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CHANGES OF THE COLOUR, TOTAL SUGAR CONTENT AND RESPIRATION RATE OF BROCCOLI HEADS DURING SHORT-TERM STORAGE

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I TEMPA ODDYCHANIA RÓŻ BROKUŁU
W CZASIE KRÓTKOTRWAŁEGO PRZECHOWYWANIA

Summary. The objective of this study was to investigate the effect of short-term storage (24–96 h) at different temperatures (3°C, 16°C and 21°C) on the colour, respiration rate and total soluble sugar content in broccoli heads. The respiration rate of broccoli heads was also measured at 21°C during 24 h. The results of the study revealed that the colour change (hue angle and L) was the highest in the broccoli heads kept at 3°C after one-day storage. On the fourth day of storage the hue value decreased at 21°C and brightness decreased at 3°C. The respiration rate, measured at 21°C during 24 h, was the highest during the first three hours, later it decreased, and between 10 and 24 h it was almost at the same level. The respiration rate measured on the day of harvest at 16°C and 21°C were almost five times higher than at 3°C. After the 24 and 96 h of storage, the respiration rate was similar at each of the three storage temperatures. There was a significant decrease in the total soluble sugar content only after 96 h of storage at the temperature of 21°C.

Key words: *Brassica oleracea* var. *italica*, storage, hue value, respiration rate, sugars

Introduction

Broccoli (*Brassica oleracea* var. *italica*) is a very common vegetable with high nutritional value. It is highly valued due to its richness in vitamins, antioxidants, anticarcinogenic compounds (Nestle, 1998) and health-promoting phytochemicals (Yuan et al., 2010), which include vitamins C and E, flavonoids, carotenoids and glucosinolates

(Podsędek, 2007). These compounds have gained attention because of their role in the prevention of cancer and cardiovascular diseases (Finley et al., 2000).

When harvested many vegetables are subjected to considerable stress due to the sudden disruption of energy, nutrient and hormone supplies (King et al., 1990). Within 24 h following the harvest expression of many senescence-related genes is induced (Eason et al., 2005; Gapper et al., 2005). The postharvest senescence of broccoli is associated with yellowing, chlorophyll degradation, accumulation of pheophytin and ascorbate decline (Costa et al., 2005; Nishikawa et al., 2003).

Respiration is at the root of compositional changes that result in deterioration of quality and closely depend on temperature (Nei et al., 2005). The suppression of respiration rate during postharvest is beneficial to maintenance of freshness and quality (Techavuthiporn et al., 2008). The respiration rate is closely related to soluble sugar content. The sugar content is dependent on the diurnal cycle. In higher plants starch is the major carbohydrate with many important functions. Starch is accumulated during the day and is remobilized at night to support continued respiration (Geiger and Servaites, 1994). According to Sulpice et al. (2009), starch is the major integrator in the regulation of plant growth. King and Morris (1994) noticed that the respiration rate of broccoli declined rapidly during the first 12 h after harvest. But in the literature on the subject there is little information about changes in the respiration rate during a several days' period of storage.

The aim of the study was to assess the influence of short-term storage (1–4 days) of broccoli heads at different temperatures on their colour, total soluble sugar content and respiration rate.

Materials and methods

Plant material

Two series of experiment on the influence of storage time (24–96 h) and temperature (3°C, 16°C and 21°C) on physiological and chemical changes in broccoli heads were conducted in 2011. Each combination consisted of five heads. The first series was carried out from 5 to 9 September, and the other one from 19 to 23 September. The research was performed on one cultivar, 'Tiburon'. The heads for the experiments were collected from the broccoli planted on 7 July at the stage of four leaves, spaced 0.5 × 0.5 m. The experiment was established on the podzolic soil, the arable layer of which was loamy sand underlying sandy loam. The soil was tested before planting and 90 $kg \cdot ha^{-1}$ of P_2O_5, 140 $kg \cdot ha^{-1}$ of K_2O and 100 $kg \cdot ha^{-1}$ of N were applied before soil cultivation. Additionally, nitrogen (100 $kg \cdot ha^{-1}$) was applied in three doses in the growing season. The plants were watered when the soil water potential had exceeded –0.04 MPa. The chemical analyses were carried out on frozen material (–20°C).

Colour

The colour of the broccoli was measured with the spectrometer StellarNet Black-Comet UV-VIS on each day of storage. The determined parameters were as follows:

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"L" – brightness, ranging from no reflection for black (L = 0) to perfect diffuse reflection for white (L = 100); "a" – redness, ranging from negative values for green to positive values for red; "b" – yellowness, ranging from negative values for blue to positive values for yellow. Four different positions on the surface of each broccoli head were measured. The colour was reported as hue angle ($h° = 180° + \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$), as previously described by Zaicovski et al. (2008).

Respiration rate

The respiration rate of broccoli was measured with the LCpro+ system (ADC BioScientific) on the basis of elimination of CO_2 in darkness after harvesting of broccoli heads from the field (day of storage) and after 24 h, and after 96 h of storage at the temperatures of 3°C, 16°C and 21°C. Additionally, the respiration rate was measured for 24 h at the temperature of 21°C. All measurements were taken every minute. During the measurement of respiration the system automatically maintained the set temperature in the chamber and the content of CO_2 in the air was kept at the level of 360 ppm. There were two repetitions of each measurement for all combinations.

Total soluble sugar content

In order to determine the total content of soluble sugar in broccoli heads the anthrone method was applied. It consists in extracting sugars from a 5 g frozen sample and adding 100 ml of distilled water. The prepared material was placed in an MPW-251 laboratory centrifuge (Med. Instruments) for 10 min and centrifuged at the rotational speed of 5000 revolutions per minute. Next, 1 ml of centrifuged solution was used for further analysis and it was filled with distilled water up to 100 ml. From the diluted solution 1 ml was taken and placed in a test tube with 5 ml of anthrone reagent. The anthrone reagent was obtained by dissolving 160 ml of anthrone in 100 ml of 80% sulphuric acid.

The extract and the reagent were placed in an MLL 547 water bath (AJL Electronic) for 10 min at a temperature of 90°C. When the solution cooled down, it was tested for absorbance at 620 nm by means of a Genesys 10VIS spectrometer (Thermo Electron Corporation).

The percentage of sugar (% CHO) was calculated according to the following formula:

$$\%\,\text{CHO} = \frac{w}{M} - \frac{y}{10}\ (\%)$$

where: w – sugar concentration ($mg \cdot l^{-1}$) based on absorbance measurement, read from glucose model curve, y – dilution (cm^3), M – weight of sample for analysis (mg).

Statistical analysis

All statistical analyses were carried out with the Stat program. The significance of the impact of temperature and duration of storage on the colour (hue angle) and brightness of broccoli and total soluble sugar content was determined with the F test. Differences between the mean values were estimated with the Duncan test at the significance level P = 0.05.

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Results and discussion

Broccoli colour is the basic visual component of senescence (Serrano et al., 2006) and can be shown as the hue angle (Tijskens et al., 2001). According to Pramanik et al. (2006), the broccoli colour is closely related to the storage temperature and duration. The broccoli stored at the temperature of 1°C for 7 and 14 days retained their dark green colour, whereas those stored at 20°C became completely yellow on the day following harvest. In our experiment during storage at the temperature of 3°C there was a high increase in the hue value and L value after the 24 hours of storage (Tables 1 and 2).

Table 1. Colour (hue angle) of broccoli heads stored at 3°C, 16°C and 21°C for 24–96 h
Tabela 1. Ton barwy róż brokułu przechowywanych w 3°C, 16°C i 21°C przez 24–96 h

Temperature Temperatura (°C)	Day of harvest Dzień zbioru	Storage time – Czas przechowywania				Mean Średnia
		24 h	48 h	72 h	96 h	
3	115.0 cd	125.0 a	122.0 ab	122.0 ab	121.0 abc	120.9 a
16	114.0 cd	120.0 abc	120.0 abc	118.0 bc	116.0 cd	117.5 b
21	118.0 bc	118.0 bc	120.0 abc	116.0 bc	110.0 d	116.5 b
Mean – Średnia	115.6 b	120.7 a	120.6 a	118.9 a	115.4 b	

*The mean values followed by the same letters do not differ significantly at P = 0.05.
*Średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0,05.

Table 2. Brightness of broccoli heads stored at 3°C, 16°C and 21°C for 24–96 h
Tabela 2. Jasność róż brokułu przechowywanych w 3°C, 16°C i 21°C przez 24–96 h

Temperature Temperatura (°C)	Day of harvest Dzień zbioru	Storage time – Czas przechowywania				Mean Średnia
		24 h	48 h	72 h	96 h	
3	56.4 d	76.3 a	66.2 bc	66.9 b	58.1 cd	64.8 a
16	58.3 cd	60.5 bcd	60.6 bcd	61.2 bcd	60.5 bcd	60.2 b
21	59.1 bcd	59.7 bcd	60.2 bcd	61.1 bcd	62.5 bcd	60.5 b
Mean – Średnia	57.9 c	65.5 ab	62.3 ab	63.02 ab	60.4 bc	

*The mean values followed by the same letters do not differ significantly at P = 0.05.
*Średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0,05.

This could have been caused by accumulation of anthocyans, the content of which increases due to the plant's response to the low temperature stress (Gilmour et al., 1988). Moreno et al. (2010) identified several basic anthocyanic compounds in the broccoli head. The content of the compounds largely depended on the broccoli cultivar. In the presented studies during the storage at the temperature of 3°C the hue value was

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similar from the 48 h to the 96 h of storage, but the L value decreased. In the period from 24 to 72 h of storage at the temperature of 21°C there were no significant changes in the hue value, but after the 96 h of storage the hue value decreased significantly. A decrease in the hue value after 72 h of storage at the temperature of 20°C was also confirmed by Hasperué et al. (2011). DeEll and Toivonen (1999) observed a rapid decrease of the hue value after 24 h of storage at 18°C. In the experiment conducted by Hasperué et al. (2011) the L value increased along with the duration of storage. Similarly, in our experiment after 96 h of storage at the temperatures of 16°C and 21°C the L value increased slightly, but the difference between its values after the individual periods of storage was not statistically significant.

Toivonen (1997) found that the change in the broccoli colour was strictly related to the respiration rate, which depended on the temperature and duration of storage. As the duration of storage at 1°C increased, the respiration rate decreased, which reflected reduced in metabolic activity of broccoli, and, in consequence, degradation of chlorophyll. According to Nei et al. (2005) the respiration rate of broccoli in the temperature range 10 and 40°C decreased as time elapsed. After 9 h after beginning of the experiment the respiration rate was 53–84% of initial values. In the research by Finger et al. (1999) the respiration rate decreased by 50% during the first 24 h of storage at the temperature of 25°C, and then it increased slightly. King and Morris (1994) arrived at similar conclusions. In their experiment, the respiration rate decreased from more than 600 mg CO_2 per 1 kg·h^{-1} to about 200 mg CO_2 per 1 kg·h^{-1} within 24 h. There was a different course of the respiration rate in our experiment (Fig. 1), where the measurements were taken at the temperature of 21°C.

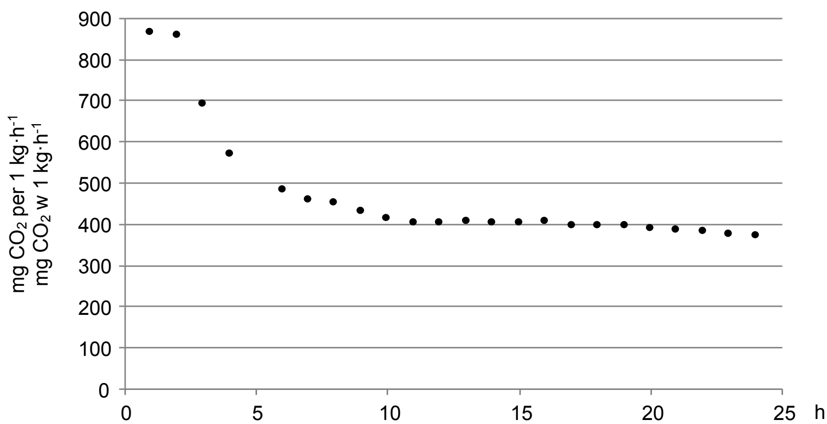

Fig. 1. Changes in the respiration rate of broccoli heads during storage at 21°C within 24 h

Rys. 1. Zmiany w tempie oddychania róż brokułu w ciągu 24 h przechowywania w temperaturze 21°C

The respiration rate of broccoli heads was the most intensive during the first three hours after harvesting (more than 800 mg CO_2 per 1 kg·h^{-1}), then it decreased and re-

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mained almost at the same level (about 400 mg CO_2 per 1 kg·h^{-1}) from 10 to 24 h of storage. The differences between our findings and those reported by King and Morris (1994), and by Finger et al. (1999) may have resulted from the different size of the broccoli heads, which were measured. This correlation was also confirmed by Tian et al. (1995), who observed that the increase in the respiration rate during the first 24 h after harvesting was inversely proportional to the size of the broccoli head. Another dependence was noticed by Irving and Baird (1996), Seefeldt et al. (2012) and Martínez-Hernández et al. (2013) who proved that the respiration rate of the broccoli depended on the cultivar. According to Seefeldt et al. (2012) the early summer cultivar had an almost three times higher average respiration rate than late summer cultivar.

Our experiment also proved that the respiration rate at 21°C (Fig. 2) after the fourth day of storage was at a similar level or there was a slight increase observed in comparison to the results after the first 24 h of storage (Fig. 1).

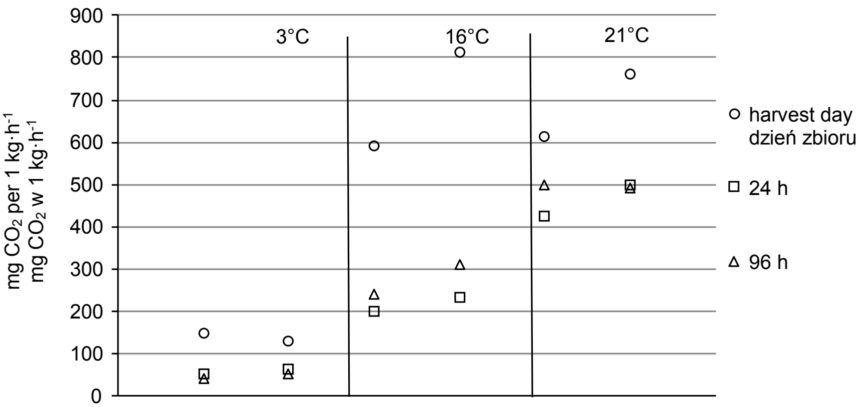

Fig. 2. Respiration rate of broccoli heads on the harvest day and after 24 and 96 h of storage at 3°C, 16°C and 21°C

Rys. 2. Tempo oddychania róż brokułu w dniu zbioru oraz po 24 i 96 h przechowywania w temperaturze 3°C, 16°C i 21°C

The respiration rate of the broccoli heads which were placed at the temperature of 3°C immediately after harvest ranged from 130 to 150 mg CO_2 per 1 kg·h^{-1}. After the 24 h of storage the respiration rate decreased and ranged from 40 to 70 mg CO_2 per 1 kg·h^{-1}. It remained at the same level until 96 h of storage. The respiration rate of the heads which were placed at the temperature of 16°C immediately after harvest was very high. Its values were similar to the respiration rate of the heads placed at the temperature of 21°C. After 24 h of storage at the temperature of 16°C the respiration rate decreased considerably and did not exceed 230 mg CO_2 per 1 kg·h^{-1}. After 96 h of storage the respiration rate was at a similar level.

In our experiment the content of sugar in broccoli heads decreased after 96 h of storage at the temperature of 21°C (Fig. 3).

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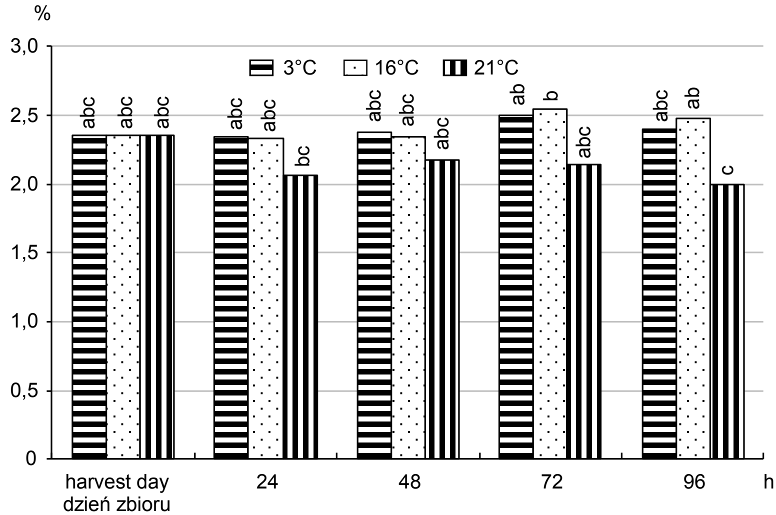

Fig. 3. Total soluble sugar content in broccoli heads during storage at 3°C, 16°C and 21°C (the mean values followed by the same letters do not differ significantly at P = 0.05)
Rys. 3. Ogólna zawartość cukrów w różach brokułu w czasie przechowywania w temperaturze 3°C, 16°C i 21°C (średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0,05)

Some authors investigated changes in the content of sugars as early as the first hours following the harvest of broccoli heads. King and Morris (1994), found a significant decrease in the content of water-soluble sugars after 6 h of storage, whereas Tian et al. (1997), the content decreased after 10 h of storage at temperature of 20°C. Tian et al. (1997) also noticed that the content of sugar decreased very slowly during the four consecutive days. Hasperué et al. (2011), stated the variation in the content of water-soluble sugar during 5 days of storage depended on the time of the day when the heads were harvested. According to these authors, variation in the content of sugar was connected with the starch content. The sugars which are produced in the process of photosynthesis during the day are metabolised into starch. The reverse process takes place at night. After harvest starch becomes degraded rapidly and the products of degradation are metabolised into soluble sugars. Consequently, the level of water-soluble sugar in the tissues is higher for a longer period of time. This might account for the findings of our experiment, where the minimal decrease in the content of sugar was observed during the storage time.

Conclusions

1. The highest hue angle value was obtained after 96 h of storage at the 3°C, whereas the brightness values after 96 h of storage in the tested temperatures were similar.

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2. The higher the temperature of storage the higher the respiration rate was of broccoli heads.

3. The total soluble sugar content significantly decreased after 96 h of storage at 21°C.

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ZMIANY BARWY, OGÓLNEJ ZAWARTOŚCI CUKRÓW I TEMPA ODDYCHANIA RÓŻ BROKUŁU W CZASIE KRÓTKOTRWAŁEGO PRZECHOWYWANIA

Streszczenie. Celem doświadczenia było określenie wpływu krótkotrwałego przechowywania róż brokułu (24–96 h) w różnych temperaturach (3°C, 16°C i 21°C) na ich barwę, tempo oddychania i ogólną zawartość cukrów. Dodatkowo tempo oddychania było mierzone co minutę w temperaturze 21°C w okresie 24 h. Na podstawie uzyskanych wyników stwierdzono, że największa zmiana barwy (ton barwy i jasność) była po 24 h przechowywania w temperaturze 3°C. Po 96 h przechowywania w 21°C zmniejszyła się istotnie wartość tonu barwy, a w 3°C – wartość jasności barwy. Tempo oddychania mierzone w 21°C przez 24 h było największe w ciągu pierwszych trzech godzin, później spadło i pomiędzy 10. a 24. godziną przechowywania pozostało na tym samym poziomie. Tempo oddychania mierzone w dniu zbioru w temperaturze 16°C i 21°C było prawie pięciokrotnie większe niż tempo oddychania w 3°C. Po 24 i 96 h przechowywania tempo oddychania miało podobną wartość w każdej z trzech temperatur przechowywania. Istotny spadek zawartości cukrów ogółem w różach brokułu stwierdzono dopiero po 96 h przechowywania w temperaturze 21°C.

Słowa kluczowe: *Brassica oleracea* var. *italica*, przechowywanie, odcień barwy, tempo oddychania, cukry

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