Int. J. Mol. Sci. **2007**, *8*, 618-627

International Journal of
Molecular Sciences
ISSN 1422-0067
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Full ResearchPaper

Irradiation Effects on Phenolic Content, Lipid and Protein Oxidation and Scavenger Ability of Soybean Seeds

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Received: 5 May 2007; in Revised Form: 31 May 2007 / Accepted: 7 June 2007 / Published: 5 July 2007

Abstract: The effect of medium doses of γ-irradiation (1-10 kGy) on total phenolic and tannin contents, lipid peroxidation, protein oxidation intensity and soluble protein content of soybean seeds, (genotype Ana), was investigated. Screening for antioxidant ability was performed using FRAP and DPPH methods. Total phenolic and tannin contents and DPPH scavenger activity were increased, while protein oxidation intensity was decreased by applied doses of γ-irradiation. Gamma irradiation provoked insignificant changes in lipid peroxidation and soluble protein content, while protein oxidation intensity was significantly decreased when dose of 10 kGy was applied. Presented results implicated that increased antioxidant capacity and protein stability of soybean seeds were increased after application of γ-irradiation.

Keywords: Phenols, protein oxidation, DPPH, γ-irradiation, soybean seeds

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Introduction

Outbreaks of food borne diseases have bean associated with consumption of poor quality food. This is due to the presence of a large number of human pathogens in food, especially in seeds. Irradiation effectively inactivates food borne pathogens in contaminated seeds. The Food and Drug Administration (FDA) has approved the use of ionizing radiation to control microbial pathogens in

seeds. High doses of irradiation may be required for the inactivation of some pathogens [1]. However, the higher doses of radiation could have adverse effects on sensorial and nutritional quality.

Soybean and its processed products have been acclaimed as health foods due to their high content of protein and essential amino acids, omega-3 fatty acids, fat-soluble vitamins, polysaccharides, and insoluble fibers [2]. Besides these constituents, soybeans also contain isoflavones that are of wide interest due to their beneficial effects on humans, such as prevention of cancer, cardiovascular diseases, osteoporosis, and menopausal symptoms [3].

Gamma irradiation causes oxidative stress and affects biomolecules by causing conformational changes, oxidation, rupture of covalent bonds and formation of free radicals [4]. The hydroxyl (HO•) and superoxide anion ($O_2^{\bullet -}$) radicals that are generated by radiation could modify the molecular properties of the proteins and lipids causing oxidative modifications of the proteins and lipid peroxidation (LP), [5]. Chemical changes of the proteins that are caused by γ-irradiation are fragmentation, cross-linking, aggregation and oxidation caused by oxygen radicals which are generated by water radiolysis [6].

Radiation processing by γ-radiation at dose of 1 kGy has been recommended for quarantine treatment of legumes including soybeans [7], whereas exposure to higher doses (up to 5 kGy) resulted in improvement in quality such as reduction in cooking time and improvement in texture without production of off-flavor [8]. Degradation of isoflavones, the major phenolic constituents in soybean seeds, has been shown to occur in soy and soy products during food processing and storage, resulting in changes in their bioactivities [9]. However, studies of the effect of irradiation on the phenolic content and the impact of these changes on the antioxidant properties of soybeans were poor and insufficient.

There are some reports showing that stress caused by low doses of γ-irradiation could induce antioxidant activities in plant material including soybean [7,10]. Our previous studies concerning antioxidant activities in stressed seeds indicated that low doses of herbicides could induce antioxidant activity in seeds of beans, lettuce and pea [11]. Therefore, the aim of our study was to investigate the effects of stress caused by medium dose of γ-irradiation (1-10 kGy) on phenolic content, lipid and protein oxidation and free radical scavenging ability of soybean.

Results and Discussion

The results obtained by investigating the influence of medium doses of γ-irradiation (1-10 kGy) on some antioxidant properties of soybean seed are presented in Figures 1-5.

Use of γ-irradiation dose up to 10 kGy did not provoke significant changes in LP (Figure 1). As shown in Figure 1, the above mentioned doses of γ-irradiation caused slight increase in LP. The maximum increase in LP was observed with a 2 kGy dose (11.0 % when compared to the unirradiated control). Other authors also established that doses up to 10 kGy caused insignificant changes in total lipids, fatty acid composition, peroxide value and trans fatty acid content [12].

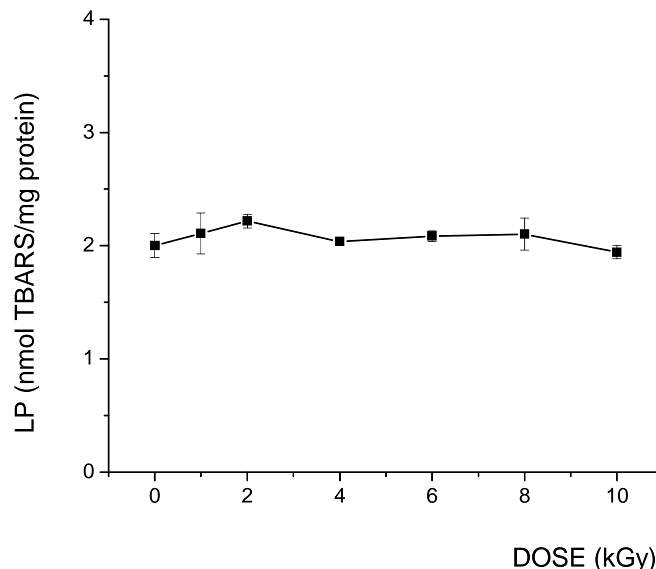

Figure 1. Effect γ-irradiation on LP intensity in soybean seeds.
Each data point represents the mean ± SD; * ANOVA, Tukey-test, $p < 0.05$.

Medium doses of γ-irradiation caused insignificant changes in soluble protein content (Figure 2a), without practical significance. Data obtained by other authors also showed that gamma irradiation, using a doses up to 10 kGy, did not induce significant loss in water soluble components such as minerals, nitrogenous constituents, sugars and proteins [13].

Quantity of carbonyl-groups in oxidatively modified proteins significantly increased only at dose of 6 kGy (5.3 %). Decrease in quantity of carbonyl groups was observed after other doses were applied, but it was significantly decreased only at dose of 10 kGy (19.3 %), (Figure 2b). Introduction of carbonyl groups into amino acid residues of proteins was a hall-mark for oxidative modification. Presented results implicated that protein oxidation intensity was more sensitive parameter of oxidative stress than the lipid peroxidation in irradiated soybean seeds, which was positively correlated with high protein content in soybean seeds. Decline in protein oxidation by dose of 10 kGy could be explain by the high and significant increase of DPPH radical scavenger capacity (Figure 5.). This observation implicate that DPPH radical scavengers, induced by dose of 10 kGy, act as protective agents against protein oxidation. Our results concerning total phenol and tannin contents (Figure 3.) and total antioxidant activity (Figure 4.) demonstrated high values of all parameters at the dose of 10 kGy, what could also provoke the decrease in protein oxidation. Other authors also established that some phenolic compounds can act as antioxidants by retarding protein oxidation reaction or by binding to the proteins [14]. The antiradical mechanism in phenol-protein aggregates may be due to ability of phenolic compounds to transfer oxidative damage from one phenolic site to other, protecting proteins from oxidation.

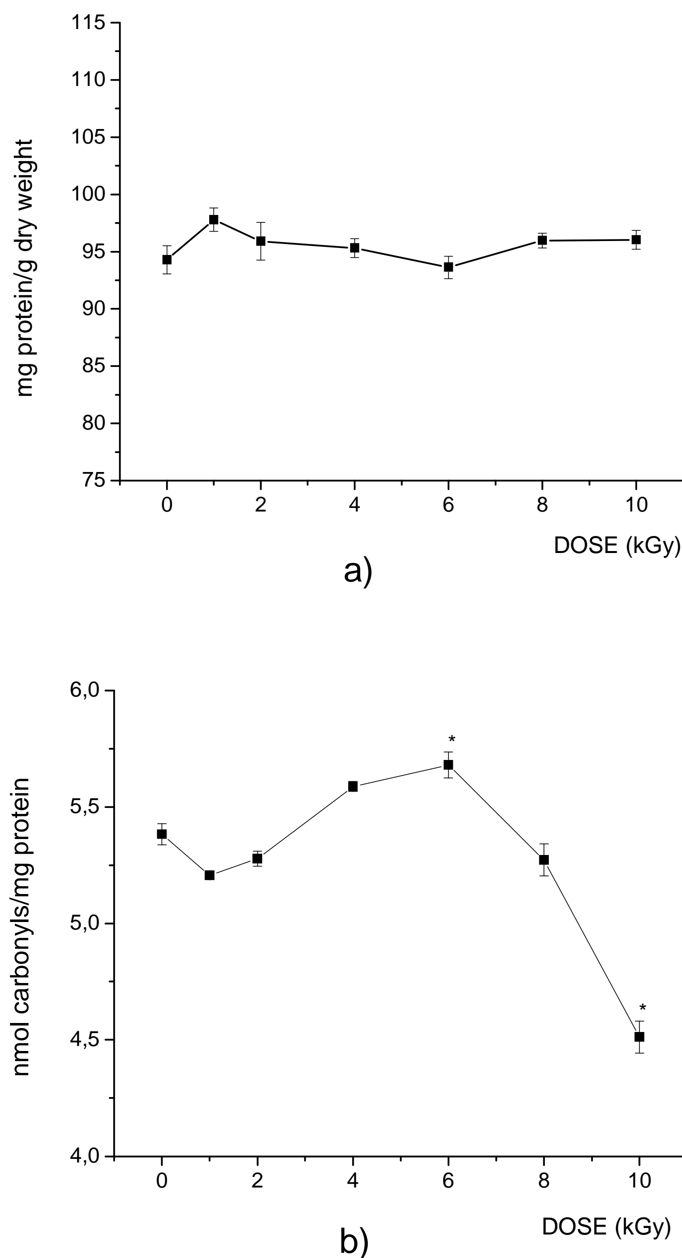

Figure 2. Effects γ-irradiation on soluble protein content (a) and protein oxidation intensity (b) in soybean seeds. Each data point represents the mean ± SD; * ANOVA, Tukey-test, $p < 0.05$.

Significant increase in total phenolic and tannin contents was caused by applied doses of γ-irradiation (Figure 3a and 3b). The maximum increase of these parameters was observed at dose of 1 kGy (10.0 % for total phenols and 21.6 % for tannins). Even at the highest dose of 10 kGy, increase in total phenolic (7.6 %) and tannin (11.0 %) contents was observed. In plant tissues many phenolic compounds are potential antioxidants: flavonoids, tannins and lignin precursors may act as ROS (reactive oxygen species) scavenging compounds. Observed increase in total phenolic and tannin contents was beneficial for antioxidant properties of soybean seeds due to polymerisation of phenolic constituents and also cross-linking and fragmentation, which were the key reactions controlling the properties of macromolecules such as proteins [15].

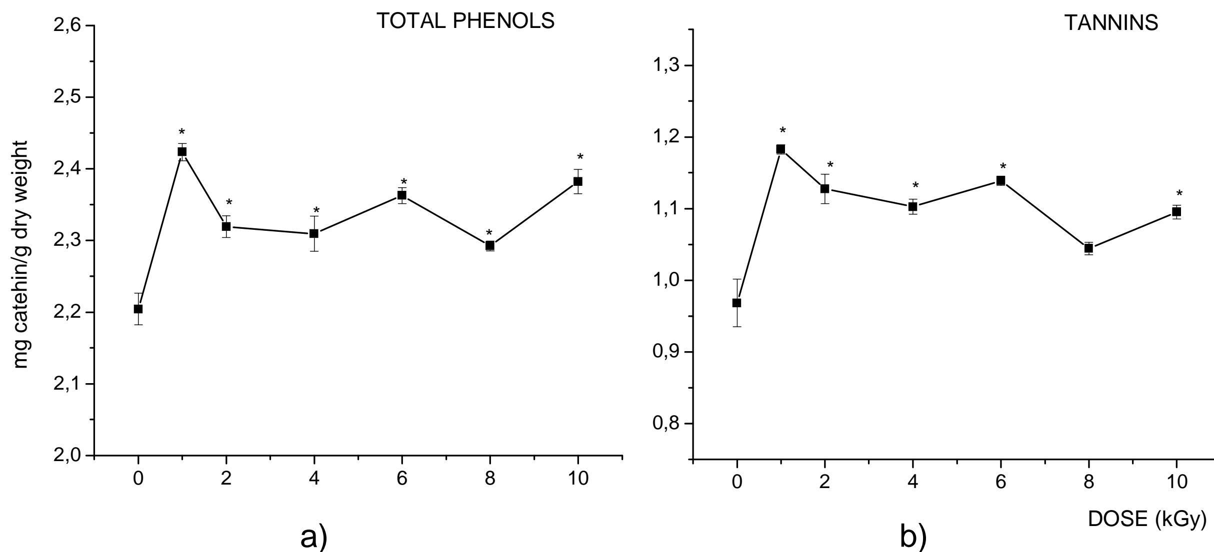

Figure 3. Effects γ-irradiation on total phenol (a) and tannin (b) contents in soybean seeds. Each data point represents the means; * ANOVA, Tukey-test, $p < 0.05$.

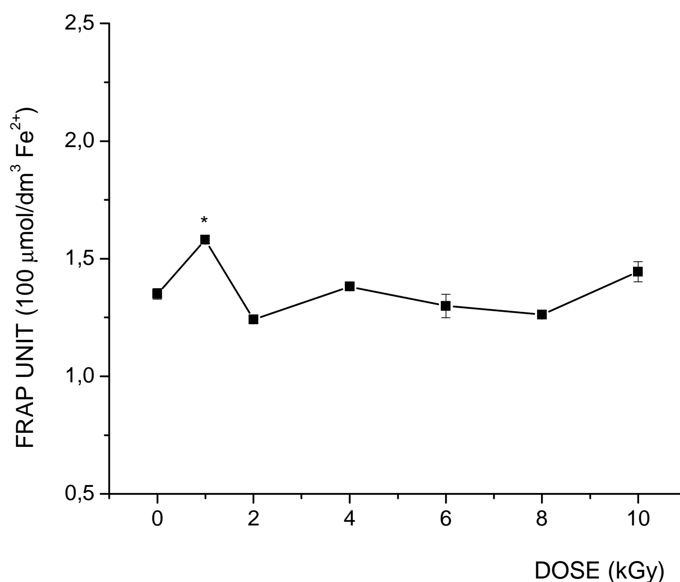

Figure 4. Effect γ-irradiation on total antioxidant activity determined by FRAP method in soybean seeds. Each data point represents the means; * ANOVA, Tukey-test, $p < 0.05$.

Total antioxidant activity determined by FRAP method was significantly increased at doses of 1 kGy (17.0 %); the increase was insignificant at 4 kGy (2.2 %) and 10 kGy (6.7 %) (Figure 4). The irradiation induced increase in total antioxidant activity at dose of 1 and 10 kGy could be the result of

high total phenol and tannin accumulation in soybean by these doses (Figure 3a,b). It is well known that among ascorbate, phenols are the most active ferric reducing agents [16].

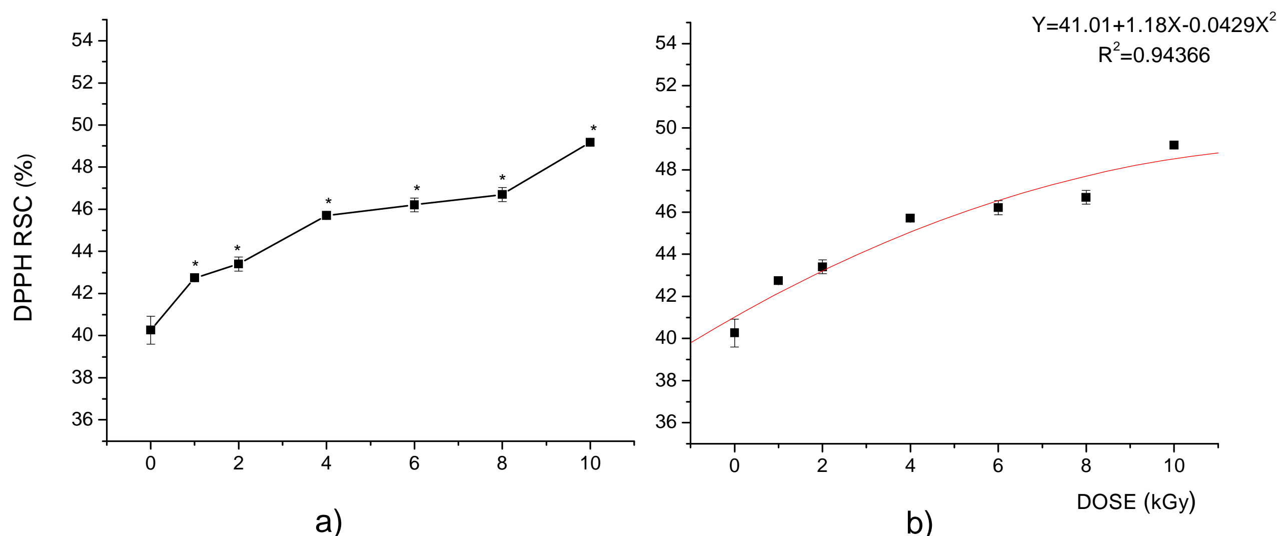

Figure 5. Effect γ-irradiation on DPPH Radical Scavenger Capacity (DPPH RSC) of soybean seeds (a) and polynomial curve fit (b). Each data point represents the mean ± SD; * ANOVA; Tukey-test, $p < 0.05$.

DPPH radical scavenger capacity (DPPH RSC) was increased corresponding to dose of γ-irradiation (up to 49.2 %). Maximum was observed at the highest irradiation dose (Figure 5a). Polynomial relationship between DPPH RSC and γ-irradiation dose ($y = 41.01 + 1.18x - 0.0429x^2$; $R^2 = 0.94366$) is presented in Figure 5b. It was also reported that γ-irradiation doses up to 5 kGy enhanced DPPH RSC of soybean butanolic extracts [7]. DPPH RSC is the measure of non-enzymatic antioxidant activity. Higher levels of DPPH antiradical activity were in correlation with no enzymatic antioxidants, especially plant polyphenols [17] what was also in agreement with results given in Figures 3a and 3b.

Conclusions

Our results indicated that medium doses of γ-irradiation (1-10 kGy) led to an antioxidant response in seed tissue of soybean genotype Ana. Antioxidant response was manifested in the increase in total phenolic and tannin contents, also in the increase of DPPH scavenger ability at all applied doses. Reduced protein oxidation intensity, was noticed when higher doses of γ-irradiation were applied. Furthermore the LP increased only slightly which could be the consequence of the appropriate antioxidant response. Therefore our results led to conclusion that γ-irradiation increased antioxidant capacity and protein stability of soybean seeds (genotype Ana), which is of great importance for health, nutritive and processing characteristics of soybean seeds.

Experimental Section

Material treatments and extraction procedures

Soybean seeds [*Glycine max* (L.) Merr.] of genotype ANA were obtained from the Institute of Field and Vegetable Crops in Novi Sad. Soybean seeds were irradiated with following doses of γ-irradiation: 1, 2, 4, 6, 8 and 10 kGy. For that purpose the ^{60}Co was used. The dose rate was 228 Gy/min. Seed irradiation was performed in the Laboratory for Radiation Chemistry and Physics ″Gama″ at the Institute of Nuclear Sciences in Vinča, Belgrade.

Irradiated and nonirradiated soybean seeds (100 seeds) were ground in a mill and reduced to a fine powder.

Plant material (1 g) was extracted with 25 ml 70 % aqueous ethanol (0.1 M HCl) under 30 min sonication in an ultrasonic bath at ambient temperature. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated. This extract was used for total phenolic and tannin contents, DPPH radical scavenger capacity and total antioxidant power determinations.

For lipid peroxidation, protein oxidation intensity and soluble protein content, phosphate buffer (pH 7) soybean extracts were used. One gram of plant material was extracted with 50 ml 0.1 M K_2HPO_4 at pH 7.0 after 30 min sonication in an ultrasonic bath at ambient temperature. After 10 minutes centrifugation at 4 ^oC and 15 000 g, the aliquots of the supernatant were used for above mentioned determinations. Extracts of nonirradiated seeds were used as the control.

Methods

Lipid peroxidation intensity was determined by the TBARS (Thiobarbituric Acid Reactive Substances) method [18]. Crushed sample (0.30 g) was homogenized in 1.25 ml trichloroacetic acid (TCA) (0.1 %)–sodium dodecyl sulfate salt (SDS) (1 %). The homogenate was centrifuged at 12,000 g for 5 min. To 300 µl aliquot of the supernatant was added 1 ml 20 % TCA containing 0.5 % TBA. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice-bath. The absorbance was read at 532 nm. TBARS quantity was expressed as nmol of TBARS mg^{-1} protein.

Soluble protein content was determined by Bradford method [19]. Soluble protein content was expressed as mg protein per g dry weight.

Protein oxidation intensity was described using the content of carbonyl groups in proteins [20]. It was determined as amount of 2,4-dinitrophenylhydrazone (DNPH) formed upon reaction with DNPH. After cells disruption and centrifugation, samples (> 1.5 mg protein) were treated with 10 mM DNPH in 2 M HCl at room temperature for 60 min. Blanks contained 2 M HCl without DNPH. Proteins were precipitated by addition of trichloroacetic acid up to final concentration 10 %, centrifuged at 4000 g for 10 min at 4 °C, and washed three times with 1 ml solution ethanol:ethyl acetate (1:1). The final pellets were dissolved in 6 M guanidine hydrochloride in 5 % (v/v) phosphoric acid. Carbonyl content was calculated using the absorbance maximum of DNPH measured at 370 nm. The results were expressed as nanomoles carbonyls per milligram protein.

Total phenols were determined by Folin-Ciocalteu procedure [21]. Aliquots (0.1 ml) of aqueous acetone extracts were transferred into the test tubes and their volumes adjusted up to 0.5 ml with distilled water. After addition of 0.25 ml Folin-Ciocalteu reagent and 1.25 ml 20 % aqueous sodium

carbonate solution, tubes were vortexed and absorbance of blue colored mixtures recorded after 40 min at 725 nm against a blank containing 0.1 ml of extraction solvent. The amount of total phenols was calculated as a catechin equivalent from the calibration curve of catechin standard solutions (covering the concentration range between 0.1 and 1.0 mg ml^{-1}), and expressed as g catechin kg^{-1} dry plant material.

Total tannin content was determined by Folin-Ciocalteu procedure as above, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone, PVPP). Insoluble, cross-linked PVPP (Sigma, Germany; 100 mg) was weighed into test tubes and 1.0 ml aqueous acetone extracts added. After 15 min at 4 ^{o}C, tubes were vortexed and centrifuged for 10 min at 4350 g. Aliquots of supernatant (0.2 ml) were transferred into test tubes and nonabsorbed phenolics determined as described. Calculated values were subtracted from total phenolic contents and total tannin contents expressed as g catechin kg^{-1} dry plant material.

Total antioxidant capacity was estimated according to the FRAP (Ferric Reducing Antioxidant Power) assay [22]. Total reducing power is expressed as FRAP units. FRAP unit is equal with 100 µmol/dm^3 Fe^{2+}. FRAP value was calculated using formula:

$$\text{FRAP value}=\Delta A_{sample}/\Delta A_{standard}$$

where ΔA_{sample} is the change in absorbance of the sample and $\Delta A_{standard}$ is the change in absorbance of the standard (100 µmol/dm^3 Fe^{2+}) after 4 min incubation at 593 nm.

DPPH radical scavenging capacity was determined using 1,1-diphenyl-2-pycril-hydrazil radical (DPPH). Reduction of DPPH radical was determined measuring disappearance of DPPH at 515 nm. DPPH RSC is expressed by percents compared to the control [23]. The percent inhibition of the DPPH radical (DPPH RSC) by the samples was calculated using the formula:

$$\text{DPPH RSC}=[(A_c-A_x)/A_c]\times 100\ \%$$

where A_c is absorbance of the control and A_x is absorbance of the sample after 30 min of incubation.

Data Analysis

All determinations were obtained from triplicate measurements. Results are expressed as mean ± standard error. Experimental data were statistically analyzed using software Origin, by one-way ANOVA. Differences between means were evaluated for significance using Tukey-test ($p < 0.05$). Polynomial regression was performed for the results obtained by RSC determination.

Acknowledgement

This research is part of the project which is financially supported by the Ministry of Science, Technologies and Development of the Republic of Serbia (N° 142036B).

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