**FOLIA**
**HORTICULTURAE**
Ann. 19/1, 2007, 15-23

# Some parameters of antioxidant capacity of red cabbage as related to different forms of nutritive nitrogen

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Key words: red cabbage, nitrogen fertilization, ascorbic acid, antioxidant capacity, peroxidase

ABSTRACT

In the three year experiment some antioxidative parameters of harvested and stored red cabbage were estimated. The effects of applied nitrogen fertilizer form on ascorbic acid (AA), total antioxidant activity (TAA), activity against hydroxyl radical (HRA) and peroxidase (POD) activity were differentiated in each year of the experiment. Long-term storage caused either decrease (2003/04) or slight increase of AA (2004/05, 2005/06) in most treatments.

TAA rose significantly after three month storage, however, a drastic reduction was observed in 2004/05. Activity against hydroxyl radical (HRA) determined in the stored plants varied distinctly in respect to both treatments and individual experimental seasons. Changes of the high POD activity affected by storage were, in most cases, negligible.

## INTRODUCTION

Antioxidative substances of hydrophilic phase, such as ascorbic acid and phenolics, are abundant in *Brassicaceae* vegetables. In our previous investigations concerning white cabbage and broccoli high level of ascorbic acid (Rożek et al. 2000, Wojciechowska et al. 2005) was observed, as well as high content of phenolic constituents (Leja et al. 2000, Leja et al. 2001, Starzyńska et al. 2001). The activity of antioxidative enzymes, peroxidase and superoxide dismutase, determined in broccoli heads (Leja et al. 1997, Starzyńska et al. 2001, Starzyńska et al. 2003) and that of white cabbage peroxidase (Leja and Wojciechowska 1998) were also very high in comparison with other plant species.

Among *Brassicaceae* vegetables, special attention should be paid to red cabbage, rich in anthocyanin rubrobrassicin (Harbourne 1967), an essential pigment in antioxidant system of vegetables (Proteggente et al. 2002). Among the three different forms of cabbage, the red one had highest vitamin C, tocopherol and phenolic content (Singh et al. 2006).

The aim of the present investigations was to evaluate the antioxidant activity of red cabbage tissue expressed both as the inhibition of lipid peroxidation and activity against hydroxyl radical, affected by different forms of nitrogen fertilizer as well as by the long-term cold storage. Additionally, ascorbic acid was estimated as antioxidative agent.

## MATERIAL AND METHODS

The three year experiment was carried out in 2003/04, 2004/05, 2005/06. Red cabbage 'Langendijker' was grown in Kraków environment on heavy soil. The commonly recommended growing conditions for this species were applied. The natural nitrogen content determined in the soil was supplemented to the level of 100 kg N $ha^{-1}$ irrespectively of the form of nitrogen fertilizer. The following forms of nitrogen treatments were used: control (natural soil nitrogen), $Ca(NO_3)_2$, $(NH_4)_2SO_4$, $NH_4NO_3$ and $(NH_2)_2CO$.

Cabbage leaves were analyzed either immediately after harvesting (the end of October) or after 4 month storage at 1-5$^oC$ in 85% of RH (the beginning of March). The mean sample consisted of 6 heads.

For the estimation of ascorbic acid, the iodate titration method was used, described by Samotus et al. (1982). Antioxidant activity expressed as the inhibition of linoleic acid peroxidation was detected according to the method given by Toivonen and Sweeney (1998). Activity against hydroxyl radical was measured as the inhibition of the deoxiribose degradation caused by the attack of hydroxyl radical (Racchi et al. 2002). The activity of non-specific peroxidase was

determined using the method given by Luck (1962) with *p*-phenyldiamine as the enzyme substrate.

All analyses were made in four replications and the results were statistically evaluated using Duncan's test for the significance p = 0.05.

## RESULTS AND DISCUSSION

### *Ascorbic acid*

The effect of nutritive nitrogen form on ascorbic acid level varied in each year of the experiment. In the freshly harvested cabbage in 2003/04 the highest and the lowest contents were observed in the control and in the plants fed with urea, respectively. In the next two years of the study, the differentiation between the treatments was less distinct and the highest level of AA was noticed just in the urea treatment (Table 1).

Table 1. Ascorbid acid level [mg 100 $g^{-1}$]

| Treatment | Ascorbid acid content | | | |
|---|---|---|---|---|
| | Year of experiment | | | |
| | Term | 2003/2004 | 2004/2005 | 2005/2006 |
| Control | Harvest | 73.5 j* | 53.2 e | 38.4 b |
| | Storage | 31.2 e | 61.2 g | 40.5 d |
| $Ca(NO_3)_2$ | Harvest | 59.4 g | 44.0 a | 33.0 a |
| | Storage | 23.3 c | 49.7 d | 39.0 bc |
| $(NH_4)_2SO_4$ | Harvest | 69.1 i | 49.7 d | 41.8 e |
| | Storage | 18.9 a | 47.1 c | 47.2 g |
| $NH_4NO_3$ | Harvest | 62.0 h | 49.7 d | 40.0 cd |
| | Storage | 21.6 b | 47.1 c | 40.8 de |
| $(NH_2)_2CO$ | Harvest | 47.5 f | 55.0 f | 45.8 f |
| | Storage | 25.1 d | 45.3 b | 38.4 b |

*numbers in columns marked with the same letters do not differ significantly

Long-term cold storage caused a drastic decrease in vitamin C level, irrespectively of the applied nitrogen form, however, only in 2003/04. In 2004/05 either an increase ($Ca(NO_3)_2$ and control) or slight decrease in this compound ($NH_4NO_3$ and $(NH_4)_2SO_4$) were found. The significant reduction of AA in the stored cabbage was observed in the case of urea only, while in the plants fed with $Ca(NO_3)_2$ and with $NH_4NO_3$ the slight decrease was noticed (2005/06).

In the previous two year investigations, concerning the effect of different nitrogen fertilizer applied either by the broadcast or by the placement technique on

ascorbic acid content in white cabbage of 'Lennox $F_1$', both the form and the application method affected its level (Rożek et al. 2000).

In white cabbage heads, stored at 5°C for four months a significant increase in ascorbic acid was observed, particularly in the case of ammonium nitrate and urea treatment, applied by both ways of fertilization (Rożek et al. 2000). The dynamics of post-harvest metabolism of vitamin C in cabbage seems to be controversial: some authors (Tadocoro et al. 1993, Sady et al. 1999) reported distinct reduction of vitamin C in the stored white cabbage, however, a significant increase of ascorbic acid in the cabbage of selected five lines stored for two months was described (Leja et al. 2000). Accumulation of ascorbic acid, followed by its rapid reduction due to the stress of harvesting and post-harvest treatment was observed in our previous study with lettuce (Rożek et al. 1994). A similar effect could be possible in the case of red cabbage, hence, the frequent estimation of ascorbic acid content during the storage period might have given a full explanation of post-harvest changes in vitamin C level.

*Total antioxidant activity (TAA) expressed as the inhibition of lipid peroxidation*

The results of antioxidant activity, according to Toivonen and Sweeney (1998) called "total antioxidant activity" (TAA), and measured as inhibition of lipid peroxidation, presented in Table 2 are differentiated, regarding both the applied form of nitrogen fertilization and the year of the experiment.

Table 2. Total antioxidant activity (TAA) expressed as the inhibition of lipid peroxidation [%]

| Treatment | Total antioxidant activity | | | |
|---|---|---|---|---|
| | Term | Year of experiment | | |
| | | 2003/2004 | 2004/2005 | 2005/2006 |
| Control | Harvest | 17.4 b | 34.8 c | 9.2 a |
| | Storage | 16.1 b | 0.0 a | 21.0 bc |
| $Ca(NO_3)_2$ | Harvest | 16.3 b | 35.6 c | 20.3 bc |
| | Storage | 34.4 c | 0.2 a | 22.6 bc |
| $(NH_4)_2SO_4$ | Harvest | 16.6 b | 36.9 c | 13.0 ab |
| | Storage | 24.4 bc | 2.7 a | 23.9 c |
| $NH_4NO_3$ | Harvest | 17.9 b | 39.2 c | 7.1 a |
| | Storage | 32.8 c | 6.8 a | 29.7 cd |
| $(NH_2)_2CO$ | Harvest | 5.2 a | 20.1 b | 35.5 d |
| | Storage | 3.6 a | 8.9 a | 25.2 c |

In 2003/04 the antioxidant activity determined after harvesting was similar in most treatments, with the exception of the urea samples where it was significantly lower. In 2004/05 the initial TAA was higher than in the previous year and did not

vary between the treatments, excepting the urea, where the lowest value was observed. In the last year of the experiment the great differentiation in respect to the nitrogen form was found: the lowest (7.1%) and the highest (35.5%) TAA were determined in $NH_4NO_3$ and in urea treatment, respectively.

Long-term cold storage of cabbage in 2004/05 caused a drastic decrease of TAA, while in 2003/04 and 2005/06 a considerable increase was noticed in most treatments (Table 2). The latter phenomen seems to be consistent with some reports concerning inhibition of lipid peroxidation by plants of *Brassicaceae*. TAA measured in five selected lines of white cabbage increased considerably after storage both at high (20°C, 10 days) and at low (5°C, 2 months) temperature (Leja et al. 2000). A similar effect was observed by Leja et al. (2001) and Toivonen and Sweeney (1998) in the stored broccoli heads.

*Activity against hydroxyl radical (HRA)*

Activity against hydroxyl radical, determined just after harvesting, was distinctly differentiated regarding particular treatments as well as the individual years of the experiment (Table 3). Similar HRA in plants fertilized with urea was observed in the three years of the study.

Table 3. Activity against hydroxyl radical (HRA) [%]

| Treatment | Activity against hydroxyl radical | | | |
|---|---|---|---|---|
| | Year of experiment | | | |
| | Term | 2003/2004 | 2004/2005 | 2005/2006 |
| Control | Harvest | 68.9 cd | 10.1 a | 22.3 b |
| | Storage | 79.2 d | 21.0 b | 62.9 g |
| $Ca(NO_3)_2$ | Harvest | 53.0 ab | 30.9 c | 13.8 a |
| | Storage | 57.5 bc | 10.9 a | 50.6 f |
| $(NH_4)_2SO_4$ | Harvest | 50.2 ab | 32.7 c | 51.9 f |
| | Storage | 43.8 a | 32.0 c | 49.9 f |
| $NH_4NO_3$ | Harvest | 40.8 a | 7.2 a | 42.0 d |
| | Storage | 44.8 ab | 29.1 c | 40.1 d |
| $(NH_2)_2CO$ | Harvest | 42.2 a | 45.3 d | 46.2 e |
| | Storage | 45.4 ab | 28.6 c | 33.7 c |

The control cabbage reacted to storage with significant increase of activity against hydroxyl radical (2004/05 and 2005/06). A similar effect was noticed in $Ca(NO_3)_2$ treatment in 2005/06 as well as in the plants fertilized with $NH_4NO_3$ (2004/05). In the case of other treatments either no significant changes or distinct decrease of HRA (urea samples in 2004/05 and 2005/06, $Ca(NO_3)_2$ application in 2004/05) were observed.

Peroxidation of PUFA (poly unsaturated fatty acids) is due to the action of hydroxyl radical (Bartosz 2003), hence, the obtained results should correspond to the activity against hydroxyl radical detected as the inhibition of deoxiribose decomposition. A great variability of HRA in the freshly harvested and stored plants of red cabbage, regarding both the applied nitrogen form and the year of experiment was not accompanied by a similar dynamics of TAA. Storage caused either an increase (control and N-$NO_3$ treatment) or, in most cases, non-significant changes of activity against hydroxyl radical. Antioxidants, engaged in neutralization of hydroxyl radical, may be different in respect to the method of measurements (inhibition of linoleic acid peroxidation or degradation of deoxiribose molecule).

*Activity of peroxidase*

High activity of peroxidase of harvested cabbage heads did not depend on the applied nitrogen form and was significantly higher (2003/04 and 2004/05) or lower (2005/06) only in the control in comparison with the other treatments. The activity of the enzyme, determined after long storage in 2003/04 did not change significantly, except for the control and $NH_4NO_3$ treatment, where decrease was observed. In the following year of the experiment either a slight increase ($NH_4NO_3$ and urea treatments) or decrease ($Ca(NO_3)_2$ treatment) were found, while in 2005/06 a significant increase of enzyme activity in the stored plants was observed (Table 4), except the reduction of POD activity in those fertilized with $NH_4NO_3$ and urea.

Table 4. Activity of peroxidase [units 100 $mg^{-1}$]

| Treatment | Term | Activity of peroxidase | | |
|---|---|---|---|---|
| | | Year of experiment | | |
| | | 2003/2004 | 2004/2005 | 2005/2006 |
| Control | Harvest | 50560 e | 53440 c | 38133 a |
| | Storage | 45706 bcd | 55760 c | 57920 e |
| $Ca(NO_3)_2$ | Harvest | 45448 bcd | 47440 b | 47413 b |
| | Storage | 48266 de | 38960 a | 52373 cd |
| $(NH_4)_2SO_4$ | Harvest | 45448 bcd | 46640 b | 54666 cde |
| | Storage | 47600 cd | 46080 b | 56373 de |
| $NH_4NO_3$ | Harvest | 47479 cd | 47600 b | 58720 e |
| | Storage | 41600 a | 53840 c | 52373 cd |
| $(NH_2)_2CO$ | Harvest | 45028 bc | 41200 a | 51840 c |
| | Storage | 43440 ab | 48960 b | 47840 b |

Stimulation of POD activity in plants fed with reduced nitrogen was observed in the previous investigations on lettuce (Leja et al. 1994, 1995) and broccoli (Leja et al. 1997). The effect of nitrogen nutritive on some antioxidative enzymes in tomato plants was reported by Shan et al. (2000). Storage of *Brassicaceae* species, such as spring cabbage (Leja and Wojciechowska 1998) and broccoli also induced the activity of these enzymes (Starzyńska et al. 2003).

In general, the effect of the applied forms of nitrogen fertilizers on some antioxidative parameters of red cabbage is difficult to interpret. The effect of the field conditions seems to be stronger than that of the used nitrogen nutritive and can determine the antioxidant capacity of the harvested and stored plants.

## CONCLUSIONS

1. The effect of nitrogen fertilizer form on some antioxidant parameters varied in each year of experiment.
2. Long-term storage affected antioxidants of red cabbage, however, the plant response differed in the individual experimental years.
3. Antioxidant capacity expressed as the inhibition of lipid peroxidation did not correspond to activity against hydroxyl radical.

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## WYBRANE PARAMETRY AKTYWNOŚCI ANTYOKSYDACYJNEJ CZERWONEJ KAPUSTY W ODNIESIENIU DO RÓŻNYCH FORM ZASTOSOWANEGO AZOTU NAWOZOWEGO

Streszczenie: Podczas trzyletniego doświadczenia oznaczono wybrane wskaźniki aktywności antyoksydacyjnej w zebranej i przechowywanej kapuście czerwonej. Stwierdzono zróżnicowany wpływ formy zastosowanego nawozu azotowego w poszczególnych latach na kwas askorbinowy (AA), całkowitą aktywność antyoksydacyjną (TAA), aktywność wobec rodnika hydroksylowego (HRA) i aktywność peroksydazy (POD). Długotrwałe przechowywanie spowodowało spadek (2003/04) lub nieznaczny wzrost (2004/05, 2005/06) zawartości AA w większości obiektów. Całkowita aktywność antyoksydacyjna (TAA) wzrastała istotnie po trzech miesiącach przechowywania, jakkolwiek w roku 2004/05 obserwowano znaczny spadek tej wartości. Aktywność wobec rodnika hydroksylowego oznaczona w przechowywanych roślinach wykazywała zróżnicowanie pomiędzy poszczególnymi obiektami oraz sezonami doświadczalnymi. Zmiany aktywności peroksydazy spowodowane przechowywaniem były w większości przypadków nieznaczne.

Received March 27, 2007; accepted June 26, 2007