ACTA Acta Sci. Pol., Technol. Aliment. 6(4) 2007, 67-74

# ANTIOXIDANT VITAMIN CONTENTS OF *CAPSICUM ANNUUM* FRUIT EXTRACTS AS AFFECTED BY PROCESSING AND VARIETAL FACTORS

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**Abstract.** The presented work defines the influence of technological process on chemical composition of peppers. The technological treatments made in this work were: lyophilization and samples evaporation. Evaporation process was conducted in two conditions of temperature and pressure: I – 35°C and 70 mbar, II – 50°C and 400 mbar. The working material consisted of four pepper cultivars, two sweet: 'King Artur' and 'Red Knight', and two hot: 'Capel Hot' and 'Robustini'. The changes of vitamins C and E, β-carotene, xanthophylls and phenolic compounds were investigated as processing and cultivar factors. Additionally antioxidant activity of phenolic compounds was evaluated by method with DPPH radical. The extracts prepared in conditions I had a significantly higher concentration of vitamin C, E, β-carotene, total phenol and antioxidant properties than the extracts obtained at the higher temperature and under the higher pressure. It was found that extracts obtained from hot fruits had a higher vitamin E and β-carotene contents than from the sweet ones. However, the extracts from sweet varieties were characterized by higher antioxidant properties and phenolic compounds than the ones obtained from hot peppers.

**Key words:** *Capsicum annuum* L., antioxidants

## INTRODUCTION

Dietary antioxidants are beneficial because of their protective roles against multiple diseases such as cancer, anemia, diabetics and cardiovascular diseases. The compounds perform their function by counteracting the oxidizing effects on lipids by scavenging highly reactive oxygen free radicals, the major oxidizing factors for the oxidative modification of low density lipoprotein and nucleic acids. Among the protective mechanism against radicals, antioxidant vitamins (vitamin E, C and β-carotene) are of special interest [Kaur and Kapoor 2001].

Pepper fruits are a rich source of antioxidants [Palevitch and Craker 1995, Howard et al. 2000, Marin et al. 2004]. They have a high level of vitamins C and E as well as

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carotenoids and xanthophylls. The total of antioxidants is completed by phenolic compounds, which occur in peppers in connection with sugars [Materska et al. 2003 a, b]. The level of antioxidants differs between cultivars, generally hot peppers are a better source of them, than the sweet ones. Additionally, hot peppers contain capsaicinoids – alkaloids specific for *Capsicum* genus, which show many pharmacological properties [Szolcsanyi 2004]. It is obvious, that fresh pepper is the best source of antioxidants and all technological factors, as freezing, lyophilization, boiling, blanching, etc., implicate degradation of those compounds. The rapidity of antioxidant degradation varies depending upon conditions of the technological process. Freeze drying is one of the most sophisticated dehydration methods. Very important advantage is that freeze drying is accomplished at relatively low temperatures and the various heat-sensitive biological compounds are not damaged [Krokida and Philippopoulos 2006].

The aim of this work was to investigate the influence preparation conditions of extracts obtained from lyophilized pepper fruit on the content of the main pepper antioxidants: vitamins C and E, phenolic compounds, β-carotene and xanthophylls.

## MATERIALS AND METHODS

### Plant material

The plant material consisted of four pepper cultivars, two sweet: 'King Arthur' and 'Red Knight', and two hot: 'Capel Hot' and 'Robustini'. The peppers were purchased from a market garden near Lublin in Poland. Fruits in full ripeness were taken for analysis. One part of plat material was analysed immediately after cutting, the other one was lyophilized and stored below –20°C prior to the analysis (2 months).

### Preparation of ethanolic extracts

Lyophilized pepper fruits of *Capsicum annuum* L. were homogenized with 80% ethanol using the Heidolph DIAX 900 homogenizer and the mixture was filtered with a filter paper. The filtrates were concentrated at the temperature of 35°C and pressure 70 mbar (extract I) and at 50°C and 400 mbar (extract II).

### Antioxidants content

**Vitamin C.** The vitamin C as L ascorbic acid content in fresh pepper fruits was determined by Tillmans method [PN-A-04019 1998] and in ethanolic extracts by the same method with modification of Roe [1967]. The pepper extracts (0.2 $cm^3$) were mixed with axolic acid (0.8 $cm^3$) and 2,6-dichlorophenolindophenol. The absorbance of these solutions were measured at $\lambda = 520$ nm. Vitamin C concentration was calculated on standard solutions of L-ascorbic acid.

**Carotenoids and xanthophylls.** Carotenoids and xanthophylls contents in the fresh pepper fruits and prepared extracts were determined according to the method described by Perucka and Materska [2003].

**Phenolic compounds.** The total phenol contents in fresh pepper fruits and prepared extracts were determined according to Swain and Hills method [1959], using Folin-Denis reagent and after measured absorbance at $\lambda = 725$ nm.

**Vitamin E.** Tocopherols were separated from carotenoid exteracts on Silica Gel (Merck) columns with anhydrous $Na_2SO_4$ layer. The column was washed with n-hexane and $\alpha$-tocopherol was elued with n-hexane and acetone (4:1) solution [Horbowicz 1989]. The content of vitamin E was determined by Müller-Mulot method [1968].

### Antioxidant activity determination

Antioxidant activity of pepper extracts was measured as scavenging free radical potential in methanolic solution of DPPH (1,1-diphenyl-2-picrylhydrazyl), as described by Burda and Oleszek [2001]. The analysis was conducted for ethanolic extracts obtained from lyophilized pepper fruits. The antiradical activity was calculated as percentage of DPPH decoloration compared to the control.

## RESULTS AND DISCUSSION

Investigations with sweet and hot pepper cultivars showed differences in dry matter content (Table 1). Hot peppers had higher dry matter level ranging from 8.18 to 16.14% in 'Capel Hot' and 'Robustini', respectively. Sweet peppers contained about 8% of dry matter. Vitamin C content in fresh fruit ranged from 101.19 to 167.54 mg/100 g of fresh weight (Table 1). It was found, that hot pepper cultivars were richer in vitamin C, than the sweet ones. The content of phenolic compounds was about 50 mg/100 g f.w. Differences in phenolic concentration in sweet and hot pepper cultivars were not so clear as in the case of vitamin C. Sweet and hot pepper cultivars differed also in β-carotene and xanthophylls contents. Hot peppers had more of those compounds than the sweet ones (Table 1). The highest level of both β-carotene and xanthophylls was found in cv. Robustini.

Table 1. Antioxidants content in fresh fruit of four pepper cultivars, $mg \cdot 100\ g^{-1}$ of fresh weight
Tabela 1. Zawartość antyoksydantów w świeżych owocach czterech odmian papryki, $mg \cdot 100\ g^{-1}$ świeżej masy

| Cultivar<br>Odmiana | Dry matter<br>Sucha masa<br>% | Vitamin C<br>Witamina C | β-carotene<br>β-karoten | Xanthophylls<br>Ksantofile | Phenolic compounds<br>Związki fenolowe |
|---|---|---|---|---|---|
| King Artur | 8.18 ± 0.049 a | 101.19 ± 3.761 a | 0.058 ± 0.005 a | 0.500 ± 0.075 a | 49.69 ± 14.496 a |
| Red Knight | 8.39 ± 0.085 a | 114.85 ± 4.405 b | 0.085 ± 0.001 a | 0.567 ± 0.061 a | 37.54 ± 19.184 a |
| Capel Hot | 9.61 ± 0.078 b | 126.74 ± 4.426 c | 0.181 ± 0.008 b | 1.582 ± 0.176 b | 45.78 ± 5.218 a |
| Robustini | 16.14 ± 0.148 c | 167.54 ± 2.828 d | 0.460 ± 0.038 c | 4.658 ± 0.257 c | 67.35 ± 10.175 a |

The results are means (n = 2) ± standard deviation; different letters in the same column indicate significant differences between means (P = 0.05).

Uzyskane wyniki są średnią (n = 2) ± odchylenie standardowe; różne litery w tej samej kolumnie oznaczają różnice istotne statystycznie między średnimi (P = 0,05).

The difference in dry matter level and antioxidants content in the investigated pepper cultivars arose from natural varietal factor. Almost always hot peppers are characterized by a higher dry matter and higher phytochemicals concentration. But sweet peppers are more popular in a daily diet, so the contribution of natural antioxidants from sweet and hot peppers to the human diet may be similar.

The goal of the presented work was to check the influence of the conditions of the technological processing on chemical composition of extracts from peppers. The technological treatments made in this work were: lyophilization and samples evaporation. Lyophilization is the kind of conservation of plant material the most neutral for its phytochemicals. Low temperature slows down redox processes and chemical composition of plant samples is almost unchangeable. Unfortunately, all kinds of next processing steps induce partial degradation of antioxidants. The rate of destruction process depends on conditions of samples preparation. In the presented work lyophilized pepper fruits were homogenized with ethanol and in the second step evaporated to solid residue. Evaporation process was conducted in two conditions of temperature and pressure: I – 35°C and 70 mbar, II – 50°C and 400 mbar. Table 2 summarises the obtained results.

Table 2. Antioxidant content of pepper fruit extracts, mg·100 $g^{-1}$ of lyophilized matter
Tabela 2. Zawartość antyoksydantów w ekstraktach z czterech odmian papryki, mg·100 $g^{-1}$ liofilizatu

| Cultivar Odmiana | Vitamin C – Witamina C | | | β-carotene – β-karoten | | | Vitamin E – Witamina E | | |
|---|---|---|---|---|---|---|---|---|---|
| | fresh świeże | extract ekstrakt I | extract ekstrakt II | fresh świeże | extract ekstrakt I | extract ekstrakt II | fresh świeże | extract ekstrakt I | extract ekstrakt II |
| King Artur | 1 237.04 ± 45.99 [b**] | 59.72 ± 10.65 [b] | 27.59 ± 12.85 [ab] | 0.71 ± 0.06 [a] | 0.64 ± 0.01 [a] | – | – | 23.57 ± 0.47 [b] | 20.42 ± 0.59 [b] |
| Red Knight | 1 368.89 ± 52.51 [c] | 138.87 ± 0.0 [c] | 15.36 ± 0.0 [a] | 1.06 ± 0.01 [a] | 0.83 ± 0.03 [c] | – | – | 13.42 ± 2.34 [a] | 11.87 ± 1.98 [a] |
| Capel Hot | 1 318.83 ± 46.58 [bc] | 192.94 ± 22.99 [d] | – | 1.89 ± 0.08 [b] | 1.20 ± 0.06 [d] | 0.76 ± 0.03 [b] | – | 61.23 ± 0.51 [f] | 43.12 ± 2.57 [d] |
| Robustini | 1 038.04 ± 17.53 [a] | 128.06 ± 22.38 [c] | – | 2.82 ± 0.23 [c] | 1.75 ± 0.0 [e] | 1.25 ± 0.0 [d] | – | 56.11 ± 0.0 [e] | 29.14 ± 1.53 [c] |

I – evaporating conditions: 35°C, 70 mbar.
II – evaporating conditions: 50°C, 400 mbar.
The results are means (n = 2) ± standard deviation; different letters in the same column indicate significant differences between means (P = 0.05), columns for extract I and II were analyzed statistically together.
I – warunki zatężania ekstraktów: 35°C, 70 mbar.
II – warunki zatężania ekstraktów: 50°C, 400 mbar.
Uzyskane wyniki są średnią (n = 2) ± odchylenie standardowe; różne litery w tej samej kolumnie oznaczają różnice istotne statystycznie między średnimi (P = 0,05), kolumny dla ekstraktu I i II były analizowane statystycznie wspólnie.

Vitamin C content decreased dramatically during pepper processing. In the mild evaporation condition (I) losses of vitamin C content were above 85% in all analysed cultivars, when compared to fresh fruits (Table 2). The intensification of the samples preparation condition (II), increased the total loss of vitamin C in hot pepper cultivars and above 98% in sweet ones.

β-carotene was the second antioxidant compound under investigation. It was more stable in the first processing conditions, than vitamin C. The evaporation of extracts obtained from lyophilized samples at 30°C and 70 mbar resulted in losing of that compound from 8.6% in cv. King Artur to above 36% in 'Capel Hot' and 'Robustini' (Table 2). The second process system influenced on the high β-carotene degradation. The total loss of this compound was recorded in sweet pepper cultivars, while in the hot cultivars above 55% lower concentration of this compound was noticed, when compared to the fresh fruits. The raising of the temperature about 15°C did not bring to significantly influence on the level of vitamin E in the extracts obtained from sweet pepper fruits. The concentrations of this antioxidant in cultivars Capel Hot and Robustini decreased about 30% and 50%, respectively (Table 2).

The changes in xanthophylls content during pepper extracts preparation were less dramatic, than of vitamin C and β-carotene (Table 3). The evaporation of the samples at condition I caused the loss of xanthophylls from 25% to 47% in cv. King Artur and Capel Hot, respectively. In the second condition (50°C and 400 mbar), the concentration of xanthophylls in the extracts was lower from 35% to 62%, when compared to the fresh fruits (Table 3).

Table 3. Antioxidant content of pepper fruit extracts, mg·100 $g^{-1}$ of lyophilized matter – continous
Tabela 3. Zawartość antyoksydantów w ekstraktach z czterech odmian papryki, mg·100 $g^{-1}$ liofilizatu – ciąg dalszy

| Cultivar<br>Odmiana | Xanthophylls<br>Ksantofile | | | Phenolic compounds<br>Związki fenolowe | | |
|---|---|---|---|---|---|---|
| | fresh<br>świeże | extract<br>ekstrakt | | fresh<br>świeże | extract<br>ekstrakt | |
| | | I | II | | I | II |
| King Artur | 6.11<br>± 0.91 [a] | 4.58<br>± 0.15 [a] | 3.95<br>± 0.45 [a] | 607.46<br>± 177.21 [a] | 221.13<br>± 26.30 [cd] | 140.99<br>± 28.50 [b] |
| Red Knight | 6.76<br>± 0.72 [a] | 4.32<br>± 0.07 [a] | 3.97<br>± 0.22 [a] | 447.44<br>± 228.65 [a] | 259.31<br>± 37.34 [d] | 176.14<br>± 16.33 [bc] |
| Capel Hot | 16.48<br>± 1.83 [b] | 8.78<br>± 0.56 [c] | 6.24<br>± 0.14 [b] | 476.38<br>± 54.29 [a] | 171.35<br>± 20.43 [bc] | 78.1<br>± 25.74 [a] |
| Robustini | 28.86<br>± 1.59 [c] | 18.2<br>± 0.74 [e] | 14.9<br>± 1.62 [d] | 417.29<br>± 63.05 [a] | 177.98<br>± 6.65 [bc] | 130.63<br>± 13.22 [ab] |

I – evaporating conditions: 35°C, 70 mbar.
II – evaporating conditions: 50°C, 400 mbar.
The results are means (n=2) ± standard deviation; different letters in the same column indicate significant differences between means (P=0.05), columns for extract I and II were analyzed statistically together.
I – warunki zatężania ekstraktów: I = 35°C, 70 mbar.
II – warunki zatężania ekstraktów: 50°C, 400 mbar.
Uzyskane wyniki są średnią (n = 2) ± odchylenie standardowe; różne litery w tej samej kolumnie oznaczają różnice istotne statystycznie między średnimi (P = 0,05), kolumny dla ekstraktu I i II były analizowane statystycznie wspólnie.

Phenolic compounds were more stable than vitamin C and β-carotene, but less than xanthophylls in two samples evaporation systems. In the first of evaporating conditions, losses of those compounds from 42% to 64% in cv. Red Knight and Capel Hot respectively, were noticed, while in the second the concentration of those compounds was lower from 60% to 84% in cv. Red Knight and Capel Hot, respectively in comparison to fresh fruits.

The rate of antioxidants degradation during technological process depends on chemical nature of antioxidant. Losses of the most active compound are noticed at first, because they participate in oxide and reduction processes and are targeting compounds for other constituents of pepper extracts.

The results of antioxidant activities of extracts determined by DPPH method are presented in Figure 1. It was found that extracts obtained from sweet pepper fruit characterized the higher antioxidant properties than from hot fruit where the higher concentrations of vitamin C, E and provitamin A were noticed. The results of these studies indicated that antioxidant activities of the extract prepared from pepper fruit depended mainly on the levels of phenolic compounds.

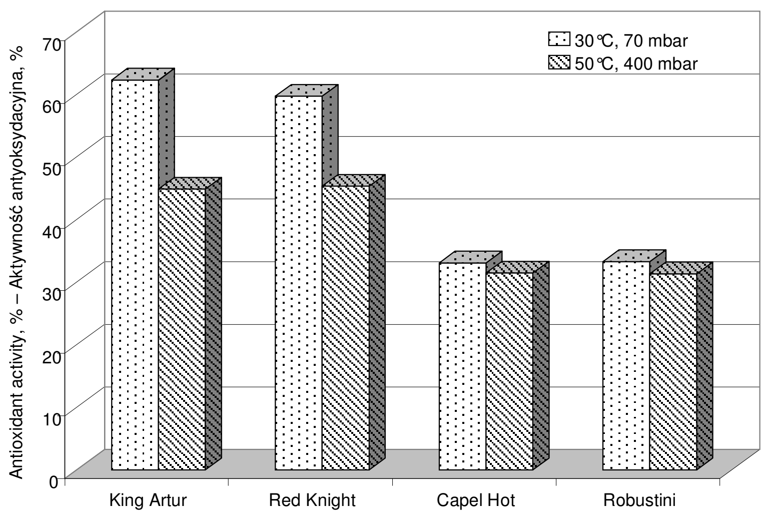


Fig. 1. Antioxidant activity of ethanolic extracts from lyophilized pepper samples, obtained in two different conditions of temperature and pressure
Rys. 1. Aktywność antyoksydacyjna ekstraktów etanolowych z liofilizowanych próbek papryki uzyskanych w dwóch układach temperatury i ciśnienia

## CONCLUSIONS

1. Extracts obtained from hot pepper fruits were characterized by a higher vitamin E and β-carotene content, than from the sweet ones. However, the extracts from sweet varieties had higher antioxidant properties and phenolic compounds concentration, than from hot.

2. Extracts prepared at 35°C and 70 mbar of pressure may function as natural antioxidants in foods systems for example in muscle foods.

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## ZAWARTOŚĆ WITAMIN ANTYOKSYDACYJNYCH W EKSTRAKTACH Z OWOCÓW *CAPSICUM ANNUM* W ZALEŻNOŚCI OD CZYNNIKÓW TECHNOLOGICZNYCH I ODMIANOWYCH

**Streszczenie.** Przedstawiona praca określa wpływ procesu technologicznego na skład chemiczny owoców papryki. Czynnikami prowadzonego doświadczenia były: proces liofilizacji oraz zatężania ekstraktów. Zatężanie próbek prowadzono w dwóch układach ciśnienia i temperatury: I – 35°C i 70 mbar oraz II – 50°C i 400 mbar. Materiałem badań były cztery odmiany papryki: dwie słodkie – 'King Artur' i 'Red Knight' oraz dwie ostre – 'Capel Hot' i 'Robustini'. W pracy określono zmiany zawartości witamin C, E, β-karotenu, ksantofili i związków fenolowych w zależności od odmiany i warunków