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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 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 biochemical (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 sensible to low temperature. Storage at 4 °C caused a depletion in polyphenols content and antioxidant capability, which was preserved at 12 °C; while stressful storage conditions did not enhance the phenolic metabolism. However, leaves stored at 12 °C showed a good amount in metabolites until the end of the storage, suggesting the possibility to extend the storability after the expiration date in order to recover bioactive compounds

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Dear Editor,
On behalf of my co-authors and myself, I am pleased to submit the manuscript entitled "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" to Food Chemistry for consideration for publication.
The objective of this study was 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). To the best of our knowledge, this is the first report regarding the effect of storage at chilling stress temperature on changes in polyphenol profile of sweet basil leaves.

We confirm that neither the manuscript nor any part of it has been published or is under consideration for publication elsewhere (abstract excluded). Any reference to or use of previously published material protected by copyright is explicitly acknowledged in the manuscript.
All authors have given a substantial contribution to conception and study design, data acquisition analysis and interpretation, handled drafting of article and revised it critically for intellectual content and participated to final approval of the final version to be published.

In my opinion, the result of the present study could be of interest for Food Chemistry's readers and I hope that the paper might be acceptable for publication.

I thank you in advance for your attention to our manuscript.

Looking forward to your reply.

Yours Sincerely,
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Highlights

- Darkened spots appeared on chilling injured basil leaves
- Respiratory metabolism was not promoted by chilling stress temperature
- Storage at 12°C preserved the polyphenols profile
- Ethylene biosynthesis might be an indicator of tissue sensitivity to chilling temperature
- Depletion in phenolic compounds was measured at chilling temperature

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 biochemical (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 showed a good amount in metabolites also at the end of the storage, suggesting the possibility to extend the storability after the expiration date, for a possible recovery of bioactive compounds.

Keywords: *Ocimum basilicum* L.; chilling injury; visual quality; polyphenols; ultra performance liquid chromatography

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, 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 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).

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77 *2.2. Plant material and experimental set-up*

78 Plants of three sweet basil (*Ocimum basilicum* L.) cultivars (*Italico a foglia larga*, Enza Zaden srl, Tarquinia, Italy;
79 *Cammeo*, Sais Sementi, Cesena, Italy; *Italiano classico*, Sementi Fuscello, Andria, Italy) were cultivated in greenhouses
80 and purchased from a local farm (Ortoflora, Fasano, Italy). Three consecutive experiments, one for each basil cultivar,
81 were performed. In each experiment and for each cultivar, 120 marketable basil plants were delivered to the Postharvest
82 Laboratory of the ISPA-CNR. Basil leaves of each cultivar were detached from plants and pooled. Then, approximately
83 200 g of detached basil leaves were placed in each polypropylene tray and packed in a macro-perforated polyethylene
84 bag, in order to avoid a modification of the atmosphere inside packages and to limit dehydration. In each experiment,
85 basil leaves bags were stored at chilling (4 °C) or non-chilling temperature (12 °C) for 9 days. Twelve bags for each
86 cultivar and experiment were prepared: two replicates for two temperatures (4 or 12 °C) for three storage time (3, 6 and
87 9 days). All plastic materials were purchased from Carton Pack (Rutigliano, Italy). Basil leaves of each cultivar at time
88 0 and after 3, 6 and 9 days were analysed to evaluate the sensory visual quality, physiological (respiration rate, ethylene
89 production and ammonium content), and biochemical parameters (antioxidant activity, total polyphenols and
90 polyphenol profile).

91

92 *2.3. Sensory visual quality, respiration rate, ethylene production and ammonium content*

93 A group of five trained researchers used a colour photographic scale associated with a brief description as a reference to
94 subjectively assess the sensory visual quality (VQ). Five quality levels, according to the following scale, were used: 5 =
95 excellent, fresh appearance; 4 = good; 3 = fair (limit of marketability); 2 = poor (just below the limit of marketability);
96 and 1 = very bad, inedible. A visual quality of 3 was considered the minimum threshold of acceptance for sale or
97 consumption, and values below 3 indicated a waste product (Cozzolino et al., 2016).
98 The respiration rate was measured using a closed system measured the respiration rate, as reported by Kader (2002).
99 Approximately 100 g of leaves for each replicate was placed into 6 L sealed plastic jars (one jar x replicate), where CO_2
100 was allowed to accumulate up to 0.1%. The time taken to reach this threshold was measured by monitoring the CO_2
101 concentration at regular time intervals. For CO_2 analysis, 1 mL of gas sample was taken from the head space of the
102 plastic jars through a rubber septum and injected into a gas chromatograph (P200 Micro GC, Agilent, Santa Clara, CA,
103 USA) equipped with a thermal conductivity detector. Carbon dioxide was analysed with a retention time of 16 s and a
104 total run time of 120 s on a 10-m porous polymer (PPU) column 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. The mixture was centrifuged for 5 min at 6,440×g, and 0.5 mL of the extract was mixed with 5 mL of nitroprusside reagent (phenol and hypochlorite in alkali reaction mixture) and heated at 37 °C for 20 min. The colour development was determined using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) reading the absorbance at 635 nm. The concentration of NH_4^+ was expressed as µmoles NH_4^+/g, using ammonium sulphate as standard (0-10 µg/mL, $R^2 = 0.99$).

2.4. Extraction of the polyphenol portion

In each experiment, basil leaves were triturated in a mixture of methanol-acetic acid-water (8: 1: 1) in a ratio 1/5 (w/v) and maintained overnight in the dark at 4 °C. The supernatants were recovered by centrifugation (11,600 x g, 15 min; Beckman Italia, Cassina de' Pecchi, Milano, Italy) and stored at -26°C until time of analysis.

2.4.1. Free radical scavenging activity

The free radical scavenging activity was determined using the stable radical DPPH assay (Brand-Williams, Cuvelier, & Berset, 1995). The analysis was performed in microplates by adding 7.5 µL of an extract to 303 µL of a methanol-DPPH solution (153 mM). Next, the absorbance at λ= 517 nm was measured (Cary 50 MPR, Varian, Palo Alto, CA, USA). The absorbance of DPPH in the absence of antioxidants (control sample) was used to determine the baseline value. The EC_{50} value defined the sample concentration (in mg) necessary to inhibit the DPPH radical activity by 50% during a 60 min incubation.

2.4.2. Total polyphenols content

The total phenolic content was determined using Folin–Ciocalteu reagent (Singleton & Rossi, 1965). The absorbance at λ= 760 nm was determined at room temperature using a Cary 73 UV/Vis spectrophotometer (Varian, Palo Alto, CA, USA). Quantification was based on a standard curve generated using gallic acid at concentrations ranging from 0.5 mM to 5 mM ($y=0.5488x+0.0148$, $R^2 = 0.9919$). The results were expressed as mg of gallic acid equivalent (GAE)/g of the fresh product ± standard deviation (SD).

2.4.3. Polyphenols profile

An ACQUITY Ultra Performance LC™ system (Waters, Milford, MA, USA) linked to a PDA 2996 photodiode array detector (Waters) was used for ultra-high-performance liquid chromatography (UPLC) analyses. The Empower software controlled the instruments and acquired and processed the data. The extracts and standards (previously dissolved in methanol) were filtered (0.45-μm; Waters) before analysis. The analyses were performed at 30 °C using a reversed phase column (BEH C_{18}, 1.7 μm, 2.1 x 100 mm; Waters) following the method of Fratianni et al. (2013). The mobile phase consisted of solvent A (7.5 mM acetic acid) and solvent B (acetonitrile) at a flow rate of 250 μL/min. Gradient elution was employed, starting with 5% B for 0.8 min, followed by 5-20% B over 5.2 min; isocratic 20% B for 0.5 min; 20-30% B for 1 min; isocratic 30% B for 0.2 min; 30-50% B over 2.3 min; 50-100% B over 1 min; isocratic 100% B for 1 min; and 100-5% B over 0.5 min. At the end of this process, the system equilibrated the column under the initial conditions for 2.5 min. The pressure ranged from 6000 to 8000 psi during the chromatographic run. The effluent was introduced into an LC detector (scanning range: 210-400 nm, resolution: 1.2 nm). The injection volume was 5 μL. Each sample was tested in triplicate, and the results were expressed as the mean values ± standard deviations.

2.5. Statistical analysis

For each experiment, a multifactor ANOVA for $P \leq 0.05$ was performed with the aim of evaluating the effect of temperature (at chilling, 4 °C, or non-chilling, 12 °C), storage (3, 6 and 9 days) and their interaction on sensory visual quality, physiological (respiration rate, ethylene production, ammonium content) and biochemical parameters (total polyphenols content and antioxidant activity).

In addition, for each experiment, individual phenolic compounds measured by UPLC were processed performing a one-way ANOVA for $P \leq 0.05$, for fresh and stored samples at chilling or non-chilling temperatures at different storage time, with data means arranged in a completely randomized design. The mean values (n=2) for individual phenols (determined by UPLC) were separated using the Student–Newman–Keuls (SNK) test ($P \leq 0.05$). Statistical analysis was performed using the Statistica 6.0 software.

3. Results and discussion

3.1 Effect of storage at chilling or non-chilling temperatures (4 or 12 °C) on basil leaves sensory visual quality and physiological parameters

Visual quality was significantly affected by temperature and storage in all cultivars (*Italico a foglia larga*, *Cammeo* and *Italiano classico*), as reported in Table 1. During storage, all samples (4 °C and 12 °C) exhibited significant reductions in VQ scores (Table 2); in addition, leaves stored at 12 °C exhibited VQ mean values 20%, 30% and 35% higher than samples stored at 4 °C for cv *Italico a foglia larga*, *Italiano classico* and *Cammeo*, respectively (Table 2). The low VQ scores assigned to basil leaves stored at 4 °C during the entire conservation was mainly due to the loss of freshness and to the appearance of slightly darkened spots, which increased on the entire leaf surface at the end of the storage. These symptoms, which were mainly observed in the *Italiano classico* basil leaves, were previously reported on other basil cultivars and represent a clear manifestation of chilling injury damage at stress temperature (Wongsheree, Ketsa & van Doorn, 2009).

Regarding respiration rate, all cultivars exhibited similar initial values of approximately 33.0 mL CO_2/kg/h (Figure 1 A-D-G), according to the data reported by Bekhradi et al. (2015) for *Dolly*, a Genovese basil cultivar. The respiration rate was exclusively affected by storage time in *Italico a foglia larga* (Table 1), showing slight changes during the storage at both temperatures (Figure 1 A). Moreover, the respiration rate was affected by temperature, storage and interaction in *Cammeo* and only by temperature in *Italiano Classico* (Table 1). In the last two cultivars, increased respiration rates were detected in leaves stored at 12 °C compared with 4 °C. This effect was showed only at the end of storage in *Cammeo* (Figure 1 D), whereas it was measured throughout the entire storage in *Italiano Classico* (Figure 1 G). Cantwell & Reid (1993) reported similar results for basil leaves stored for 3 days at 0 °C or 10 °C. Thus, it seemed that the storage of sweet basil leaves at chilling stress temperature (4 °C) did not cause an increase in respiratory metabolism, which is consistent with previous reports on chilling-sensitive vegetables (Saltveit & Morris, 2000).

As for ethylene production, the three cultivars of basil exhibited a different pattern (Figure 1 B-E-H). In particular, ethylene production was affected only by storage in *Italico a foglia larga*, which showed low values with slight changes during storage at both temperatures (Figure 1 B), according to the respiration rate results. Whereas, ethylene was affected by temperature, storage and their interaction in the other two cultivars studied (Table 1). In detail, in *Cammeo* very low ethylene values were measured until 6 days of storage (both at 4 °C and 12 °C). Then, at 9th day of storage, an increase in ethylene production of approximately 2-fold was detected in leaves stored at 12 °C respect to the ones stored at 4 °C (Figure 1 E). In *Italiano classico*, a severe increase in ethylene content in leaves stored at 4 °C was measured starting from the 3th day in storage, whereas no ethylene accumulation was noted in leaves stored at 12 °C (Figure 1 H).

194 This last result is consistent with data reported by Aharoni et al. (2010) on sweet basil leaves stored at 6 or 12 °C for 1
195 week. The increase in ethylene biosynthesis during storage has been suggested as an indicator of tissue sensitivity to
196 chilling temperatures in different vegetables (Lafuente, Zacarias, Martínez-Téllez, Sanchez-Ballesta & Dupille, 2001;
197 Megías et al., 2016). The induction of ethylene production is always associated with an accumulation of transcripts for
198 the ethylene biosynthesis enzymes (Lado, Rodrigo, Cronje & Zacarías, 2015). The different patterns in ethylene
199 production revealed by the different cultivars might lead to consider *Italico a foglia larga* as a genotype with low
200 sensitivity to chilling temperature of 4 °C, whereas *Italiano classico* could be considered a cultivar that has a high
201 sensitivity to injury at low temperature. Thus, the sensibility to chilling injury exhibited by different basil cultivars
202 could be considered as a genotypic trait, as previously highlighted by Cantwell &Reid (1993).
203 Ammonium content was affected only by interaction in *Italico a foglia larga* and by temperature, storage duration and
204 interaction in *Cammeo* and *Italiano Classico* (Table 1). As reported in Figure 1 C-F-I, during storage, starting from an
205 initial value of approximately 0.5 µg NH_4^+/g, an increase in ammonium was measured in basil leaves of cv *Italico a*
206 *foglia larga*, *Cammeo* and *Italiano classico* stored at 4 °C. Similar NH_4^+ accumulation was observed in vegetables that
207 were stored in stressful conditions (Cefola, Amodio, Rinaldi, Vanadia, & Colelli, 2010; Cefola, Pace, Colelli, &
208 Cantwell, 2015). On the contrary, during storage at 12 °C, the ammonium content exhibited a slight reduction in *Italico*
209 *a foglia larga* and *Cammeo* (Figure 1 C-F) or remained almost constant in *Italiano Classico* (Figure 1 I). Generally,
210 during storage low ammonium accumulation is associated with a good preservation of overall quality for leafy
211 vegetable (Tudela, Marín, Garrido, Cantwell, Medina-Martínez & Gil, 2013; Cefola & Pace 2015).
212
213 *3.2 Effect of storage at chilling or non-chilling temperatures (4 or 12 °C) on basil leaves antioxidant activity and*
214 *total polyphenols*
215 Antioxidant activity and total phenols content were affected by temperature, storage duration and their interaction in
216 *Cammeo* and *Italiano Classico* cultivars (Table 1).
217 Fresh leaves of all three cultivars exhibited an excellent antioxidant activity, with EC_{50} values ranging between 0.78 mg
218 (*Italiano classico*) and 1.05 mg (*Italico a foglia larga*) (Figure 2 A-B-C). Such values are of remarkable interest, mainly
219 compared with previous works that reported EC_{50} values between 1 and 4 mg (extraction in ethanol and water,
220 respectively) (Sulaiman, Sajak, Ooi, Supriatno & Seow 2011). Our results were more similar to those of Hakkim, Gowri
221 Shankar & Girija (2007), which, however, worked using previously dried leaves. Storage at chilling or non-chilling
222 temperatures (4 or 12 °C) affected the antioxidant activity in different modes (Figure 2 A-B-C). At the end of storage at
223 4 °C, cv *Italico a foglia larga* and *Italiano classico* exhibited a nearly 100% loss of antioxidant capability respect to

fresh samples (2.015 mg *versus* 1.05 mg and 1.34 mg *versus* 0.78 mg, respectively, Figure 2 A and 2 C); the loss was even more evident in the case of cv *Cammeo*; in fact, about 7 times more of extract (5.99 mg of extract instead of the initial 0.836 mg, Figure 2 B) was necessary to inhibit the activity of the stable radical DPPH (Figure 2 B). Storage at 12°C effectively preserved the antioxidant activity of the three cultivars for 9 days, and the loss of antioxidant power was minimal. Both considering the antioxidant activity observed at the 9th day of storage and the trend exhibited throughout storage, cv *Italiano classico* could be considered to exhibit superior quality compared with the other two varieties and probably related to its different chilling sensibility (Wongsheree, Ketsa & van Doorn, 2009).

The content of total polyphenols in the fresh detached leaves was approximately 2.5 mg GAE /g in all basil cultivars studied (Figure 2 D-E-F). Such data are consistent with Dogan, Turan, Dogan, Arslan, & Alkan (2005), and Shiga, Shoji, Shimada, Hashida, Goto & Yoshihara (2009). At the 9th day of storage at 4°C, cv *Italico a foglia larga* and *Italiano classico* exhibited respectively losses of 46% and 20% respect to fresh leaves (Figure 2 D and 2 F); at the end of the storage, the loss of polyphenols was much more marked in cultivar *Cammeo* basil leaves (78%) stored at 4°C. On the other hand, storage at 12°C seemed strongly limit this event in all cultivars (Figure 2 D-E-F). Anyway, data of antioxidant activity and polyphenols content resulted highly associated; a reduction in total phenol content generally corresponded to a loss in antioxidant capability, mainly highlighted at chilling temperature.

3.3 Effect of storage at chilling or non-chilling temperatures (4 or 12 °C) on polyphenol profile

The polyphenol profile obtained by UPLC analysis is reported in Table 3, which shows the results of the three one-way ANOVA carried out on the three experiments (*Italico a foglia larga*, *Cammeo* and *Italiano classico*) for fresh samples (0 day) and basil leaves stored at chilling (4 °C) or no chilling temperature (12 °C) at different storage time (3, 6 and 9 days). Rosmarinic, caffeic, gallic acid and apigenin were found in all the three cultivars starting from time zero. However, some differences characterising the cultivars, as well as the time and temperature of storage, complemented the scenario, confirming the results obtained by Jayasinghe, Gotoh, Aoki, & Wada (2003). Rosmarinic acid was the most abundant polyphenol (1.5-1.4 mg/gr of fresh product) detected in all the three cultivars. At the end of storage, chilling (4 °C) caused different loss of rosmarinic acid respect to fresh samples (analysed at 0 day) depending on cultivar, ranging from 30% (*Italiano classico*) to 85% (*Cammeo*), with an intermediate behaviour in *Italico a foglia larga* (52%). This reduction is probably due to the antioxidant role of rosmarinic acid (Jayasinghe et al., 2003) in chilling stressed basil leaves. On the other hand, the non-chilling temperature allowed to preserve this important molecule, reporting a decrease of the amount of rosmarinic acid oscillating from 6% (*Cammeo*) to 19% (*Italiano Classico*) until 47% (*Italico a foglia larga*). As regard caffeic acid, present just after detaching from plants, in the highest quantities in *Cammeo* (508.7 ±33.0 µg/g fw) and

Italiano Classico (547.0 ±36 µg/g fw), the trend was different, depending on the storage temperature used. At 12 °C an oscillating trend was measured in all cultivars; moreover at the end of the storage *Italiano classico* and *Italico a foglia larga* showed an amount in caffeic acid similar to fresh leaves, whereas a slight reduction was observed in *Cammeo* leaves (Table 3). Despite, storage at 4 °C led to a linear decrease of the amount of caffeic acid, and this was particularly evident in the *Cammeo* cultivar (Table 3).

Just after detachment, gallic acid was measured in the highest amount in the *Italico a foglia larga* (117 ± 16 µg/g fw), whereas a content 4-fold lower were detected in the other two cultivars analysed (Table 3). In all cultivars, storage at 12 °C preserved the initial content, although with some fluctuations; a similar behaviour was also showed in basil leaves of cv *Cammeo* and *Italiano Classico* stored at 4 °C. Differently, in *Italico a foglia larga* storage at 4 °C caused a severe reduction of about 4-fold respect to initial value at the end of storage (Table 3).

Apigenin was the only flavonoid initially present in all the three cultivars; in particular it was detected in *Italiano classic*o in an amount of 42.0 (± 2.8) µg/g fw, 7-fold-higher than the content measured in the other two cultivars (Table 3). Moreover, at the end of storage, in *Italico a foglia larga* an increase in the content of apigenin of 28% (12° C) and 41% (4° C), respect to the fresh product, was measured. The increase of apigenin turned more marked in *Cammeo* (5.4 and 4.1 times more after storage of 9 days at 12° and at 4 °C, respectively). At the end of the storage, it is to emphasize the strong increase of apigenin found in the *Italiano Classico* stored at 12 °C compared to the storage at chilling temperature, which maintained a level of apigenin similar to that observed in the fresh product (Table 3).

The three cultivars exhibited, although in a specific way, other secondary metabolites, not always present in the fresh product, the synthesis of which was dependent on the temperature and the time of storage. Among the phenolic acids, at time zero we found chlorogenic acid in cultivars *Italico a foglia larga* and *Italiano classico*, but not in *Cammeo*. After 9 days of storage in *Italico a foglia larga* chlorogenic acid increased of about 3 and 4-fold in samples stored at 4 and 12 °C, respectively. Therefore, in the *Italiano classico* a similar increase was measured only at 12 °C, whereas no significant changes were detected in leaves stored at 4 °C respect to fresh samples (Table 3). Chicoric acid was found in the cultivar *Italico a foglia larga* starting from the 6^{th} day and the 9^{th} day of storage at chilling and no-chilling temperatures, respectively. Cultivar *Cammeo* resulted to contain chicoric acid from the 3^{rd} day of storage. However, while the *Italico a foglia larga* was probably able to synthesize chicoric acid during storage, in cultivar *Cammeo* the metabolite disappeared at the 9^{th} day of storage at 4 °C, but continued to be present in the samples stored at 12 °C (Table 3). Caftaric acid, was initially detected only in *Cammeo* (16.0 ± 1.5 µg/g fw) and *Italiano Classico* (24.5 ± 0.8 µg/g fw). During storage at 4 °C, it was detected only at 3^{rd} day in *Italiano Classico*, while it was never detected in *Cammeo*. Differently during storage at 12 °C the amount in caftaric acid doubled in both cultivars respect to fresh samples. As regard cv *Italico a foglia larga*,

caftaric acid was shown only at the end of the storage at 12 °C (Table 3). Among flavonoids, quercetin was detected in cultivar *Cammeo* only after 9 days at 12 °C, while in *Italico a foglia larga* starting from the 3rd day of storage at both temperatures (Table 3). The loss of basil leaves membrane structure, under chilling stress temperature, involves an overproduction of radical oxygen specie (ROS) including $^{\cdot}O_2^-$, HOOH and hydroxyl radical and singlet oxygen, leading to an oxidative stress. There is evidence to confirm that phenolic compounds can detoxify ROS by donating a hydrogen and decomposing in peroxides. Thus, the reduction in phenolic compounds, such as rosmarinic, gallic, chlorogenic and caffeic acids, in basil leaves stored at chilling stress temperature, might be considered the result of the scavenging of ROS (Dua, Agrawal, Singh & Mahajan, 2016). On the other hand, at 12 °C, the polyphenols content was preserved and in many cases enhanced as consequence of the biochemical mechanisms of biosynthesis, which are promoted after the detachment of basil leaves from the plants as well as in wounded vegetable (Saltveit, 2000). Thus, the presence of rosmarinic, caffeic and caftaric acids in important amount, even after 9 days, in basil leaves stored at 12 °C, could give an important "functional" meaning, suggesting the possibility of a recovery of bioactive compounds at the end of the storage. Rosmarinic acid might be metabolised by the microbiome to produce caffeic acid and derivatives, including caftaric acid (Bel-Rhlid, Crespy, Pagé-Zoerkler, Nagy, Raab & Hansen, 2009). Caffeic and caftaric acid can be subsequently metabolised by the microbiome, producing simpler molecules with a positive impact on both the microbiome and organism (Gonthier et al., 2006).

4. Conclusion

Postharvest storage of detached basil leaves at chilling temperature (4 °C) caused the manifestation of dark spots on leaf surface. Moreover, chilling temperature did not enhanced the respiratory metabolism, while it caused a significant increase in ethylene production and ammonium content. Thus, these two last parameters could be indicator of basil cultivar chilling sensibility. In addition, storage at 4 °C caused a depletion in antioxidant activity and polyphenols content. The significant reduction in the main phenolic compounds (rosmarinic, gallic, chlorogenic and caffeic acids) measured in chilling injured basil leaves might be associated to the no-enzymatic antioxidant action performed by these metabolites. On the other hand, the good preservation or the enhancement in phenolic compounds measured even after 9 days of storage at 12 °C, led to consider basil leaves as a good source of bioactive molecules also after the expiration date.

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Figure Captions

Figure 1. Effect of storage at chilling (4 °C) or non-chilling temperatures (12 °C) on respiration rate, ethylene production and ammonium content of basil leaves belonging to cultivar *Italico a foglia larga* (A, B, C), *Cammeo* (D, E, F) and *Italiano classico* (G, H, I).

Figure 2. Effect of storage at chilling (4 °C) or non-chilling temperatures (12 °C) on antioxidant activity and total phenol content of basil leaves belonging to cultivar *Italico a foglia larga* (A, D), *Cammeo* (B, E) and *Italiano classico* (C, F).

Table 1

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 (µmole 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≤ 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 x 2 storage temperatures) were used. Fw: fresh weight.

Table 2

Table 2. Effect of temperature (4 or 12 °C) and storage (3, 6 and 9 days) on visual quality score (5 = excellent, fresh appearance, 4 = good, 3 = fair, limit of marketability, 2 = poor, just below the limit of marketability, 1 = very bad, inedible) of basil leaves from *Italico a foglia larga*, *Cammeo and Italiano Classico* cultivars

Cultivar name	**Temperature (°C)**		*Significance*	**Storage (days)**			*Significance*
	4	**12**		**3**	**6**	**9**	
Italico a foglia larga	2.8 b	3.6 a	**	4.1 a	3.2 b	2.2 c	***
Cammeo	2.5 b	3.9 a	***	4.4 a	3.5 a	1.8 b	***
Italiano Classico	2.4 b	3.4 a	**	4.2 a	2.6 b	1.8 b	***

For each cultivar and for each factor (temperature or storage time) different letters indicates mean significantly different for the following levels: **($P \leq 0.01$), *** ($P \leq 0.001$). For temperature mean values of 6 data (2 replicates x 3 storage times, after 3, 6, 9 days); for each storage time mean values of 4 data (2 replicates x 2 temperature at 4 and 12 °C). Method: 95.0 percent Student–Newman–Keuls (SNK).

Table 3

Table 3. Phenolic compounds of basil leaves from *Italico a foglia larga* (Experiment 1), *Cammeo* (Experiment 2) and *Italiano Classico* (Experiment 3) cultivars identified by UPLC in the fresh samples (just after detachment, at 0 days, d), and in leaves stored (for 3, 6 and 9 d) at chilling (4 °C) or no chilling temperature (12 °C)

Phenolic compounds (µg/g)	**STORAGE CONDITION**							
	0d	**3d-12°C**	**3d-4 °C**	**6d-12°C**	**6d-4°C**	**9d-12°C**	**9d-4°C**	*P*
Experiment 1: *Italico a foglia larga*								
gallic acid	117.27^{a}	25.26^{b}	114.57^{a}	23.42^{b}	49.23^{b}	117.16^{a}	35.55^{b}	***
caftaric acid	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	7.95^{a}	0.00^{b}	**
chicoric acid	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	16.03^{c}	147.18^{a}	99.10^{b}	****
cholorogenic acid	52.93^{d}	167.89^{b}	177.87^{b}	106.30^{c}	107.56^{c}	229.45^{a}	154.48^{b}	***
catechin	19.55^{a}	0.93^{bc}	1.28^{bc}	1.04^{bc}	2.07^{b}	0.00^{c}	0.00^{c}	****
caffeic acid	285.04^{a}	129.66^{d}	168.03^{bc}	87.16^{de}	119.38^{de}	200.82^{b}	136.81^{cd}	****
rosmarinic acid	1508^{a}	895^{b}	856^{bc}	483^{d}	717^{c}	812b^{c}	732^{bc}	****
apigenin	6.66^{ab}	7.07^{ab}	7.44^{ab}	6.47^{ab}	4.07^{b}	8.15^{a}	9.64^{a}	*
quercetin	0.00^{c}	8.31^{b}	12.41^{b}	7.31^{b}	22.22^{a}	7.12^{b}	0.00^{c}	***
Experiment 2: *Cammeo*								
gallic acid	37.17^{bc}	57.11^{b}	91.50^{a}	58.91^{b}	32.50^{c}	59.00^{b}	41.64^{bc}	**
caftaric acid	24.46^{b}	0.00^{d}	0.00^{d}	9.29^{c}	0.00^{d}	52.84^{a}	0.00^{d}	****
chicoric acid	0.00^{e}	27.13^{d}	40.66^{c}	108.77$^{^{\circ}}$	0.00^{e}	83.39^{b}	0.00^{e}	****
cholorogenic acid	0.00^{b}	10.51^{b}	0.00^{b}	4.62^{b}	4.06^{b}	36.35^{a}	0.00^{b}	**
caffeic acid	508.7^{a}	49.8^{d}	98.9^{d}	259.5^{c}	84.9^{d}	388.4^{b}	91.3^{d}	****
rosmarinic acid	1405^{a}	1094^{b}	511^{c}	1451^{a}	484^{cd}	1321^{ab}	206^{d}	****
apigenin	4.98^{cd}	8.56^{cd}	0.00^{d}	15.92^{abc}	11.21^{bc}	27.01^{a}	20.58^{ab}	**
quercetin	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	2.65^{a}	0.00^{b}	*
Experiment 3: *Italiano Classico*								
gallic acid	46.16^{c}	86.82^{a}	41.78^{c}	29.17^{d}	63.41^{b}	48.65^{c}	51.35^{c}	***
caftaric acid	16.08^{c}	7.99^{d}	36.15^{a}	27.71^{b}	0.00^{e}	29.77^{b}	0.00^{e}	****

cholorogenic acid	44.36^{c}	36.60^{c}	79.43^{b}	90.44^{b}	80.94^{b}	138.70^{a}	21.44^{c}	***
catechin	0.00^{b}	48.65^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	****
caffeic acid	547^{a}	241^{e}	455^{bc}	407^{cd}	313de	541^{b}	318de	**
rosmarinic acid	1672^{ab}	1692^{a}	1593^{ab}	811^{d}	485^{e}	1365^{bc}	1180^{c}	****
apigenin	42.00^{b}	42.76^{b}	54.88^{b}	37.12^{b}	46.74^{b}	230.50^{a}	45.46^{b}	****
rutin	0.00^{d}	81.14^{b}	0.00^{d}	42.03^{c}	96.85^{a}	0.00^{d}	0.00^{d}	****

For each experiment and for each compound identified, mean values (n=2) followed by different letters (a-e) are significantly different for $P \leq 0.05$.
*for $P \leq 0.05$; ** ≤ 0.01; ***≤ 0.001; **** ≤ 0.0001. Method: 95.0 percent Student–Newman–Keuls (SNK).

Figure 1

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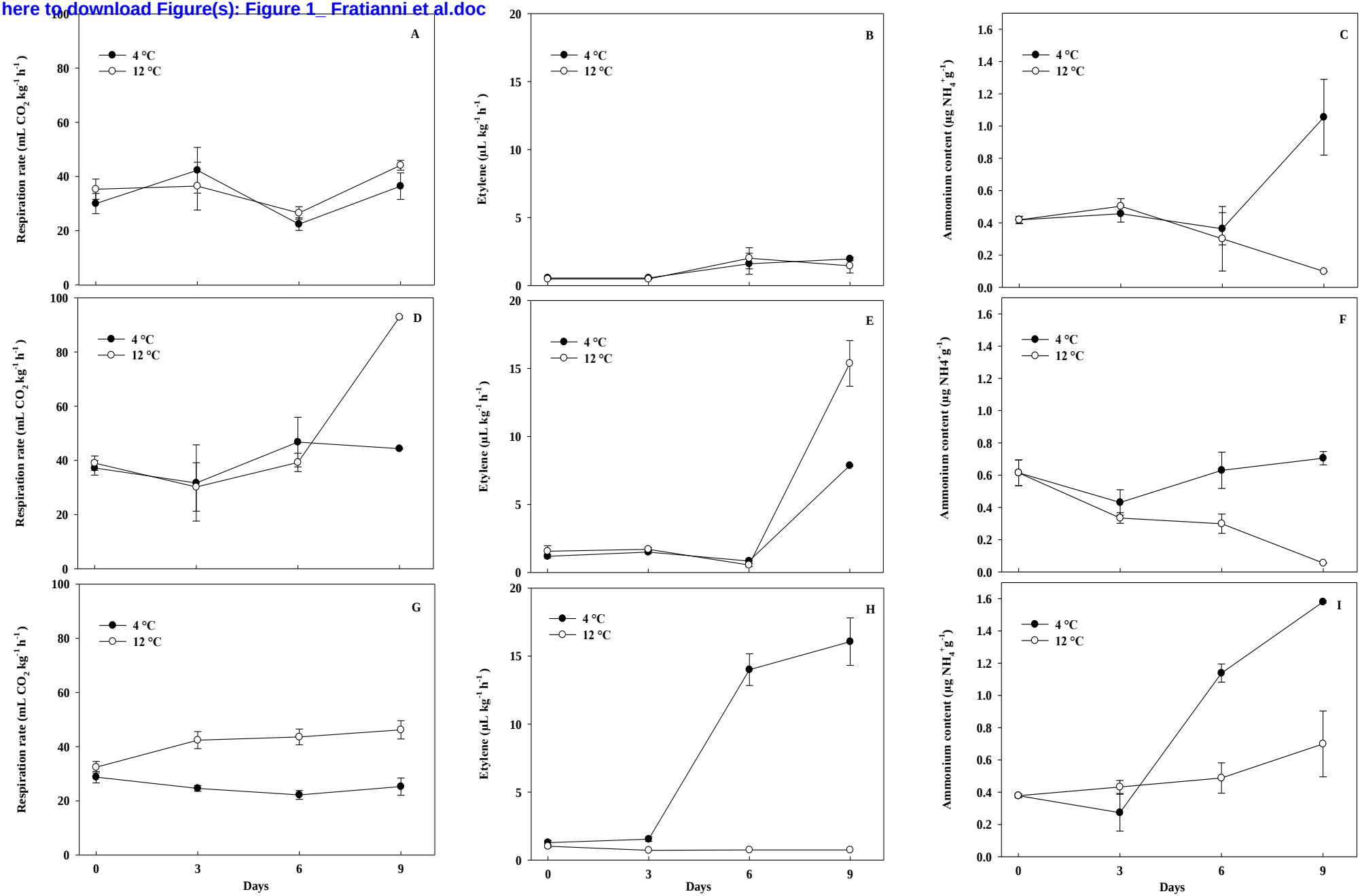

Figure 1

Figure 2

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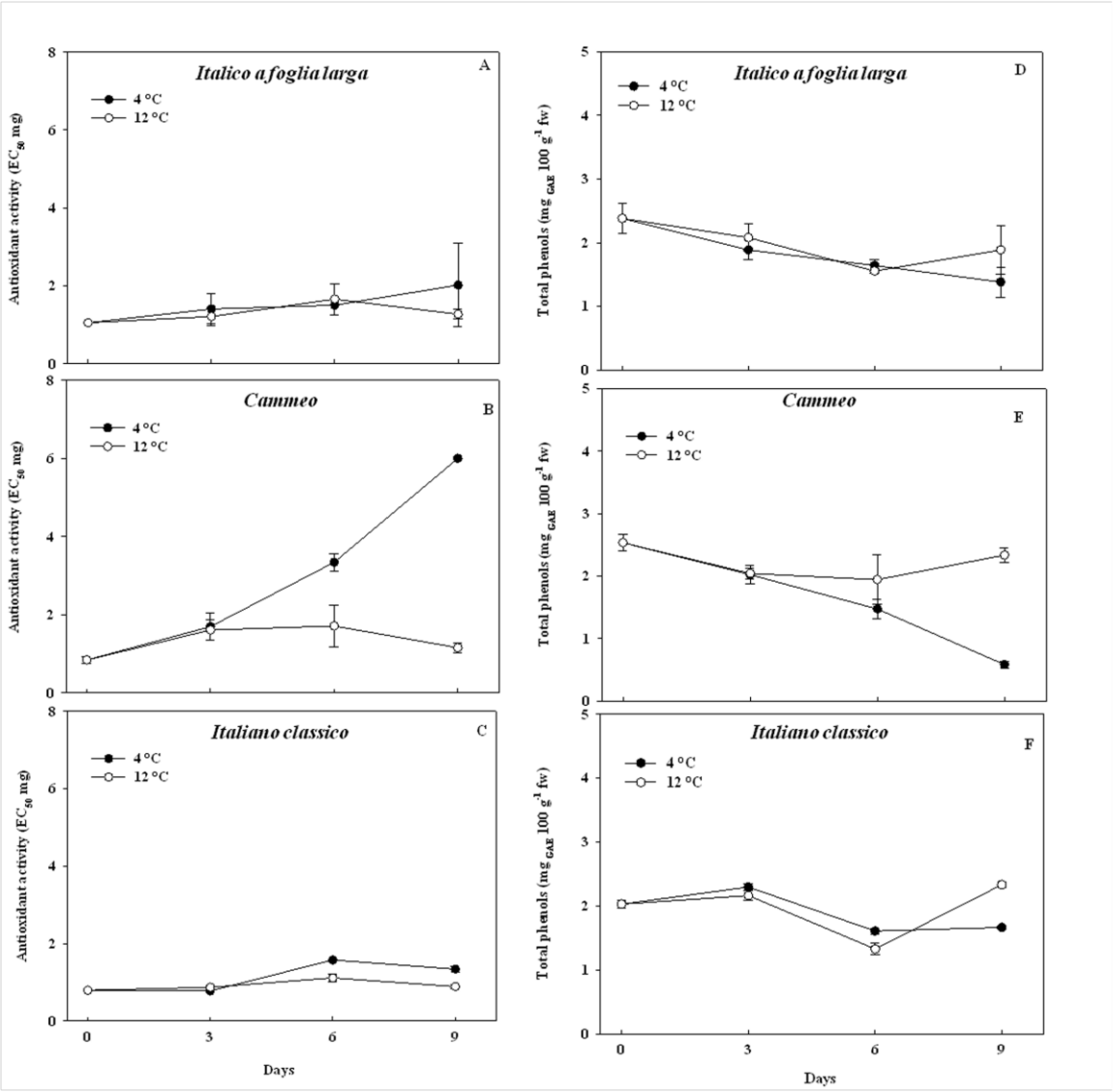

Figure 2