Effect of thermal treatment and storage on antioxidant activity of some spices

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Summary

Effect of heat treatment and storage on antioxidant activity of black pepper, allspice and oregano was studied by determination of antiradical activity, reducing power, thiobarbituric acid number and content of total phenolic substances. Thermal treatment at 130 °C for 5 minutes caused significant decrease of all antioxidant activity parameters with the exception of increased content of phenolic substances in black pepper. Found differences in antioxidant activity between thermally treated and untreated samples were essentially retained during the storage of all spices. Spices storage of half a year caused significant decrease of antioxidant activity; the decrease was more pronounced in the case of thermally treated spices. Found relative degradation of the majority antioxidant attributes of spices are characteristic for food systems with short heat treatment with initial reduction in the overall antioxidant activity due to the thermal degradation of naturally occurring antioxidants and formation of early products of the Maillard reaction by reducing components.

Keywords

black pepper; oregano; allspice; thermal treatment; antioxidant activity

Food processing involves changes in structural integrity of the plant material and this produces both negative and positive effects on their antioxidant activity. The antioxidant activity is diminished owing to inactivation of antioxidant compounds caused by different chemical reactions enhanced by the effect of heat. The positive effects of food processing include in some cases transformation of antioxidants into more active compounds, such as the deglycosylation of onion quercetin, as well as an increase in the antioxidant activity owing to inhibition of enzymes [1]. An important primary effect of food browning caused by the Maillard reactions may be the formation of antioxidants in a thermal treatment conditions as well [2, 3]. Thermal processing of tomatoes enhances their nutritional value by increasing the bioaccessible lycopene content as well as the total antioxidant activity [4-6]. Cooking sweet corn increases its antioxidant activity, despite decreasing its vitamin C content [7]. Antioxidant activity of beets processed under typical commercial processing conditions remained constant despite an 8% loss of vitamin C. In contrast, vitamin C and dietary folate content of green beans remained constant, whereas a 32% reduction in phenolic compounds occurred after typical commercial processing conditions. The antioxidant activity of green beans was reduced by 20% [8]. Among some fresh and thermally treated vegetables, shallot showed the highest total antioxidant activity, followed by spinach, swamp cabbage, cabbage and kale. Except for shallot and cabbage, the antioxidant activities of kale, spinach and swamp cabbage were significantly decreased ($p < 0.05$) after thermal treatment. Moreover, this study revealed that a one minute thermal treatment significantly decreased ($p < 0.05$) the total phenolic content of all vegetables studied [9]. Effects of microwave and conventional cooking methods were studied on the total phenolics and antioxidant activity of pepper, squash, green beans, peas, leek, broccoli and spinach. Total phenolics content of fresh vegetables ranged from 1.832 to 13.447 $mg.g^{-1}$ (as gallic acid equivalent) on the dry weight basis. The total antioxidant activity ranged from 12.2% to 78.2%. With the exception of spinach, cooking affected total phenolics content significantly ($p < 0.05$). The effect of various cooking methods on total phenolics was significant ($p < 0.05$) only for pepper, peas and broccoli. After cooking, total antioxidant activity increased or remained unchanged depending on

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the type of vegetable but not type of cooking [10]. Soy isoflavones daidzein and genistein in model solutions (pH 7 and 9) were thermally treated at 120 °C or incubated at 70, 80, and 90 °C. The antioxidant activity of incubated isoflavone solutions, followed by the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) test, decreased rapidly at pH 9 for genistein, whereas only moderate reduction was observed for daidzein (pH 7 and 9) or genistein at pH 7 [11]. In honey heated at different temperatures (50, 60 and 70 °C) for up to 12 days antioxidant activity assessed by the 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical increased with the temperature and time of treatment [12]. Antioxidant activity and the total phenolic content of ethanolic extracts of mango seed kernel products increased to a maximum after heating to 160 °C [13]. The flavonoids of red apples showed a higher antioxidant activity in fresh state, and their activity diminished under heat (cooking) [14]. The maximum antioxidant activity of catechin and resveratrol solutions was higher and occurred in a shorter time as the storage temperature was increased or the solvent polarity was decreased. The maximum values of the latter variable in the catechin and resveratrol cases (reaching an antiradical activity value higher than 50% in comparison with the initial one) were detected when oxidation was carried out in ethanol at 60 °C after 6 and 24 h of storage, respectively. Such variations were due to different reaction pathways. In fact, oxidative polymerization and oxidative formation of hydroxyl groups were found to be responsible for the enhancements of antiradical activity in catechin and resveratrol, respectively. A similar trend with variations of temperature in the different media was also observed in the case of grape extract [15].

After 8 hour storage of black currant and black aronia berry juice concentrates at 60 °C the amount of polyphenols decreased by 46% and 22%, anthocyanins by 31% and 35%, respectively. Antioxidant activity decreased by 26% and 56%, respectively [16]. Blood orange segments were subjected to brief thermal treatments before transformation into juice. Thermal treatment generally induced an increase in the main phenolic substances of orange juice, such as anthocyanins and total cinnamates, while ascorbic acid underwent a decrease. Antioxidant properties, evaluated in a lipoxygenase-linoleic acid system, were higher in thermally-treated samples, while free radical scavenging activity, evaluated by ESR spin trapping of hydroxyl radical and DPPH radical quenching, were enhanced in untreated juices [17]. The stability of water-soluble antioxidant in orange and carrot juice was studied on a kinetic basis during thermal (75–120 °C) and high pressure (100–800 MPa; 30–65 °C) treatments. The water soluble antioxidant capacities of both orange and carrot juice, determined using the Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity TEAC number, increased for the first 60 min and 90 min of thermal treatment respectively [18]. Free radical scavenging capacity of the extracts from red and white grape pomace peels (RGPP, WGPP) and as a reference for two commonly used antioxidants (BHA (synthetic) and DL-tocopherol (natural) heated at processing temperatures (80, 100, and 120 °C) was evaluated using the DPPH reagent. Kinetic behavior of the sample extracts did not change when they were heated, following a general multiplicative model in which remaining DPPH was more affected by temperature than the time needed to reach the steady state [19].

Spices and vegetables possess antioxidant activity that can be applied for preservation of lipids and reduce lipid peroxidation in biological systems. The potential antioxidant activities of selected extracts of spices (water and alcohol 1:1) were investigated on enzymatic lipid peroxidation. Water and alcoholic extract (1:1) of commonly used spices (garlic, ginger, onion, mint, cloves, cinnamon and pepper) dose-dependently inhibited oxidation of fatty acid, linoleic acid in presence of soybean lipoxygenase. Among the spices tested, cloves exhibited the highest while onion showed the lowest antioxidant activity. The relative antioxidant activities decreased in the order of cloves, cinnamon, pepper, ginger, garlic, mint and onion. Spice mix composed of ginger, onion and garlic; onion and ginger; ginger and garlic showed cumulative inhibition of lipid peroxidation thus exhibiting their synergistic antioxidant activity. The antioxidant activity of spice extracts were retained even after boiling for 30 min at 100 °C, indicating that the spice constituents were resistant to thermal denaturation. Antioxidant activity of these dietary spices thus suggests that in addition to imparting flavour to the food, they possess potential health benefits by inhibiting the lipid peroxidation [20]. The effect of heating on antioxidant effectiveness and the chemical composition of basil, cinnamon, clove, nutmeg, oregano and thyme essential oils was studied on scavenging of DPPH radical. When heated up to 180 °C, nutmeg oil (but not the other essential oils under study) showed a significantly higher free radical-scavenger activity and evident changes in its chemical composition [21]. Although dietary spices are resistant to thermal denaturation, interestingly, when powders and oils were analysed, turmeric showed an increase in antioxidant activity on heating (120 °C for 1 hour) while in the case of ginger this activity was reduced [22].

These findings along with earlier results suggest that the effects of thermal processing on the antioxidant activity of foods and their components vary with the respective produce crop type, with the individual spice and with the heating procedure. In order to make valid conclusions more research into antioxidant activity concerning all types of food matrix will be very useful and required. This article brings some experimental results about antioxidant activity changes caused by thermal treatment of grounded raw black pepper, oregano, and allspice - spices which are the most used spices in our country.

MATERIALS AND METHODS

Materials and sample preparation

For the antioxidant activity, black pepper (density 550 $g.l^{-1}$, dry matter 87.9% w/w) from Vietnam, oregano K. B., N. Bükey, from Cambidi, Izmir, Turkey (dry matter 90.1% w/w), and allspice from Mexico (dry matter 86.9% w/w) were used. All raw and thermally non-treated spices harvested in 2005 year were obtained from wholesale distributor Mäspoma, Dvory nad Žitavou, Slovakia. All samples of spices were ground at this company. Next day the thermal treatment of spices was carried out at the VÚP Food Research Institute (Bratislava) by heat treatment at 130 °C for 5 minutes in 25 ml closed glass bottles, placed in a metal block thermostat (Liebisch, Bielefeld, Germany). These laboratory conditions well simulated the sterilization of spices, whereby the internal temperature of spices in bottles achieved 96 °C, similar to the commercial practice. Raw and thermally treated samples of spices were packed in 75 g polyethylene bags and stored in closed glass container at laboratory conditions (25 °C, relative humidity 40%). Dry matter content of these spices increased by about 4% upon 6 months storage. Determination of some antioxidant properties was made with extracts prepared from 2 g spices extracted for 1 hour with 50 ml of water-methanol solution 80% (v/v) using a laboratory shaker (Innova 2000, New Brunswick Scientific, Edison. New Jersey, USA) at 3,3 Hz. Water-methanol solution was chosen on the basis of our experimental results and is the most frequently used solvent for antioxidants extraction from herbs and spices [23].

DPPH radical scavenging assay

DPPH radical scavenging assay was modified according to Bandonienė [24]. Methanol spice extract (0.5 ml black pepper, or 0.05 ml oregano, or 0.05 ml allspice) was placed into 25 ml of DPPH (6.10^{-5} $mol.l^{-1}$) methanol solution, thoroughly mixed and absorbance at 515 nm was measured after 5 min. Radical scavenging activity was calculated as:

$$\% = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100$$

The spice extracts dilution caused by different extract doses in DPPH solution was calculated for the correct comparison of spices antiradical activity.

Thiobarbituric acid number

Thiobarbituric acid reactive substances were determined according to method of Zin [25]. To 1 ml of methanol spice extract, 20% (w/w) aq. trichloroacetic acid (2 ml) and of 0.67% (w/w) aq. thiobarbituric acid solution (2 ml) were added. This mixture was then placed in a boiling water bath for 10 min. After cooling it was centrifuged at 50 Hz for 20 min. Thiobarbituric acid number was determined as the absorbance of supernatant at 532 nm.

Reducing power

Determination of reducing power was carried out according to Chyau et al. [26]. Spice methanol extract (2 ml black pepper, or 0.1 ml oregano, or 0.1 ml allspice) was mixed with 2 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2 ml of 1% (w/w) potassium ferricyanide, and the mixture was incubated at 50 °C for 20 minutes. After 2 ml of 10% (w/w) trichloroacetic acid was added, the mixture was centrifuged at 50 Hz for ten minutes. Upper layer 1 ml was mixed with 1 ml of distilled water and 0.2 ml of 0.1% (w/w) ferric chloride, and the absorbance at 700 nm was read after 1 minute. The spice extracts dilution caused by different extract doses in this assay was calculated for the correct comparison of spices reducing power.

Total phenolic compounds

Total phenolics were determined using the Folin-Ciocalteau modified method [27]. 100 μl of the methanol spice extract was diluted to 15.9 ml with water and 1 ml of Folin-Ciocalteau reagent (Merck, Darmstadt, Germany) was added. After 10 min, 3 ml of 20% of sodium carbonate was added and the content was mixed thoroughly. The colour was developed and after 60 min absorbance measured at 755 nm. The same procedure was used for a standard solution of gallic acid. The results were expressed as mg gallic acid/litre of extract.

Spectrophotometric measurements

For the spectrophotometric measurements of DPPH radical-scavenging activity, thiobarbituric acid value and reducing power the UV-VIS Specord M40 (Carl Zeiss, Jena, Germany) was used at the following conditions: spectral bandwidth 20 cm^{-1}, integration time 1 s, and gain 3. For all measurement a square cell with path length of 1 cm was used.

Statistical analysis

For the statistical significance determination the ANOVA - Analysis of Variance (one factor) at the significance level of 0.05 was used. In all assays three runs of the full procedure were carried out, including the weighing, extraction and analysis of each sample. For the visualization and classification of all thermally treated and untreated spices, the Multiple Canonical Discriminant Analysis (MCDA) was applied using of statistical programme Unistat® (Unistat, London, United Kingdom) with standardized criteria of convergence.

RESULTS AND DISCUSSION

All experimental results of antioxidant activity, characterized by the DPPH antiradical activity, thiobarbituric number, reducing power and total polyphenolics content are summarized and visualized in Figs. 1–4. These figures show average values of antioxidant parameters obtained from triplicate measurement of each spices.

In the Fig. 1 we can see results of spice methanol extracts scavenging activity on stable DPPH radical. From the tested spices this activity was the highest in oregano, followed by allspice; it was lowest in the black pepper methanol extract. Oregano had approximately twenty times higher DPPH antiradical activity than black pepper, allspice activity was slightly lower activity than that of oregano. Thermal treatment caused significant decrease of antiradical activity; mainly in allspice (9% immediately after thermal treatment, and 32% after 6 months storage) and oregano (6% immediately after thermal treatment, and 12% after 6 months storage). In the case of black pepper storage caused more significant changes of antiradical activity (about 18% decrease of both thermally treated and untreated black pepper) than thermal treatment. The storage alone of the thermally untreated allspice and oregano caused practically no changes of their scavenging activity, but in the case of treated samples decrease of DPPH activity reached about 5% at oregano, and up to 25% at allspice.

Fig. 2 shows changes of the thiobarbituric acid number which represents the content of total oxidative products in both thermally treated and untreated methanol spice extracts. The highest content of naturally present oxidative reactive substances was found in allspice, a slightly lower content was found in oregano, while in black pepper the content was rather low. In the case of allspice and oregano, thermal treatment caused significant decrease of the content of oxidative reaction substances. Immediately after heat treatment the changes achieved 24% at allspice and 8% at oregano. The decrease of thiobarbituric number probably relates to the formation of some early Maillard reaction products, arisen during the short heat treatment. Most major Maillard reactions are represented by sugar-amino acid groups and during heat treatment or storage of food the reaction

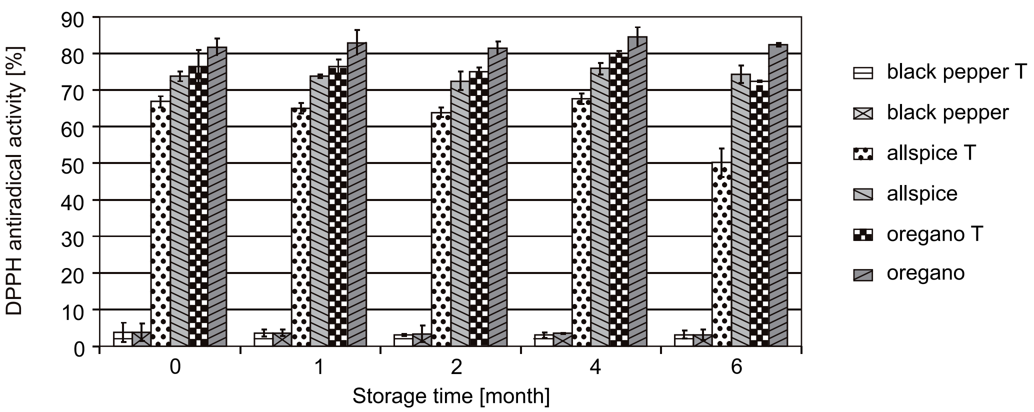

Fig. 1. Effect of thermal treatment and storage on DPPH antiradical activity of black pepper, allspice and oregano.
T – thermally treated spices.

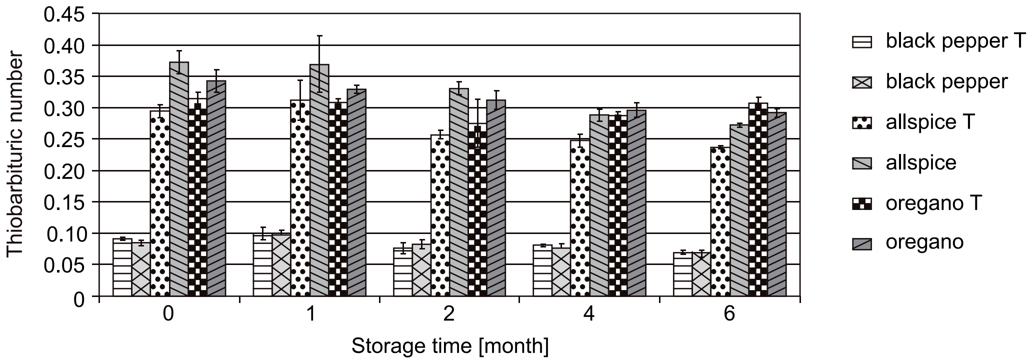

Fig. 2. Effect of thermal treatment and storage on thiobarbituric number of black pepper, allspice and oregano.
T – thermally treated spices.

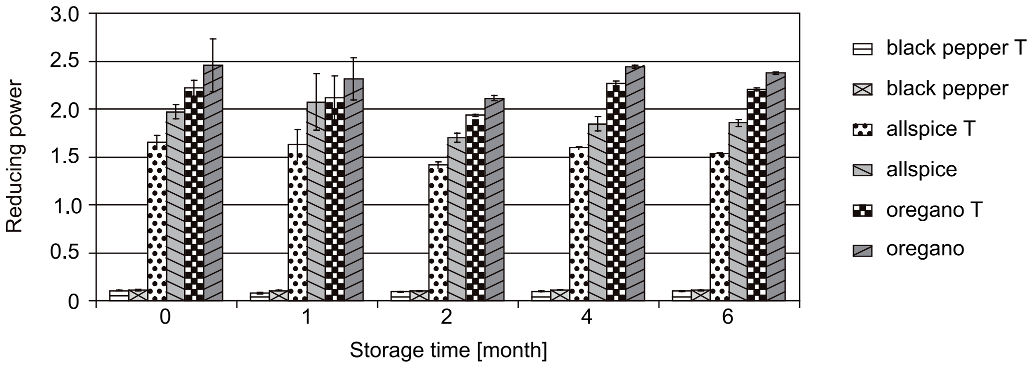

Fig. 3. Effect of thermal treatment and storage on reducing power of black pepper, allspice, and oregano.
T – thermally treated spices.

between reducing saccharides and amino acids or proteins produced strong reducing materials such as amino reductants [28-32]. The found differences in thiobarbituric number of spices moderately decreased during the storage of spices. Effect of storage also significantly changed the content of oxidative products in spices; the effect was more pronounced in thermally untreated allspice and oregano. After six months of storage the thiobarbituric number decreased by about 27% in allspice and by 15% in oregano. In the case of thermally treated allspice the decrease of this value was only 19% after 6 months of storage. After four months of storage the decrease in oregano had reached about 9%, later the thiobarbituric number moderately increased. The effect of temperature on black pepper caused no significant differences between treated and untreated samples. Storage of black pepper effected significant decrease of the thiobarbituric number, 18% in untreated, and 24% in the treated black pepper. In comparison to allspice and oregano, thermally treated spices showed higher decrease in the thiobarbituric number.

Determination of the reducing power (Fig. 3) showed similar differences among spices as it was found in the case of DPPH antiradical activity observation. The black pepper extract showed the lowest and oregano the highest tendency to reduce Fe^{3+} to Fe^{2+}. Reducing power of all spices was significantly affected by thermal treatment. The biggest change of this value was determined in allspice, where immediately after thermal treatment the reducing power decreased by about 16%. Insignificant changes of reducing power were found in the case of black pepper. Differences among thermally treated and untreated spices have been intensified during the storage of spices. During the two months of allspice and oregano storage reducing power moderately decreased and then slowly increased.

Fig. 4 shows the determined content of total phenolic compounds both in thermally treated and untreated spices. The highest content of phenolics expressed as gallic acid was found in oregano, less

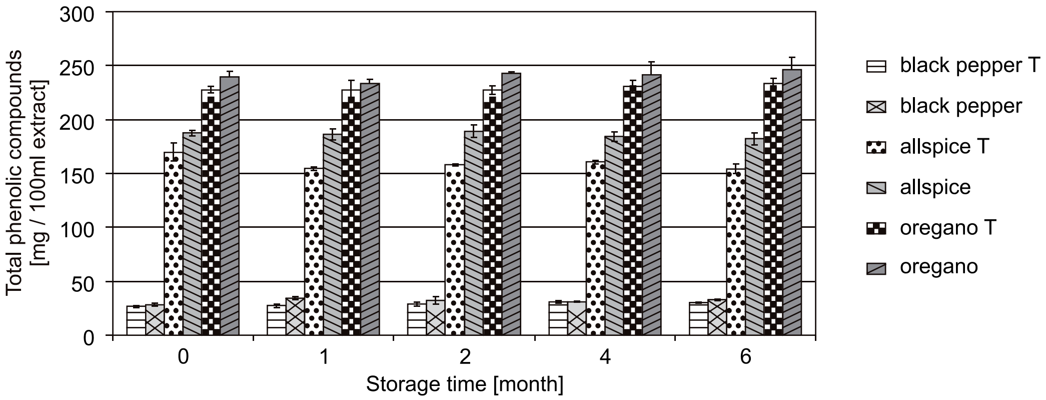

Fig. 4. Effect of thermal treatment and storage on total phenolics content (as gallic acid) of black pepper, allspice and oregano.
T – thermally treated spices.

in allspice, and the lowest content in black pepper. In case of allspice and oregano sterilization temperature caused significant phenolic compounds decrease (9% at allspice, 5% at oregano), and significant increase of these compounds at black pepper after one month storage (26%). These differences did not change much during the storage of spices. Storage similarly affected phenolics content of spices and after 6 months, decreasing their content by about 10% in treated allspice, increasing it by about 13% in untreated and by 17% in thermally treated black pepper, while it was practically unchanged at oregano.

Fig. 5 shows the results of the canonical discriminant analysis of average values obtained from all antioxidant activity determinations of thermally treated, untreated, and spices stored for 6 months. This procedure very well statistically differentiated all the spices according to their species and more, with 94% correctness separated thermally treated from untreated spices on the basis of their classification by case.

CONCLUSIONS

According the results found, we can state that thermal treatment caused significant decrease of antiradical activity, reducing power, content of oxidative and phenolic substances in all spices with the exception of black pepper, where temperature induced an increase in the content of phenolic

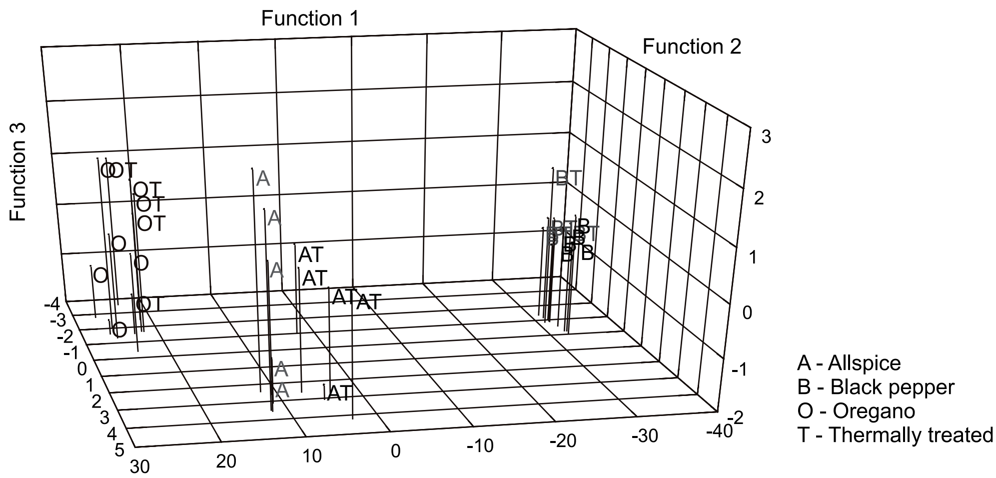

Fig. 5. Canonical discriminant analysis of thermally treated and stored spices.

substances. This finding about phenolics supports the thesis, that an increase in polyphenolics due to thermal treatment may partially enhance antioxidant activity of some food matrix, presented by many authors [2, 3, 10, 13]. Found differences in antioxidant activity between thermally treated and untreated samples of all spices were hardly affected by subsequent storage. With the exception of black pepper as mentioned above, storage itself caused significant decrease of antioxidant activity, which was more pronounced in the case of thermally treated spices. Found relative degradation of the majority of antioxidant attributes of spices are characteristic for systems after short heat treatment with initial reduction in the overall antioxidant activity due to the thermal degradation of naturally occurring antioxidants.

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