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SUSCEPTIBILITY OF CARROT ROOTS AND APPLE FRUITS TO ENZYMATIC BROWNING PROCESSES

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Summary. The present study of three vegetation periods evaluated the enzymatic browning process of two different commodities, carrot roots and apples. The influence of the genetic potential (effect of genus and cultivar), of magnesium fertilization is case of carrot and of storage (in case of carrot 6 months in a traditional natural mound and in case of apple 6 months under ultra low oxygen (ULO) conditions) were investigated. In the case of carrot 5 different cultivars were investigated, middle late 'Berjo', late: 'Flacoro', 'Karotan', 'Koral', 'Perfekcja', and magnesium doses (0, 45, 90 kg·ha^{-1} of MgO) by constant fertilization with nitrogen (70 kg·ha^{-1}), phosphorus (80 kg·ha^{-1} of P_2O_5), and potassium (100 kg·ha^{-1} of K_2O). In October harvested roots were stored for 6 months in the traditional natural mound. In the case of apple fruits seven different cultivars were used: 'Elstar', 'Gloster', 'Honeygold', 'Idared', 'Jonagored', 'Ligol', and 'Szampion', from the commercial Agricultural and Fruit Farm Klimkiewicz, Wtelno (Kuyavian-Pomeranian Voivodeship) using integrated horticulture production procedures. Apple fruits were stored under ULO conditions for 6 months (>2% O_2, <2% CO_2, 1.5–2.0°C and 95–96% RH). Susceptibility of commodities was evaluated directly after harvest and after the 6 months of storage using collorimetrically determination method at 475 nm. The evaluation of the enzymatic browning process in carrot roots and apple fruits revealed significant dependency on the used plant material (genus). Carrot roots were generally less susceptible to enzymatic browning compared to the apple fruits. Leaf application of magnesium fertilization, especially at the dose of 90 kg MgO per 1 ha inhibited the browning process. Storage time of 6 months in the mound promoted discoloration of carrot roots and thus negatively influenced their quality. In the case of apple fruits the appropriate choice of cultivar was most important for limiting the enzymatic browning process, which is especially important when selecting raw material for juice production. A low susceptibility to enzymatic browning was found in fruits of cv. 'Elstar', whereas the cv. 'Honeygold' was most susceptible. Changes in the susceptibility to the browning processes of fruit flesh after storage were statistically significant, however quite small in comparison to the values measured directly after harvest.

Key words: enzymatic browning, carrot roots, apples, storage, fertilization, ULO

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INTRODUCTION

Colour is a physical product property, which to a great extent influences its positive or negative perception by the consumer [Kidoń and Czapski 2010, Keutgen et al. 2014]. Colours of fruits and vegetables are crucial factors of plant products. Because they change after harvest due to senescence processes, colours are very important quality criteria for the retailer and consumer [Keutgen 2000]. With colour changes several processes are combined, for instance biosynthesis and degradation of valuable intrinsic and storage compounds, which determine the quality of the produce. Especially the quality of horticultural commodities for the fresh market and direct consumption is combined with the visual appearance, which represents the primary function for trade classifications, quantification of freshness, and purchase decision. Colour intensity is also a very important indicator during storage and processing and undergoes constant changes during aging, damages and stress factors such as water, nutrient, cold, temperature or light stress. It influences the consumer's perception of palatability and popularity. In the commodity the colour is mainly a result of pigmentation, which is determined genetically and developmental-physiologically in the pre- and postharvest period. Besides the desired colour development, enzymatic browning processes may occur in fresh commodities. Browning processes of raw materials is often combined with its decay and, thus, negatively influences the perception of the produce. The browning process occurs as a consequence of the reaction of polyphenolic compounds with polyphenol oxidases (PPO) in the presence of oxygen, usually initiated by the enzymatic oxidation of monophenols into o-diphenols and o-diphenols into quinones (Fig. 1), which undergo further non-enzymatic polymerization leading to the formation of dark coloured, non-soluble pigments such as melanins [Perera 2005, Gliszczyńska-Świgło 2010].

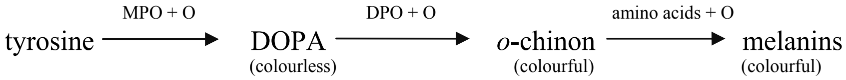

Fig. 1. Reaction of the enzymatic browning processes [Sikorski 2007]

Rys. 1. Reakcja procesu ciemnienia enzymatycznego [Sikorski 2007]

The browning process occurs during senescence or after damaging the plant tissue e.g. as a consequence of size reduction, cutting, peeling, and defrosting. Enzymatic browning depends on the physiological stage of the commodity, the activity of PPO [Kowalska et al. 2007, Sikorski 2007] and on the presence of polyphenolics, mainly catechin, chlorogenic and caffeic acids, which are the most reactive ones [Jihong et al. 2007]. However, the intensity of the process depends not only on the polyphenolics, but also on the presence of other naturally occurring antagonists of the process, such as carotenoids or citric and ascorbic acids. The largest, and at the same time the most diverse group due to their structure and properties represents the group of polyphenolics, with generally very high antioxidant activities [Nijveldt 2001]. Because different fruits and vegetables, such as apple, potato, lettuce, pear, banana, carrots and peach are rich in polyphenolic substances, they are susceptible to enzymatic browning during processing and storage, and, thus, may be refused by the consumers. Restriction of the enzymatic browning process is therefore a very important aim in food industry. Several methods are used for the decrease of enzymatic browning, such as the use of antioxidants and inhibitors, pascalisation, ultrafiltration, treatment with superficial CO_2, blanching, freezing, cooling, changing of pH, irradiation and others, but not all of them can be applied on fresh items. Therefore, the aim of the study was to evaluate the enzymatic browning processes in two different commodities, apples and carrot roots with respect to their genetic potential, magnesium fertilization that may promote process antagonists, and the impact of storage.

MATERIAL AND METHODS

Different plant material susceptible to enzymatic browning was used for the experiments, the apples as an example for fruits and carrot roots for vegetables. In the case of carrot roots the plant material derived from field experiments performed at the Mochełek Experimental Station of the Faculty of Agriculture and Biotechnology at the UTP University of Science and Technology in Bydgoszcz (Kuyavian-Pomeranian Voivodeship) during 2007–2009. Field experiments were conducted after the cultivation of barley, on a light soil with a typically slightly acidic pH, low concentrations of available forms of P and K and very low amounts of Mg. Experiments were conducted in a factor-dependent split-plot design with three replications and comprised the following factors: evaluation date (directly after harvest and after 6 months of storage), cultivars (middle late 'Berjo', late: 'Flacoro', 'Karotan', 'Koral', 'Perfekcja'), and Mg doses (0, 45, 90 $kg \cdot ha^{-1}$ of MgO) by constant fertilization with N (70 $kg \cdot ha^{-1}$), P (80 $kg \cdot ha^{-1}$ of P_2O_5), and K (100 $kg \cdot ha^{-1}$ of K_2O). Leaf application of magnesium was performed once or twice during the period of intensive plant growth (July, August) at a concentration of 3% in the form of magnesium sulphate (16%). Cultivation and plant protection measures were performed in line with the requirements for carrot plants: the seeds were treated with Funaben T (a.s. thiuram – 45%, carbendazym – 20%); each year before emerging the herbicide Stomp 330 EC (a.s. 33% pendimethalin) was applied. Furthermore, during the vegetation period a manual weed control was performed. Carrot roots were harvest at the stage of full maturity (1st decade of October), where the mean samples from each parcel were taken for further analytical and storage considerations. Harvested roots were stored for 6 months in the traditional natural mound. Discoloration potential or enzymatic browning susceptibility was performed using the colorimetric method after Dean et al. [1993]. Samples of 25 g from the apical and basal ends were homogenized for 30 s in 25 ml of 0.02 $mol \cdot l^{-1}$ phosphate buffer and then left to oxidize for 24 h under room temperature conditions. The browning processes were quantified by measuring the colour of the sample solution at 475 nm with an SHIMADZU UV-1800, UV-Vis spectral photometer system (Japan). The samples were diluted with the extraction buffer at a ratio of 1 : 3 before photometric measurements. The presented results are the means of three measurements as absorbance units at 475 nm (AU_{475}). The results were evaluated as susceptibility classes from resistant (0.00) to very susceptible (over 0.80) as presented in Table 1.

Table 1. Susceptibility classes on enzymatic browning processes in raw material evaluated by the homogenization method of Dean et al. [1993]

Tabela 1. Klasy podatności na procesy ciemnienia enzymatycznego w świeżym materiale określne zgodnie z homogenizacyjną metodą według Dean i innych [1993]

Susceptibility classes Klasy podatności		Homogenization method Metoda homogenizacyjna (AU_{475})
1	Resistant to browning processes Odporne na procesy ciemnienia	0.00–0.20
2	Moderately resistant to browning processes Średnio odporne na procesy ciemnienia	0.21–0.40
3	Moderately susceptible to browning processes Średnio podatne na procesy ciemnienia	0.41–0.60
4	Susceptible to browning processes Podatne na procesy ciemnienia	0.61–0.80
5	Very susceptible to browning processes Bardzo podatne na procesy ciemnienia	>0.80

In the case of apple fruits seven different cultivars were used: ‘Elstar’, ‘Gloster’, ‘Honeygold’, ‘Idared’, ‘Jonagored’, ‘Ligol’, and ‘Szampion’, which were produced in an orchard in line with integrated horticulture production procedures at the Agricultural and Fruit Farm Klimkiewicz, Wtelno (Kuyavian-Pomeranian Voivodeship). The fruit plantation comprised 2400 trees per 1 ha fertilized with 50 $kg \cdot ha^{-1}$ of N and 50 $kg \cdot ha^{-1}$ of K_2O, phosphorus fertilization was not applied. During the cultivation the following pesticides were used: fungicides: Merpan 80WG (a.s. captan) total dose 3 $l \cdot ha^{-1}$, Delan 700 WG (a.s. dithianon) 0.75 $l \cdot ha^{-1}$; insecticides: Corogen 200 SC (a.s. chlorantraniliprole) 0.175 $l \cdot ha^{-1}$, Envidor 240 SC (a.s. spirodiclofen) 0.4 $l \cdot ha^{-1}$, Affirm 095 SG (a.s. emamectin benzoate) 3 $l \cdot ha^{-1}$; herbicides: Roundap (glyphosate 360 $g \cdot l^{-1}$ as izopropoloamine salt) 0.1 $l \cdot ha^{-1}$; adjuvant: Silwet Gold (a.s. organosilicone-modified polyalkylenoxide heptamethyltrisiloxane) 5 $l \cdot ha^{-1}$. Apple fruits were harvest in October at the day with no precipitation. Susceptibility of apple mark to enzymatic browning processes were performer using the method of Dean et al. [1993] as described above with some modifications of Wichrowska directly after harvest as well as after 6 months of storage in an ULO storage room under reduced content of oxygen of not less than 2% O_2 and not more than 2% CO_2 at a temperature of 1.5–2.0°C and 95–96% RH.

Data were analysed with the FRANAL statistical program of Prof. Rudnicki. All data sets were tested for analysis of variance and variance homogeneity ($p = 0.05$) by Tukey’s multiple range tests.

RESULTS AND DISCUSSION

Extensive research [Keutgen et al. 2014] about the factors influencing the susceptibility to enzymatic browning have shown the main influence of cultivar, location with the corresponding growing conditions (e.g. fertilization, water availability, weather conditions), maturity at the time of harvest, mechanical load, environmental stress, storage conditions, and compounds relevant to the occurring enzymatic processes, e.g. free phenolic amino and organic acids, enzyme activities or mineral nutrients.

The statistical analysis of the results revealed that the investigated carrot cultivars, contrary to expectations, did not differ in their susceptibility to enzymatic browning (Table 2). This observation might be explained by the selection of quite stable, modern cultivars of uniform, bright orange colour, which according to the classification of Dean et al. [1993] can be rated as a raw material resistant to enzymatic discolouration. However, some differences were observed. The cultivars 'Berjo' and 'Perfekcja' directly after harvest were characterized by the highest susceptibility to the enzymatic browning processes, whereas the industrial cv. 'Karotan' was quite resistant (Table 2).

The intensity of the browning process is dependent on the amount and kind of fertilizer, for example high nitrogen fertilization may results in higher concentrations of polyphenolics and tyrosine as well as in the decrease of citric acid content. In consequence, the susceptibility of the raw material to the enzymatic browning process may increase. The opposite effect was found when potassium fertilizers were applied [Ciećko et al. 2005].

The evaluation of the influence of magnesium fertilizers revealed that an additional leaf application statistically significantly decreased the susceptibility to enzymatic browning processes, particularly in the case 90 kg·ha^{-1} of MgO were supplied. The positive influence of magnesium was combined with an increase in the contents of carotenoids

Table 2. Susceptibility of fresh carrot roots to enzymatic browning processes

Tabela 2. Podatność świeżych korzeni marchwi na procesy ciemnienia enzymatycznego

Sampling time Czas poboru (A)	Cultivar Odmiana (B)	Fertilization MgO Nawożenie [kg·ha^{-1}] (C)			Mean value Średnia
		0	45	90	
Direct after harvest Bezposrednio po zbiorze	Berjo	0.088 ±0.10	0.084 ±0.14	0.079 ±0.16	0.084 ±0.36
	Flacoro	0.075 ±0.46	0.072 ±0.19	0.069 ±0.21	0.072 ±0.23
	Karotan	0.056 ±0.13	0.051 ±0.22	0.045 ±0.18	0.051 ±0.24
	Koral	0.068 ±0.08	0.061 ±0.09	0.057 ±0.27	0.062 ±0.50
	Perfekcja	0.092 ±0.03	0.089 ±0.12	0.084 ±0.24	0.088 ±0.20
	Mean value Średnia	0.076 ±0.22	0.071 ±0.37	0.067 ±0.31	0.071 ±0.49
After storage Po przechowywaniu	Berjo	0.156 ±0.07	0.153 ±0.11	0.148 ±0.27	0.152 ±0.21
	Flacoro	0.149 ±0.12	0.142 ±0.08	0.138 ±0.16	0.143 ±0.14
	Karotan	0.131 ±0.21	0.125 ±0.12	0.123 ±0.39	0.126 ±0.33
	Koral	0.147 ±0.19	0.140 ±0.32	0.134 ±0.33	0.140 ±0.38
	Perfekcja	0.153 ±0.15	0.147 ±0.21	0.145 ±0.15	0.148 ±0.19
	Mean value Średnia	0.131 ±0.38	0.125 ±0.29	0.123 ±0.35	0.142 ±0.34
Mean value – Średnia		0.112 ±0.40	0.106 ±0.27	0.102 ±0.38	0.107 ±0.49
NIR $_{p=0.05}$ *LSD* $_{p=0.05}$		A = 0.131 B/A = n.s. A/C = 0.108	B = n.s. A/B = n.s. C/B = n.s.	C = 0.156 C/A = 0.115 B/C = n.s.	

n.s. – non significant / nieistotne.

and ascorbic acid [Domaradzki et al. 2010, Wszelaczyńska and Pobereżny 2011], which directly impair the browning reaction due to their antioxidative properties [Keutgen et al. 2014]. In addition, the magnesium fertilization may also increase the content of citric acid and decrease that of chlorogenic acid and, thus, may lower the tendency to discoloration of raw material [Grudzińska 2009]. In contrast, magnesium deficiency as a stress factor may increase the content of polyphenolics during the vegetation period and negatively influence the colour of plant material.

Changes of phytochemical composition as well as of antioxidant capacity of plant commodities continue after harvest and, hence, their levels are directly a result of the treatment after harvest and storage conditions. During the storage period the decrease of carotenoids, but also of other antioxidants such as ascorbic and citric acid were found [Grudzińska 2009, Wszelaczyńska and Pobereżny 2011]. At the same time, due to the intensive physiologically processes, among others the content of polyphenolics, particularly that of free tyrosine, may increase [Grudzińska 2009, Keutgen et al. 2014].

The most important factor influencing the degree of unfavourable discolouration in carrot roots was the long period of storage (Table 2). The most susceptible to enzymatic browning after 6 month of storage were the cultivars 'Berjo' and 'Perfekcja', the resistant one was cv. 'Karotan'. It is generally accepted that the most important agent inhibiting the development of browning processes represents ascorbic acid. Its losses during storage are very high and may reach even a level of 80%. In consequence, discolouration may occur at a higher extent [Wszelaczyńska and Pobereżny 2011].

In the case of apple fruits the conducted research revealed large differences between the investigated cultivars in their susceptibility to enzymatic browning. Among the investigated apple cultivars cv. 'Szampion' was characterized by the lowest susceptibility to discolouration (Table 3). The present results agree with those presented by Biller et al. [2007] and Zaremba et al. [2007], who evaluated the changes of several colour parameters of apple fruits during the drying process. Although cv. 'Szampion' is very rich in polyphenolic compounds, the results of Sieliwanowicz et al. [2005] attributed the generally low susceptibility of apple fruits to their high antioxidant capacity.

A low susceptibility to enzymatic browning was also found in the fruit flesh of cv. 'Elstar'. Fruits of this cultivar can be recommended for production of naturally cloudy juices and mark products without the necessity of antioxidants application to the technological process. Today, due to the consumers' demand, on the market the offer of fruit juices without any additional agents and additives, even without ascorbic acid, is significant and constantly growing. A high quality of such juices is determined not only by the correct technological process, but first and foremost by the selection of the appropriate raw material.

In the presented research the most susceptible cultivar was cv. 'Honeygold' (Table 3). In the case of processing the addition of ascorbic acid for colour stabilization at a concentration of not higher than 250 $mg \cdot kg^{-1}$ raw material is urgently recommended [Oszmiański and Wojdyło 2006]. Changes in the susceptibility to the browning processes of fruit flesh after storage were statistically significant, however quite small in comparison to the values measured directly after harvest.

Table 3. Susceptibility to enzymatic browning processes of chosen apple cultivars direct after harvest and after 6 month of ULO storage

Tabela 3. Podatność na procesy ciemnienia enzymatycznego wybranych odmian jabłek bezpośrednio po zbiorze i po 6 miesiącach przechowywania w ULO

Cultivar Odmiana	Susceptibility to enzymatic browning processes Podatność na procesy ciemnienia enzymatycznego (AU_{475})		
	Direct after harvest Bezpośrednio po zbiorze	After storage Po przechowywaniu	Mean value Średnia
Elstar	0.140 ±0.001	0.149 ±0.003	0.144
Honeygold	0.621 ±0.003	0.690 ±0.002	0.655
Ligol	0.210 ±0.012	0.234 ±0.004	0.222
Szampion	0.059 ±0.003	0.100 ±0.002	0.079
Gloster	0.287 ±0.005	0.300 ±0.001	0.293
Idared	0.589 ±0.003	0.622 ±0.001	0.605
Jonagored	0.534 ±0.002	0.550 ±0.002	0.542
Mean value Średnia	0.348	0.378	0.363

NIR (test Tukeya): I – odmiana 0,014; II – czas przechowywania 0,005; Interakcje I/II 0,020; II/I 0,012.

LSD (Tukey test): I – cultivar 0.014; II – storage time 0.005; Interactions I/II 0.020; II/I 0.012.

CONCLUSIONS

The evaluation of the enzymatic browning process in carrot roots and apple fruits revealed significant dependency on the used plant material (genus). In the case of the generally less susceptible carrot roots the most important factors determining the discoloration processes were long term storage and magnesium leaf application at a MgO concentration of 90 $kg \cdot ha^{-1}$. In the case of apple fruits the appropriate choice of cultivar was most important for the development of the enzymatic browning process and, thus, for the assurance of the technological quality of raw material for juice production.

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PODATNOŚĆ KORZENI MARCHWI I JABŁEK NA PROCESY CIEMNIENIA ENZYMATYCZNEGO

Streszczenie. Przedstawione trzyletnie doświadczenia dotyczyły oceny procesów ciemnienia dwóch różnych rodzajów produktu, korzeni marchwi (5 odmian) i jabłek (7 odmian). Przebadano wpływ potencjału genetycznego (wpływ rodzaju i odmiany), nalistnego nawożenia magnezem (45 i 90 kg·ha^{-1}) u marchwi oraz przechowywania (w przypadku marchwi tradycyjne kopcowanie przez 6 miesięcy, a w przypadku jabłek 6 miesięcy w warunkach ULO, czyli >2% O_2, <2% CO_2, 1.5–2.0°C oraz 95–96% wilgotności względnej. Przeprowadzona ocena wykazała, że procesy ciemnienia w korzeniach marchwi i owocach jabłoni zależały od gatunku. Korzenie marchwi podlegały mniej procesom ciemnienia, przy czym nalistne nawożenie magnezem ograniczało ciemnienie, a przechowywanie sprzyjało temu procesowi. W przypadku jabłek dobór odmiany stanowił najważniejszy czynnik w ograniczeniu procesów ciemnienia, co odgrywa znaczącą rolę w wyborze surowca do produkcji soków czy suszu jabłkowego. Ponadto podczas przechowywania zwiększało się ciemnienie, ale mniej w porównaniu do korzeni marchwi.

Słowa kluczowe: ciemnienie enzymatyczne, korzenie marchwi, jabłka, przechowywanie, nawożenie, ULO