

Classification and prediction of γ -irradiation of ten commercial herbs and spices by multivariate evaluation of properties of their extracts

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Summary

Antioxidant activity, dry matter content, extractability, total phenolic compounds content and CIE $L^*a^*b^*$ colour characteristics of methanolic extracts of 10 spices exposed to γ -irradiation doses from 0 kGy (reference) to 30 kGy were evaluated in different time intervals after the irradiation by means of UV-VIS spectrophotometry in order to assess the influence of γ -irradiation and post-irradiation storage on spices quality. Experimental data revealed that γ -irradiation itself did not cause so dramatic changes as the subsequent post-irradiation storage, or, that the changes were, at least, comparable. Multivariate statistical methods (factor analysis, canonical discriminant analysis and k^{th} -nearest neighbour classification) facilitated differentiation of irradiated spices from the corresponding references and γ -radiation dose prediction on the basis of processing of experimentally determined characteristics of their extracts. In case of samples exposed to 0 kGy, 10 kGy and 30 kGy, differentiation and classification correctness higher than 85% was reached. In case of 0 kGy and 30 kGy samples differentiation, for 8 spices absolutely correct recognition was achieved and, for remaining 2 spices, still sufficient recognition correctness of 75% and 92.8%, respectively, was achieved. By the statistical models used, more than 90% correctness of dose prediction was achieved in the majority of cases.

Keywords

spices; γ -irradiation; antioxidant properties; dose estimation; discrimination; classification

Food processing frequently leads to changes in structural integrity of plant materials, having thus both negative and positive effects on its antioxidant activity. Processing by ionizing radiation is currently internationally recognized as an effective sterilization method to maintain the quality of spices unchanged for a long time. Foodstuffs authorized for irradiation treatment at a maximum overall average absorbed radiation dose of 10 kGy include dried aromatic herbs, spices and vegetable seasonings [1]. According to Codex Alimentarius General Standard for irradiated foods, the maximum absorbed dose applied to a food should not exceed 10 kGy, with an exception for cases when higher dose application is necessary to achieve a legitimate technological purpose [2]. United States Food and Drug Administration has approved irradiation of a variety of foods, including fresh fruits, vegetables and spices. Herbs, spices and vegetable seasonings can be legally irradiated up to the dose of 30 kGy [3].

Currently, herbs and spices are the most extensively examined raw materials for the occurrence and possibilities of isolation of antioxidants. In this context, the effect of γ -irradiation on antioxidant properties of herbs and spices or on their individual components has been also extensively studied by many authors. SJÖVALL et al. [4] investigated the γ -irradiation induced changes in 11 pure aroma compounds typically present in spices, applying gas chromatography coupled to mass spectrometry. As a result of γ -irradiation, their total amounts decreased by 4–13%, different results depend on whether the spices were irradiated individually or in a mixture.

CALUCCI et al. [5] found that γ -irradiation resulted in a general increase in the concentration of quinone radicals in nine aromatic herbs and spices, and in a significant decrease in concentrations of total ascorbate and carotenoids in some spices. The effect of irradiation on antioxidant properties of seven dessert spices irradiated at 1,

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3, 5 and 10 kGy was evaluated by MURCIA et al. [6]. This study did not show any significant differences in the antioxidant activity of water extracts of spices evaluated by several assays.

GLC analysis of powdered black pepper from Egyptian markets γ -irradiated at doses of 5 kGy and 10 kGy performed by EMAM et al. [7] confirmed that, the original number of 19 different compounds, including monoterpenes, sesquiterpenes or their oxygenated derivatives were identified in reference sample, was reduced by γ -irradiation to 16 (at 5 kGy) and 15 (at 10 kGy), respectively.

PIGGOTT and OTHMAN [8] investigated the composition of steam-distilled volatile oils of black pepper from three sources treated by γ -radiation at doses of 10, 20 and 30 kGy and subsequently stored. Although the changes in the content of volatile oil were recognized as negligible, principal components analysis of the data revealed clear differentiation of the control and irradiated samples of two pepper cultivars immediately after irradiation.

Minor effect of γ -irradiation at 10 kGy on content and composition of volatile oil in ground black and white pepper was noticed also by SHIGEMURA et al. [9]. On the other hand, higher radiation doses caused a decrease in saffrole content in black pepper to approximately 10% [10].

The influence of heat treatment and γ -irradiation on the composition of black pepper volatile oils was studied by SADECKÁ et al. [11, 12]. The results proved that both heat treatment and γ -irradiation at 30 kGy caused a significant decrease of some volatiles, e.g. of β -elemene, α -guaiene, α -humulene or β -farnesene. The volatile and essential oils of clove, cardamom and nutmeg γ -irradiated at 10 kGy were analysed by VARIYAR et al. [13, 14]. While no changes were observed for clove and cardamom, for γ -irradiated nutmeg, a 6-fold increase in the content of myristicin accompanied with a decrease in elemicin content was found.

In addition, γ -irradiation of clove and nutmeg caused significant changes in the contents of phenolic acids. No significant qualitative or quantitative changes in the aroma constituents of ginger rhizomes γ -irradiated at a dose of 60 Gy were detected [15].

ANDREWS et al. [16] found that a dose of 5 kGy caused the significant reduction, and a dose of 10 kGy total elimination of microbial contamination of dry ginger, without affecting its flavour quality. The same finding was earlier published by WU and YANG [17], reporting also on practically negligible effect of γ -irradiation at the low dose of 50 Gy on flavour components of fresh ginger. In this context, SIRIKULVADHANA and PROMPUKESERA

[18] revealed that irradiation at a dose of 40 Gy affected sprouting of ginger rhizomes.

The effects of irradiation and heat treatment on composition and antioxidant properties of some culinary herbs and spices were recently reviewed by POLOVKA and SUHAJ [19]. As concluded, the effect of γ -irradiation varied with the crop type, its origin, harvesting method, pre-treatment, and even with the method used for the respective impact assessment. It was pointed out also to some common characteristics (markers) of radiation treatment, e.g. the increased content of polyphenols, decrease in volatile oils and decrease in some vitamins in irradiated samples.

The above-presented information reflects the diverse effects of γ -irradiation on composition and microbial contamination of herbs and spices. In this contribution, the impact of ionizing radiation (at doses of 10 kGy and 30 kGy) and subsequent storage on antioxidant properties of selected herbs and spices (ground black pepper (*Piper nigrum*), oregano (*Origanum vulgare*), allspice (*Pimenta dioica*), clove flower buds (*Syzygium aromaticum*), ginger roots (*Zingiber officinale*), caraway (*Carum carvi*), bay leaves (*Laurus nobilis*), curcuma (*Curcuma longa*), onion (*Allium cepa*) and garlic (*Allium sativum*)) is presented. As an original contribution to the research focused on the possibilities of the detection of irradiation of food, in particular herbs and spices, methods of multivariate statistics, i.e. factor analysis, canonical discrimination analysis and k^{th} -nearest neighbour classification, were utilized to distinguish the irradiated spices from non-irradiated ones, and to classify them according to the absorbed dose of γ -radiation. The results obtained could be effectively utilized by food control authorities, extending their capability to control γ -irradiation of food, as there is still a lack of methods suitable for tracing γ -irradiation and its dose.

MATERIALS AND METHODS

Samples and preparation of extracts

Dried ground black pepper, oregano, allspice, clove flower buds, ginger roots, caraway, bay leaves, curcuma, onion and garlic were obtained from local distributors or producers (refer to Tab.1 for further details on samples origin).

Spice samples were packaged in polyethylene bags (approximately 100 g each) in a number corresponding to the number of doses to which samples were to be exposed. Samples were irradiated by a standard procedures in Artim (Prague, Czech

Tab. 1. Dry matter content and extraction yields of methanolic extracts of spices and their changes resulting from γ -irradiation and 6 months post-radiation storage at ambient conditions.

Spice	Country of origin (Distributor)	Dose of γ -radiation [kGy]	Dry matter content [%]		Extraction yield [g·kg ⁻¹]	
			Immediately	6 months of storage	Immediately	6 months of storage
Allspice	Mexico (Mäspoma, Zvolen, Slovakia)	0	86.9 ± 0.1	86.9 ± 0.1	5.8 ± 0.1	5.6 ± 0.2
		10	86.9 ± 0.1	86.9 ± 0.1	5.9 ± 0.1	5.7 ± 0.1
		30	86.9 ± 0.1	86.9 ± 0.1	5.5 ± 0.4	5.7 ± 0.2
Bay leaves	Turkey (J. Kotányi, Wolkersdorf im Weinviertel, Austria)	0	92.5 ± 0.1	93.2 ± 0.2	11.9 ± 0.1	12.8 ± 0.2
		10	93.2 ± 0.1	93.3 ± 0.2	11.7 ± 0.1	12.7 ± 0.1
		30	93.1 ± 0.1	93.2 ± 0.1	11.6 ± 0.1	12.8 ± 0.1
Black pepper	Vietnam (Mäspoma, Zvolen, Slovakia)	0	88.1 ± 0.1	91.8 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
		10	87.8 ± 0.1	92.0 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
		30	87.9 ± 0.1	92.1 ± 0.1	5.3 ± 0.1	5.3 ± 0.7
Caraway	Turkey (Mäspoma, Zvolen, Slovakia)	0	92.1 ± 0.8	92.6 ± 0.1	8.4 ± 0.2	9.3 ± 0.1
		10	92.9 ± 0.3	92.6 ± 0.1	8.0 ± 0.1	9.2 ± 0.2
		30	92.8 ± 0.2	92.9 ± 0.6	7.9 ± 0.1	9.2 ± 0.1
Clove	India (Mäspoma, Zvolen, Slovakia)	0	81.6 ± 0.1	87.2 ± 0.4	12.8 ± 0.1	13.9 ± 0.3
		10	81.8 ± 0.1	88.2 ± 0.7	13.0 ± 0.1	13.4 ± 1.4
		30	81.7 ± 0.1	88.4 ± 1.4	13.3 ± 0.1	13.8 ± 1.0
Curcuma	India (J. Kotányi, Wolkersdorf im Weinviertel, Austria)	0	90.9 ± 0.1	92.0 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
		10	90.9 ± 0.1	91.7 ± 0.1	3.8 ± 0.1	4.1 ± 0.1
		30	90.9 ± 0.1	91.8 ± 0.4	3.9 ± 0.1	4.1 ± 0.1
Garlic	Austria (J. Kotányi, Wolkersdorf im Weinviertel, Austria)	0	94.0 ± 0.1	92.6 ± 0.1	15.6 ± 0.1	15.1 ± 0.7
		10	94.0 ± 0.1	92.5 ± 0.1	15.9 ± 0.1	16.2 ± 1.3
		30	94.0 ± 0.1	92.2 ± 0.1	17.3 ± 0.1	17.9 ± 0.9
Ginger	India (Mäspoma, Zvolen, Slovakia)	0	89.4 ± 0.1	91.4 ± 0.1	4.8 ± 0.2	5.9 ± 0.2
		10	88.9 ± 0.1	92.0 ± 0.7	4.9 ± 0.1	6.3 ± 0.4
		30	81.7 ± 0.1	90.3 ± 0.2	5.2 ± 1.3	6.1 ± 0.5
Onion	India (J. Kotányi, Austria)	0	93.8 ± 0.1	92.8 ± 0.1	34.3 ± 0.1	33.1 ± 0.1
		10	93.8 ± 0.1	93.1 ± 0.1	33.5 ± 0.5	33.5 ± 0.5
		30	93.8 ± 0.1	92.7 ± 0.1	33.0 ± 0.1	33.2 ± 0.1
Oregano	Turkey (J. Kotányi, Wolkersdorf im Weinviertel, Austria)	0	90.1 ± 0.1	90.3 ± 0.1	9.8 ± 0.2	9.8 ± 0.2
		10	90.2 ± 0.1	90.2 ± 0.1	9.8 ± 0.3	9.9 ± 0.3
		30	90.1 ± 0.1	90.1 ± 0.1	9.9 ± 0.2	9.9 ± 0.2

Numerical values are presented as mean ± standard deviation ($n = 3$).

Republic). Irradiation was performed in a closed square chamber equipped with a set of ⁶⁰Co bars used as a γ -source, with automatic position and radiation dose check. Performance of the γ -source reached (2 ± 0.1) kGy·h⁻¹. Spices were exposed at doses of (10 ± 0.5) kGy and (30 ± 0.7) kGy, respectively. Dose precision of exposure was controlled by inner alanine dosimeter placed at the top of the samples batch directly in the chamber.

The applied doses were chosen with respect to the Directive EC 1999/3 as well as FDA limits for spices irradiation [1, 3]. As a reference, respective non-irradiated (0 kGy) sample of the same quality and origin was used. Between the experiments, samples were stored in closed paper and polyethylene bags in laboratory conditions (25 °C, rela-

tive humidity 40%). Their dry matter content was determined as a basic characteristic (Tab. 1).

Extracts from individual spices were prepared by mixing exactly 2 g of the spice with 50 ml 80% (v/v) aqueous methanolic solution. The mixture was shaken for 1 h at 25 °C using a laboratory shaker (Innova 2000; New Brunswick Scientific, Edison, New Jersey, USA) at 3.3 Hz and, subsequently, the solid phase was removed by filtration through a folded paper filter (604½ Whatman, Schleicher and Schuell, Dassel, Germany). Extraction yields of individual spices were determined as another basic characteristic (Tab. 1).

Extracts were prepared 2 times, immediately after the respective spice irradiation and after 6 months of their post-radiation storage at

ambient conditions, in order to assess the influence of both irradiation and storage on selected qualitative characteristics, as indicated below. All chemicals used in experiments were of analytical purity grade.

Spectrophotometric measurements

Measurements were performed using UV-VIS Specord M40 spectrophotometer (Carl Zeiss, Jena, Germany), typically under the following conditions: spectral band width 10 nm; integration time 1 s; gain 1. If not specified otherwise, a square cell with a path length of 1 cm was used. Measurements were carried out in duplicates at a laboratory temperature ranging from 22 °C to 25 °C (in case of •DPPH assay, the temperature was kept at (25 ± 1) °C).

•DPPH radical-scavenging assay

A modified version of 2,2-diphenyl-1-picrylhydrazyl (•DPPH) free radical-scavenging assay of BANDONIENÉ et al. [20] was used. A quantity of 0.5 ml (caraway, ginger, black pepper) or 0.05 ml (bay leaves, clove, allspice, oregano) of methanolic extract was added to 25 ml methanolic solution of •DPPH and absorbance at 515 nm was recorded after a 5 min reaction at 25 °C. Radical-scavenging activity (RSA) was expressed as the percentage of inhibition of •DPPH free radical calculated as:

$$RSA = \frac{(A_C - A_S)}{A_C} \times 100 \quad (1)$$

where A_C is absorbance of control and A_S is absorbance of sample.

Results were corrected for different volumes of methanolic extracts of spices used in the scavenging assay.

Thiobarbituric acid reactive substances (TBARS)

Oxidative reaction products determined as thiobarbituric acid number were analysed by the method of ZIN [21]. To 1 ml of methanolic spice extract, 20% aqueous solution of trichloroacetic acid (2 ml) and thiobarbituric acid aqueous solution (2 ml) were added. This mixture was then placed to a boiling water bath for 10 min. Subsequently, after cooling down to room temperature, the mixture was centrifuged at 500 × g for 20 min. Thiobarbituric acid number was determined as absorbance of supernatant at 532 nm.

Ferric reducing power (FRP)

Determination of ferric reducing power of spice extracts, as their ability to reduce the Fe^{3+} to Fe^{2+} , was done by the method of CHYAU et al.

[22]. Briefly, spice methanolic extract (100 µl) was mixed with 1.9 ml of distilled water, 2 ml of 0.2 mol·l⁻¹ sodium phosphate buffer (pH 6.6) and 2 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After that, 2 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 500 × g for 10 min. Upper layer (1 ml) was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride, and the absorbance at 700 nm was read after 1 min.

Total phenolic compounds (TPC) concentration

The concentration of total phenolic compounds was determined using the Folin-Ciocalteu modified method [23]. A volume of 100 µl of spice methanolic extract was diluted in 15.9 ml of distilled water and 1 ml of Folin-Ciocalteu reagent was added and the mixture was mixed. After 3 min, 3 ml of 20% of sodium carbonate was added and the mixture was mixed. As a result of the reaction, colour was developed and absorbance at 755 nm was measured after 60 min, and related to the absorbance of blank experiment. The same procedure was repeated using a standard solution of gallic acid. The results were expressed as milligrams of gallic acid equivalents (GAE) per litre of extract.

Colour characteristics

CIE $L^*a^*b^*$ colour values of spice extracts were estimated using Illuminant A (according to The International Commission on Illumination, utilizing the spectrophotometer accessory) as a source of light, and standard observer for a 2° viewing angle. A square cell (path length, 1 cm) was used for the absorbance spectra measurements in the spectral range from 380 nm to 780 nm, using a step of 10 nm.

Statistical analysis

Multidimensional pattern recognition techniques including factor analysis (FA) and canonical discriminant analysis (CDA) were used to characterize the mutual relationship between the individual experimental characteristics, and to differentiate and classify spice samples according to the absorbed γ-radiation dose. The methods are designed in a way that enables the enhancement of hidden properties of the original data set and allows the reduction of multi-dimensional data set to only a few dimensions, which can sufficiently explain all the original data and relationships between them. CDA enables the recognition and prediction ability determination as the percentage of correctly classified samples. The classification rules achieved by canonical classification functions

were validated by means of a cross-validation procedure, which was performed by dividing the complete data set into a training set and an evaluation set. Samples were assigned randomly to a training set, consisting of 75% of them, and the test set, composed of the remaining 25% of the samples. The recognition ability was also examined by the k^{th} -nearest neighbour discrimination and classification procedure. Statistical calculations were performed by means of the statistical package Unistat v. 5.6 (Unistat, London, United Kingdom).

RESULTS AND DISCUSSION

Effects of γ -irradiation and post-irradiation storage on some characteristics of spices and their methanolic extracts

As the first characteristic parameter of spices under study, their dry matter content and its alterations following either from irradiation or post-irradiation storage, were evaluated. Data presented in Tab. 1 clearly indicate that, for individual spices, the dry matter content in reference spice samples determined immediately after the spices irradiation ranged from 81% (clove) to 94% (garlic). At

the same time, no significant differences were found between the appropriate reference sample and samples exposed at 10 kGy and 30 kGy, respectively.

After 6 months of storage at ambient conditions, slightly increased dry matter content was determined in the majority of samples, with the highest difference in clove and black pepper, (7% and 4%, respectively). As there were practically no differences in dry matter between the reference and irradiated samples, it can be concluded that these changes were not caused by irradiation and were just the result of continuous water lost during the storage.

Results demonstrate the significant impact of γ -radiation on extraction capability of some spices. Immediately after irradiation, extraction yields of individual spices were slightly increased – as a result of exposure at the dose of 30 kGy, the increase for garlic reached 10%, for ginger 8%, and for clove 4% (Tab. 1). In other samples, the changes were only negligible or within the measurement uncertainty. The changes in extractability could follow from the degradation of the cell membranes and cell walls by the action of ionizing radiation. The affected cells released substances at

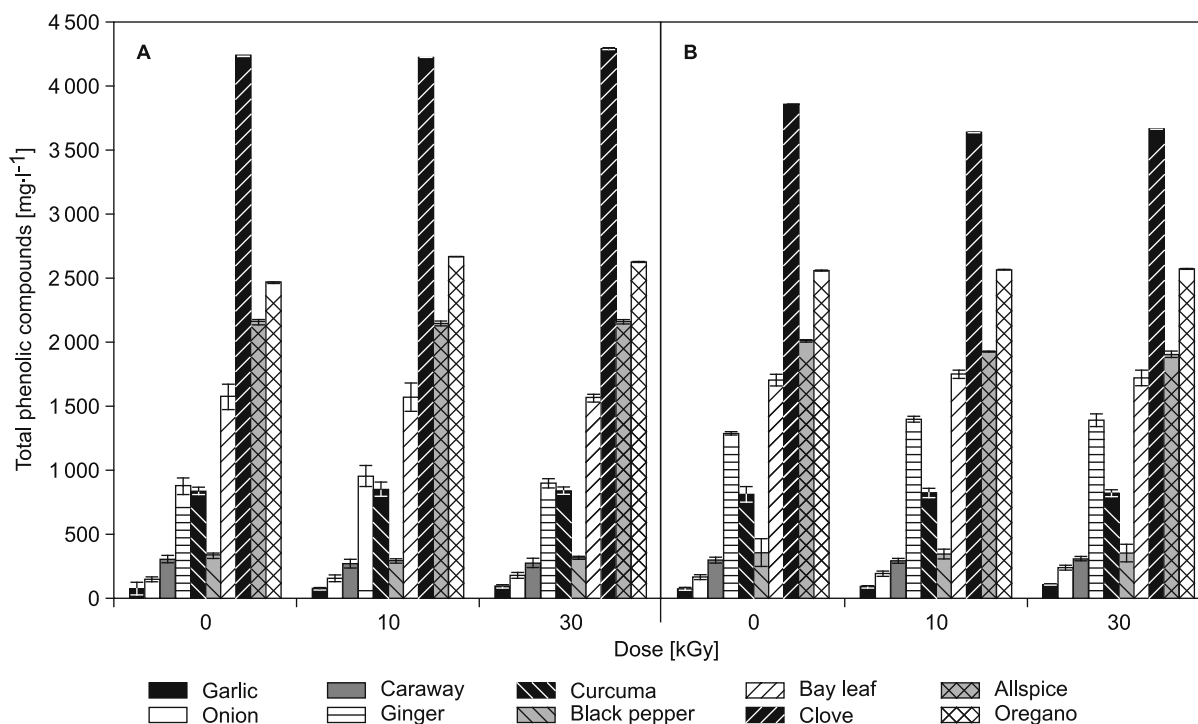


Fig. 1. Effect of γ -irradiation and 6 months post-irradiation storage on total phenolic compounds of extracts prepared from spices under study.

A – γ -irradiation, B – 6 months post-irradiation storage.

Results presented are expressed as averaged gallic acid equivalents in milligrams per litre of extract ($n = 3$).

Tab. 2. Basic characteristics of methanolic extracts of spices evaluated in different time intervals after the exposure of spice samples to γ -irradiation.

Spice	Immediately after irradiation			After 6 months of post-irradiation storage		
	0 kGy	10 kGy	30 kGy	0 kGy	10 kGy	30 kGy
Total phenolic compounds [mg·l ⁻¹]						
Allspice	2033 ± 28	2024 ± 54	2037 ± 27	1893 ± 59	1816 ± 35	1796 ± 27
Bay leaves	1466 ± 20	1467 ± 17	1459 ± 11	1596 ± 105	1647 ± 36	1619 ± 66
Black pepper	269 ± 19	232 ± 26	254 ± 21	289 ± 19	286 ± 20	294 ± 18
Caraway	239 ± 48	212 ± 10	216 ± 12	233 ± 12	234 ± 8	249 ± 5
Clove	4039 ± 96	4032 ± 108	4097 ± 30	3684 ± 45	3478 ± 31	3504 ± 59
Curcuma	758 ± 28	773 ± 54	762 ± 27	731 ± 59	749 ± 35	746 ± 27
Garlic	15 ± 5	21 ± 10	41 ± 12	17 ± 12	36 ± 8	54 ± 5
Ginger	794 ± 29	872 ± 33	819 ± 35	1193 ± 21	1304 ± 18	1298 ± 16
Onion	87 ± 20	102 ± 26	123 ± 21	105 ± 19	137 ± 20	183 ± 18
Oregano	2336 ± 60	2528 ± 79	2487 ± 35	2426 ± 14	2438 ± 22	2443 ± 47
DPPH radical-scavenging ability [%]						
Allspice	77.1 ± 2.7	75.2 ± 1.9	75.6 ± 1.9	72.5 ± 2.4	72.1 ± 0.7	71.2 ± 2.4
Bay leaves	44.1 ± 1.6	47.1 ± 0.9	48.4 ± 1.4	46.4 ± 0.7	46.5 ± 0.3	46.2 ± 0.2
Black pepper	37.7 ± 0.9	34.6 ± 0.6	32.6 ± 0.7	34.3 ± 2.3	32.7 ± 1.1	31.5 ± 0.6
Caraway	4.5 ± 0.3	4.1 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
Clove	68.1 ± 1.5	69.7 ± 2.7	69.3 ± 2.2	70.9 ± 0.9	71.7 ± 1.9	71.2 ± 1.2
Curcuma	11.2 ± 0.1	11.3 ± 0.1	11.3 ± 0.1	10.7 ± 0.2	10.6 ± 0.1	10.6 ± 0.1
Garlic	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.5 ± 0.1
Ginger	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.6 ± 0.1	7.6 ± 0.3	7.8 ± 0.1
Onion	0.6 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	1.1 ± 0.0	1.4 ± 0.0
Oregano	81.9 ± 2.1	80.6 ± 1.2	80.9 ± 1.0	81.5 ± 1.9	82.5 ± 1.7	80.3 ± 2.6
Thiobarbituric acid reactive substances number						
Allspice	0.33 ± 0.02	0.33 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.32 ± 0.02
Bay leaves	0.51 ± 0.01	0.51 ± 0.08	0.52 ± 0.03	0.47 ± 0.04	0.48 ± 0.05	0.43 ± 0.01
Black pepper	0.06 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.10 ± 0.02
Caraway	0.03 ± 0.01	0.14 ± 0.04	0.11 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Clove	0.29 ± 0.02	0.30 ± 0.02	0.34 ± 0.02	0.26 ± 0.04	0.27 ± 0.02	0.28 ± 0.03
Curcuma	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Garlic	0.51 ± 0.04	0.72 ± 0.02	0.92 ± 0.01	0.26 ± 0.01	0.51 ± 0.01	0.63 ± 0.01
Ginger	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
Onion	0.21 ± 0.01	0.44 ± 0.01	0.67 ± 0.01	0.83 ± 0.06	1.10 ± 0.01	1.25 ± 0.03
Oregano	0.29 ± 0.02	0.31 ± 0.01	0.34 ± 0.01	0.31 ± 0.02	0.32 ± 0.01	0.33 ± 0.02
Ferric reducing power						
Allspice	1.77 ± 0.05	1.70 ± 0.15	1.73 ± 0.06	1.70 ± 0.05	1.65 ± 0.04	1.63 ± 0.12
Bay leaves	0.29 ± 0.05	0.24 ± 0.04	0.26 ± 0.02	0.91 ± 0.01	0.98 ± 0.05	0.96 ± 0.08
Black pepper	1.97 ± 0.03	1.82 ± 0.04	1.80 ± 0.03	1.98 ± 0.05	1.88 ± 0.08	1.80 ± 0.08
Caraway	0.15 ± 0.02	0.12 ± 0.03	0.12 ± 0.03	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
Clove	1.99 ± 0.04	1.95 ± 0.06	1.94 ± 0.07	1.91 ± 0.01	1.88 ± 0.09	1.81 ± 0.03
Curcuma	0.28 ± 0.01	0.31 ± 0.01	0.32 ± 0.03	0.34 ± 0.01	0.33 ± 0.01	0.33 ± 0.02
Garlic	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.08 ± 0.03
Ginger	0.54 ± 0.04	0.54 ± 0.04	0.53 ± 0.03	0.67 ± 0.04	0.67 ± 0.03	0.68 ± 0.04
Onion	0.11 ± 0.01	0.11 ± 0.02	0.14 ± 0.01	0.11 ± 0.02	0.15 ± 0.01	0.18 ± 0.01
Oregano	2.11 ± 0.09	2.10 ± 0.06	2.30 ± 0.20	2.10 ± 0.03	2.10 ± 0.02	2.20 ± 0.03

Results are presented as mean ± standard deviation ($n = 3$).

Total phenolic compounds are expressed in milligrams of gallic acid equivalent per litre of extract. Thiobarbituric acid reactive substances number (TBARS) is expressed as absorbance of supernatant at 532 nm. Ferric reducing power is expressed as absorbance of supernatant at 700 nm.

extraction easier than the non-treated ones [24]. Our results correlated well with the trends in extractability observed by other authors [25, 26].

Great variability in the concentration of polyphenols of spice samples was confirmed (Fig. 1A), without respect on whether the samples were exposed to γ -radiation, or not. The highest *TPC* concentration was determined in clove extracts and, in a descending order, in extracts of oregano > allspice > bay leaves > ginger > curcuma > black pepper > caraway > onion and garlic (Tab. 2, Fig. 1). Their contents in respective spice extracts were in good agreement with those previously reported by HINNEBURG et al. [27]. As regards the irradiation effects, it is obvious that exposure of spices to γ -irradiation had the most significant impact on onion and garlic, for which the 15% and 30%, and even 40% and 173% increase was noticed, respectively, taking into account values for the respective reference, and samples exposed at 10 kGy and 30 kGy. In

case of oregano, ginger and clove, only moderate and slight increase in *TPC* of about 7%, 5% and 2% was observed, whereas for the other spices under study, only some negligible changes within the measurement uncertainty were noticed. Similar findings were previously reported by PÉREZ et al. [28] for dry rosemary leaf powder γ -irradiated at 30 kGy. An increase in soluble phenols in some other spices or foodstuffs caused by irradiation was also previously noticed by other authors [13, 14, 25, 29, 30]. Regarding the influence of post-irradiation storage on *TPC* concentration (Tab. 3, Fig. 1B), its slight decrease was noticed, do not exceeding 10%, in case of allspice, caraway, curcuma and clove extracts, comparing the values of corresponding references. On the other hand, increased *TPC* values were found in extracts of other spices, most significantly in ginger and onion (by 49% and 21%, respectively). When 10 kGy and 30 kGy processed samples are compared to the reference, an increase was obvious in majority of samples,

Tab. 3. Classification of spices according to the doses of irradiation and the validation characteristics (recognition and prediction ability).

Spice	Correctly classified cases [%]			
	Canonical discriminant analysis		k^{th} -nearest neighbour discrimination – recognition*	
	Recognition	Prediction	$k = 2$	$k = 3$
Discrimination of samples exposed at 0 kGy vs 10 kGy vs 30 kGy				
Allspice	88.9	78.0	55.7	44.4
Bay leaves	59.5	54.8	61.8	55.9
Black pepper	100.0	90.0	72.2	72.2
Caraway	61.9	60.5	66.7	66.7
Clove	94.5	94.5	50.0	55.7
Curcuma	73.3	75.0	53.3	66.7
Garlic	100.0	90.0	66.7	55.6
Ginger	72.2	66.7	66.7	61.1
Onion	86.7	83.3	80.0	86.7
Oregano	94.4	93.3	66.7	66.7
Discrimination of samples exposed at 0 kGy vs 30 kGy				
Allspice	100.0	91.7	66.7	75.0
Bay leaves	75.0	77.8	79.2	79.2
Black pepper	100.0	100.0	83.3	75.0
Caraway	92.8	82.2	78.6	82.1
Clove	100.0	100.0	50.0	58.3
Curcuma	100.0	80.0	80.0	80.0
Garlic	100.0	90.0	90.0	90.0
Ginger	100.0	91.7	66.7	75.0
Onion	100.0	90.0	100.0	100.0
Oregano	100.0	100.0	83.3	91.7

* for $k = 1$, 100% correct recognition was achieved in all cases.

most significantly for ginger, garlic and onion (by 64/60%, 57/110% and 140/260% for extracts prepared from samples exposed at 10 kGy/30 kGy, respectively). Explanation of the observed trends is somewhat difficult, as it cannot be attributed only to extractability changes as previously discussed by AYED et al. [24], because in case of e.g. oregano or ginger, these changes were minimal and those observed for e.g. onion or garlic did not correspond from the quantitative point of view to the observed trend in *TPC*. A better way out would be to assign them to the destructive oxidation process taking place during irradiation, which is capable of breaking the chemical bonds of polyphenols. Thereby, release of low molecular weight soluble phenols could occur [31]. The \cdot DPPH radical-scavenging ability (*RSA*) test represents a routine assay for characterization of antioxidant activity of different foods and biosystems including spices. As depicted on Fig. 2, the highest *RSA* was found for oregano extract, followed by those of allspice, ginger and clove prepared from the respective references. In contradiction, the lowest *RSA* was observed for caraway, onion and garlic extracts. These results are in good correlation with the *TPC* concentration and in agreement with those previously reported by KHATUN et al. [32], SHOBANA and NAIDU [33] and SHAN et al. [34]. As regards the effect of irradiation on *RSA*, immediately after irradiation the \cdot DPPH *RSA* was significantly changed only in case of black pepper, bay leaves and onion extracts. In case of the first mentioned, a decrease in *RSA* by 8% and 13.5%, for the second an increase by 6% and 10%, whereas for the last mentioned, 30.6% and even 54% increase was observed for extracts prepared from samples exposed at 10 kGy and 30 kGy, respectively. The other extracts revealed either only slight or negligible changes of their *RSA* following from irradiation. The observed decrease for black pepper as well as the increase for bay leaves extracts could not be sufficiently attributed neither to the dry matter, extractability nor *TPC* concentration changes resulting from irradiation. However, in particular in case of black pepper, the observed decrease can be effectively explained by the reduction of the content of some other non-phenolic bioactive compounds, as suggested by EMAM et al. [7]. The decrease of antioxidant components in some other spices, e.g. coriander and bay leaves irradiated at doses up to 50 kGy by 4–13%, was observed also by SJÖVALL et al [4]. AHN et al. [35] reported that immediately after irradiation, the scavenging ability of Chinese cabbage was reduced as a result of irradiation at a dose of merely 2 kGy. In case of onion extracts, there exists a clear positive correlation between

TPC and *RSA*. On the other hand, an increase in *RSA* was previously reported for extracts of dry rosemary leaf powder subjected to 30 kGy [28], green tea treated at 10 kGy and 20 kGy [36], citrus pomaces electron-beam irradiated at 40 kGy [26], soybean, carrot or kale juice γ -irradiated at 5 kGy [29]. The enhancement of *RSA* induced by thermal treatment of some of the spices was observed previously as well [32]. As a result of 6-months post-irradiation storage, in case of black pepper, bay leaves and onion, a practically identical trend as described above was observed. Besides them, a notably increased \cdot DPPH *RSA* of the extract from garlic exposed at 30 kGy was confirmed, reaching 4 times the value of the extract from the corresponding fresh reference. Increased *RSA* values resulting from storage was also determined for ginger, bay leaves and clove extracts. Decreased values were determined for the allspice, caraway and curcuma extracts. However, in all these cases the differences between the “fresh reference” extracts values and those of stored spices did not exceed 10%.

The contents of thiobarbituric acid reactive substances in the extracts from respective reference spice samples differed in accordance with expectations regarding the spice type. These were determined in the following descending order: bay leaves \geq garlic $>$ allspice $>$ oregano \geq clove $>$ onion $>$ ginger $>$ black pepper \geq curcuma $>$ caraway (presented in Fig. 3 and in detail in Tab. 2). Majority of the studied samples were characterized by the γ -dose-dependent increase in *TBARS* values attributed to oxidative products formation induced by γ -radiation. When compared to the respective reference, the greatest difference in the content of oxidative products was observed for irradiated caraway (increased approximately 5 times and 4 times), followed by onion, where the increase reached on average 2 times and 3 times the reference value, for samples exposed at 10 kGy and 30 kGy, respectively. *TBARS* concentration in the extracts of garlic and curcuma exposed at 30 kGy increased immediately after irradiation by approx. 80% and, for black pepper extracts, by approx. 67%. Extracts of allspice, bay leaves, ginger and oregano did not show any changes in the oxidation products formation, or the changes were within the measurement uncertainty. Thus it can be concluded that these three spices are, regarding resistance to oxidation stress, the best from the examined group of spices. As a result of 6-months post-irradiation storage, extracts from reference samples of the majority of investigated spices retained their *TBARS* values or only small fluctuations within the measurement uncertainty

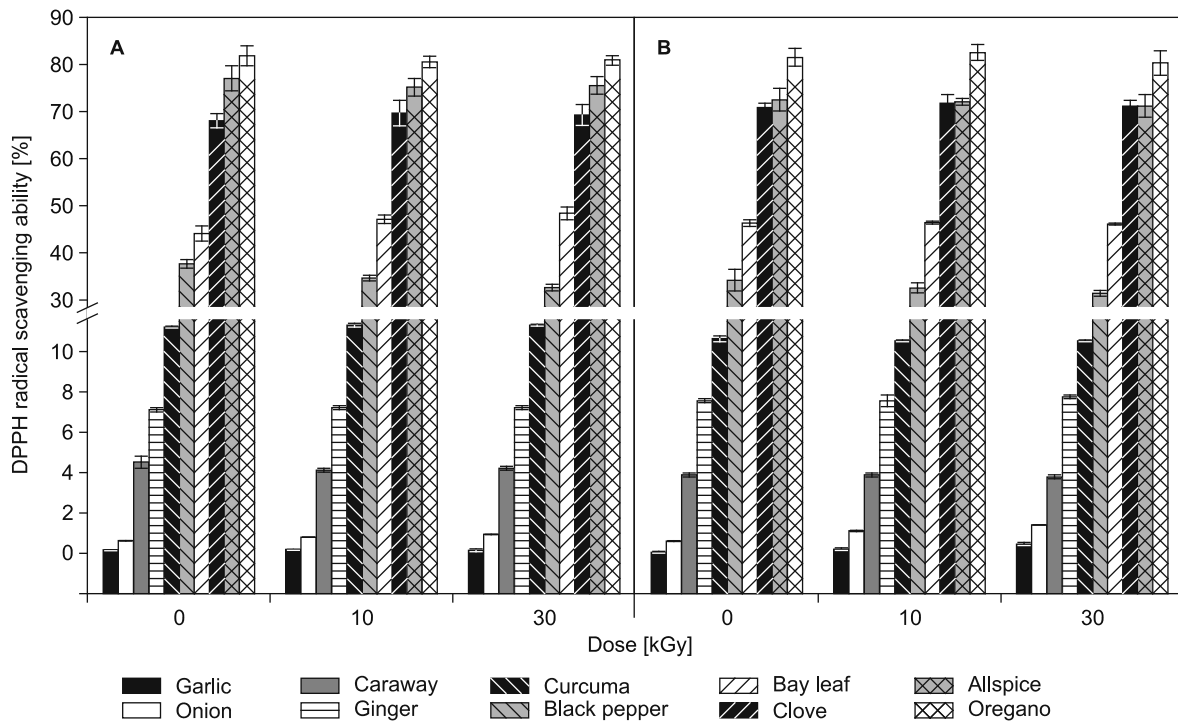


Fig. 2. Effect of γ -irradiation and 6 months post-irradiation storage on *DPPH scavenging activity of extracts prepared from spices.

A – γ -irradiation, B – 6 months post-irradiation storage.
Results presented are expressed as mean \pm standard deviation ($n = 3$).

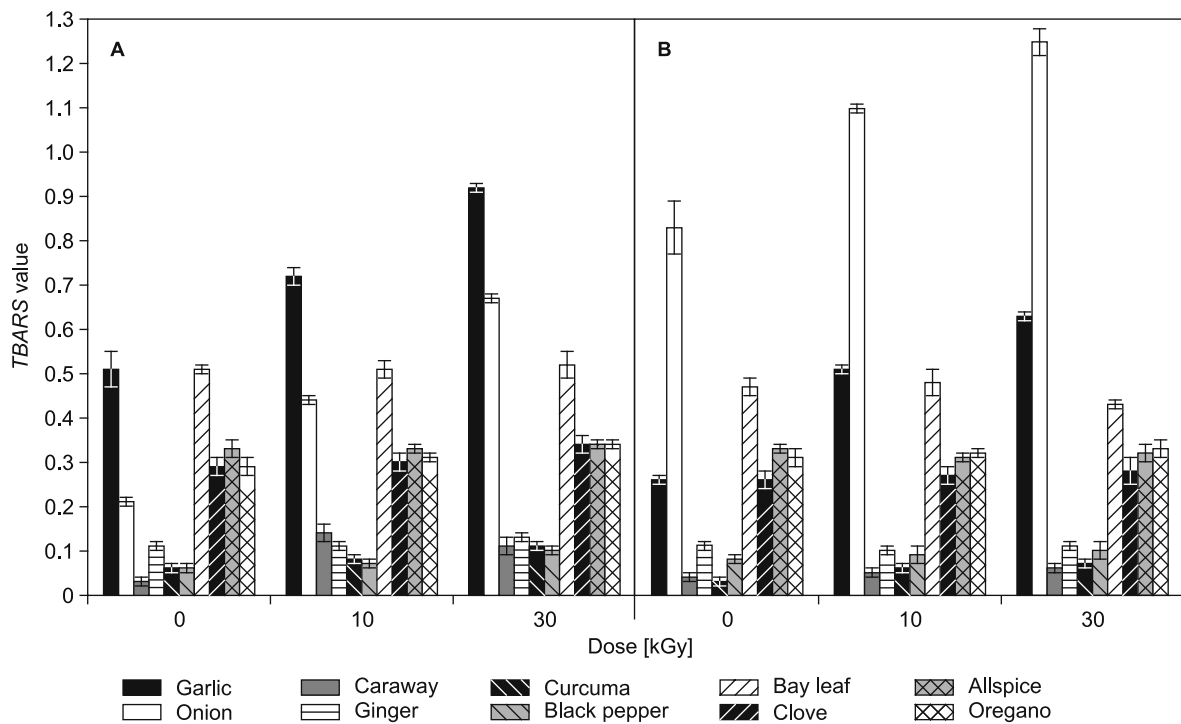


Fig. 3. Effect of γ -irradiation and 6 months post-irradiation storage on the formation of oxidative products expressed as TBARS values in extracts prepared from spices.

A – γ -irradiation, B – 6 months post-irradiation storage.
Results presented are expressed as mean \pm standard deviation ($n = 3$).

were noticed, with exception for garlic and onion extracts, for which significant changes were observed. For the former, a decrease by approx. 50% was detected and for the latter, 4-times increased *TBARS* value was detected. These changes were even much more pronounced than those detected immediately after irradiation. As regards the irradiated samples, their extracts showed either non-significant or only small changes in *TBARS* value upon the storage period.

Ferric ions reducing power of the methanolic extracts of spices was assessed by the potassium ferricyanide reduction method based on monitoring of the ability of individual spice extracts to reduce Fe^{3+} /ferricyanide complex. The formation of ferrous products is accompanied by the development of colour of characteristic wavelength. The following descending order of *FRP* values was found for spice extracts under study: oregano > clove > allspice > ginger > black pepper ≥ curcuma > caraway > onion > bay leaves and garlic, which possessed *FRP* values in fact close to zero (Tab. 2, Fig. 4). Irradiation itself had only minor impact on *FRP* of majority of spices, however, its dose-dependent increase by 10–25% was noticed for garlic, onion and oregano extracts, in par-

ticular for those prepared from samples exposed at 30 kGy. The increase of *FRP* by the action of γ -irradiation has also been reported by AHN et al. [35] in Chinese cabbage, by SONG et al. [29] in carrot juice and by HUANG and MAU [25] in extracts from the mushroom *Agaricus blazei*. Post-irradiation storage at ambient conditions resulted in slight changes of *FRP* in both directions, whereas for black pepper and oregano extracts, the *FRP* values remained unchanged or altered only within the measurement uncertainty, when compared to those of reference extracts prepared immediately after γ -irradiation. The most significant *FRP* increase was observed for bay leaves extracts, reaching three times the value of extracts prepared just after irradiation. Considerable 20–100% increase was noticed also for caraway, curcuma, garlic and ginger extracts. On the opposite, decreased *FRP* values were determined in allspice extracts. It can be concluded that, in majority of cases, the alterations in *FRP* values resulting from post-irradiation storage of spices were in a close relationship with the changes in extraction ability discussed above.

Colour of spices and its changes is considered to be one of the most significant markers indicating any way of treatment and is also accepted as

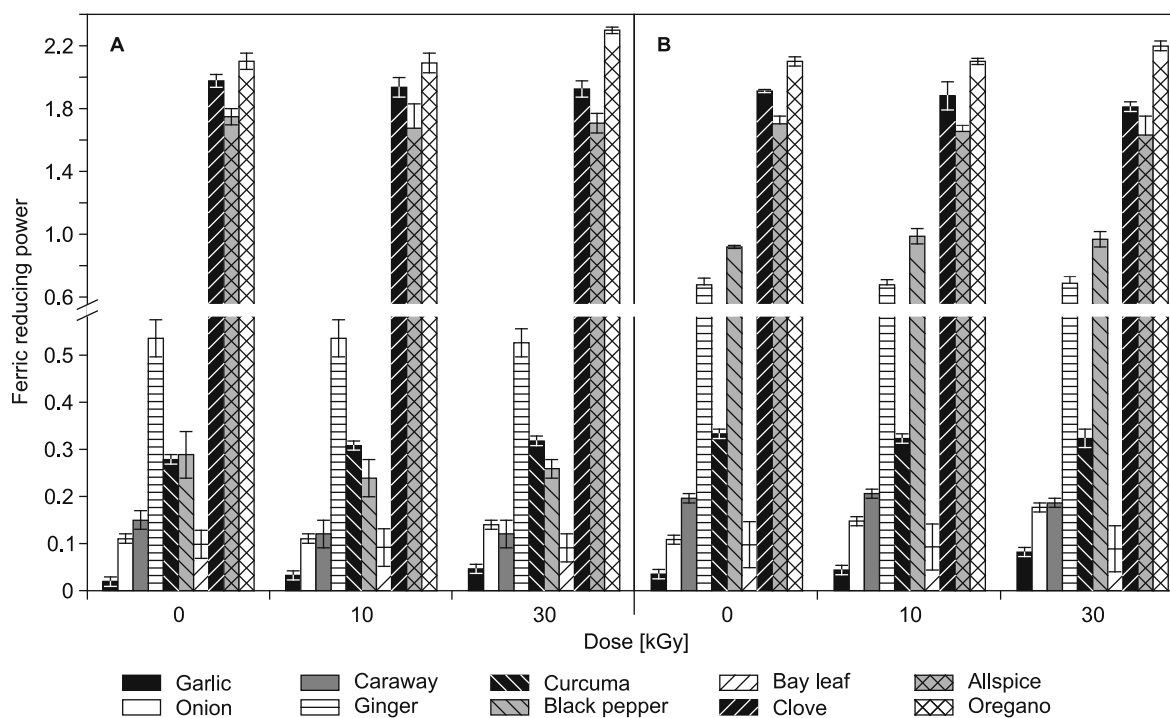


Fig. 4. Effect of γ -irradiation and 6 months post-irradiation storage on ferric ions reducing power of extracts prepared from spices.

A – γ -irradiation, B – 6 months post-irradiation storage.
Results presented are expressed as mean \pm standard deviation ($n = 3$).

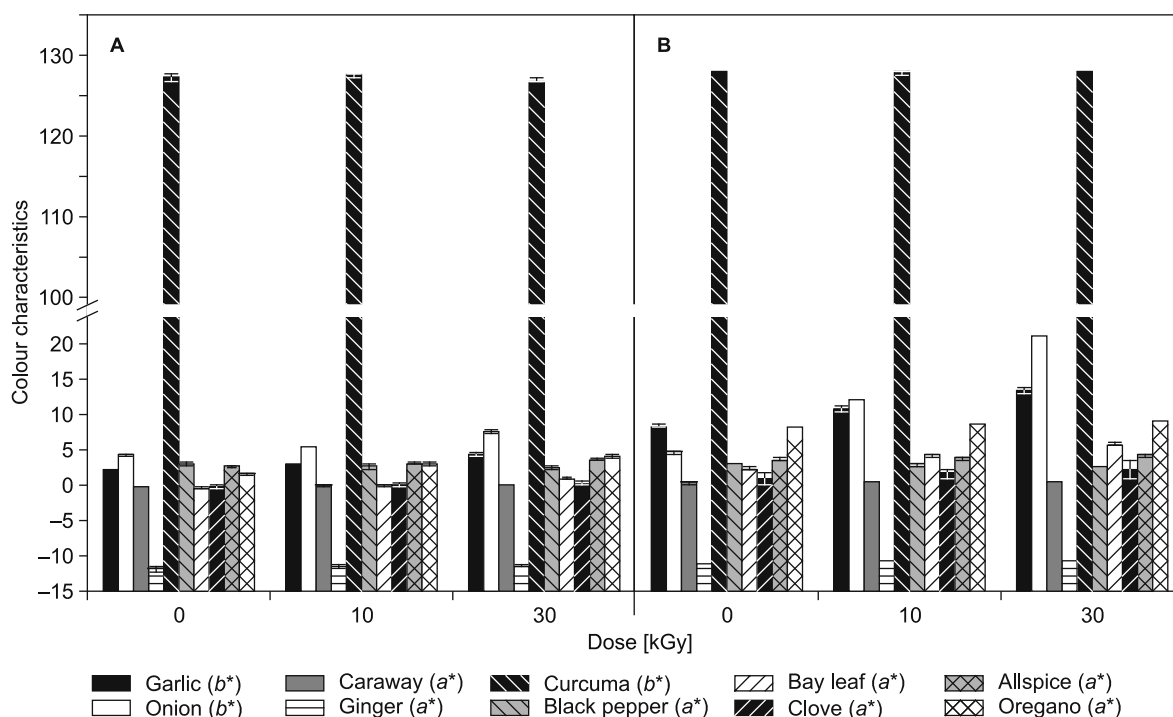


Fig. 5. Effect of γ -irradiation and 6 months post-irradiation storage on redness and yellowness of spices.

A – γ -irradiation, B – 6 months post-irradiation storage.

Redness – colour parameter a^* , yellowness – colour parameter b^* .

Results presented are expressed as mean \pm standard deviation ($n = 3$).

one of qualitative parameters. Series of experiments performed with individual spice extracts were focused on the determination of their colour values in CIE $L^*a^*b^*$ colour space. As regards lightness (L^* parameter), its changes were considered as non-significant for all spice extracts under study, or its changes revealed ambiguous trend with differences either from irradiation or post-irradiation storage within the standard deviations. However, this was not the case of the remaining colour characteristics a^* and b^* . Data presented on Fig. 5 clearly demonstrate that with exception for extracts of curcuma, garlic and onion, the most significant changes were observed for chromaticity layer a^* (negative values indicate green while positive values red colour), without respect to whether it was the effect of γ -irradiation or post-radiation storage. For all black pepper extracts, the decrease in redness was observed. The same effect was previously reported for chlorophyll or green tea leaves extracts exposed to γ -irradiation [36]. In all other cases, the value of a^* gradually increased with the radiation dose absorbed, at maximum by 20% for oregano, followed by bay leaves and allspice samples. As already mentioned, in case of curcuma, garlic and onion, significant

changes of colour value b^* were observed, visible even by eye. It reached 15% and 11% for onion and garlic extracts, respectively. These findings are consistent with data of other authors [37–40]. Even more intensive changes in colour characteristics were noticed in extracts prepared after a half year storage of spices at ambient conditions. In all cases, with exception for curcuma and black pepper, a marked increase of either a^* or b^* colour parameter was proved, ranging from 50% (e.g. for allspice or onion extracts) up to 500% (oregano, bay leaves extract). The observed changes in colour characteristics of irradiated foods and their extracts were previously suggested to be associated with the creation of coloured compounds by Maillard reaction, by the non-enzymatic browning reactions, or by enzymatic oxidation of some phenolic compounds present in food matrix [41, 42].

To summarize, the above-presented results revealed that γ -irradiation itself did not cause so dramatic changes as the subsequent post-irradiation storage, or that the changes were at least comparable. Looking on antioxidant activity changes of individual spice extracts, it is obvious that the changes observed immediately after irradiation were often smaller than the variance of the values

obtained for corresponding reference samples. It indicates that the real experimental antioxidant activity changes induced by γ -irradiation were without practical significance and would not have any negative impact on the consumers' health. In addition, trends in changes of the investigated characteristics of all spices were not significant enough, or even contradictory, to be used alone for the purposes of clear differentiation of respective reference spice samples from samples exposed to γ -irradiation.

Statistical evaluation of experimental data

Many authors reported on different correlations between the results of different antioxidant activity assays and phenolic compounds contents in different food matrices. The same phenomenon was fully confirmed by here presented experimental data. When the entire group of investigated spices was evaluated, strong correlation between the phenolic compounds concentration and \bullet DPPH radical-scavenging ability and ferric reducing power were found, with correlation coefficients $r = 0.846$ and $r = 0.899$, respectively. Similar correlations for the same antioxidant activity characteristics were observed previously for extracts of irradiated rosemary [28]. When individual spices were evaluated, best correlation ($r > 0.9$) between \bullet DPPH and TPC was found for allspice, ginger and clove extracts. Besides that, strong correlation between TPC and FRP ($r > 0.9$) was found for ginger, caraway and bay leaves. On the other hand, only negligible correlations were observed between TBARS and other antioxidant activity characteristics. On the other hand, moderate negative correlation between the TBARS value and colour value a^* ($r = -0.65$) was found.

However, these pair correlations do not facilitate complex evaluation of the relationship between all experimental characteristics. At the same time, they do not allow neither differentiation of the reference from irradiated samples, nor classification according to the absorbed γ -dose. To do so, methods of multivariate statistics need to be involved, as was previously illustrated on γ -irradiated caraway and bay leaves [43]. In the current study, the above-mentioned approach was further extended. Method of principal component factoring (factor analysis) with varimax rotation was used for the data visualization and comparison, taking into consideration the complete dataset of all antioxidant properties characteristics and colour values. This orthogonal rotation maximizes the variance of the loadings within factors across the variables and thus offers the best opportunity of factor interpretation. As clearly depicted on

Fig. 6, first two factors with eigenvalues higher than 1 explained cumulatively more than 78.9% of the total dataset variance. As also follows from the results (Fig. 6A), the first factor was related to the antioxidant properties descriptors (\bullet DPPH, ferric reducing power, concentration of polyphenolic compounds), and the second one to the TBARS values. The latter factor is also negatively associated with colour parameters. Plot of factor scores (Fig. 6B) facilitates the comparison of examined spices according to all the antioxidant data (\bullet DPPH, TBARS, FRP), TPC concentration and colour values a^* and b^* , as well as their changes induced by γ -radiation and post-irradiation storage. As presented in the score plot of above-mentioned two most significant factors, the entire group of ten analysed spices was well separated into several isolated clusters, with exception for garlic and onion or clove and oregano, which are plotted in mixed (common) clusters due to some similarities in the examined descriptors. Curcuma in the plot of factor score is in an extra position, most probably due to its colour differences from other spices.

More effective differentiation of the studied spices was achieved by canonical discrimination, by means of which, more than 90% correct classification of the samples according to their species was reached, without respect to whether they were irradiated or not. Herein, the correctness of classification in terms of spices recognition was negatively influenced by mis-classification of some garlic and onion samples.

From the perspective of backward detection (identification) of irradiated spices and the absorbed dose estimation, very important are the results of data discrimination and classification according to the absorbed dose of γ -radiation, presented in Tab. 3. For these purposes, two different approaches were used, namely, canonical and k^{th} -nearest neighbour discriminations. For both of them, the recognition ability was calculated (on the basis of experimental characteristics, the ability of the model to correctly classify samples of known absorbed dose). In case of canonical analysis, also the prediction ability was calculated (the ability to correctly classify the samples of unknown properties into the group according to the absorbed dose). As a logical consequence of what is written above, the recognition ability test gives usually a higher score than the prediction one.

In order to cover both legislation limits that are usually used for spices irradiation – the safety limits of EC (10 kGy) [1] and US FDA (30 kGy) [3] and to test the capability of the statistical method to correctly recognize also spice samples exposed

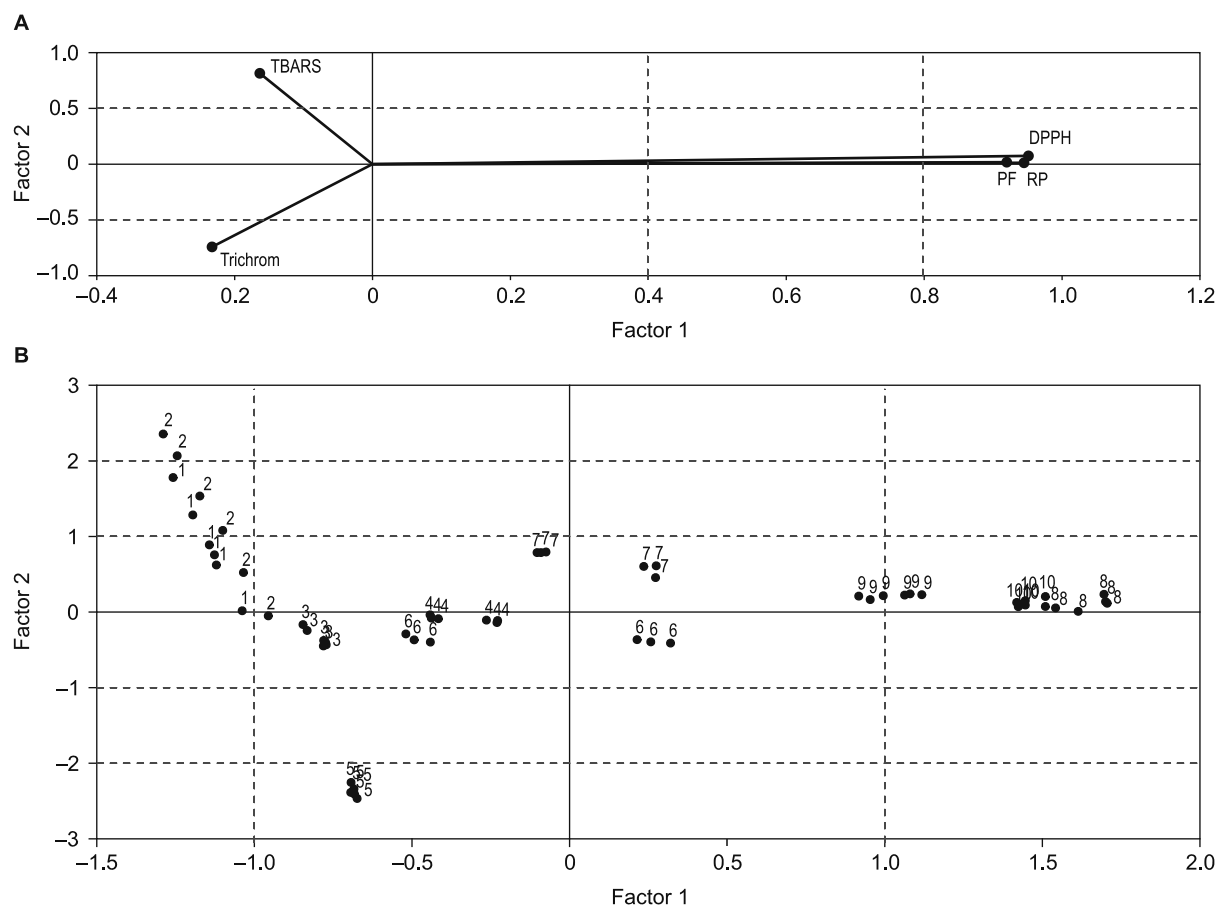


Fig. 6. Factor analysis with varimax rotation of spices under study illustrating the relationship between the antioxidant properties of extracts and their colour characteristics.

A – plot of factors, B – plot of factor scores.

TBARS – thiobarbituric acid number, Trichrom – colour parameters a^* or b^* of respective spice extract (refer to Fig. 5 for further details), DPPH – antiradical activity, PF – polyphenolic substances, RP – reducing ability.

Spices: 1 – garlic, 2 – onion, 3 – caraway, 4 – ginger, 5 – curcuma, 6 – black pepper, 7 – bay leaves, 8 – clove, 9 – allspice, 10 – oregano.

to lower radiation doses, two classification procedures were applied. The first one focused on classification, mutual recognition and prediction, of respective reference sample and samples of the same spice irradiated at 10 kGy and 30 kGy. As clearly demonstrated in Tab. 3, in case of six spices, more than 86% and, for garlic and onion, even 100% correct recognition was achieved by means of canonical discriminant analysis. On the other hand, in case of curcuma and ginger, the recognition score reached approx. 72% and, in case of caraway and bay leaves samples, the recognition score reached only 61.9 and 59.5%, respectively. The reason for such relatively bad results follows from the mis-classification of individual spices into groups according to the absorbed dose, caused by the similarities in experimental data sets in particular for samples exposed at 10 kGy and the re-

spective reference. As expected, the prediction ability was in all cases lower than the recognition ability, with an exception for curcuma samples. Regarding the last mentioned, there is not a satisfying explanation for the observed phenomenon, it can only be supposed that it is caused by the small number of samples and their variability. It should be noted here that for the above-described discrimination, the results of *DPPH radical-scavenging tests, TBARS values as well as TPC concentration were found to be the most efficient markers.

k^{th} -nearest neighbour discrimination and classification was performed for $k = 1$, $k = 2$ and $k = 3$. By this method, the samples were classified into groups (corresponding to radiation doses) on the basis of comparison of experimental characteristics of the classified object to those of one, two and three nearest, already classified samples,

respectively. While for $k = 1$, an absolutely correct classification was obtained, increasing the number of mutually compared objects decreased significantly the classification score. An interesting comparison offer the results of $k = 2$ and $k = 3$. For black pepper, caraway and oregano samples, no difference was observed, whereas in case of all-spice, bay leaves, garlic and ginger, a worsening of classification score by 5–10% occurred as a result of expanding the number of mutually compared objects for clove, curcuma and onion.

When only respective reference and samples exposed at 30 kGy were discriminated, the recognition ability evaluated by means of canonical discrimination analysis reached 100% in all cases, with exception for caraway and bay leaves, for which 92.8% and only 75% correct recognition was found, respectively (Tab. 3). Again, as expected, the prediction ability was lower in a majority of cases. However, these results are still sufficient to treat the method as effective for distinguishing the spices according to the applied dose of γ -radiation. k^{th} -nearest neighbour discriminant analysis demonstrated at $k = 1$ similar recognition capability as the canonical discrimination. For the higher numbers of neighbours compared (higher k -values), the recognition correctness decreased. From a simple comparison of the discrimination efficacy for $k = 2$ and $k = 3$ follows the better applicability of this method for $k = 3$.

These results clearly confirmed the existing great variability of experimental results even for a single spice. Non-significant role, in particular in case of k^{th} -nearest neighbour classification, plays the randomness of the selection of objects for comparison notable for higher values of parameter k . At the same time, results of multivariate statistics processing showed that antioxidant characteristics and colour descriptors can be effectively used to develop the model for spices classification into categories according to radiation dose. This approach can also find further application in the decision-making process of food safety offices or authorities dealing with monitoring of application of γ -irradiation as a toll to sterilization of foods.

CONCLUSION

γ -Irradiation as well as the post-irradiation storage caused some perceptible changes of antioxidant properties, which varied with type of spices. However, it was proven that post-irradiation storage caused more dramatic changes in the monitored characteristics than γ -irradiation itself, or that the changes were at least compara-

ble. The observed changes in antioxidant activity induced by γ -irradiation were without practical significance and would not have any negative impact on the health of the consumer. The antioxidant activity characteristics as well as colour coordinates of methanolic extracts of spices irradiated at doses of 0 kGy (reference), 10 kGy and 30 kGy can be effectively processed by multivariate statistics. Canonical discrimination analysis as well as k^{th} -nearest neighbour classification offered sufficient discrimination of irradiated samples from the reference ones, as well as facilitated estimation of the absorbed dose, even after 6 months of post-irradiation storage, when majority of commonly used markers of irradiation disappear.

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