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HYDROCOOLING AS A TECHNIQUE TO MAINTAIN POSTHARVEST QUALITY AND PROLONG THE SHELF-LIFE OF SWEET BASIL BRANCHES AND LEAVES

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ABSTRACT

Sweet basil is known for its rapid deterioration. Consequently, hydrocooling may serve as a method for preserving the quality and extending the shelf life of vegetables. This study evaluated the impact of hydrocooling on the postharvest quality and shelf life of basil leaves and branches. For this purpose, basil seedlings were produced through vegetative propagation in trays filled with substrate. Once fully rooted, the plants were transplanted into experimental beds. Ninety days of post-transplantation, basil leaves and branches were harvested and transported to the laboratory. In the lab, samples (25 g) of the leaves and branches were selected, weighed, and then immersed in ice water at 4 ºC for 0, 3, 6, and 9 minutes. The samples were wrapped in polyvinyl chloride film and stored at 5 ºC for periods of 2, 4, and 6 days. The results indicated that basil leaves and branches subjected to hydrocooling for 6 minutes experienced less fresh weight loss and maintained their color and visual appeal for up to 4 days. In contrast, those subjected to hydrocooling for 9 minutes exhibited reduced titratable acidity and lipid peroxidation. Hydrocooling durations of 6 and 9 minutes proved to be the most effective in maintaining basil quality for up to 4 days at 5 °C.

Keywords: *Ocimum basilicum* L., hydrocooling, leaves, post-harvest quality, shelf-life.

HIDRORESFRIAMENTO COMO TÉCNICA PARA MANTER A QUALIDADE PÓS-COLHEITA E PROLONGAR A VIDA ÚTIL DE MANJERICÃO

RESUMO

O *Ocimum basilicum* L. é conhecido por sua rápida deterioração pós-colheita. Nesse contexto, o hidroresfriamento pode ser utilizado como uma estratégia para preservar a qualidade e prolongar a vida útil de hortaliças. Este estudo teve como objetivo avaliar o efeito do hidroresfriamento sobre a qualidade pós-colheita e a vida útil de folhas e ramos de manjericão. Para isso, mudas de manjericão foram produzidas por propagação vegetativa em bandejas contendo substrato e, após o completo enraizamento, transplantadas para canteiros experimentais. Noventa dias após o transplante, folhas e ramos foram colhidos e transportados ao laboratório. As amostras (25 g) de folhas e ramos foram selecionadas, pesadas e submetidas à imersão em água gelada a 4 °C por 0, 3, 6 e 9 minutos. Em seguida, foram acondicionadas em filme de policloreto de vinila e armazenadas a 5 °C por períodos de 2, 4 e 6 dias. Os resultados indicaram que folhas e ramos de manjericão submetidos ao hidroresfriamento por 6 minutos apresentaram menor perda de massa fresca, além de melhor manutenção da coloração e do aspecto visual por até 4 dias de armazenamento. Por outro lado, o hidroresfriamento por 9 minutos resultou em menores valores de acidez titulável e peroxidação lipídica. Os tempos de

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hidroresfriamento de 6 e 9 minutos mostram-se mais eficazes para manter a qualidade do manjericão por até 4 dias a 5 °C.

Palavras-chaves: *Ocimum basilicum* L., hidroresfriamento, folhas, qualidade pós-colheita, vida de prateleira.

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INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is an annual or perennial herb that belongs to the Lamiaceae family and features numerous species with different varieties and cultivars (Brindisi and Simon, 2023). Indigenous to India, sweet basil is widely used for garden ornamentation, bee forage, and the production of fresh, dried, and frozen products, as well as essential oils for the food, cosmetic, and pharmaceutical industries. Its remarkable range of appealing aromas and health benefits makes it particularly valuable to consumers in the central tropical and subtropical regions of Asia, Africa, Central America, and South America (Brindisi and Simon, 2023).

Dried sweet basil is widely preferred due to easier transport, storage, and lower post-harvest losses (Sęczyk et al., 2022). However, demand for fresh basil has increased with consumer preference for fresh foods (Abidoye et al., 2022), making proper storage essential to extend shelf life and maintain quality. Fresh basil is highly perishable, showing rapid deterioration that challenges distribution (Akbari et al., 2018; Ciriello et al., 2023). Like other herbs, such as watercress (Nasturtium officinale W.T. Aiton) and mint (*Mentha* spp.), fresh basil shelf life improves at lower temperatures, lasting up to 4 weeks at 0 °C, but only 2 - 4 days at 20 °C (Hruschka and Wang, 1979). A similar effect has been reported for parsley, which remains marketable for 21 - 36 days at 0 °C compared to just 3 days at 20 °C.

In this context, there are numerous post-harvest techniques, including refrigerated storage and hydrocooling, that serve as valuable alternatives for maintaining quality and extending the storage and marketing period of sweet basil. Hydrocooling is a pre-cooling technique that effectively removes field heat from freshly harvested produce, thereby preserving the quality and extending the shelf life of basil sprigs and leaves (Guimarães et al., 2018).

Hydrocooling is a low-cost postharvest technique that can be implemented directly on the farm, contributing to the preservation of vegetable quality and increasing its market value. Cold storage is also an efficient and cost-saving method for preserving fresh produce, as it slows metabolism and reduces moisture loss and deterioration (Chitarra and Chitarra, 2005). Basil stored at 5 °C shows lower weight loss and higher water content than at 25 °C (Guimarães et al., 2018), but temperatures below 5 °C can cause chilling injury, with damage also occurring below 10 °C (Akbari et al., 2018). Symptoms include leaf necrosis, browning, wilting, loss of turgidity, and decay (Brindisi and Simon, 2023). Chilling sensitivity remains a significant challenge for sweet basil growers, distributors, and retailers, as it is often stored and transported alongside other fresh produce at low temperatures to reduce the risk of disease and decay (Aharoni et al., 2010). Furthermore, Aharoni et al. (2010) reported a significant increase in water loss, wilting, and infection by *Botrytis* spp. and *Erwinia* spp. in basil plant tissues stored at temperatures above 12 °C.

The commercialization of fresh basil branches and leaves presents challenges due to their high perishability and rapid post-harvest deterioration. According to Costa et al. (2013), fresh basil can be stored at room temperature for 4 to 5 days. After this period, the leaves begin to wilt, primarily because of elevated respiration and transpiration rates. This deterioration is further evidenced by the appearance of dark spots, which result from oxidative stress on cell membranes caused by reactive oxygen species (ROS).

Thus, the combination of post-harvest techniques, such as refrigerated storage and hydrocooling, emerges as an alternative to extend shelf life and maintain the quality of basil after harvest. These strategies, particularly hydrocooling, can contribute to reducing respiration and transpiration rates, thereby slowing physiological processes responsible for senescence and product deterioration. However, despite advances in this field, there are still significant gaps in scientific knowledge regarding the isolated or combined application of these techniques, especially with respect to their efficiency, economic feasibility, and adaptability to the conditions of small-scale producers. Therefore, the present study aimed to evaluate the quality of basil branches and leaves subjected to different hydrocooling times and subsequently stored under refrigerated conditions, seeking to generate information that may support the development of more efficient and accessible post-harvest conservation strategies.

MATERIALS AND METHODS

Sweet basil planting and harvesting

Sweet basil seedlings were established using herbaceous cuttings approximately 10 cm long, placed in expanded polystyrene trays filled with a substrate composed of 30% vermiculite, 20% *Pinus* spp. bark, 25% coconut fiber, and 25% rice husk. These trays were kept in a greenhouse until the seedlings were fully rooted. Afterwards, they were transplanted into small beds measuring 2 × 2 m, using soil that had been amended and fertilized according to the crop's nutritional requirements.

Ninety days of post-transplantation, fresh branches and leaves were harvested in the early morning, placed in plastic bags, and transported to the laboratory for analysis. In the laboratory, the leaves and branches were sorted, weighed, and prepared for sampling, with ~ 25 g of branches and leaves. This procedure followed the commercialization standard of this species in Brazil (Guimarães et al., 2018).

Initial analysis and treatments

Before administering the treatments, three samples (~ 25 g of sweet basil branches and leaves) were randomly selected and analyzed for epidermal color (*L*, *C*, and *h*°), fresh weight (g), titratable acidity (%), soluble solids content (°Brix), vitamin C content (mg 100 g^{-1}), antioxidant activity (measured by ABTS and DPPH, µmol min^{-1} mg $protein^{-1}$), membrane lipid peroxidation (nm m^{-1}), and the activity of the enzyme ascorbate peroxidase (µmol min^{-1} mg $protein^{-1}$) to establish a baseline characterization of the plants. Subsequently, the branch and leaf samples were immersed in cold distilled water at 4 ºC and subjected to the following treatments: 3, 6, and 9 minutes (hydrocooling time). The basil stems and leaves in the control treatment were immersed in water at 25 °C for the same times as the other treatments. Afterward, the samples were left to dry at 25 °C. Following this, the samples were placed in expanded polystyrene trays, wrapped with polyvinyl chloride (PVC) plastic film with a density of 1.4 g cm^{-3}, and stored in a biochemical oxygen demand (BOD) chamber at 5 ± 0.2 °C with a relative humidity of 90 – 95% for periods of 2, 4, and 6 days. A preliminary test was conducted with the aim of identifying the maximum storage period during which the basil still exhibited acceptable quality. PVC films were employed for basil packaging due to their elasticity, self-adherence, low cost, and ease of application directly on the farm or at the packing facility. Moreover, a 25 µm PVC film exhibits water vapor transmission rate (WVTR) and oxygen transmission rate (OTR) values of 2.2 ± 0.9 g h^{-1} m^{-2} and 150–400 cc O_2 m^{-2} day^{-1}, respectively, under temperatures ranging from 23 to 38 °C and 90% relative humidity. These characteristics indicate that PVC promotes the formation of a modified atmosphere which, when combined with appropriate refrigeration and handling practices, can be sufficient to preserve the quality of fresh leafy vegetables.

Post-treatment and post-storage analyses of sweet basil leaves

Leaf epidermis color

The color of the leaf epidermis was assessed based on lightness (*L*), chromaticity (*C*), and hue angle (*h*º) using a colorimeter (Delta Vista 450G) with D65 illuminant and d/8 diffuse lighting system. Measurements of the leaf epidermis color were taken on one side of the main vein in the central region of the leaves. For each treatment (hydrocooling time and storage time), 15 sweet basil leaves were used.

Fresh weight loss

The fresh weight loss (FW) of sweet basil was calculated for each treatment level by comparing the differences in fresh weight of leaves and branches before hydrocooling and after storage. This was determined using the equation FW = [(initial weight – final weight) / final weight] × 100. The results were presented as percentages (%) of fresh weight loss (Álvares et al., 2010).

Titratable acidity

The titratable acidity (TA) of sweet basil leaves and branches was determined using the potentiometric method. For this analysis, 0.5 g of samples from the sweet basil leaves and branches were homogenized and macerated, then mixed with 25 mL of distilled water. Continuous stirring was maintained while titrating with 0.1 M sodium hydroxide until a pH of 8.1 was reached, as confirmed by a pH meter (Instrutherm). The results were expressed as a percentage and calculated using the formula $TA_{(\%)} = [(V \times N)/P] \times 100$, where: *V* represents the sodium hydroxide volume used in titration, N denotes the molarity of the solution, and *P* refers to the volume (mL) of the sample employed in the titration.

Soluble solids (SS)

The soluble solid (SS) content of leaves and branches was measured using a digital refractometer (Edutec model EEQ9029) with a temperature correction at 20 °C, and the results were expressed in °Brix.

Vitamin C content

The vitamin C content in sweet basil was determined using the method proposed to Tillmans et al. (1932), which is based on the reduction of the indicator 2,6-dichlorophenolindophenol (DCPI) by ascorbic acid. The results were expressed in milligrams of ascorbic acid per 100 g of sweet basil leaves and branches.

Antioxidant activity

The antioxidant activity of sweet basil was assessed using two methods: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-picrylhydrazyl (DPPH). Initially, the ABTS radical was diluted in 96% ethanol. An aliquot of 2.940 μL of this diluted radical + 60 μL of the sample was pipetted into the tubes. These were shaken, and the absorbance was measured using a spectrophotometer (Quimis) at a wavelength of 754 nm after 6 minutes. For the DPPH antioxidant activity, the method was adapted from Kim et al. (2002). In this procedure, 2.9 mL of DPPH radical was mixed with 0.1 mL of the sample in test tubes, shaken, and the absorbance was measured at a wavelength of 517 nm after 30 minutes. The results for both the ABTS and DPPH analyses were expressed at mg 100 mL^{-1}.

Membrane lipid peroxidation

Membrane lipid peroxidation in sweet basil was assessed using the method described by Heath and Packer (1968), which quantifies malondialdehyde (MDA) through its reaction with 2-thiobarbituric acid (TBA). To prepare the samples, 160 mg of sweet basil leaves and branches were macerated in 2 mL of a 0.1% trichloroacetic acid solution. The mixture was then transferred into 2 mL Eppendorf microtubes and centrifuged at 10,000 rpm for 15 minutes at 4 °C. Subsequently, 500 mL of the supernatant was removed and added to microtubes containing 1.5 mL of 20% trichloroacetic acid and 0.5% thiobarbituric acid. The samples were then analyzed using a spectrophotometer at wavelengths of 440, 532, and 600 nm. The results were expressed as nmol of malondialdehyde formed per gram of fresh weight.

Activity of the enzyme ascorbate peroxidase

The activity of the enzyme ascorbate peroxidase was measured using the method proposed by Sekita (2012). For this procedure, 0.3 g samples of sweet basil leaves were macerated with mortar and pestle and mixed with 2 mL of an extracting solution consisting of dibasic phosphate (0.1M) and monobasic phosphate (0.1M) at pH 6.8, along with ethylenediaminetetraacetic acid (0.1 M) and phenylmethylsulfonyl fluoride (0.1 M) in a mortar. The resulting mixture was then transferred to microtubes and centrifuged at 12,000 rpm for 15 minutes at 4 °C. To determine the ascorbate peroxidase activity, 0.8 mL of enzyme extract was added to the reaction medium containing 2.2 mL of the buffer solution [dibasic phosphate (0.1 M) + monobasic phosphate (0.1 M) at pH 6.8 + ethylenediaminetetraacetic acid (0.1 M) + ascorbic acid (0.1 M) + hydrogen peroxide (0.1 M)]. This mixture was analyzed using a spectrophotometer at a wavelength of 290 nm for 2 minutes.

Experimental design and data analysis

The assay was conducted using a completely randomized design following a factorial scheme of 4 × 3 [four hydrocooling times (0, 3, 6, and 9 minutes) × three storage times (2, 4, and 6 days)] with four repetitions (samples of sweet basil leaves and branches of 25 g). Initially, the data underwent the Bartlett test to assess the homogeneity of variances, followed by the Shapiro-Wilk test to evaluate the normality of the residuals. Since all data met the assumptions of the standard model, the interaction of explanatory variables (hydrocooling time × storage time) was analyzed. In cases of significant interaction between the explanatory variables, the "*fat2.crd*" function from the "*ExpDes*" package (Ferreira et al., 2014) was used for interaction separation and means were compared using Tukey's test (P < 0.05) along with the adjusted P-values. All analyses were performed using the statistical software "*R*" version 4.3.1 (R Development Core Team, 2023).

RESULTS AND DISCUSSION

Physicochemical analyses at harvest

At the time of harvest, the sweet basil leaves exhibited values of L (47.7 ± 2.61), C (24.5 ± 1.68), and $h°$ (112.6 ± 1.27), indicating a dark yellowish-green color with minimal lightness. The initial C vitamin content was measured at 26.2 ± 0.42 mg 100

g^{-1}. For comparison, studies by Roberto (2018) reported C vitamin levels of 4.01 mg $100g^{-1}$ and 2.6 mg 100 g^{-1}, respectively. At harvest, the membrane lipid peroxidation content in the basil leaves was recorded at 0.6 ± 0.06 nm m^{-1}. The initial levels for total acidity (TA), pH, and soluble solids (SS) in basil leaves were 4.3% ± 0.75, 6.38, and 3.9 ± 0.26 ºBrix, respectively. These findings are consistent with those obtained by Henrique et al. (2017), who reported pH and SS values of 6.43 and 3.0 °Brix, respectively. However, these same authors noted a lower total acidity (0.19%) in basil tissues compared to the results of the present study.

In the initial assessment, the inhibition of antioxidant activity measured by the ABTS and DPPH methods was 0.5 and 0.4 µmol min^{-1} mg $protein^{-1}$, respectively. Regarding the activity of the ascorbate peroxidase enzyme, the initial average was 4.3 ± 2.09 µmol min^{-1} mg $protein^{-1}$.

In preliminary tests, storing bundles of basil at a temperature of 25 °C, both with and without plastic covering, led to significant water losses and leaf darkening in less than 2 days after being placed in the chamber. A similar outcome was observed for basil stored without a modified atmosphere at 5 ºC. Additionally, symptoms of necrosis and chilling injury appeared in under 2 days of storage. Based on these preliminary findings, it was decided to conduct experiments using a modified atmosphere, four hydrocooling durations (0, 3, 6, and 9 minutes), and three storage periods (2, 4, and 6 days) at a temperature of 5 °C.

Preservation and changes in fresh weight, total acidity, and soluble solids during storage

The duration of hydrocooling and storage in the chamber significantly influenced the fresh weight loss and preservation of basil leaves and branches. No statistically significant differences were observed in fresh weight loss between basil branches and leaves after 2 and 4 days of storage, regardless of the hydrocooling duration. However, samples that underwent storage and hydrocooling for 6 days and 9 minutes, respectively, experienced an average weight loss of 18.8% ± 3.12%, which was statistically different from those that underwent hydrocooling for 0, 3, and 6 minutes (Figure 1A). This phenomenon can be attributed to the continued respiration and transpiration processes of the plant post-harvest, resulting in the loss of water and turgor pressure in the tissues (Chitarra and Chitarra, 2005).

At both zero (control) and 3 minutes, the most significant fresh weight losses occurred at 4 and 6 days of storage. For hydrocooling durations of 6 and 9 minutes, the most notable loss was observed at 6 days of storage, while the other treatments showed no statistically significant differences (Figure 1A). The branches and leaves submerged in ice water for 6 and 9 minutes likely retained a higher water content within the cells, which, by lowering field heat, reduced metabolic and respiratory processes and helped maintain a lower product temperature. The samples that underwent hydrocooling at 4ºC and were subsequently stored at 5 ºC experienced minimal temperature fluctuations between hydrocooling and storage, resulting in lower levels of water loss. This preservation of turgidity in leaf tissues lasted for up to 4 days of storage, with noticeable weight losses occurring only after this period.

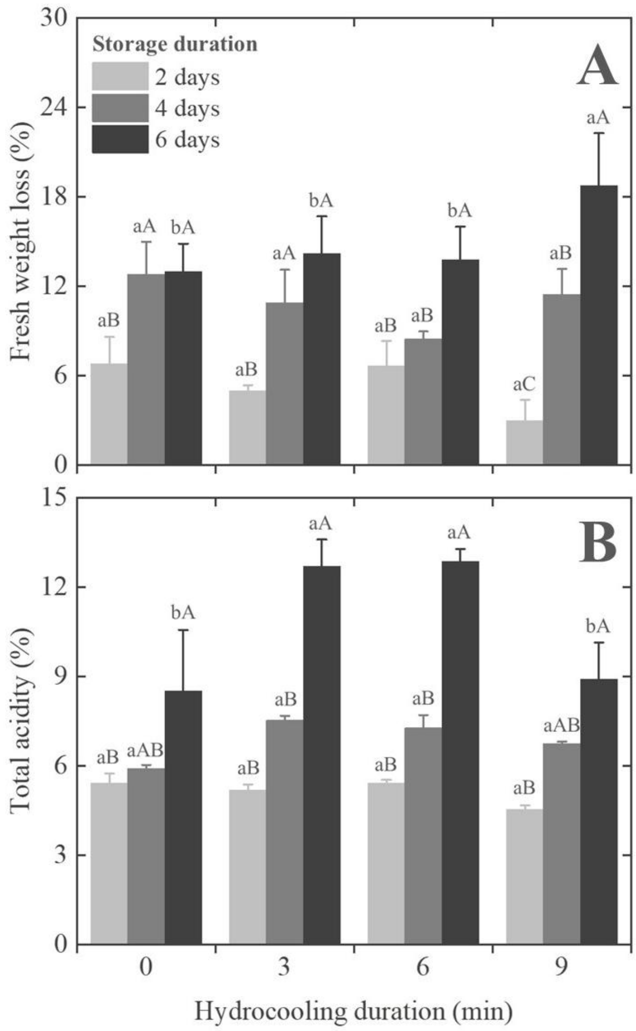

Figure 1. Percentage of fresh weight loss (**A**) and total titratable acidity (**B**) in sweet basil leaves and branches subjected to hydrocooling for 0, 3, 6, and 9 minutes and stored for 2, 4, and 6 days at 5 °C. Means followed by the same uppercase letters (storage duration) and lowercase letters (hydrocooling duration) do not differ statistically according to Tukey's test ($P < 0.05$).

According to Guimarães et al. (2018), basil branches and leaves that underwent hydrocooling for 5 minutes and were subsequently stored at 5 °C exhibited lower fresh weight losses compared to those that were not subjected to hydrocooling and stored at room temperature (25 °C). A similar outcome was observed in parsley. When pre-cooled and stored at 5 °C for 24 hours, it demonstrated reduced fresh weight compared to treatments without pre-cooling and stored at 25 °C (Álvares et al., 2010). Fresh weight loss results from the discrepancy in the water pressure gradient between the product and its environment, leading to wilting and water loss from the product (Taiz and Zeiger, 2004). Consequently, pre-cooling techniques effectively lower the product's temperature and metabolic rates immediately after harvest. When combined with cooler storage temperatures, these methods help reduce the gradient, thereby minimizing fresh weight loss during the critical initial stages (Álvares et al., 2010).

After 6 days of storage, basil leaves and branches that underwent hydrocooling for 3 and 6 minutes exhibited a higher percentage of total acidity than those subjected to hydrocooling for 0 and 9 minutes. No significant differences were observed in the other storage durations, regardless of the hydrocooling durations (Figure 1B). The hydrocooling durations of 0 and 9 minutes showed lower and higher titratable acidity at 2 and 6 days, respectively, but they did not differ from the acidity levels observed at 4 days of storage. In contrast, both hydrocooling durations of 3 and 6 minutes were comparable, displaying the highest percentage of titratable acidity at 6 days, followed by values at 2- and 4-day post-storage (Figure 1B).

Acidity percentages tend to decrease due to the consumption of sugars and organic acids during cellular respiration and metabolism (Chitarra and Chitarra, 2005). The present study observed variations in the titratable acidity (TA) content, with both increases and decreases occurring based on hydrocooling and storage durations. In studies involving chives (*Allium fistulosum* L.) and coriander (*Coriandrum sativum* L.) that were subjected to hydrocooling for 0, 5, 10, 15, and 20 minutes, and then stored for 9 days, variations in TA concentrations were noted, observed across different hydrocooling and storage durations (Betin et al., 2018). However, the findings from this study contrast with those of Nascimento et al. (2017), who reported decreases in TA levels in lettuce (*Lactuca sativa* L.) stored at 7 °C for 4 and 10 days. These authors suggest that lower acidity values in the samples correlate with higher levels of product deterioration. During the 6 days of storage, basil samples subjected to hydrocooling for 0 and 9 minutes exhibited the lowest TA values, likely because the absence of hydrocooling, or immersion for 9 minutes in water at 4 °C, may have induced stress in the tissue, leading to the consumption of some organic acids and, consequently, a reduction in TA.

Soluble solids (SS) serve as a quality parameter indicating the levels of sugars, organic acids, vitamins, phenolics, and other compounds present in the leaves (Chitarra and Chitarra, 2005). In the present study, however, no significant differences were observed in the SS content, which remained around 4.2 °Brix, regardless of hydrocooling and storage durations. Some research suggests a decline in SS contents starting from the 4th day of storage in hydrocooled caruru (*Amaranthus* spp.), which may indicate a consumption of metabolic reserves (Silva, 2016). In contrast, other studies report an increase in SS contents due to water loss, as observed in hydrocooled chives (*A. fistulosum*) stored for 9 days (Betin et al., 2018).

Changes in biochemical parameters

The C vitamin content in the basil samples exhibited statistically significant differences only 4 days after storage. During this initial period, basil bunches subjected to hydrocooling for 6 and 9 minutes showed lower vitamin C contents than the control group, which had a content of 20.9 mg 100 g^{-1}. Conversely, the basil that underwent hydrocooling for 3 minutes showed no significant differences in C vitamin content when compared to the other treatments (Figure 2B). However, for the other storage durations (2 and 6 days), no differences were observed for this parameter, regardless of the hydrocooling duration.

Basil branches and leaves subjected to hydrocooling for 0 and 3 minutes did not exhibit significant changes in C vitamin content, regardless of the storage duration (Figure 2A). However, hydrocooling for 6 and 9 minutes resulted in the highest C vitamin contents observed after 2 days of storage, followed by a subsequent decline (Figure 2A). Ascorbic acid, commonly known as C vitamin , is naturally found in fruits and vegetables. However, it is particularly sensitive to processing and storage conditions, which can lead to considerable reduction and degradation. Therefore, it is frequently used as a conservation index and quality parameter in various food analyses (Hoehne and Marmitt, 2019).

Conversely, C vitamin is present as an ascorbic acid, primarily slowing cellular oxidation while inhibiting and sequestering free radicals and reactive oxygen species (ROS) (Chitarra and Chitarra, 2005). Consequently, the lowest levels of vitamin C were observed in basil that underwent hydrocooling for 6 and 9 minutes and was stored for 4 and 6 days at 5 °C. This may be due to the plant tissues utilizing a portion of the ascorbic acid as a mechanism to alleviate oxidative stress within the cells and combat ROS, which in turn leads to a decrease in vitamin C levels and mitigates the degradation of cell membranes through lipid peroxidation (Figure 2A).

At 2 and 6 days of storage, no differences in lipid peroxidation were observed, regardless of the hydrocooling duration. However, at 4 days of storage, the least membrane degradation occurred in basil that underwent hydrocooling for 6 and 9 minutes (Figure 2B). In contrast, lipid peroxidation levels at 2 days of storage were higher than those observed at 4 days. However, this difference was not statistically significant when compared to the results from 6 days of storage (Figure 2B). During storage, stress conditions can arise, leading to tissue injury, mechanical damage, and microbial attack, which may disrupt the metabolism of leafy vegetables and generate ROS, including hydrogen peroxide (H_2O_2), singlet oxygen, superoxide, and hydroxyl radicals. These ROS can cause oxidative damage to plant cell membranes (Ogweno et al., 2008), resulting in the formation of a toxic compound known as malondialdehyde (MDA), a secondary product of polyunsaturated fatty acid oxidation. MDA is typically used as an indicator of oxidative stress levels and plasma membrane integrity, since it reflects the extent of lipid peroxidation within the membrane (Posmyk et al., 2005).

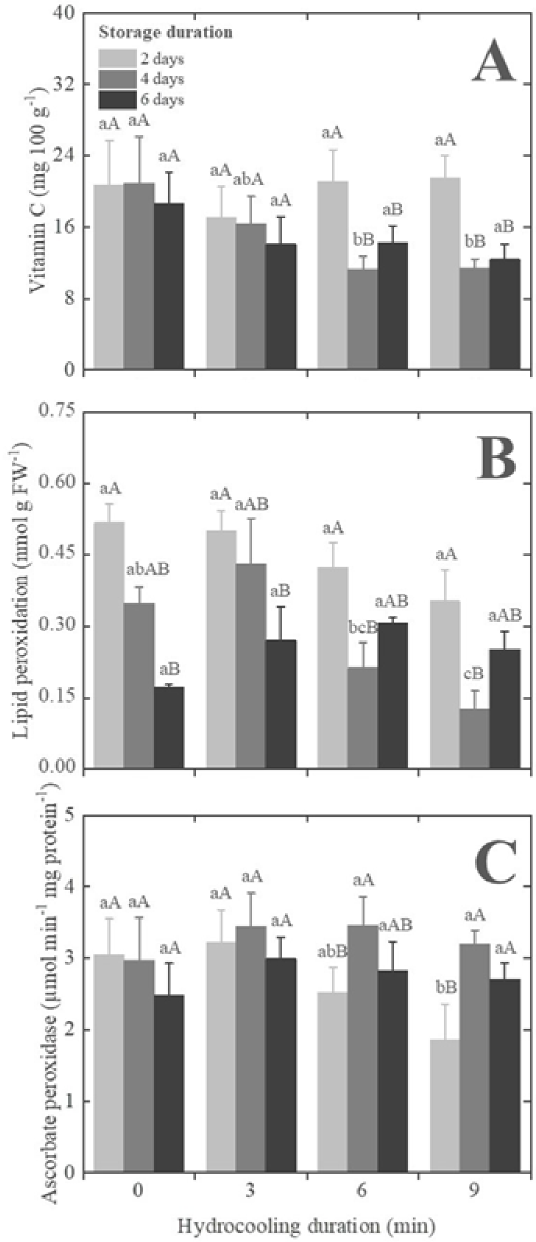

Figure 2. Vitamin C content (**A**), plasma membrane lipid peroxidation (**B**), and activity of the ascorbate peroxidase enzyme (**C**) in sweet basil leaves subjected to hydrocooling for 0, 3, 6, and 9 minutes, and stored for 2, 4, and 6 days at 5 °C. Means followed by the same uppercase letters (storage duration) and lowercase letters (hydrocooling duration) do not differ statistically according to Tukey's test ($P < 0.05$).

The lowest lipid peroxidation values at 4 days of storage were observed in basil subjected to hydrocooling for 6 and 9 minutes. This may be explained by the fact that, in order to reduce reactive oxygen species and lipid peroxidation, the basil tissues consumed part of their ascorbic acid content (Figure 2B). Thus, basil hydrocooled for 9 minutes and stored for 4 days showed a significant reduction in ascorbic acid levels. Ascorbic acid serves as a powerful antioxidant, protecting cells from oxidative damage. The highest activity of ascorbate peroxidase enzyme was recorded at 4 days of storage, particularly at hydrocooling durations of 3 and 6 minutes. The lowest enzyme activity was noted at 9 minutes after 2 days of storage. Statistical differences between the hydrocooling durations were not pronounced, with the most significant variation in activity observed between the different storage durations (Figure 2C).

Reactive oxygen species are known to cause oxidative damage to the plasma membranes of cells (Ogweno et al., 2008), degrade plant tissue, and diminish the quality of vegetables. These plants are frequently exposed to various factors that contribute to increased ROS, including both biotic and abiotic stresses. In response, vegetables often enhance the activity of antioxidant stress enzymes, such as ascorbate peroxidase, to mitigate ROS levels. Notably, basil subjected to hydrocooling for 3 minutes and subsequently stored for 4 days showed an increase in the activity of this enzyme. This rise in activity may be attributed to the elevated stress levels experienced by basil during storage and hydrocooling, leading to higher ROS levels. Consequently, the basil used some of its antioxidants and increased ascorbate peroxidase activity to counteract ROS, thereby preserving the integrity of cell membranes and maintaining the quality of its leaves and branches.

Antioxidant activity (ABTS and DPPH)

No interaction was observed between hydrocooling and storage durations regarding antioxidant activity, as measured by the ABTS and DPPH methods (P = 0.985 and P = 0.051, respectively). For the ABTS method, a significant difference was detected only in the days of storage (P = 0.015) (Table 1). In contrast, the DPPH method revealed a significant difference related to hydrocooling duration (P = 0.041), with the most pronounced antioxidant activity occurring in basil subjected to hydrocooling for 3 minutes, compared to other treatments (Table 1). Antioxidants are substances capable of neutralizing or reducing the reactivity of free radicals and reactive oxygen species (ROS) (Alves, 2020). Consequently, a decrease in antioxidant activity measured by the ABTS method was noted after 2 days of storage. In contrast, the DPPH method indicated higher antioxidant activity at 3 minutes and lower activity at 6 minutes of hydrocooling (Table 1). The increase in hydrocooling duration may have reduced respiration and metabolic processes in tissues, thereby diminishing ROS and oxidative damage. After 4 days of storage, a decrease in antioxidant content (as measured by the ABTS method) was observed, attributed to the plant’s use of antioxidants to combat reactive species that could jeopardize the integrity of the cell’s plasma membrane.

Table 1. Antioxidant activity by ABTS and DPPH methods in sweet basil leaves subjected to hydrocooling for 0, 3, 6, and 9 minutes and stored for 2, 4, and 6 days at 5 °C.

Hydrocooling duration (minutes)	Storage duration (days)[1]			
	2	4	6	Mean ± SE
	ABTS (µmol trolox 100 mL of extract^{-1})			
0 (control)	0.6 ±0.01	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02 a
3	0.6 ± 0.02	0.5 ± 0.02	0.5 ± 0.03	0.5 ± 0.02 a
6	0.6 ± 0.01	0.5 ± 0.01	0.5 ± 0.02	0.5 ± 0.01 a
9	0.5 ± 0.02	0.5 ± 0.01	0.5 ± 0.07	0.5 ± 0.03 a
Mean	0.6 ± 0.02 A	0.5 ± 0.02 B	0.5 ± 0.04 B	
	DPPH (µmol trolox 100 mL of extract^{-1})			
0 (control)	0.94 ± 0.01	0.89 ± 0.01	0.93 ± 0.01	0.92 ± 0.01 ab
3	0.94 ± 0.01	0.93 ± 0.01	0.94 ± 0.01	0.94 ± 0.01 a
6	0.90 ± 0.01	0.92 ± 0.01	0.92 ± 0.04	0.91 ± 0.01 b
9	0.93 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	0.93 ± 0.01 ab
Mean	0.93 ± 0.01 A	0.92 ± 0.01 A	0.93 ± 0.01 A	

[1]Data (mean ± SE) followed by the same uppercase letters in the columns (storage duration) and lowercase letters (hydrocooling duration) in the rows do not differ statistically according to Tukey's test (P < 0.05).

Leaf color

After 2 days of storage, the 3-minute and 6-minute hydrocooling treatments exhibited higher L values than the other treatments (Table 2). For the other storage days, no significant differences were found among the treatments (P = 0.224). Conversely, the leaves subjected to various hydrocooling durations demonstrated statistical differences. Specifically, the 3-minute treatment showed higher values at 2 days of storage, while lower averages were observed at 6 days. However, for the 6-minute and 9-minute treatments, no statistical differences were found (Table 2).

For the *C* values, which serve as parameters indicating the shade (light/dark) of plant tissues, significant differences were observed across various storage and hydrocooling durations. After 2 days of storage, the highest *C* values were found in the 3-minute hydrocooling treatment, closely followed by the control and 9-minute treatments. However, these differences were not statistically significant (Table 2). On the 4th day of storage, the control exhibited the highest average, while the other treatments had lower values. After 6 days of storage, the darkest shades were observed in the control and 3-minute hydrocooling treatment, with the lightest shade recorded in the 6-minute treatment, which was not statistically different from the 9-minute hydrocooling treatment. Notably, basil leaves that were not subjected to hydrocooling demonstrated greater chromaticity after 4 days of storage (Table 2). For hydrocooling durations of 3 and 6 minutes, the highest average *C* values were observed at 2 days of storage, followed by those at 4 and 6 days of storage. In contrast, for the 9-minute hydrocooling treatment, the highest *C* values were noted after 2 and 4 days of storage, with the lowest at 6 days of storage.

Table 2. Skin color (lightness, chroma, and hue angle) of sweet basil leaves subjected to hydrocooling for 0, 3, 6, and 9 minutes and stored for 2, 4, and 6 days at 5 °C.

Hydrocooling duration (minutes)	Storage duration (days)[1]		
	2	4	6
	Lightness (*L*)		
0 (control)	47.7 ± 2.36 Bab	49.0 ± 2.23 Aa	47.5 ± 2.55 Ab
3	49.6 ± 2.19 Aa	48.5 ± 2.25 Aab	47.9 ± 2.31 Ab
6	48.8 ± 2.22 Aa	48.5 ± 2.23 Aa	47.6 ± 2.45 Aa
9	48.1 ± 2.27 Ba	48.4 ± 2.32 Aa	47.7 ± 2.24 Aa
	Chroma (*C*)		
0 (control)	24.5 ± 1.36 Bb	25.5 ± 1.39 Aa	23.7 ± 2.14 Ab
3	26.2 ± 1.38 Aa	24.4 ± 1.51 Bb	23.3 ± 1.55 Ac
6	25.3 ± 1.62 ABa	24.0 ± 1.46 Bb	22.0 ± 1.36 Bc
9	25.0 ± 1.19 Ba	24.2 ± 1.45 Ba	22.8 ± 1.67 ABb
	Hue angle (*h*°)		
0 (control)	112.6 ± 1.01 Aa	111.0 ± 1.18 Ab	107.6 ± 3.23 Bc
3	111.0 ± 1.97 Ba	111.3 ± 1.41 Aa	107.8 ± 2.28 Bb
6	112.3 ± 1.10 Aa	111.2 ± 1.41 Ab	110.2 ± 1.95 Ab
9	112.1 ± 1.07 ABa	110.6 ± 1.20 Ab	107.1 ± 2.49 Bc

[1]Data (mean ± SE) followed by the same uppercase letters in the columns (storage duration) and lowercase letters (hydrocooling duration) in the rows do not differ statistically according to Tukey's test ($P < 0.05$).

After 2 days of storage, the highest and lowest hue angle values were recorded at 0 and 6 minutes, and at 9 minutes, respectively, with no statistically significant differences compared to the other treatments. However, after 4 days of storage, no notable differences were observed, regardless of the hydrocooling duration (Table 2). At 6 days of storage, the basil subjected to hydrocooling for 6 minutes exhibited a greenish-yellow coloration (high *h*°). In contrast, basil leaves subjected to hydrocooling for 0 and 9 minutes showed the highest hue values at 2 days of storage, followed by measurements taken at 4 and 6 days. For basil leaves subjected to 3 minutes of hydrocooling, the peak readings were observed at both 2 and 4 days. Similarly, samples subjected to 6 minutes of hydrocooling exhibited the highest hue value at 2 days of storage. There were no statistically significant differences in leaf color among the hydrocooling durations. However, noteworthy changes occurred throughout the storage period. During storage at 5 °C, branches and leaves exhibited a loss of luster, darkening, and a transition to a yellowish hue. An exception was observed with leaves subjected to hydrocooling for 6 minutes. After 6 days of storage, they displayed a dark yellowish-green hue with a shiny intensity, with no significant losses compared to the initial analyses (Figure 3). The color of plant tissues is influenced by chlorophyll pigments, carotenoids, and phenolic compounds such as anthocyanins and flavonoids found within plant cells (Chitarra and Chitarra, 2005). Nevertheless, after the harvest, the processes of respiration and metabolism persist, leading to the depletion of reserves and the loss of green color due to chlorophyll degradation, as well as the breakdown of lipids, proteins, and RNA. This promotes the visibility or formation of carotenoids in the leaves, which are responsible for the orange and/or yellowish colors that arise during the natural senescence of plant tissues throughout storage (Repke et al., 2009).

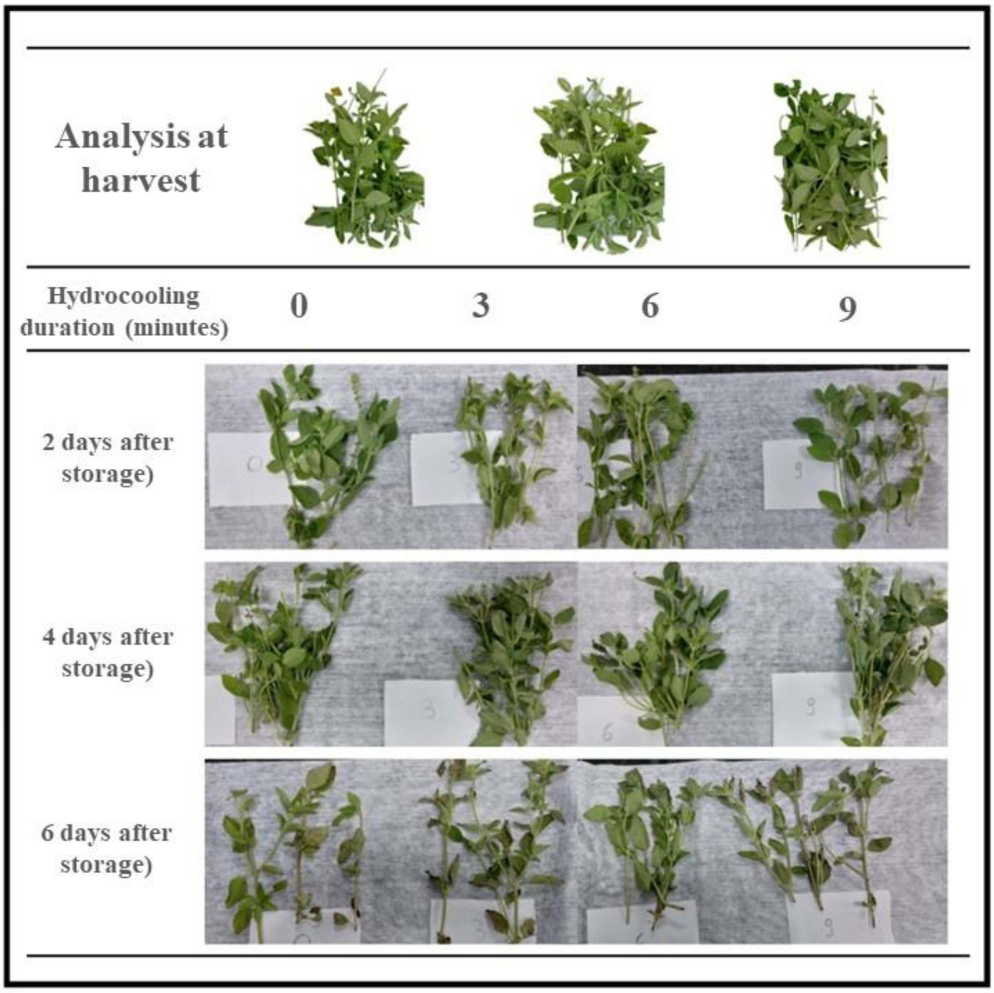

Figure 3. Evolution of the appearance of sweet basil branches and leaves at harvest and after 0, 3, 6, and 9 minutes of hydrocooling and stored for 2, 4, and 6 days at 5 ºC.

CONCLUSIONS

Hydrocooling has been shown to be an effective technique for preserving basil branches and leaves for up to 4 days when stored at 5 °C, contributing to the maintenance of product quality and visual characteristics. Basil stored for this period exhibits lower fresh mass loss and less change in leaf color when subjected to hydrocooling for 6 and 9 minutes. The lowest lipid degradation is observed in basil subjected to 9 minutes of hydrocooling and stored for 4 days at 5 °C. Conversely, higher acidity levels are noted after 6 days of storage when the basil branches and leaves have undergone hydrocooling for 3 and 6 minutes. It can be concluded that hydrocooling durations of 6 and 9 minutes are more effective in maintaining the postharvest quality of basil when stored under refrigeration at 5 °C for up to 4 days.

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