PHYSICAL CHEMISTRY 2021

15th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume I

The Conference is dedicated to the

30th Anniversary of the founding of the Society of Physical Chemists of Serbia

and

100th Anniversary of Bray-Liebhafsky reaction

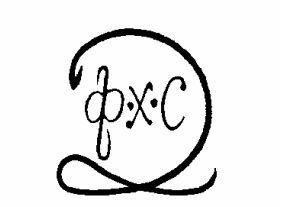
September 20-24, 2021
Belgrade, Serbia

Title: Physical Chemistry 2021 (Proceedings) **ISBN** 978-86-82475-40-8
Volume I: ISBN 978-86-82475-38-5
Editors: Željko Čupić and Slobodan Anić
Published by: Society of Physical Chemists of Serbia, Studentski Trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: ″Jovan″, <Printing and Publishing Company, 200 Copies
Number of pages: 6+344, Format A4, printing finished in December 2021

Text and Layout: ″Jovan″

200 - *Copy printing*

CONTENT



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Organized by

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in co-operation with

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INFLUENCE OF ULTRAVIOLET B (UV-B) IRRADIATION ON ANTIOXIDANT CAPACITY AND FLUORESCENCE CHARACTERISTICS OF SOYBEAN (*GLYCINE MAX* L.) SEEDS

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ABSTRACT

UV-B light, plays a crucial role as a signal for inducing plant response and development of specific photomorphogenic responses. The UV radiation may have a damaging effect on cellular components and macromolecules in seeds, which may influence seed quality. We compared *Glycine max* L. seeds exposed to UV-B radiation for 1 h with non-irradiated seeds (control). The antioxidant activity was estimated using a DPPH assay. The seeds' fluorescence characteristics were studied by fluorescence excitation-emission matrices. According to the obtained results the UV-B irradiated seeds possess a significantly higher free radical scavenging activity compared to the control. The fluorescence analysis showed differences in the spectral emission profiles of irradiated seeds compared to the control seeds. The results imply that 1h seed exposure to UV-B increases free radical content which may alter the structures of cellular macromolecules resulting in degradation of some of the fluorophores.

INTRODUCTION

Plants are constantly exposed to ultraviolet radiation from the sunlight, UV-A (320–390 nm) and UV-B (280–320 nm) [1]. The damaging effect of ultraviolet radiation is visible in cellular components and macromolecules due to the production of reactive oxygen species (ROS) and their free radical reactions [2, 3]. Increasing interest in the effects of UV-B radiation on plants as well as important mechanisms of defense to stress and damage caused by the radiation is a topic of numerous research studies.

Soybean (*Glycine max* L.) is a widespread annual legume and one of the most important plant protein sources from its edible seeds. Soybean varieties have expressed differences in their antioxidant activities [4]. Total phenolic composition, anthocyanin and flavonoid content influence the antioxidative properties of soybean seeds. It was demonstrated that antioxidants from some varieties of soybean seeds provide some health benefits, particularly in their seed hulls [4].

In the present work, the antioxidant activity and fluorescence of soybean seeds were investigated before (control) and after 1h of UV-B radiation treatment.

METHODS

Soybean (*Glycine max* L.) seeds were purchased from the local market in Belgrade, Republic of Serbia. We compared soybean (*Glycine max* L.) seeds exposed to UV-B radiation for 1 h with non-irradiated seeds (control). The seeds were irradiated by a UV-B lamp (312 nm, 15 W Hg, Sankyo Denki, Japan) for 1h. UV light was positioned at 50 cm distance from the seeds.

After grinding in a mill, soybean seeds were further homogenized with liquid nitrogen to obtain a fine powder. Antioxidant activity (AA %) was measured using a 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA) assay. The sample was added in the reaction mixture containing 0.1 mM DPPH in 70 % ethanol and shaken in the dark. The aliquots were taken after 30 min and placed in microplate wells where absorbance was measured at 517 nm in UV-VIS microplate reader (Tecan Infinite M Nano+, Switzerland). Pure deionized water was used as blank while 0.1 mM DPPH was

considered as a control. Antioxidant activity was calculated as a percentage of depleted DPPH-reagent.

The fluorescence steady-state measurements were recorded using an Fl3-221 P spectrofluorimeter (JobinYvob, Horiba, French Republic). The ranges of the excitation spectra were 260 nm to 380 nm, while the range for the recorded fluorescence emission spectra was 270 nm to 515 nm. The spectral bandwidth of 2 nm was applied for both the excitation and emission slits.

RESULTS AND DISCUSSION

The antioxidant capacity of soybean seeds non-irradaited (control) and UV-B irradiatided are presented in **Figure 1**. Our results showed that treating the soybean (*Glycine max* L.) seeds with UV-B light for 1h significantly increased the DPPH$^{\bullet}$ scavenging activity. Several studies revealed that the UV-B irradiation caused changes in the production of some compounds (flavonoids and phenolic) in several plant species. It has been reported that the UV-B irradiation induced changes in the plants' response regarding the increased content of radical-scavenging compounds, such as antioxidant enzymes and non-enzyme antioxidant compounds [5, 6].

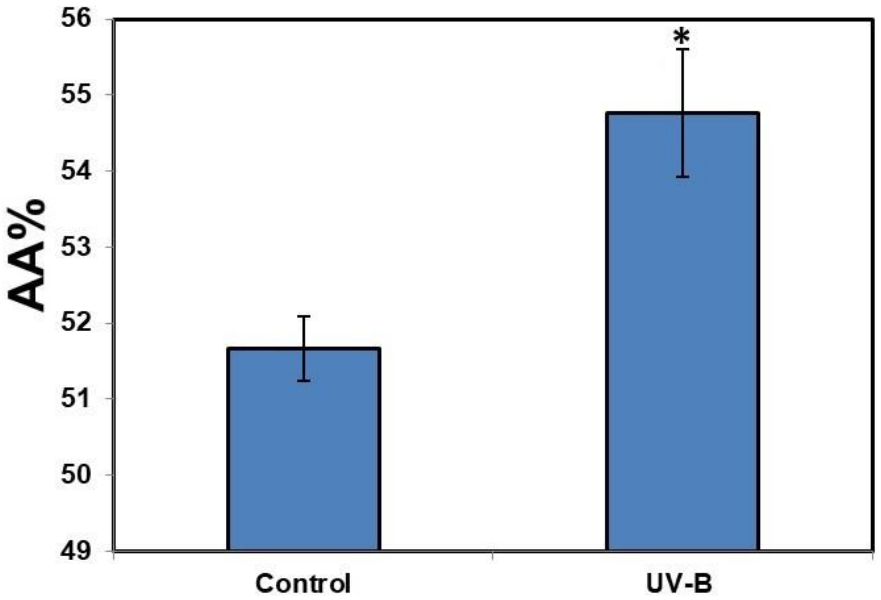

Figure 1. Effects of 1 h- UV-B irradiation on antioxidant capacity in soybean seeds. Each value is expressed as the means ± SD (n = 3). Asterisk (*) indicates a significant difference at $p<0.05$.

Representative excitation-emission matrices (EEMs) of the non-irradiated and the UV-B irradiated soybean seeds are shown in **Figure 2**. The comparison of the EEMs reveals the characteristic high fluorescence regions with excitation-emission wavelength peaks at 280/355 nm, 290/400 nm, and 370/465 nm. This could arise from different types of fluorophores, such as proteins and phenolics. The averaged emission spectra of the analyzed seeds are shown in **Figure 3**. Differences were observed concerning the spectral shape, emission peak positions, as well as intensity. Our results showed that the peak intensity at 290/400 nm was higher in the control samples and decreased after being exposed to UV-B light for 1h. This could be explained by the degradation of some of the seeds' fluorophores under UV-B stress conditions.

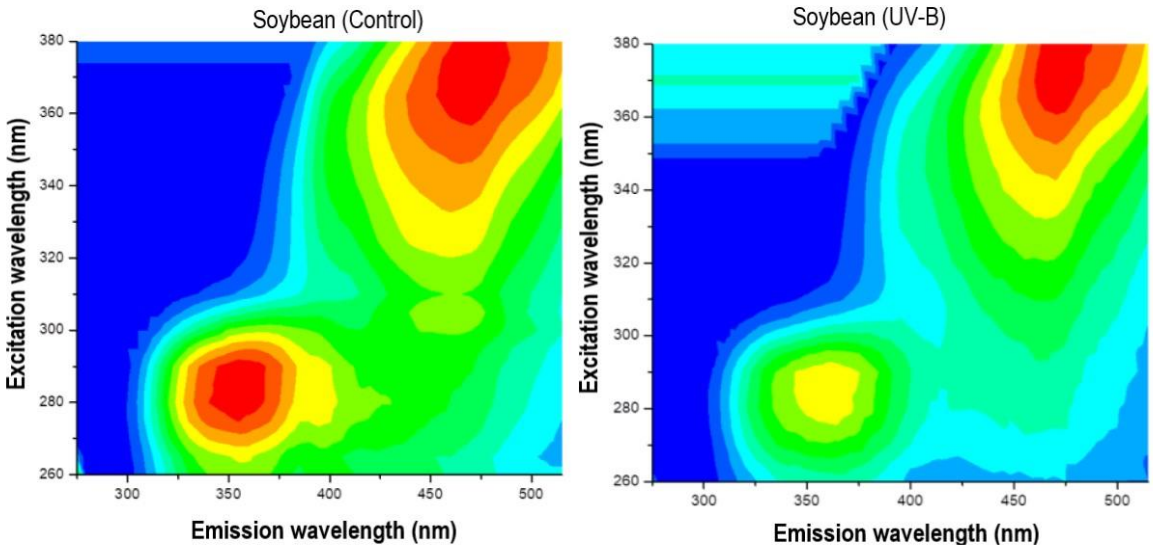

Figure 2. EEMs of the control and UV-B irradiated soybean seeds.

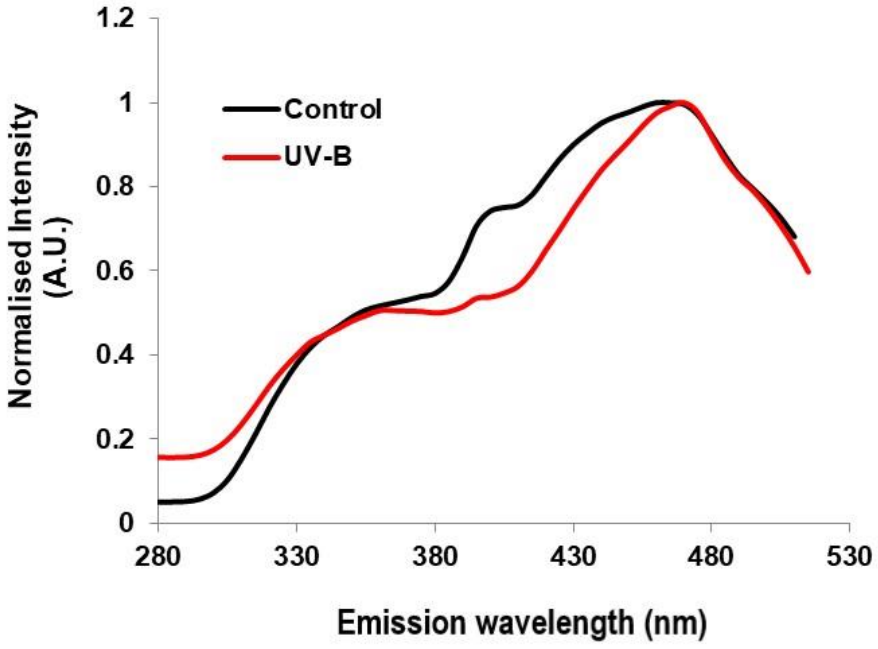

Figure 3. Overlay of the normalized emission spectra for control (dark line) and UV-B irradiated (red line) soybean seeds. The spectrum of each sample is an average of the 25 spectra recoreded for various excitation wavelengths.

CONCLUSION

Our results show that 1h- UV-B irradiation induces the increase of antioxidant capacity in soybean seeds. The effect of UV-B irradiation is visible in the decreased fluorescence peak intensities of the treated seeds compared to the control. The production of ROS and free radical reactions after exposure to UV light may alter the structures of cellular macromolecules resulting in degradation of some of the fluorophores. It may be useful for the improved soybean seeds quality. The results of this study

indicate that EEM fluorescence analysis could contribute to a better understanding of fluorescence species in UV-B-irradiated seeds.

Acknowledgement
This work was supported by the Ministry for Science of the Republic of Serbia (Grant no. 451-03-9/2021-14/200053).

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