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Distribution pattern of antioxidants in white cabbage heads (*Brassica oleracea* L. var. *capitata* f. *alba*)

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Abstract The aim of this paper has been to investigate the occurrence of health-promoting antioxidants, such as vitamins C and E as well as antioxidative enzymes, e.g. superoxide dismutase, catalase and peroxidase in different parts of the cabbage head (the outer one green leaves, the central leaf layer and the inner layer young, etiolated leaves). Vitamin C content in both the central (209 mg/dm^3) and inner (202 mg/dm^3) layers of cabbage heads was higher in comparison to the outer one (163 mg/dm^3), while vitamin E content (α-tocopherol) in the outer layer (4 μg/g FW) was higher by about 26 and 34% in comparison to the central and inner layers, respectively. The cooperation between vitamins and enzymatic antioxidants led to the highest H_2O_2 level in the outer layer, while the O_2^- level was the highest in the inner part of the cabbage head. We conclude that leaf layers in cabbage heads reflect the different potential of bioactive compounds accumulation, related to the stage of tissue development, as well as tissue-specific stress response.

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Abbreviations
APX Ascorbate peroxidase
AsA L-Ascorbic acid
CAT Catalase
ROS Reactive oxygen species
SOD Superoxide dismutase

Introduction

The *Brassicaceae* family includes more than 350 genera and 3,500 species and is characterised by a short life cycle and wide adaptability. As such they are suited for cultivation in different seasons as well as in a variety of environments. Many *Brassicaceae* crops (e.g. cabbage, broccoli, Brussels sprouts, cauliflower), commonly known as crucifers, are recognised for their contribution to human nutrition (Salunkhe and Kadam 1998).

The white cabbage (*Brassica oleracea* L. var. *capitata* f. *alba*), originating from the Mediterranean region, was known to the ancient Greeks and Romans. Today, due to consumer preferences and being cheap and available, it is among other crucifers one of the most important dietary vegetables consumed in Europe and in the world (Kusznierewicz et al. 2007). The white cabbage provides significant levels of vitamins (A, C, E, K), and other phytochemicals, such as glucosinolates or sulphur-containing compounds (Kurilich and Juvik 1999; Kopsell et al. 2004). Moreover, many epidemiological studies report a relationship between the consumption of *Brassicaceae*

plants and protection against numerous chronic diseases, including several types of cancer (Byers and Perry 1992), cardio- and cerebrovascular, ocular and many neurological diseases (Block et al. 1992; Podsędek 2005). It is known that vitamins, such as vitamins C and E (Byers and Perry 1992; Cao et al. 1996; Singh et al. 2007) may stimulate the immune system and they can retard several degenerative processes (Hofius and Sonnewald 2003). Therefore, a more accurate characterisation of the content of these antioxidants in white cabbage is needed.

It is well established that antioxidants scavenge and regulate the level of reactive oxygen species (ROS) generated in metabolic processes and produced at a higher level under various stress conditions (Halliwell and Gutterridge 2007). Similarly to animals and humans, plant tissues enclose antioxidant systems including enzymatic (e.g. superoxide dismutase SOD, catalase CAT, ascorbate peroxidase APX) and non-enzymatic antioxidants, such as ascorbic acid (Asc, vitamin C) and tocopherols (vitamin E) (Mittler 2002). Antioxidants, such as vitamins C and E, are essential for human and animal health, but they are synthesised only in photosynthetic organisms (Prior and Cao 2000; DellaPenna 2005). Vitamin C protects the hydrophilic space of the cell (Havaux et al. 2005), and acts as a photoprotective agent during photosynthesis, being involved in plant development and response of plant tissues against stress factors (Mittler 2002; Prior and Cao 2000; De Gara 2003; Smirnoff 2000; Kornas et al. 2010; Latowski et al. 2010). Alpha-tocopherol is the major vitamin E compound found in leaf chloroplasts, where it is located in the chloroplast envelope, thylakoid membranes and plastoglobuli. This antioxidant deactivates photosynthesis-derived ROS (mainly $^1O_2$ and OH^-), and prevents the propagation of lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes (Kamal-Eldin and Appelqvist 1996). Vitamin C also plays an important role in the regeneration of α-tocopheryl radicals produced during the reduction of lipid peroxyl radicals (Surówka et al. 2009; Mène-Saffrané and DellaPenna 2010).

Among enzymatic antioxidants, superoxide dismutase, catalase and peroxidases play a key role in the regulation of O_2^- and H_2O_2 levels. SODs, found in almost all cellular compartments, disproportionate O_2^- to H_2O_2 (Alscher et al. 2002). Hydrogen peroxide may be scavenged by catalases or peroxidases. CAT is mainly associated with peroxisomes where it scavenges H_2O_2 generated during photorespiration (Niewiadomska and Miszalski 2008; Passardi et al. 2005). Hydrogen peroxide may be also scavenged in the ascorbate–glutathione cycle, where in the first step of this pathway H_2O_2 is reduced to water by APX with the ascorbate as the electron donor (Asada 1999; Shigeoka et al. 2002).

The aim of this paper has been to study the distribution of vitamins C and E as well as activities of certain antioxidative enzymes, such as SODs, CATs and peroxidases, located in different white cabbage head layers. To our knowledge, this has been the first investigation of the occurrence of certain enzymatic and non-enzymatic antioxidants in different cabbage leaf layers and we have tried to correlate their levels or activity with some ROS compounds, such as O_2^- or H_2O_2. The comparison of the distribution of primary antioxidants (vitamins) inside the cabbage head is interesting due to their bioactive properties and for nutritional and physiological aspects.

Materials and methods

Plant material

White cabbage seeds of the Counter F_1 cultivar were sown in a greenhouse to multipots filled with peat substrate. About 1 month old seedlings were planted into the field under natural environmental conditions near Kraków (in the southern part of Poland) and harvested in October. After 24-h adaptation to room conditions, leaves from three different layers of cabbage heads: outer (1st–3rd leaves), central (6th–8th leaves) and inner (14th–16th leaves) (Fig. 1) were frozen in liquid nitrogen and kept at −70°C for further analysis. Analyses were performed on pooled material collected from appropriate leaves (outer, central and inner) of six cabbage heads.

Standards and reagents

3,3-diaminobenzidine tetrachloride (DAB) and nitroblue tetrazolium (NBT) staining reagents were prepared as solutions of 2 and 0.5 mg per mL of potassium phosphate buffer, respectively. The titanium reagent was prepared as a 1:5 (v/v) solution of titanium tetrachloride in concentrated HCl. HPLC determination of vitamin E content was performed with use of α-tocopherol standard. All reagents were provided by Sigma–Aldrich Chemie GmbH, Munich, Germany.

Chlorophyll determination

Chlorophyll $a + b$ content was determined using light absorption by aqueous acetone chlorophyll extracts (80%) according to Arnon (1949). The tissue (0.5 g) was homogenised in chilled acetone (1 mL). After centrifugation at $14,000×g$ for 5 min at 4°C supernatant was collected. The concentrations of chlorophylls a and b were determined with a DU Beckman spectrophotometer at 663 and 645 nm using simultaneous equations based on the specific absorption coefficients for chlorophylls a and b.

Fig. 1 Indication of leaf layers: outer (light-exposed), central and inner taken for analysis from the white cabbage head (*Brassica oleracea* L. var. *capitata* f. *alba*)

OUTER layer
(1st - 3rd leaf)

CENTRAL layer
(6th - 8th leaf)

INNER layer
(14th - 16th leaf)

Histochemical detection of H_2O_2 and $O_2^{\cdot-}$

H_2O_2 detection was performed by an endogenous peroxidase-dependent in situ histochemical staining procedure, using DAB according to Thordal-Christensen et al. (1997). Leaf discs ($\varnothing = 12$ mm) collected from leaves of different layers of cabbage heads were infiltrated for 24 h in the dark at room temperature in a solution containing 2 mg/mL DAB (pH 5.5). Control leaves were infiltrated in a solution without DAB. In situ $O_2^{\cdot-}$ presence was estimated using the NBT staining method according to Fryer et al. (2002) with the following modifications: leaf discs ($\varnothing = 12$ mm) collected from leaves of different layers of cabbage heads were infiltrated for 24 h in the dark at room temperature in solution of 0.5 mg/mL NBT prepared in 10 mM potassium phosphate buffer (PBS), pH 7.2. Control leaves were infiltrated in a solution without NBT. After incubation with DAB and NBT the samples were cleared in a solution containing 4:1 ethanol/chloroform (*v/v*) and 0.15% trichloroacetic acid until the chlorophyll was completely removed.

Spectrophotometric analysis of H_2O_2 content

The H_2O_2 level was investigated according to the procedure described by Ślesak et al. (2008). The absorbance of the resulting solution was read at 415 nm against water blank. The concentration of hydrogen peroxide in the extract was determined by comparing the absorbance against a standard curve representing the titanium—H_2O_2 complex, over the range from 0.05 to 0.3 mmol in 1 mL.

Extraction and determination of soluble proteins

The extraction of soluble proteins was completed in 100 mM pH 8.0 Tricine-Tris buffer containing 100 mM $MgSO_4$, 1 mM dithiotreitol (DTT) and 3 mM EDTA. Leaves (1.0 g) were homogenised in 1.5 mL of the extraction buffer and centrifuged for 2 min at $12,000 \times g$. The whole procedure was carried out at 4°C. The level of soluble proteins was determined using the Bradford (1976) method.

The analysis of SOD isoforms and total peroxidase activity

The activity of SOD was estimated by the semi-quantitative polyacrylamide gel electrophoresis (PAGE) method. Gel staining was performed according to the protocol previously described by Miszalski et al. (1998). For each lane, 15 μg of the protein extract was applied. For the identification of SOD isoforms, native gels were stained in buffers containing 5 mM H_2O_2 or 2 mM KCN.

The total activity of peroxidases (POX) was estimated by the semi-quantitative method using PAGE, and the staining of the native gel was performed according to the protocol previously described by Barcelo et al. (1987). For each lane 30 μg of protein was applied.

Gels were scanned and densitometric analyses were performed with Image J (GPL license).

Spectrophotometric measurements of antioxidant enzyme activities

Catalase (CAT) activity was measured according to the method described by Aebi (1984). The disappearance of

hydrogen peroxide [initial concentration: 0.4% H_2O_2 in phosphate buffer (50 mM KH_2PO_4, 50 mM Na_2HPO_4, pH 7.0)] was monitored at 240 nm. Enzyme activity was defined as 1 mmol of H_2O_2 decomposed by 1 mg of total soluble proteins per minute. For the calculation of the absorbance coefficient for H_2O_2 a value of 42.6 $(mmol/L)^{-1}cm^{-1}$ was used.

Ascorbate peroxidase was measured spectrophotometrically according to Nakano and Asada (1981). Ascorbate peroxide activity was determined via the loss of the ascorbate level at a wavelength of 240 nm in a reaction mixture containing pH 7.0 phosphate buffer (KH_2PO_4/K_2HPO_4), 1 mM EDTA, 15 mM L-AsA and 40 mM H_2O_2. Enzyme activity was defined as 1 mmol of AsA decomposed by 1 mg of protein per minute. For the calculation of the absorbance coefficient for AsA a value of 2.8 $(mmol/L)^{-1}$ cm^{-1} was used.

Vitamin C determination

Cell sap from selected leaves was centrifuged for 15 min at $14,000 \times g$ and 4°C. Ascorbate content in the cell sap was measured reflectometrically, using a Merck RQflex 10© device. Ascorbic acid reduces yellow molybdophosphoric acid to phosphormolybdenum blue which is determined reflectometrically. Each experiment was conducted in three replicates.

Extraction of tocopherols and determination of α-tocopherol content using an HPLC method

For the extraction of tocopherols, a modified method described by Heudi et al. (2004) was used. 5 mL of 96% (v/v) ethanol was added to 0.5 g of powdered leaf tissue. Samples were incubated in a water bath at 70°C. After 15 min incubation, 80% (w/v) KOH solution was added and the samples were left in the water bath for the next 30 min. After incubation, probes were centrifuged for 15 min at 4,000g in 4°C and supernatant was mixed with water in 1:1 (v/v) proportion and passed through XTR columns (Chromabond®). The columns were rinsed twice with 25 mL of N-hexane which was then collected. The N-hexane from the samples was evaporated and the precipitate was dissolved in 1.5 mL of 96% methanol.

The separation of hydrophobic compounds was performed by HPLC using an Agilent 1100 apparatus. A 10 μL sample was separated on a Zorbax Eclipse XBD C18 4.6×75 mm 3.5 μm column. As a mobile phase, methanol (A) and acetonitrile/dichloromethane 1/1 v/v (B) was used. Analysis was performed under a gradient of 0–4 min 100% A, 4–13 min 100–30% A with 1 mL/min flow rate. The eluate was analysed with a fluorescence detector with an excitation wavelength of 220 nm and

emission wavelength of 333 nm. Linearity was verified for 0–100 μg/mL of the test substance.

Statistical analysis

Two-way ANOVA followed by Tukey's HSD multiple range test was used to determine the individual treatment effects at $P \leq 0.05$.

Results

Chlorophyll and ROS compounds content

Moving from the outer towards the inner parts of cabbage heads (Fig. 1), their leaves differ in colour, morphology and, as shown on Fig. 2a, the total chlorophyll content decreases. The outer green leaves show the highest H_2O_2 level of about 12 μmol H_2O_2/g FW, approximately twofold higher than in the central and inner parts of cabbage heads (Fig. 2b, c), while the level of superoxide radicals was the highest in the inner layer (Fig. 2c). The veins of the leaf discs showed more intensive staining than interveined parts in both of the test ROS compounds (Fig. 2c).

Activity of certain antioxidative enzymes

In the leaves of white cabbage heads, five SOD isoforms were detected (Fig. 3b) and their activity pattern showed dependence on leaf layers (Fig. 3). In the inner layer, MnSOD activity was about 30% higher in comparison to the outer and central layers (Fig. 3a). An FeSOD isoform was identified only in the outer layer. Three CuZnSOD isoforms (CuZnSODI, CuZnSODII, CuZnSODIII) were found. CuZnSODIII activity gradually decreased from the outer to the inner layers, while those of CuZnSODI and CuZnSODII were identified only in central and inner layers (Fig. 3a). Their activities were higher in the inner than in the outer layer. The highest CuZnSODIII activity coincides with the high level of total chlorophyll in green leaves, as well as with the highest H_2O_2 level (Figs. 2a, b, 3a).

The analysis of total peroxidase activity was similar to APX activity with a gradual loss from the outer to the inner layers (Figs. 4, 5b). In contrast to this, the highest APX activity at a level of 205 μmol AsA reduced/min/mg protein in the outer layer was determined and it was about fivefold higher than in central and in inner layers (Fig. 5b). Both the outer and inner layers of cabbage heads showed similar catalase activity (10.8 and 9.55 μmol H_2O_2 decomposed/min/mg protein, respectively), and higher than that measured in the central layer (4.56 μmol H_2O_2 decomposed/min/mg protein; Fig. 5a).

Fig. 2 Level of chlorophyll (Chl) and ROS compounds in cabbage ▶
leaves (*Brassica oleracea* L. var. *capitata* f. *alba*). Total chlorophyll
(*a* + *b*) (**a**); hydrogen peroxide content (**b**); values represent
means ± SD (*n* = 3). The *same letters above bars* indicate no
differences at the significance level ($P \leq 0.05$), according to the
Tukey HSD (high significant differences) test. Detection of superox-
ide radicals and hydrogen peroxide in leaf discs (Ø = 12 mm) of
cabbage heads using NBT or DAB staining, respectively (**c**). No
specific coloration was observed in parallel controls

Content of low molecular antioxidants

Vitamin C showed a higher content in both central
(209 mg/L) and inner (202 mg/L) layers of the cabbage
head, in comparison to the outer leaf (163 mg/L) (Fig. 5c).
The content of vitamin E (α-tocopherol) measured in the
outer layer was higher by about 26 and 30%, respectively,
in comparison to the central and inner layers (Fig. 5d).

Discussion

The outer leaves of cabbage heads contain chlorophyll
(Chl), which allows them to perform photosynthesis
(Solymosi et al. 2005). The foliar concentration of the
photosynthetic pigments is related to the amount of
absorbed solar radiation in the PhAR range (Curran et al.
1990). Towards the inner part of the cabbage head, leaves
are etiolated due to the limited light access (Kruk 2005). As
shown by Mittler (2002) photosynthesis as well as other
metabolic processes, such as photorespiration or respira-
tion, can lead to the generation of excess ROS and oxi-
dative stress. Our results indicate that in the white cabbage
head the production/removal of superoxide radicals and
hydrogen peroxide is leaf layer dependent, and photosyn-
thetically competent tissues are the most important source
of H_2O_2 (Fig. 2). According to Shao et al. (2008) super-
oxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) act as
secondary messengers in many processes associated with
plant metabolism and development; thus, we suppose that
the high H_2O_2 level in the outer layer and the superoxide
radical level in the inner layer (Fig. 2b, c) may be primarily
related to the stage of tissue development and to the type
and level of ROS molecules needed for ROS-related signal
induction.

The excess of ROS in plant cells is scavenged with
enzymatic (SODs, catalases, APXs) and non-enzymatic
(vitamins E and C) antioxidants (Havaux et al. 2005;
Hofius et al. 2004; Munné-Bosch and Peñuelas 2005). The
first line of cell defence against ROS is SODs classified
into three groups: iron SOD (FeSOD), manganese SOD
(MnSOD) and copper–zinc SOD (CuZnSOD) (Alscher
et al. 2002). In *B. oleracea*, we identified five forms of
SODs (Fig. 3) and the activity of FeSOD was identified
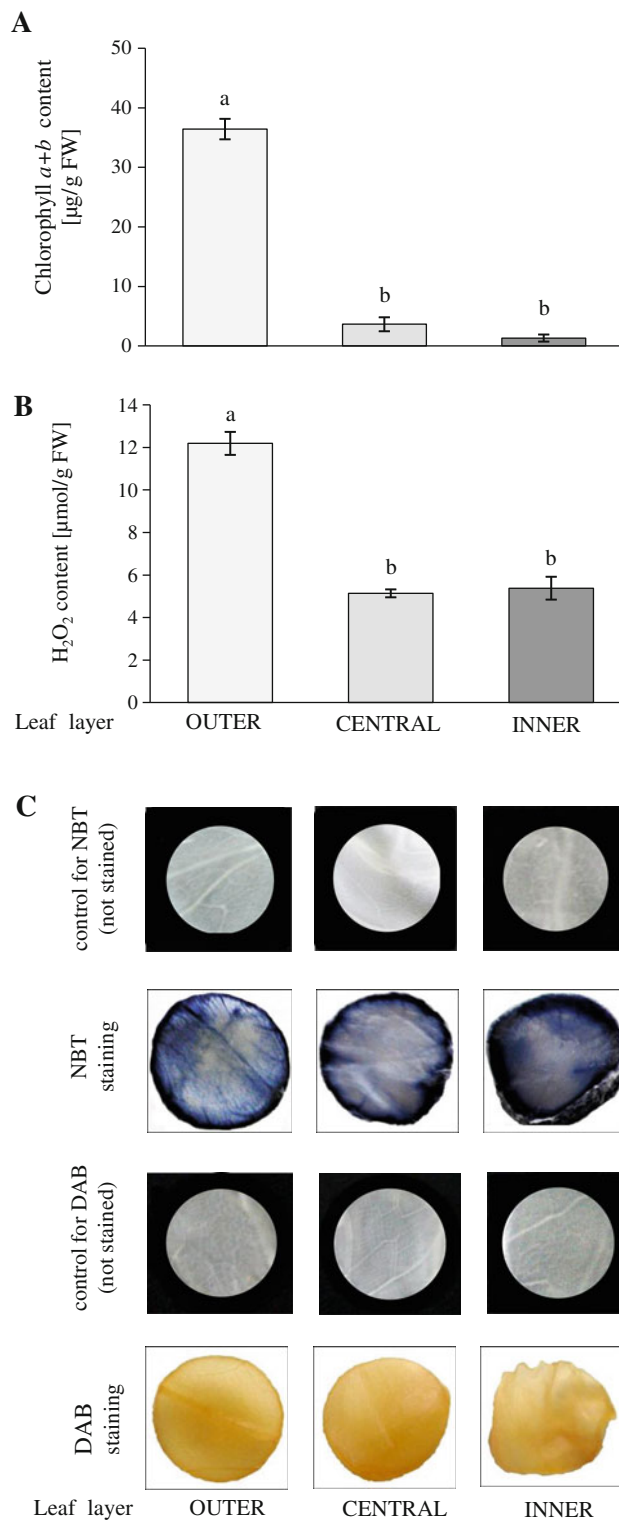
only in the layer of green leaves. These data are in
agreement with Alscher et al. (2002) indicating mainly
chloroplastic localisation of the FeSOD form. Moreover,
our results lead to the suggestion that CuZnSODIII is also
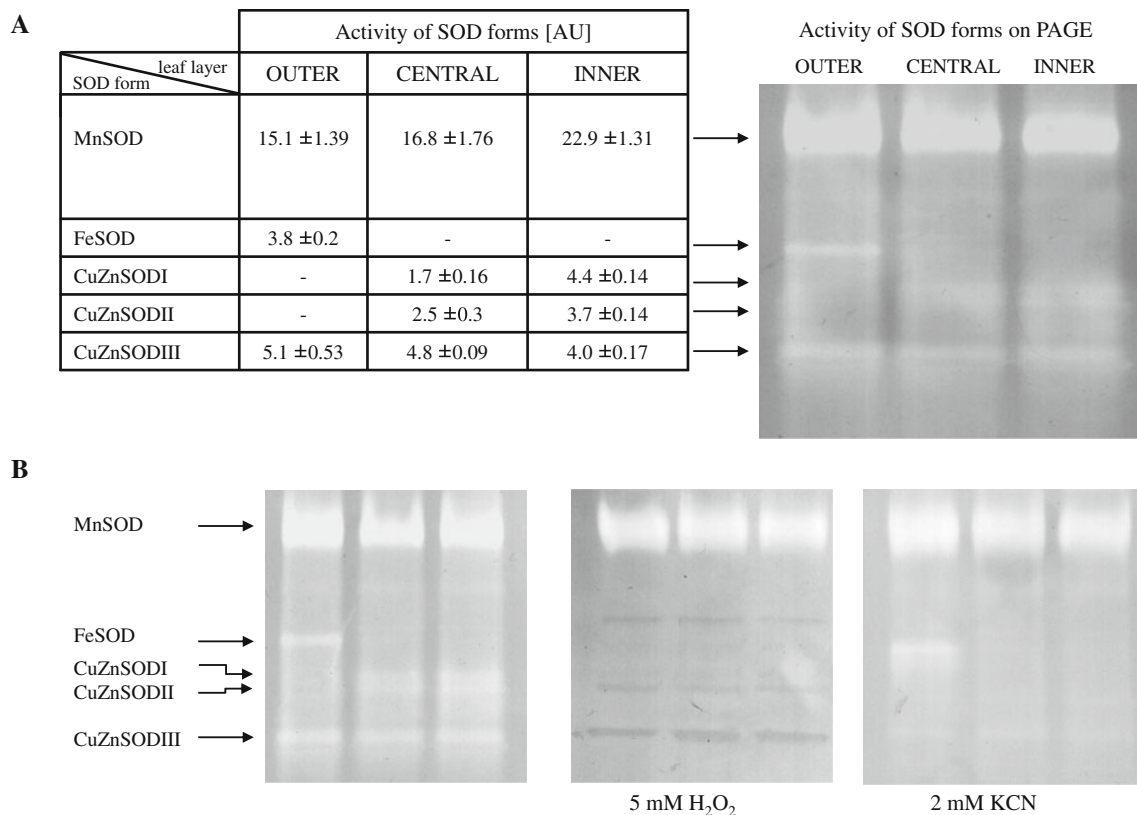associated with chloroplasts (Fig. 3), while the other two

A

SOD form \ leaf layer	Activity of SOD forms [AU]		
	OUTER	CENTRAL	INNER
MnSOD	15.1 ±1.39	16.8 ±1.76	22.9 ±1.31
FeSOD	3.8 ±0.2	-	-
CuZnSODI	-	1.7 ±0.16	4.4 ±0.14
CuZnSODII	-	2.5 ±0.3	3.7 ±0.14
CuZnSODIII	5.1 ±0.53	4.8 ±0.09	4.0 ±0.17

B

Fig. 3 PAGE activity staining of superoxide dismutase (SOD) isoforms in leaf extracts of *Brassica oleracea* cv. *capitata*. Extracts of soluble proteins containing 15.0 μg protein were loaded to each well. The gel shown is a typical example of three replicates. The area corresponding to the sum of activities included inside the defined peak of the SOD isoform is presented as mean ± SD (**a**). Identification of SOD forms stained with nitroblue tetrazolium (NBT) in the presence of 5 mM H_2O_2 or 2 mM KCN (**b**)

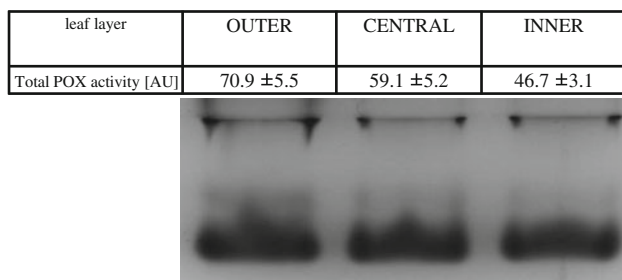| leaf layer	OUTER	CENTRAL	INNER
Total POX activity [AU]	70.9 ±5.5	59.1 ±5.2	46.7 ±3.1

Fig. 4 Peroxidases visualized on native polyacrylamide gels stained with 3,3-diaminobenzidine (DAB). Extracts of soluble proteins containing 30.0 μg protein were loaded to each well. The gel shown is a typical example of three replicates

CuZnSODs isoforms (CuZnSODI and CuZnSODII) with the highest activity in central and inner layers (Fig. 3a) are more related to processes occurring outside the chloroplasts. The highest activity of the CuZnSODI and CuZnSODII isoforms in the inner leaves corresponds to the highest level of superoxide radicals and the highest MnSOD activity (Figs. 2, 3a) localised mainly in mitochondria (Alscher et al. 2002). We associate these alterations in antioxidative enzyme activity and superoxide radical level with vigorous respiratory processes in developing leaves inside the cabbage head. These data are in agreement with Azcón-Bieto (1983), showing that respiratory activity in young leaves is higher than in mature tissues, and with Lennon et al. (1995) who presented a general decline of respiration with age and with the development of photosynthetic activity.

The concentration of hydrogen peroxide may be regulated by catalases and peroxidases. As suggested by Mittler (2002), CAT might be involved in the removal of excess ROSs, while APX is probably responsible for the modulation of the ROS level for signalling purposes, since they have different affinities for H_2O_2 (CAT at mM and APX at μM range). In the experiments we point out that in green leaves of cabbage heads the H_2O_2 content (Fig. 2b) in comparison to interior layers was also the highest, parallel to the high catalase activity (Fig. 5a) as well as the highest total peroxidase (Fig. 4) and APX activities (Fig. 5b). These results indicate that different enzymatic antioxidants are involved in the regulation of H_2O_2 concentration in different leaf layers and the H_2O_2 level is dependent on the part of leaves in the cabbage head. Since the higher H_2O_2 content was measured in chlorophyll-rich leaves, it could
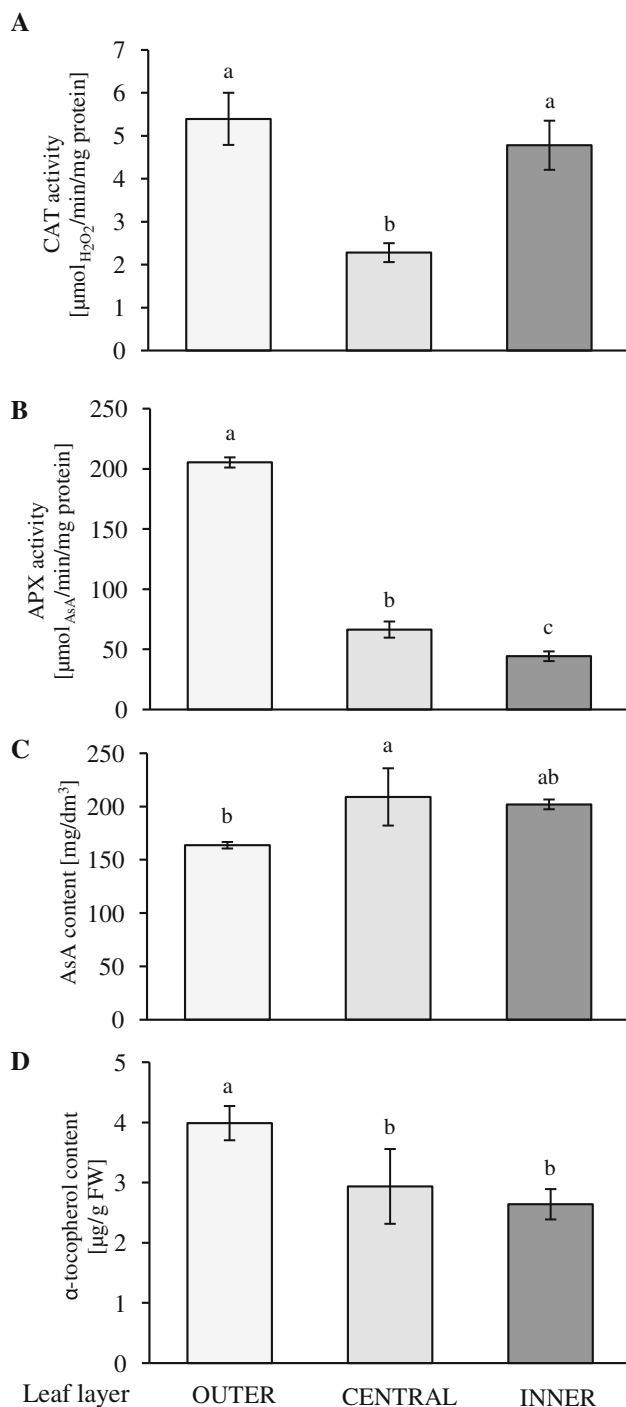
A

B

C

D

Fig. 5 Activity of catalase (**a**), ascorbate peroxidase (**b**) and content of low molecular antioxidants—vitamin C (**c**) and vitamin E (**d**) in *Brassica oleracea* cv. *capitata* leaves. Values represent mean ± SD (n = 3). The *same letters above bars* indicate no differences at the significance level ($P \leq 0.05$), according to the Tukey HSD (high significant differences) test

be speculated that the H_2O_2 generated during photosynthesis acts as signalling molecules and plays a crucial role in the control of metabolic and defence processes in cabbage heads. It is also worthy to note that in the light-exposed layer, APX activity was nearly twice as high as in the etiolated leaf layers (Fig. 5b) and parallel to the lowest vitamin C content (Fig. 5b, c). This indicates that APX is strongly involved in the control of the H_2O_2 level generated in photosynthetic and photorespiratory processes in the chlorophyll rich layer, and the rapid induction of APX in intense light could compensate the slow response of the ascorbate pool itself, as previously shown by Foyer and Noctor (1999).

In the inner part of cabbage heads, the relatively high activity of total peroxidises peroxidases and catalases (Figs. 4, 5a) and a similarly lower H_2O_2 level in comparison to the outer layer (Fig. 2b, c) lead to a conclusion that in etiolated leaves H_2O_2 is removed with higher efficiency and peroxidases may play a regulatory role in the regulation of H_2O_2 content. It seems that peroxidases in the inner part may be involved in the regulation of tissue growth and development of e.g. cell elongation. In the etiolated part of cabbage heads, APX activities are probably potentially inhibited by ascorbate (Figs. 4, 5c), as previously shown by Cordoba-Pedregosa et al. (1996) in experiments made on onions.

The significant alteration of antioxidative enzyme activities (CAT, APX and SOD) measured in different leaf layers is probably related to the changes in non-enzymatic antioxidant content. Our results point out that in the white cabbage head vitamins C and E are located in different proportions in outer, central and inner layers (Fig. 5c, d) and this is probably related to the metabolic state of leaves as well as to the functions of these compounds in metabolic processes as well as in antioxidative defence. Since vitamins C and E are part of information-rich redox buffers and enzymatic cofactors (Foyer 2005), they may play an important role in redox cell signalling, they may regulate gene expression and influence tissue growth. As demonstrated by Smirnoff and Pallanca (1996), vitamin C may accumulate at high concentrations in photosynthetic tissues, in which it is intimately involved in the regulation of photosynthesis and protection of chloroplasts against damage caused by ROS, such as $O_2^{.-}$, H_2O_2, hydroxyl radicals ($OH^{.}$) and singlet oxygen ($^1O_2$). In contrast to this, our results indicate an about 25% decrease in vitamin C content in the green layer in comparison to the interior parts of cabbage heads (Fig. 5c). This may be due the involvement of vitamin C in many protective processes. Vitamin C may be used up in the ascorbate–glutathione cycle and act as a co-factor of violaxanthin de-epoxidase in the xanthophyll cycle; it may also be used up for vitamin E regeneration (Müller-Moulé et al. 2002). However, in inner layers the high content of ascorbate similar to the high activities of MnSOD, CuZnSODI and CuZnSODII as well the high level of superoxide radicals (Figs. 2c, 3a, 5c) seem to be related to the very active metabolism in young tissues

Fig. 6 HPLC chromatograms of tocopherol standards and extracts of *Brassica oleracea* leaves. Other details are given in "Materials and methods"
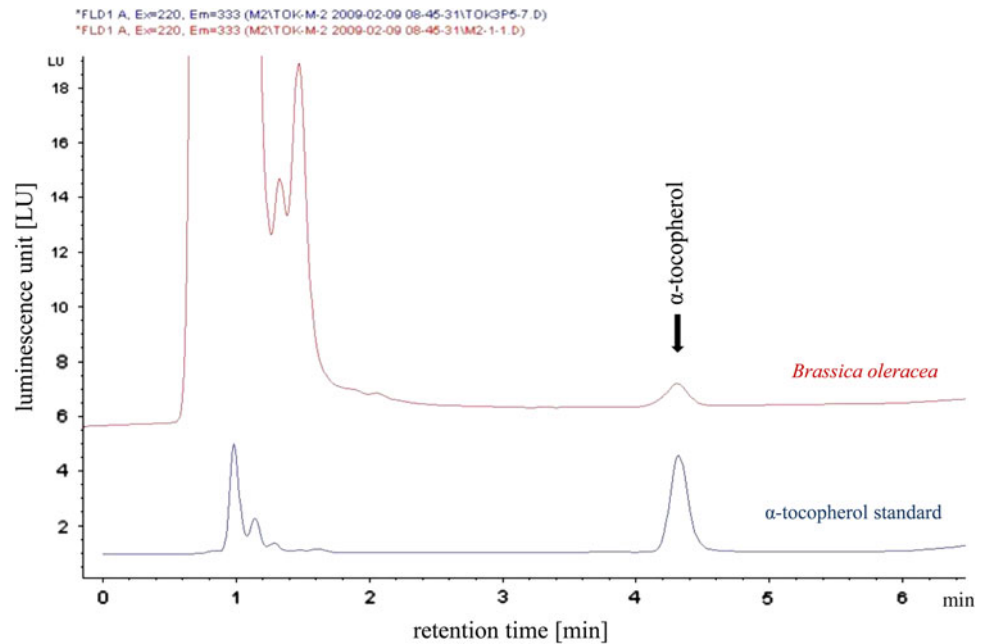
and plant growth processes, such as cell division and cell elongation, as demonstrated earlier on an *Arabidopsis vtc-1* mutant by Veljovic-Jovanovic et al. (2001). Our results are also in agreement with earlier data (De Gara et al. 1991, 1996; Borraccino et al. 1994; Lennon et al. 1995) showing that developing tissues have higher mitochondrial activity/amount as well as a higher vitamin C level than mature tissues.

The most extensively described function of α-tocopherol is its involvement in various mechanisms of the protection of chloroplast membranes and polyunsaturated fatty acids against ROS (especially singlet oxygen) action (Maeda and DellaPenna 2007; Mène-Saffrané and DellaPenna 2010). Our results show that *B. oleracea* leaves have a relatively low content of total α-tocopherol when compared with leaves of other species (Szymańska and Kruk 2008a, b). Moreover, the distribution pattern of α-tocopherol found in white cabbage head leaves is rather different than that in lettuce heads showing the lowest concentration of α-tocopherol in the intermediate part (Szymańska and Kruk 2008a). Our results (Figs. 5d, 6) point out that the occurrence of vitamin E is not restricted only to green leaves. Vitamin E is also localised in etiolated parts of cabbage heads and these results are in agreement with Szymańska and Kruk (2008a) showing localisation of α-tocopherol in different parts of lettuce heads. Moreover, our results indicate the highest α-tocopherol content in the outer leaves (Fig. 5d). Such localisation of vitamin E may be due to the organelles in which it is synthesised, such as chloroplasts and chromoplasts, as shown by Horvath et al. (2006). Moreover, our results point out that the dietary properties of the investigated parts of cabbage heads are different.

Conclusions

The synchronization of antioxidative processes occurring in cabbage leaves (among outer, central and inner layers) lead to different levels and types of ROS in tissues at different stages of their development and physiological functions. Moreover, cabbage head leaves represent heterogeneity of the biological material.

The alteration of vitamins C and E content inside the cabbage head suggests that they play not only an antioxidative role and they may be somehow involved in metabolic regulation and/or plant signalling.

The significant variations in vitamins C and E levels among the different leaf layers are responsible for differences in the health-promoting properties of cabbage head leaf layers. This suggests differentiated utilisation of these parts of cabbage heads by consumers according to their demand.

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