## BIOTIC AND ABIOTIC FACTORS AFFECTING THE CONTENT OF THE CHOSEN ANTIOXIDANT COMPOUNDS IN VEGETABLES

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#### Summary

Vegetables are a rich source of biologically active substances, which support the body's defense mechanisms. A large group of these substances are compounds with antioxidant properties. Apart from vitamins (A, C and E), tocopherols, carotenoids, glutathiones and thiocyanates, polyphenols are also classified as the compounds of antioxidant properties being found in plants. They include: phenolic acids, flavonoids and hydroxycinnamic acid and among them a large group of anthocyanins. These compounds inhibit DNA damage in cancer cells, induce the production of insulin in the pancreas and protect the human brain from aging. They have also high antioxidant activity, which determines the defense mechanisms of plants under stress, such as temperature variations, UV radiation, attacks by pests and mechanical damage. Their content may vary among individual plants of the same species, which is associated with a number of internal and external conditions, such as genetic factors, environmental and agronomic. The contents of anthocyanins determines plant species, botanical variety and breeding and biological processes associated with ontogenesis. Also climatic and soil factors, the factors influencing the content of antioxidants and agronomic factors such as method, place and date of planting, fertilizing, mulching, salinity may contribute to the formation of stress conditions during plant growth and increase the content of antioxidants in plants. This review focused on the content, composition, and antioxidant capacity of vegetables.

key words: polyphenols, anthocyanins, antioxidant activity

#### **INTRODUCTION**

Many chronic and degenerative diseases, such as cancer, Alzheimer, Parkinson and heart disease are partly caused by oxidative stress, caused by free radicals. Free radicals are the reactive forms of oxygen which emerge in living cells as a result of the oxidoreduction process (Bartosz 2003). These compounds react with the components of living cells, modifying them and damaging them on the DNA level, which in consequence leads

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to the emergence of various diseases such as tumours, diabetes, joint inflammation and heart attack (Shahidi 1997, Bartosz 2003). The scientific research indicates that the frequent consumption of plant-derived products decreases the risk of many dangerous civilisation illnesses, in the emergence of which oxidative stress plays a major role (Duthie et al. 2000). Plants, including vegetables, are a rich source of biologically active substances which support the organism's immune mechanisms (Bartosz 2003). The antioxidant compounds constitute a numerous group among these substances (Grajek 2004). Known as antioxidants, these substances protect cell molecules against the changes connected with the activity of oxidative stress (Davies 2000). Apart from vitamins (A, C and E), tocopherols, carotenoids, glutathiones and thiocyanates, polyphenols are also classified as the compounds of antioxidant properties being found in plants (Grajek 2004, Szajdek & Borowska 2004). In comparison to vitamins E, C and carotenoids, they are considered compounds whose antioxidant activity is stronger (Dai & Mumper 2010).

### Polyphenols as an important antioxidants

Polyphenols are the most numerous group of secondary metabolites and are characterised by high antioxidant activity which condition the immune mechanisms of plants in such stress conditions as: temperature fluctuations, UV radiation, pests' attacks and mechanical damages (Dixon & Pavia 1995). Polyphenols commonly occur in plant consumption products, including fruit and vegetables as well as grains, coffee, tea or cocoa (Erdman et al. 2007).

They are a chemically diverse group of compounds, in which there are, among others, phenolic acids, hydroxycinnamic derivatives and flavonoids (Ho 1992). It is estimated that the most numerous group of polyphenols in vegetables is composed of phenolic acids which are in the majorhydroxycinnamic derivatives ity whereas the content of flavonoids fluctuates within a wide range. According to Chun et al. (2005), this is from 4% in white cabbage to 39% in red cabbage while Bahorun et al. (2004), during the analysis of 10 species of vegetables, indicated the scope of flavonoid content in the entire polyphenol content to be from 51% to 79%.

Scalbert & Williamson (2000) state that a daily intake of polyphenols reaches on average 1 g per person whereas according to Ovaskainen et al. (2008), for the citizens of Finland, it is located within the range of 863-415 mg per day, for Spanish people from 2590 mg per day to 3016 mg per day (Saura-Calixto et al. 2007). The contribution of phenolic acids in this value amounts to 75% of the total intake, proanthocyanidins 14% and flavonoids 10%. The most significant sources of polyphenols, on the basis of the inhabitants of Finland, are coffee, tea, cereal products and fruit. It is estimated that vegetables are less significant source of these components (Ovaskainen et al. 2008). Approximately 48% of the polyphenols from the diet are bioabsorbable in the small intestine and 42% in the large intestine. Small quantities (10%) are inaccessible and are moved out from the organism within the process of digestion.

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Flavonoids can be divided into groups, depending on the level of oxygen heterocyclic oxidation as well as the number and location of hydroxyls. There are the following groups: flavones, flavonones, flavanones, isoflavones, chalcones and anthocyanins as well as proanthocyanidins (Małolepsza & Urbanek 2000).

In plant tissues, flavonoids are frequently colored to flower petals and fruit. So far, approximately 6467 flavonoid compounds have been identified (Harborne & Baxter 1999). They protect plants from UV radiation and they have antiviral, antimycotic and antibacterial properties. They are also attractants to symbiotic organinsects deterrents to isms and (Małolepsza & Urbanek 2000). As polyphenol compounds, flavonoids undergo oxidation and therefore they can reduce the reactive forms of oxygen and other free radicals (Cao et al. 1997). From among flavonoids, the dominating components of vegetables are quercetin, myricetin and kaempferol derivatives, most frequently in the form of glycosides. Anthocyanins are present in not many vegetables: red cabbage, eggplant, lettuce, radicchio chicory, red bean, red onion, red species of broccoli, kale, radish, black carrot, potatoes (Piccaglia 2002, Sudha & Ravishankar 2003, Łata & Wińska-Krysiak 2006, Wu et al. 2006, Llorach et al. 2008, Mulabagal et al. 2009) (Table 1).

The content of polyphenols in plants depends on many biotic and abiotic factors. The most important ones are genome and ontogenesis. The polyphenols present in plants can be divided into two groups: the so-called primary ones (which are synthesised during the normal development of plant tissues) and the induced ones, that is, produced mostly as a result of biotic and abiotic stress, for example, the activity of elicitors such as the salts of heavy metals, physical injuries, infections, temperature, UV rays (Lattanzio *et al.* 2006).

The content of polyphenols in plants quoted in various studies also depends on the methods of its determination (the Folin Ciocalteu method and the chromatography method) as well as the manner of extraction from plant material. The majority of polyphenols in the glycosidic form are soluble in water whereas aglycones are less soluble. With rare exceptions, solubility in water increases along with the number of hydroxyl groups in a molecule. Polyphenols with a few hydroxyl groups are soluble in ether, chloroform, methanol, ethanol and ethyl acetate. Hence, next to water, methyl and ethyl alcohol, in various proportions with water, are used for the extraction of these compounds.

Most often used for extraction is 50% methanol (Vinson *et al.* 1998), 70% methanol (Proteggente *et al.* 2002), 80% methanol (Bahorun *et al.* 2004) or 70%, 80% acetone or 80% acetic acid-acidified acetone (Wu *et al.* 2006). The contents given by particular authors are significantly different, which also results from the manner of calculating into a given standard – which most often is gallic acid or (+)-catechin.

### Properties and occurrence in vegetables of anthocyanins

From among flavonoids, an important group with strongly antixodiative properties is compounds of anthocyanins. They are violet, blue, pink dyes dissolved in the sap of cell vacuoles mostly in flowers and fruit as well as in leaves, sprouts and roots. Depending on the pH, they can exist in multi-colour or colourless chemical forms (Goto & Kondo 1991). They are typical dyes of higher plants; however, their presence has also been identified in certain gymnosperm plants, among others, in Picea obovata and Bryum (Muszyński & Guzewski 1976). Anthocyanins play an essential role in plant pollination through attracting insects. Moreover, they also protect plants from the negative consequences of UV radiation as well as from such stressors as drought or low temperatures (Mazza & Minati 1993, Chalker-Scott 1999, Harborne & Williams 2001). So far, about 500 various structures of these compounds have been identified (Mazza & Miniati 1993). Their presence has been noted at least in 27 families and 73 species of plants used in food industry (Bridle & Timberlake 1997). Anthocyanins are the compounds which are very well soluble in water and in alcohol solutions. They are more stable at the low pH of the solution (Brouillard & Dangles 1994). An anthocyanin molecule is a glycoside, which include a sugar molecule and anthocyanidin molecule. Particular anthocyanidins are different from one another by the number of hydroxyl groups, which decides upon their colouring (Muszyński & Guzewski 1976). In the compositional structure of anthocyanins, 17 different anthocyanidins have been distinguished. The six most popular ones are: pelarcyanidin, malvidin, pegonidin, tunidin, delphinidin, peonidin (Kozłowska et al. 2007). In 247 examined plant species, it was found that pelargonidin was responsible for the colour orange, cyanidin was responsible for the colour red and delphinidin for the colours violet and blue (Muszyński & Guzewski 1976). The interest in anthocyanins results primarily from the positive influence they have on the functioning of the human organism (Pascual-Teresa & Sanchez-Ballesta 2008). Anthocyanins, among others, hinder DNA damage in cancerous cells, induce the production of insulin in the pancreas, protect the human brain from the ageing processes (Hou 2003, Jayaprakasam et al. 2005, Lau et al. 2006). Their function is to eliminate the reactive forms of oxygen and to "sweep away" free radicals, thanks to which they protect the human organism from heart diseases, atherosclerosis and hypertension. Another important role of these substances is connected with their use in the technological industry, they are used as natural food dyes (Bridle & Timberlake 1997, Pascual-Teresa & Sanchez-Ballest 2008). A daily intake of anthocyanins depends on the type and quantity of products consumed. In the United States, the biggest quantities of these antioxidant compounds are provided in fruit and vegetables whereas in the Netherlands their main source is tea and chocolate (Chun et al. 2005). The consumption of anthocyanins in the USA was estimated to be 215 mg daily in summer months and 180 mg in winter months. In Italy, however, it fluctuated from 25 mg to 215 mg daily (Kuhnau 1976, Alberti-Fidanza et al. 1996).

Vegetable species	Anthocyanisn content $(mg \cdot 100 g^{-1} \text{ fresh weight})$	References		
Black bean	24.1 - 44.5	Macz-Pop et al. 2006		
Red onion	23.3 - 48.5	Ferreres et al. 1996,		
Red offion	23.3 - 48.3	Wu et al. 2006		
Red chicory	130 - 280	Mulabagal et al. 2009		
		Biesiada et al. 2010,		
Red cabbage	10 - 322	Wu et al. 2006,		
		Mazza & Miniati 1993		
Eggplant	8 - 85	Wu et al. 2006,		
Eggplant	8 - 85	Koponen et al. 2007		
Rhubarb	4 - 200	Timberlake 1988,		
Kilubalb	4 - 200	Koponen et al. 2007		
Radish	11 - 60	Giusti & Wrolstad 1996		
Rauisii	11-00	Piccaglia et al. 2002		
Lettuce 'Lollo rossa'	45.6	Llorach et al. 2007		
Kale	12.1 - 86.4	Łata & Wińska-Krysiak 2006		

Table 1. The anthocyanins content in selected species of vegetables

The content of anthocyanins can be different in particular plants of the same species, which is connected with a number of internal and external conditions such as genetic, environmental and agronomic factors (e.g. light intensity and type, temperature, humidity, soil type, fertilisation as well as plant processing and storage). The influence of environmental factors, in particular light and temperature is one of the best known factors conditioning the accumulation of anthocyanins. However, the agronomic factors, the manipulation of which may contribute to the emergence of stress conditions in the period of plant growth and, at the same time, to the increase of the content of antioxidants in plants – are also responsible for this phenomenon (Pascual-Teresa & Sanchez-Ballesta 2008).

# Biotic factors – species, cultivars and othogenesis

The species of the plant as well as its botanic and agronomic variety are

conditioned by the content of phenol compounds, including flavonoids (Table 2) (Ehlenfeldt & Prior 2001). The total content of flavonoids in the twelve varieties of the onions with white, yellow and violet scale under analysis fluctuated from 1.2 to 980  $mg \cdot kg^{-1}$  and depended on the colour. Its highest concentration was in the yellow-scale varieties and the lowest one in white-scale varieties (Marrotti & Piccaglia 2002). The number of antioxidant compounds also depends on the part of the plant. Nicoletto & Pimpini (2010) noted the highest number of polyphenols in chicory roots, as compared to the stems. These compounds were also present in leaves, in which their level depended on the place of growth. Slightly higher values of these compounds were identified in the middle leaves, as compared with the external and internal leaves (Nicoletto & Pimpini 2010).

Among the vegetables which contain the highest quantities of anthocyanins, there are radicchio chic-

ory, in which, depending on the cultivar, the content of these compounds can fluctuate from 130 to 280 mg·100 g<sup>-1</sup> of fresh matter, red cabbage 25-495 mg·100 g<sup>-1</sup> of fresh matter (Mazza & Miniati 1993, Mulabagal et al. 2006, Wu et al. 2006). The highest content of anthocyanins is to be found in the 'Indigo' cultivar of radicchio chicory (280 mg $\cdot$ 100 g<sup>-1</sup> of fresh matter) and red cabbage (322 mg·100 g<sup>-1</sup> of fresh matter) (Mulabagal et al. 2006, Wu et al. 2006) (Table 1). In basil leaves, there might be approximately 18 mg·100 g<sup>-1</sup> of fresh matter of those compounds, in red radish from 12 to 185 mg·100 g<sup>-1</sup> whereas in grapevine skins from 25 to 102 mg·100 g<sup>-1</sup> (Piccaglia et al. 2002). From among the cultivars, the smallest numbers of dyes are to be found in the greenleaved varieties, as compared with those red-leaved ones. This is confirmed by the research of Łata & Wińska-Krysiak (2006) who, in redleaved kale, found 725  $\mu g \cdot g^{-1}$  anthocyanins more as compared with the green-leaved variety. Llorach et al. (2008) showed that the green cultivars of lettuce did not contain anthocyanins whatsoever whereas in the red cultivars, the quantity of anthocyanins fluctuated from 25.9 to 45.6 mg $\cdot$ 100 g<sup>-1</sup> of fresh matter. The differences in the level of anthocyanins for the specified plant can be significant, which is confirmed by the research into red cabbage, onion, basil and chicory (Mazza & Miniati 1993, Phippen & Simon 1998, Piccaglia et al. 2002, Hallmann & Rembiałkowska 2006, Mulabagal et al. 2009). As far as red cabbage is concerned, within one variety, the quantities, expressed in dry matter, can fluctuate from 10.0 to 15.4 g·kg<sup>-1</sup>

of fresh matter, depending on the year of cultivation, and from 12.4 to 19.1, depending on the location of the plantation (Piccaglia *et al.* 2002). An interesting potential source can also be a corn – anthocyanins extracted from its red seed coat colour at high pH.

The content of polyphenols altogether in dill leaves was increasing along with the growth of the plants while in sprouts, it fluctuated to a small degree (Lisiewska et al. 2006). The level of polyphenols showed an increasing tendency related with plant maturation in broccoli and Agastacha rugosa (Vallejo et al. 2003). According to Navarro et al. (2006), antioxidant activity increased along with the ripening of pepper fruit. Similar results were also obtained by Anttonen et al. (2006) in strawberry fruit. However, in rosemary plants, the level of these compounds was the highest in the earliest development phases (Vallejo et al. 2003). Gajewski et al. (2006) found the highest number of anthocyanins in eggplant fruit which were physiologically underripe, as compared with the ripe fruit. Chutichudet et al. (2011), who examined the influence of the developmental stage on the activity of polyphenols in the leaves of lettuce, noted the highest level of these compounds in the youngest developmental phase of plants (28 leaves); in the next phases (42 and 59 leaves), their level decreased and increased again in the old plant (73 leaves). Similarly, Gamon & Surfus (1999) claim that the leaves of young plants have the higher content of anthocyanins. Many authors pay attention to the phenomenon of seedling reddening. This may be the effect of a transitory accumulation of anthocyanins resulting from photoinduction (Christie *et al.* 1994, Gitz *et al.* 1998). Bieżanowska-Kopeć & Pisulewski (2006) noted the increase in the content of antioxidants during the process of bean seed germination. The content of polyphenols in the seeds under analysis increased every day after germination has been completed.

The content of polyphenols can undergo quantity as well as quality changes in the course of plant ontogenesis and ripening of edible parts. These transformations have been better studied in fruit. The antioxidant activity and content of polyphenols altogether in underripe (white and pink) and ripe fruit of bilberry are comparable but the latter have more anthocyanins (Kalt *et al.* 2003). However, in the case of strawberry and blackcurrant, the highest antioxidant activity was determined in underripe fruit (Wang & Lin 2000).

# Abiotic factors - climate, soil and agrotechnical conditions

The plants grown in the fields are often exposed to adverse climatic conditions. Temperature fluctuations can occur during a day or even within an hour. Such changes, in connection with the changes of other conditions such as light intensity or water availability, can lead to irreversible damage to plant cells and this is a response to stress conditions (Kacperska 1989, Huner et al. 1996). This is confirmed by the research of Solecka et al. (1999) who, in the plants of winter rape subjected to the activity of low temperatures, noted the increase of the content of phenolic acids. In eggplant, on the other hand, low temperature slows down the process of anthocyanin synthesis in fruit (Nothmann et al. 1978).

An important factor affecting the content of anthocyanins is light intensity. In the research carried out by Richards et al. (2004) into various varieties of lettuce grown in strictly controlled conditions, the increase in light intensity caused the increase in the content of anthocyanins. However, Ordidge et al. (2010), who examined the chemical composition of, among others, red lettuce cultivated in plastic tunnels of various ultraviolet ray permeability, observed the highest content of anthocyanins in the plants in plastic tunnels of the highest ray permeability. Toor et al. (2006) point to the influence of solar radiation on the content of antioxidant compounds in the fruit of greenhouse tomato. In the research, it was shown that the fruit picked up in summer contained more polyphenols than those picked up in spring. It was connected with the weather conditions during plant growth, in particular with bigger solar radiation. The authors are of the opinion that higher temperature and plant age could also influence the accumulation of those substances. Similarly, Hunt & Barker (1980) noted that light stimulated the production of phenols and flavonoids in tomato skin. The most visible effect was observed in red light. It is related to the speed of the biosynthesis of phenol compounds through the increase of the activity of enzymes, in particular of phenylalanine ammonia-lyase, which is responsible for transforming phenylalanine into coumarin acid which is involved in the formation of phenol compounds (Smith 1973). Similarly, the application of light in the cultivation of potato increases the quantity of anthocyanins in the bulbs; however, in some varieties, these compounds emerge without its involvement (Lewis *et al.* 1998).

The type of soil also conditions the content of antioxidants. The research of Łata & Wińska-Krysiak (2006) showed that the plants of kale cultivated in lessive soils contained more anthocyanins, glutathione and ascorbate in comparison to those plants cultivated in mud. At present, the compounds with antioxidant properties, in particular humus substances, can determine the biological activity of soil, and what follows, the course of the processes of oxidation and reduction which affect the form of interaction between soil and the plant (Schepetkin et al. 2002, Rimmer 2006). The results of other authors' research indicate that the plants grown in the humus-abounding soils produce more quantities of antioxidants than those grown in less rich soils (Shiow & Wang Hsin-Shan 2003, Jarosz 2006, Rimmer 2006).

The method of cultivating plants has also an influence on the content of anthocyanins and other antioxidant compounds. The research carried out by Hallmann & Rembiałkowska (2006) proved that ecologically cultivated onions contained by 6.67% more phenol compounds in comparison with conventionally cultivated onions. They were also characterised with a bigger content of vitamin C by 6.95% and of anthocyanins by 6.24%. Similar correlations were found by Hamouz et al. (2005) in the analysed varieties of potatoes. The plants from the ecological fields were characterised by higher quantities of polyphenols, on average by 10.2%, in comparison with the plants from conventional fields. The accumulation of antioxidant compounds in the ecologically cultivated vegetables can be the result of a different fertilisation and, what follows, a different nutrient management or the response to the preying of the pests which attack the ecologically cultivated plants more often than the ones grown in the conventional manner (Young et al. 2005, Hallmann & Rembiałkowska 2006). In the research of Young et al. (2005) the increase of phenol compounds in leafy vegetables cultivated in ecological ways was just the response to the pests' attacks. The induction of anthocyanin biosynthesis resulting from the attacks of pathogens occurs most frequently by the participation of jasmonic acid and its methyl ester which are the elements of plant immune system (Creelman & Mullet 1997, Saniewski & Czapski 1999). The damage caused by pathogens as well as the one formed mechanically cause the release of linoleic acid from the phospholipids' membranes, which is then transformed into jasmonic acid. The increased concentration of this compound is responsible for the initiation of genes which take part in the immune mechanisms of plants. This acid also takes part in the biosynthesis anthocyanins (Franceschi & of Grimes 1991).

The higher content of active compounds in ecologically cultivated vegetables confirms the Growth-Differentiation Balance Hypothesis which holds that in the ecological systems when organic fertilisers are used, plants change metabolism and synthesise in the first place the compounds which have in their chemical compositions mainly carbon [C]: sugars, phenol compounds, dyes, some vitamins such as vitamin C (Brandt & Mølgaard 2001). This theory is confirmed by the research of Toor et al. (2006) who noted higher antioxidant activity as well as a higher content of antioxidant compounds in tomatoes fertilised with hen manure, as compared to the ones fertilised with mineral fertilisers with the ammonium form. Heimler et al. (2009), who examined the influence of biodynamic and conventional cultivation on the content of polyphenols and antioxidant activity in Cichorium intybus, claim that the type of cultivation is not as significant as the environmental conditions as regards the content of antioxidant compounds. They demonstrated that the plants exposed to stress connected with insufficient water intake and big solar exposure contained more polyphenols than those not exposed to stress and the type of cultivation played a secondary role.

Deficiency of nutrients, in particular phosphorus and nitrogen, contributes to the accumulation of anthocyanins. This is confirmed in the research with bean plants by Jaszczuk et al. (2004). In the bean plant with the deficit of phosphorus, the intensity of photorespiration increases, as does the level of glycolic acid. This metabolic track is responsible for the increase of hydrogen peroxide as a reactive form of oxygen, hence the increase of anthocyanins has the purpose of protecting plants against oxidative stress (Foyer et al. 1997, Kondracka & Rychter 1997). Bongue-Bartelsman & Philips (1995) are of the opinion that in tomatoes, the deficit of nitrogen causes the increase of flavonoids through the influence on the expression of the genes coding the anthocyanin biosynthesis enzymes. According to Taiz & Zeiger (1998), the deficit of nitrogen can cause the synthesis of anthocyanins. However, this thesis is not confirmed by research of Politycka & Golcz (2004) who did not report the influence of a nitrogen dose on the content of anthocyanins in basil leaves. On the other hand, Nguyen & Niemeyer (2008) observed that the basil fertilised with the smallest dose of nitrogen contained more antioxidant compounds than when bigger doses of this component were provided. Similarly, Biesiada et al. (2010) noted the biggest content of anthocyanins in red heads of 'Langendijker' cabbage fertilised with the smallest dose of nitrogen (50 kg N·ha<sup>-1</sup>). The same tendency occurred in the case of narrow-leaved lavender. In the research into this variety of lavender, it was demonstrated that intensive fertilisation with nitrogen decreases the content of polyphenols in plants (Biesiada et al. 2008). Calcium ions cause the increase of the content of anthocyanins too. This correlation was observed by Sudha & Ravishankar (2003) who noted the increase of anthocyanins production along with the increase in the level of calcium in the callus cultures of carrot. Piccaglia et al. (2002) noted the smallest content of anthocyanins with the biggest doses of potassium and phosphorus.

The place and the date of plant cultivation also influence the content of antioxidants. Davies & Hobson (1981) reported bigger contents of these compounds in the tomatoes cultivated in the fields in comparison to the greenhouse ones. Howard *et al.* (2002), on the other hand, noticed the

decrease of the content of polyphenols by 50% in the leaves of spinach cultivated in autumn, as compared to the one cultivated in spring and so did Toor *et al.* (2006) in tomatoes from summer crops in comparison to the ones from spring crops. Similarly, Gazula *et al.* (2007) reported bigger contents of anthocyanins in the leaves of nine varieties of lettuce planted in late summer (August, September) in comparison to the plants planted in the early summer months (June, July).

The next factor determining the quantity of antioxidant compounds in plant is mulching. Anttonen *et al.* (2006) reported bigger contents of anthocyanins in the plants of strawberry bedded with brown foil, as compared to the ones bedded with white foil, and the bigger contents of polyphenols in the plants mulched with white foil.

Salinity has also a big influence on the accumulation of antioxidant compounds. Navarro *et al.* (2006) reported the increase of polyphenol content along with the increase of salinity in red fruit of *Capsicum annuum*; however, in the green and ripening fruit, salinity remained at the same level or slightly decreased. In the opinion of Keutgen & Pawelzik (2007), the level of anthocyanins in the conditions of salinity depends on plants' sensitivity to salinity: the decrease of anthocyanins can be noticed in plants which are salinity-sensitive.

Li *et al.* (2010) observed that red lettuce watered with water with the addition of abscisic acid contained definitely more polyphenols and anthocyanins, as compared to the control.

#### **Processing and storage**

Phenol compounds can undergo degradation during the processes of food preparation and processing. Heat treatment has a significant influence on the total content of polyphenols and although they are more stable than vitamin C, the losses of these compounds may reach approximately 70%. Cieślik et al. (2007) concluded that during cauliflower cooking, the losses of polyphenols were as follows: of flavonoids 81.25%, of phenolic acids 40%. Short-term heat treatment of plant products, that is, blanching, also decreased the level of polyphenols in green cauliflower and broccoli by 12.2% and 17.9%, respectively and in Brussels sprout and kale 28.9% and 25.2%, respectively. The lowest decrease of product antioxidant activity occurs after cooking the previously frozen products and in the case of the vegetables of the cabbage family and the difference between AA measured with the ABTS test and DPPH of fresh vegetables and the ones cooked after previous freezing can reach 51%. Analysing the time of blanching the vegetables of the cabbage family in terms of AA, Ismail et al. (2004) concluded that it is differentiated in terms of species; the biggest losses in AA were found in Chinese cabbage (40%) and the smallest ones - in red cabbage (4%) after 15-minute blanching. Stasiak & Ulanowska (2008) showed that the cooking of bean seeds caused the decrease of the content of polyphenols by several dozen per cent in comparison to raw seeds.

Koukounaras & Siomos (2010) reported a significant decrease of polyphenols in the plants of radicchio in the first week of storing at the temperature of 0°C and humidity >90%. During later period of storage, however, their quantity remained unchanged.

The process of forcing has also an influence on the content of antioxidant compounds. Nicoletto & Pimpini (2010) noticed that the longer the process of forcing the plants of chicory lasted, the fewer phenol compounds they contained.

Due to the common use of anthocyanins as natural dyes, it is essential to shorten the time of extracting them from plant tissues. It has been proved that the extraction of anthocyanins depends strictly on temperature and the pH. Anthocyanins are most stable at the pH=2. This is confirmed by the research of Türker & Erdoğdu (2006) who showed that the coefficient of anthocyanins diffusion from black carrot tissues was increasing along with the increase of temperature and along with the simultaneous decrease of the pH and reached the highest level at the temperature of 50°C and at the pH=2. It was related to the increased permeability of cell membranes at the low pH and the higher solubility of anthocyanins at higher temperatures.

The technological processes and plant storage are essential factors conditioning the quality and chemical composition of vegetables. Although the objective of storage technology is such manipulation of plant metabolism that their shelf life is prolonged and their quality is almost not lowered, some of these treatments lead to the decrease of the content of anthocyanins in plants (Gil *et al.* 1997). The research of Gajewski *et al.* (2006) showed that storing the fruit of eggplant in optimal conditions caused the decrease of anthocyanins content, which was connected to the quality changes occurring in those plants. Bridle & Timberlake (1997) also point to the not stable enough character of anthocyanins during storage. Similarly, Kidoń & Czapski's (2009) research demonstrated that as long as storing the juices produced from black carrot at the temperatures of 20°C and 35°C brings about a significant degradation of anthocyanins, storing them at low temperatures does not contribute to the decomposition of those dyes. It was also shown that vegetable processing affects the stability of anthocyanins through induction or hindering their biosynthesis. Lavelli et al. (2009) proved that in the fragmented leaves of red chicory stored in a modified atmosphere there was an increase of the content of anthocyanins and antioxidant activity; however, in the leaves packed in normal conditions, their level decreased. Ferreres et al. (1996) are of the opinion that such dye losses are dependent upon the structure of anthocyanins.

#### Vegetable antioxidant activity

Most authors stress the fact that the assessment of total polyphenols content is a good indicator of plant raw material antioxidant activity (AA). Some authors stated that the antioxidant activity of fruit and vegetables is positively correlated with the high total content of polyphenols as well as flavonoids and anthocyanins (Jamroz *et al.* 2006). According to Karadenüz *et al.* (2005) a high and significant correlation between AA and total phenolic content was determined in fruit (r 2 = 0.9307, P < 0.01) and vegetable (r 2 = 0.9361, P < 0.05).

However, flavonoid content was not significant correlated with antioxidant activity in vegetables. According to Kähkönen et al. (1999) antioxidant activity does not necessarily correlate with high amount of phenolics, and that is why both phenolic content and antioxidant activity information must be discussed when evaluating the antioxidant potential of extracts. Several methods have been used to measure antioxidant activity in different articles expressed as "total antioxidant activity" (Rice-Evans 2000), or "total antioxidant capacity" (Young 2001), or "total antioxidant potentials" (Simonetti et al. 1997). According to Ou et al. (2002) the authors treat the "total antioxidant power" as the "total reducing power". The antioxidant activity is then interpreted as the reducing capability. These methods are based on either single electron transfer (SET) reaction or a hydrogen atom transfer (HAT) reaction between an oxidant and a free radicals. SET assays include the ABTS/TEAC (Trolox Equivalent Antioxidant Capacity), CUPRAC, DPPH (using diphenyl-ppicrylhydrazyl radical) and FRAP (Ferric Reducing Antioxidant Power)

methods, each using different chromogenic redox reagents with different standard potentials. As an example of HAT- based assays, oxygen radical absorbance capacity (ORAC- Oxygen Radical Absorbance Capacity) assays (Cao et al. 1996) applies a competitive reaction scheme in which antioxidant and substrate kinetically compete for thermally generated peroxyl radicals through the decomposition of azo compounds such as BAP (2,2'azobis (2-aminopropane) dihydrochloride). According to Awika et al. (2003) ABTS and DPPH methods, which are more cost effective and simpler were demonstrated to have similar predictive power as ORAC on sorghum antioxidant activity. At present there are relatively few reports on the systemisation of vegetables in terms of their AA. It is measured in various ways, among others, by means of such tests as FRAP. ABTS. DPPH and others. In the above - mentioned methods, the level of absorbance change is determined. The most widely used solvents for the extraction of anthocyanins are aqueous solutions of acetone, methanol, and ethanol (Horbowicz et al. 2008).

Research factor	Vegetable species	References		
Nitrogen dose	▶ Basil	Politycka & Golcz 2004 Phippen & Simon 1998		
Variety 🔶	►			
Variety 🔶	▶			
Cultivation method:	Onion	Hallamnn & Rembiałkowska		
ecological		2006		
conventional	<b>↓</b>			
Variety 🗸 🗸	▶			
Storage:				
modified atmosphere	Chicory	Mulabagal <i>et al.</i> 2009 Lavelli <i>et al.</i> 2009 Heimler <i>et al.</i> 2009		
air				
Cultivation method:				
conventional	•			
biodynamic 🛛 🔸	▶			
Variety 🔶	*			
Soil type:	Kale	Łata & Wińska-Krysiak 2006		
lessive soil		Edu & Whiska Riyslak 2000		
mud	ŧ			
Nitrogen dose:				
$50 \text{ kgN} \cdot \text{ha}^{-1}$	Red cabbage	Biesiada et al. 2010		
250 kgN·ha <sup>-1</sup>	<u>+</u>			
Stage of maturity	➡ Egg plant	Gajewski et al. 2006		
Storage		•		
Salinity	Annual pepper	Navarro et al. 2006		
Type of fertilizer:		-		
organic	Tomato	Toor <i>et al.</i> 2006		
mineral	•			
Light intensity		Richards et al. 2009		
Variety 4	Lettuce	Llorach <i>et al.</i> 2008 Li <i>et al.</i> 2006		
Abscisic acid				
Low temperature	Winter rape	Solecka et al. 1999		
Cultivation term:				
spring	Spinach	Howard et al. 2002		
autumn	♥			
Cultivation method:				
ecological	Potato	Hamouz et al. 2005		
conventional	▼			

Table 2. Factors a	affecting the	content	of	antioxidative	compounds	in	some	kinds	of
vegetabl	les								

I increase of antioxidant compounds,
 ➡ independence of test factor

Determining by means of DPPH and ABTS tests consists in sweeping away the free stable radical whereas the FRAP test involves the reduction of  $Fe^{+3}$  ions to  $Fe^{+2}$  ions (Benzie & Strain 1996, Re *et al.* 1999, Kim *et al.* 2002). The FRAP method is simple, relatively cheap and can be used in the analysis of all products not showing absorption in the same analytic

range (593 nm). The FRAP method involves the biggest number of reactive antioxidant components present in a sample while the DPPH method, only part of the most reactive ones (the ABTS method - the medium values). Therefore, the numerical values obtained in the DPPH method are the lowest. The final result depends on the time, adopted by a given author, after which the measurement of absorbance was taken. It creates problems in the comparison of the data obtained by two separate researchers (Bartoń et al. 2005). Determining AA by means of the above-mentioned methods facilitates the interpretation of the obtained data since these are complementary methods.

Table 3 presents the antioxidant activity of selected vegetable species. The results presented come from the studies analysing a larger group of vegetables in comparable conditions. The highest AA is characteristic for spinach, chilli pepper, red beet and then broccoli, radicchio leaves, artichoke and asparagus. The lower AA is characteristic of such vegetables as cucumber or carrot (Pellegrini *et al.* 2003, USDA 2011).

In antioxidant tests carried out by TEAC/ABTS, ORAC and FRAP methods on regulary consumed fruits and vegetables available on the U.K. market, fruits and vegetables rich in anthocyanins (e.g., strawberry, raspberry and red plum) showed the highest AA values, followed by those rich in flavones (orange and grapefruit) and flavonols (onion, leek, spinachi and green cabbage), while the hydroxycinnamic acids rich ones (apple, tomato, pear and peach) exhibited the lower values. The antioxidant capaci-

ties (in TEAC units, on fresh weight basis) followed the hierarchic order strawberry >> raspberry = red plum >> red cabbage >>> grapefruit = orange > spinach > broccoli > green grape  $\approx$  onion > green cabagge > pea > apple > cauliflower  $\approx$  pear > tomato  $\approx$ peach = leek > banana  $\approx$  lettuce (Proteggente et al. 2002). A total of 927 freeze-dried vegetable sample from American market were analysed using the ORAC and FRAP methods, and the rank order with ORAC was: green pepper > spinach > purple onion > broccoli > beet > cauliflower > red pepper > white onion > snap bean > tomato > white cabbage > carrot > pea, whereas with FRAP, the order was: red pepper > green pepper > beet > spinach > cauliflower > tomato > broccoli > white cabbage > purple onion > carrot > snap bean > white onion > pea (Ou *et al.* 2002).

On the basis of ORAC findings, Ou et al. (2002) concluded that green pepper, spinach, purple onion, broccoli, red beet and cauliflower were the leading source of antioxidant activity against peroxyl radicals. Cao et al. (1996) reported that based on the fresh weight of the vegetable, garlic had the highest ORAC antioxidant activity ( $\mu$ mol TE·g<sup>-1</sup>) against peroxyl radicals (19.4) followed by kale spinach (12.6), Brussels (17.7),sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant (9.8-3.9), cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery and cucumber (3.8-0.5), but the green and black teas had much higher antioxidant activity against peroxyl radicals than all these vegetables.

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		F1 · 1			OD A C**	
	Phenolics	Flavonoids mg CE		TEAC/ABTS*	ORAC**	
Vegetable species	GAE/catechol	$\cdot 100g^{-1}$	µmol Fe <sup>2-</sup> ·kg <sup>-</sup>	µmol TE∙kg⁻¹	µmol TE ·100 g <sup>-1</sup>	
	UAL/catechoi	FW	<sup>1</sup> FW	FW	FW	
Artichoke	321.3 <sup>4</sup>	1 00	11.09	1.55	6552	
Arugula	90-102.7 <sup>3</sup>		14.3	3.55	1950	
0		2			2252	
Asparagus	$14.5^4 - 64.1^2$	$7.66^{2}$	10.6	3.92	292	
Bean green	$10.0^4 \ 35.5/4.51^2$	$0.66^{2}$	2.35	1.27		
Brussels sprouts	257.1 <sup>4</sup> /68.8 <sup>1</sup>					
Red beet	327/323 <sup>1</sup>		15.31	2.94	1776	
Broccoli	25.02 <sup>2</sup> 98.9 <sup>4</sup> 87.5 -	$2.35^{2}$	11.67	2.04	1510	
Broccoll	$101.7/87.5^{1}$		11.67	3.04		
Cabbage	$40^3$ , $45.28^2$ - $92.0^1$	$1.20^{2}$	5.79	1.15	529	
Cabbage red	139.3 - 178 <sup>3</sup>			23.2	2496	
Carrot	8.40 <sup>2</sup> , 10.1 <sup>4</sup> 96.0/55.0 <sup>1</sup>	1.07	1.06	0.44	697	
Cauliflower	$10.4^5, 4/96.0^1$	$1.54^{2}$	4.27	1.10	870	
Celery	$17.9^2, 98.0^4$	$0.28^{2}$	1.16	0.49	552	
Chicory	14.7 <sup>4</sup> , 102.7		6.72	1.86		
Cucumber	$48^{1}$		0.71	0.43	232	
Eggplant	$45^3, 65.6^4$		3.77	1.10	932	
Garlic	47.6 <sup>2</sup> , 59. <sup>4</sup> 4, 87/145 <sup>1</sup>	$5.43^{2}$			5708	
Leek	27.7 - 32.7 35.7 <sup>4</sup>		2.15	0.72	569	
Lettuce green	$9.82^2, 124.5^2$	4.57	4.94	1.33	1532	
Lettuce red	$170.1^{3}$				2426	
Onion yellow	24.27 <sup>2</sup> , 76.1 <sup>4</sup>	4.73 <sup>2</sup>	5.28	1.82	913	
Onion red					1521	
Deducan	$26.8^4$ , 119 - 173.2 <sup>3</sup> -		20.09	0.22	001	
Red pepper	67.3 <sup>1</sup>		20.98	8.32	821	
Graan nannar	$18.2^4$ , $52.4^2$ , $119^3$ ,	1.12			935	
Green pepper	$131.0 - 246.7/115.0^1$	1.12			955	
Pepper chili	115		23.64	7.62		
Parsley	$280.2^4$				1301	
Pumpkin	15.93 <sup>2</sup>	$0.84^{2}$	4.00	3.71	483	
Radicchio		11.39		3.24		
Radish red	29.45 <sup>2</sup> , 160.0/54.5 <sup>1</sup>	$1.36^{2}$	3.77	2.22	1750	
Spinach	32.54 <sup>2</sup> , 208.9	$1.42^{2}$	26.94	8.49	1513	
Sweet corn	50.37 <sup>2</sup>	5.03 <sup>2</sup>			728	
Squash	$12.10^2$	$0.42^{2}$				
Tomato	$13.7^4, 23.69^2  76.9/68.0^1$	$1.77^{2}$	5.12	1.65	387	
Zucchini	18.9 <sup>4</sup>		3.33	2.86	180	

Table 3. Antioxidant activity in s	selected vegetables
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[<sup>1</sup>-Kaur & Kapoor 2002, <sup>2</sup>-Chun *et al.* 2005, <sup>3</sup>-Hassimotto *et al.* 2005, <sup>4</sup>-Brat *et al.* 2006, <sup>5</sup>-Marinova *et al.* 2005]

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#### CZYNNIKI BIOTYCZNE I ABIOTYCZNE WPŁYWAJĄCE NA ZAWARTOŚĆ WYBRANYCH ZWIĄZKÓW ANTYOKSYDACYJNYCH W WARZYWACH

#### Streszczenie

Warzywa są bogatym źródłem substancji biologicznie aktywnych, które wspomagają mechanizmy obronne organizmu. Liczną grupę spośród tych substancji stanowią związki o działaniu antyoksydacyjnym. Zalicza się do nich m.in.: niektóre witaminy (A, C i E), związki polifenolowe, tokoferole, karotenoidy, glutationy i tiocyjaniany. Związki te hamują uszkodzenia DNA w komórkach nowotworowych, indukują produkcję insuliny w trzustce, chronią ludzki mózg przed procesami starzenia. Spośród nich najliczniejszą grupę stanowią polifenole, posiadające wysoką aktywność antyoksydacyjną, która warunkuje mechanizmy obronne roślin w warunkach stresowych, takich jak: wahania temperatury, promieniowanie UV, ataki szkodników i uszkodzenia mechaniczne. W skład polifenoli wchodzą m.in.: kwasy fenolowe, kwas hydroksycynamonowy oraz flawonoidy, a wśród nich duża grupa antocyjanów. Ich zawartość może się różnić u poszczególnych roślin tego samego gatunku, co jest związane z szeregiem wewnętrznych i zewnętrznych uwarunkowań, takich jak czynniki genetyczne, środowiskowe i agrotechniczne. Zawartość antocyjanów może zależeć od: gatunku rośliny, odmiany botanicznej i hodowlanej oraz procesów biologicznych związanych z ontogeneza. Również czynniki klimatyczne (szczególnie światło i temperatura), glebowe i agrotechniczne, takie jak: sposób, miejsce i termin uprawy, nawożenie, mulczowanie, zasolenie, mogą przyczynić się do powstawania warunków stresowych w okresie wzrostu roślin i tym samym zwiększać zawartość antyoksydantów w roślinach.

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