

Antioxidant Potential of Spinach, Peas, and Sweetcorn in relation to Freezing Period

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Abstract

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We evaluated changes in the content of total polyphenols and antioxidant activity of frozen spinach, peas, and sweet corn in relation to the storage period. Total polyphenol content and antioxidant activity in analysed samples were determined by colorimetric methods. Both parameters were analysed in frozen samples monthly and the changes were monitored during storage in a freezing box at a temperature of -18°C for 10 months. Freezing had a different influence on the levels of total polyphenol content and antioxidant activity in individual analysed samples. The greatest decrease in antioxidant activity during the entire period of freezing was recorded in spinach (79.4%), while the lowest decrease was observed in peas (26.8%). A relatively significant decrease in antioxidant activity was also found in sweet corn (62.7%). On the contrary, the greatest decrease of total polyphenol content throughout the period of freezing was found in peas (62.0%), and lowest decrease was recorded in sweet corn (only 5.0%). The total polyphenol content in spinach decreased by 43.1% after 10 months of storage.

Keywords: antioxidant activity; frozen; phenolic content; food raw material

The human diet contains a huge variety of non-nutrient components whose implications in metabolism have beneficial health significance. The interest in the investigation of active components, especially phenolic compounds, from natural sources has greatly increased in recent years. Polyphenol compounds are widely diversified, these compounds are classified into different groups as a function of the number of phenol rings and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans (TIMORACKÁ *et al.* 2010a; JURÍKOVÁ *et al.* 2012).

Vegetables, legumes and some cereals are important sources of phenolic compounds. Maize is particularly rich in phenolic acids and anthocyanins are the primary pigments in coloured maize (DEL POZO-INSFRAN *et al.* 2006). TIMORACKÁ *et al.* (2010b) mentioned that phenolic acids, flavones, and flavonols are typical polyphenols in peas. Spinach is a rich source of polyphenols. It contains high levels of flavonols unique to spinach. They are mainly quercetagen derivatives. Other compounds in spinach were scarcely studied, although some like *p*-coumaric acid contribute significantly to its antioxidant activity (EDENHARDER *et al.*

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2001). PATTHAMAKANOKPORN *et al.* (2008) stated that among the factors which can affect the level of phenolic compounds in raw food materials could be the species, size, and texture of raw foods, the prepared form of the samples and mainly the conditions of storage (e.g. time, temperature). Polyphenols are reactive compounds which can be degraded and polymerised through both enzymatic and non-enzymatic reactions during processing. Polyphenols can be oxygenated and degraded by various enzymes that are released when plant cells are broken down. The resulting products are important in determining the organoleptic properties (for example, the appearance and taste) of foods and beverages (SHOJI 2007).

A large part of the population cannot eat fresh food every day, and therefore frozen food is often used, mainly for practical reasons, as its preparation is rapid (NINFALI & BACCHIOCCA 2003). Freezing is considered as one of the best methods of treatment of seasonal raw foods, because it maintains biologically important nutrients and original taste during the storage period (KOPEC 2002). Freezing has only a minor effect on the nutrient content of foods, but only if they are frozen correctly and at the appropriate time (FELLOWS 2000). Several operations, such as selection, washing, peeling, and cutting, blanching and other necessary operations influence the actual process of freezing of raw foods. These operations can greatly affect the quality of frozen raw food materials (BAHÇEÇI *et al.* 2005). Relatively few works investigating the effect of freezing on the polyphenol content and antioxidant activity of raw food materials have been published.

Therefore the aim of this study was to evaluate the content of total polyphenols and antioxidant activity of chosen raw food materials (spinach, peas and sweet corn) in relation to a freezing period.

MATERIAL AND METHODS

Chemicals and instruments. Both studied parameters – total polyphenol content and antioxidant activity were analysed by colorimetric methods (Shimadzu UV/VIS-1240 spectrophotometer; Shimadzu, Kyoto, Japan). The chemicals used for all analyses were as follows: Folin-Ciocalteu reagent, monohydrate of gallic acid, p.a., anhydrous sodium carbonate, p.a., ethanol, p.a., methanol, p.a., 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical, p.a., and Trolox (97%).

Samples. Fresh samples of peas (cv. Torda), spinach (cv. Matador) and sweet corn (cv. Sundance) were obtained from Vinica a.s., Vinica, Slovakia. Productive parts of crops were washed with distilled water several times, gently dried and immediately stored in plastic bags at -18°C . Only spinach was homogenized by mixing before freezing. Ethanol extracts were prepared after sample defrosting and homogenization by addition of 100 ml of ethanol to 50 g of the fresh sample. The mixture was shaken at room temperature for 8 h at 250 rpm. Samples were then filtered through filter paper (130 g/m²; Filtrak Brandt GmbH, Thermalbad Wiesenbad, Germany) and kept at 8°C for further analysis.

Total polyphenol content determination. Total polyphenol content (TPC) was determined by a modified method described by LACHMAN *et al.* (2003). 0.2 ml of the sample extract was pipetted into a 50-ml flask and diluted with 5 ml of distilled water. An amount of 2.5 ml Folin-Ciocalteu reagent was added to the diluted mixture and after 3 min 7.5 ml of 20% aqueous solution of Na₂CO₃ was added. Then the sample was added distilled water to the volume of 50 ml and after mixing left at room temperature for 2 hours. Blank and calibration solutions of gallic acid were prepared using the same procedure. The absorbance of sample solutions was measured against the blank at 765 nm. Total polyphenol (TP) content in samples was calculated as the amount of gallic acid equivalent (GAE) in mg/kg of sample fresh weight.

Antioxidant activity determination. Free radical scavenging antioxidant activity (AA) was assessed by the method of BRAND-WILLIAMS *et al.* (1995) using 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical. For the analysis, 3.9 ml of DPPH solution in methanol (0.025 g/l) was added to a cuvette and the absorbance was measured at 515.6 nm. Subsequently, 0.1 ml of the sample extract was added to the cuvette with DPPH solution and the absorbance was measured after 10 minutes. An increasing amount of antioxidants present in the sample extract reduced DPPH and faded; the colour of the solution was proportionately in correlation with the antioxidant concentration. The calibration curve was constructed with the standard Trolox in the concentration range of 0–800 $\mu\text{mol/l}$, which was used as positive reference. Antioxidant effectiveness was expressed in mmol Trolox/g of sample fresh weight and/or in % inhibition of DPPH (quantitative ability of the tested compound to remove a part of DPPH radical in a certain period).

Table 1. Antioxidant activity (AA) in mmol Trolox/g and total polyphenol content (TPC) in mg GAE/kg in relation to freezing period in months

| Time (h) | Spinach | | Sweetcorn | | Pea | |
|----------|-----------------------------|---------------------------|-----------------------------|---------------------------|-----------------------------|--------------------------|
| | AA | TPC | AA | TPC | AA | TPC |
| 0 | 0.834 ± 0.016 ^a | 285.1 ± 7.2 ^{ab} | 0.970 ± 0.018 ^a | 179.7 ± 2.4 ^a | 0.682 ± 0.015 ^a | 85.9 ± 2.4 ^a |
| 1 | 0.792 ± 0.009 ^b | 293.0 ± 2.4 ^a | 0.942 ± 0.021 ^{ab} | 215.6 ± 7.2 ^{bc} | 0.778 ± 0.014 ^b | 99.2 ± 2.4 ^{bc} |
| 2 | 0.756 ± 0.011 ^c | 293.7 ± 4.8 ^a | 0.908 ± 0.014 ^{bc} | 224.2 ± 4.8 ^c | 0.836 ± 0.029 ^{bc} | 110.5 ± 4.8 ^c |
| 3 | 0.614 ± 0.024 ^d | 282.1 ± 2.4 ^b | 0.886 ± 0.017 ^c | 213.5 ± 2.4 ^{bd} | 0.802 ± 0.005 ^c | 97.2 ± 2.4 ^b |
| 4 | 0.486 ± 0.015 ^e | 271.3 ± 2.4 ^c | 0.728 ± 0.011 ^d | 206.5 ± 4.8 ^{de} | 0.740 ± 0.031 ^{ab} | 82.5 ± 2.4 ^a |
| 5 | 0.434 ± 0.013 ^f | 270.1 ± 4.8 ^c | 0.702 ± 0.010 ^e | 201.3 ± 4.8 ^{ef} | 0.694 ± 0.015 ^a | 74.7 ± 2.4 ^d |
| 6 | 0.362 ± 0.015 ^g | 270.4 ± 2.4 ^c | 0.606 ± 0.013 ^f | 195.9 ± 2.4 ^f | 0.676 ± 0.016 ^a | 59.4 ± 4.8 ^e |
| 7 | 0.274 ± 0.012 ^h | 260.4 ± 4.8 ^d | 0.556 ± 0.020 ^g | 189.6 ± 2.4 ^g | 0.626 ± 0.011 ^d | 51.8 ± 2.4 ^f |
| 8 | 0.238 ± 0.017 ⁱ | 231.3 ± 2.4 ^e | 0.494 ± 0.011 ^h | 182.7 ± 4.8 ^{ag} | 0.594 ± 0.018 ^{de} | 37.5 ± 2.4 ^g |
| 9 | 0.173 ± 0.010 ^{j*} | 199.9 ± 2.4 ^f | 0.406 ± 0.008 ⁱ | 177.5 ± 2.4 ^{ah} | 0.568 ± 0.017 ^e | 35.3 ± 2.4 ^g |
| 10 | 0.159 ± 0.011 ^j | 162.3 ± 2.4 ^g | 0.352 ± 0.008 ^j | 170.7 ± 2.4 ^h | 0.496 ± 0.009 ^f | 32.6 ± 2.4 ^g |

^{a-j} values with different letters denote significant differences ($P < 0.05$) among times of storage; values of AA and TPS are expressed as arithmetic mean ± standard deviation; *different storage freezing period in month of DPPH and total polyphenol content (TPC) in mg GAE/kg

Statistical analysis. Statistical analyses were performed using the Statistica 6.0 software (StatSoft, Boston, USA) and the results were evaluated by analysis of variance (ANOVA). Differences in data were evaluated by the post hoc *LSD* test at a 5% significance level.

RESULTS AND DISCUSSION

Both studied parameters – antioxidant activity and the content of total polyphenols of spinach, sweet corn, and peas in relation to the freezing period are described in Table 1.

The highest level of antioxidant activity of fresh samples was observed in sweet corn (0.970 mmol

Trolox/g = 49.3% inhibition of DPPH), while the lowest was found out in peas (0.682 mmol Trolox/g = 34.4% inhibition of DPPH). AA of spinach was 0.834 mmol Trolox/g (42.5% inhibition of DPPH). On the other hand, the highest level of total polyphenol content of fresh samples was found out in spinach (285.1 mg GAE/kg) and the lowest in peas (85.9 mg GAE/kg). TPC in sweet corn was 179.7 mg GAE/kg. ISMAIL *et al.* (2004) found out a significantly higher level of AA in fresh spinach (66.4% inhibition of DPPH). TURKMEN *et al.* (2005) determined TPC in fresh spinach to be 589 mg GAE/kg. LOPEZ-MARTINEZ *et al.* (2009) evaluated the AA and TPC of 18 maize varieties. The levels of AA in maize were in the range of 40–100% inhibition of DPPH. The highest value of AA was recorded

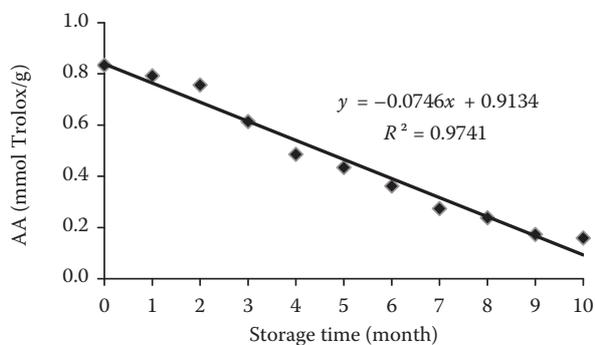


Figure 1. Antioxidant activity (mean) of spinach in relation to the storage period

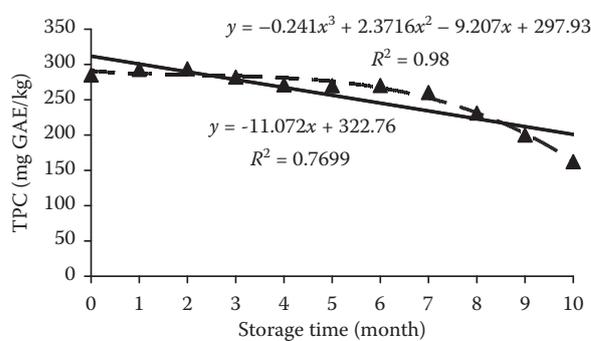


Figure 2. Total polyphenol content (mean) of spinach in relation to the storage period

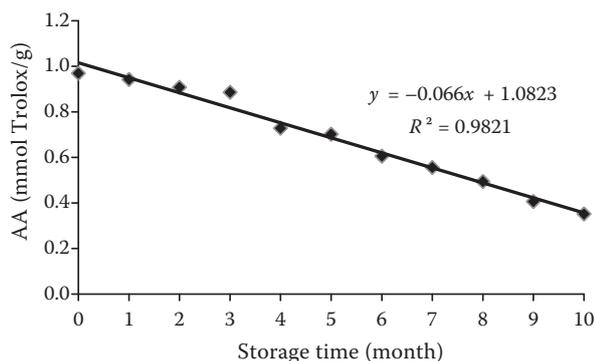


Figure 3. Antioxidant activity (mean) of sweet corn in relation to the storage period

in purple varieties (100% inhibition of DPPH) and relatively high values of AA were also found in black and red varieties (69–92% inhibition of DPPH). Non-pigmented varieties exhibited AA in the range of 50–90% inhibition of DPPH. The same authors found out TPC in maize in the range of 170–3400 mg GAE/kg. The lowest TPC was recorded in colourless varieties, which is in correlation with our results. TURKMEN *et al.* (2005) determined the AA level in peas as 21.3% inhibition of DPPH, which is significantly less than our result. HALVORSEN *et al.* (2002) also observed that AA in peas was relatively low among legumes and vegetables. TIMORACKÁ (2007) determined a much lower average level of TPC (14.5 mg GAE/kg DW) in different varieties of peas.

The AA level in spinach during the freezing period decreased relatively rapidly and linearly (Figure 1). Its value was about 79.4% lower after 10 months of storage (Figure 7). A decrease of the AA value was statistically significant from the beginning of storage until the 9th month.

TPC in spinach increased slightly in the first two months, then a slight decrease was recorded

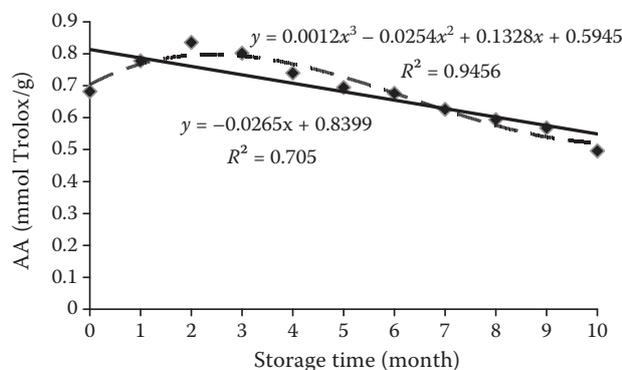


Figure 5. Antioxidant activity (mean) of peas in relation to the storage period

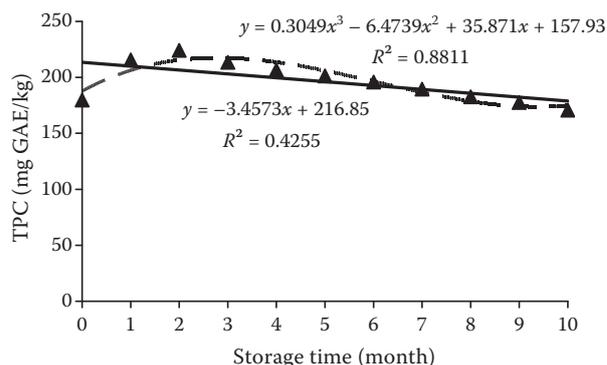


Figure 4. Total polyphenol content (mean) of sweet corn in relation to the storage period

until the 7th month. Subsequently, a relatively large, statistically significant decrease of TPC was recorded until the end of storage (Figure 2). TPC in spinach decreased about 43.1% after 10 months. The time dependence of TPC had rather a polynomial ($R^2 = 0.980$) than linear ($R^2 = 0.770$) character. Analyses of AA and TPC in spinach are the subjects of many research works, but the studies of a long-term effect of freezing on AA and TPC in spinach are missing.

The level of AA in sweet corn during freezing decreased relatively rapidly and linearly, similarly to spinach. A statistically significant decrease of AA was observed from 3rd to 10th month (Figure 3). The recorded decrease of AA in sweet corn (about 62.7%) was less significant compared to spinach.

TPC in sweet corn increased in the first two months, then a slight decrease was recorded until the end of storage (Figure 4). TPC in sweet corn decreased only about 5.0% after 10 months. The time dependence of TPC had rather a polynomial ($R^2 = 0.881$) than linear ($R^2 = 0.426$) character, similarly to spinach. AA and TPC in different varieties of maize are the subjects of many research works,

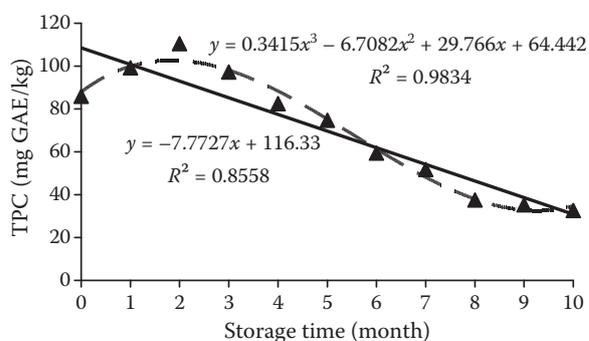


Figure 6. Total polyphenol content (mean) of peas in relation to the storage period

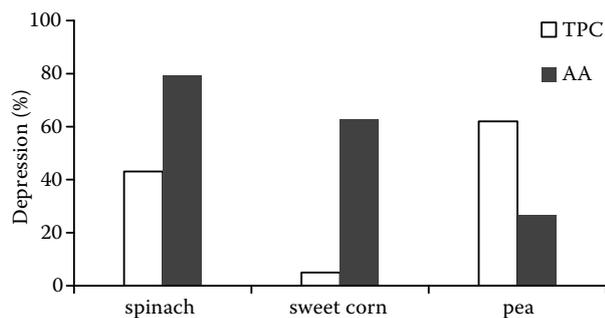


Figure 7. Depression (in %) of antioxidant activity (AA) and the total polyphenol content (TPC) after 10 months of freezing

but the studies of a long-term effect of freezing on AA and TPC in sweet corn are missing.

The time dependence of AA in peas had a different trend compared to spinach and sweet corn. The dependence of AA had a significantly polynomial character ($R^2 = 0.947$). The AA level in peas increased in the first two months, then a slight decrease was recorded until the end of storage (Figure 5). The recorded decrease of AA level in peas after 10 months of storage (about 26.8%) was not very significant and it was the lowest among all analysed crops. PUUPPONEN-PIMIA *et al.* (2003) observed a higher decrease (41.7–55.6%) of AA in peas during 12 months freezing.

TPC in peas increased significantly in the first two months, then a rapid decrease was recorded until the 8th month. Finally a slight decrease of TPC was found out from the 8th month to the end of storage (Figure 6).

The decrease of TPC in peas (about 62.0%) was the highest among all analysed crops. PUUPPONEN-PIMIA *et al.* (2003) observed a much lower decrease (12.5–33%) of TPC in peas during 12 months freezing. The time dependence of TPC had rather a polynomial ($R^2 = 0.983$) than linear ($R^2 = 0.856$) character, similarly to spinach and sweet corn. TIMORACKÁ (2007) recorded an even 50% decrease of selected flavonoids in different varieties of peas during 6 months freezing.

CONCLUSIONS

Freezing had a different influence on the levels of TP content and AA in individual analysed samples. A great decrease in AA levels during the period of freezing (10 months) was recorded in spinach (79.4%) and sweet corn (62.7%), while the lowest decrease

was observed in peas (26.8%). On the contrary, the greatest decrease of TP content throughout the period of freezing was found in peas (62.0%), and the lowest decrease of TP content was recorded in sweet corn (only 5.0%). The TP content in spinach decreased by 43.1% after 10 months of storage. The continuous decrease in AA during the storage period (10 months) was found in spinach and sweet corn. For the peas, AA increased in the first two months, then its level fell steadily after 10 months. For all three studied raw food samples, TP content increased in the first two months, then we recorded a continuous decrease of TP content. While the decrease in sweet corn was very slight, in peas and spinach it was quite significant. Among the factors which can affect the levels of antioxidant activity and total phenolic compounds in food samples could be the species, size and texture of raw foods, the prepared form of the samples and mainly the conditions of storage (e.g. time, temperature).

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