

PHYSIOLOGICAL CHANGES DURING POSTHARVEST SENESCENCE OF BROCCOLI¹

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ABSTRACT - The objective of this work was to determine the early physiological changes throughout shelf life of fresh broccoli (*Brassica oleracea* L. var. *italica*) cv. Piracicaba Precoce at 25°C and relative humidity of 96% in the dark until complete senescence. Head inflorescences showed lack of turgidity and commercial value when weight loss reached up to 5%, coinciding with 48 hour after harvest. Chlorophyll content was stable until 24 hours after harvesting; afterwards, an intense degradation phase took place. At 72 hours, total head yellowing was observed when chlorophyll content dropped to 30% of its initial content. Peroxidase activity increased by 1.4 fold during the first six hours, dropping to its lowest level approximately 24 hours after harvesting. However, from this time on, an increment of activity was observed until 72 hours. At 24 hours after harvesting, respiration was reduced by 50%. At later stages of senescence, respiration of florets was stable, but in a lower level than that determined at harvest. Sharp reduction of starch and reducing sugars was observed within 24 hours after harvesting, followed by continuous period of decline in starch and non-reducing sugars.

Index terms: chlorophyll, peroxidase, respiration, starch, soluble sugars.

ALTERAÇÕES FISIOLÓGICAS DA SENESCÊNCIA PÓS-COLHEITA DE BRÓCOLIS

RESUMO - Alterações fisiológicas ocorridas durante a vida de prateleira em inflorescências de brócolis (*Brassica oleracea* L. var. *italica*) cv. Piracicaba Precoce foram avaliadas durante armazenamento a 25°C e umidade relativa de 96%. A perda de turgidez e de valor comercial das inflorescências ocorreu 48 horas após a colheita, totalizando 5% de perda da matéria fresca. O conteúdo de clorofila permaneceu inalterado até 24 horas após a colheita, seguindo-se um período de intensa degradação. As inflorescências estavam completamente amarelas 72 horas após a colheita, quando apresentavam 30% do teor inicial de clorofila. A atividade peroxidativa elevou-se em 1,4 vezes nas seis horas subseqüentes à colheita, diminuindo até 24 horas, seguido de aumento da atividade até 72 horas pós-colheita. A respiração foi reduzida em 50% nas primeiras 24 horas após a colheita, e permaneceu estável nos estádios finais do amarelecimento, mas com taxas inferiores às observadas na colheita. Os teores de amido e açúcares redutores sofreram quedas acentuadas nas 24 horas subseqüentes à colheita, bem como uma fase de persistente declínio do amido e de açúcares não-redutores.

Termos para indexação: clorofila, peroxidase, respiração, amido, açúcares solúveis.

INTRODUCTION

Fresh broccoli deteriorates quickly once harvested, mainly due to relatively high respiration

and susceptibility to wilting when stored at room temperature (Gillies & Toivonen, 1995). Since freshly harvested broccoli is composed by immature tissues, the inflorescence head shows rapid senescence and chlorophyll degradation (Clarke et al., 1994; Corcuff et al., 1996). Wang (1977) studied the length of broccoli shelf life and observed that after two or three days at 20°C the florets showed intense yellowing caused by sepal chlorophyll degradation. In addition, during the early hours after harvest, broccoli branchlets stored at 20°C showed sharp reduction of glucose and fructose

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contents (King & Morris, 1994). This might be related to the high respiration rate observed in this vegetable (Kader, 1987; King & Morris, 1994; Tian et al., 1995).

In Brazil several harvested fresh vegetables, such as broccoli, cauliflower and cabbage are not subjected to pre-cooling or refrigeration until they reach the consumer, which usually takes on average from one to three days. During this period, significant composition alterations might occur, affecting their quality and nutritional value.

The aim of this work was to investigate some physiological changes in the early shelf life of broccoli stored in the dark at room temperature (25°C) and high relative humidity (96%).

MATERIAL AND METHODS

Inflorescences of broccoli (*Brassica oleracea* L. var. *italica*) cv. Piracicaba Precoce were harvested when the heads were completely developed and without opened florets. The harvest of the inflorescences was carried out between 7 and 8 a.m. and immediately transported to the laboratory for disinfection in 1% sodium hypochloride solution for 15 minutes, followed by repeated washing with distilled water. Then, the heads were kept chambers under air flow of 60 liter hour⁻¹ at 25°C and relative humidity of 96% until complete senescence. The storage was done under dark conditions, in order to avoid any interference of photosynthesis on chlorophyll breakdown and CO₂ evolution.

The heads were weighed at harvest, 6, 12, 24, 48 and 72 hours after harvesting, and the losses of weight were expressed as percentage of fresh weight at harvest. The chlorophyll content of florets was evaluated according to Arnon (1949) and expressed as percentage of the harvest content.

Peroxidase activity in the florets was determined according to Lagrimini & Rothstein (1987).

For respiration determination, samples of florets with approximately 15 g of fresh weight were obtained from the heads and the CO₂ production evaluated with an IRGA equipped with a 1.5 liter chamber and a flow rate of 36 liter hour⁻¹ at 20°C in the dark.

For soluble sugar analysis, 2 g of florets were extracted three times with boiling 80% ethanol. The ethanolic fraction was used for soluble and reducing sugars determinations (Hodge & Hofreiter, 1962). The pellet resulting from the ethanol extraction was treated with 30% perchloric acid for starch determination (McCready et al.,

1950). For dry weight determinations, samples of florets were oven dry at 70°C. All measurements were carried out in four replicates, composed by one head per replicate. Data from carbohydrates changes during storage were submitted to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The rate of fresh weight loss of the inflorescence head was somewhat constant throughout shelf life (Fig. 1A), this behavior shows that, in broccoli, like many other vegetables, the rate water loss is mainly dependent of temperature and humidity of the storage room (Finger & Vieira, 1997). This constant rate on weight loss was also observed in broccoli stored at 13°C and 95% relative humidity, in wrapped or unwrapped heads with micro-perforated film (Toivonen, 1997). The loss of inflorescence turgidity, evaluated by the presence of wilting, occurred when the dehydration reached up to 5%, coinciding with 48 hours after harvest.

There was no significant chlorophyll degradation up to 24 hours after harvesting. However, an intense degreening in the florets took place after this period. At 72 hours of shelf life, the chlorophyll accounted for only 30% of its initial content (Fig. 1B) and complete yellowing of the inflorescence florets was observed. Clarke et al. (1994) also observed similar trend of chlorophyll degradation in florets during shelf life of broccoli cv. Shogun.

Specific activity of peroxidase had 1.4 fold increment over the first six hours after harvest, decreasing to its lowest level at 24 hours (Fig. 2). The initial increment in broccoli peroxidase activity seems to be caused by the stress of harvest. Simon & Ross (1970) observed an increase of peroxidase activity following the injury of tobacco plants, which was spread out systemically throughout non damaged tissues. The intense drop in peroxidase activity after six hours from harvest, might reflect the end of the harvest stress effects.

The period following the first 24 after harvest was characterized by continuous increment of the peroxidase activity (Fig. 2), this may indicate the initial loss of membrane integrity (Thomas & Stoddart, 1980). It has been proposed that several degrading reactions occur during organelles destruc-

tion, including the synthesis of hydrogen peroxide (Elstner, 1982). Moreover, the presence of hydrogen peroxide increases the degreening in the flavedo tissues of satsuma mandarin fruits by peroxidase chlorophyll degrading pathway (Yamauchi et al., 1997). Therefore, in broccoli, the increase in peroxidase activity might represent a mechanism to eliminate hydrogen peroxide produced during later stages of degreening.

The results presented here show that weight loss, chlorophyll degradation and peroxidase activity seemed to be good indicators of broccoli senescence.

Florets respiration rate dropped by 50% in the first 24 hours after harvest, followed by slight

increase at later stages of shelf life (Fig. 3). The behavior of respiration observed here was similar to that observed by King & Morris (1994) working broccoli cv. Green Belt stored at 20°C. On the other hand, Tian et al. (1995) observed an increase of respiration up to 24 hours after harvesting for cv. Shogun, and this was inversely proportional to floret size. Afterwards, the respiration declined up to 48 hours after harvesting, followed by a period of slight increase or stabilization, depending on the floret size. However, Irving & Baird (1996) observed marked increments in the respiration of floret for the same cultivar stored at 20°C between 24 and 96 hours after harvesting. Therefore, it seems possible that the postharvest changes of respiration, in broccoli, might be cultivar dependent.

High contents of carbohydrates in detached organs are related to longer postharvest shelf life periods in vegetables like lettuce, cabbage and kale (Lipton, 1987; Amarante & Puschmann, 1993). Here, starch content degraded immediately after harvest, showing total loss (significant at $P \leq 0.05$) of 67% after 72 hours of storage (Fig. 4). A similar result was reported for broccoli cv. Shogun, where starch con-

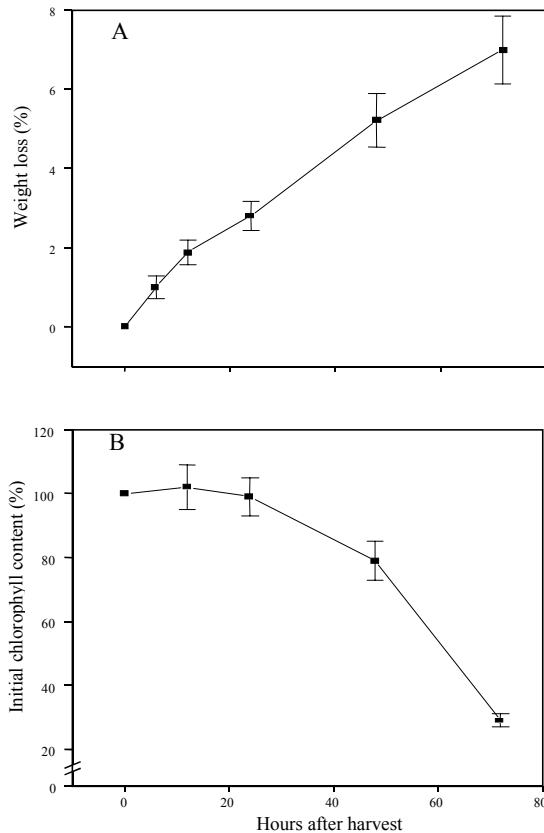


FIG. 1. Weight loss (A) and changes in chlorophyll content (B) of broccoli inflorescences throughout storage at 25°C and 96% relative humidity in the dark. Bars represent standard error.

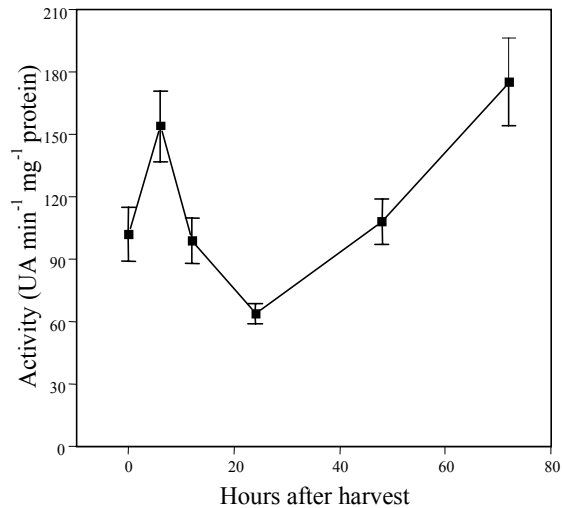


FIG. 2. Peroxidase specific activity during storage of broccoli inflorescences in the dark at 25°C and 96% relative humidity. Bars represent standard error.

tent decreased by 86% after 24 hours of storage at 20°C (Tian et al., 1997).

Associated with the starch drop, there was an increase of 31% in non-reducing sugars concentration

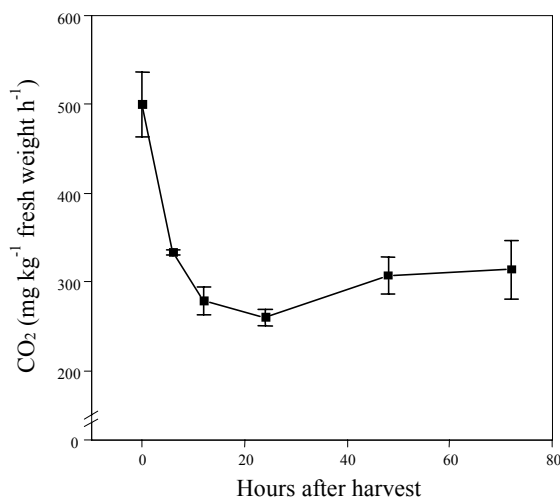


FIG. 3. Respiration of broccoli florets during storage in the dark at 25°C and 96% relative humidity. Bars represent standard error.

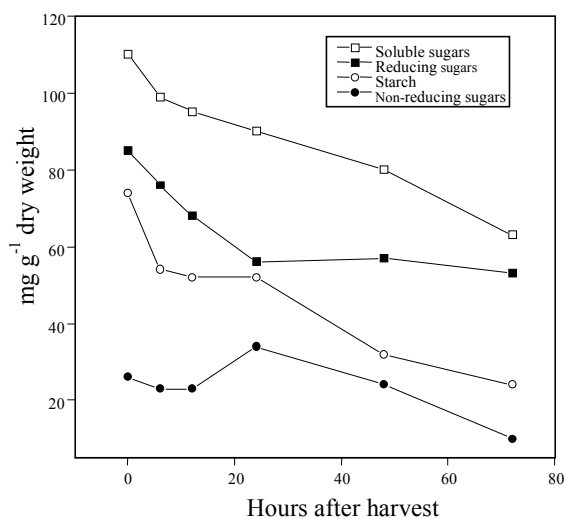


FIG. 4. Changes in starch, non-reducing sugars, total soluble sugars and reducing sugars content in broccoli florets stored in the dark at 25°C and 96% relative humidity.

(significant at $P \leq 0.05$) at 24 hours after harvesting (Fig. 4). This clearly shows that starch breakdown keeps the pool of soluble sugars in the florets. Towards the end of storage life, the non-reducing sugars showed persistent drop, and at 72 hours, the florets had consumed 71% its content at 24 hours after harvesting (significant at $P \leq 0.05$) (Fig. 4). However, it has been reported by Downs et al. (1997) that sucrose content in broccoli cv. Shogun declined by 50% during the first six hours after harvesting.

As starch and reducing sugars contents are quickly reduced within 24 hours after harvesting, the decrease of reducing sugars seems to be associated with the losses of total soluble sugars (Fig. 4). The relationship between the changes of starch and soluble sugars showed that starch and reducing sugars are the main source of respiratory substrate during the initial postharvest life of broccoli, while at later stages, starch and non-reducing sugars have been more intensely metabolized. Our results showed that the short shelf life of broccoli is related to a marked depletion of carbohydrates, probably induced by intense respiration rates.

CONCLUSIONS

1. Inflorescences of broccoli show a short shelf life due to intense weight loss and chlorophyll degradation throughout storage at room temperature.
2. Inflorescences have high respiratory rate and show increase on peroxidase activity at later stages of senescence.
3. Carbohydrates are intensely metabolized throughout shelf life due to high respiratory activity.

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