

Respiratory activity and pigment metabolism in fresh-cut beet roots treated with citric acid

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ABSTRACT

Beet roots cv. Early Wonder were graded for firmness, color and size, and were peeled inside a cold room (10°C). Roots were then shredded (2 mm thick), sanitized for 6 minutes (NaClO/200 mg L⁻¹), rinsed and centrifuged. Fresh-cut beet roots were then treated for 5 minutes with citric acid in the following concentrations: 0 (control), 500; 1,000; 1,500 and 2,000 mg L⁻¹. The material was centrifuged again, placed in trays, wrapped with PVC plastic film, and stored at 5°C and 85-90% HR for 10 days. Every two days, treatments were analyzed for respiratory activity and betacyanin and betaxanthin contents. The application of citric acid caused the reduction of respiratory rate in the fresh-cut tissue. In the second day of storage, control showed the highest respiratory activity among treatments, reaching around 77 mL CO₂ kg⁻¹ h⁻¹. Citric acid solution dips in a concentration higher than 500 mg L⁻¹ contributed to a decrease in respiratory activity and no peak in CO₂ evolution was observed. There were significant differences among all treatments during the storage period for the contents of betacyanin, which were around 40; 45; 48; 51 and 55 mg 100 g⁻¹ for the fresh-cut material treated with 0; 500; 1,000; 1,500 and 2,000 mg L⁻¹ of citric acid, respectively. Also, levels of betaxanthin were around 25; 29; 33; 35 and 39 mg 100 g⁻¹ for the material treated with 0; 500; 1,000; 1,500 and 2,000 mg L⁻¹ of citric acid, respectively. The application of citric acid after minimal processing apparently have the ability to reduce respiratory rate and the degradation of pigments, which contributes to extend the shelf life of the fresh-cut product.

Keywords: *Beta vulgaris* L., respiration, betacyanin, betaxanthin, minimal processing.

RESUMO

Atividade respiratória e metabolismo dos pigmentos de beterrabas minimamente processadas tratadas com ácido cítrico

Beterrabas 'Early Wonder' foram selecionadas quanto à firmeza, cor e tamanho e descascadas em câmara fria (10°C). As raízes foram cortadas (2 mm de espessura), sanitizadas, enxaguadas e centrifugadas. As beterrabas foram tratadas com ácido cítrico nas concentrações: 0 (controle), 500; 1000; 1500 e 2000 mg L⁻¹. O produto foi novamente centrifugado, colocado em bandejas envoltas com filme de PVC e armazenado a 5°C e 85-90% de umidade relativa durante 10 dias. A cada dois dias, foram avaliados a atividade respiratória e os teores de betacianina e betaxantina nos tratamentos. A aplicação de ácido cítrico nas diferentes concentrações reduziu a taxa respiratória nos tecidos minimamente processados. No segundo dia de armazenamento, o tratamento controle apresentou a maior atividade respiratória alcançando valores próximos a 77 mL CO₂ kg⁻¹ h⁻¹. A aplicação de ácido cítrico igual ou maior a 500 mg L⁻¹ contribuiu para o decréscimo da atividade respiratória e o pico na evolução de CO₂ não foi observado. Foi verificada diferença significativa entre os tratamentos durante todo o armazenamento para os teores de betacianina, tendo sido observados valores ao redor de 40; 45; 48; 52 e 55 mg 100 g⁻¹ para os produtos tratados com 0; 500; 1.000; 1.500 e 2.000 mg L⁻¹, respectivamente. Os níveis de betaxantina para beterrabas minimamente processadas foram ao redor de 25; 29; 33; 35 e 39 mg 100 g⁻¹ para os tratamentos de 0; 500; 1.000; 1.500 e 2.000 mg L⁻¹, respectivamente. A aplicação de ácido cítrico é sugerida durante o processamento mínimo como forma de reduzir a taxa respiratória e a degradação de pigmentos, contribuindo assim para o aumento da vida de prateleira de produtos minimamente processados.

Palavras-chave: *Beta vulgaris* L., betalaína, betacianina, betaxantina, processamento mínimo.

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During the past few years fresh-cut products have grown rapidly both in the foodservice sector and at the retail level. Focusing consumer convenience, they are prepared and handled aiming at offer a fresh and healthy product (Moretti & Sargent, 2002). The obtaining of fresh-cut products involves many steps such as cleaning, washing, trimming, coring, slicing, shredding, and other related operations (Cantwell, 2000).

Fresh-cut business is an activity showing exponential increase in many countries, especially in large metropolitan areas, such as São Paulo, one of the biggest cities in Brazil, where supermarkets sell around US\$ 4 million of fresh-cut products monthly, considering both fruits (54%) and vegetables (46%) (Rojo & Saabor, 2002).

In general, fresh-cut products have a short shelf life, which is mainly due to the

mechanical stresses. In the cut surface, cells and membranes are damaged leading to alterations in the whole tissue metabolism. Many authors have observed increasing in carbon dioxide and ethylene evolution (Brecht, 1995; Moretti *et al.*, 2000, 2002a), water loss (Moretti & Sargent, 2000), alterations in flavor and aroma (Moretti *et al.*, 2002b), decline in levels of nutrients such as ascorbate (Gil *et al.*, 2006), development of off-odors

(Beaulieu, 2006), and increase in the activity of enzymes related to enzymatic browning, e.g., phenylalanine-ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) (Degl'Innocenti *et al.*, 2005). Kato-Noguchi & Watada (1997) observed that respiratory activity of fresh-cut carrots was drastic reduced by the addition of citric acid, which is related to the inhibition of phosphofruktokinase, which catalyzes the phosphorylation of fructose 6-phosphate into fructose 1,6-bisphosphate, in the glycolytic pathway of the respiratory metabolism.

Among many fruit and vegetable crops that are being processed in Brazil, beet roots are increasing in importance. They are pink-reddish in color due to the presence of a group of pigments named betalain, which was misdesignated as anthocyanin due to the presence of nitrogen in their molecule. These pigments were soluble in water and are divided in two groups: betacyanin (related with the red color) and betaxanthin (related with the yellow color). These two groups of pigments are present in beet roots and are involved with the characteristic color of this vegetable crop (Fenema, 1995).

One of the main technological problems found by processors dealing with fresh-cut beet roots is the significant discoloration and dehydration of the minimally processed material. Washing and rinsing operations carried out after slicing have favored the loss of betacyanin and betaxanthin, since these pigments are soluble in water (Nilson, 1970).

The factors mentioned above, the economical importance of this vegetable crop, as well as the lack of information in the consulted literature regarding the study of fresh-cut beet roots focusing pigments degradation, the present work was carried out aiming to study the effects of citric acid in the respiratory activity and in the content of betalain in fresh-cut beet roots.

MATERIAL AND METHODS

Beet roots cv. Early Wonder were obtained from a local grower in Piracicaba, São Paulo State, Brazil, and taken to the postharvest laboratory.

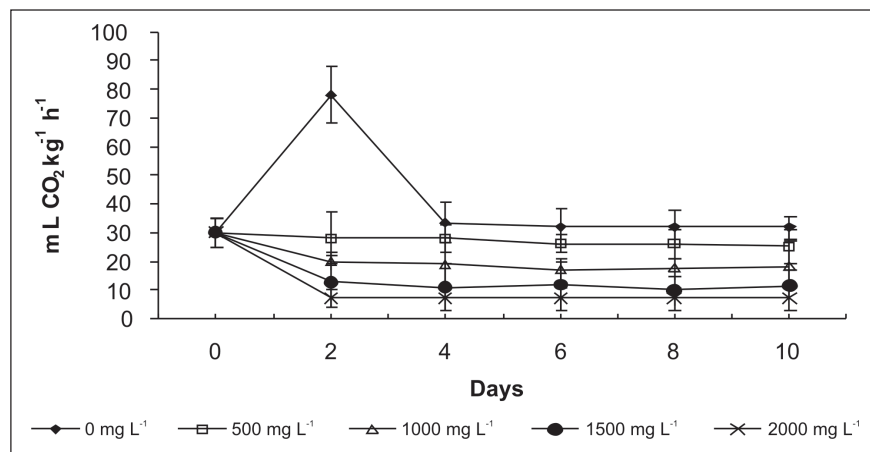


Figure 1. Respiratory activity of fresh-cut beet roots treated with citric acid and stored at 5°C for 10 days. Vertical bars represent \pm SD. (atividade respiratória de beterrabas minimamente processadas tratadas com ácido cítrico e armazenadas a 5°C por 10 dias. Barras verticais representam o desvio padrão da média). Piracicaba, ESALQ, 2007.

Roots were graded for firmness, color and size.

Roots were peeled inside a cold chamber (10 \pm 1°C) and immersed in cold water (5 \pm 0,5°C) for 2 minutes to reduce the abrasion stress. Roots were then shredded (2 mm thick), sanitized for 6 minutes (NaClO/200 mg L⁻¹) in order to reduce the risks of microbiological contamination, and rinsed for 1 minute (3 mg L⁻¹ free chlorine) to remove chlorine in excess. The fresh-cut material was centrifuged at 2,200 rpm for 1 minute to remove the water excess.

Fresh-cut beet roots were then treated for 5 minutes with citric acid in the following concentrations: 0 (control, immersion in water), 500; 1,000; 1,500 and 2,000 mg L⁻¹. The material was centrifuged again in the same conditions mentioned above. Minimally processed beet roots were placed in trays and wrapped with PVC plastic film, 14 mm thick, and stored at 5°C and 85-90% RH for 10 days.

Every two days, the treatments were analyzed for respiratory activity and betacyanin and betaxanthin contents. For the respiratory activity analysis, 180 g of fresh-cut beet roots were placed in a hermetic sealed jar (0,6 L), which was tightly closed and stored at 5C for 1 hour. Air samples were taken from the glass jars using a syringe and the respiratory activity was analyzed in a CO₂ analyzer. Betacyanin and betaxanthin were determined according to Kluge *et al.*

(2006). Two grams of the previous frozen samples were grinded with 5 mL of distilled water. The homogenate was placed in tublets and centrifuged (15,000 rpm) at 4°C for 40 minutes. One mL of the supernatant was added to 24 mL of distilled water in test tubes. The solution was then homogenized in a Vortex and absorbance was read at 476, 538 and 600 nm.

Calculations were carried out according to the following equations: $x = 1,095 (a-c)$; $y = b-z-x/3.1$; and $z = a-x$, where: a = absorbance at 538 nm; b = absorbance at 476 nm; c = absorbance at 600 nm; x = absorbance of betacyanin; y = absorbance of betaxanthin; and z = absorbance of impurities.

Analyses were performed using a completely randomized design, with 30 treatments arranged in a factorial scheme (five concentrations of citric acid and 6 sampling times), with 5 replications. Data were subjected to analysis of variance and the least significance difference (Fisher L.S.D.) procedure was carried out. Differences between any two treatments larger than the sum of two standard deviations were always significant ($pd < 0.05$).

RESULTS AND DISCUSSION

The application of citric acid in different concentrations caused the reduction of CO₂ evolution in the fresh-

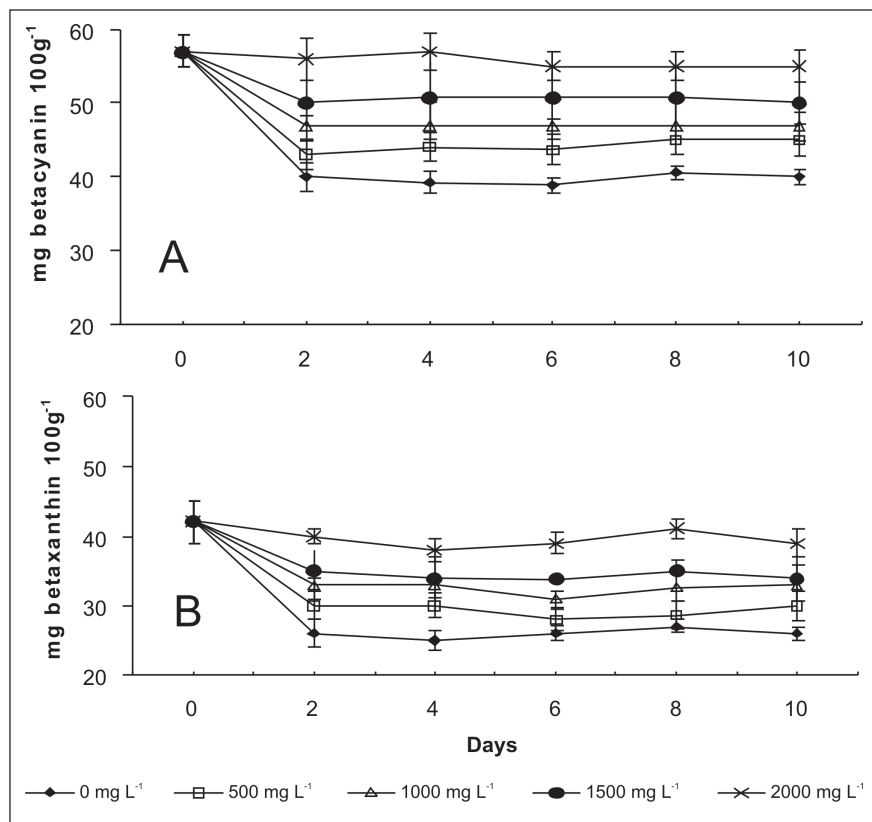


Figure 2. Betacyanin (A) and betaxanthin (B) content of fresh-cut beet roots treated with citric acid and stored at 5°C for 10 days. Vertical bars represent \pm SD. (conteúdo de betacianina (A) e betaxantina (B) de beterrabas minimamente processadas tratadas com ácido cítrico e armazenadas a 5°C por 10 dias. Barras verticais representam o desvio padrão da média). Piracicaba, ESALQ, 2007.

cut tissue (Figure 1). In the second day of storage, control showed the highest respiratory activity among treatments, reaching around 77 mL CO₂ kg⁻¹ h⁻¹. After the fourth day of storage, carbon dioxide evolution of the untreated tissue stabilized around 30 mL CO₂ kg⁻¹ h⁻¹, but remained continuously higher than beet root treated with 1,000 to 2,000 mg L⁻¹ of citric acid (Figure 1).

A citric acid application higher than 500 mg L⁻¹ contributed to a decrease in respiratory activity and no peak in CO₂ evolution was observed (Figure 1). After the second day of storage, carbon dioxide evolution stabilized at 25; 18; 10 and 3.5 mL CO₂ kg⁻¹ h⁻¹ for 500, 1,000; 1,500 and 2,000 mg L⁻¹ of citric acid treatment, respectively.

Results are similar to the findings of Kato-Noguchi & Watada (1997) who observed that the respiratory activity of fresh-cut carrots was reduced when citric acid concentration was increased. The peak in CO₂ evolution verified in

the untreated material was probably related to the stress occurred by the time of processing, which caused a disruption in cell tissues, allowing respiratory substrates reaction with their respective enzymes. This kind of result in minimally processed beet root no treated with citric acid was also observed by Vitti *et al.* (2003). In treated beet roots the stress was reduced as the concentration of citric acid increased. On the other hand, immersion in citric acid solutions caused probably a reduction in the activity of phosphofrutokinase, with subsequent reduction in the synthesis of pyruvic acid in the glycolysis pathway (Kato-Noguchi & Watada, 1997). The subsequent reduction in the respiratory activity observed in the untreated material after the fourth day was probably related to the self-regulation of the respiratory activity due to the high production of ATP (Purvis, 1997).

There were significant differences among treatments during the storage

period for the contents of betacyanin, which were around 40; 45; 48; 51 and 55 mg 100 g⁻¹ for the fresh-cut material treated with 0; 500; 1,000; 1,500 and 2,000 mg L⁻¹ of citric acid, respectively (Figure 2A). On the other hand, the levels of betaxanthin in fresh-cut beet roots were around 25; 29; 33; 35 and 39 mg 100 g⁻¹ for the material treated with 0; 500; 1,000; 1,500 and 2,000 mg L⁻¹ of citric acid, respectively (Figure 2B).

Nilson (1973) observed the content of betacyanin and betaxanthin is cultivar dependent, and the levels of these pigments are around 45 to 210 and 20 to 140 mg 100 g⁻¹, respectively. Thus, due to the processes of sanitation and rinsing, there is a significant loss of color in the fresh-cut material. The immersion in citric acid solutions or other antioxidant appears to protect the tissue against pigment degradation. As the concentration of the citric acid solution increased, the pigment loss decreased (Figure 2). Salgado (1997) verified that treatments with an antioxidant, such as ascorbic acid, increased the stability of betalain in beet roots. The author suggested that 400 mg L⁻¹ was the best concentration to prevent pigment degradation. The combination of citric and ascorbic acids had no synergistic effect in preventing pigment degradation when compared to the application of one antioxidant alone (Attoe & Elbe, 1985).

According to our results, it is suggested that the application of citric acid after minimal processing have the ability of reducing carbon dioxide evolution and minimizing the degradation of pigments, which contributes to extend the shelf life of the fresh-cut product.

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