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Changes in visual quality, physiological and biochemical parameters assessed during the postharvest storage at chilling or non-chilling temperatures of three sweet basil (*Ocimum basilicum* L.) cultivars

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ABSTRACT

Leaves of three different sweet basil (*Ocimum basilicum* L.) cultivars (*Italico a foglia larga*, *Cammeo*, and *Italiano classico*) packed in macro-perforated polyethylene bags were stored at chilling (4 °C) or non-chilling temperature (12 °C) for 9 days. During storage, visual quality, physiological (respiration rate, ethylene production, ammonium content) and chemical (antioxidant activity, total polyphenols and polyphenol profile) parameters were measured. Detached leaves stored at chilling temperature showed visual symptoms related to chilling injury, while ethylene production and ammonium content resulted associated to cultivar sensibility to damage at low temperature. Storage at 4 °C caused a depletion in polyphenols content and antioxidant capability, which was preserved at 12 °C. Regarding the polyphenols profile, stressful storage conditions did not enhance the phenolic metabolism. However, leaves stored at 12 °C did not loss a significant amount of metabolites respect to fresh leaves, suggesting the possibility to extend the storability after the expiration date, for a possible recovery of bioactive compounds.

1. Introduction

Sweet basil *(Ocimum basilicum* L.) is a perennial crop that is widely diffused in Asia, Africa, South America, and in the Mediterranean region. Sweet basil represents a rich source of phytochemicals and, similar to other culinary herbs, it is extensively used for its organoleptic properties. In medicine, the importance of sweet basil is related to its antioxidant, antimicrobial, and antiviral properties (Chiang, Ng, Cheng, Chiang, & Lin, 2005). Indeed, the consumption of basil leaves markedly increase glutathione S-transferase activity that partly controls chemical carcinogens in the stomach, liver, and oesophagus (Aruna & Sivaramakrishnan, 1990). Sweet basil contains acidic phenolic compounds, such as cinnamic, caffeic, sinapic, caftaric, rosmarinic and ferulic acid, and different flavonoids, such as apigenin and catechin (Baritaux, Amiot, & Nicolas, 1991). These compounds act as strong antioxidants, free radical scavengers, and metal chelators (Cook & Samman, 1996). Moreover, chicoric acid, present in different parts of basil (Lee & Scagel, 2009), is thought to behaviour as an antioxidant, anti-inflammatory, antiviral, and immune-stimulator (Tsai, Chiou, Chi Chan, Sung, & Lin, 2012).

Fresh sweet basil, as well as fresh spices and culinary herbs, are considered perishable commodities with a very short shelf life (Loughrin & Kasperbauer, 2001). In sweet basil leaves, it is well documented that storage at low temperature (below 12 °C) causes chilling injury, characterized by brown discoloration of the middle areas of the leaf, stem browning and collapse, wilting of the leaves and loss of glossy appearance and characteristic aroma (Cozzolino et al., 2016). In addition, chilling stress temperature might cause changes in the polyphenolic compounds and antioxidant activity of stored leaves. Many papers showed that chilling stress at a critical low temperature enhances phenolic metabolism, which varies among commodities (Lattanzio, 2003). However, based on

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the authors' knowledge, this is the first report regarding the effect of storage at chilling temperature on changes in polyphenol profile of sweet basil leaves. Starting from these findings, this paper aims to study changes in visual quality, physiological (respiration rate, ethylene production, ammonium content) and biochemical (antioxidant activity, total polyphenols and polyphenol profile) parameters taking place during postharvest storage of three basil cultivars at chilling (4 °C) or non-chilling temperatures (12 °C).

2. Materials and methods

2.1. Chemicals and reagents

Caffeic, ferulic, p-coumaric, gallic, caftaric, rosmarinic, and chlorogenic acids, epicatechin, rutin, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butyl-parahydroxybenzoate, high pressure liquid chromatography (HPLC)-grade methanol, sulphuric, metaphosphoric, acetic and formic acids, acetonitrile, ethanol and acetone were purchased from Sigma-Aldrich (Milano, Italy). Apigenin, luteolin and hyperoside were purchased from Extrasynthese (Genay, France).

2.2. Plant material and experimental set-up

Plants of three sweet basil (*Ocimum basilicum* L.) cultivars (*Italico a foglia larga*, Enza Zaden srl, Tarquinia, Italy; *Cammeo*, Sais Sementi, Cesena, Italy; *Italiano classico*, Sementi Fuscello, Andria, Italy) were cultivated in greenhouses and purchased from a local farm (Ortoflora, Fasano, Italy). Three consecutive experiments, one for each basil cultivar, were performed. In each experiment and for each cultivar, 120 marketable basil plants were delivered to the Postharvest Laboratory of the ISPA-CNR. Basil leaves of each cultivar were detached from plants and pooled. Then, approximately 200 g of detached basil leaves were placed in each polypropylene tray and packed in a macro-perforated polyethylene bag, in order to avoid a modification of the atmosphere inside packages and to limit dehydration. In each experiment, basil leaves bags were stored at chilling (4 °C) or non-chilling temperature (12 °C) for 9 days. Twelve bags for each cultivar and experiment were prepared: two replicates for two temperatures (4 or 12 °C) for three storage time (3, 6 and 9 days). All plastic materials were purchased from Carton Pack (Rutigliano, Italy). Basil leaves of each cultivar at time 0 and after 3, 6 and 9 days were analysed to evaluate the sensory visual quality, physiological (respiration rate, ethylene production and ammonium content), and chemical parameters (antioxidant activity, total polyphenols and polyphenol profile).

2.3. Sensory visual quality, respiration rate, ethylene production and ammonium content

A group of five trained researchers used a colour photographic scale associated with a brief description as a reference to subjectively assess the sensory visual quality (VQ). Five quality levels, according to the following scale, were used: 5 = excellent, fresh appearance; 4 = good; 3 = fair (limit of marketability); 2 = poor (just below the limit of marketability); and 1 = very bad, inedible. A visual quality of 3 was considered the minimum threshold of acceptance for sale or consumption, and values below 3 indicated a waste product (Cozzolino et al., 2016).

The respiration rate was measured using a closed system, as reported by Kader (2002). In detail, 100 g of leaves for each replicate was placed into 6 L sealed plastic jars (one jar x replicate), which were closed and CO_2 was allowed to accumulate up to 0.1%. The time taken to reach this threshold was measured by monitoring the CO_2 concentration at regular time intervals. For CO_2 analysis, 1 mL of gas sample was taken from the head space of the plastic jars through a rubber septum and injected into a gas chromatograph (P200 Micro GC, Agilent, Santa Clara, CA, USA) equipped with a thermal conductivity detector. Carbon dioxide was analysed with a retention time of 16 s and a total run time of 120 s on a 10-m porous polymer (PPU) column (Agilent, Santa Clara, CA, USA) at a constant temperature of 70 °C. The respiration rate of the leaves of each basil cultivar was measured on detached leaves at 0 day (after the exposure for 12 h at 4 and 12 °C) and after 3, 6 and 9 days of cold storage at 4 and 12 °C and results were expressed as mL CO_2/kg/h.

Ethylene production (μL C_2H_4/kg/h) was measured using a closed system (Kader, 1992). Fresh produce (about 50 g for each replicate), was placed into 4.20 L sealed plastic-jars, where ethylene was allowed to accumulate until 0.1 ppm (standard concentration). The time taken to reach this threshold was measured by monitoring the CO_2 concentration at regular time intervals. Then, the gas sample was taken from the headspace through a rubber septum and measured using ethylene analyser (Easy-1 Absoger, Les Barthes, France). The ethylene concentration was then referred to the sample weight, the headspace volume in the jars, and the elapsed time. Ethylene from the leaves of each basil cultivar was measured at 0 day (after the exposure for 12 h at 4 and 12 °C) and after 3, 6 and 9 days of cold storage at 4 and 12 °C.

For ammonium (NH_4^+) analysis five grams of chopped basil leaves was homogenised (Ultra-Turrax T-25, IKA, Staufen, Germany) for 2 min in 20 mL of deionised water (Cefola & Pace, 2015). The mixture was centrifuged for 5 min at 6440g, and 0.5 mL of the extract was mixed with 5 mL of nitroprusside reagent

Table 1
Effects of temperature (at 4 or 12 °C), storage (3, 6 and 9 days) and their interaction on sensory visual quality, physiological and biochemical parameters of the leaves of *Italico a foglia larga*, *Cammeo* and *Italiano Classico* basil cultivars.

Parameters	Experiment 1: *Italico a foglia larga*			Experiment 2: *Cammeo*			Experiment 3: *Italiano Classico*		
	Temperature (A) (4 or 12 °C)	Storage (B) (3, 6 and 9 days)	A x B	Temperature (A) (4 or 12 °C)	Storage (B) (3, 6 and 9 days)	A x B	Temperature (A) (4 or 12 °C)	Storage (B) (3, 6 and 9 days)	A x B
Visual Quality score (5–1)	**	***	ns	***	***	ns	**	***	ns
Respiration rate (mL CO_2 kg^{-1} h^{-1})	ns	*	ns	*	**	**	***	ns	ns
Ethylene production (μL kg^{-1} h^{-1})	ns	**	ns	***	***	**	***	***	***
Ammonium content (μg NH_4^+ g^{-1} fw)	ns	ns	*	***	***	***	***	***	**
Antioxidant activity (EC_{50}, mg)	ns	ns	ns	***	***	***	***	***	***
Total Phenols (mg of gallic acid equivalent GAE g^{-1})	ns	ns	ns	***	*	***	*	***	***

ns: not significant; * for $P \leq 0.05$; ** ≤ 0.01; *** ≤ 0.001. For storage temperature dataset of 6 samples (2 replicates x 3 storage durations), for storage duration dataset of 4 samples (2 replicates × 2 storage temperatures) were used. Fw: fresh weight.