

## Some antioxidative properties of selected white cabbage DH lines

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### ABSTRACT

Twenty five double haploid (DH) lines of white cabbage were examined and compared with their parental lines as well as with two cabbage cultivars ('Lennox F<sub>1</sub>' and 'Attraction F<sub>1</sub>') regarding their antioxidative capacity. Ascorbic acid, total phenols and radical scavenging activity (RSA) were determined both in freshly harvested cabbage heads and after long-term (4 months) storage.

The great variability of ascorbic acid and phenol contents was observed in plants of DH lines. The RSA was relatively low, however, differed among the investigated lines and cultivars. Cold storage caused the significant increase of all detected parameters. High content of soluble phenolics and ascorbic acid was accompanied by high antiradical activity only in some of the investigated DH lines and in the 'Lennox F<sub>1</sub>' cultivar.

## INTRODUCTION

*Brassicaceae* species are important vegetable crops that contain high levels of health promoting substances such as anti-carcinogenic glucosinolates (Verhoeven et al. 1997) and antioxidant constituents. Vitamin C, phenolic compounds, tocopherols and carotenoids are present in the *Brassica* vegetables, being beneficial in human diet (Larson 1988). In the breeding of white cabbage to receive material of good biological quality, estimation of the antioxidant capacity seems to be particularly important.

In modern plant breeding, a highly homozygous and genetically stable material such as doubled haploids (DH lines) has been commonly used. To receive doubled haploids, the chromosome number in haploid plants is doubled, hence the offspring of the individual DH plant obtained by its self-pollination is genetically identical and homozygous.

The aim of the present study was to determine the antioxidant properties of 25 DH lines of white cabbage in comparison with two parental lines and two cultivars ('Lennox F<sub>1</sub>' and 'Attraction F<sub>1</sub>') considered high quality standards. Contents of ascorbic acid and soluble phenolics as well as the antiradical activity were determined both in freshly harvested and stored cabbage heads.

## MATERIAL AND METHODS

The field experiment was carried out in 2004 in the Kraków area. Twenty five DH lines of white cabbage were obtained by pollination at the bud stage of DH received previously by the androgenesis of F<sub>1</sub> generation originated from two inbreeding lines crossing. The initial inbreeding lines differed in respect to their chemical composition and the length of the vegetation period. Two white cabbage cultivars ('Lennox F<sub>1</sub>' and 'Attraction F<sub>1</sub>') were included into the experiment as the standards. The plants were cultivated in randomized blocks in three replications. Cabbage was harvested at full maturity.

The freshly harvested cabbage heads were either analysed immediately or stored for four months in commercial cold chamber, at 1-2°C and at 80-85% of relative humidity. The mean sample of each replication consisted of 9 heads. All analyses were made in 4 replications.

In order to determine total phenols (soluble phenolics), extracts in 80% methanol were prepared. Total phenols were measured by the photometric method with Folin's reagent, described by Swain and Hillis (1959). Ascorbic acid content was determined by iodate-titration method of Samotus et al. (1982). Radical scavenging activity (RSA) was estimated by the method given by Pekkarinen et al. (1999) and expressed as the percent of DPPH free radical neutralized for 30 minutes.

The obtained results were statistically evaluated, using Duncan's test, at the significance level 0.05.

## RESULTS

Ascorbic acid

Great discrepancies in ascorbic acid content (Table 1) were found in the freshly harvested cabbage. The lowest levels of this compound were observed in 4019 and 4027 (18.75 and 19.65 mg 100 g<sup>-1</sup>) DH lines, the highest content was found in 4011 (51.48 mg 100 g<sup>-1</sup>) DH line. The low and medium level of vitamin C was found in the parental lines, the medium content of this compound was noted in both examined cultivars.

Table 1. The antioxidant properties of white cabbage – ascorbic acid content (mg 100 g<sup>-1</sup> f.w.)

Pedigree	Ascorbic acid content (mg 100 g <sup>-1</sup> f.w.)	
	Freshly harvested cabbage	Long-term stored cabbage
'Lennox F <sub>1</sub> '	35.61 lm *	55.88 y
'Attraction F <sub>1</sub> '	38.72 op	45.32 st
Parent 1	33.44 ij	40.96 u
Parent 2	21.12 c	30.80 h
DH lines		
4005	29.48 gh	46.20 t
4006	35.21 klm	36.32 mn
4007	27.28 f	38.28 op
4008	30.36 h	33.00 i
4009	25.60 e	43.56 r
4011	51.48 w	45.32 st
4012	20.82 bc	35.64 lm
4013	29.94 h	48.88 s
4014	26.61 ef	43.57 r
4015	38.30 op	39.17 p
4016	34.82 kl	33.44 ij
4017	49.44 v	55.00 xy
4018	35.19 klm	41.80 q
4019	18.75 a	35.64 lm
4020	34.52 jkl	43.56 r
4021	20.68 bc	38.72 op
4022	28.60 g	36.32 mn
4023	37.55 no	41.80 q
4024	40.88 s	46.64 t
4025	39.14 p	44.88 s
4026	27.28 f	46.20 t
4027	19.65 ab	35.20 klm
4028	23.32 d	33.88 ijk
4029	35.64 lm	41.80 q
4031	34.76 jkl	54.12 x

\* Data followed by the same letter do not differ significantly

Long-term storage caused considerable increase of ascorbic acid content, excepting the only two cases (4011 and 4016 DH lines) in which the decrease was noted. The lowest and the highest level of ascorbic acid in the stored cabbage heads was observed in one of the parental line – ‘Parent 2’ (30.80 mg 100 g<sup>-1</sup>) and in ‘Lennox F<sub>1</sub>’ cultivar (55.88 mg 100 g<sup>-1</sup>) samples, respectively.

### Total phenols

Total phenol content (Table 2), determined in cabbage heads after harvesting was ranged between 43.15 (4019) and 66.03 mg 100 g<sup>-1</sup> (4027). In the leaves of parental lines and both cultivars a similar, relatively low level of soluble phenolics was noted.

Table 2. The antioxidant properties of white cabbage – total phenols (mg 100 g<sup>-1</sup> f.w.)

Pedigree	Total phenols (mg 100 g <sup>-1</sup> f.w.)	
	Freshly harvested cabbage	Long-term stored cabbage
‘Lennox F <sub>1</sub> ’	48.87 cd *	71.99 y
‘Attraction F <sub>1</sub> ’	48.69 c	66.62 uvw
Parent 1	48.65 c	65.62 tuv
Parent 2	49.50 cde	62.56 opqrs
DH lines		
4005	62.43 nopqr	61.12 mnopq
4006	53.55 ghij	64.01 qrstu
4007	59.13 lm	54.95 ijk
4008	55.98 jk	45.35 ab
4009	55.04 ijk	65.42 stuv
4011	62.09 mnopqr	53.15 ghij
4012	49.27 cd	63.37 pqrst
4013	52.65 fgghi	76.08 z
4014	45.94 b	70.49 xy
4015	59.68 lmno	87.66 B
4016	52.79 fgghi	59.44 lmn
4017	60.64 mnop	79.82 A
4018	55.31 ijk	75.90 z
4019	43.15 a	57.60 kl
4020	54.23 hij	64.68 rstu
4021	54.68 hijk	60.81 mnop
4022	53.33 ghij	61.39 mnopq
4023	52.29 efghi	60.40 lmnop
4024	52.65 fgghi	72.61 y
4025	49.59 cde	51.80 defgh
4026	54.36 hij	80.63 A
4027	66.03 tuvw	68.74 wx
4028	63.09 pqrst	60.41 lmnop
4029	50.74 cdefg	67.74 vw
4031	49.91 cdef	65.90 tuvw

\* Data followed by the same letter do not differ significantly

In the stored plants a significant accumulation of phenolic compounds was found, except of three DH lines (4007, 4008, 4011) where total phenol content decreased slightly. The variability of phenol level (45.35-87.66 mg 100 g<sup>-1</sup>) was more distinct after storage than after harvesting. The highest level was observed in 4013, 4015, 4017, 4018, 4026 DH lines. Cabbage heads of parental lines and of 'Attraction F<sub>1</sub>' cultivar accumulated the medium content of total phenols, in the case of the standard 'Lennox F<sub>1</sub>' cultivar a high level of these compounds was observed.

#### Radical scavenging activity

RSA, determined in cabbage leaves after harvesting, was relatively low, however differed in investigated lines and cultivars (Table 3). The lowest values were noted in one of the parental lines 'Parent 2' and in 4005, 4012, 4019, 4021, 4022 DH lines (2.76-4.30% of free radical neutralization), the highest exceeded 10% of antiradical activity (4017 and 4031 DH lines). Slightly lower but not significantly different was RSA observed in DH lines 4018, 4024, 4029. Cold storage of cabbage caused, in most cases, a significant increase of RSA, except DH lines 4005 and 4008 where radical scavenging activity was reduced in comparison with its initial value. The lowest and the highest antiradical activities were determined in 4005 and in 4013 DH lines – 2.49 and 17.40% respectively. A considerable discrepancy was also observed in parental lines ('Parent 2' – 5.23% and 'Parent 1' – 13.79%), as well as in cabbage of examined cultivars (6.72 and 15.14% for 'Attraction F<sub>1</sub>' and 'Lennox F<sub>1</sub>', respectively).

The purpose of the present investigations was to characterize the DH cabbage lines as the source of health promoting substances, participating in the neutralization of active oxygen species. According to the obtained results, the most promising lines seem to be 4017 (high level of ascorbic acid and phenolics correlated with high antiradical activity, determined both after harvesting and after storage), as well as lines 4018 and 4026 of high RSA and high phenol level in the freshly harvested as well as the stored cabbage. Among the lines of good antioxidative properties, particularly distinct after long term storage, special attention should be paid to DH lines 4013, 4014, 4024 of high RSA accompanied with high content of soluble phenolics. The above DH lines should be selected and recommended for the further breeding investigations.

Table 3. The antioxidant properties of white cabbage – radical scavenging activity (RSA), (%)

Pedigree	Radical scavenging activity (RSA) (%)	
	Freshly harvested cabbage	Long-term stored cabbage
'Lennox F <sub>1</sub> '	6.41 jkl *	15.14 C
'Attraction F <sub>1</sub> '	5.73 hijk	6.72 klm
Parent 1	5.19 fghi	13.79 AB
Parent 2	3.20 abc	5.23 fghi
DH lines		
4005	4.26 cdef	2.49 a
4006	5.74 hijk	6.75 klm
4007	4.87 fgh	5.82 hijk
4008	6.72 klm	3.57 abcd
4009	4.56 defg	13.89 B
4011	6.17 ijkl	9.78 rstu
4012	3.35 abc	7.28 lmn
4013	6.41 jkl	17.40 D
4014	5.83 hijk	10.85 uvwx
4015	7.79 mno	9.15 pqr
4016	7.12 lmn	6.76 klm
4017	10.33 stuv	13.53 AB
4018	9.71 rst	12.75 zA
4019	4.30 cdef	9.88 rstu
4020	8.15 nop	11.19 vwx
4021	2.76 ab	9.05 pqr
4022	4.26 cdef	11.89 xyz
4023	5.47 ghij	8.46 opq
4024	9.46 qrs	12.70 yzA
4025	7.77 mno	13.49 AB
4026	4.72 efgh	13.09 AB
4027	4.56 defg	9.78 rstu
4028	4.82 efgh	3.71 bcde
4029	9.66 rst	11.73 wxyz
4031	10.75 tuvw	11.64 wxy

\* Data followed by the same letter do not differ significantly

## DISCUSSION

As described previously, *Brassicaceae* plants are abundant in health promoting components of human diet. Among them, ascorbic acid and phenolic substances are considered effective antioxidants of the hydrophilic phase. Radical scavenging activity concerning the ability of antioxidative substances to neutralize free radicals, can be treated as an efficient characteristics of the antioxidant capacity of plant tissue. It is the reason why just these parameters have been measured in the present study.

According to the obtained results, ascorbic acid content determined in the freshly harvested cabbage of parental lines differed significantly, while a similar level of this compound was observed in both examined cultivars. The variability of vitamin C content was more distinct in the DH lines than in the parental lines. Differentiation of the ascorbic acid level in cabbage of parental and DH lines as well as in both cultivars was noted after long term storage.

Ascorbic acid content increased significantly during long-term storage of cabbage heads. A similar phenomenon was observed by Rožek et al. (2000) in white cabbage of 'Lennox F<sub>1</sub>' cultivar stored for 4 months at low temperature (1-2°C), particularly in treatments with ammonium nitrate and urea. The slight increase of ascorbic acid in cabbage of five selected lines stored for 2 months at 5°C was reported by Leja et al. (2000). However, in investigations of Tadokoro et al. (1993) the decrease in vitamin C level in the stored winter cabbage was found. Moreover, the distinct reduction of this compound was noted in leaves of the stored spring cabbage (Sady et al. 1999).

The initial level of phenolics was similar in plants of parental lines and in those of both cultivars, however was significantly differentiated in DH lines. This variability was maintained during the storage period.

In most cases, long-term cold storage caused accumulation of soluble phenols. The effect of storage, both at low and high temperature on accumulation of phenolics was observed in our previous studies, concerning *Brassicaceae* species, such as broccoli (Leja et al. 2001, Starzyńska et al. 2001) and white cabbage (Leja et al. 2000). The post-harvest increase of phenolics in broccoli flower buds was caused by increase of phenylpropanoids and flavonoids (Starzyńska et al. 2003). On the other hand, four month storage at low temperature produced a significant decrease in phenol compounds (total, phenylpropanoids, flavonols and anthocyanins) of red cabbage (Leja et al. 2005).

Antiradical activity of the freshly harvested cabbage was relatively low, however, varied slightly in plants of parental lines. As in the case of vitamin C and phenolics, RSA was more differentiated in plants of DH lines in comparison with parental lines and the investigated cultivars. In the stored cabbage heads great differentiation of antiradical activity was found in parental lines, both cultivars, and DH lines.

In cabbage leaves of investigated lines and cultivars the poor antiradical activity (RSA), determined after harvesting, increased during storage. Post-harvest storage of plants of *Brassicaceae* species usually induced their antioxidative ability. A considerable increase in the activity of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase in stored broccoli flower buds was observed by Toivonen and Sweeney (1998). The increase of the activity of superoxide dismutase and peroxidase in broccoli stored at room temperature and at 5°C was reported by Starzyńska et al. (2003), peroxidase activity rose also

distinctly in broccoli stored for 2 weeks at low temperature (Leja et al. 1997), as well as in the stored spring cabbage (Mareczek et al. 2000).

Antioxidant activity, expressed as the inhibition of lipid peroxidation increased during a short-term storage of broccoli (Toivonen and Sweeney 1998, Leja et al. 2001) and as the result of two month storage at 5°C of five white cabbage lines (Leja et al. 2000). Radical scavenging activity (RSA) determined in broccoli flower buds stored at room temperature rose distinctly, however low temperature treatment did not lead to any significant changes of this parameter (Starzyńska et al. 2003). A similar effect concerned the antiradical activity of red cabbage stored for 4 months at low temperature (Leja et al. 2005).

According to the present results, antioxidative properties of studied cabbage lines expressed as ascorbic acid and soluble phenol contents, as well as their antiradical activity were more distinct in plants treated with low temperature (four month cold storage). To estimate the biological quality of selected cabbage lines regarding their antioxidant capacity, the above parameters should be determined not only in the freshly harvested plants but also in the stored ones.

The antioxidative function of ascorbic acid and phenolic constituents in plant tissue seems to be evident, however not always a direct dependence between the level of antioxidants and antiradical activity is found. In the parental lines examined in the present study, low and high level of vitamin C corresponded to low and high antiradical activity (particularly after storage), however no correlation with soluble phenols was observed. In stored cabbage heads of 'Lennox F<sub>1</sub>', deemed as the standard cultivar, high content of ascorbic acid was accompanied with high RSA and high level of phenol compounds. In the investigated DH lines, the relatively high RSA (e.g. over 10%) was connected with high content of ascorbic acid and high level of phenol compounds in some cases only. Similar findings were reported previously. According to Velioglu et al. (1998) who examined 28 plant products, in many cases the high antioxidative activity was not correlated with the phenol content. In cabbage heads kept at room temperature no effect of ascorbic acid on antioxidant activity was found, however in the case of low temperature treatment a certain antioxidative action of vitamin C was possible (Leja et al. 2000).

The antioxidative capacity of DH cabbage lines examined in the present study, seemed to be differentiated and connected both with ascorbic acid and soluble phenolics. To select the lines of health promoting substances for further investigations, just these parameters should be estimated both in freshly harvested and in stored cabbage heads.

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#### ANTYOKSYDACYJNE WŁAŚCIWOŚCI WYBRANYCH LINII DH KAPUSTY BIAŁEJ GŁOWIASTEJ

Streszczenie: Przebadano 25 linii podwojonych haploidów (DH) kapusty białej i porównano je z liniami rodzicielskimi, jak również z dwoma odmianami ('Lennox F<sub>1</sub>' i 'Attraction F<sub>1</sub>') pod względem ich właściwości antyoksydacyjnych. Kwas askorbinowy, sumę fenoli i zdolność zmiatania wolnego rodnika (RSA) oznaczono zarówno w świeżo zebranych główkach kapusty jak tuż po długotrwałym ich przechowywaniu (4 miesiące). Zaobserwowano znaczne zróżnicowanie zawartości kwasu askorbinowego i związków fenolowych w liniach DH. Wartość RSA była względnie niska, jakkolwiek wykazywała zróżnicowanie w obrębie badanych linii i odmian. Przechowywanie w niskiej temperaturze spowodowało istotny wzrost wszystkich badanych parametrów. Wysoka zawartość rozpuszczalnych związków fenolowych i kwasu askorbinowego korespondowała z wysoką aktywnością antyrodnikową tylko w niektórych badanych liniach DH oraz w przypadku odmiany 'Lennox F<sub>1</sub>'.

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