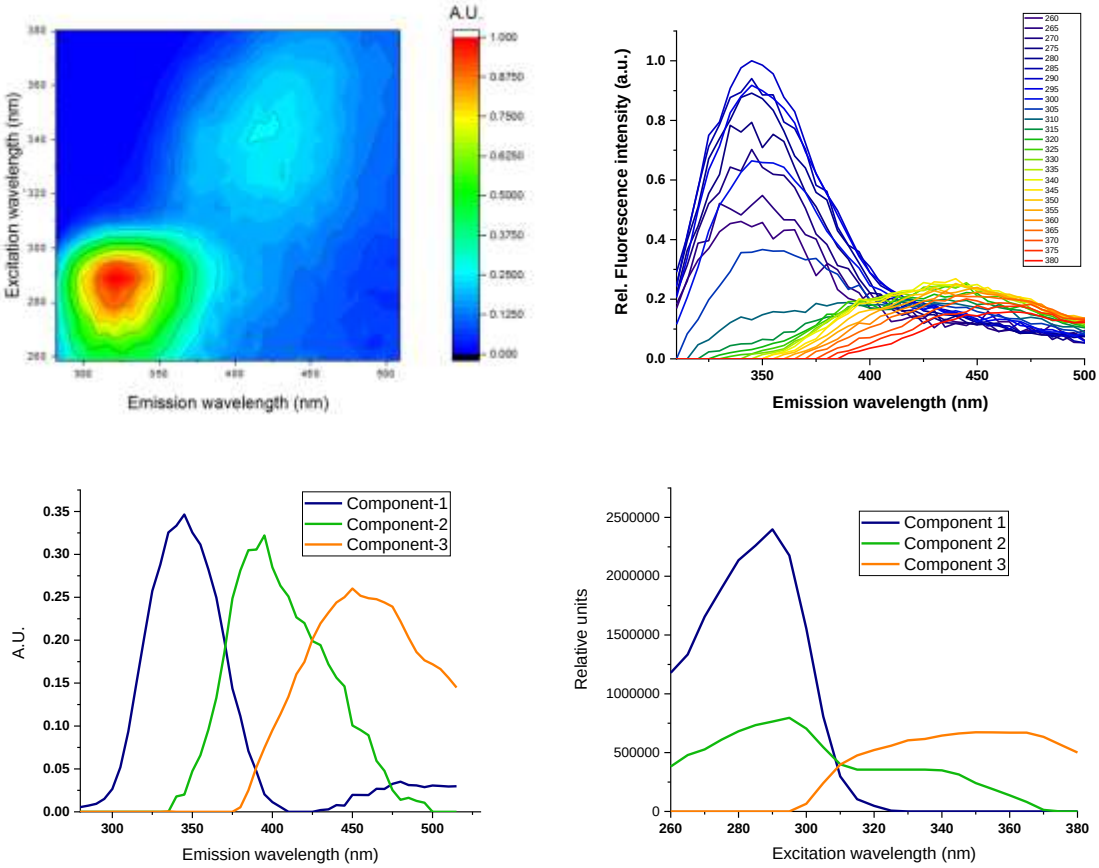

Figure 1 Normalised EEM of mung bean seed samples (a) contour map and (b) spectra; (c) emission and (d) excitation spectral profiles of the spectral components obtained by multivariate analysis (MCR-ALS).

Results of MCR-ALS analysis showed three optimal fluorescence components with the following positions of the excitation/emission peak maxima, component 1 (C1) – Ex 290/Em 345 nm, component 2 (C2) – Ex 295/Em 395 nm, and component 3 (C3) - Ex 350/Em 450 nm. Emission wavelengths of the obtained components displayed in Figure 1c correspond to the emission maxima of proteins (component 1) and phenolic compounds (components 2 and 3) (Stanković et al. 2019).

Conclusions

Legumes are considered functional food ingredients and a major source of dietary antioxidants. Fluorescent spectroscopy combined with statistical analysis has been proved as a useful combination for the identification of protein and phenolic spectral components. Secondary metabolites were determined as a good parameter for the estimation of seed quality and an indicator of tolerance to different types of stress. The advancement in this research lies in collecting information about bioactive compounds, such as (poly)phenols, that are useful in improving the functional and antioxidant properties of quality seeds used in daily diet.

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