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Study of gamma radiation effects on free radicals generation and antioxidant potential of beebread

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Introduction: Gamma irradiation could be used as a safe method for disinfection and shelf-life prolongation of beebread. By means of the using of ionizing radiation can be avoided proteins and sugars deterioration, no changes to flavor, taste and texture also were observed. Beebread (also called fermented pollen, pergolas and perga) is considered a functional food, which increase nutritional value and it is known with its antioxidant characteristics which is supposed protection of living organisms from oxidative damage, resulting in the prevention of various diseases. The biological activity of bee pollen is related to its high antioxidative potential and radical scavenging activity due to the presence of polyphenols, including flavonoids.
The effect of irradiation on induced radicals and antioxidant properties of beebread were not yet investigated. The aim of the present study was to evaluate the effect of different doses irradiation on antioxidant activity of beebread and its main phenolic constituents by EPR and HPLC analysis. Analyzing the radical components induced by gamma rays and their impact on DPPH free radical scavenging activity was also done.

Used research methods

- *Electron Paramagnetic Resonance (EPR) spectroscopy* . It is a unique technique that can detect selectively paramagnetic species (free radicals) and it is considered to be one of the most effective and accurate techniques for determining of free radical scavenging activity (FRSA).

Estimation of FRSA – by EPR using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).
The portion of beebread extracts was added to 1 ml 0.002 M ethanol solution of DPPH. The percent of the DPPH radicals scavenged by pollen extract was calculated according to the equation:

Scavenged DPPH radicals (%) = $[(I_0 - I) / I_0] \times 100$

The IC_{50} values for beebread samples were determined (Table 1). The antioxidant properties of the analyzed sample are inversely proportional to the value of the IC_{50}.

Determination of Trolox Equivalent Antioxidant Capacity (TEAC)
For TEAC determination of beebread extracts the previously used method (Bartoszek and Polak, 2012, Mladenova et al, 2019) was applied. To quantify the antioxidant activity of the beebread samples Trolox solution was used. The regression equation for the linear relationship between the percent scavenged DPPH• and concentration of Trolox was assessed as: y = 1.05x – 10.37 , where y is the percentage of scavenged DPPH• calculated on the basis of the regression equation for each one of the beebread samples (data are given in Table 1, where x is the volume of the sample [ml]).

- *Spectrophotometrically determination of total phenolic (TPC) and flavonoids content (TFC)*

The Folin–Ciocalteu method was used to determine total phenolic content of the beebread extracts (Singleton et al., 1999). The aluminium chloride method was employed for the total flavonoids content assay.

- *Determining of ultra-high performance liquid chromatography (UHPLC) profile*

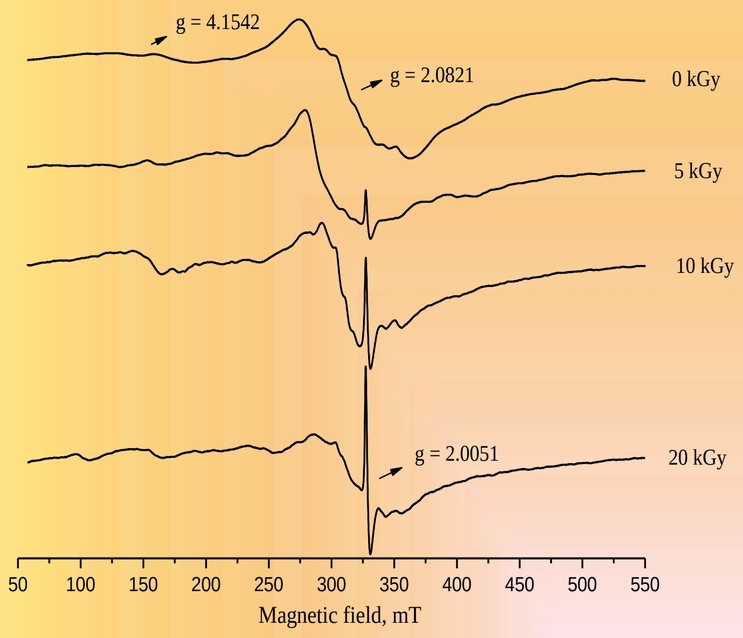
To study the effect of gamma sterilization on the main constituents of beebread the HPLC analysis was used. Change in the concentration of bioactive compounds belonging to three main polyphenol groups after irradiation was observed.

Results and discussion

Features of EPR spectra

The spectra of beebread samples exhibit:

- Fe^{3+} in tetrahedral (g=4.1542) and octahedral surrounding (g=2.0821);
- The narrow signal at g=2.0051 is appeared after gamma sterilization. It is attributed to O-centered free radicals. Their intensity gradually increases at doses of 5 up to 20 kGy.



Study on the EPR signal fading kinetics of the radiation-induced free radicals

The kinetic curves for 5 and 10 kGy are almost identical, the difference is more pronounced between 10 and 20 kGy. As can be seen from the Fig. 2, the intensity gradually decreases with time, faster for samples irradiated with 20 kGy. Free radicals recombine over time after irradiation or were quenched by antioxidant molecules. Thus they can only be measured for 4 months as for that period identification of previous sterilization is possible.

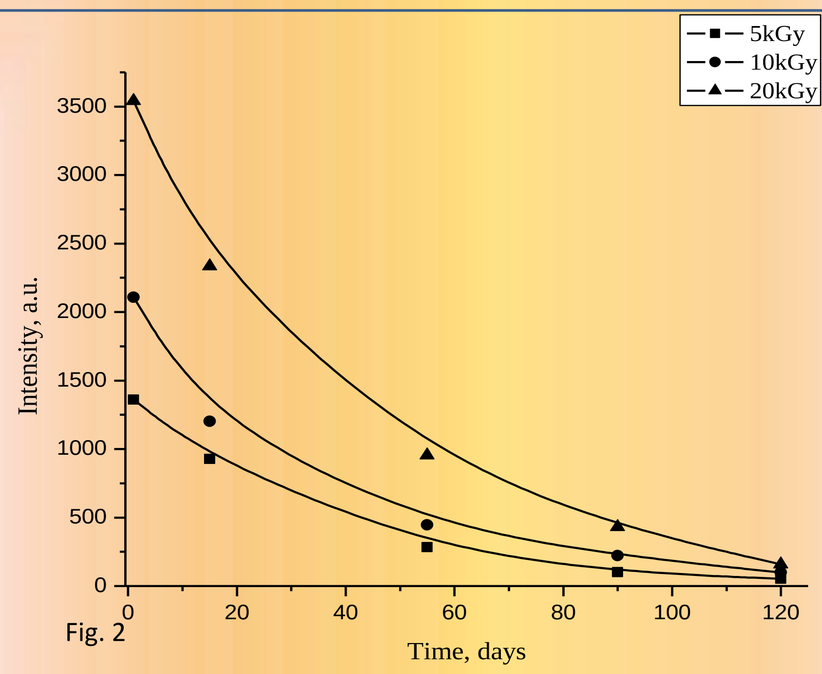
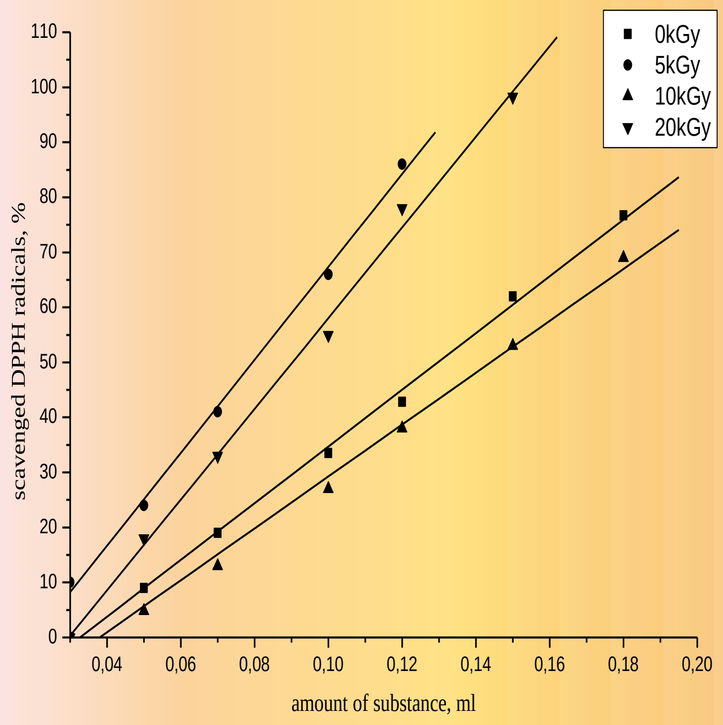


Fig. 2

Study on irradiation effect on FRSA of beebread by EPR

The EPR signal intensity reduction of DPPH• is monitored after addition of the beebread extracts. The signal intensity decreases with the increasing volume of added samples. The decrease in free radical concentration is proportional to the radical scavenging capacity of the tested antioxidant.

The dependences between amount of beebread extracts (irradiated with different doses) and percent scavenged DPPH radicals was made (Fig. 3).



FRSA avarage increased for 5 kGy with approximately 27 % and for 20 kGy irradiated samples with 19 %).

FRSA slight decreased (6%) at beebread irradiated with 10 kGy.

The data presented in Table 1 about values of IC_{50} and TEAC confirmed the positive effect of irradiation with doses from 5 and 20 kGy on antioxidant capacity of beebread.

Table 1.

Dose (kGy)	IC_{50} (ml)	Regression equation	TEAC (µmol/ml)	TPC	TFC	
				mg GAE/g sample	mg QE/g sample	mg CE/g sample
0	0.13	y=515.45x-16.8	484.8±1.5	10.42±0.15	1.74±0.04	1.32±0.14
5	0.079	y=844.36x-17.1	797.8±1.8	14.14±0.26	3.01±0.11	2.86±0.17
10	0.144	y=471.6x-17.9	442±2.4	13.04±0.07	2.57±0.10	2.39±0.14
20	0.091	y=823.9x-24.36	771.3±2.4	13.50±0.19	2.67±0.05	2.70±0.22

TPC and TFC analysis

The results for TPC and TFC (Table 1) show that after irradiation their values increased. Both increased the most in samples irradiated with 5 kGy (TPC and TFC increase 1.4 times for GAE, 1.7 for QE, and 2.2 for CE). The formation of more antioxidant compounds probably is due to degradation of some higher molecular weight polyphenols into smaller ones by gamma irradiation.

Study of HPLC profile before and after irradiation

The phenolic compounds detected in beebread samples in our study were identified as flavonols (catechin standart was used), phenolic acids (p-coumaric acid, caffeic acid and siringic acid standarts were used) and quercetine glycosides (rutin and quercetin standarts were used). The obtained data from HPLC analysis for samples before and after irradiation are presented in Table 2.

Table 2.

Dose [kGy]	Flavonols [mg/g]	Phenolic acids [mg/g]	Quercetin glycosides [mg/g]
0	0.29±0.012	0.28±0.012	15.62±0.656
5	0.47±0.020	0.88±0.037	17.48±0.734
10	0.30±0.013	0.45±0.019	15.38±0.646
20	0.32±0.013	0.80±0.034	14.40±0.605

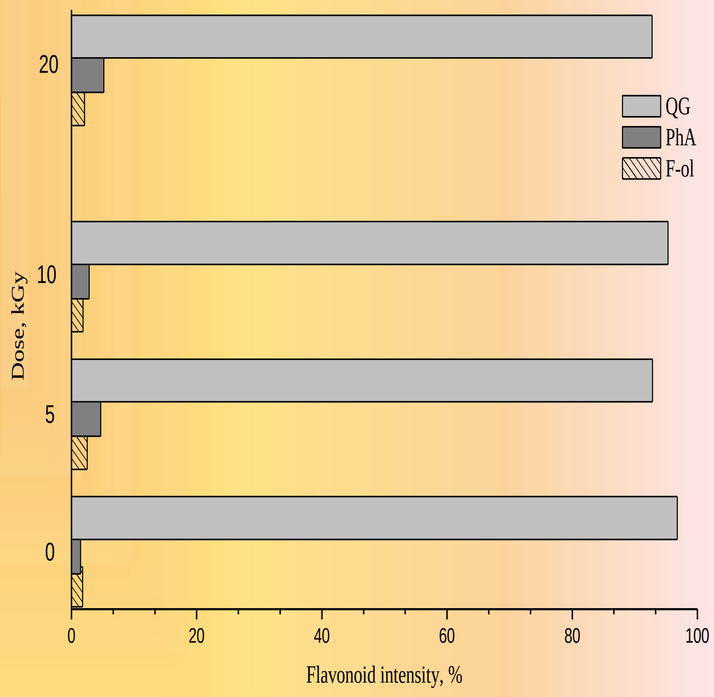


Fig.4. Dependence between irradiation dose (kGy) of beebread and intensity of flavonoids (%) from the different groups: QG-Quercetin glycosides; PhA-Phenolic acids; F-ol-Flavonols.

Conclusions

The EPR study shows that ionizing radiation induced O-centered free radicals in beebread samples. Their relative concentration gradually increases at irradiation with doses of 5 up to 20 kGy. The O-centered radicals are stable for 4 months in perga and the frame of this period irradiation could be identified.
Gamma treatment has been found to affect the free radical scavenging activity of beebread. Gamma sterilization of studied samples has a positive effect on antiradical capacity after irradiation with 5 and 20 kGy, accord to non-irradiated beebread. These results are in agreement with the increased concentration of polyphenolic compounds after gamma treatment. The determined concentration of identified group flavonoid compounds by HPLC show that the increasing of flavonols and phenolic acids is correspond to increased antioxidant activity at samples irradiated with 5 and 20 kGy. Perga extracts possessed the strongest activity after irradiation at 5 kGy with respect to DPPH radicals and obtained data from TPC, TFC and HPLC assay.

References:
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