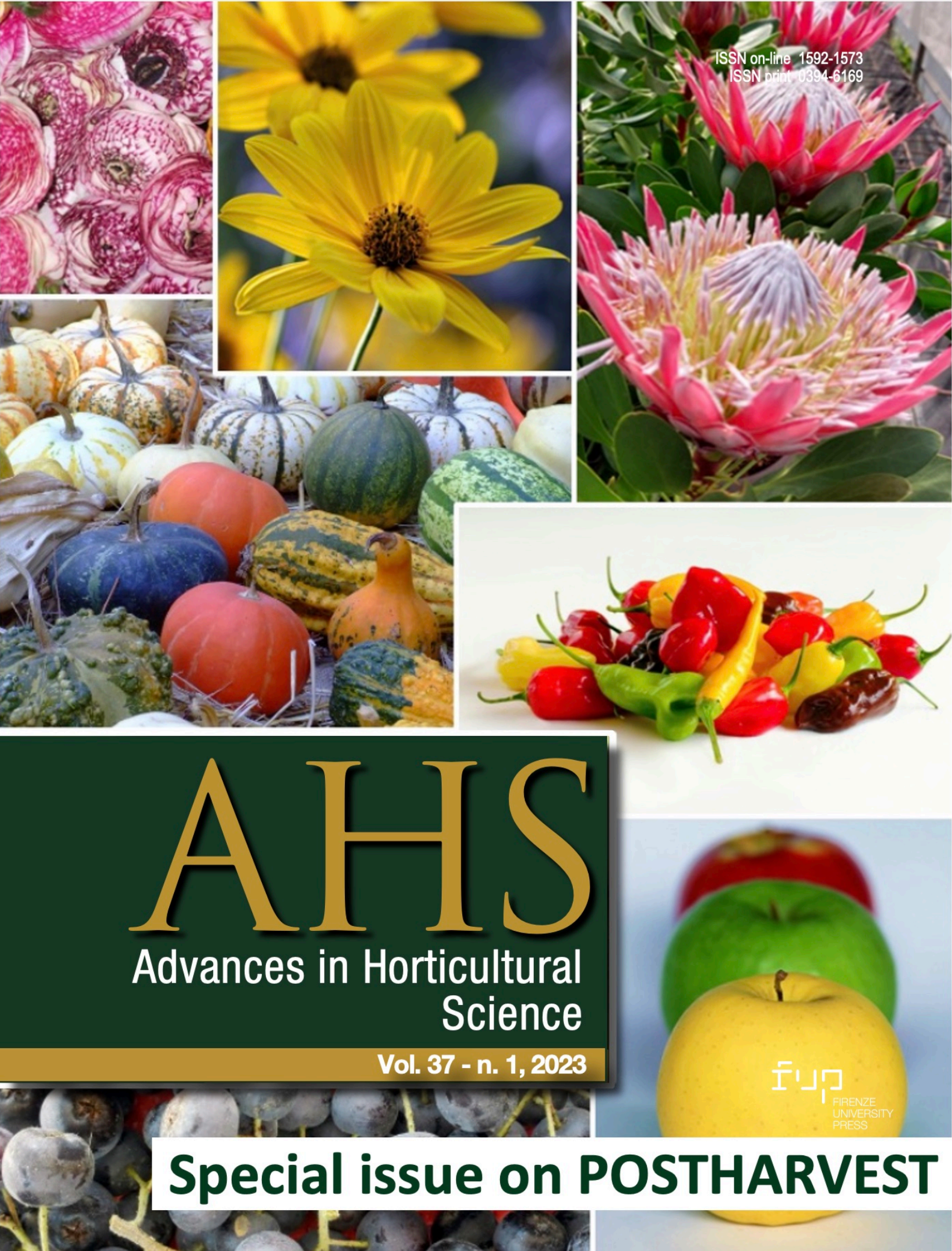

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Foreword

In a world in which perishable products, such as fruits and vegetables, flowers and ornamental plants, must be stored for long periods before being sold and must make long journeys from the place of production to that of consumption, often even from one continent to another, the development of appropriate technologies that allow these products to preserve their qualitative, product and commercial characteristics as much as possible becomes an essential strategy for the productive sector. Postharvest technology is an area in which Italy occupies a leading position worldwide both in R&D and in the manufacturing industry for sorting, grading, packaging technologies, and refrigeration and controlled atmosphere storage facilities. A strong cooperation between the world of research, both public and private, that of agricultural production and that of product conservation technologies is the key point of this predominant position of our country in this specific sector.

The *Postharvest Working Group (PWG)* of the ***Italian Society for Horticultural Science (SOI)*** organised **Postharvest 2022**, the **8th Postharvest Conference** in Pescia, on September 29-30, 2022. Previous edition was held in October 2019 (shortly before the pandemic) in Milan when the 25th anniversary of PWG was celebrated.

Like previous conferences, Postharvest 2022 aimed to bringing together scientists, students, and Industry representatives, with different background, to discuss the state of the art in R&D and technological development in the postharvest physiology and technology of fresh fruits, vegetables, and ornamentals, analysing perspectives and future needs, and indicating possible synergies.

Over 70 delegates participated in the Conference from prestigious Italian and Foreign Institutions and companies, with 25 oral presentations and 25 Posters.

A policy of maintaining relatively low costs for students and scholarship holders allowed the participation of numerous young researchers who had the opportunity to present their results and interact with leading national and international postharvest experts and industry representatives.

Two technical visits (to one of the most important companies in the Pistoia Nursery District and to the Pescia Flower Market) and one cultural event (the splendid Historic Garden of Villa Garzoni in Collodi) completed the Program, together with the social dinner and the Business meeting of SOI-PWG, and allowed further interactions among participants.

Our heartfelt thanks go to Ilaria Mignani and Stefania Nin, Associate Editors of this “Postharvest” Special Issue, whose precious work in editing definitely led to the success of this event.

Gianluca Burchi
Co-convener of the 8th Postharvest Conference

Giancarlo Colelli
Co-convener of the 8th Postharvest Conference, Coordinator of the SOI-PWG, and Chair of the ISHS Division Postharvest and Quality Assurance

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Fruit maturity and antioxidant activity affecting superficial scald development in 'Abate Fétel' pears

A. Bonora [1 (*)], A. Venturoli [1, 2], M. Venturi [1], A. Boini [1], L. Corelli Grappadelli [1]

[1] *Department of Agricultural and Food Science, University of Bologna, Viale Giuseppe Fanin, 46, 40127 Bologna, Italy.*

[2] *Current affiliation: Terremerse Soc. Coop., Via Cà del Vento, 21, 48012 Bagnacavallo (RA), Italy.*

Key words: Antioxidant capacity, fruit quality, preharvest factors, Pyrus communis, superficial scald, total phenolic content.

(*) **Corresponding author:**
a.bonora@unibo.it

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Abstract: Superficial scald (SS) is one of the main physiological disorders affecting postharvest of pears. Its onset is linked to oxidative processes. Antioxidant compounds such as ascorbic acid and phenolics could play a key role in preventing SS. Growing environment and fruit quality also have an influence on SS symptoms occurrence. The aim of this project is to understand the relationship between antioxidant activity, phenolic content, and development of SS in 'Abate Fétel' pear. Moreover, the effect on SS of fruit maturity at harvest was assessed using multivariate statistical approach. Data were collected in thirty orchards in the Emilia-Romagna region (Italy) in three seasons (2018, 2019 and 2020), and the fruit were stored in a regular atmosphere for 120 days. Antioxidant capacity was determined by 2,2-diphenyl-1-picrylhydrazy (DPPH) method and total phenol content by Folin-Ciocalteau colorimetric protocol. The results showed that 340 mg of ascorbate/100 g of FW and 300 mg of gallic ac./100 g of FW at least provide good protection against SS. Multivariate analysis indicated that pulp firmness and index of absorbance difference (I_{AD}) seem to keep low the SS occurrence, when at harvest are higher than 6.3 kg and 1.9, respectively. In conclusion, it would be possible to build a forecasting model to control SS that considers pre-harvest data and content of antioxidants in different orchards, to improve the postharvest management of 'Abate Fétel'.

1. Introduction

Superficial scald (SS) is one of the main physiological storage disorders of European pears (*Pyrus communis L.*). SS is a skin disorder that appears as brown or black patches on the fruit. SS is considered a chilling injury which induces a damage and death within the surface layers of cells in localized regions (Lurie and Watkins, 2012). During SS development necrosis of the hypodermal cortical tissue seems to be induced by oxidation products of the sesquiterpene (E, E)-α-farnesene (Bain and Mercer, 1963; Rowan *et al*., 2001). α-farnesene, accumulates at a relatively high

level in the fruit peel during low-temperature storage (Whitaker *et al*., 2009; Yazdani *et al.*, 2011; Lu *et al*., 2013; Calvo *et al*., 2015). The observation that SS could be inhibited by certain antioxidant treatments and low oxygen in the storage rooms atmosphere has provided evidence that development of the disorder was associated with oxidative processes (Huelin and Coggiola, 1970; Whitaker, 2004; Vanoli *et al.*, 2015). Thus, the conjugated trienols (CTols) that result from the oxidation of α-farnesene are assumed to play a causal role in the occurrence of SS (Whitaker, 2007; Giné Bordonaba *et al*., 2013). Nevertheless, it is generally accepted that the accumulation of both α-farnesene and CTols may be mediated by ethylene which is effectively correlated with SS development (Bai *et al*., 2009; Lu *et al*., 2013; Xie *et al.*, 2014; Yazdani *et al*., 2011). Therefore, it has been suggested that α-farnesene oxidations is a direct consequence of free radical reactions occurring during chilling injury and α-farnesene is not always required for the induction of SS but rather in aggravating the symptoms in fruit already compromised by oxidative stress (Rao *et al*., 1998; Rupasinghe *et al*., 2000). In this context, it has been suggested that superficial scald mainly results from an imbalance between the fruit capacity to generate antioxidants and the reactive oxygen species (ROS) produced during cold stress (Ahn *et al*., 2007; Guerra *et al*., 2012; Ju and Bramlage, 2019). Nevertheless, the antioxidant system in fruit includes an enzymatic and a non-enzymatic component that play an important role modulating oxidative damage to cell walls (Ahn *et al*., 2007; Lurie and Watkins, 2012; Li *et al*., 2016). Furthermore, non-enzymatic antioxidants can prevent oxidation-linked damages responsible for superficial scald through biosynthesis of phenolics that are involved in protective redox-linked pathways under cold stress (Larrigaudière *et al*., 2016; Sarkar *et al*., 2018). The nonenzymatic scavengers of reactive oxygen species include low molecular mass antioxidants with high-reducing potentials, such as ascorbic acid (AA) and glutathione (GSH). Ascorbic acid acts as an antioxidant compound since it can protect fruit membranes from lipid peroxidation (Shewfelt and Del Rosario, 2000) and acts against reactive O_2 species in concert with α-tocopherol (Jimenez *et al.*, 1997). Nevertheless, AA tends to decrease during storage and processing of fruit and vegetables (Haffner *et al*., 1997). A relationship was found between AA content and the susceptibility to browning during experimental storage under various brown core-inducing conditions (Pintó *et al*., 2001). In pears the antioxidant capacity is well explained by phenolics content (Galvis Sánchez *et al*., 2003). Several studies have demonstrated that these compounds are associated with resistance to SS development in apples and pears (Ju *et al*., 1996; Zhao *et al.*, 2016). Phenolic compounds are particularly sensitive to storage factors such as controlled atmosphere (Amiot *et al.*, 1993). Variability of phenolics in plant tissues depends on many pre-harvest factors, such fruit maturity and environmental conditions, including temperature, UV light, and nutrition (Markham *et al*., 1998; Rivero *et al*., 2001; Rühmann *et al*., 2002). Casero *et al*. (2004) used the partial least squares regressions (PLS), a multivariate technique, and found correlations between fruit quality attributes, such as fruit acidity and firmness, and storage disorders with nutrients such as calcium, potassium and phosphorus, both in the leaf and fruit. Moreover, PCA biplots were helpful in showing the segregation between SS classes and their associations with the various physicochemical attributes (Cronje *et al*., 2015). In pear, pulp firmness is one of the most relevant quality parameters (Saquet, 2019). Softer fruit had rounder cells separated by larger intercellular spaces than firmer fruit. On the other hand, firmer fruit have smaller cells with less interspace which means denser tissues and longer storage than soft fruit (Johnston *et al*., 2002). Moreover, the DA-meter, a handheld device that measures chlorophyll concentration several millimetres into the flesh of fruit providing the index of absorbance difference (I_{AD}) (Ziosi *et al*., 2008), can discriminate the ripening stage of climacteric fruit for postharvest tailored cold storage (Bonora *et al*., 2013; Gagliardi *et al*., 2014; Sadar and Zanella, 2019). Fruit ripeness is also well predicted by starch degradation using a multivariate statistical approach (Zude-Sasse *et al*., 2002). Conversely, in 'Abate Fétel' pear fruit the starch index is not always employed even if some studies have reported the use of this procedure to predict pear storability and postharvest issues (Kingston, 1992; Le Lezec and Belouin, 1994; Agar *et al*., 1999; Calvo *et al.*, 2011). In pears starch pattern degradation can be influenced by environmental and management factors such as temperatures, harvest date and deficit irrigation affecting the kinetics of starch accumulation and degradation (Watkins *et al*., 1982; Kramer, 1983; Lopez *et al*., 2013; Lindo-García *et al*., 2019). Total sugar content is an internal fruit quality trait that is crucial for consumer acceptance (Osorio

and Fernie, 2014). Total soluble solids in 'Abate Fétel' and 'Forelle' pear are mainly fructose, glucose and sucrose (Mesa *et al*., 2016), and they increase in concentration after storage since starch is converted via hydrolysis into sugars over time (Visser *et al*., 1968; Crouch and Huysamer, 2011; Rizzolo *et al*., 2015). Additionally, sorbitol accumulates in the fruit still attached to the tree (Mesa *et al*., 2016), acting as cryoprotectant in cellular structures during cold storage by preventing dehydration of membranes and proteins through an osmotic adjustment process (Busatto *et al*., 2018). Therefore, the aim of this work was to research relations between antioxidant activity, phenolic content, and SS development on 'Abate Fétel' pears. Furthermore, preharvest maturity and non-destructive postharvest quality parameters, as well as antioxidant activity and phenolic content, influencing the occurrence of superficial scald using multivariate analysis and regression trees were investigated to develop new reliable hypotheses of their effects in SS development, without compromising consumer acceptance and nutritive value.

2. Materials and Methods

Fruit material and superficial scald evaluation

Fruit were harvested during three consecutive seasons (2018, 2019 and 2020) from different 'Abate Fétel' orchards located in the Emilia-Romagna Region, Italy. Fruit from 30 and 23 farmers were collected and their maturity assessed in 2018 and in seasons 2019 and 2020, respectively. The farmers were indicated by three digit-numbers. In all seasons, two orchards with historical higher SS and two with lower SS were subjected to biochemical analysis at harvest and during storage. In 2018, eighteen 15 kg boxes for each farm were placed in a regular atmosphere (0.5°C and >90% of relative humidity - RH). After 3 (T1), 4 (T2), and 5 months (T3) of storage, the room was opened, following the calendar normally applied by the company. In 2019 and 2020 only six 15 kg boxes per orchard were harvested and placed with a regular atmosphere in a cold room which was opened after 4 months (T2). Afterwards, the presence of superficial scald was assessed in 30 fruits per farm. We defined four classes depending on the severity of symptoms in the skin of pears: class 0 where there was no peel browning, class 1 from 0% to 25% fruit peel showing SS, class 2 from 25% to 50% SS, and class 3 over 50% SS after shelf life. A SS index was computed as follows (Bonora *et al*., 2021):

$$\text{SS index} = \sum_{0}^{4} \frac{\text{(index level) x (fruit at this level)}}{\text{Total number of fruit}}$$

Analysis of the physical characteristics

In all seasons, 30 fruits per orchard at harvest (T0) were subjected to qualitative analysis such as fruit size, index of absorbance difference (I_{AD}), pulp firmness, soluble solid content and starch content. Moreover, non-destructive fruit quality such as size and I_{AD} after 4 months (T2) of cold storage were considered. Weight and dimensions (diameter and height) of each fruit were measured with an automatic caliper (S_Cal WORK, Sylvac, Switzerland) and an electronic balance (KB 1200-2N, KERN, Germany) connected to a notebook. Individual fruit ripeness expressed as I_{AD} was measured with the DA-meter 53500 (Sinteleia, Bologna, Italy) on the fruit side most exposed and less exposed to the sun. Individual fruit flesh firmness (FFF) was determined by FTA (Fruit Texture Analyser, Güss Instruments, Strand, Western Cape, South Africa) fitted with an 8 mm diameter tip, after removing the fruit peel from opposite sides at 180°. The mean value of fruit ripeness and firmness, from the two sides, was calculated. Soluble solid concentration (SSC;°Brix) was determined by measuring the refractive index of the juice for each fruit with a digital refractometer (PAL-1, Atago). The stage of starch hydrolysis was determined by dipping half-cut pears into a Lugol solution and scoring the fruit according to the Ctifl-EUROFRU scale (1-10; 1 = minimum, 10 = maximum starch hydrolysis) (Planton, 1995). Finally, at harvest (T0) and during storage (T1, T2, T3) pieces of the same size with pulp and peel of fruit from all the orchards in 2018 and from four representative farmers in 2019 and 2020 were frozen in liquid nitrogen and stored at -80°C. These plant materials have been used for quantification of antioxidant activity and total phenolic content.

Quantification of antioxidant activity

To estimate the antioxidant activity, the 2,2-diphenyl-1-picrylhydrazy (DPPH) method was used (Adapted from Brand-Williams *et al*., 1995). The DPPH working solution was prepared in 70% acetone (v/v), with a final concentration of 0.02 mg/mL (w/v) and stored at 4°C until needed. Afterwards, antioxidant compounds from 0.5 g of pear (flesh and peel) were extracted in 10 mL of acetone 70%. The frozen

material (0.5 g of pear) was homogenised in a Ultraturrax (IKA T25 digital ULTRA-TURRAX) with 10 mL of extraction solution (acetone 70%) for 2 minutes on ice. After vortexing, the tubes were sonicated in a bath-type sonicator for 15-20 minutes and the homogenates were centrifuged at 1,500 x *g* for 20 minutes at 5°C. Fruit extracts (0.1 mL) were allowed to react with 3.9 mL of the DPPH solution for 30 minutes in the dark, and the absorbance at 515 nm by UV-VIS spectrophotometer (Libra S80PC VBW UV/Vis, Biochrom), was measured. The DPPH working solution was considered as the blank and the calibration curve was made using ascorbic acid.

Total phenolic content

Phenolic compounds quantification was performed using the Folin-Ciocalteau colorimetric method (Adapted from Vieira *et al*., 2009). Total phenolics from 0.5 g of pear (flesh and peel) were extracted in 10 mL of 70% acetone. The frozen material (0.5 g of pear) was homogenised in a Ultraturrax (IKA T25 digital ULTRA-TURRAX) with 10 ml of extraction solution (acetone 70%) for 2 minutes on ice. After vortexing, the tubes were sonicated in a bath-type sonicator for 15-20 minutes. The homogenates were centrifuged at 1,500 x *g* for 20 minutes at 5°C. 250 µL of supernatant were added to 2 mL of deionized water and 250 µL of Folin reagent. After mixing, samples were incubated for 5 min and 5 mL of sodium carbonate (Na_2CO_3) and 5 mL of distilled water were added. Following 1 h incubation in the dark, absorbance was measured at 750 nm by UV-VIS spectrophotometer (Libra S80PC VBW UV/Vis, Biochrom). The phenolic concentrations were determined using gallic acid as a standard.

Data treatment and statistical analysis

All the results of antioxidants and phenolics were statistically evaluated by analysis of variance (ANOVA). Furthermore, these data were presented considering four key producers at harvest (T0), after 3 (T1), 4 (T2) and 5 months (T3) of regular air storage. These producers were selected according to the incidence of SS: two had a high incidence of SS (131 and 432) and the others had a low development of SS (272 and 351). Moreover, the fruit quality data were subjected to multivariate analysis to highlight which among the factors considered appears to be more related to the onset of superficial scald. Multivariate statistical analyses, such as canonical correspondence analysis (CCA) and recursive partitioning and regression trees (rpart) analysis, were performed using the statistical software R (R core team, 2020), by addition of packages "vegan" (Oksanen *et al*., 2019) and "rpart" (Therneau and Atkinson, 2019). CCA was used to estimate the interactions between the frequencies of SS classes and the numeric variables. The blue vector indicates the increase of the factors in a certain direction (SS class). Finally, we considered the total variability explained by two components (CCA1 and CCA2) and how each variable affects the first and the second component. Therefore, maturity data at harvest and SS after 4 months in all seasons were considered to elaborate the overall picture. Finally, rpart analysis was applied to detect which factors could contribute more to SS and to understand their thresholds. Green and red lights indicate a decrease or an increase in SS index, respectively.

3. Results

In 2018 antioxidant capacity in fruit during storage decreased significantly (Fig. 1 and Table 1). Regarding phenolic compound content in fruit of different producers, the differences were not statistically significant at harvest and during conservation (Table 1). This can be explained looking at the different producers' behaviour (Fig. 2). Indeed, two different trends can be observed during the first 3 months of storage: in 272 and 351 phenols tend to increase, while in 131 and 432 they decrease. Thereafter, phenols in 131, 432 and 351 increase from T1 to T2

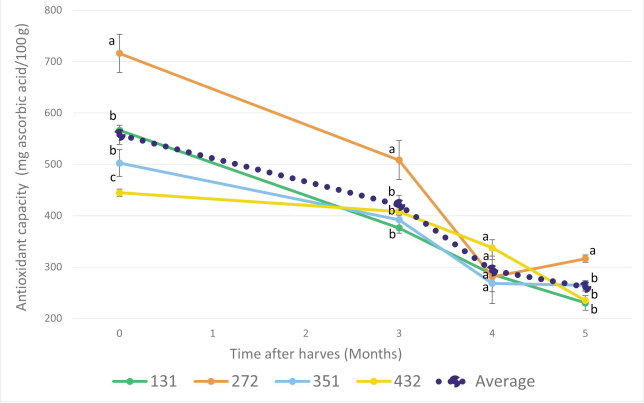

Fig. 1 - Evolution of antioxidant capacity in season 2018 (mg ascorbic acid/100 g of fresh fruit) of four farmers (131, 272, 351, 432) and their average trend. Bars represent standard error of the mean (±SEM). Points followed by the same letter in every sampling point are not significantly different from each other. Mean separation by LSD test (P≤0.05).

Table 1 - Mean and standard error of the mean (SEM) of SS index, antioxidant capacity, total phenolic content in season 2018 at harvest (T0), after 3 months (T1), 4 months (T2), 5 months (T3) in cold storage

Epochs	SS index	Antioxidant capacity (mg ascorbic acid/100 g of fresh fruit)	Total phenolic content (mg gallic acid/100 g of fresh fruit)
T0			
Mean	/	480.92 a	281.99
SEM	/	19.04	15.91
T1			
Mean	5.89 b	370.40 b	312.62
SEM	0.80	11.62	17.85
T2			
Mean	35.46 a	300.38 c	299.37
SEM	2.52	7.85	12.90
T3			
Mean	43.08 a	264.41 c	263.16
SD (%)	2.66	13.05	18.95
Significance (p<0.05)	***	***	NS
Levene test	NS	NS	NS

Data represent the average of fruit quality of 30 producers between epochs for each variable. Values followed by the same letter in columns are not significantly different from each other. Means separation by LSD test (P<0.05).
*** Significant at P≤0.001; NS = not significant.

before decreasing notably again. On the other hand, in 272 we note only a slightly decrease from T1 to T2.

In our study there is a clear distinction between T1, T2 and T3 in terms of SS occurrence in the first season (Table 1). In addition, figures 3, 4, and 5 confirms the great variability of the incidence of SS among the different producers in T2 in all seasons. The evolution of SS index in 2018 of the 30 producers is also shown in Table 1. At T1 the index is low while

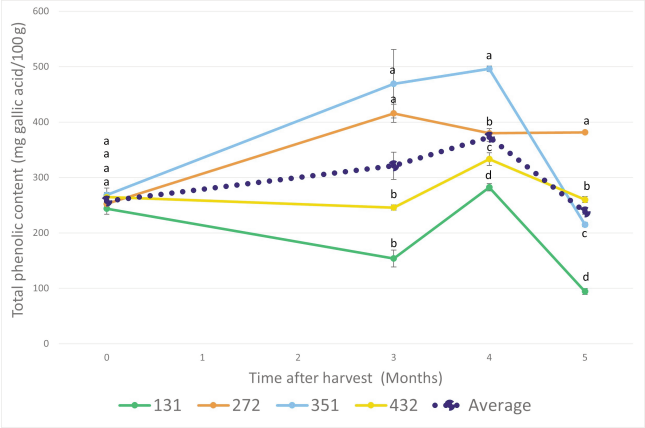

Fig. 2 - Evolution of total phenolic content in season 2018 (mg gallic acid/100 g of fresh fruit) of four selected farms (131, 272, 351, 432) and their average trend. Bars represent standard error of the mean (±SEM). Values followed by the same letter in every sampling point are not significantly different from each other. Mean separation by LSD test (P≤0.05).

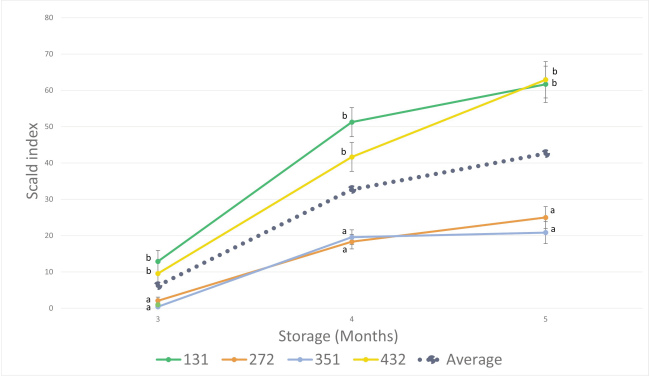

Fig. 3 - Evolution of superficial SS index of four selected farms (131, 272, 351, 432) and their average trend during storage in 2018. Bars represent standard error of the mean (±SEM). Values followed by the same letter in every sampling point after harvest are not significantly different from each other. Mean separation by LSD test (P≤0.05).

there is a considerable increase of SS incidence at T2 and at T3, while antioxidants decrease significantly. Among the key producers of the first season in figure 3, two farmers had a higher SS index (131, 432), while two producers had a lower SS index (272, 351). In detail, the results show that the producers with the lowest SS (351, 272) are those in which phenols increase during the first three months of storage (Fig. 2). Therefore, has been hypothesized that fruit were able to initially react and use these substances to protect themselves from oxidative stress.

Particularly, 351 accumulated phenols till 4 moths which drop from T2 to T3 even below 431, probably, consuming their reducing power instead of antioxidants avoiding polyphenol oxidase activity and browning. On the other hand, the producers (131, 432) with the greatest SS are those in which the phenols drop during the first three months of storage, even if they rise again in the following months (Fig. 2). Probably, the damage caused by oxidative stress is already underway. Notably, we found a drastic decrease of antioxidants between T1 and T2 in producer 272, even if denoted the highest initial antioxidant values at harvest (Fig. 1). Nevertheless, 272 had a low incidence of SS and this could be explained by the fact that during the first three months the antioxidants were high, and phenols increase reaching and keeping a certain threshold value till T3.

Weather and physiological factors in the second and the third season appear to also influence the average nonenzymatic scavengers' level and the SS occurrence (Fig. 4 and Fig. 5). Thus, we found a general high presence of antioxidants and low SS in 2019, characterized by a rainy and cold season. On the contrary, the protective compounds decreased, and SS increased in all producers in 2020 when the temperatures and yields were higher. Moreover, the data shows that antioxidants drop in the first three months of storage in all the four producers considered (Fig. 4 and Fig. 5). However, in both seasons the incidence of SS in producers 131 and 432 was higher

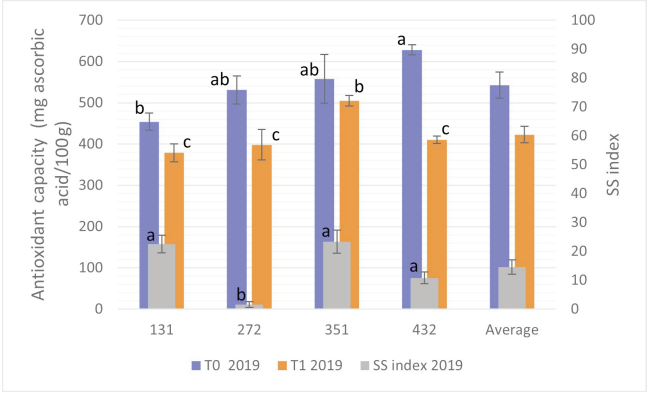

Fig. 4 - Evolution of antioxidant capacity (mg ascorbic acid/100 g of fresh fruit) and SS index after 4 months of cold storage (T2) in seasons 2019 of four farmers (131, 272, 351, 432) and their average trend. Bars represent standard error of the mean (±SEM). Values followed by the same letter between four producers are not significantly different from each other considering DPPH values at T0 and T1 or SS index during storage. Mean separation by LSD test (P ≤ 0.05).

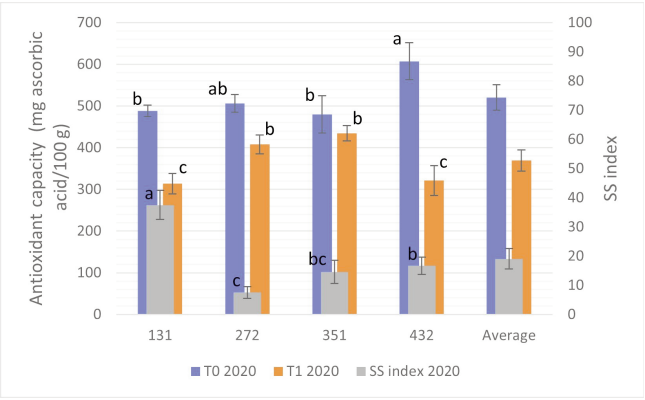

Fig. 5 - Evolution of antioxidant capacity (mg ascorbic acid/100 g of fresh fruit) and SS index after 4 months (T2) of cold storage in seasons 2020 of four farmers (131, 272, 351, 432) and their average trend. Bars represent standard error of the mean (±SEM). Values followed by the same letter between four producers are not significantly different from each other considering DPPH values at T0 and T1 or SS index during storage. Mean separation by LSD test (P≤0.05).

when the antioxidants decrease drastically after 3 months of cold storage, regardless of the level at harvest.

In figure 6 and figure 7, CCA and rpart analysis are applied to study the effects of maturity of 'Abate Fétel' pear at harvest and during storage against SS development at T2 during three consecutive seasons (2018, 2019 and 2020). The multivariate model

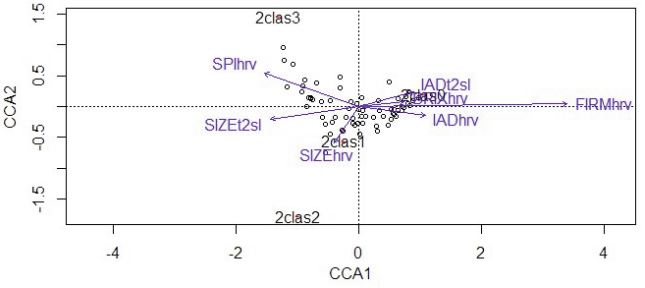

Fig. 6 - Canonical correlation analysis (CCA) of superficial scald classes in 'Abate Fétel' pear after 4 months of cold storage (clas0 0%, clas1 1%-25%, clas2 26-50%, and clas3 51-100% of peel symptoms) against qualitative orchard features at harvest during three seasons 2018, 2019 and 2020 (blue vectors) and the scores of producers (black circles). Total variability explained (53%): CCA1 (90%); CCA2 (8%). The following abbreviations have been used: weight of the fruit at harvest (SIZEhrv), weight of the fruit after 4 months of cold storage (SIZEt2sl), pulp firmness at harvest (FIRMhrv), soluble solid content at harvest (BRIXhrv), IAD-meter values at harvest (IADhrv), IAD values after 4 months of cold storage (IADt2sl), starch pattern index at harvest (SPIhrv).

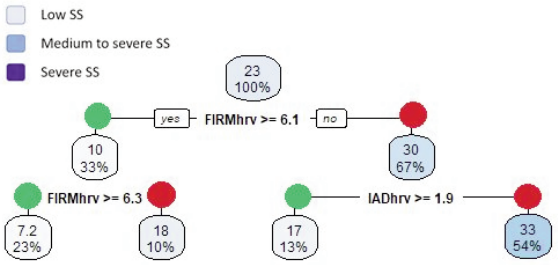

Fig. 7 - Recursive partitioning and regression tree (rpart) analysis, correlation between quality factor and Scald Index. Towards green point hypothesis (FIRMhrv≥ 6.1; FIRMhrv≥6.3; IADhrv≥1.9) is confirmed, to red point is not satisfied. Numbers in the circle represent Scald Index and the percentage of producer that are included in that value of scald index. The colour of the boxes represents the severity of SS: low SS (scald index: 0-15; most fruits do not show SS or show slight symptoms), medium to severe SS (scald index: 16-30; occurrence of progressively more severe symptoms), severe SS (scald index: >30; most fruit show severe symptoms and other very severe symptoms). The following abbreviations have been used: pulp firmness at harvest (FIRMhrv), index of absorbance difference at harvest (IADhrv).

explains 27% of the observed SS variability (CCA1 89% and CCA2 7%). In our study we found that flesh firmness at harvest can prevent SS after cold storage considering all seasons and its contribution to component 1 is 0,95 against SS (Fig. 6). The orchards (23%) with pulp firmness at harvest higher than 6.3 Kg developed low SS (7.2 SS index), while the SS index increased three times in the farms (67%) which, at harvest, scored less than 6.1 Kg of firmness (Fig. 7). However, in figure 6 we noted that bigger fruit at harvest and after storage are more prone to SS (its contribution to principal component is 0.11 at harvest and 0.40 after storage towards SS). In our research starch content at harvest in different producers and seasons influences SS during cold storage with an important contribution to component 1 and component 2 (0.43 and 0.51 respectively towards class 3 after 4 months). The non-destructive I_{AD}-meter values also contribute to preventing SS (Fig. 6), although its contribution to component 1 is lower than firmness and SPI (0.30 and 0.25, at harvest and during storage respectively against SS). Furthermore, in figure 7 we found a specific value of I_{AD} which contributed to SS occurrence for three consecutive years. Among the farms which scored firmness value lower than 6.1 (67%), a fraction (13%) with I_{AD} higher than 1.9 developed an average SS index of 17. The 54% with firmness and I_{AD} lower than 6.1 and 1.9 respectively denoted a SS index higher than 33. Moreover, we found that °Brix promotes resistance to SS during storage of 'Abate Fétel' pears in Emilia Romagna (Fig. 6) and Its contribution to component 1 is remarkable (0.20 against SS).

4. Discussion and Conclusions

As shown in our research, several studies confirm that antioxidant capacity, in particular ascorbic acid, drops during storage (Lee and kader, 2000; Franck *et al*., 2003), promoting a variable SS development in pear between orchards located in different environment (Bonora *et al.*, 2021). Indeed, Silva *et al*. (2010) reported that storage reduced differences in antioxidant capacity between producers at harvest. About phenolic content, fruit may react and produce more phenols when stored for few months. This behaviour is reported in apples by Leja *et al.* (2003) who showed that phenolic compounds are synthesised during storage. Moreover, Calvo *et al*. (2015) highlighted that in addition to the initial value of antioxidants, it is important the level of protective compounds be maintained.

Regarding quality factors affecting SS, Wang and Arzani (2019) also reported a good and negative correlation between high flesh firmness at harvest and SS development in 'd'Anjou' pears. Nevertheless, fruit with a high flesh firmness are more unripe (Stow, 1988) and more prone to contain less antioxidants (Kaur *et al*., 2021). Furthermore, larger fruit generally ripe faster and are characterised by lower firmness and dry matter after storage, by probably increased respiration rate, oxidative stress, and water loss as consequence (Gwanpua *et al.*, 2013). Accelerated senescence, and increased susceptibility to chilling injury have been reported to result from weight loss (Prange and Wright, 2023). On the other hand, the higher surface-volume ratio of larger fruit seems to prevent SS by a reduced evapotranspiration and weight loss during storage (Pasquariello *et al.*, 2013). Although Stow (1988) described starch pattern index as an unreliable method to determine optimum harvesting date of pears, Szczesniak and Ilker (1988) reported that parameters influencing storability and fruit textural characteristics of 'Forelle' pears include the starch content. In contrast with our study, the incidence of superficial scald in apple

declines when the starch pattern index advances (Watkins *et al*., 1982; Mditshwa *et al*., 2015). Concerning I_{AD} meter values, a three-year study by DeLong *et al*. (2014) to develop optimal harvest time for 'Honeycrisp' in Nova Scotia (Canada) led to fruit with a low incidence of disorders after 3 months of storage. Indeed, 'Abate Fétel' pears with higher I_{AD} values at harvest ripen less over 6 months of cold air storage (Rudell *et al*., 2017). In fact, the content of primary photoassimilates certainly supports the production of secondary metabolites such as antioxidants (Mellidou *et al*., 2021).

To conclude, the development of SS seems to be the consequence of the occurrence of many quality and biochemical traits. Therefore, it is important to highlight that it is not possible to consider only one variable at a time to find a solution in pears. We explored the possibility to use multivariate analyses to help understand the relationships between all the factors that may influence SS. Antioxidant capacity is essential in 'Abate Fétel' pear to prevent SS occurrence. Moreover, good pulp firmness, increased I_{AD} values, high total soluble solids and low starch degradation at harvest seems to have a positive impact on SS development. Furthermore, rpart analysis of fruit maturity at harvest confirms the importance of reaching threshold values, as indicators of potential fruit susceptibility to SS during storage, in addition to the absolute trends in multivariate analysis. Therefore, pre-harvest quality and antioxidant values at harvest can be compared with threshold values to discriminate batches of fruit based on their potential to develop SS symptoms. However, it is important to consider that for application purposes it would be necessary to develop faster systems for the quantification of fruit maturity and antioxidant capacity at harvest in the orchards or during storage, using reliable, non-destructive methods. Accordingly, the fruit industry may consider a predictive software to help manage the storage, minimising SS in pears and improving cold room fulfilment and energy efficiency, by recording at harvest antioxidant data and fruit maturity indexes in different 'Abate Fétel' orchards.

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AHS
Advances in Horticultural Science

Effects of cold storage on quality parameters and nutraceutical compounds of pomegranate fruits (cv. Acco)

A. Cirillo [1] (*), A. Magri [2, 3], M. Petriccione [2], C. Di Vaio [1]

[1] *Department of Agricultural Sciences, University of Naples Federico II, Via Università, 100, 80055 Portici (NA), Italy.*

[2] *Research Centre for Olive, Fruit and Citrus Crops, Council for Agricultural Research and Economics (CREA), Via Torrino, 3, 81100 Caserta, Italy.*

[3] *Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Via Vivaldi, 43, 81100 Caserta, Italy.*

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Key words: Bioactive compounds, cold storage, *Punica granatum* L., quality fruit.

Abstract: *Punica granatum* L. contains several bioactive compounds with antioxidant activity that have a positive effect on human health. This study aims to investigate the changes in the chemical-physical and qualitative parameters of pomegranate fruits cv. Acco from harvest up to +90 days of cold storage (+4°C and 95% RH). Morphological parameters, juice yield, weight loss, total soluble solids content (TSS), pH, titratable acidity, the color of the epicarp (L*, a*, b*), content of polyphenols, anthocyanins, flavonoids, and antioxidant activity were analyzed. The results showed an increase (about 29%) in the juice content (%) at +60 days of cold storage. Cold storage has also shown positive effects on some bioactive compounds. Flavonoids and anthocyanins content increased from 287.98 mg CE/100 ml of juice to 389.23 mg of CE/100 ml of juice and from 8.32 to mg/100 ml of juice to 11.13 mg/100 ml of juice at + 90 days of cold storage, respectively. On the basis of our results that confirmed the literature data, the pomegranate fruit is rich in bioactive compounds that exert beneficial actions on human health, and it has also been demonstrated that such nutraceutical compounds increased during cold storage, allowing the fruit to be preserved a long term.

(*) Corresponding author:
aurora.cirillo@unina.it

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1. Introduction

The pomegranate (*Punica granatum* L.) is generally cultivated in the Mediterranean Basin, in the regions of Southern Asia, in India and in North and South America, where long and hot summer favor an optimal fruits ripening (Erkan and Dogan, 2018). Its adaptation to the Mediterranean climate has favored its spread in several countries giving rise, over the centuries, to numerous local genotypes. The fruits are generally harvested when fully ripe and displayed a smooth shining leathery skin with a color varies from green, to pink, reddish, or dark red (Love *et al.*, 2014). The pomegran-

ate fruits have a low respiration rate and a non-climacteric respiratory pattern (Ben-Arie *et al.,* 1984). The edible part of the fruit is called arils and constitutes about 52% of total fruit (w/w), comprising 78% juice and 22% seeds. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins (El-Nemr *et al.*, 1990) and has also been reported to be a rich source of antioxidants (Gil *et al.,* 2000; Kulkarni *et al.,* 2004). The pomegranate is highly valued for health-promoting benefits of its fruit and processed product as demonstrated by numerous *in vivo* and *in vitro* studies (de Nigris *et al.,* 2007; Bell and Hawthorne*,* 2008; Davidson *et al.,* 2009). The consumers pose more attention to the nutraceutical value of fruits, in fact several studies aim to valorize this aspect, as reported by Graziani *et al.* (2020, 2021). The recent growing awareness of consumers to health aspects of fresh fruits and processed products greatly increased the interest in consumption of this fruit and its processed products; consequently, worldwide pomegranate production expanded considerably. Apart from its demand as fresh fruit and juice, the processed products such as carbonated drinks, syrup, wine, and candy are also gaining importance in world trade (Dak and Pareek*,* 2014). The fruit juice of pomegranate was found to have an exceptionally high antioxidative capacity (Reddy *et al.,* 2007; Cirillo *et al.*, 2022). The shelf-life of pomegranate is about 12-14 days at ambient conditions (Naveena *et al.*, 2008); but cold storage with recommended temperature from 0 to 10°C can be used from 2 weeks to 5 months influencing in different manner cultivars storability (Ehteshami *et al.*, 2019). Storage method is very important because physiological and enzymatic processes cause the loss of quality with browning of the skin, necrotic pitting, pallor of the arils that depreciate the product during storage (Fawole and Opara, 2013 a; Dorostkar and Moradinezhad, 2022). In the present study, cv. Acco pomegranate storability, one of the main cultivars marketed in Italy, was analyzed, evaluating physical-chemical and biochemical changes during refrigerated storage up to 90 days from harvest to define the maximum storage time so that the fruits are still appreciated by consumers both organoleptic and nutritional point of view.

2. Materials and Methods

Experimental orchard and sampling

The experiment was conducted in 2019 in Eboli (Salerno, Italy) (40° 33’ 29” N; 14° 58’ 28” E a 15 m a.s.l.) at “Improsta” Regional Experimental Farm, where were cultivated the pomegranate trees of cv. Acco. The trees were trained to sapling system and spaced 3.5 m on the rows and 4 m between the rows. Pomegranate fruits were hand-harvested 3th September 2019 according to a randomized block design from homogeneous trees, for grown and production load. Fruits (n=120) were collected, and 12 lots (10 fruits each) were prepared. Three lots were analyzed at harvest while the others were stored in the cold chamber at a temperature of 4±1°C and 95±1% relative humidity, and analyzed after 30, 60 and 90 days of storage. In this work, 0 indicates the harvest time and +30, +60 and +90 the days of refrigerated storage.

Pomological and physico-chemical traits of fruits

Pomological and physico-chemical traits of the fruits were carried out at the Pomology Laboratory of the Department of Agriculture of the University of Naples “Federico II”. Fruit and seed weight (g) was determined by an electronic digital balance (Precisa Instruments AG, model XB220A, Dietikon, Switzerland) to monitor the weight loss of fruits during cold storage. Equatorial diameter (mm) and fruit length without calyx (mm) were measured by electric digital caliper with ±0.01 mm accuracy (Mitutoyo, Kawasaki, Japan). Color of epicarp was determined with a colorimeter (Minolta, model CR-400, Tokyo, Japan) that was capable of quantifying colors according to international standards and expressed in defined color spaces. The instrument was calibrated with “white” managed by the light source on a white tile, before each measurement. The L* a* b* (CIELAB) color space is the most common method for measuring the color of an object or materials of different origins and it is widely used in all sectors. In this color space, L* indicates brightness, while a* and b* the chromaticity coordinates: +a* is the direction of red, - a* is the direction of green, +b* is the direction of yellow, and - b* is the direction of blue. The measuring was repeated four times in different points of the fruit. The pomegranate seeds were hand-separated from the epicarp and carpellary membranes, counted, and squeezed using a small press. A juice yield of 300 g of pomegranate seeds was measured and expressed as a percentage (w:v). Juice (200 mL) was stored at -20°C and afterwards used for the evaluation of physico-chemical and nutraceutical traits. The pH, titratable acidity (TA) and total soluble solids (TSS) were assessed on the arils juice. The TSS con-

tent was determined with a HI 96.814 digital refractometer of Hanna instruments and results were expressed as °Brix. The pH was determined with a pH meter by the Hanna Instruments laboratory and total acidity was evaluate by acid-base titration, with 0.1N sodium hydroxide standard solution and the results were expressed as g citric acid 100 mL^{-1}.

Determination of total phenolic content

Total phenolics content in juice was determined according to a Folin-Ciocalteu procedure (Singleton and Rossi, 1965). The assay was carried out in duplicate for each sample using 5 µL of extract, 100 µL of Folin-Ciocalteu reagent and 300 µL of 7.5% (w/v) Na_2CO_3 solution; the mixture assay was left in the dark for 2 hours at room temperature, then the absorbance was determined at the wavelength of 765 nm The results were expressed as mg of gallic acid equivalent/100 mL of juice (mg of GAE/100 mL of juice).

Determination of flavonoids content

The assay was carried out according to Zhishen *et al.* (1999) by the aluminum chloride colorimetric method using 20 µL of juice and the absorbance was determined at 510 nm. Results were expressed as mg of catechin equivalent/100 mL of juice (mg CE/100 mL of juice).

Determination of anthocyanins content

The assay was carried out according to Magri *et al.* (2020) by a pH-differential method using 50 µL of juice in KCl pH 1 and CH_3COONa pH 4 buffer. The absorbances at 510 and 700 nm were determined. Results were expressed as mg of cyanidin-3-glucoside equivalent/100 mL of juice (mg C_3G/100 mL of juice).

Antioxidant activity

The assay was conducted as described by Petriccione *et al.* (2015) using 20 µL of juice and 1480 µL of 1,1-diphenyl-2-picril-hydrazyl (DPPH). The change in absorbance was observed at 515 nm and the results were expressed as mg of Trolox equivalent/100 mL of juice (mg Teq/100 mL of juice).

Statistical analysis

Analysis of variance (ANOVA) on the complete randomized block design on the data and mean separation by Duncan's multiple range test ($p<0.05$) and Principal component analysis (PCA) were carried out using XLSTAT, version 2013, statistical software package (New York, NY, USA).

3. Results

Fruit pomological characterization

The changes of the pomological parameters in the cv. Acco pomegranate fruits during the refrigerated storage at + 30, + 60 and + 90 days are shown in table 1. There is a significant reduction in the fruit weight loss about 10.5% already after 30 days of cold storage, up to 23.7% at +90 days, while no significant differences are highlighted for the equatorial diameter, the fruit length, and the seeds weight. One of the most important commercial parameters is the juice content in the seeds, our study showed a significant increase in the percentage of juice from collection to storage in the fridge, after +60 days about 63.5% compared to harvest (Table 1).

Juice quality parameters and fruit color characterization during cold storage

The results of the physico-chemical properties of cv. Acco during cold storage are shown in table 2. A significant reduction in total soluble solids (TSS) was highlighted, from 14.25°Brix at harvest to 11.85°Brix already at +60 days of refrigerated storage, up to a

Table 1 - Pomological parameters (fruit weight, equatorial diameter, fruit length, seed weight (n=100), % weight loss, % juice) at harvest and during the refrigerated storage (+30, +60, +90 days) of pomegranate fruits (cv. Acco). 0 days is the time of harvest

Days	Fruit weight (g)	Equatorial diameter (mm)	Fruit length (mm)	Seed weight (g)	Weight loss (%)	Juice (%)
0	216.38 ± 9.86 a	80.38 ± 1.53 a	63.5 ± 4.12 a	113.88 ± 5.76 b	0 ± 0.00 a	47.60 ± 0.47 c
30	187.18 ± 6.68 b	78.63 ± 0.92 a	65.5 ± 0.79 a	111.44 ± 4.86 b	10.5 ± 0.85 b	54.43 ± 1.55 b
60	171.27 ± 8.84 b	80.31 ± 1.35 a	67.0 ± 1.32 a	113.38 ± 6.70 b	19.6 ± 1.76 bc	63.50 ± 1.69 a
90	167.13 ± 7.48 b	80.88 ± 1.15 a	69.56 ± 0.73 a	119.87 ± 4.70 b	23.7 ± 2.10 c	61.35 ± 1.63 a
Significance	***	NS	NS	NS	**	***

All the data are expressed as mean ± SE (standard error). The same letter indicates not significant differences according to Duncan's multiple range test ($p<0.05$). Level of significance at the ANOVA are indicated as NS (not significant), * ($0.01<P<0.05$), ** ($0.01> P> 0.001$), and *** ($P<0.001$).

Table 2 - Total soluble solids (TSS), titratable acidity (TA), pH and color attributes (L*, a* and b*) at harvest and during cold storage (+30, +60 and +90 days) of pomegranate fruits (cv. Acco). 0 Days is the time of harvest

Days	TSS (Brix°)	TA (g citric acid 100 mL^{-1})	pH	L*	a*	b*
0	14.25 ± 0.25 a	8.75 ± 0.25 a	3.00 ± 0.00 b	47.08 ± 0.66 a	47.14 ± 0.88 a	26.98 ± 0.49 a
30	13.78 ± 0.09 a	8.10 ± 0.11 a	3.10 ± 0.09 b	40.51 ± 1.68 b	43.47 ± 1.32 b	20.66 ± 0.62 b
60	11.85 ± 0.72 b	6.60 ± 0.14 b	3.53 ± 0.07 a	39.09 ± 0.87 b	44.14 ± 0.55 b	20.63 ± 0.74 b
90	10.58 ± 0.21 c	5.63 ± 0.31 c	3.63 ± 0.07 a	41.21 ± 1.08 b	40.03 ± 0.56 c	20.68 ± 0.75 b
Significance	***	***	***	***	***	***

All the data are expressed as mean ± SE (standard error). The same letter indicates not significant differences according to Duncan's multiple range test (p<0.05). Level of significance at the ANOVA are indicated as NS (not significant), * (0.01<P <0.05), ** (0.01> P> 0.001), and *** (P <0.001).

final reduction of about 25.75% at +90 days while the pH showed an increase at +60 days of refrigerated storage equal to 17.66%. Color coordinates (L*, a*, b*) of pomegranate peel during the refrigerated storage are shown in Table 2. L* and fruit peel redness (*a**) showed a reduction with progressed refrigerated storage. The highest a * index was found at harvest (47.14), while after + 90 days of refrigerated storage this parameter was reduced to 40.03. b* was 26.98 at harvest while after + 30 days of refrigerated storage a reduction of about 23.42% was observed, without significant differences up to 90 days of storage.

Content of bioactive compounds and antioxidant activity during cold storage

The current use of pomegranate fruit regards especially the nutritional and, still potential, health benefits that come out from the various parts composing this one (carpellary membranes, arils, seeds and bark). Indeed, the phytochemical composition of the fruit abounds in compounds (flavonoids, ellagitannins, proanthocyanins, mineral salts, vitamins, lipids, organic acids) presenting a significant biological and nutraceutical value. Table 3 shows the bioactive compounds of the pomegranate fruits cv. Acco during the refrigerated storage. The polyphenol content did not display significant differences up to 60 days of storage, with average values of 272 mg GAE/100 ml of juice, while at 90 days an increase in the polyphenol content was shown up to a value of 389.23 mg GAE/100 mL juice; the flavonoid content showed a small increase at 30 days of storage (124.58 mg EC/100 mL juice), without statistical significance, for the other two periods considered with an average content of 103 mg CE/100 mL juice; the anthocyanin content displayed a slight decrease up to 60 days of refrigerated storage with average values of about 3 mg C_3G/100 mL of juice, followed by an increase after 90 days, up to 11.13 mg C3G/100 mL of juice. The antioxidant activity of pomegranate

Table 3 - Total polyphenol, anthocyanins, flavonoids content and antioxidant activity (D) at harvest and during cold storage (+30, +60 and +90 days) of pomegranate fruits (cv. Acco). 0 Days is the time of harvest

Days	Polyphenols (mg GAE/100 mL juice)	Anthocyanins (mg C_3G/100 mL juice)	Flavonoids (mg CE/100 mL juice)	Antioxidant activity (µmol TE/ 100 mL juice)
0	287.98 ± 8.86 b	8.32 ± 1.67 a	99.49 ± 3.25 a	271.63 ± 4.13 a
30	285.25 ± 26.07 b	3.53 ± 2.67 b	124.58 ± 9.27 a	268.11 ± 5.56 a
60	244.15 ± 23.92 b	2.72 ± 3.36 b	102.99 ± 1.90 a	267.67 ± 3.49 a
90	389.23 ± 29.01 a	11.13 ± 4.71 a	104.21 ± 10.46 a	267.97 ± 3.21 a
Significance	*	**	NS	NS

All the data are expressed as mean ± SE (standard error). The same letter indicates not significant differences according to Duncan's multiple range test (p<0.05). Level of significance at the ANOVA are indicated as NS (not significant), * (0.01<P <0.05), ** (0.01> P> 0.001), and *** (P <0.001).

juice showed no significant differences during the refrigerated storage with average values of 268 µmol TE/100 mL juice.

Principal component analysis (PCA)

To obtain a broad overview of all parameters evaluated in cv. Acco fruit following the refrigerated storage a principal component analyses (PCA) was conducted. Figure 1 shows the PCA of the changes of nutraceutical compounds and qualitative parameters, during refrigerated storage. The first two principal components (PCs) disclosed 88.39% of the cumulative variance with PC1 detailing for 55.55% and PC2 for 32.83%. At harvest this cultivar showed a higher antioxidant activity, while a higher flavonoids content is shown between +30 and +60 days of refrigerated storage, at +90 days of refrigerated storage a higher polyphenols and anthocyanins content was highlighted. The qualitative parameters showed higher values at +30 days of refrigerated storage for TSS and TA, while at + 60 days there was a higher juice yield.

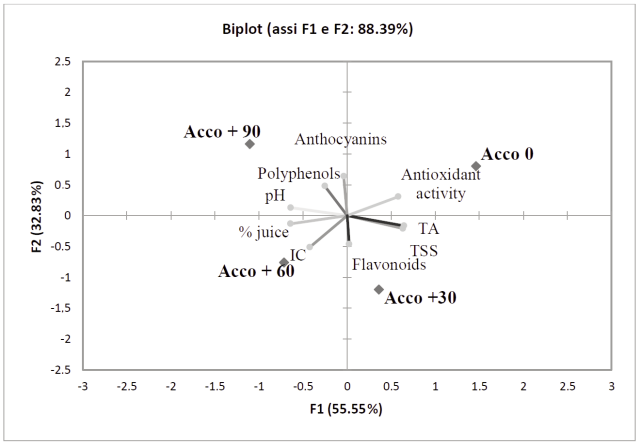

Fig. 1 - Principal component analysis (PCA) based on quality parameters and bioactive compounds at harvest and during cold storage (+30 +60 and +90 days) of pomegranate fruits (cv. Acco). 0 is the time of harvest.

4. Discussions and Conclusions

Both fresh market and processing industry drive pomegranate consumption, and it is crucial to acknowledge all fruit characteristics to not only classify varieties from a botanical point of view, but also to meet the current market demand for quality fruits (Martìnez *et al.*, 2006). In the recent years, the demand and the consumption of pomegranate fruit have shown an extensive growth in several countries worldwide becoming a valuable commodity due to high levels of nutraceutical compounds and greater awareness of health-promoting benefits (Asgary *et al.*, 2022). The major postharvest losses can occur among harvest and consumption affecting either fruit quantity or quality at any stage in the postharvest chain. Freshly harvested agricultural products are a living thing that breathes and undergoes changes during postharvest handling (Kiaya, 2014; Erkan and Dogan, 2018; Siddiqui *et al.*, 2022). Postharvest diseases caused by bacteria, fungi, and other microorganisms are compromised factors and a consistent problem for long term storage of pomegranate fruits (Munhuweyi *et al.*, 2016; Akhila *et al.*, 2022; Gurtler and Garner, 2022). These spoilages greatly affect the appearance, aroma and taste of fruits and the control of postharvest diseases represents the most significant economic challenges in agriculture. High water and sugars content combined with soft and delicate texture make pomegranate fruits susceptible to weight loss, mechanical damage, and attack of pathogens (Sayarri *et al.*, 2012). Physiological and enzymatic disorders are responsible to qualitative decay and storage life in pomegranate like fruit wrinkling, browning, and drying of skin and seeds, seeds paleness and the pathogens often that cause damage to the tissues, thereby making the fruit unsaleable (Caleb *et al.*, 2012 a; Nazoori *et al.*, 2022). As previously mentioned, an important aspect is the juice content. Our results reported an increase in the juice content during refrigerated storage most likely due to the "softening" of the tissues of the arils increasing the extraction of the juice. At harvest, pomological and physico-chemical traits in cv. Acco fruit showed a slight difference compared to what is reported by Ferrara *et al.* (2014); these differences may be ascribed to different cultural practice, climatic and soil conditions (Fadavi *et al.*, 2005; Ferrara *et al.*, 2014). Weight loss is one of the major problems associated with stored pomegranate fruit which cause hardening of the skin and browning of the rind and seeds (Artés *et al.*, 2000 a; Caleb *et al.*, 2012 b; Pareek *et al.*, 2015). Several studies have demonstrate that weight loss increased with increasing temperature and prolonged storage in different pomegranates cultivars and it is due to water being lost through natural porosity of the skin (Al-Mughrabi *et al.*, 1995; Al-Yahyai *et al.*, 2009; Wasker, 2011; Fawole and Opara, 2013 b). Our results, on the physico-chemical parameters of pomegranate fruits during cold storage, are in agree with those reported in the

literature where a reduction in TSS and TA is shown. According to a similar study on 'Mollar' pomegranate after only 7 days of storage at 4°C, the TSS content is reduced about 11% (Gil *et al.*, 1996), while Artes *et al.* (2000 b) showed a significant reduction in the acidity of pomegranate fruit juice in cv. Molla de Elche stored at 5°C for 90 days and subsequently, held at 20°C for six days. The decrease in TSS content could be a result of the degradation of sugars with prolong storage period and the changes in TA levels are strong indications of the ongoing metabolism in the fruit during storage since pomegranate is a non-climacteric fruit (Fawole and Opera, 2013 a). The decrease in fruit acidity can be attributed to the cumulative effects of the increase in juice content and the use of organic acids which act as a substrate for cellular respiration that occurs during fruit ripening (Diakou *et al.*, 2000). Organic acids present in pomegranate include citric, malic, acetic, fumaric, tartaric and lactic acid, but citric acid is the main one which represents the titratable acidity of pomegranate fruits (Melgarejo *et al.*, 2000). The results obtained on the peel color changes during refrigerated storage are in agreement with the results obtained by other studies, where similar storage conditions on 'Ganesh' pomegranate fruit induced the slight change in stored fruit color over a 12-week duration (Nanda *et al.*, 2001). Furthermore, during cold storage our findings highlighted a reduction of fruit lightness and a darker and saturated red color of the skin. In addition to being appreciated for its qualitative aspects the pomegranate is known for the high nutritional value of its fruits and for its beneficial effects on health, and the medicinal properties of the different parts of the tree are also well known. The main phytochemicals responsible for these beneficial health effects are polyphenols which include ellagic acid, ellagitannins (eg punicalagin), punicic acid, anthocyanins, flavonols, flavan-3-oils and flavones. Ellagic acid can be free or condensed with different sugars (glucose, rhamnose and arabinose), with different concentrations between various cultivars (Zaouay *et al.,* 2012). Although ellagitannins are the main polyphenols in pomegranates, punicalagin and punicalin are the compounds most characterized for their antiatherogenic properties (Seeram *et al.*, 2005). Anthocyanins are the pigments responsible for the typical pomegranate fruit color and include delphinidin, cyanidin and pelargonidin 3-glucoside and 3,5-diglucosides. The changes in the composition and concentration of anthocyanins have been shown in the different cultivars, the main anthocyanin in the Spanish sweet pomegranate cultivar "Mollar de Elche" is cyanidin 3-glucoside, while cyanidin 3,5-diglucoside has been found as the main compound in sour pomegranate cultivars (Sayyari *et al.*, 2011 a, b). Several studies highlighted the changes in the bioactive compounds content during the refrigerated storage of the pomegranate (Zaouay *et al.,* 2012; Ehteshami *et al.*, 2020). Fawole and Opara (2013 a) have shown that the polyphenol content did not vary during the first 4 weeks of storage in the 'Bagwa' fruit but increased after 8 weeks due to an accumulation of anthocyanins; variations of bioactive compounds during cold storage are also shown in apples fruits (Graziani *et al.,* 2020). In our study, no decrease in polyphenol content was observed during refrigerated storage in agreement to Baltacioglu *et al.* (2011) and this is attributed to the action of oxidative enzymatic activities following low temperature stress (Ehteshami *et al.*, 2020). The anthocyanin content in the pomegranate showed different trends related to the cultivars and storage temperatures (Fawole and Opara, 2013 a), which suggests that varietal differences represent a key factor in the post-harvest biosynthesis of anthocyanins (Turfan *et al.*, 2011). Furthermore, several studies have shown that the antioxidant activity is linked to the bioactive compounds content and that a decrease in this activity is observed in the pomegranate during refrigerated storage after 4 weeks compared to harvest (Fawole and Opara, 2013 a). Our study showed during refrigerated storage a significant weight loss, while the juice percentage increased, with maximum values at +60 days. Acidity (TA) and TSS decreased during refrigerated storage, but the reduction in acidity was more significant than that of TSS, therefore overall good organoleptic characteristics were showed. The polyphenols and anthocyanins increased during refrigerated storage, while the flavonoids and antioxidant activity were constant. On basis of our results suggest that pomegranate fruits cv. Acco have a maximum storage time in the fridge of 60 days for fresh consumption while they can exceed 60 days to produce the juice, as the chemical-nutraceutical characteristics remain optimal. During the cold storage the pomegranate fruits of the cv. Acco cultivar did not show the appearance of plant diseases (molds or rot fungi), thus allowing a good conservation of the product.

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Phytonutritional and aromatic profiles of *Tulbaghia simmleri* Beauv. edible flowers during cold storage

I. Marchioni [1], B. Najar [1, 2 (*)], A. Copetta [3], B. Ferri [2], B. Ruffoni [3], L. Pistelli [2], L. Pistelli [1, 4]

[1] *Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali (DISAAA-a), Università di Pisa, Via del Borghetto, 80, 56124 Pisa, Italy.*

[2] *Dipartimento di Farmacia, Università di Pisa, Via Bonanno Pisano, 12, 56126 Pisa, Italy.*

[3] *CREA Centro di Ricerca Orticoltura e Florovivaismo, Corso Inglesi, 508, 18038 Sanremo (IM), Italy.*

[4] *Centro Interdipartimentale di Ricerca Nutraceutica e Alimentazione per la Salute (Nutrafood), Università di Pisa, Via del Borghetto, 80, 56124 Pisa, Italy.*

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(*) **Corresponding author:**
basmanajar@hotmail.fr

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Abstract: Edible flowers are appreciated due to their aesthetic features, nutritional value and antioxidant properties. *Tulbaghia simmleri* Beauv. (Amaryllidaceae family) flowers are characterized by a pleasant garlic taste and are consumed both as fresh and dried products. The aim of this work was to assess the effect of chilling temperature (+4°C) on the visual quality, nutritional content, and aroma profile of *T. simmleri* flowers after two (T2) and six (T6) days of storage. Colorimetric analysis highlighted a reduction in petal brightness at T6 and hence their darkening, due to a significant increase in *a coordinate and the decrease in the *b** one. Total polyphenols and flavonoids content remained unchanged until the end of the experiment, while total anthocyanins increased at T2. Flowers antioxidant activity (DPPH assay) decreased progressively during cold storage, while catalase (CAT) and ascorbate peroxidase (APX) activities increased. The aroma profile was analyzed by HS-SPME associated with GC-MS, underlining that fresh flowers were dominated by high content in monoterpenes (around 80%), with 1,8-cineol as main compound (53.1%). Cold storage reduced this class of volatiles while sesquiterpenes and non-terpenes increased; between them, benzyl benzoate reached 12%.**

1. Introduction

Edible flowers (EFs) are traditionally consumed since ancient times (Mlcek and Rop, 2011). Some of them are commonly recognised as vegetables (e.g. artichokes, broccoli, capers), while others are still considered

"unusual food" (reviewed in Pires *et al*., 2019). EFs straights rely on their colours, shapes, flavours, tastes, and nutrients (e.g. carbohydrates, proteins, vitamins, phytochemical compounds with antioxidant and healthy properties) (Fernades *et al*., 2017). Their market is constantly expanding, and new species with attractive sensorial features and good storage attitude are required.

Tulbaghia (common name: wild garlic) is a genus of monocotyledonous plants (Amaryllidaceae family) indigenous to South Africa (Lyantagaye, 2011). Herbaceous perennial bulbs, corms or rhizomes characterize its species. *Tulbaghia* spp. flowers, held in umbels in groups of ten or more, are strongly fragrant and characterised by tubular shape (Zschocke and Van Staden, 2000). A raised crown-like structure or a fleshy ring at the centre of the flower tube are distinctive features of this genus (Vosa, 2000). The colours are different, mainly white, pink or mauve. Flowers and rhizomes produce cysteine-derived sulphur compounds (e.g. marasmicin), which confer to this organs a pleasant alliaceous smell, especially when bruised or during senescence (Aremu and Van Staden 2013; Kubec *et al*., 2013). The peculiar aroma and the pungent garlicky taste of flowers make several *Tulbaghia* spp. interesting for the food industry (Kubec *et al*., 2013).

T. simmleri Beauv. is mainly known as ornamental plant, which flowers consist of six tepals and a central crown of six lobes, fused for more than a third of their length to form a tube. The lobes have pointed tips, giving the crown a fringed edge (Vosa, 2000). In the southern hemisphere, its period of blooming ranges between April to October, even though, with particular climate conditions, it could be extended until early spring (Zschocke and Van Staden, 2000). In the northern hemisphere, however, its period of blooming ranges between October to April. Several bioactive compounds characterize this plant, since it is used to treat fever, colds, headaches, asthma, and tuberculosis in South African traditional medicine (Zschocke and Van Staden, 2000). *T. simmleri* has been severely neglected when compared to the most common *T. violacea*, for which several culinary uses are known, also concerning flowers (Aremu and Van Staden, 2013; Rivas-García *et al*., 2022). Further investigation on *T. simmleri* worth to be performed, since this species produce deep mauve, long lasting edible flowers, which period of bloom does not overlap the one of *T. violacea* (not available in autumn and winter). This will ensure the availability of EFs with garlic taste for most of the year. Moreover, Takaidza *et al*. (2018) highlighted good total polyphenolic and flavonoid content, and hence good antioxidant activity, in *T. simmleri* plants, in comparison with other seven *Tulbaghia* species, *T. violacea* included.

Postharvest technologies are common methods to extend EFs shelf-life, as it is generally rather short (2-10 days) (Fernandes *et al.*, 2019, 2020). Flowers are high value products, which must be picked with care, packaged properly to protect them from any mechanical damage, and stored at proper temperature until consumption (Fernandes *et al*., 2020). Improperly handled/stored edible flowers suffer tissue browning, flower wilt, dehydration, petal discoloration, and abscission. The senescence process is associated with physiological changes and catabolism, which are linked to accelerated respiratory levels, weight reduction, and/or plant hormone response (Kou *et al*., 2012; Landi *et al*., 2018). To address these concerns, fresh edible flowers are often stored under low temperatures, generally at chilling ones (4-5°C) (Fernandes *et al*., 2020). Since different EFs species showed different behaviour at cold storage (Landi *et al*., 2018; Marchioni *et al*., 2020 a, 2020 b), postharvest studies should be performed for each flower, in order to elucidate their physiological response to low temperature and hence their shelf-life.

The aim of this work was to evaluate the phytonutritional and aromatic profile of *T. simmleri* EFs stored at 4°C for 0, 2 and 6 postharvest days. Spectrophotometric and chromatographic analyses were performed in order to highlight any changes in polyphenolic content (flavonoids and anthocyanins included), antioxidant activity, and volatile organic compounds (VOCs) during cold storage.

2. Materials and Methods

Plant material and postharvest conditions

Tulbaghia simmleri plants were provided by the Chambre d'Agriculture des Alpes-Maritimes (CREAM, Nice, France) and were grown at Research Centre for Vegetable and Ornamental Crops (CREA, Sanremo, Imperia, Italy, GPS: 43.816887, 7.758900). Details on plant cultivation is reported in Najar *et al.* (2019). Full open flowers were picked in April, weighed and cold stored as described in Marchioni *et al*. (2020 b), for two (T2) and six (T6) postharvest days. Fresh flowers

were considered as control (T0).

Weight loss and colour determination

Flowers weigh was measured (Ohaus® analytical Standard Series™ Model AS60S, Ohaus Corporation, Florham Park, N.J. USA) before cold storage (T0) and at the end of each experimental point (T2 and T6) to calculate their weight loss (formula reported in Fernandes *et al*., 2018). Once flowers had been weighed, their colour was evaluated with a spectrophotometer SP60 series (X-Rite Incorporated, Michigan, USA). *L** (lightness), *a** (redness) and *b** (yellowness) colour coordinates (CIELAB scale, CIE 1976) were measured in different point of at least ten flowers, in order to best describe their colour variations.

Biochemical analyses

Biochemical analyses were performed using frozen samples. Total phenolic, flavonoid and anthocyanins content were determined as reported by Marchioni *et al*. (2020 b). Data were reported as mg gallic acid equivalents (GAEq)/g fresh weight (FW) (polyphenols), mg catechin equivalents (CEq)/g FW (flavonoids), and mg malvin chloride equivalents (MEq)/g FW (anthocyanins). Radical scavenging activity (DPPH assay) of each sample was determined as described by Brand-Williams *et al.* (1995). Data was expressed in IC_{50}, which represent the concentration of the sample able to inhibit by 50% the radical DPPH. All absorbance were read in a UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Enzymatic activities

Frozen flowers (200 mg) were pulverized and homogenized in 2 mL of extraction buffer, consisting of 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2% (w/v) insoluble polyvinylpolypyrrolidone (PVPP), as reported by Pistelli *et al*. (2017). Samples were centrifuged at maximum speed for 30 min at 4°C and the supernatant was used for enzyme activities. The soluble protein content was determined according to Bradford (1976) using bovine serum albumin as standard.

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decomposition of hydrogen peroxide (H_2O_2), recording the decline in absorbance per minute at 240 nm (Zhang and Kirkham, 1996). The reaction started by adding 20 µL of extract to 980 µl of 8.8 mM H_2O_2 solution in 50 mM sodium phosphate buffer. One unit of CAT is determined as the amount of enzyme required to detoxify 1 µmole of H_2O_2 (ε= 394 M^{-1} cm^{-1}) per minute. Data were expressed as unit of CAT per mg of soluble proteins (µmol min^{-1} mg^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by following the decrease in absorbance at 290 nm (ε = 2.7 mM^{-1} x cm^{-1}) due to enzymatic ascorbate oxidation (Nakano and Asada, 1981). The reaction started by the addition of 50 mM H_2O_2 solution to the reaction mixture (20 µl of extract, 0.15 mM disodium EDTA and 0.37 mM ascorbic acid in 50 mM sodium phosphate buffer). A unit of APX is defined as the amount needed to oxidize 1 µmole of ascorbic acid per minute. Data were expressed as unit of APX per mg of soluble proteins (µmol min^{-1} mg^{-1}).

Spontaneous emission analysis

The spontaneous emission analysis was performed as reported in our previous work (Marchioni *et al*., 2020 b). Briefly, and after the chosen storage time had elapsed (0, 2 and 6 days at 4°C), 1g of *T. simmleri* was properly weighted to be sealed in a 25 mL glass flask and kept at laboratory temperature (around 21°C) for 15 min (equilibration time). Once the time expired, the 100 µm polydimethylsiloxane PDMS fiber (Supelco, Bellefonte, PA, USA), was exposed to the flask headspace for 10 min, to be than transferred into the GC-MS instrument.

Statistical analysis

The normal distribution of the residuals and the homogeneity of variance was determined and then data were statistically analyzed by one-way analysis of variance (ANOVA) (Past3, version 3.15), using Tukey Honestly Significant Difference (HSD) with a cut-off significance of p<0.05 (letters).

3. Results and Discussion

Weight loss and chromatic changes during cold storage

The visual quality of *T. simmleri* flowers has been almost entirely maintained up to the sixth days of cold storage (T6) (Fig. 1, Table 1). The main changes observed during postharvest treatment were the decrease in flowers fresh weight, brightness (*L**) and bluish parameter (*b**), along with the increase in the reddish parameter (*a**) (Table 1). Taken together, these variations resulted in a slight darkening of the petals at the end of the experiment, without any evi-

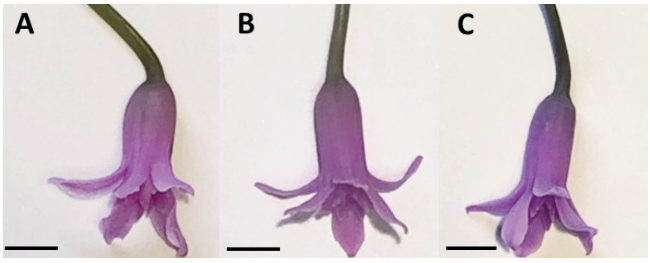

Fig. 1 - Visual appearance of *T. simmleri* flowers after different times of cold storage (4°C): freshly picked flowers (A); after 2 days of cold storage (T2) (B); and after 6 days of cold storage (T6) (C). Bar scale: 1 cm.

dent loss of flower firmness.

The decrease in fresh weight is due to the loss of cell turgor, which is correlated to flower shape. Significant water loss can determine decreased floral diameter, as well as petals curling and crumpling (Kou *et al*., 2012; Ahmad and Thair, 2016; Marchioni *et al*., 2020 b). Nevertheless, the weight loss in *T. simmleri* flowers was very limited (around 7%), showing, therefore, a good aptitude to cold storage. Moreover, the latter was observed to reduce the brightness of seven different EFs (Landi *et al*., 2018), as well as *T. simmleri* flowers (Table 1). This decrease in *L** values is indicative of tissue darkening, commonly associated with the oxidation of phenolics and their polymerization into dark brown pigments, as a result of the activities of polyphenol oxidase (PPO), peroxidase and phenylalanine ammonia lyase (PAL) (Landi *et al*., 2018; Hu and Shen, 2021). The same process could also be responsible for the changes in the color coordinates *a** and *b**, which turn towards darker hues (Table 1).

Table 1 - Weight loss and chromatic changes of *T. simmleri* flowers at 0 (T0), 2 (T2), and 6 (T6) postharvest days (storage at 4°C)

Parameters	Days		
	0	2	6
Weight loss (%)	0 c	3.21 ± 0.04 b	7.34 ± 0.69 a
L*	56.01± 1.25 a	55.54 ± 1.19 a	49.45 ± 0.68 b
a*	22.71 ± 0.64 c	25.78 ± 0.65 b	27.84 ± 0.46 a
b*	-14.16 ± 1.08 a	-19.73 ± 0.69 b	-20.02 ± 0.51 b

Data are reported as mean ± standard error (weight loss, n = 4; L*, a*, b*, n = 15). Different letters indicate statistically significant differences (p<0.05; Tukey's HSD test).

Antioxidant compound and enzyme activities

Polyphenols are considered as the most important and widest natural compounds with antioxidant activity (Cavaiuolo *et al*., 2013). Thanks to their bioactive potential, these molecules can help to prevent chronic degenerative diseases, cardiovascular disorders, and different types of cancer (Pires *et al*., 2019; Skrajda-Brdak *et al*., 2020). Postharvest treatment should maintain unaltered flowers polyphenols concentration to guarantee health benefit until flowers consumption. Our results satisfied this statement, because no changes were observed up to T6 for polyphenol and flavonoids amounts (Table 2). Indeed, a short increase in the total anthocyanins content was quantified already after 2 days (T2) that could be correlated to the interchange between bluish and reddish parameters (Table 1). Despite this positive trend, it should be noted that *T. simmleri* fresh flowers are characterized by low amount of phenolic compound than other well-known and currently consumed EFs (Li *et al*., 2014; Chen *et al*., 2018). Moreover, higher quantities of polyphenols and flavonoids were also reported in other species of the same genus, such as *T. cominsii* and *T. violacea*, probably connected to the use of different extraction methods (Landi *et al*., 2018; Rivas-García *et al*., 2022). Nevertheless, maintaining the levels of phenolic compounds in *T. simmleri* flowers could indicate that this species did not show substantial signs of decay up to the end of the experiment. As regards total anthocyanins content, their increase was previ-

Table 2 - Antioxidant compounds, radical scavenger activity (DPPH assay), catalase (CAT) and ascorbate peroxidase (APX) activities of T. simmleri flowers at 0 (T0), 2 (T2), and 6 (T6) postharvest days (storage at 4°C)

Parameters	Days		
	0	2	6
Total polyphenols (mg GAEq/g FW)	1.22 ± 0.01 a	1.30 ± 0.04 a	1.32 ± 0.03 a
Total flavonoids (mg CEq/g FW	0.30 ± 0.01 a	0.32 ± 0.01 a	0.29 ± 0.01 a
Total anthocyanins (mg MEq/g FW)	0.21 ± 0.02 b	0.29 ± 0.01 a	0.24 ± 0.02 a
DPPH assay (IC_{50} mg/ml)	4.20 ± 0.28 a	3.46 ± 0.19 a	5.22 ± 0.09 b
CAT activity (µmol min^{-1} mg^{-1})	12.68 ± 0.41 b	9.05 ± 0.24 c	21.25 ± 0.27 a
APX activity (µmol min^{-1} mg^{-1})	0.66 ± 0.04 b	0.64 ± 0.04 b	0.83 ± 0.04 a

Data are reported as mean ± standard error (n = 6). Different letters indicate statistically significant differences (P<0.05; Tukey's HSD test).

ously observed also in other EFs stored at low temperature, but the regulatory mechanisms in flowers are still under debate (Shvarts *et al*., 1997; Landi *et al*., 2015; Marchioni *et al*., 2020 b).

Senescence and flowers exposure to low temperatures are tightly associated with a rise in reactive oxygen species (ROS) level in the cells, whose production is accompanied by the activation of several enzymes involved in ROS scavenging (Cavaiuolo *et al*., 2013; Darras, 2020). Polyphenolic compounds also take part to this process, as demonstrated by the reduction of flowers antioxidant activity observed at T6 (Table 2). In this work, the attention was paid to the ROS scavenging enzymes that use hydrogen peroxide (H_2O_2) as substrate, namely catalase (CAT) and ascorbate peroxidase (APX). *T. simmleri* flowers showed that CAT activity is higher than the one of APX (Table 2), suggesting a greater involvement of CAT in H_2O_2 inactivation. Moreover, both the enzymes increased their activity at T6 (Table 2). To the best of our knowledge, very few papers investigated ROS scavenging enzymes activity in EFs stored at chilling temperature as single postharvest treatment. In fact, Chrysargyris *et al*. (2018, 2019) combined the conservation at 5°C with preharvest salinity treatment and modified atmosphere packaging to observe the storage aptitude of *Tagetes patula* and *Petunia* × *hybrida* flowers. Nevertheless, in agreement with our results, APX activity was lower than the one of CAT in *T. patula* flowers, after both 7 and 14 postharvest days (Chrysargyris *et al*., 2018). CAT activity was also investigated by Rizzo *et al*. (2019), highlighting different trend depending on the species and the polypropylene (PP) film used. In the control thesis (comparable with our experiment), CAT activity increases significantly after 6 days of cold storage only in half out of the four studied flowers (*Malva sylvestris* and *Papaver rhoeas*), similarly to what we observed for *T. simmleri*.

Aroma profile

Monoterpenes were the main class of compounds, regardless the storage time and their percentage, that represented at least 50% of the identified fraction (Table 3). Interesting to note is the drastically decrease in oxygenated hydrocarbons content which was of 77% (passing from 0- to 2-day conservation) and 60% (passing from 0- to 6-day conservation) respectively. On the contrary, this decrease was somehow compensated by the increase in the monoterpene hydrocarbons after 2-day storage (an increase of about 2-folds) and by non-terpene compounds after 6-day storage (an increase of about 2.5-folds).

In detail of composition, the fresh flower (T0) was rich in linalool and 1,8-cineol and these compounds almost completely disappear after 2 days of storage. A decrease of linalool content was observed also in papaya "Golden" fruit stored at low temperature (Gomes *et al*., 2016). Interestingly is also the increase of limonene content, about 5-folds, from T0 and T2 (3.01% *vs* 14.78%, respectively), the same compound conserved the latter percentage even at T6. Worthy to note, the presence of benzyl-benzoate in the flowers is only noticeable after 2- and 6-days of refrigeration, and its quantity is tripled during this time.

This work reported for the first time the chemical composition of spontaneous emission of the studied species. Also noteworthy is the absence of sulfur compounds. Almost similar behavior has been seen in *T. violacea*, where such compounds were present in a negligible amount, which were around 1.2% in leaves and do not exceed 4% in roots detected using the same analysis technique (HS-SPME) (Staffa *et al*., 2020). Rhizomes' essential oil (EO) of a South African species of *T. violacea* was also reported to be rich in 2,4-dithiapentne, which represent more than the half of the identified fraction (Soyingbe *et al*., 2013). Hydrocarbons were the major compounds in the hexane extract of *T. violacea* calli from Cairo (Egypt) (55.0%), while the flowers were rich in oxygenated compounds (74.6%) (Eid and Metwally, 2017). On the contrary, the EO from the same species studied by the same team but published two year before underline the prevalence of sulfur compounds in both leaves and flowers and represented 79.7% and 57.5%, respectively (Eid, 2015).

4. Conclusions

Cold storage can reduce some biochemical reactions, although stress conditions increase the reactive species of oxygen (ROS) inside plant tissues. *Tulbaghia simmleri* flowers maintain almost unaltered their visual quality, and their content in antioxidant compounds, up to 6 postharvest days. Moreover, cells counteract ROS production increasing CAT and APX activity. The aroma profiles changed during the cold treatment, even if monoterpenes remained the most represented class of volatile compounds. Looking at the main characteristics of the flowers we can conclude that *T. simmleri* showed a good aptitude to chilling temperature, suggesting the need to test longer period of storage.

Table 3 - Aroma profile of *T. simmleri* flowers detected by headspace solid phase microextraction (HS-SPME) at 0 (T0), 2 (T2), and 6 (T6) postharvest days

N°	Class	Component	L.R.I	Days		
				0	2	6
1	nt	(E)-3-hexen-1-ol	866	2.37 ± 0.10		
2	mh	α-Thujene	932		0.20 ± 0.00	tr
3	mh	α-Pinene	939	0.19 ± 0.02	3.36 ± 0.83	1.78 ± 0.12
4	mh	Camphene	953		0.38 ± 0.08	0.19 ± 0.01
5	nt	Benzaldehyde	961		0.93 ± 0.18	
6	mh	Sabinene	976		0.53 ± 0.11	0.26 ± 0.00
7	nt	1-octen-3-ol	978	4.89 ± 0.16		
8	mh	β-Pinene	980		1.18 ± 0.27	0.59 ± 0.02
9	nt	3-Octanone	988	2.90 ± 0.16		
10	om	2,3-dehydro-1,8-cineole	991	0.96 ± 0.08		
11	mh	Myrcene	992		1.48 ± 0.61	
12	nt	3-Octanol	993	2.09 ± 0.13		0.80 ± 0.21
13	mh	δ-3-Carene	1011		0.44 ± 0.00	
14	mh	α-Terpinene	1018	0.18 ± 0.04	1.03 ± 0.37	0.84 ± 0.08
15	mh	*p*-Cymene	1026	0.12 ± 0.01	7.26 ± 2.92	4.72 ± 0.70
16	mh	Limonene	1031	3.10 ± 0.08	14.74 ± 6.42	14.80 ± 1.13
17	om	1,8-Cineole	1033	53.10 ± 0.08		10.38 ± 0.25
18	om	(*Z*)-β-ocimene	1033		0.20 ± 0.06	0.14 ± 0.03
19	mh	(*E*)-β-ocimene	1040		0.62 ± 0.00	0.38 ± 0.08
20	nt	Phenyl acetaldeyde	1043	1.30 ± 0.04		
21	mh	γ-Terpinene	1062	0.63 ± 0.08	5.74 ± 0.12	3.82 ± 0.02
22	om	*cis*-Sabinene hydrato	1068	0.82 ± 0.16		
23	mh	Terpinolene	1088	0.32 ± 0.15	1.23 ± 0.39	1.05 ± 0.02
24	mh	Linalool	1098	15.51 ± 0.10	1.32 ± 0.68	0.92 ± 0.04
25	nt	Phenyl ethyl alcohol	1110		1.31 ± 0.03	
26	om	*trans*-Limonene oxide	1139		1.24 ± 0.06	
27	om	*trans*-Pinocarveol	1140			0.83 ± 0.10
28	om	Camphor	1143		1.89 ± 0.44	1.95 ± 0.14
29	om	Menthone	1154		0.46 ± 0.03	0.51 ± 0.01
30	om	Isomenthone	1164		0.40 ± 0.18	0.22 ± 0.01
31	om	Borneol	1165		0.76 ± 0.06	0.59 ± 0.02
32	om	δ-Terpineol	1167	tr		
33	om	*trans*-linalool oxide	1172	0.47 ± 0.05		0.32 ± 0.02
34	om	neo-Menthol	1174		0.76 ± 0.14	
35	om	*cis*-Pinocamphone				0.71 ± 0.06
36	om	4-Terpineol	1177	0.24 ± 0.08	1.57 ± 0.40	1.19 ± 0.09
37	om	α-Terpineol	1189	5.27 ± 0.08	0.79 ± 0.15	0.26 ± 0.04
38	nt	Decanal	1204	0.64 ± 0.06		
39	om	Verbenone	1205			0.30 ± 0.05
40	om	Lilac alcohol B	1210	1.08 ± 0.11		
41	nt	Methyl 4-nonenoate		0.36 ± 0.14		
42	om	*trans*-Carveol	1217	0.32 ± 0.08		
43	om	Methyl carvacrol	1244		0.68 ± 0.30	0.68 ± 0.12
44	om	Linalyl acetate	1257		2.14 ± 0.33	1.95 ± 0.35
45	om	Isobornyl acetate	1285		2.11 ± 0.49	1.98 ± 0.35
46	om	Myrtenyl acetate	1325	1.43 ± 0.16	0.30 ± 0.00	
47	om	Methyl perillate		0.15 ± 0.07		
48	sh	α-Cubebene	1351		0.23 ± 0.00	
49	sh	α-Longipinene	1352			1.98 ± 0.35
50	sh	α-Copaene	1376		0.63 ± 0.11	0.27 ± 0.07
51	sh	β-Caryophyllene	1418	0.99 ± 0.06	2.48 ± 0.49	2.89 ± 0.21
52	sh	α-Guaiene	1439			0.35 ± 0.04
53	sh	Aromandrene	1442		0.12 ± 0.00	0.17 ± 0.02
54	ac-12	(*E*)-geranyl acetone	1453	tr		

Data are reported as mean ± standard deviation (SD) (n=2).

... to be continued

Table 3 - Aroma profile of *T. simmleri* flowers detected by headspace solid phase microextraction (HS-SPME) at 0 (T0), 2 (T2), and 6 (T6) postharvest days

N°	Class	Component	L.R.I	Days		
				0	2	6
55	sh	α-Humulene	1454		0.21 ± 0.00	0.22 ± 0.01
56	sh	Alloaromandrene	1461		0.30 ± 0.06	0.43 ± 0.01
57	sh	Viridiflorene	1493		0.57 ± 0.06	0.66 ± 0.06
58	sh	(*E,E*)-α-farnesene	1508	tr	1.72 ± 0.10	
59	sh	*trans*-γ-cadinene	1513			0.55 ± 0.02
60	om	Geranyl isobutyrate	1514	tr		
61	sh	*trans*-Calamenene	1532		1.35 ± 0.14	
62	os	Caryophellene oxide	1581		0.15 ± 0.00	2.36 ± 0.02
63	sh	Cadalene	1674	0.30 ± 0.07	0.59 ± 0.08	0.44 ± 0.06
64	nt	Benzyl benzoate	1762		12.58 ± 2.85	35.30 ± 0.02
		monoterpene hydrocarbons (mh)		18.95 ± 0.02	37.55 ± 0.67	24.80 ± 1.83
		oxygenated monoterpenes (om)		63.82 ± 0.01	14.46 ± 3.01	25.32 ± 0.66
		Total monoterpenes		82.77 ± 0.01	52.01 ± 3.68	50.12 ± 0.17
		sesquiterpenes hydrocarbons (sh)		1.29 ± 0.13	7.90 ± 0.64	9.67 ± 0.52
		oxygenated sesquiterpenes (os)		-	0.15 ± 0.00	0.55 ± 0.02
		Total sesquiterpenes		1.29 ± 0.13	7.97 ± 0.75	10.22 ± 0.54
		non terpenes (nt)		15.54 ± 0.39	14.81 ± 3.00	35.73 ± 0.08
		Total identified		98.59 ± 0.26	74.79 ± 0.06	96.06 ± 0.71

Data are reported as mean ± standard deviation (SD) (n=2).

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Effect of microwave mild heat treatment on postharvest quality of table grapes

M.L.V. de Chiara (*), N. De Simone, G. Spano, M.L. Amodio, G. Colelli
Department of Sciences of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli, 25, 71122 Foggia, Italy.

Key words: *Botrytis cinerea*, external appearance, microbiological quality, microwave energy, mold development, ready-to-eat grape.

(*) **Corresponding author:**
maria.dechiara@unifg.it

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Abstract: Table grapes are characterized by high susceptibility to mold development during post-harvest, mostly due to *Botrytis cinerea*. Microwave application on ready-to-eat product can represent an alternative to antifungal treatment. With the aim of identifying the maximum energy that can be applied on grape without detrimental effects a Central Composite Design was developed testing the application of 10 combinations of treatment time (seconds) and microwave power (Watt). As a result, energies above 8000 kJ negatively affected the sensorial quality of fresh product, both in the presence and absence of *B. cinerea* inoculum (10^6 log CFU g^{-1}). The physico-chemical parameters did not show significant differences, but two time/power combinations improved sensory quality of table grape, being selected for the subsequent packaging trial: 14 s/100 W and 80 s/100 W. Treatments were applied before or after packaging in polypropylene bags. At the end of storage period, 100 W applied for 80 seconds before packaging led to a better external appearance of the product than the other treatments, maintaining an intermediate level of mesophilic bacterial load and no significant differences in terms of nutritional quality. 80 seconds at 100 Watt combined with packaging can represent a valuable starting point for further experiments.

1. Introduction

Table grapes are characterized by high susceptibility to mold and rot development during prolonged postharvest storage and commercialization, often leading to a general decrease in overall bunch quality. Among other factors, fungal decay, mainly caused by *Botrytis cinerea*, is the principal responsible for deterioration with grey mold development (Williamson *et al.*, 2007; Ahmed *et al.*, 2018). After harvest, a favorable environment for the germination of fungal spores is created on berries surface, mainly for damaged fruits (De Simone *et al.*, 2020). For this reason, during post-harvest life of fruits and vegetables, processing technologies and biotechnologies aimed to provide physical, chemical, and biological hurdles to limit the development of undesired microorganisms

(Capozzi *et al.*, 2009). Conventional thermal processes can result in the reduction of nutritional and sensory quality of the product, due to slow heat transmission within the plant tissue. Application of mild thermal treatments aimed to control postharvest disease by means of microwave heating (Dar *et al.*, 2020), could allow to avoid the use of chemical compounds and therefore residues in the treated product and ensuring at the same time minimal environmental impact, thus representing a valuable alternative to traditional thermal processing. Microwaving ensures instead a fast and effective heat treatment reducing risk of injuries and decrease of nutritional compounds. However, oversized intensity of the treatment can induce an excessive temperature increase, resulting in a damage to the fresh plant tissue. Moreover, high temperatures could affect grape biochemical characteristics, for example losses of aroma-related compounds and development of oxidative processes (Modesti *et al*., 2020). To date, a small number of studies deals with the use of microwave treatment on fresh produce (Karabulut and Baykal, 2002; Zhang *et al.*, 2004, 2006; Sisquella *et al.*, 2013), showing its efficiency in prolonging postharvest life of peaches, in which microwave inhibited growth of inoculated pathogens after 2 minutes, being also effective in controlling endogenous microflora with a very low decay incidence. As for nectarines, brown rot incidence was significantly reduced by microwaving to less than 14% versus 45% of untreated product. Treatment caused a delay of softening and internal damage. Zhang *et al*. (2006) observed that in pears treated for 2 or 3 min *Penicillium expansum* population was significantly lower than control samples without impairing quality of the fruits. Fresh-cut carrots, apples, and minimally processed bok choy were subjected to high power/short time treatments showing promising results. Microwave treatments maintained physical, chemical and sensory quality of fresh-cut carrots over storage period, reducing surface whitening and avoiding firmness modification, also enhancing bioactive compounds concentration. However, microbial growth was greater than control samples during shelf-life (Martínez-Hernández *et al.*, 2016). Application of 454 W for 5 s on bok-choy decreased respiration rate, decay occurrence and etiolation, while improving integrity of cell membrane with a final better quality of product (Song *et al.*, 2018). As for fresh cut apples, a significant microbial reduction was observed for treatment at 300 W for 35 s, with no detrimental effect on nutritional parameters and a slight decrease of visual quality (Colelli *et al.*, 2021). However, there is the need to deeply study this technology and its effect on the species of interest with the aim to keep intact the fresh-like characteristics of the product. Moreover, microwave application as a part of a hurdle technology and its application in combination with packaging could be recommended to avoid recontamination, thus the objective of this paper is to provide preliminary information concerning the effect of microwave heating on table grape quality, in terms of efficacy in maintaining physical and microbiological quality.

2. Materials and Methods

A preliminary test to select maximum microwave energy output to be applied on fresh table grape was carried out by means of a Central Composite Design (CCD). Treatment time (seconds) and microwave power levels (Watt) were considered as CCD factors, ten combinations were identified using the software StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA) (Table 1). Table grape was divided into 100 g-batches and treated according to the experimental design, using a solid-state microwave oven at a laboratory scale (2450 MHz, maximum power 1000 W). Processed products were stored in air at 5±1°C and 95% RH up to 14 days after treatment. Each combination was performed once, being the statistical variability already considered during designing of the experimental CCD plan. The experiment was also conducted on samples previously inoculated by

Table 1 - Treatment time (seconds) and microwave power levels (Watt) according to an experimental plan based on Central Composite Design 2^{2+} star with two central points

Run	Treatment time (seconds)	Microwave power levels (Watt)
1	14	100
2	47	32
3	94	265
4	47	265
5	47	498
6	0	265
7	14	430
8	47	265
9	80	430
10	80	100

immersion in a $1x10^6$ spores/mL solution of *Botrytis cinerea* CECT 20973 purchased from the Spanish Type Culture Collection (CECT, Paterna, Spain), and stored at low temperature for 7 days+7 days of room temperature shelf life. For not inoculated samples, immediately after the treatment and after 7 and 14 days, the main physicochemical and microbiological parameters were evaluated, while inoculated samples were evaluated only for external aspect due to the massive presence of *B. cinerea* that could affect in a non-realistic way the quality of these latter samples. Obtained results were subjected to the specific statistical analysis for CCD using the software StatGraphics, to create estimated response surface plots. The second trial was subsequently performed on uninoculated samples using the two most effective combinations to understand the best moment for microwave heating application throughout the minimal processing. Ready-to-eat table grape bunches (100 g) were subjected to treatments before or after packaging within polypropylene (PP) bags (10x15 cm), and subsequently stored at 5°C up to 14 days. During storage (at initial time and after 7 and 14 days), physicochemical, microbiological, and organoleptic evaluations were carried out and results were subjected to statistical analysis. The treatments were as follows: CTRL: samples not treated; *LowMW*: microwave treatment at 14 s/100 W and subsequent application of the packaging; *HighMW*: microwave treatment at 80 s/100 W and subsequent application of the packaging; *LowMW PP*: microwave treatment at 14 s/100 W on packed samples; *HighMW PP*: microwave treatment at 80 s/100 W on packed samples. All the treatments were performed in triplicate.

Ranking test

First of all, ranking test was performed on samples treated as described for CCD to select the maximum microwave output energy to be applied on the products without detrimental effect. Seven trained panelists were asked to rank the samples according to their preference, evaluating the fresh-like appearance of the product and its organoleptic properties and the presence of typical fresh-like flavor, by assigning a score (from 1 to 10 considering each sample as a whole, based on the following characteristics: fresh-like appearance and organoleptic properties and the presence of typical fresh-like flavor). The sum of the score given by each panelist was compared with preset values to statistically evaluate the differences between the samples in terms of the tested parameters.

Fungal strain and growth condition

Botrytis cinerea CECT 20973, purchased from the Spanish Type Culture Collection (CECT, Paterna, Spain), was used to inoculate samples. Cryopreserved cultures were plated on Potato Dextrose Agar (PDA, Oxoid), and incubated at 25°C for 5 days. Fungal spore suspension was prepared by brushing the plates surface with saline solution (8.6 g L^{-1} NaCl) supplemented with 0.01% Tween 80 using a sterile swab and stored at 4°C for short-term uses. Fungal spores concentration was determined by plating serial dilution on PDA plates and adjusted to approximately 1 x 10^6 spores/mL.

Microbial load determination

Grape berries from each replicate were diluted (1:10) with NaCl 8.6 g L^{-1} solution, and homogenized in a blender (Bag Mixer, Interscience, Saint-Nom-la-Bretéche, France) for 2 min. Then, samples were submitted to tenfold serial dilution. Mesophilic microorganisms were enumerated by plate counting on Plate Count Agar (PCA) and incubated at 25 for 48 h. Yeasts and molds were plated on Potato Dextrose Agar (PDA) (Oxoid) added with chloramphenicol (100 mg L^{-1}) and incubated at 30°C for 48 h.

Ascorbic acid, dehydroascorbic acid and total vitamin C determination

Ascorbic acid, dehydroascorbic acid and total vitamin C amounts were assessed homogenizing 5 grams of fruit tissue with 5 ml of extraction medium (MeOH: H_2O (5:95) plus citric acid (21 g L^{-1}) with EDTA (0.5 g L^{-1}) and NaF (0.168 g L^{-1}). The homogenate was filtered, centrifuged, and the supernatant was recovered. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined through HPLC analysis (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) as described by Zapata and Dufour (1992) with some modifications. AA and DHAA contents were expressed as mg of ascorbic or dehydroascorbic acid per 100 g of fresh weight. Vitamin C content corresponds to the sum AA+DHAA and was expressed as mg 100 g fw^{-1}.

Color analysis and determination of the berries' temperature

Color of berries surface was measured on 5 berries per each replicate using a spectrophotometer (CM 2600d, Konica Minolta, Japan) in the reflectance mode with the CIE L*a*b* color scale. Immediately after the treatment, berries surface temperature was acquired using thermal imaging camera Flir C5 (Teledyne Technologies, Wilsonville, Oregon, USA).

Texture

Berries firmness evaluation on 5 berries from each replicate was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England, UK) by measuring the maximum compression force at a rate of 1.5 mm s^{-1}.

Sensorial quality

A panel of six trained people evaluated external appearance and overall rating of bunches, berries, and rachis of the stored product from each replicate at each sampling day. It was used a hedonic scale associated to a brief description corresponding to a score from 1 to 5, where 1= really poor and 5=excellent, being 3 the limit of marketability and 2 the limit of edibilit.

Gas analysis

Oxygen (O_2) and carbon dioxide (CO_2) concentrations, expressed as kPA, inside plastic bags containing table grapes were monitored over storage time by using a hand-held gas analyzer (CheckPoint, PBI Dansensor) to measure gases concentration in 15 ml of headspace.

Statistical analysis

Data were subjected to a two-way ANOVA (for treatment and sampling time), and means were separated by Tukey's test at $P<0.05$ (5% significance level) using Stat Graphics Centurion XVI.I software. Mean values within each sampling were separated applying Tukey's test with significant difference when $P\leq0.05$.

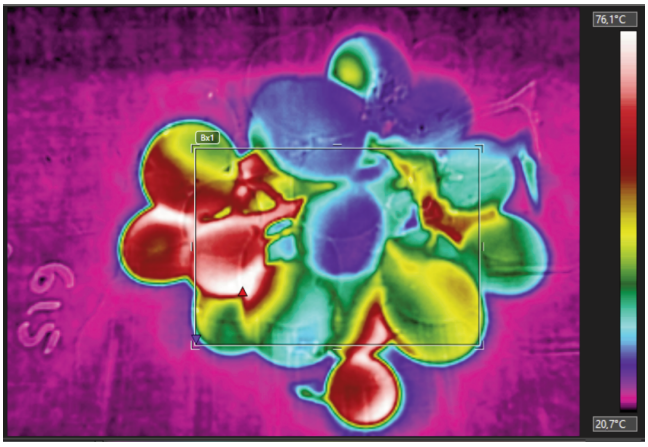

Fig. 1 - Example of temperature distribution on the bunch after microwave heating.

Table 2 - Minimum, maximum, and average temperature for samples treated as described by the experimental plan of the CCD

Run	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)
Initial T	20.3	15.2	17.5
1	29.7	20.4	22.6
2	47.3	20.9	23.3
3	65.9	22.9	37.7
4	68.7	21.7	30.9
5	68.0	23.1	37.7
6	22.2	17.4	19.1
7	42.5	19.9	24.3
8	70.8	21.3	29.5
9	81.1	23.2	44.7
10	60.5	21.1	27.3

3. Results and Discussion

First of all, as from ranking test results, it was observed that samples from treatment 1 and 9, treated with 14 s/100 W and 80 s/430 W, respectively, were significantly different from the other, showing better and worse characteristics than the other treatments, respectively. Specifically, sample number 9 reached, after treatment, a maximum temperature of 81°C, with an average of 44.7°C, consequently the panelists evaluated this sample negatively, highlighting the significant presence of cooked flavor. In general, grape temperature during treatment increased very heterogeneously among all samples (Fig. 1, Table 2). In fact, as observed by many researchers, microwave heating often leads to the creation of hot spots in several product zones, depending on its geometry, thickness and dielectric properties, which are in turn dependent on the moisture content, and starting temperature of the food (Ho and Yam, 1992; Buffler, 1992; Zhou *et al.,* 1995; Campanone and Zaritzky, 2005; Vadivambal and Jayas, 2010). The increase in temperature was progressive, even if not proportional, to the increase in the total energy supplied to the product as expected by the treatment. Consequently, the results of the CCD showed that the more the microwave energy, the more the damage to the fresh product, leading to a worsening of the organoleptic quality of ready-to-eat table grapes for energies above 8000 kJ, both in the presence and absence of *B. cinerea* inoculum (10^6 log CFU g^{-1}). However, the chemical and physical parameters of

uninoculated samples, did not show significant differences (data not shown), for this reason the following best treatments from a sensory point of view, as reported in figure 2, were selected for the subsequent packaging trials: 14 s/100 W and 80 s/100 W, respectively, treatment 1 and 10. Moreover, this latter evidence was associated with a slight reduction of the total mesophilic bacterial load of the selected samples treated with combination 1 and 10 (14/100 and 80/100), equal to 0.2 log and 0.6 log respectively, compared to the untreated sample, even if not statistically significant (data not shown). The selected treatments allowed to maintain a high visual quality score, above the limit of marketability, up to 14 days of storage. It is possible to observe that treatments 3, 5 and 9 caused instead a severe deterioration of table grape appearance, resulting not to be suitably applied on fresh product. There is a great lack of existing literature regarding the application of microwaves on grapes intended for fresh consumption, therefore these preliminary data were used as starting point for the next experiment, and it is difficult to compare them with other data for the same product. However, similar results were observed for different products such as fresh cut carrots and apples as reported by Martínez-Hernández *et al.* (2016) and Colelli *et al.* (2021). Authors stated that high energy treatments could detrimentally affect quality of fresh product. For this reason, it became crucial to carry out the reported preliminary screening, preferably based on a statistical approach such as that of the CCD which allows to test a large number of treatments at different energies to obtain a complete picture of the effects of microwaving on the product.

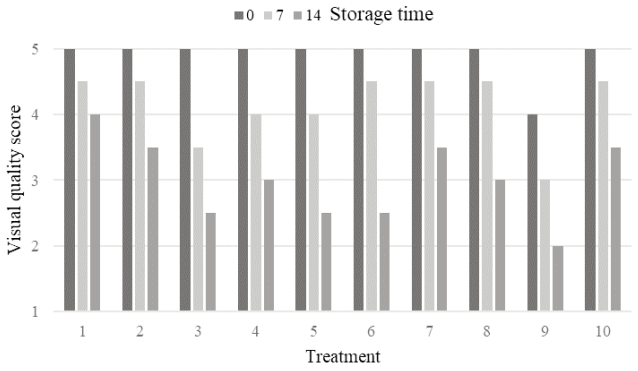

Fig. 2 - Visual quality score evolution during storage time for uninoculated table grape bunches treated as described in Table 1.

As for the second trial, Table 3 shows the results of the two-way analysis of variance, pointing out how storage time significantly influenced all the evaluated parameters while microwave intensity, combined or not with packaging, affected the sensory aspects described as visual appearance of bunches, berries and rachis, and some of the physico-chemical parameters. Moreover, the interaction between the two factors influenced several quality aspects of table grape subjected to microwave heating and stored up to two weeks. On the other hand, however, as described below, the significant effects of the treatment on the qualitative aspects of the product are lost during storage and, for most of the evaluated

Table 3 - Effect of microwave treatment, storage time and their interaction on physico-chemical, sensory and microbiological attributes of table grape stored up to 14 days

	Control	LowMW	LowMW PP	HighMW	HighMW PP	Treatment (A)	Storage time (B)	Interaction (A X B)
Total mesophilic load (logCFU g^{-1})	3.05±0.57	3.02±0.48	3.02±0.56	3.16±0.30	2.97±0.29	NS	****	NS
Yeasts and molds (logCFU g^{-1})	3.25±0.36 b	3.32±0.23 b	3.40±0.35 ab	3.55±0.24a	3.23±0.41 b	**	****	*
Ascorbic acid (mg $100g^{-1}$)	0.62±0.39	0.58±0.22	0.48±0.35	0.62±0.43	0.72±0.39	NS	****	NS
Dehydroascorbic acid (mg 100 g^{-1})	2.04±0.32	1.95±0.17	1.74±0.24	1.98±0.20	1.75±0.41	*	*	*
Vitamin C (mg $100g^{-1}$)	2.66±0.64	2.54±0.32	2.23±0.47	2.60±0.50	2.48±0.73	NS	****	NS
O_2 (kPa)	11.02±8.38	10.14±8.31	10.40±8.52	10.62±7.95	10.83±7.98	NS	****	***
CO_2 (kPa)	6.00±4.79 bc	6.72±5.07 a	6.65±5.39 ab	6.60±5.08 abc	5.95±4.56c	**	****	***
Bunch appearance (Score)	4.17±0.48 b	4.50±0.43 a	4.50±0.41 a	4.50±0.28 a	4.33±0.57 ab	**	****	***
Berries appearance (Score)	4.08±0.61 b	4.58±0.47 a	4.58±0.33 a	4.58±0.35 a	4.25±0.48 b	***	****	**
Rachis appearance (Score)	4.42±0.56 ab	4.50±0.43 a	4.42±0.35 ab	4.50±0.43 a	4.33±0.66 b	**	****	****
Firmness (N)	0.47±0.11 ab	0.57±0.17 a	0.47±0.09 b	0.50±0.12 ab	0.45±0.05 b	*	****	**

Mean values ± standard deviations of 9 samples are reported (3 replicates x 3 storage times). ****= P≤0.0001; *** = P≤0.001; **= P≤0.01; *= P≤0.05; NS, not significant.
LowMW = 14 s/ 100 W; HighMW = 80 s/100 W; PP= polypropylene bags.

parameters, the differences resulted to be not significant after 14 days.

The most interesting results were reached in terms of maintenance of the sensorial quality of the fresh product. Specifically, all the samples, including not treated one, were characterized by a very high sensorial quality even after two weeks of storage within passive modified atmosphere packaging. However, HighMW sample, treated at 100 W for 80 sec and then packed, showed the highest rating due to the very fresh-like appearance of its berries and globally, its bunches, at the end of storage time. It is widely recognized that ready-to-eat and fresh-cut products should be visually free from defects, clean, with no presence of soil or off odor up to the end of the storage time, moreover, the entire bag content should be edible without no further requirement before consumption (Barrett *et al.*, 2010). Consequently, visual appearance of fresh table grapes and fresh product in general, represents the first aspect influencing the consumers decision, and size, color, visual quality, and external appearance in general are used to describe it (Musacchi and Serra, 2018). In this context, it was observed in the present work that microwave treatment was able to better maintain the visual appearance of table grape during storage when packed and stored as ready-to-eat product, representing a starting point for subsequent applications and optimization of this technique on fresh produce. From a microbiological point of view, no statistically differences were observed between samples at the end of storage time, neither due to the different intensity of the treatments nor to the presence/absence of the packaging film during microwave treatment. In figure 3 it is possible to observe how, concerning mesophilic microorganisms, a lower load, even if not significant, was maintained after 14 days of storage for all the treated samples, compared to untreated, reaching the latter the highest value equal to 3.59 log CFU g^{-1}. Final values for LowMW, LowMW PP, HighMW, HighMW PP were 3.29, 3.42, 3.34 and 3.06, respectively, with HighMW PP sample showing the highest difference compared to control (0.54 log CFU g^{-1}). Low efficacy of the treatment in terms of reduction of the microbial content can be related to non-uniform heating process, thus leading to the incomplete action on to the microorganisms due to uneven distribution of temperature (Vadivambal and Jayas, 2010). Little literature existing on ready-to-eat samples in order to compare results. Colelli *et al.* (2021) observed that 35 s/300 W

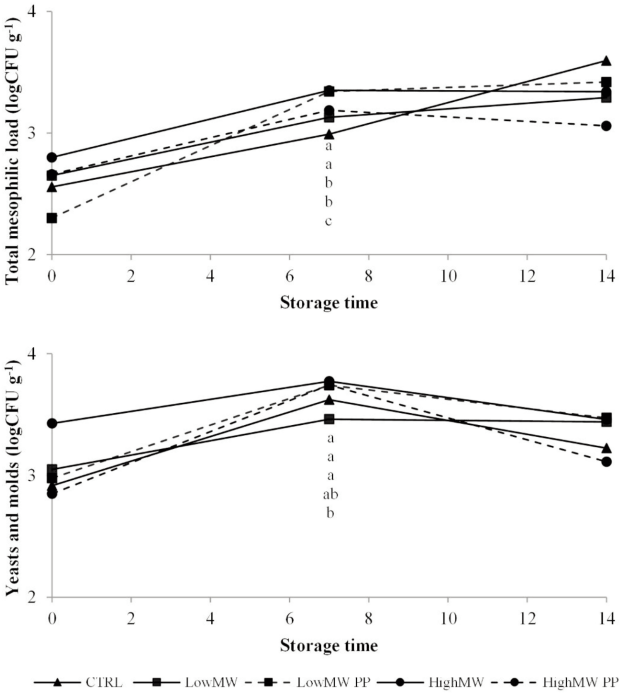

Fig. 3 - Total mesophilic and yeasts and moulds loads evolution over time on table grape samples untreated (CTRL) and subjected to low (14 s/100 W) and high (80 s/100 W) microwave treatment before (LowMW, HighMW) and after polypropylene packaging application (LowMW PP, HighMW PP). Means with different letters at the same time of storage are significantly different according to Tukey's test (P≤0.05).

on fresh-cut apples allowed to reach a 2-log reduction in mesophilic load at the end of storage time, however, the higher microwave intensity resulted in the appearance of side effects on nutritional quality. Otherwise, as reported by Martínez-Hernández *et al.* (2016) 60 s/900 W microwave treatment applied on fresh-cut carrots led to an initial microbial reduction, followed by an increment during storage mainly due to the detrimental effect on plant tissue caused by an excessive temperature increase. As demonstrated by the present experiment, using a well-modulated microwave energy, allowed at least to maintain a good visual quality of ready-to-eat table grape (Fig. 4), without significant reduction of nutritive compounds and firmness. Moreover, it was not possible to correlate the differences in the visual appearance with the different gaseous concentration within the plastic bags, in fact about 3 kPa of oxygen and 11 kPa of CO_2 were reached at the end of storage time for all the samples. This probably contribute to the maintenance of grape quality, also Cefola and Pace (2016) reported the beneficial effect of high CO_2 concentra-

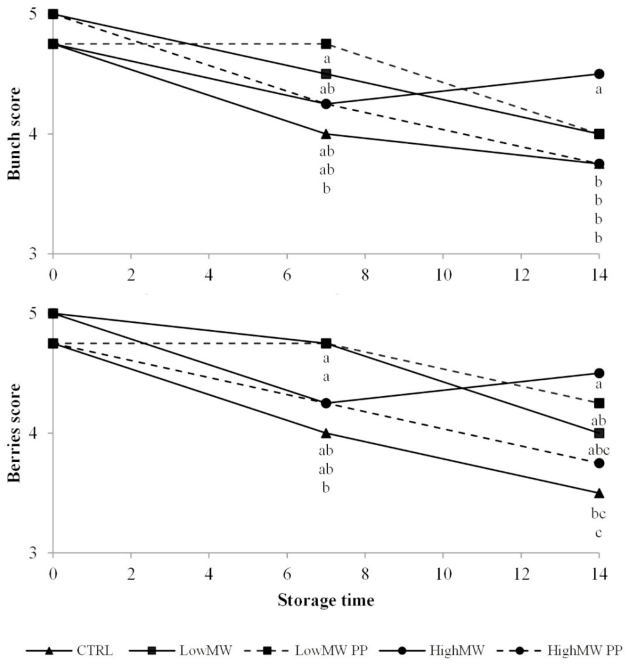

Fig. 4 - Bunches and berries sensorially evaluated score over storage time for table grape samples untreated (CTRL) and subjected to low (14 s/100 W) and high (80 s/100 W) microwave treatment before (LowMW, HighMW) and after polypropylene packaging application (LowMW PP, HighMW PP). Means with different letters at the same time of storage are significantly different according to Tukey's test (P≤0.05).

tion during storage on 'Italia' table grapes, both in terms of sensory quality preservation and decay control. It is therefore possible to state that the treatments, at the applied intensities, did not influence the respiratory rate of table grape and consequently there are no differences concerning metabolic activity and physiological aging. As for ascorbic and dehydroascorbic acid, it was observed a physiologically slightly decreasing trend, without however differences due to the intensity of the treatment (data not shown). Similar results were also observed for different table grape varieties when stored as minimally processed products (Nicolosi *et al.*, 2018).

4. Conclusions

At the end of the storage period, sample subjected to 100 W microwave power for a treatment time of 80 seconds and subsequently packed, showed a better external appearance than the other treatment and the control samples, however maintaining an intermediate level of mesophilic bacterial load. No significant differences in terms of nutritional quality were observed. The time/power combination identified with this preliminary experiment and its combination as hurdle technology with packaging can represent a valuable starting point for further experiments aiming at identifying a mild microwave treatment to be applied to improve table grapes quality and safety. Their combination with other treatments aimed to maximize the antifungal activity should be better investigated.

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Quality of cold stored lemon fruit from orchards consociated to ancient olive trees

M. Allegra (*), F. Ferlito, B. Torrisi, S. Trovato, G. Cicciarello, M.C. Strano
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Centro di Ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Corso Savoia, 90, 95024 Acireale (CT), Italy.

Key words: Agroecology, agroforestry, cold storage, consociation, lemon fruit, postharvest quality.

(*) **Corresponding author:**
maria.allegra@crea.gov.it

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Abstract: In the hilly area of Gioia Tauro (Calabria, Southern Italy), lemon orchards are grown in consociation with centuries-old olive trees. Lemons are partially shaded by olive canopies and the microclimate at the level of their canopies is suitable for plants growth and quality productions. Under these conditions, lemon trees are grown even without irrigation, providing, despite this limitation, a quality product. This study aimed to i) investigate the qualitative characterisation of two clonal selections of the lemon cultivar Femminello, F. Siracusano (S) and F. Zagara bianca (ZB), from the described intercropping, on irrigated (I) and non-irrigated (NI) crops; ii) assess the quality preservation during cold storage, in order to evaluate the availability of lemons for marketing in a period of shortage such as the summer season. Fruits were harvested at commercial maturity, and cold stored at 10±1°C and RH 85-90%, for 60 days. Decay incidence, physiological disorders, weight loss, and the main physical-chemical parameters were assessed at harvest (T0) and every 15 days (T15, T30, T45, T60). The absence of decay and physiological disorders was observed throughout the 60-day storage period, in both clonal selections under the two management conditions. The weight loss was greater in fruits from irrigated lemon groves of both S and ZB. S_I showed significantly lower fruits weight and higher titratable acidity than S_NI. Total soluble solids and titratable acidity were statistically lower for ZB_I than for NI fruits.

1. Introduction

Several research have highlighted that some agri-food models are not sustainable from an economic, social and environmental point of view (IPES, 2016; FAO, 2017). For many local agricultural contexts, the main perspective to survive is the design and set up of agricultural systems more at the service of the right to food (food sovereignty), enhancing territoriality and reducing ecosystems degradation (Loker and Francis, 2020; Ciaccia *et al.*, 2021). In some cases these objectives are reached by the cultural consociation, adopted among crops with the aim of fostering

agro-ecological services thus providing both environmental and economic benefits (Las Casas *et al.*, 2022). This represents an agroforestry model, mixed and multifunctional by definition, that should be promoted, empowering marginalized actors and farmers by creating something different (Rossi, 2020) and feasible only by a holistic approach that embrace a long-term vision, such as agroecology (Barrios *et al.*, 2020). These models are not purely productive, as the presence of trees also provides environmental services such as soil improvement, surface runoff reduction, and conservation of biodiversity. At the same time, agroforestry systems provide aesthetic services as well (Katsoulis, 2022).

In the hilly area of Gioia Tauro (Calabria, Southern Italy), lemon orchards are planted in consociation with centuries-old olive trees over 20 meters high. The general distance between olives is wide (10x10 m) according to their large and rarely pruned canopy, ensuring that the lemons are planted both in intra- and inter-rows, in a 5x5 m design. With this regular agroforestry model, olives play a key role in terms of the agroecological service, characterizing the landscape and preserving the fragile area, preventing soil erosion, and increasing biodiversity. Moreover, thanks to its long history in the area and management, compared to the other intensive woody crops, does not require high external inputs, thus contributing to reducing environmental pollution (De Graaff and Eppink, 1999). The microclimate and the adequate lighting conditions at the level of the lemon canopy is suitable for the growth of this culture and for obtaining high quality productions. Under these conditions, lemon trees can be grown rainfeed, although irrigation may influence fruit quality at harvest and during the postharvest process (Asrey *et al.*, 2018; Tadayon and Hosseini, 2020). To the best of our knowledge no studies were carried out about the effect of the consociation of olive-lemon cultivations and on postharvest performance of lemon fruit, which quality could also be prolonged by proper cold storage (Strano *et al.*, 2022, 2023). The extension of the availability to market of high quality fruit, in a period of shortage such as the summer season, would allow to obtain the maximum economic profit for the growers. This study aimed to assess i) the physical-chemical parameters of two clonal selections of lemon cv Femminello: F. Siracusano (S) and F. Zagara bianca (ZB), with high commercial value, consociated with olive cultivations, from irrigated (I) and non-irrigated (i.e., rain-fed, NI) crops; ii) the qualitative response to the cold storage of lemons coming from the two different management techniques.

2. Materials and Methods

Study site and fruit sample

Fruits of lemon (*Citrus limon* (L.) Osbeck) cultivar Femminello, clones Siracusano (orchard 1) and Zagara bianca (orchard 2), both grafted on sour orange (*C. aurantium* L.), planted in a 5x5 m design on a loam soil, from irrigated and non-irrigated crops of the corresponding orchard, were grown in the hills of Gioia Tauro (Calabria, Italy; lat. 38,32 N; long. 15,97 E; elevation 200 m a.s.l.).

According to the USDA (2017) texture triangle, soil of the two orchards had a loam texture (orchard 1: 462 g kg^{-1} sand, 317 g kg^{-1} silt, 222 g kg^{-1} clay; orchard 2: 602 g kg^{-1} sand, 231 g kg^{-1} silt, 167 g kg^{-1} clay), and an organic matter content of 97 (orchard 1) and 88 g kg^{-1} (orchard 2). Plants of irrigated crops were fully irrigated, corresponding to 95-98% of crop demand, while non-irrigated crops had no irrigation system, relying on rainfall for water (Ferlito *et al.*, 2014). Fruits were harvested at commercial maturity in April 2022, transported to CREA laboratories (Acireale, Sicily, Italy), selected accordingly to their uniformity in size, absence of defects and alterations (lesions and/or rot symptoms) and, washed with tap water. Three replicates of 100 fruits for each sample, were randomly placed in plastic boxes, for a total of 300 fruits, then air-dried at 20°C and stored for 60 days at 10±1°C and 85-90% relative humidity (RH).

Physical-chemical changes

Fruits were evaluated at harvest (T0), for the following parameters: weight, rind thickness, carpel axis, peel and pulp color, firmness, juice yield, total soluble solids (TSS), pH, titratable acidity (TA), maturity index (TSS/TA) and, after 15 (T15), 30 (T30), 45 (T45) and 60 (T60) days of storage, also for weight loss, decay incidence and severity of physiological disorders (chilling injury and aging).

Fruit weight loss, expressed as percentage, was evaluated on 30 fruits per sample (10 fruits/replicate) by weighting the same fruit at T0 and at each control. The results were calculated as follows: = [(m0-m1) / m0] × 100; where: m0 = the initial weight; m1 = the weight measured at each control.

The juice was extracted using an electric citrus fruit squeezer and the pooled juice of five fruits per replicate was analyzed. The juice yield was calculated

as follows: juice yield (%) = (juice weight/fruit weight) x 100. TSS content was determined with a digital refractometer (ATAGO RX-5000, Atago, Japan) and results expressed as °Brix. Titratable acidity (TA), expressed as % (w/v) of citric acid equivalent, was determined by potentiometric titration (T50 Automatic Titrator, Mettler Toledo) with 0.1 N sodium hydroxide solution (AOAC, 1995; Ladaniya, 2008). Vitamin C (ascorbic acid, mg·100 mL^{-1}) concentration was determined by liquid chromatography (Rapisarda and Intelisano, 1996). The technological index 'TI' was calculated according to Kluge *et al.* (2003) by the equation:

TI = (Total soluble solids x Juice percentage) / 100.

TI is an important variable linked to the quality of the juices destined for the citrus industry. Higher TI values correspond to better quality (Chitarra and Chitarra, 1990).

Decay incidence (%) was determined by a visual evaluation of fruit infected by rots. The severity of chilling injury was determined by the visual examination of the fruit pericarp, using a four-grade scoring system to estimate the damage of the rind surface: 0 = none, 1 = less than 5% (light), 2 = 5% to 25% (moderate), and 3 = over 25% (severe). The incidence of the fruits affected by aging (%) was determined by the presence of fruits with a necrotic area of rind tissue around the stem-end button.

Firmness measurement

Firmness was tested by a texture analyser (Zwick/Roell DO-FB0.5 TS model 2002, Genoa, Italy) using an 8 mm flat probe (Mitcham *et al.*, 1996). Two measurements were made on two opposite of the equatorial zones of 15 fruits per sample. The results were reported as the peak force in Newton (N) (Nasrin *et al.*, 2020).

Colour evaluation

Fifteen fruits for each sample were taken, at each time interval, for the determination of peel and pulp colour by CIELAB coordinates *L** (lightness), *a** (red-green component) and *b** (yellow-blue component) using a Minolta Spectrophotometer CM-2500d (Minolta, Milan, Italy). Three color measurements were made for each sample fruit and the results were expressed as Citrus Colour Index (CCI) = (1000 × *a**)/(*L** × *b**) (Jiménez-Cuesta *et al.*, 1981).

The effect of cold storage at 10°C on the color of lemon fruits was also evaluated, at each time interval, through the Chroma (*C**), which defines the color intensity (higher *C** values indicate brighter yellow color); the Hue angle (*h°*), with values closer to 90° for the more yellow fruits and increasing for the greener fruits; and Browning index (BI), both for peel and pulp (Kluge *et al.,* 2003). The browning index (BI) was used as an indicator of the intensity of brown discoloration. BI was calculated as follows (Palou *et al.*, 1999; Olivas *et al.*, 2007):

BI = [100(x−0.31)] / 0.172

where: x = [(*a** +1.75)] / [5.646 L + *a** -3.012 *b**].

Statistical analysis

One-way and factorial analysis of variance (ANOVA) was performed using STATISTICA 6.0 software (Stat Soft Italia srl) according to a completely randomized experimental design. Data are means of three independent determinations. Means comparisons were performed by Tukey's HSD test at $p \leq 0.05$, 0.01, 0.001, based on the F-test significance.

3. Results

Physical-chemical changes

Observation of the fruits revealed the absence of decay and physiological disorders in both clonal selections under the two management conditions (I and NI) throughout the 60-day storage period (data not showed).

As shown in figure 1, the weight loss was significantly greater in fruits from irrigated lemon groves of both clonal selections, with average values of 23.7%

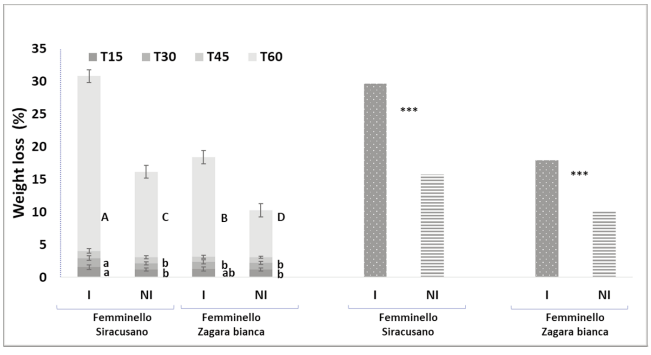

Fig. 1 - Weight loss percentage of 30 lemon fruits from each clonal selection (Femminello Siracusano and F. Zagara bianca) and irrigation management (I: Irrigated; NI: Non-irrigated) monitored during cold storage at 10±1°C and RH 85-90%. Textured bars represent total weight loss from T0 to T60 ($p \leq 0.001$ ***). Means of the same time interval indicated by different letters are significantly different (lowercase $p \leq 0.05$, uppercase $p \leq 0.001$) based on Tukey's HSD test. Error bars show the standard deviation.

(I) *vs* 12.9% (NI), in the interval from T45 to 60 days of cold storage, although it remained 3.6% (I) and 3% (NI) up to T45.

The response to irrigation management resulted clonal selection-specific. The response of each clonal selection over time is reported. The width of the carpellar axis did not reveal significant differences in the comparison between the irrigation managements of each clonal selection, except at T15 in the case of S, and at T30 in the case of ZB, in favor of NI (Fig. 2).

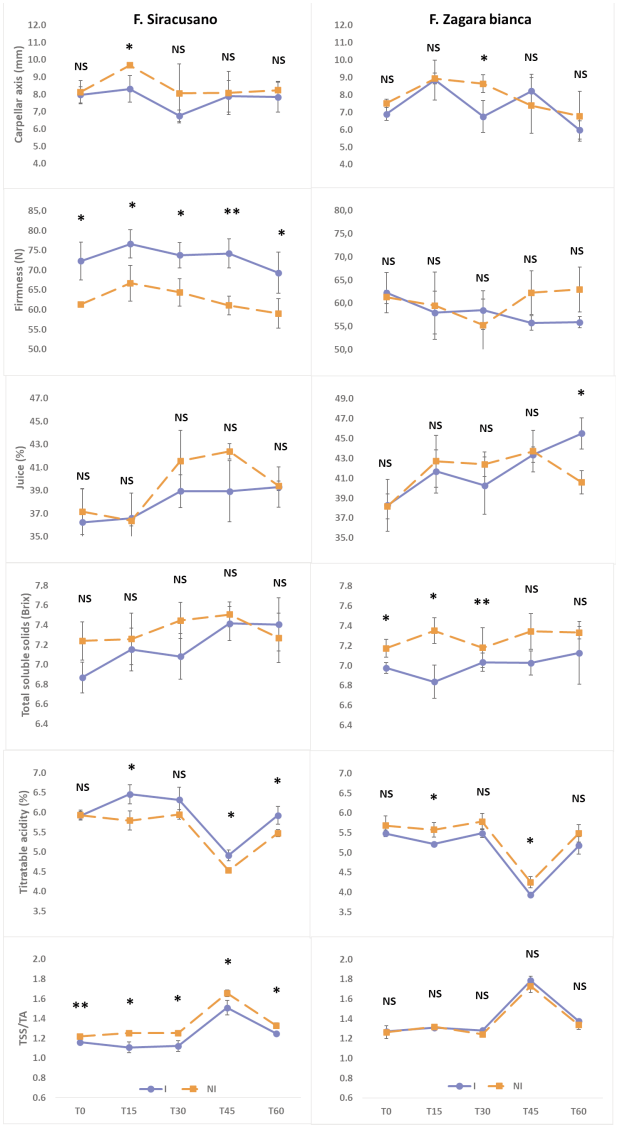

Fig. 2 - Response of the physicochemical parameters (carpellar axis, mm; firmness, N; juice, %; total soluble solids, TSS °Brix; titratable acidity, TA %; TSS/TA ratio) monitored during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano and F. Zagara bianca) to irrigation management (I= Irrigated; NI= Non-irrigated). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05*, uppercase p≤0.01** and p≤0.001***; NS, no significant differences) based on Tukey's HSD test. Error bars show the standard deviation.

No significant difference between managements in the percentage of juice and TSS for S. Only at T60, the juice yield of ZB_I was significantly higher than ZB_NI, while TSS already differed at T0 up to T30 in favor of ZB_NI (Fig. 2). The titratable acidity had a different behavior for the two clonal selections, with I statistically higher for S at T15, T45 and T60, and NI for ZB at T15 and T45 (Fig. 2). TSS/TA was statistically higher for S_NI, compared to S_I, while no difference was recorded for ZB (Fig. 2).

Peel percentage and peel to pulp ratio did not show significant differences. Only at the end of the storage (T60) ZB_NI showed significantly higher values (Fig. 3).

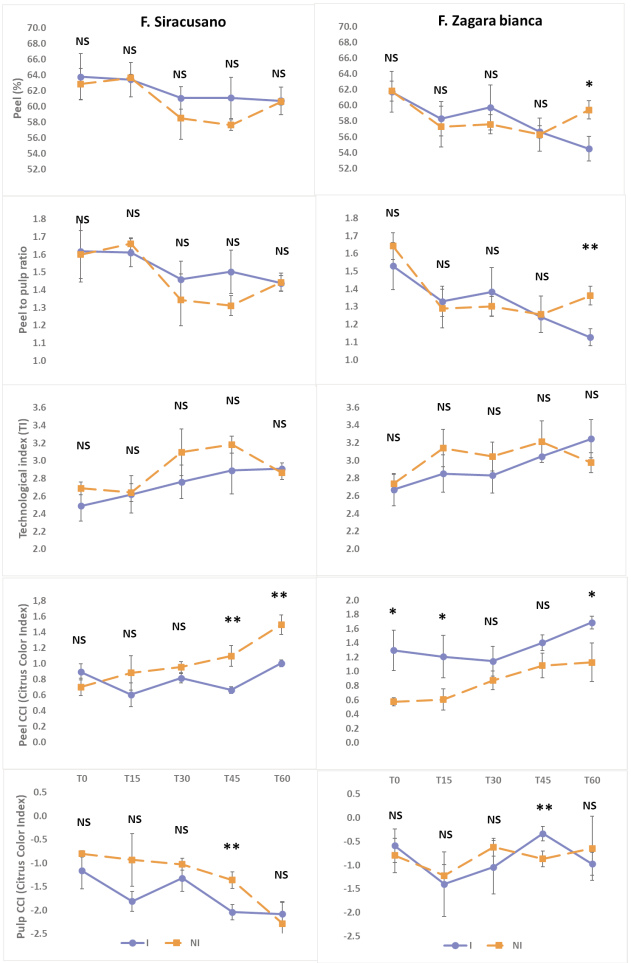

Fig. 3 - Response of the physicochemical parameters (peel, % w/w; peel to pulp ratio; Technological index, TI; peel and pulp Citrus Color Index, CCI) monitored during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano and F. Zagara bianca) to irrigation management (I= Irrigated; NI= Non-irrigated). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05*, uppercase p≤0.01** and p≤0.001***; NS, no significant differences) based on Tukey's HSD test. Error bars show the standard deviation.

Vitamin C values were significantly higher for S, while no significant differences emerged between I and NI management (Fig. 4).

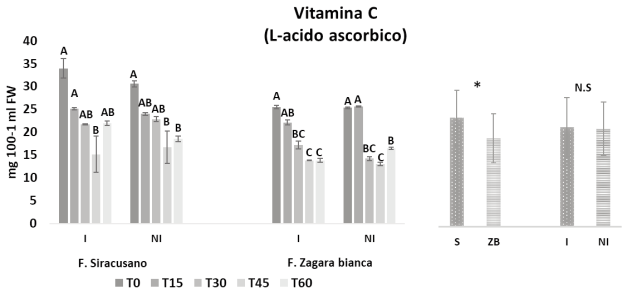

Fig. 4 - Variation of the vitamin C content during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano 'S' and F. Zagara bianca 'ZB') and two irrigation management (I= Irrigated; NI= Non-irrigated). Means observed at each time interval relative to each irrigation management of the clonal selections indicated by different letters are significantly different (p≤0.001) based on Tukey's HSD test. Textured bars represent the results of the factorial ANOVA (p≤0.05*; NS, no significant differences). Error bars show the standard deviation.

Firmness measurement

No significant difference during storage regarding firmness for ZB, while S showed higher values for I as early as T0 (Fig. 2).

Colour evaluation

While the color parameters and indices relating to the pulp very rarely showed significant differences between irrigation managements I and NI (Fig. 5), those relating to the peel show in some cases significant, and often antithetical, differences between the two clonal selections. This is the case of CCI, Chroma and BI, for which S_NI had statistically higher values than S_I, while the opposite occurred for ZB. The peel *h*° had a specular behavior with respect to what was described for the other indices (Fig. 5).

Differences at harvest (T0) occurred in the case of S, for firmness (Fig. 2), peel CCI (Fig. 3) and peel BI (Fig. 5) with higher values for I, which was probably later in ripening, as the lower TSS/TA value seems to demonstrate (Fig. 2).

Zagara bianca showed differences already at harvest (T0) in TSS (Fig. 2) and peel *h*° (Fig. 5), higher in NI, while peel CCI (Fig. 3), C* and BI (Fig. 5) were higher in I.

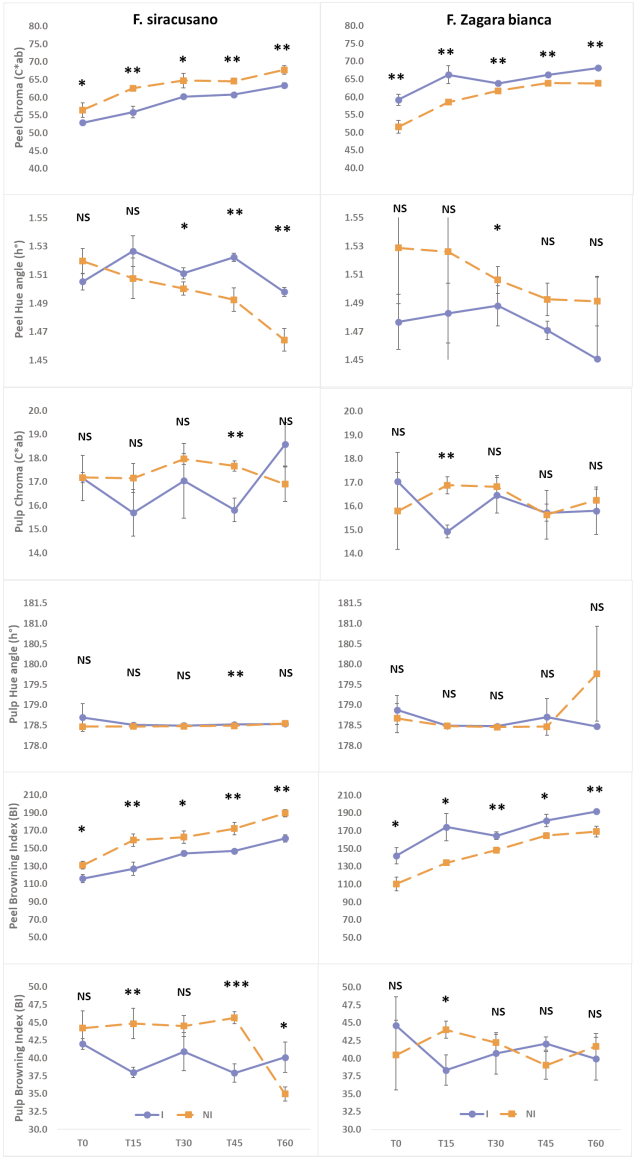

Fig. 5 - Response of the colour parameters of fruit peel and pulp (Chroma, C*ab; Hue angle, h°; Browning index, BI) monitored during cold storage at 10±1°C, RH 85-90%, of lemon fruits from two clonal selections (Femminello Siracusano and F. Zagara bianca) to irrigation management (I= Irrigated; NI= Non-irrigated). Means of the same time interval indicated by different letters are significantly different (lowercase p≤0.05*, uppercase p≤0.01** and p≤0.001***; NS, no significant differences) based on Tukey's HSD test. Error bars show the standard deviation.

4. Discussion and Conclusions

The modern woody crops systems are generally characterized for a strong modification on agro-ecosystem structure and functioning. In a climate change scenario also a modification of the related

agronomical processes such as the water and mineral availability, the solar radiation and the spontaneous flora growth could be affected (Ciaccia *et al.*, 2019). In the present work the response of the particular consociation between the lemon and the old-century olives" in Calabria was investigated, assuming that it could allow to lengthen the lemon quality traits and shelf life throughout the response to long periods of cold storage (Fung *et al.*, 2019). Furthermore, the response of irrigated and non-irrigated lemon groves was compared.

Understanding these linkages, the obtained data can predict how the effect of a single agronomic practice at the micro and macro-area scales can reduce the water input for irrigation. Moreover, the lemon storage could drastically reduce the lengthen of the irrigation season. Moreover, from the economical point of view, the proposed study can be an evaluable way for the marketing of fresh lemons. In fact, as observed by Ciriminna *et al.* (2020), the fresh lemons have a constant demand from consumers which in some seasons cannot be entirely satisfied by Italian production. Finally, the consociated model could be diversified also adopting other species that are well adapted to shade such as berries (Cicala *et al.*, 2002).

The microclimatic effects induced by the presence of the olive trees are reflected on the preservation of the fruit quality, on the protection from natural adversities such as excessive insolation or hail, and seems to result in a postponement of ripening, particularly in non-irrigated lemon groves (Brunori *et al.*, 2019).

The results of this study seem to support the hypothesis of the effectiveness of the shading effect and of the microclimate induced by the presence (cover, protection) of the olive trees, on the quality of the production of both clonal selections studied, and on the sustainability of non-irrigated crops, given the comparability of many of the qualitative parameters with those of irrigated crops. Therefore, despite the considered period is not too long to draw general conclusions the preliminary results of the research show that the Agroecological system and the use of the non-economical valuable olive as an Agro-ecological service crop could realize a mutual relationship into the agroecosystem.

Storage at 10°C avoided the development of decay and physiological disorders regardless of clonal selection and irrigation management.

Management strategies that increase the productivity of existing agricultural land are increasingly needed (Salmon *et al.*, 2015). The diffusion of the consociation here studied could allow the exploitation of large tracts of land that host centuries-old olive groves, which must be preserved for their environmental and cultural importance, but no longer usable at a production level, given the very high management and harvest costs (Brunori *et al.*, 2019). Additionally, the possibility of eliminating or reducing the water supply makes this intercropping particularly interesting.

Increased agricultural productivity is generally achieved by increasing inputs. Irrigation, which currently accounts for about 70% of global freshwater withdrawals, is one of the most important and widespread means of achieving this goal (Lobell *et al.*, 2009). Rain-fed croplands, also called dryland farming, include all cropland where no water from any storage or delivery mechanism is utilized, but crops are not flooded, and where harvesting occurs at least once per year (Salmon *et al.*, 2015).

As a result of the present work, the comparable quality of the fruits at harvest, both with and without irrigation, and their qualitative maintenance with cold storage would allow to have a good product on the market even in periods of shortage.

The comparison with lemon groves without olive trees will be investigated in order to estimate any qualitative improvement induced by the two crops consociation.

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Treated sediment as substrate component of three containerized ornamental species: effects on marketable and qualitative traits

M. Castellani [1], D. Bonetti [2], M. Antonetti [2], D. Prisa [2], G. Burchi [2], S. Nin [2 (*)]

[1] *Flora Toscana, Via di Montecarlo, 81, 51017 Pescia (PT), Italy.*

[2] *Council for Agricultural Research and Economics, Research Centre for Vegetables and Ornamental Crops, Via dei Fiori, 8, 51012 Pescia (PT), Italy.*

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Key words: Calla lily, cherry laurel, growing media, protea, sustainability.

Abstract: Carried out within the LIFE17ENV/IT/000347-SUBSED project, this research aimed at investigating the effect of a treated sediment (TS) as substrate component on the quality and marketability of three widespread containerized ornamental species: cherry laurel (*Prunus laurocerasus*) cv. Novìta, calla lily (*Zantedeschia aethiopica*) and protea (*Protea cynaroides*) cv. Little Prince. The TS was mixed with soilless substrates as sphagnum peat, coir, and bark in different proportion (0%, 25% and 50%). In cherry laurel, the TS used in 25 - 50% proportions reduced plant height, slightly altering its attractive vibrant foliage. A positive effect of the TS was evidenced on calla lily, where both tested sediment-based mixtures allowed a copious blooming and flower quality raised as the sediment content increased (TS 50% > TS 25% > TS 0%). Post-harvest longevity and colour of flowers were not affected by substrate composition. The effect of sediment-based substrates on protea growth and blooming showed an opposite trend (TS 0% > TS 25% > TS 50%), with plants grown on 50% *v/v* TS exhibiting a considerable reduction in plant growth and production of flower clusters, with brighter tones turning towards purple. Based on sale values, the TS proved to be a sustainable alternative for the production of potted ornamentals if properly mixed with other organic matrixes, such as peat and coir.

(*) Corresponding author:
stefania.nin@crea.gov.it

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All relevant data are within the paper and its Supporting Information files.

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1. Introduction

The ornamental plant industry in Tuscany is a strategic sector, representing approx. 5% of the entire national agricultural production, which derives for about 75% from potted plants and nurseries (trees and shrubs) and for the remainder from fresh cut flowers and foliages (Martorana, 2021). This segment produces a giant assortment of ornamental and flower species in about 6,500 hectares of land, concentrated in the provinces of Pistoia and Lucca. With over 3,300 nursery enterprises, 2,060 of which are ornamental producers and 1,900 flower growers, this sector has a

large impact on employment and induced economy, as well as a strong export vocation (Pagliantini, 2020). The power of this sector lies in the variety of the assortment offered for sale and therefore nursery industries are constantly looking for new species and cultivars. Another important factor for the expansion of this sector is the adaptation of new production technologies to the concept of sustainable development. Great deal of the ornamental and flowering plant production is carried out in containers and depends almost entirely on the quality of soilless media derived from both organic and inorganic constituents. Indeed, since successful container cultures depend on producing a stable finished plant, different components in the growing substrate can pose significant problems if they affect growth rate, nutrition or plant shape and aesthetics (Salachna, 2022).

Its unique physical properties and large availability have led peat to be the dominant organic constituent of growing media in many parts of the world in recent decades. Nevertheless, other organic materials have been recently attracting more attention as a partial substitute for sphagnum peat in a container growing medium (Fascella, 2015; Barrett *et al*., 2016). Among these is coir, regarded as a rapidly renewable resource, as it is a by-product of coconut harvest. Moreover, especially in Europe, innovative approaches have involved an increasing use of locally available organic materials such as bark (wood fibre) and transformation of composted wastes as next-generation constituents of growing media. Perlite or vermiculite components are often used to improve drainage and aeration (Gruda, 2019).

Recent studies have focused on the possibility of reusing sediments in agriculture by combining them with standard growing media or biochar amendment (Renella, 2021). This practice could be best successfully applied to ornamental species grown in containers, where health risk due to the presence of toxic residues are minimized. Thus, in this study we investigated the effect of a treated sediment as a substrate component on the quality and marketability of three widespread containerized ornamental species: cherry laurel, calla lily and protea.

Prunus laurocerasus 'Novìta', commonly known as cherry laurel, an evergreen ornamental with a very fast growing and plant development, is one of the most commercially important outdoor ornamental species for the Italian nursery. The cultivar 'Novìta', selected in Holland, has now become widely established and has replaced the previous cultivar 'Caucasica', compared to which it has longer, thicker, and shinier leaves, and above all, is more resistant to diseases and water stress (Ilnitsky *et al*., 2019). Nowadays, container-grown cherry laurels, as all outdoor nursery plants, can be sold at any time of year with two major peaks in spring and autumn. Its commercial value is a function of the size of the plant, first of all its height.

Calla lily (*Zantedeschia aethiopica*) is a very popular flower of great world economic importance, grown as cut flower, potted or garden plant. This species flowers from late summer into spring in frost free areas and prefers partially illuminated environments (De Hertogh, 1996). Calla cultivation is spread from central to southern Italy, where it is performed indoor under greenhouse conditions to protect the crop from October to the end of April (Janowska and Andrzejak, 2022). On the market, cut flowers are classified into grades based on the size of the stem and the flower (spathe). Furthermore, the sale price varies throughout the year, reaching the highest values in the winter period and the lowest values in the month of April, when the quantity of flowers offered on the market is highest.

The protea used in this experiment was *Protea cynaroides* dwarf 'Little Prince', a patented variety, bred for flowerpot production at the National Research Council of South Africa. This cultivar differs from the well-known protea King, national flower of South Africa, for its compact habit, which makes it suitable for expanding the potted plant floral market. Protea is an unusual plant for Europe, where it is considered a niche product much appreciated by fans of exotic plants. Commercial growing of proteas for the cut flower market is increasing also in other European regions with mild winter, especially Portugal and Canary Islands (Lewis and Matthews, 2002; Claassens, 2017). At the marketing stage, protea potted plants are graded and priced according to the number of flowers.

These species were used as model plants of the ornamental plant sector (evergreen shrubs, flowerpot, cut flower) to assess the commercial potential of sediment-based growing media combined with sphagnum peat, coir and wood fibre on the existing floriculture market.

2. Materials and Methods

This study used the marine sediment dredged

from Leghorn port (central Italy, 43°33'25''N, 10°17'39''E) in 2008. To reduce the level of contaminants, the sediment was recovered through three years of phytoremediation process under the frame of the AGRIPORT project (Agricultural Reuse of Polluted Dredged Sediments, Eco-innovation EU Project n. ECO/08/239065ECO/08/239065) (Masciandaro *et al*., 2014; Doni e*t al*., 2015). The phytoremediated sediment underwent landfarming, consisting in a periodical aeration by mechanical handling for three months, before using it.

The treated sediment (TS) was then used as a partial substitute of standard substrates, commonly used for ornamental and flowering crop cultivation in Tuscany. In addition to sphagnum peat, other materials that are already being used for commercial productions as an alternative to peat, such as coconut coir and bark, were employed as organic materials, while pumice was added as inorganic matrix in all mixtures. Sphagnum peat, coir, bark and pumice were mixed separately with the TS to create several media containing 25 or 50% by volume of TS (Table 1). Mixes were used as growing media for soilless cultivation of three ornamental crops: *P. laurocerasus* 'Novìta' (outdoor ornamental shrub), *Z. aethiopica* (cut flower), *P. cynaroides* 'Little Prince' (flowering potted plant). Thanks to its unique microporous properties, the standard peat-based substrate (60% peat, 40% pumice *v/v*) was used as control in all cultivation trials.

The experiments were performed in private facilities of Central Italy (Tuscany). Plants were cultivated under greenhouse conditions for one year growing season over 2020-2021 (cherry laurel and calla) and 2021-2022 (Protea) (Fig. 1). Standard commercial nursery management practices were followed, except those plants received different water amounts depending upon the species, as better specified below in the experimental design of each considered species. The efficiency of soilless cultivation was evaluated both in terms of quantity and quality of marketable products and commercial value. The commercial expected value was obtained by multiplying each product category by the corresponding sale price of a given selling season and market demand. Data on prices for the studied species were given by two Tuscan's leading ornamental trading centres (Ornamental Nursery District, Pistoia, Italy; Flora Toscana, Pescia (PT), Italy) during 2021 for potted cherry laurel plants and cut calla flowers, and during 2022 for potted proteas.

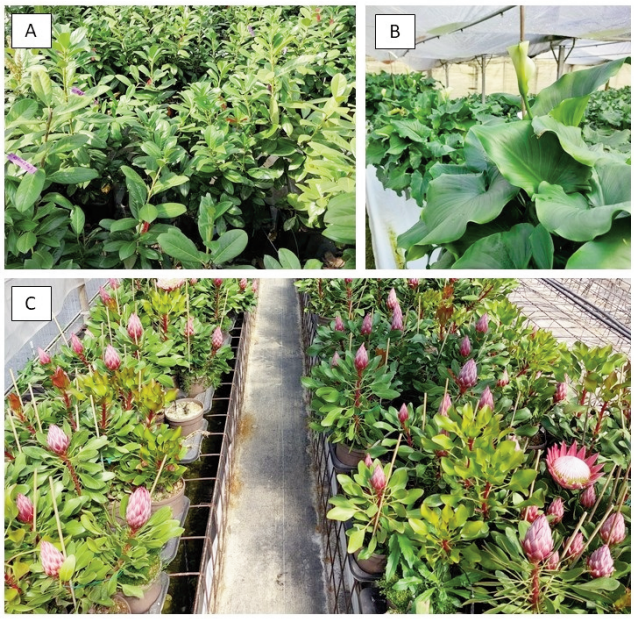

Fig. 1 - Greenhouse soilless culture of *Prunus laurocerasus* (A), *Zantedeschia aethiopica* (B), and *Protea cynaroides* (C) in Tuscany (Central Italy).

*Cherry laurel (*Prunus laurocerasus*) 'Novìta'*

Cherry laurel is a very common shrub in Italian gardens, widely used as evergreen ornamental barrier plant (hedge). It is easily propagated by cutting and sold as potted plant at different stages of growth in different pot sizes. Totally 336 cherry laurel rooted cuttings were grown in 8.5-L drip-irrigated pots. The experimental design consisted of seven growing media (GM) and two water regimes (WR) as described in Table 1. Growing media were prepared by mixing defined volumes of previously homogenized TS and standard substrates based on peat, coconut coir, and bark, each one containing a certain amount of inert pumice. GM containing commercial peat-based substrate alone was considered as the control. Plants were watered by an automated drip irrigation system providing two irrigation rates: normal (WR1 = 250 mL/day of water per pot on average) and reduced by 20% (WR2= 200 mL/day of water per pot on average). Each GM*WR combination was replicated in three blocks, each containing four pots, each one consisting of two cuttings (8 cuttings × 14 treatments × 3 blocks = 336 cuttings). All plants were supplied with a slow-release fertilizer (Nitrophoska Gold®). Growth in height of all cherry laurel plants was measured at the end of the growing cycle and the corresponding sale price was considered as index of production quality. Leaf colour (i.e., the coordi-

Table 1 - Composition of the tested growing media

GM			Peat	Treated sediment	Coir		Bark (wood fiber)	Pumice
Cherry laurel	Calla	Protea			Fiber	Peat		
LMix 1	CMix 1	PMix 1	60					40
LMix 2	CMix 2	PMix 2	45	25				30
LMix 3	CMix 3	PMix 3	30	50				20
LMix 4				25	45			30
LMix 5				50	30			20
LMix 6				25			45	30
LMix 7				50			30	20
		PMix 4		25	34.2	22.8		18
		PMix 5		50	22.8	15.2		12
		PMix 6		25	17.1	39.9		18
		PMix 7		50	11.4	26.6		12

nates L* [brightness], a* [redness], and b* [yellowness]) was measured with a colorimeter (Chromameter, Minolta CR 200) on three different leaf points shortly after growth measurements. The chroma index (Chroma) was calculated using the coordinates a* and b* (Chroma = [(a*2 + b*2) 1/2]).

*Calla lily (*Zantedeschia aethiopica*)*

Calla lily is a species of great economic importance worldwide thanks to the beauty of its flowers. In Italy, calla is grown as outdoor garden and potted plant, but also largely raised for cut flower production under greenhouse conditions. Totally 378 calla rhizomes were planted in 30-L containers placed on three raised benches. Three different GM combining different proportions of the TS with a standard peat-based substrate were tested (Table 1). Benches were served by different water regimes (WR) by a drip irrigation system: i) WR1, high water regime=WR2+30% (1220 mL/day of water per pot on average); ii) WR2, normal water regime (930 mL/day of water per pot on average); iii) WR3, low water regime=WR2-30% (650 mL/day of water per pot on average). Each GM*WR combination was replicated in three blocks, each containing seven pots, each holding 2 rhizomes (14 rhizomes x 9 treatments x 3 blocks = 378 rhizomes). Plants were fed with a nutrient solution commonly adopted for the cultivation of soilless calla and the experiment was stopped at the end of the first flower flush. Flower number and flower length were monitored during plant life cycle (once a week from September to February, and twice a week from March to May) by collecting, counting and measuring all the flowers produced in the experimental test. Moreover, flowers were graded by stem length and sale price of each flower category was calculated during the whole harvest season. Marketable senescence (number of days to get pollen on spathe) and final senescence (number of days to spathe browning) were determined by storing 15 cut flowers (5 for each replicated block) in water at room temperature. On the same flowers, spathe colour was measured on three different floral leaf points as described above.

*Protea (*Protea cynaroides*) 'Little Prince'*

National Flower of South Africa, protea is commercially relevant for the flower industry and in Italy it is extensively cultivated as flowering pot and exported all over the world. Crop protection is a fundamental requirement for its cultivation in Tuscany; moreover, water needs are high when this species is grown under soilless conditions. Totally 210 rooted cuttings were transferred in 2-L pots and cultivated until full blooming.

The TS was mixed in different proportion with peat and coir to obtain seven growing media, as described in Table 1. Each substrate mix was replicated three times with 10 pots for each replicated plot. Plants were supplied with 150 mL/day of water per pot on average. All plants were fertilized with Nutricote timed release fertilizer (NPK 18:6:8 - 360 day releasing time) and watered with daily drip irrigation. Number and length of stems, number and dimension of flowers of all experimental plants were measured after the full opening of the flowers, and the sale price of each flowering potted plant was considered. The cluster colour of all flowering plants was measured on three different bracts as described above.

Statistical analysis

Pots in the three greenhouse experiments were arranged according to a randomized complete block design. All collected data were subjected to analysis of variance (ANOVA) to determine treatment effects. Where significant effects were determined, Tukey's test was used to separate differences among treatment means at the 99% ($p<0.01$) level of confidence, applying SPSS v27 software (SPSS Inc., Chicago, Il, USA).

3. Results

*Cherry laurel (*Prunus laurocerasus*) 'Novìta'*

The GM had a clear effect on final plant height (Fig. 2A), while WR and GM*WR interaction were without effect on plant development. LMix 6 and LMix 7, containing TS mixed with wood fiber, were the most limiting for *P. laurocerasus* growth. Cherry laurel grown in TS and peat (LMix 2 and LMix 3) did not statistically differ from the control, while plants obtained on mixes containing TS and coir (LMix 4 and LMix 5) exhibited intermediate height values. Selling values followed the same trend as the plant development (Fig. 2B). As regards leaf blade colour, leaves of cherry laurel showed a significant yellowing compared to the control when grown mixes containing TS and bark (Table 2).

Fig. 2 - Plant height (A) and selling value (B) of *P. laurocerasus* at marketing stage. Mean separation among bars by Tukey's test. Means (n = 24) followed by different letters are significantly different ($p<0.01$).

Table 2 - Effect of growing media (GM) on the colour of cherry laurel leaves

GM	Chroma
LMix 1	18.5 a
LMix 2	18.8 a
LMix 3	17.6 abc
LMix 4	17.3 abc
LMix 5	17.2 abc
LMix 6	16.2 bc
LMix 7	15.8 c

Mean separation within column by Tukey's test. Means (n= 24) followed by different letters are significantly different ($p<0.01$).

*Calla lily (*Zantedeschia aethiopica*)*

Number of flowers and selling price were significantly affected by the GM and WR, while the GM*WR interaction did not affect productive traits. The treated sediment had a clear positive effect on plant blooming, since the number of flowers showed increasing values as the content of the sediment in the mixture increased (Table 3). Regarding water supply, a 30% water reduction diminished calla blooming, being the number of flowers per plant on average 21% smaller than that obtained under normal water supply (4.5 vs 5.7). In general, the produc-

Table 3 - Main effect of growing media (GM) and water regime (WR) on the number of calla lily flowers and their selling value

Factor	Flowers/plant (n)	Selling value/flower (€)
GM		
CMix 1	4.6 b	1.8 b
CMix 2	5.5 a	2.6 a
CMix 3	5.8 a	2.8 a
WR		
WR1	5.7 a	2.9 a
WR2	5.7 a	2.6 a
WR3	4.5 b	1.6 b

Mean separation within columns by Tukey's test. Means (n= 63) followed by different letters are significantly different ($p<0.01$).

tion of flowers increased over time reaching the maximum peak in March and April. Flowers were subdivided according to the stem length, which varied from 50 to 100 cm. Plants cultivated on CMix 3 produced a consistently higher number of quality flowers reaching 80, 90 and 100 cm of final length compared to the control (72 *vs* 45, 43 *vs* 6 and 29 *vs* 0, respectively). Thus, the selling value averaged over the entire harvest season was found to be greater for flowers obtained on CMix 3 consisting of 50% TS (Table 3). No differences in the colour of the spathe were evidenced (Fig. 3), whatever growth conditions (GM and WR) were used (Table 4). Regarding petal senescence, inferior cut flower performance during vase life was observed when flowers were cultivated on CMix 1 and CMix 2 with reduced irrigation (Table 4).

Fig. 3 - Calla lily cut flowers at harvest time.

*Protea (*Protea cynaroides*) 'Little Prince'*

ANOVA showed that all considered parameters of protea were significantly affected by the growth media. In particular, protea grown on the control (PMix 1) exhibited significant higher average values of stem number and length (Table 5), along with an anticipated flower induction and development (data non shown) compared to plants grown on sediment-based media. The incorporation of 50% *v/v* TS in the growing media drastically reduced plant growth and flower production in all tested mixes (Fig. 4A). On the other hand, protea grown on mixes containing 25% *v/v* TS had a more compact shape, but developed a good number of flowers, except for PMix 6 containing a higher percentage of coir dust. Selling prices of potted flowering proteas were in line with the values expressed by plant vegetative and reproductive behaviour under the different tested soilless conditions (Fig. 4B). Once flowers opened, they were similar in terms of size (Table 6; Fig. 5), except for flower length which was smaller in PMix 5, PMix 6 and PMix 7 essentially related to the lesser flower development at the time of the data collection. Some differences in the bract colour of protea clusters were perceptible as indicated by the Chroma Index reported in table 6. In general, control flowers were deep pink, while those obtained on sediment-based substrates had brighter tones and tending towards purple. In

Table 4 - Effect of GM*WR interaction on the senescence and colour of calla lily flowers

GM*WR	Flower senescence		Chroma
	Marketable	Final	
CMix 1 WR1	11.8 ab	15.0 ab	6.6 NS
CMix 1 WR2	14.8 a	19.0 a	6.1 NS
CMix 1 WR3	8.2 c	15.4 ab	6.2 NS
CMix 2 WR1	13.2 ab	16.6 ab	6.2 NS
CMix 2 WR2	13.8 ab	18.4 ab	6.1 NS
CMix 2 WR3	7.6 c	14.0 b	6.0 NS
CMix 3 WR1	13.4 ab	17.2 ab	5.9 NS
CMix 3 WR2	13.8 ab	17.2 ab	6.2 NS
CMix 3 WR3	12.0 ab	17.4 ab	6.1 NS

Mean separation within columns by Tukey's test. Means (n= 15) followed by different letters are significantly different (p<0.01). NS = not significant.

Table 5 - Effect of growing media (GM) on stem number and lenght of *P. cynaroides*

GM	Stem/plant (n)	Stem lenght (cm)*
PMix 1	2.9 a	31.6 a
PMix 2	2.8 ab	23.0 b
PMix 3	2.1 bc	15.5 cde
PMix 4	2.5 ab	18.9 bc
PMix 5	1.3 c	9.6 e
PMix 6	2.7 ab	15.8 cd
PMix 7	1.9 bc	13.9 de

*Values are the average of all plant stems.
Mean separation within column by Tukey's test. Means (n= 30) followed by different letters are significantly different (p<0.01).

Fig. 4 - Bloomed plants (A) and selling value (B) of *P. cynaroides* at marketing stage. Mean separation among bars by Tukey's test. Means (n = 30) followed by different letters are significantly different ($p<0.01$).

Table 6 - Effect of growing media (GM) on protea flower size and colour

GM	Flower size			Chroma
	Length (mm)[z]	Width (mm)[z]	Diameter (mm)[y]	
PMix 1	97.3 a	38.7 a	22.5 NS	32.7 a
PMix 2	97.3 a	38.4 a	22.3 NS	30.0 ab
PMix 3	97.9 a	39.3 a	21.4 NS	28.3 ab
PMix 4	97.8 a	40.3 a	23.1 NS	32.2 a
PMix 5	81.0 b	32.9 b	22.7 NS	27.5 b
PMix 6	74.7 b	38.6 a	22.3 NS	30.9 ab
PMix 7	81.6 b	33.9 b	22.0 NS	28.9 ab

[z] measured with closed flower on March 2022; [y] measured with fully open flower on May 2022.
Mean separation within columns by Tukey's test. Means (n = 30) followed by different letters are significantly different ($p<0.01$). NS = not significant.

particular, the flowers collected on PMix 5 exhibited the lowest chroma index mainly associated to the a* component more shifted towards green and the b* component towards yellow.

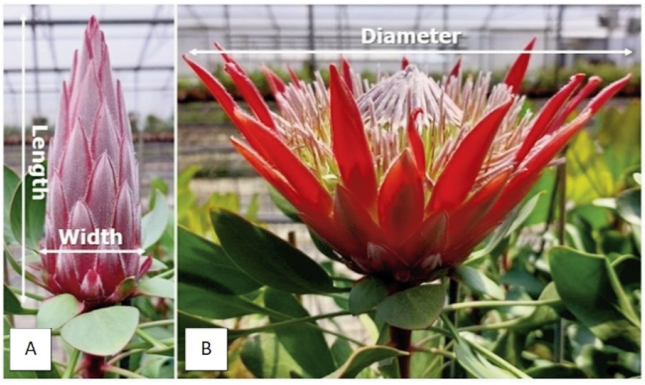

Fig. 5 - Size measurement on Protea flower. Length and width (A), diameter (B).

4. Discussion and Conclusions

Results showed that the incorporation of the treated sediment into growing media for soilless cultivation affected both plant growth and blooming differently according to the considered ornamental species.

In the case of evergreen cherry laurel, the TS used in proportions of 25-50% reduced plant height growth, especially when combined with wood fibre, slightly altering its attractive vibrant foliage. This species is easy to care and adapts to most types of soils, from light and sandy to heavy clay; thus, the reduced plant development was assumed to be primarily associated to the combined properties of the used matrices (Tozzi *et al*., 2022).

A positive effect of the TS was evidenced on calla lily, notoriously known as a water demanding species. In fact, due to its clayey silt composition, the sediment can increase water retention capacity when added to traditional soilless growing media, although the effect appears to depend primarily on the amount of sediment added to the medium (Martínez-Nicolás *et al*., 2021). In our study, both tested sediment-based mixtures allowed a copious blooming, while a 30% water reduction determined a highly significant decrease in flower production. The highest number of flowers and of best quality (stem size between 80-100) were obtained from plants grown in the presence of 50% of TS. On the other hand, petal senescence appeared to be mainly influenced by the water amount that plants receive during cultivation, while no significant differences were

detected in the colour of calla flowers among the considered treatments.

The effect on protea plants grown on different sediment-based substrates was almost opposite. Plants grown on mixes containing 50% *v/v* TS exhibited a considerable reduction in plant growth and flower production (Fig. 6). Nevertheless, when 25% *v/v* TS was added to peat and coir fibre, only 7-13% of potted plants failed to bear flowers and displayed a nice compact habit. In this regard, it should be noted that a compact behaviour might represent a valuable feature for this species, when cultivated as a flowering pot, since growth regulators are being used to induce branching and improve compactness (Ben-Jaacov and Silber, 2007). Suggested factors limiting plant development and flower production could be related to high pH values and nutrient content, especially phosphorus, inherited from the sediment matrix. In fact, this perennial flowering species develops proteoid roots to increase nutrient absorption, being therefore adapted to poor soils of low nutritional status, particularly phosphorus, with a pH 4-6, and a clay content of less than 20% (Griebenow *et al*., 2022; Walter *et al*., 2022). Concerning other aesthetic aspects of the plant, the amount of TS incorporated in the substrate mixes affected the colour of clusters but not their size.

Ultimately, based on sale values, the TS can be a sustainable alternative for soilless production of ornamental crops, both for environmental protection and economic development. Recommended for calla cut flower production, the TS can be also used successfully for potted cherry laurel and protea cultivation if properly combined in the correct proportions (up to a maximum of 50% and 25%, respectively) with other organic matrixes, such as peat and coir.

A large number of consumers are changing their way of life and the products they buy in an effort to live more sustainably. It's clear that nursery industries need to place sustainability in the heart of their business strategy. However, the cost of eco-friendly plants is often higher than conventional products, due to expensive raw materials which are necessary to produce with lower environmental impact. So, the issues are: is such discrepancy really that significant? Are costumers willing to pay more for a plant obtained with the utmost respect for the environment?

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Fig. 6 - Protea plants grown on control substrate (1 = PMix 1), mixes containing 25% TS (2 = PMix 2, 4 = PMix 4, 6 = PMix 6), and mixes containing 50% TS (3 = PMix 3, 5 = PMix 5, 7 = PMix 7

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Use of xanthan gum and calcium ascorbate to prolong cv. Butirra pear slices shelf life during storage

E. Guccione (*), A. Allegra, V. Farina, P. Inglese, G. Sortino
Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, 4, 90128 Palermo, Italy.

Key words: Browning, edible coating, fresh-cut, pear, respiration.

Abstract: After cut, pear fruit (*Pyrus communis* L.) during shelf life can be subjected to color and flavor changes. To maintain flesh colour and firmness, different technologies could be employed during shelf life, such as chemical, physical and edible coating treatments. In the present study, the effects of two edible coating formulations containing xanthan gum and calcium ascorbate on fresh-cut pear fruit were investigated. After harvest, 200 fruits were cut and coated with Xanthan Gum (XAN) and distilled water or Xanthan gum + Calcium Ascorbate (ASC), respectively, while control (CTR) pear fruit slices were soaked in distilled water and lastly packed in polyethylene terephthalate (PET) packages sealed with a composite film (PP-PET). All samples were stored at 5±0.5°C with RH 90% for 10 days. Measurements were carried out at 3, 5, 7 and 10 days of storage evaluating visual quality score, browning index, color, total solid soluble content (TSS), flavor, ascorbic acid content and total phenols content. The results showed that ASC treatment was the most efficient treatment in terms of color changes, ascorbic acid content, visual quality score and browning index, until the 7th day of storage. Moreover, ASC treatment reported lower mean values in terms of taste and flavor score if compared to CTR and XAN treatments. Untreated pear slices (CTR) kept good values concerning flavor score until the 3rd day of storage while on the 5th and 7th day off-flavor values were the same as treated samples.

(*) **Corresponding author:**
eugenia.guccione@unipa.it

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All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

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1. Introduction

'Butirra' pear fruit is cultivated in southwestern Sicily and belongs to sicilian MIPAAF PATs (traditional agricultural products). After harvest, usually occurring between the second decade of July and the first decade of August, they must be consumed as they are easily rotten and are subjected to fast decay. It's easy to understand how fresh-cut 'Butirra' poor shelf-life is a key barrier to its commercialization especially for the fast weakening of tissues and surface's browning that happens after cut due to the action of polyphenol oxidase (PPO) (Amiot *et al.*, 1995; Hodges and Toivonen, 2008). Edible coatings are widely employed since they prevent the loss of quality acting like a selective barrier to gas exchanges between

food and external environment. Xanthan gum enhances all these properties and also controls the rheology of the final food product exhibiting pseudoplastic properties in solutions (Palaniraj and Jayaraman, 2011). As a generally recognized as safe (GRAS) molecule, xanthan gum is an exopolysaccharide produced by the fermentation of a carbohydrate by cultures of *Xanthomonas campestris*. It is then refined by extraction with ethanol or 2-propanol, dried, and powdered (FDA). Calcium ascorbate is the calcium salt of ascorbic acid that is widely used as an antioxidant whose reducing action against quinones and diphenols prevents browning of unprocessed fruit as it only produces colorless derivatives; it is a reducing agent, capable of promoting the chemical reduction of the pigment precursors responsible for browning, acting by reducing o-benzoquinone or dihydroxyphenol or irreversibly inactivating PPO, promotes the regeneration of antioxidants and acts synergistically with complexing agents (Araújo, 2004). Furthermore, this cation can maintain cell wall structure by binding to pectins and forming calcium pectate (Vilas Boas *et al.*, 2009). Calcium stabilizes the membranes and cell walls, preserving their integrity and functionality and protecting them from being cleaved by hydrolytic enzymes that cause fruit softening (Poovaiah, 1986; Vilas Boas *et al.*, 2009). Xanthan gum combined with antioxidant agents had positive effects on the reduction of weight loss and browning, preventing the loss of firmness, and the growth of psychotropic microorganisms, molds and yeasts in minimally processed apples and pears (Sharma and Rao, 2015; Allegra *et al.*, 2022). The aim of the present study was to evaluate the effectiveness of edible coating based on xanthan gum and xanthan gum enriched with calcium ascorbate on fresh-cut cv 'Butirra' pear fruit stored in passive atmosphere.

2. Materials and Methods

The experiment was carried out in 2021. 'Butirra' pear fruit (Quince BA29 rootstock and intermediate 'Butirra Hardy' graft) were harvested during the second week of august in a commercial orchard located in Zafferana Etnea (Catania, Italy), Italy, (730 m above sea level). The soil is a sandy clay loam (63% sand, 19% silt, 18% clay), with pH 6.9 and active carbonates lower than 5%, trees were trained as a free palmette. Fruits were hand-picked at an optimal ripening stage tested with Lugol solution. All trees received the same conventional cultural cares from planting until the end of the current experiment. After harvest, fruit were cold stored and transported at University of Palermo and stored at 5±1°C in cold room the night before the analysis.

Experimental design

Two hundred fruit were harvested from 20 trees and brought to the laboratory where they were dipped in chlorinated water (100 ppm of free chlorine) for 360 s to sanitize them. Defective fruit were discarded and the remaining were sorted by firmness (4.1±1 kg/cm^2) and average weight (120± 20.2 g). Quality indexes were calculated the first day of analysis, particularly, color (CIELab), flesh compactness and total solid soluble content (TSS).

Fruits were selected for weight, maturation index, caliber and absence or presence of defects and sanitized with OxVirin 200 ppm and H_2O by soaking for 30 minutes. Then, they were peeled and cut. Edible coatings were applied by dipping and solutions were formulated as follows:

i) Control (CTR): fruits were dipped in distilled water and used as control;
ii) XAN: the solution was made by mixing 3% of xanthan gum in distilled water using a magnetic stirrer;
iii) ASC: the solution was made by mixing 3% of xanthan gum and 2% of calcium ascorbate in distilled water using a magnetic stirrer.

After treatments, fruit were packed in PET boxes, sealed with a composite PP-PET film and stored at 5±1°C with 95% relative humidity (RH) for 10 days. Trials were carried out at 3, 5, 7 and 10 days of storage evaluating visual quality score, browning index, color, total solid soluble content (TSS), sensorial analysis, ascorbic acid content and total phenols content.

Weight loss

The following formula was adopted to determine weight loss during storage

$$\text{Weight loss (\%)} = [(W1-W2)/(W1)] \times 100$$

Where *W1* and *W2* represent initial weight (T0) and measured weight at 3, 5, 7 and 10 days of storage with a precision balance (Gibertini, Italy), respectively. At the beginning of trial period all boxes had homogeneous weight (100 ± 2.1 g).

Color

Flesh color was measured throughout the experiment on the first day of analysis (0) and on the 3rd,

5th, 7th and 10th day of storage. Color was measured through a portable colorimeter (Chroma Meter CR 400, Konica Minolta Sensing Inc., Tokyo, Japan) equipped with an 8 mm measuring head and a C illuminant (6774 K). The white standard plate of the manufacturer was used for calibration. Chromatic difference (ΔE) was calculated using the following formula to express the magnitude of difference between the non-aged pulp and stored samples:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

All trials were carried out in triplicate and data were reported as ± mean standard error (SE n=3).

Browning index

Browning index (BI) was determined following the equation of Ruangchakpet and Sajjaanantakul (2007):

$$(BI) = [100\ (x - 0.31)]/0.17$$

where $x = (a^* + 1.75\ L^*)/(5.645\ L^* + a^* - 0.3012b^*)$.

Firmness

Fruit firmness was tested with a texture analyzer equipped with a 2.5 cm flat-tip (Instron 5564, MA, USA). The maximal force was expressed in kg/cm^2 and slices were compressed with a speed of 5 mm/s to a depth of 4 mm.

Total solid soluble

Total solid soluble content was determined on pear fruit juice extracted from samples at each storage time using a hand-held refractometer (ATAGO Palette PR-32).

Total phenols content

Total phenols content was quantified according to Sortino *et al.* (2022). 30 grams of fresh tissue for each replication was homogenized with methanol on 1:10 ratio and then filtered through a Whatman grade N.1 filter, the application of reduced pressure allowed the concentration of methanolic extracts and the residue was then suspended in 50% aqueous methanol and used for phenolic content quantification. Phenols content was determined through a spectrophotometrical analysis at the wave lenght of 700 nm and results were expressed in gallic acid equivalent (mg kg^{-1} fresh weight).

Ascorbic acid

Ascorbic acid content was analyzed at each sampling date with the Megazyme kit (Bray Business Park, Bray, Co., Wicklow, Ireland) as reported by Allegra *et al.* (2015).

Sensorial analysis

Sensorial analysis was carried out by a panel of 12 specifically trained panelists (Sortino *et al.*, 2017). All samples were subjected to a panel made up of 14 descriptors: external color uniformity (ECU), compactness (COM), pulp color intensity (PCI), odor (O), herbaceous odor (HO), floral odor (FO), sweetness (SW), sourness (S), bitterness (B), juiciness (J), pear flavor (PF), herbaceous (HF) and floral flavor (FF) and overall rating (OR). The graduated scale went from 1 (absence of descriptor) to 9 (descriptor at its fullest intensity). Sensorial analysis was carried out from day 0 to day 10.

Visual quality score

Edible coatings effect on 'Butirra' fresh-cut slices was evaluated at each storage time on six slices used as single replicates, for each treatment. Six trained judges used a list of descriptors wrote down in preliminary meetings. Descriptors involved the medium value of color, integrity and appearance on pear fruit slices as reported by Allegra *et al.* (2022). Descriptors were quantified using a 5 points hedonic scale where 5= very good, 4= good, 3= sufficient (limit of marketability), 2= poor (limit of usability) and 1= very poor (inedible).

CO_2 and O_2 inside packaging

O_2 and CO_2 content inside packages was analyzed at each sampling date using a PBI Dansensor Checkpoint O_2-CO_2 analyzer (Ametek Mocon, MS, USA) equipped with infrared detectors.

Statistical analysis

The experimental design consisted in two treatments and one control, observed at 0, 3, 5, 7 and 10 days at 5°C after treatment. Nine slices were used as single replicates and analyzed at each sampling date. Analysis of variance was applied (Systat 13.0 for Windows was used as statistical software) and the significance of data ($p \leq 0.05$) was evaluated with Tukey's test.

3. Results and Discussion

Total solid soluble content

Our results showed a general increase in TSS during storage; control slices content increased about 29.03% during the 10 days of storage while XAN and ASC treatments scored an increase of 16.30% and 15.45%, respectively. CTR slices increase is probably

due to the greater water loss of the untreated samples, which also results in higher percentages of weight loss (Fig. 1). XAN and ASC treatments were more efficient than CTR in limiting the increase in TSS content, this phenomenon is due to ripening processes that result in the hydrolysis of starch into mono- and disaccharides (Mahajan *et al.*, 2004) and in the activation of respiration processes where sugars are the main substrate used (Dong *et al.*, 2004). Significant differences occurred between treatments on 5th and 7th day while on 10th day XAN and ASC treatment recorded the same value.

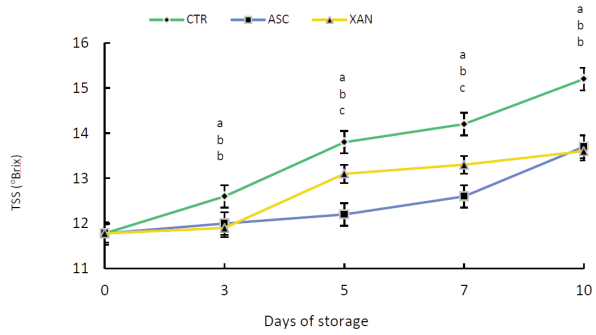

Fig. 1 - Total solid soluble content of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significative differences between treatments. p≤0.05 was used in the Tukey's test. The data are provided as the mean ± SE (n = 3).

Weight loss and firmness

Weight loss during cold storage showed an increase in all sample slices (Fig. 2). CTR samples showed a higher percentage of weight loss if compared to other treatments. ASC treatment showed lower weight loss than XAN and control pear slices. The effectiveness of ascorbate calcium-can be attributed to the ability of calcium to preserve the compactness of cell structures by limiting the action of pectolytic enzymes. In other work on fresh-cut pear the use of calcium ascorbate could be responsible of the maintenance of cellular wall structure since calcium maintains glycosidic bindings stable avoiding the collapse of cellular wall and the subsequent loss of liquids (Akhtar *et al.*, 2010). Grant *et al.* (1973), on the other hand, showed that the maintenance of firmness in calcium-treated fruits may be due to its accumulation in cell walls, which facilitates the crossing of pectic polymers by increasing wall strength and cell cohesion. Obtained results for weight loss are

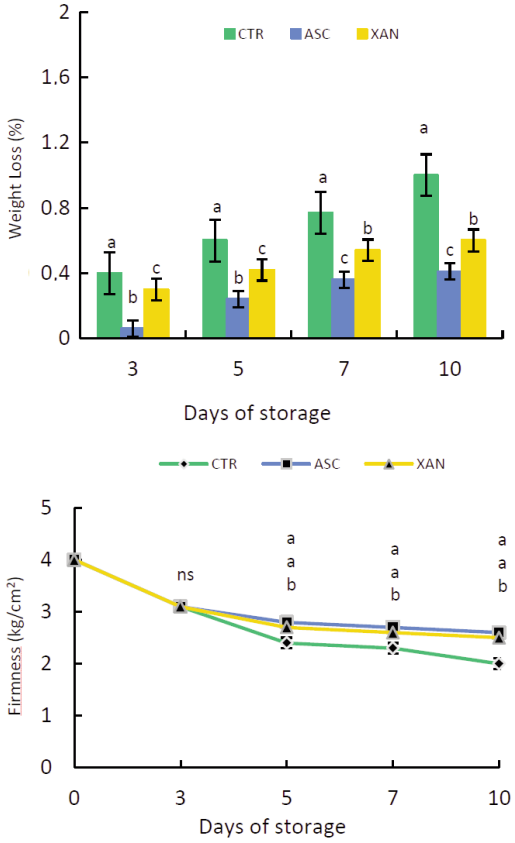

Fig. 2 - Weight loss (%) and firmness (kg/cm²) of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. 'Butirra' fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significative differences between treatments. p ≤ 0.05 was used in the Tukey's test. The data are provided as the mean ± SE (n= 3).

closely linked, as previously discussed, with TSS content data.

Color changes and browning index

Color monitoring during cold storage showed a change in L*, a* and b* values in 'Butirra' in all treatments (Table 1). A low decrease in L* values occurred in XAN treatment. Color changes are more limited in ASC treated samples.

At the end of cold storage period (T10), ΔE reached its highest in CTR samples and its lowest in ASC treated samples. Untreated 'Butirra' fruit slices recorded the highest browning values at each sampling date, and it began to increase sharply from day five, while XAN and ASC treatments recorded the lowest values. Significant differences occurred between ASC and XAN treatments from 5th day of storage while no significant differences were record-

Table 1 - Color slices (CIELab index) and color variation (ΔE) of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C

Days of storage	Treatments	L*	a*	b*	ΔE
T0	CTR	63.89	-13.57	36.94	-
T3	CTR	64.64 a	-0.32 b	24.30 b	18.33 NS
	ASC	66.27 b	-0.38 b	24.98 b	17.96
	XAN	64.82 a	0.61 a	26.16 a	17.84
T5	CTR	66.97 a	-0.03 c	20.82 NS	27.27 a
	ASC	63.96 b	-0.11 b	19.25	22.23 b
	XAN	57.51 c	0.51 a	19.25	23.50 b
T7	CTR	65.12 a	-0.22 a	17.4 b	28.69 a
	ASC	63.02 b	-0.07 b	16.72 b	24.33 b
	XAN	52.39 c	0.01 b	20.02 a	24.55 b
T10	CTR	45.53 c	-0.02 b	12.20 b	33.66 a
	ASC	62.65 b	0.03b	14.90 a	25.93 b
	XAN	65.24 a	-0.36 a	14.37 a	26.19 b

At each sampling date, different letters indicate significative differences between treatments. NS = not significant. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean ± SE (n= 3).

ed at 10th day between the two edible coatings formulations (Fig. 3). A similar trend occurred in Sharma and Rao (2015) on fresh-cut pear treated with Xanthan gum for 8 days of cold storage.

Sensorial analysis and visual quality score

Sensorial analysis was carried out at each storage time and at harvest time high values were recorded for all descriptors except for bitterness, sourness, herbaceous and floral odor and flavor. A slower decrease in treated pear slices was recorded. All treatments recorded higher values than CTR one at each sampling time and no off-flavors or negative descriptors were found in edible coating treated samples until 10th day of storage. In fact, 'Butirra' pear slices treated with ASC and XAN recorded positive values for descriptors concerning sweetness, compactness, external color uniformity, juiciness and pear flavor during all storage time while, on the contrary, CTR samples recorded increasing values of negative descriptors such as bitterness and sourness as early as the 5th day of analysis. Overall rating was quite positive in treated samples until 10th day (Fig. 4).

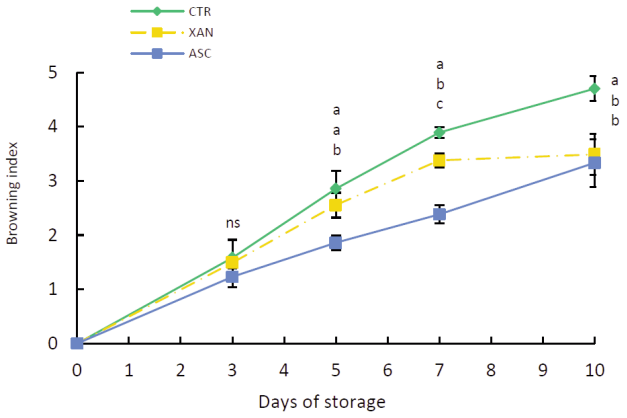

Fig. 3 - Browning index of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significative differences between treatments. p≤0.05 was used in the Tukey's test. The data are provided as the mean ± SE (n = 3).

Visual quality score test enhanced that a decrease in mean values occurred in all treatments registering significant differences since the 3rd day of analysis (Fig. 5). Since day 3, CTR samples recorded mean values under 3 (limit of marketability). Instead, the judges evaluated ASC samples on 10th day at the limit of marketability (score=3). Xanthan coating had a positive effect on visual score of fresh-cut pear (Sharma and Rao, 2015) extending their marketability until the 10th day.

Total phenols and ascorbic acid content

No significant differences were showed between treatments on pear slices ascorbic acid content. During storage, total phenol content increases slowly in both CTR and XAN treatment. After 7 days a sharp increase was observed in treated and untreated samples and significant differences occurred between

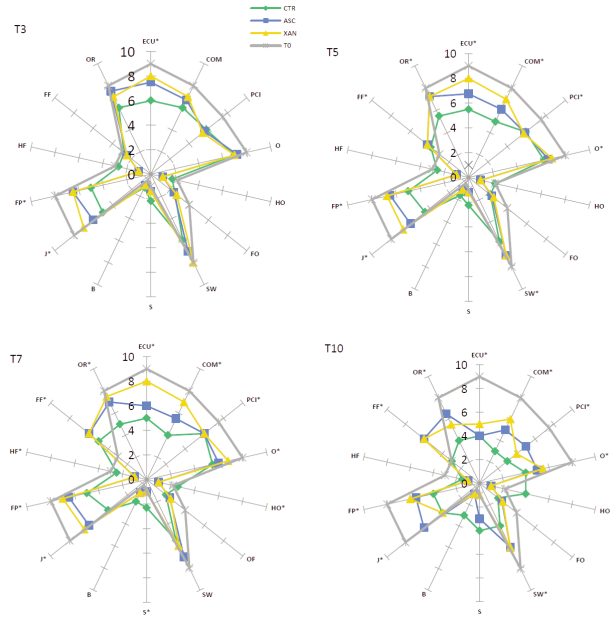

Fig. 4 - Sensorial analysis of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices after cut (0) and at 3, 7 and 10 days of storage at 5°C. At each sampling date, * indicates substantial changes between treatments. At each sampling date, NS indicate no changes between treatments. p≤0.05 was used in the Tukey's test. Legend: external color uniformity (ECU), compactness (COM), pulp color intensity (PCI), odor (O), herbaceous odor (HO), floral odor (FO), sweetness (SW), sour (S), bitter (B), juiciness (J), pear flavor (PF), herbaceous flavor (HF), floral flavor (FF) and overall rating (O).

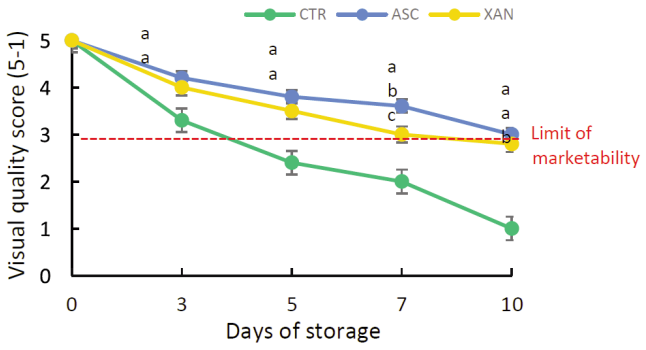

Fig. 5 - Visual quality score of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5 °C. At each sampling date, different letters indicate significative differences between treatments. p≤0.05 was used in the Tukey's test. The data are provided as the mean ± SE (n = 3).

ASC and other treatments (Fig. 6). An increase of total phenol content is possible in stress conditions after cutting or in low temperature (Amodio *et al.*, 2014).

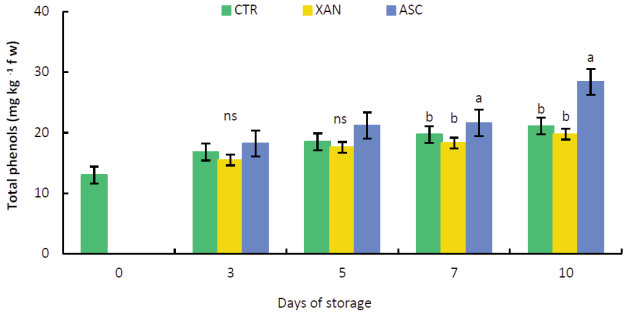

Fig. 6 - Total phenols content (mg kg^{-1} fw) of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after storage for 3, 5, 7, 10 days at 5°C. At each sampling date, different letters indicate significative differences between treatments. p≤0.05 was used in the Tukey's test. The data are provided as the mean ± SE (n = 3).

CO_2 and O_2 inside packaging

A limited O_2 consumption and CO_2 production occurred during storage in both XAN and ASC treatments while higher values were registered in CTR samples (Fig. 7). Significant differences occurred from the 3rd day between treated and untreated samples, particularly, CTR samples registered a value of 1.5 kPa for O_2 and a value of 21 kPa for CO_2 at 10th day of storage. Both XAN and ASC kept the two parameters more stable. Similar trends were observed on fresh-cut peach treated with calcium lactate and ascorbic acid and on breba fig fruit stored in passive atmosphere (Allegra and Colelli, 2015; Allegra *et al.*, 2015).

4. Conclusions

The two different formulations based on calcium ascorbate and Xanthan gum preserved pear slices of 'Butirra' during the 10-day storage at 5°C. Positive effects were observed on browning, weight loss and firmness up to the 10th day, furthermore, the two edible coating formulations preserved the sensory attributes of fresh cut 'Butirra'. Our results showed that Xanthan gum with calcium ascorbate treatment improved the retention of firmness, browining and weight loss than control slices. This result was confirmed by the sensorial analysis in which positive descriptors showed positive values until the 10th day of storage while untreated samples began to develop off-flavor and off-color since day 3.

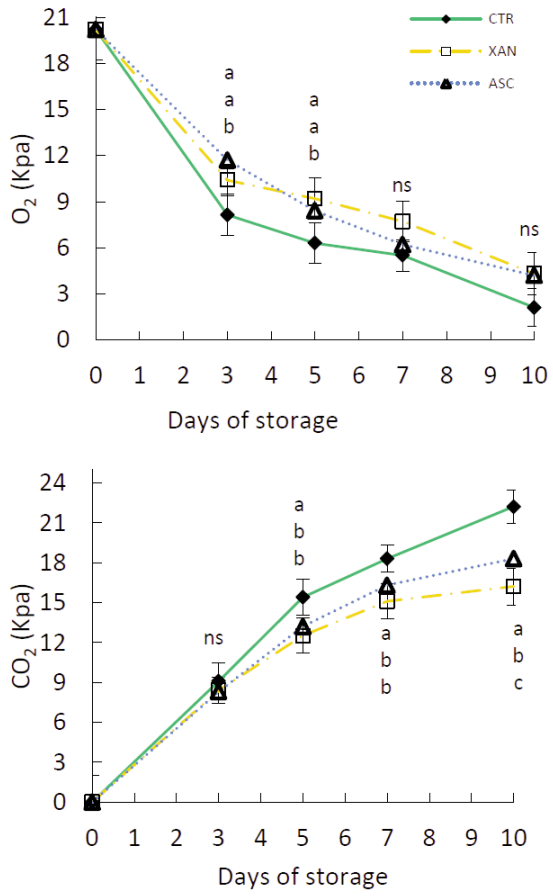

Fig. 7 - O_2 and CO_2 inside packaging of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significative differences between treatments. p≤0.05 was used in the Tukey's test. The data are provided as the mean ± SE (n = 3).

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CO_2 modified atmosphere packaging: stress condition or treatment to preserve fruit and vegetable quality?

M. Cefola [1], I. Capotorto [1], V. Lippolis [2], S. Cervellieri [2], A. Damascelli [2], R. Cozzolino [3], B. De Giulio [3], B. Pace [1(*)]

[1] *Institute of Sciences of Food Production, National Research Council (CNR) c/o CS-DAT, Via M. Protano, 71121 Foggia, Italy.*

[2] *Institute of Sciences of Food Production, National Research Council (CNR), Via G. Amendola, 122/O, 70126 Bari, Italy.*

[3] *Institute of Food Science, National Research Council (CNR), Via Roma, 64, 83100 Avellino, Italy.*

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Key words: Carbon dioxide, fermentative metabolites, modified atmosphere packaging, respiration rate, short-term treatment.

Abstract: In addition to the adoption of proper temperature and relative humidity, the selection of an atmosphere surrounding packaged fresh produce with reduced O_2 and/or increased CO_2 is one of the most widely used and useful tools to prolong the shelf-life of horticultural crops. However, as O_2 and/or CO_2 values that might cause injury are strictly related to the commodity, they should be optimized for each product. Here three study cases are reported about the application of modified atmospheres (MA), with different CO_2 concentrations (0-40 kPa), to table grapes (cv. Italia) and sweet cherries (cv. Ferrovia) and, as a short-term treatment (48 h at 0°C), to fresh-cut artichokes (cv. Violet de Provence). In each trial, the effect of high CO_2 treatment on quality parameters was observed during cold storage. Concerning table grape 'Italia', our results show that low CO_2 (up to 10 kPa) MA preserved the quality and sensory parameters of the fruit, whereas high CO_2 (> 20 kPa) caused a fermentative metabolism. As for sweet cherries 'Ferrovia', 20 kPa CO_2 MA helped to maintain the quality traits during storage. On the other hand, this fruit proved to be sensitive to CO_2 accumulation (over 20 kPa) in hypoxic conditions, since it caused an increase in respiration rate and the biosynthesis of volatile fermentative metabolites. Finally, for fresh-cut artichokes, a short-term CO_2 treatment, up to 10kPa, reduced respiration rate and browning index, preserving the volatile profile, while high CO_2 (40 kPa) may have caused fermentative metabolism. In conclusion, the application of a MA enriched in CO_2 has been shown to have different effects on the quality parameters of the three products, in agreement with the fact that CO_2 sensibility depends on each specific fruit or vegetable under study.

(*) Corresponding author:
bernardo.pace@ispa.cnr.it

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Fruits and vegetables are perishable products, and extending the keeping quality during their postharvest life represents one of the main

goals of researchers in this field. It is widely known that, together with the proper temperature and relative humidity management, the gas composition surrounding the product during storage, is one of the main factors that affect the quality of horticultural crops (Kader, 2003). In general, the decrease in oxygen, the increase in CO_2, or the association of both conditions are useful to preserve the physiological state of fruits and vegetables, reducing the rate of respiration, oxidative processes, and decay, thus prolonging their shelf-life. In contrast, inappropriate gas concentrations outside safe limits can cause stress conditions that lead to physiological disorders, development of off-odours due to fermentative metabolism, or increases in susceptibility to decay (Mangaraj and Goswami, 2009). Although low O_2 and high CO_2 have similar effects, under modified atmosphere packaging (MAP) conditions, elevated CO_2 is a major factor influencing the quality of fruits and vegetables (Watkins, 2000). In addition, the sensitivity to elevated high CO_2 and/or low O_2 levels depends on the commodity (Toivonen and DeEll, 2002). It is influenced by pre and postharvest factors, such as cultivars or stage of maturity, and by processing, since the oxygen consumption and the consequent CO_2 accumulation in fresh-cut produce is faster than in corresponding intact produce (Francis *et al*., 2012). To obtain the beneficial effect of MAP, gas conditions should be optimized for each product. Starting from these considerations, the aim of the present work was to compare the effect of different CO_2 concentrations in MAP on the quality of table grapes, sweet cherries and fresh-cut artichokes as case studies.

2. Materials and Methods

Table grapes (*Vitis vinifera* L., cv. Italia), sweet cherries (*Prunus avium* L., cv. Ferrovia), and artichokes (*Cynara cardunculus* (L.) subsp. *scolymus* Hayek, cv. Violet de Provence) were provided by local farms located in Noicattaro and Foggia (Italy) and processed at the Postharvest Laboratory of CNR-ISPA the day of harvest. Selected bunches of table grapes (about 1 kg each) were placed in polyethylene terephthalate (PET) trays (model CL1/135 Carton Pack, Rutigliano, Italy). They were sealed with a vacuum sealer (model Boxer 50 Lavezzini Vacuum Packaging System, Fiorenzuola d'Arda, Italy) in 30 x 40 cm polyamide/polyethylene (PA/PE) bags (Orved S.p.A., Musile di Piave, Italy) applying two modified atmosphere (MA) mixtures with different initial CO_2 concentrations plus 1 kPa of O_2: 1.0/0.03 O_2/CO_2 kPa (1 kPa-O_2), and 1.0/20.0 O_2/CO_2 kPa (1 kPa-O_2 + 20 kPa-CO_2). Unsealed bags were used as control (Air). All samples (4 replicates per treatment) were analyzed at harvest and after 20 days of storage at 5°C for respiration rate (RR), rachis browning (rB), ethanol, and acetaldehyde contents.

For sweet cherries, about 200 g of fruits, without defects or diseases, were placed in PET trays and sealed in 30 x 40 cm PA/PE bags with three MA mixtures: 1.0/0.03 O_2/CO_2 kPa (1 kPa-O_2), 16.0/20.0 O_2/CO_2 kPa (16 kPa-O_2 + 20 kPa-CO_2), and 1.0/20.0 O_2/CO_2 kPa (1 kPa-O_2 + 20 kPa-CO_2). Samples stored in unsealed bags (Air) were used as controls. All samples (3 replicates per treatment) were analyzed at harvest and after 21 days of storage at 5°C for RR, relative water content of peduncles (RWC), and volatile organic compounds (VOCs).

As for artichokes, the heads were trimmed, eliminating the external bracts and cutting the stem. The obtained artichoke hearts were then cut into quarters and dipped for 5 min in a solution of 1% ascorbic acid + 0.2% citric acid (w:v), drained, and randomly selected for different treatments. In particular, three replicates of 16 artichoke quarters were kept for the initial determinations, while the remaining quarters were closed in 50 x 50 polypropylene (PP) bags (Carton Pack® Rutigliano, Italy), about 600 g per bag, applying 4 MA mixtures with different initial CO_2 concentrations plus 10 kPa of O_2: 10.0/10.0 O_2/CO_2 kPa (CO_2-10kPa), 10.0/20.0 O_2/CO_2 kPa (CO_2-20kPa), 10.0/30.0 O_2/CO_2 kPa (CO_2-30 kPa), 10.0/40.0 O_2/CO_2 kPa (CO_2-40 kPa). Unsealed bags were used as control (Air). After 48 h of storage at 0°C, all bags were opened, artichoke quarters were placed in open polyethylene bags and analyzed after 48 h at 0°C plus 7 days of storage at 5°C for RR, browning index (BI) and VOCs profile. The headspace gas composition (O_2 and CO_2) within each MA package was monitored daily using a gas analyzer (CheckPoint, PBI Dansensor, Ringsted, Denmark). RR was measured initially (Fresh) and at the end of the storage for each product using a closed system, as reported by Kader (2002 a). Samples were put into 6 L sealed plastic jars, allowing the accumulation of CO_2 up to 0.1 kPa. For CO_2 analysis, 1 mL of gas sample was collected from the headspace of each jar and injected into a gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA, USA) equipped with dual columns and 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. RR was expressed as mL CO_2 kg^{-1} h^{-1}. In table grapes, rB was scored on a rating scale from 1 to 5 (1= absence, 2= light; 3= moderate; 4= severe; 5= extreme) as reported by Lichter *et al*. (2011), whereas for acetaldehyde and ethanol analysis, the procedure reported by Cefola *et al*. (2018) was used.

In sweet cherries, the RWC of peduncles was calculated in percentage, as reported by Cefola *et al*. (2018), while the VOCs analysis was carried out, as reported by Cozzolino *et al*. (2019).

In fresh-cut artichokes, BI and VOCs were evaluated, as reported by Capotorto *et al*. (2020).

3. Results and Discussion

Starting from the gas composition inside MA packages described above for each product, during storage the concentrations of O_2 and CO_2 changed due to the respiration of the products and gas permeation through packaging material, and the final gases composition were reported in Table 1.

As for table grapes, O_2 concentrations decreased from 1 kPa to about 0.2 kPa or 0.3 kPa, in 1k Pa-O_2 and in 1 kPa-O_2 + 20 kPa-CO_2 respectively, while CO_2 concentrations increased from 0.03 kPa to roughly 10 kPa in 1 kPa-O_2 packages, and from 20 kPa to about 30 kPa in 1k Pa-O_2 + 20 kPa-CO_2 MA.

For sweet cherries, in 16 kPa-O_2 + 20 kPa-CO_2 bags, the O_2 concentration gradually decreased, reaching the mean value of about 1 kPa after 21 days of storage. In 1 kPa-O_2 and 1 kPa-O_2 + 20 kPa-CO_2 samples, the initial O_2 concentration remained unchanged during the storage. On the other hand, the amount of CO_2 increased during conservation, reaching the final mean values of 25.7 kPa, 45.3 kPa and 42.4 kPa in 1 kPa-O_2, 16 kPa-O_2 + 20 kPa-CO_2 and 1 kPa-O_2 + 20 kPa-CO_2 packages, respectively. Finally, as for fresh-cut artichokes, no significant changes in gas composition inside bags were observed.

Results on table grapes are reported in Table 2. In the Fresh samples the RR measured was equal to 4.2 (± 0.4) mL CO_2 kg^{-1} h^{-1}; after 20 days of the storage, a reduction in RR was measured in air samples (3.0 ± 0.2 mL CO_2 kg^{-1} h^{-1}), while it remained almost constant in table grapes samples treated with 1 KPa O_2 (4.7 ± 0.6 mL CO_2 kg^{-1} h^{-1}). On the contrary the use of high CO_2 concentrations (>20 kPa) in the MA mixture (1 kPa-O_2 + 20 kPa-CO_2) increased the value of RR resulting more than a 2-fold higher than Fresh sample. Significant differences were observed, for sample stored in air and 1 kPa-O_2 + 20 kPa-CO_2 (Table 2). As shown in Table 1, browning of the table grapes rachis was found in all treatments after 20 days of storage. However, higher browning was observed in air and in 1 kPa-O_2 + 20 kPa-CO_2 samples, whereas the use of 1 kPa-O_2 was able to keep a light to moderate browning of the rachis (mean value of 2.5). After storage ethanol and acetaldehyde concentrations did not change from their initial values (4.2 and 0.6 mg L^{-1}, respectively) in the Air samples, whereas they significantly increased in table grapes exposed to low (<10 kPa) or high CO_2 (>20 kPa) concentrations (Table 2). Moreover, samples exposed to 1 kPa-O_2 + 20 kPa-CO_2 showed higher accumulations of ethanol and acetaldehyde than table grapes packed in 1 kPa-O_2.

Table 1 - Initial and final concentration of O_2 and CO_2 for each treatment on table grape

Treatment	Initial kPa		Final kPa	
	O_2	CO_2	O_2	CO_2
1 kPa-O_2	1.0	0.04	0.2	10.0
1 kPa-O_2 + 20 kPa-CO_2	1.0	20.0	0.3	30.0
Air	20.0	0.03	20.0	0.03

Table 2 - Effect of CO_2 treatments on respiration rate, rachis browning, ethanol and acetaldehyde contents of table grapes (*Vitis vinifera* cv. Italia) after 20 days of storage at 5°C

Treatment	Respiration rate (mL CO_2 kg^{-1} h^{-1})	Rachis browning (1-5)	Ethanol (mg L^{-1})	Acetaldhyde
Fresh	4.2 b	1.0 c	4.2 c	0.6 c
1 kPa-O_2	4.7 b	2.5 b	2142 b	8.9 b
1 kPa-O_2 + 20kPa-CO_2	9.0 a	3.6 a	3606 a	17.6 a
Air	3.0 c	4.4 a	5.8 c	0.6 c

Mean values followed by different uppercase and lowercase letters indicate significant differences between fresh and treated sample, and within treatments at day 20, respectively, according to LSD test (P≤0.05).

Sweet cherries (Table 3) showed an initial respiration rate of 8.2 (± 0.3) mL CO_2 kg^{-1} h^{-1} which increased 1.5 fold in air and more than 5 times in the other MA treatments. The highest RR was observed in 1 kPa-O_2 + 20 kPa-CO_2 (48.9 ± 0.7 mL CO_2 kg^{-1} h^{-1}) followed by 16 kPa-O_2 + 20 kPa-CO_2 (44.4 ± 0.6 mL CO_2 kg^{-1} h^{-1}) and 1 kPa-O_2 (43.2 ± 0.1 mL CO_2 kg^{-1} h^{-1}).

The RWC % of peduncle increased in all MA treatments, maybe due to the high relative humidity inside the packages. The highest RWC % values were observed in low O_2 treatments, probably thanks to the lower respiration rate of these samples.

Among VOCs analysed, 1-pentanol, marker of sensory alteration, was closely associated with negative aroma intensity which resulted directly described as pungent, and fermented flavour. Whereas the reduction of hexenal and 2-hexenal were indicators of lost in freshness (Cozzolino *et al*., 2019).

As reported in Table 2, 1-pentanol was not detected in Fresh and Air samples, while a significant increase of this alcohol was observed in the other MA treatments. Samples treated with 1 kPa-O_2 + 20 kPa-CO_2 had the highest value of 1-pentanol, while 1 kPa-O_2 and 16 kPa-O_2 + 20kPa-CO_2 MA treatments had similar values (Table 2). As for hexanal, (Table 3) statistical analysis did not show significant changes after 21 days at 5°C excepted for the treatment, 1 kPa-O_2 + 20 kPA-CO_2 which showed a reduction with respect to the fresh sample. In contrast, 2-hexenal decreased significantly during storage, but no differences in its concentration were observed when comparing MA treatments (Table 3).

Results of fresh-cut artichokes are reported in Table 4. RR of the fresh sample was 120.8 (± 0.2) mL CO_2 kg^{-1} h^{-1}. After the short-term CO_2 treatments (48 h, 0°C) and 7 days of storage at 5°C, RR decreased significantly in all samples, except in artichokes treated with 40 kPa of CO_2. The lowest RR was detected in fresh-cut artichokes treated with CO_2-10 kPa (44.5 ± 4.3 mL CO_2 kg^{-1} h^{-1}), followed by CO_2-20 kPa and CO_2-30 kPa, which reported similar values (68.1 ± 1.1 and 63.9 ± 5.2 mL CO_2 kg^{-1} h^{-1}, respectively), and Air (93.6

Table 3 - Effect of CO_2 treatments on respiration rate, relative water content (RWC) of peduncles, 1-pentanol, hexanal and 2-hexenal relative peak area (RPA) contents of sweet cherries (*Prunus avium* cv. Ferrovia) after 21 days of storage at 5°C

Treatment	Respiration rate (mL CO_2 kg^{-1} h^{-1})	Relative water content of peduncles (%)	1-Pentanol relative peak area (%)	Hexanal	2-Hexenal
Fresh	8.2 B	53.9 B	0.0 B	112.3 NS	366.6 A
1kPa-O_2	43.2 Ac	65.4 Aa	6.0 Ab	47.3 NS a	118.0 B NS
16kPa-O_2 + 20kPa-CO_2	44.4 Ab	58 Ab	7.6 Ab	26.4 NS ab	99.9 B NS
1kPa-O_2 + 20kPa-CO_2	48.9 Aa	60.5 Ab	18.6 Aa	14.3 Bb	55.7 B NS
Air	12.1 Ad	53.6 NS c	0.0 NS c	40.5 NS ab	125.2 B NS

Mean values followed by different uppercase and lowercase letters indicate significant differences between fresh and treated sample, and within treatments at day 21, respectively, according to LSD test (P≤0.05).

Table 4 - Effect of short-term CO_2 treatments on respiration rate, browning index, ethanol and hexanal relative peak area (RPA) contents of fresh-cut artichokes (*Cynara cardunculus* cv. Violet de Provence) after 7 days of storage at 5°C

Treatment	Respiration rate (mL CO_2 kg^{-1} h^{-1})	Browning index	Ethanol relative peak area (%)	Hexanal
Fresh	120.8 A	0.0 B	34.5 NS	41.7 NS
CO_2-10 kPa	44.5 Bd	131 Ad	114.0 NS c	5.4 NS a
CO_2-20 kPa	68.1 Bc	137 Abc	379.1 Ab	5.0 NS a
CO_2-30 kPa	63.9 Bc	135 Acd	482.5 Ab	4.3 NS a
CO_2-40 kPa	121.5 Aa	140 Ab	363.5 Ab	2.1 Bb
Air	93.6 Bb	152 Aa	886.3 Aa	3.8 NS a

Mean values followed by different uppercase and lowercase letters indicate significant differences between fresh and treated sample, and within treatments at day 7, respectively, according to LSD test (P≤0.05).

± 1.7 mL CO_2 kg^{-1} h^{-1}), while the highest RR was observed in CO_2-40 kPa (121.5 ± 0.2 mL CO_2 kg^{-1} h^{-1}). As expected, all samples developed browning after 7 days of storage, regardless of treatment. However, differences in the severity of browning were observed when comparing treatments at the end of the storage: the application of short-term CO_2 treatments (from 10 kPa to 40 kPa) significantly reduced the incidence of browning compared to Air samples and, among the CO_2 treatments, CO_2-10 kPa had the lowest browning index (Table 4). Considering all the VOCs identified by HS-SPME/GC-MS analysis, ethanol and hexenal were, respectively, the most representative compounds of negative and positive aspects of fresh-cut artichokes (Capotorto *et al*., 2020).

As shown in Table 4, ethanol significantly increased during storage, except for the CO_2-10kPa treatment, where its concentration was similar to that of fresh samples. The highest ethanol concentration was found in Air samples, followed by treatments added with CO_2 at 20, 30, and 40 kPa that showed similar values. As for hexanal, it was significantly lower only in fresh-cut artichokes treated with CO_2-40 kPa (Table 4).

For table grapes, data related to ethanol and acetaldehyde, together with the RR results, indicate that high CO_2 concentrations (>20 kPa) on this commodity may cause physiological injury and the induction of anaerobic metabolism.

The present results are supported by similar findings on the effect of high CO_2 concentrations (>20 kPa) on table grapes by Cefola and Pace (2016). High CO_2 concentrations also negatively influence the acceptability of table grapes by consumers: rachis browning, is, in fact, the main issue that limits the acceptability of table grapes by consumers (Cefola *et al*., 2018). A similar effect of high CO_2 concentrations (>20 kPa) on the acceleration of rachis browning was previously observed on table grapes (Crisosto *et al*., 2002; Deng *et al*., 2006) and is a consequence of the stress induced by exposure to high CO_2 concentrations (Crisosto *et al*., 2002; Liguori *et al*., 2015).

For sweet cherries, considering that the highest RR was observed when MA with 20kPa CO_2 was applied, these results indicate that this CO_2 concentration, especially when associated with low oxygen, can cause stress, as confirmed by VOC analysis. Similar behaviour in the production of C5 volatiles, such as 1-pentanol, was previously observed (Contreras *et al*., 2017; Mastrandrea *et al*., 2017), and it seems to be favoured under low O_2 and high CO_2 atmospheres. The present results on the cv. Ferrovia are in contrast with previous results on other sweet cherries cultivars (Kader *et al*., 1989; Esturk *et al*., 2012), but those cultivars have better tolerance to high CO_2.

It has been stated that the physiological susceptibility of commodities to high CO_2 can be cultivar-dependent, and is generally seen with vegetables and other fruit (Watkins, 2000).

Results on fresh-cut artichokes indicate that the application of high CO_2 concentrations (around 40 kPa) has a negative effect on the shelf-life.

Similar results on the detrimental effect of high CO_2 were previously observed on fresh-cut artichokes during storage (La Zazzera *et al*., 2012, 2015). As observed for table grapes, sweet cherries, and fresh-cut artichokes, the exposure to elevated CO_2 atmospheres can stimulate respiration and ethylene production rates, indicating a stress response (Kader, 2002 a). These increases in respiration might be related to the inhibition by high CO_2 of several enzymes of the Krebs cycle, including succinate dehydrogenase, which triggers anaerobic respiration or causes the accumulation of succinic acid, which is potentially toxic to cells (Kays, 1991; Varoquaux, 1991; Kader, 2002 b). Furthermore, for fresh-cut artichokes, the intolerance to high CO_2 concentration and mechanical wounding enhances a different array of enzymatic pathways, many of which are associated with volatile accumulation, which lead to development of off-flavors (Salunkhe *et al*., 1976; La Zazzera *et al*., 2015).

4. Conclusions

For table grapes, the storage in high CO_2 (>20 kPa) caused a severe increase in respiration rate, ethanol and acetaldehyde accumulation, and a decline in sensory quality due to the rachis browning, all probably consequences of the induction of the anaerobic metabolism. The application of CO_2 up to 10kPa was able, instead, to keep the good quality table grapes during storage.

Sweet cherry (cv. Ferrovia) is very sensitive to high CO_2 when it is applied together with low oxygen in MA, as indicated by responses in respiration rate, relative water content of the peduncles, and VOC emissions, with some of these responses being considered positive and some negative in relation to quality.

Short-term treatment with high CO_2 (around 40 kPa) caused an increase in respiration rate and the induction of fermentative metabolism in fresh-cut artichoke. The application of CO_2 concentrations up to 10 kPa reduced respiration rate and tissue browning during storage in air at 5°C and preserved the fresh VOC profile. Application of short-term CO_2 might be a promising postharvest treatment to preserve the quality and the volatile profile of fresh-cut artichokes during storage.

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Non-destructive determination of ripening in melon fruit using time-resolved spectroscopy

M. Vanoli [1 (*)], G. Cortellino [1], V. Picchi [1], M. Buccheri [1], M. Grassi [1], F. Lovati [1], L. Marinoni [2], P. Levoni [2], A. Torricelli [2, 3], L. Spinelli [3]

[1] *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca Ingegneria e Trasformazioni Agroalimentari (CREA-IT), Via G. Venezian, 26, 20123 Milano, Italy.*

[2] *Politecnico di Milano, Dipartimento di Fisica, Piazza Leonardo da Vinci, 32, 20133 Milano, Italy.*

[3] *Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche (IFN-CNR), Piazza Leonardo da Vinci, 32, 20133 Milano, Italy.*

Key words: Carotenoids, *Cucumis melo* L., ethylene, juiciness, quality, TRS.

Abstract: **The aim of this work was to explore the feasibility of time-resolved reflectance spectroscopy (TRS) in determining the ripening degree and the quality of orange-fleshed melons. Sixty 'Honey Moon' melons were measured by TRS in the 540-1064 nm range and classified as less (LeM), medium (MeM), and more (MoM) mature according to increasing values of μ_a540. MoM fruit showed yellower peel color, slightly more orange pulp, higher juiciness and higher carotenoid contents than LeM ones. MoM fruit also showed higher internal ethylene concentration and lower firmness than LeM ones, even if the differences were not significant. The μ_a540 was positively related to internal ethylene, carotenoid accumulation, and juiciness, indicating that μ_a540 was linked to different ripening processes in melons. However, the relationship between μ_a540 and total carotenoid content was not as high as expected due to the low variability of pulp color and of carotenoid content. Changes in flesh color toward a more orange shade were accompanied by increased juiciness and ethylene production and by carotenoid accumulation, while changes in peel color were associated with changes in flesh firmness and juiciness. In conclusion, the absorption coefficient measured at 540 nm (μ_a540) by TRS could be used to sort melons in different ripening degrees; however, its applicability will need to be evaluated on a larger number of fruits and on other varieties.**

[(*)] Corresponding author:
maristella.vanoli@crea.gov.it

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1. Introduction

Melon (*Cucumis melo* L.) fruit are particularly appreciated by consumers for their sweetness, flavour, texture, and attractive flesh color, as well as for their nutritional and phytochemical properties. Melons are a good source of carotenoids, ascorbic acid, and phenolic compounds (Gómez-García *et al*., 2020; Manchali *et al*., 2021; Singh *et al*., 2022). Melon quality depends on the balance between sugars, organic acids, volatiles, and texture characteristics (Kyriacou *et al*., 2018). These traits are strongly affected by genotype, agro-climate conditions, harvest matu-

rity, post-harvest handling, and storage conditions (Kyriacou *et al*., 2018). Melons picked at the optimal harvest maturity have premium quality as maturity at harvest has a large impact on sugars, volatiles, and texture. During ripening, rind color changes from green to yellow, orange, or creamy yellow, depending on melon genotype; mesocarp color turns from pale green to orange in orange-fleshed melons, along with carotenoid accumulation; firmness and acidity decrease, while sucrose as well as soluble solids, vitamin C and pH markedly increase (Beaulieu and Lea, 2007; Tristan *et al*., 2022). Melons harvested mature develop the strongest flavour, whereas fruit harvested early develop a less aromatic flavour: esters are predominant in ripe melons, aldehydes are present at higher concentration in immature fruit while a sharp increase of alcohols is typical of overripe melons (Senesi *et al*., 2005; Beaulieu and Lea, 2007; Vallone *et al*., 2013; Lignou *et al*., 2014). During ripening, the sensory scores for color intensity, fruity aroma, juiciness, and sweetness increased while the sensory scores for firmness and sourness generally decreased (Vallone *et al*., 2013). In climacteric melons, ethylene was produced at higher levels in fully ripe fruits and was negatively related to firmness and positively related to sensory juiciness and fruity flavour and aroma (Senesi *et al*., 2005; Vallone *et al*., 2013).

For more than two decades, several efforts have been made to measure quality characteristics of melons in a nondestructive way (Zeb *et al*., 2021). In industrial fruit sorting, accuracy, cost, and detection speed are important factors. Thus, spectroscopic techniques being fast, simple, and cost-effective, have been widely studied. In melons, Vis-NIR spectroscopy (Sanchez *et al*., 2014; Lu *et al*., 2015; Khurnpoon and Sirisomboon, 2018; Zeb *et al*., 2021), computer vision (Calixto *et al*., 2022) and hyperspectral imaging (Sun *et al*., 2017; Cho *et al*., 2022) were studied with less or more successful results for predicting soluble solids, moisture, pulp color, and firmness and for classifying fruit according to different sweetness degree or as suitable or not for harvesting. Among spectroscopic techniques, time-resolved reflectance spectroscopy (TRS) is gaining increasing interest in assessing fruit quality. Due to its accuracy in measuring optical properties in deep tissues it allows the evaluation of maturity and internal defects of horticultural products with a relatively thick surface layer (Lu *et al*., 2020). TRS allows the complete optical characterization of a diffusive medium through the measurements of the absorption (μ_a) and of the scattering (μ_s) coefficients by probing flesh at a depth of 1-2 cm with no or limited influence from the skin (Cubeddu *et al*., 2001; Rizzolo and Vanoli, 2016). While scattering is related to the structural properties of fruits and vegetables, absorption depends on the chemical composition of the tissue, mainly on the presence of pigments such as chlorophylls, anthocyanins, and carotenoids (Lu *et al*., 2020). TRS has been mainly applied in post-harvest studies on fruit and vegetables for estimating fruit ripening, for the detection of internal defects, and for discriminating fruit with different texture and sensory characteristics (Rizzolo and Vanoli, 2016). The absorption coefficient measured at 670 nm (μ_a670) at harvest was shown to be a maturity index for various fruit, such as peaches, nectarines, plums, pears, and apples, as it declines with fruit ripening (Vanoli and Buccheri, 2012). The μ_a540 (carotenoid tail) has been used as a non-destructive maturity index for mangoes, as it was able to classify intact mangoes of different cultivars according to pulp color and to the contents of total and individual carotenoids (Rizzolo and Vanoli, 2016; Vanoli *et al*., 2018).

TRS has not yet been studied on melons, so the aim of this work was to explore the feasibility of TRS in determining the ripening degree and the quality of orange-fleshed melons.

2. Materials and Methods

Fruit

The experiment was carried out on 'Honey Moon' (*Cucumis melo* L. cantalupensis) melons. 'Honey Moon' fruits have round shape, medium size, a smooth grey-green skin that turns yellow when fully ripe. Fruits has firm, deep orange flesh and show high sugar content and a pleasant and almost exotic aroma. In order to have high variability in fruit maturity, 60 melons were picked and selected in a packinghouse in Sermide (Mantova-Italy) on a peel color basis (15 fruit for four color stages: blue, gray-green, yellow-green and yellow). Then fruit were immediately transported to the CREA-IT lab in Milan, measured by TRS in the 540-1064 nm range and classified as less (LeM), medium (MeM) and more (MoM) mature according to increasing μ_a540 values (low μ_a540=less mature fruit; high μ_a540=more mature fruit). All the 60 melons were also individually evaluated for color (peel and flesh) and for standard matu-

rity indices (flesh firmness, soluble solids content, acidity). Among the 60 melons, 20 fruits covering the whole range of μ_a540 (i.e., the highest, the lowest and 18 intermediate values of μ_a540), were chosen for internal ethylene concentration, total carotenoid content and juiciness analyses.

Time-resolved reflectance spectroscopy (TRS)

Whole melons were measured by TRS on two opposite sides in the fruit equatorial region. A portable compact setup working at discrete wavelengths developed at Politecnico di Milano (Zhao *et al*., 2022) was employed. The light source is a supercontinuum fiber laser (SC450-6W, Fianium, UK) providing white-light pulses, with duration of a few picoseconds. Two custom-made filter wheels loaded with overall 14 band-pass interference filters (MaxLine series, Semrock, NY, USA, and TECHSPEC series, Edmund Optics, New Jersey, USA) are used for spectral selection in the range 540-1064 nm. Light is delivered to the sample by a 200 µm core step-index fiber and collected by a 1 mm core graded-index fiber; interfiber distance was set to 1.5 cm. A pair of filter wheels identical to the previous one is used for cutting off the fluorescence signal originated from the sample when it is illuminated in the visible spectral region. The light then is detected by a customized Silicon PhotoMultiplier module (Martinenghi *et al.*, 2016) and the photon time-of-flight distribution is measured by a time-correlated single-photon counting board (SPC-130, Becker&Hickl, Germany). The instrumental response function has a full width at half maximum of about 100 ps and the typical acquisition time is 1 s per wavelength. A semi-infinite model for photon diffusion in a turbid medium was used to analyze TRS data to assess the bulk optical properties of the samples (Martelli *et al*., 2009) to obtain the estimates of μ_a and μ_s at each wavelength.

Peel and flesh color

Color was measured on the equatorial region on two opposite side of whole fruit and, after cutting longitudinally the melons, on two opposite portions of the flesh, 1.5 cm from the fruit edge. A CM2600D spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) with the primary illuminant D65 and 2° observer, was used to perform the color measurements in the L^* (lightness) a^* (green-red), b^* (yellow-blue) color space. From a^* and b^* values, hue ($h°$) and chroma (C^*) were computed according to $h° = \text{arctangent}(b^*/a^*) \times 360/(2\times3.14)$ and $C^* = (a^{*2} + b^{*2})^{-2}$. In the flesh also the color spectra (350-740 nm) were considered. Color readings were averaged for each fruit.

Standard maturity indices and juiciness

Flesh firmness

After having cut the melons into two parts along the longitudinal axis, flesh firmness was measured on two opposite sides in the equatorial part of the fruit (around 1,5 cm from the fruit edge) using an 8 mm diameter plunger mounted on a TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) at a crosshead speed of 200 mm min^{-1}. The two measurements were averaged for each fruit.

Soluble solids content (SSC) and titratable acidity

Two longitudinal slices around 4 cm thick, taken from two opposite sides of each fruit (in the same places of the firmness measurements) were frozen for the evaluation of soluble solids content (SSC) and of titratable acidity. After thawing, SSC was determined on few juice drops from each slice by using an automatic refractometer (RFM81, Bellingham-Stanley Ltd., England). Titratable acidity (TA) was measured by titrating 10 g of juice plus 50 mL of distilled water with 0.1 N NaOH to pH 8.

Juiciness

Juiciness was measured on pulp cylinders (diameter = 15 mm; height = 10 mm) taken from two opposite sides of the equatorial part of the fruit. Each cylinder was compressed between two plates with a TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) at a deformation rate of 100 mm min^{-1} by a compression of 50% of the original height of the cylinder (Eccher Zerbini *et al*., 1999). This method correlated with sensory analysis, and only the juice that could be easily and quickly released by the pulp cylinder was measured. Juiciness was calculated as the ratio between the juice weight spilled out after compression and the cylinder's original weight.

Internal ethylene concentration

1 mL sample of internal gas was taken from the seed cavity of each melon using a syringe equipped with a 15 cm long, 15-gauge needle. The sample was injected in a DANI GS 86.10 gas chromatograph and analyzed for the ethylene content following the conditions reported by Rizzolo *et al*. (2005), using a deactivated aluminum oxide F1 (80-100 mesh) column (1/8 in × 200 cm Alltech Italia, Sedriano, Italy), column temperature 100°C, injection temperature 100°C, and a flame ionization detector temperature

of 225°C. Ethylene was identified and quantified by relating the peak area of the sample to that of a 10 μL L^{-1} external standard and was expressed as ppm.

Total carotenoid content

Two slices per fruit were frozen for total carotenoid content analysis. 1 g of frozen sample was rapidly homogenized (Ultra-Turrax, IKA-Werk, Germany) in 5 ml of an ice-cold solution of hexane: acetone:ethylacetate (2:1:1) containing 1% of 1% butylated hydroxitoluene (BHT) in methanol. The homogenate was sonicated for 10 min, centrifuged at 10000 *g* for 15 min at 4°C and the organic phase was collected. The absorbance of the organic phase, after proper dilution, was measured spectrophotometrically at 450 nm. Total carotenoid content was estimated using the molar extinction coefficient for b-carotene in hexane (139200 l mol^{-1} cm^{-1}), as reported in Craft and Soares (1992).

Statistical analysis

Data were submitted to analysis of variance (Statgraphics ver. 7, Manugistic Inc., Rockville, MD, USA) considering TRS maturity class as a source of variation, and means were compared by Tukey's test at P≤0.05%. The relationships between μ_a540 and quality characteristics and the relationships among quality parameters were studied using regression analysis. For each parameter, the model with the higher performance was considered. Only the models with correlation coefficient r>0.50 were considered.

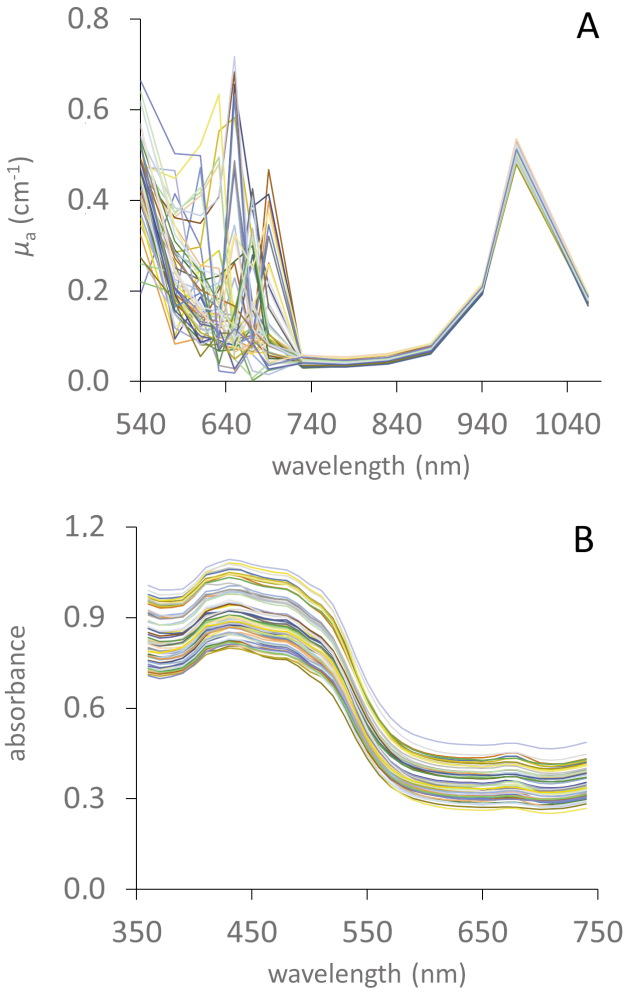

Fig. 1 - TRS absorption spectra (A) and pulp color spectra (B) of 'Honey Moon' melons.

3. Results

TRS absorption spectra

TRS absorption spectra of 'Honey Moon' melons (Fig. 1A) showed a maximum at 980 nm (corresponding to water), high absorption values at 540 nm (corresponding to the tail of carotenoids) and very high variability in the chlorophyll absorption range (610-690 nm).

The variability in the chlorophyll absorption was not linked to the presence of chlorophyll in the pulp but depended on the presence of a green layer (about 3.5-7 mm) between the rind and the pulp, which interfere with TRS measurements that pass through the pulp, up to 2 cm. The color spectra of the pulp confirmed the TRS data, showing a very low absorption in the chlorophyll range and a very high absorption in the carotenoid range (400-500 nm) (Fig. 1B). Then, the absorption coefficient measured at 540 nm (μ_a540) was chosen as a possible ripening index for melons due to its correlation to the carotenoid content in the pulp, as previously found by Rizzolo and Vanoli (2016) and Vanoli *et al*. (2018) in mangoes. The μ_a540 ranged from 0.194 to 0.663 cm^{-1}. Melons were ranked according to increasing μ_a540 (increasing maturity) and sorted into three maturity classes: low (μ_a540 = 0.383±0.008 cm^{-1}), medium (μ_a540 = 0.451±0.004 cm^{-1}) and more mature (μ_a540 = 0.,537±0.014 cm^{-1}).

Peel and flesh color

Peel and pulp color significantly changed with TRS maturity classes (Table 1). Peel color turned from green to yellow-green with advancing TRS maturity class, as b^* and C^* values increased and $h°$ values decreased from LeM to MoM melons. Pulp color became slightly more orange, as b^* and $h°$ were lower in MoM fruit than in LeM ones. There was no

Table 1 - Peel and pulp color parameters of 'Honey Moon' melons in relation to TRS maturity classes

Maturity stage	*L**	*a**	*b**	*C**	*h°*
Peel					
Less mature	72.4 a	-6.1 a	23.3 b	24.2 b	106.5 a
Medium mature	73.4 a	-5.4 a	26.0 ab	26.7 ab	103.4 ab
More mature	76.0 a	-5.4 a	28.8 a	29.4 a	101.2 b
Pulp					
Less mature	62.1 a	14.0 a	33.9 ab	36.7 a	67.6 a
Medium mature	63.2 a	14.4 a	34.7 a	37.6 a	67.5 a
More mature	60.0 a	14.4 a	33.3 b	36.3 a	66.6 b

Means in the same column followed by different letters are statistically different at P≤0.05 (Tukey's test).

main change in the pulp color of melons, as *b** ranged from 29.3 to 38.1 and *h°* from 64.5 to 70.2.

Standard maturity indices, juiciness, internal ethylene and total carotenoids content

Flesh firmness ranged from 7.09 to 27.55 N, SSC from 7.4 to 12.2% and acidity from 1.70 to 5.57 g l^{-1} citric acid. Flesh firmness, SSC and acidity were not significantly affected by TRS maturity classes (Fig. 2 A, B, C). However, firmness and acidity were lower and SSC was higher in MoM fruit than in LeM ones. Juiciness ranged from 6.86 to 22.27% and was significantly higher in MoM fruit than in the LeM ones (Fig. 2 D). Internal ethylene concentration ranged from 51.9 to 153.3 ppm. It did not significantly change with TRS maturity class, even if the LeM fruit showed the lowest concentration and MoM ones the highest (Fig. 2E). Total carotenoids content ranged from 16.95 to 28.25 mg Kg^{-1} fw; it was significantly higher in MoM fruit than in LeM ones (Fig. 2F).

Regression analysis

The results of regression analysis between μ_a540 and quality parameters are summarized in Table 2. The μ_a540 was significantly related to juiciness (r=0.53), internal ethylene concentration (r=-0.86) and total carotenoid content (r=0.66). The μ_a540 was also positively related to skin color (except for *a**)

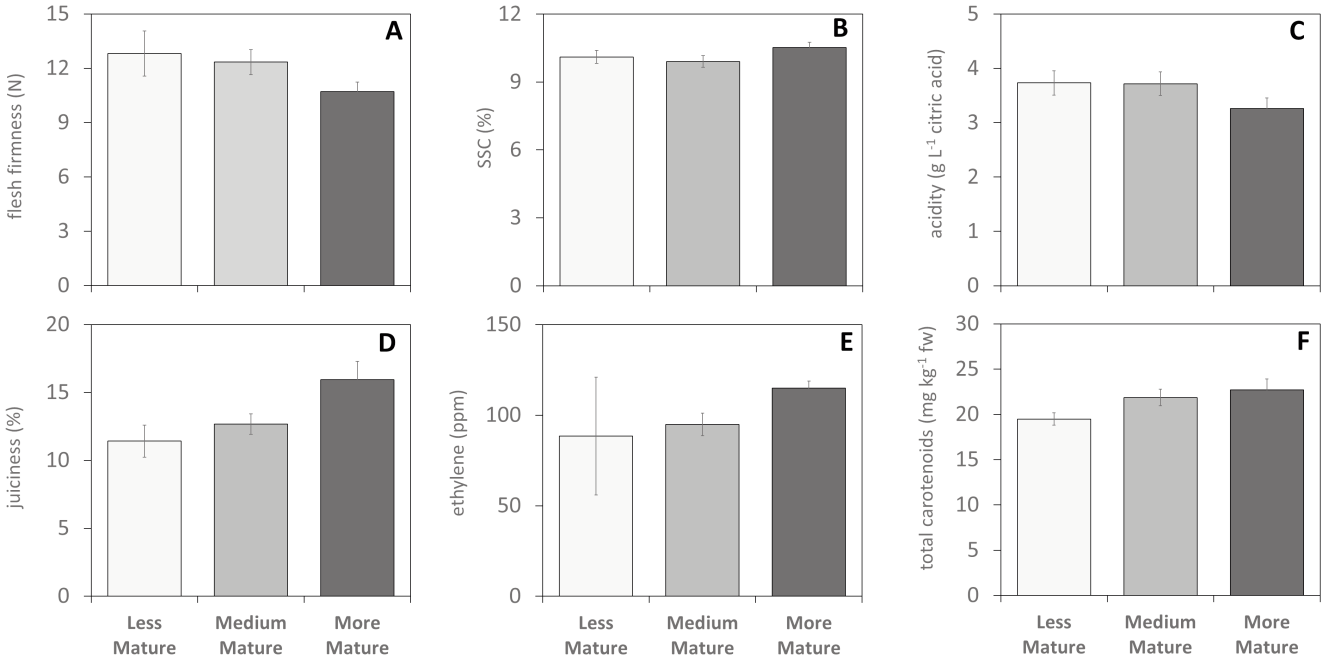

Fig. 2 - Flesh firmness (A), soluble solids content (B), acidity (C), juiciness (D), internal ethylene concentration (E) and total carotenoid content (F) in 'Honey Moon' melons in relation to TRS maturity classes. Bars refer to SE.

Table 2 - Regression models between μ_a540, juiciness, internal ethylene concentration and total carotenoid contents

	r	P	Model type
Juiciness	0.531	*	DR
Internal ethylene	-0.864	***	RX
Total carotenoids	0.664	**	DR

For each significant regression, the following data are given: r= correlation coefficient; P=significance of the model (***, P<0.001; **, P<0.01; *, P<0.05), and model type.
DR= double reciprocal; RX=reciprocal X.

Table 3 - Regression models between firmness, juiciness, internal ethylene concentration, total carotenoid content and pulp color

	r	P	MT
Firmness			
*b** peel	0.616	***	RY
*C** peel	0.604	***	RY
h° peel	0.576	***	LIN
Juiciness			
firmness	0.733	***	RX
*b** peel	0.565	*	DR
*C** peel	0.548	*	DR
h° peel	0.538	*	RX
h° pulp	0.577	*	RX
Ethylene			
*a** pulp	-0.617	*	RY
Carotenoids			
juiciness	0.640	*	DR
*a** pulp	0.679	**	DR
h° pulp	-0.881	***	LIN

For each significant regression, the following data are given: r= correlation coefficient; P=significance of the model (***= P<0.001; **= P<0.01; *= P<0.05) and MT= model type.
RY=reciprocal Y; LIN=linear; RX=reciprocal-X; DR=double reciprocal.

with r<0.5. No significant correlation was found between μ_a540 and pulp color, flesh firmness, SSC and acidity.

Significant relationships were also found among peel color, firmness and juiciness (Table 3). Firmness was related to *b** peel (r=0.62), *C** peel (r=0.60) and to *h*° peel (r=0.58); juiciness was related to *b** peel (r=0.57), *C** peel (r= 0.55) and to *h*° peel (r= 0.54). As for pulp color, total carotenoid content was positively related to *a** pulp (r=0.68) and negatively to *h*° pulp (r= −0.88); internal ethylene was related to *a** pulp (r= −0.61) and juiciness to *h*° pulp (r= 0.58). Significant relationships were also found between juiciness and total carotenoids (r= 0.64) and between juiciness and firmness (r= 0.73).

4. Discussion and Conclusions

'Honey Moon' melons used in this experiment showed pulp color similar to that of fruit of the same cultivar picked at commercial maturity, even if firmness, acidity and SSC had lower values (Cavicchi and Pasotti, 2004). Total carotenoid content showed values typical of orange-fleshed fruit (Saladie *et al*., 2015; Singh *et al*., 2022). Internal ethylene concentration confirms that 'Honey Moon' is a climacteric genotype, as there was an increase in ethylene production with advancing fruit maturity (Senesi *et al*., 2005; Vallone *et al*., 2013; Saladie *et al*., 2015).

TRS measurements of melons showed some problems. In fact, the absorption in the chlorophyll range cannot be used as a maturity index as already done for other fruits, such as apples, pears, peaches and nectarines (Rizzolo and Vanoli, 2016), since chlorophyll was almost absent in the melon pulp while it was present in a layer just under the peel. On the other hand, μ_a540 was able to distinguish MoM melons from LeM ones, as MoM fruit showed yellower peel color, slightly more orange pulp, higher juiciness and higher carotenoid contents, in agreement with Kyriacou *et al*. (2018), Senesi *et al*. (2005) Beaulieu and Lea (2007), Vallone *et al*. (2013), Saladie *et al*. (2015) and Tristan *et al*. (2022). MoM fruit also showed higher internal ethylene concentration and lower firmness than LeM ones, even if the values showed high variability and the differences were not significant. The μ_a540 was also positively related to internal ethylene, carotenoid accumulation and juiciness, indicating that μ_a540 is linked to different ripening processes in melons. However, the relationship between μ_a540 and total carotenoid content was not as high as expected (r=0.66) and as previously found in mangoes when r ranged from 0.78 to 0.91 depending on the cultivar (Vanoli *et al*., 2018). It seems that in 'Honey Moon' melons, the range of μ_a540 (0.194-0.663 cm^{-1}) is quite similar to that of mangoes (0.117-0.835 cm^{-1}), while the variability in total carotenoids

(16.95-28.25 mg Kg^{-1} fw) and in pulp color ($h°$ pulp= 64.5-70.2) was narrower in melons than in mangoes (total carotenoids= 5-56 mg Kg^{-1} fw; $h°$ pulp=71-104). Moreover no correlation was found in melons between μ_a540 and pulp color while in mangoes μ_a540 was negatively related to $h°$ pulp with r= 0.83-0.98 (Vanoli *et al*., 2018). In 'Honey Moon' fruit, with advancing TRS ripening degree, the changes in flesh color toward a more orange shade were accompanied by increased juiciness values and ethylene production and by carotenoid accumulation, while changes in peel color toward a yellow shade were associated with fruit softening.

In conclusion, the absorption coefficient measured at 540 nm (μ_a540) by the TRS technique could be used to sort melons in different ripening degrees; however, its applicability will need to be evaluated on a larger number of fruits and on other varieties.

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Image analysis to predict the maturity index of strawberries

A. Corvino [1], R. Romaniello [2], M. Palumbo [1, 2], I. Ricci [1], M. Cefola [1], S. Pelosi [1], B. Pace [1]

[1] *Institute of Sciences of Food Production, National Research Council (CNR) c/o CS-DAT, Via Michele Protano, 71121 Foggia, Italy.*

[2] *Department of Agriculture, Food and Natural Resources and Engineering, University of Foggia, Via Napoli, 25, 71122 Foggia, Italy.*

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(*) **Corresponding author:**
antonia.corvino@ispa.cnr.it

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Abstract: Traditionally, strawberries are harvested manually when the typical colour of the cultivar does not reach at least 80% of the surface. The focus of this research activity is to develop an automatic system based on image analysis in order to objectively define the optimal harvest time. Strawberries (cv. Sabrosa), with different degrees of maturation, were analyzed in four different harvesting periods and subsequently selected and classified, based on the ripening percentage, in three maturity classes: R0-25, R50-70 and R75-100. Each class of 10 strawberries, evaluated in triplicate, was subjected to image analysis and physiological and qualitative evaluation by measuring the following parameters: respiration rate, pH, total soluble solids content, and titratable acidity. The images, captured by a digital camera, were processed using Matlab® software and all the data found were supported by multivariate analysis. The image processing has made it possible to create an algorithm measuring objectively the percentage and the saturation level of red assigning the fruit to each class. Principal component analysis (PCA) shows that discriminating parameters are the Chroma and the red Area, then used in a Partial Least Square Regression (PLSR) model to predict the TSS/TA ratio with R^2 of 0.7 and 0.6 for calibration and validation set, respectively.

1. Introduction

Strawberries are fruit, belonging to the family of the Rosaceae and genus *Fragaria*. Only at the end of 1600 strawberry is no longer considered an ornamental plant, but rather a fruit to be cultivated and marketed for its delicacy. *Fragaria chiloensis*, coming from Chile, arouses the interest of many farmers for its unusual size differing from other strawberry species. The current strawberry, called *F. ananassa*, comes from the random hybridization, that occurred in the second decade of 1700, of *F. virginiana* (coming from the eastern United States) with *F. chiloensis* (coming from the Chilean coasts of the Pacific) (Angelini, 2010). The

obtained species is consumed and appreciated all over the world for sensorial and nutritional quality. Strawberries are usually consumed as fresh fruit and they represent a healthy food choice for their richness in vitamin C, micronutrients and bioactive compounds, mostly natural antioxidants such as phenols known even for anti-inflammatory action. This fruit, expressing better its potential, needs to be harvested at the right ripening because it is not climacteric. In general ripening influences the appearance, texture, flavour, and aroma due to physiological, biochemical and structural modifications. Strawberry is hand-picked evaluating visually the product when the characteristic colour is reached. As consequence, the mistake of collecting an overmature or immature fruit, by presenting a poor product on the market, is very likely to occur.

Generally, the qualitative parameters of fresh products are determined by destructive analytical techniques which involve a sample preparation phase, time-consuming and can be performed on a limited number of samples, often reducing their representativeness. In addition, the environmental impact, and the contact of the operator with the chemicals should not be overlooked, especially if they are not properly trained and experienced. For these reasons, it is important to consider eco-friendly and objective non-destructive methods that can quickly assess the proper harvest time by evaluating the quality of the product at hand. Numerous studies have investigated various non-destructive techniques and their applicability in the field for the determination of the main qualitative parameters of fruit and vegetables. Image analysis (IA) has proven to be a successful contactless tool in fruit and vegetables quality assessment. This technology captures images in the electromagnetic spectrum and extracts the most discriminating external characteristics (shape, colour and defects) and the next phase of data processing can allow, through predicting models, the estimation of chemical and physical properties of samples (Palumbo *et al.,* 2022). The objectives of this study were (1) to implement a standardized computer vision system to characterize quantitatively colour changes during the ripening of strawberries using the *L**, *a**, *b** colour space, (2) to identify features of interest that can be related with ripening stages, such as colour saturation (Chroma) and Hue angle, (3) to develop a statistical model using selected features to identify the ripening stages of strawberries from samples previously classified by expert visual inspection.

2. Materials and Methods

Plant material

Candonga strawberries (*Fragaria × ananassa Duch.*) var. Sabrosa, which have different degrees of ripeness, were provided by a cooperative company of fresh fruit (APOFRUIT Italia Soc. Coop., Scanzano Jonico, Italy) in four different consecutive harvest times from February to May (one harvest per month) called H1, H2, H3 and H4. Then, they were transported in cold conditions to the Postharvest Laboratory of CNR-ISPA of Foggia to be processed. Fruits were selected by eliminating damaged sample and were grouped into three classes, based on the visual evaluation of colour: R0-25 (from 0 to about 25% of red colour on fruit surface), R50-70 (from 50 to about 70% of red colour on fruit surface) and R75-100 (from 75 to about 100% of red colour on fruit surface) (Fig. 1). Each class, consisting of 10 strawberries, was evaluated in triplicate; each replicate was subjected to IA analysis, physiological (respiration rate) and physical-chemical (pH, total soluble solids and titratable acidity) characterization as below reported.

Computer vision system

Digital Camera AP-3200T-PGE (JAI Ltd., Yokohama, Japan), positioned inside a Photo studio box HPB-60D (HAVOX®, Vendôme, France), was used to image a batch containing 10 strawberries for each replicate. In total, for each class, three replicates were considered, for a total of 30 berries. The camera sensor was an RGB CMOS type, providing a spatial resolution of 3.2 MP at 2 fps and a colour depth of 24 bit/pixel. The lens used was a 12 mm focal length and F1.8 (KOWA Lens mod. LM12NC3 1/2) allowing a field of view (FOV) of (35 × 30 cm). The lighting was supplied by two LED handrails consisting of 20 diodes (HAVOX HPB-60, 5500K, 13,000 100 lumen CRI 93+). A Colour Checker Passport Photo 2 (X-rite Italy srl, Prato Italy)

Fig. 1 - Maturity class selected for the experiment.

with 24 known colour stains was placed in the camera's FOV as a chromatic reference. The images captured by the digital camera were processed using Matlab® R2021b (MathWorks Inc., Natick, MA, USA).

Image segmentation

Each raw image of the strawberry was separated from the background, generating a binary image. In detail, the algorithm processed the raw images by cropping the unnecessary image border and separating the three colour-components: red, green and blue (RGB). The background was thresholded using the R image, since showing the highest contrast between the object of interest (strawberry) and the background. The coarse segmentation of the strawberries was carried out by a threshold method (Gonzalez *et al.,* 2004). On the resulting binary images, a morphological filter was applied to erode the strawberry rim and a flood- filling operation was carried out to overcome the threshold defects. Using this primary mask (binary image), the total area and the red area of each strawberry were calculated to get the percentage of red coverage. In the red area, colour features have been extracted to get information on Chroma and Hue angle needed to correlate them with the analytical data.

Destructive chemical analysis

Titratable acidity (TA) and pH using a semi-automatic titrator/pH meter (PH-Burette 24 - Crison Instrument, Barcelona, Spain), were measured on about 100 g of homogenized strawberries (for each class and replicate) as reported by Cozzolino *et al*. (2021). Similarly, the total soluble solids value (TSS) was determined using a digital refractometer (DBR35-XS Instruments, Carpi, Italy) and results were expressed in °Brix. The maturity index (MI) was calculated as the ratio of TSS and TA for each class (Melgarejo *et al*., 2017).

Respiration rate

The respiration rate (RR) of strawberries was determined at 4°C using a closed system as reported by Kader (2002). Thoroughly, each replicate, about 250 g of product, was put into a 3.6 L sealed plastic container to let CO_2 accumulate up to 0.1% as the CO_2 standard concentration. At regular time intervals CO_2 concentration was monitored until the reference value is reached. A Gas sample (1 mL) was drained from the headspace through a rubber septum and injected into the gas chromatograph (p200 micro-GC-Agilent, Santa Clara, CA, USA) equipped with dual columns and 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 RR was expressed as mL CO_2/kg h.

Statistical analysis

The data obtained were analyzed by multifactor ANOVA for $p \leq 0.05$ to evaluate the effects of the maturity class and harvest time (fixed factors) on pH, total soluble solid, titratable acidity, colour parameters and RR (variables). Parameters affected only by maturity class were subjected to a post-hoc test (Fisher), using Statgraphics (version 18.1.12, Warrenton, VA, USA).

A principal component analysis (PCA) was performed using the software Statistica version 6.0. (Statsoft Inc., Tulsa, OK, USA) (Jolliffe, 2022) with the aim of selecting the parameters able to discriminate the maturity classes. Based on the results obtained a Partial Least Square Regression (PLSR) was applied to develop a predictive method using the Unscrambler 10.0 software (CAMO Software, Oslo, Norway). In detail, 70% of the data was used in the calibration step and the remaining 30% was used to validate the obtained model.

3. Results and Discussion

Among the analytical data, RR and the MI were affected by the interaction of the two factors (maturity class x Harvest time) as reported in figure 2. MI showed all classes were different at first harvest with values of 6.61 (± 1.22), 7.38 (± 0.09) and 9.02 (± 0.84) for R0-25, R50-70 and R75-100, respectively; at the second harvest R75-100 reported higher value than the other samples, and this difference was measured also in the last two harvests (Fig. 2A). A similar trend was observed for the RR (Fig. 2B); in detail, at the first harvest time R0-25, R50-70 and R75-100 reported values of 5.12 (± 0.06), 7.73 (± 0.45) and 12.30 (± 0.45) mL CO_2/kg h, respectively. Then, at the second harvest, the RR increased at values around 10 mL CO_2/kg h for the fruit coming from R0-25 and R50-70 maturity classes, remaining almost constant in the last two harvests. On the other hand, for the full maturity class (R75-100), an increase in RR was found during the harvest time reaching at the last the values of about 20 mL CO_2/Kg h (Fig. 2B). Regarding the classes R50-70 and R75-100, similar results were found by Cozzolino *et al*. (2021) on strawberries (cv.

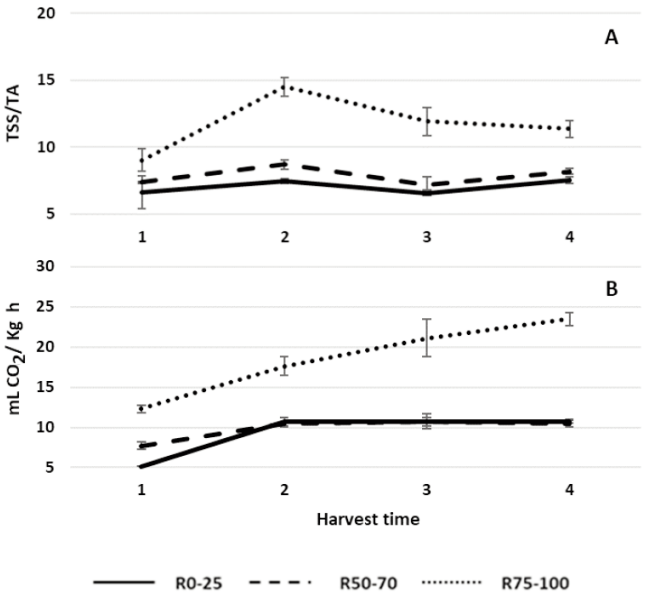

Fig. 2 - Effect of the maturity class and harvest time on maturity index (TSS/TA) (A) and respiration rate (mL CO_2/Kg h) at different harvest time (B).

Sabrosa) collected at two different ripening stages, namely half-red (in ripening phase, fully expanded and 50% red) and red (in ripening phase, fully expanded and 100% red) in three consecutive harvests.

The image processing allowed us to measure the percentage of red (Area red) and the colour parameters Chroma and Hue angle, which enabled the three classes' differentiation as indicated in figure 3.

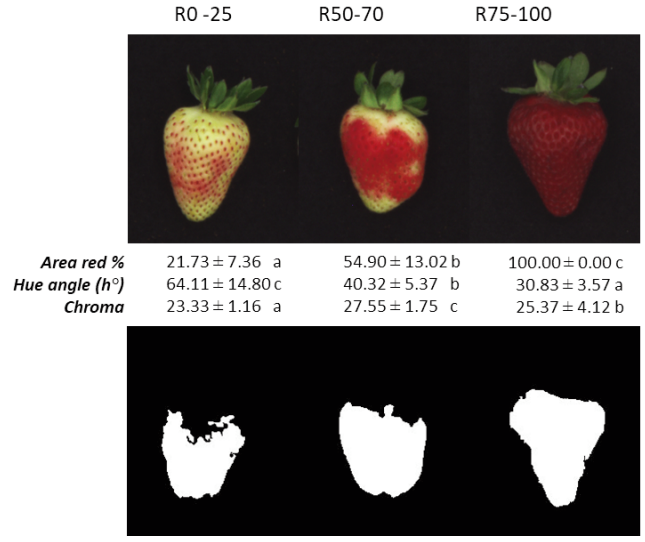

Fig. 3 - Strawberries image, mask, and correlated colour data (Area red , Hue angle and Chroma). Different letters indicate statistically significant differences according to the least significant difference (LSD) Fisher's test.

Since, harvest time affected only two quality parameters, data coming from the different harvests were collected and used for the multivariate analysis (PCA and PLSR). Regarding PCA, also confirming data of ANOVA analysis, Area red and Chroma were able to discriminate the maturity class on the first component, which accounted for 94% of the variability (Fig. 4A). Thus, these two parameters were used as predictors of maturity index TSS/TA, in a resulted PLS model, which showed R^2 of 0.7 in calibration and 0.6 in validation (Fig. 4B).

On the basis of these findings, an algorithm was developed using the Matlab® software, for the objective measurement of the percentage of red (Red Area) and the saturation level (Chroma) of a strawberry starting from the acquired images to automatically and non-destructively attribute the fruit to each class, applying the PLSR model.

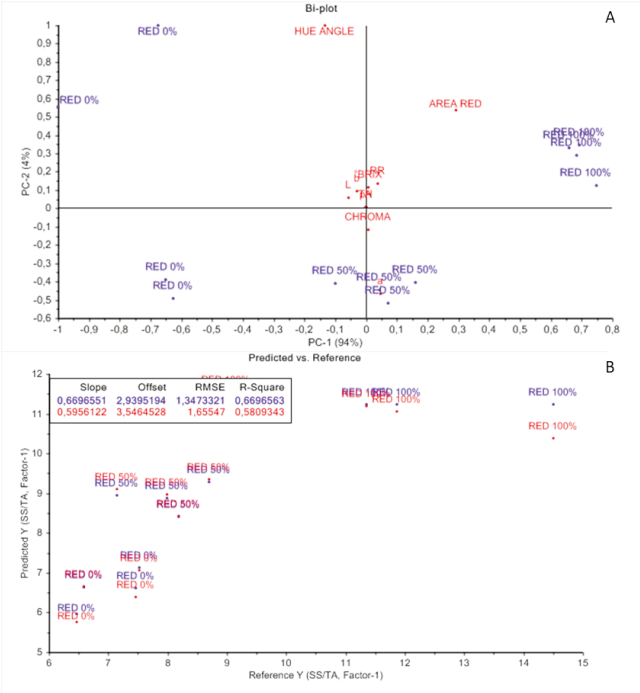

Fig. 4 - Principal Component Analysis (PCA) (A), and Partial Least Square Regression (PLSR) (B) model.

4. Conclusions

Results demonstrated that is possible to predict TSS/TA index starting by colour parameters extracted by IA on strawberry (cv. Sabrosa), collected in four consecutive harvests. The performance of the predictive model obtained might be improved by increasing the number of samples and extending the analysis

also to other cultivars trying to build an algorithm available for handheld devices used in the field or in general for applications available to consumers to consciously buy the product.

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Ethanol fermentation- and ethylene physiology-related gene expression profiles in Red Delicious apples stored under variable hypoxic conditions and protocols

E. Salamé [1], S. Brizzolara [1] (*), M. Rodrigues [2], M. Iob [3], P. Tonutti [1], B. Ruperti [2]

[1] *Crop Science Research Center, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà, 33, 56127 Pisa, Italy.*

[2] *Department of Agronomy, Food, Natural Resources, Animals and Environment, Via dell'Università, 16, 35020 Legnaro (PD), Italy.*

[3] *Marvil Engeneering, Zona Produttiva Schwemm, 8, 39040 Magré Sulla Strada del Vino (BZ), Italy.*

(*) **Corresponding author:**
stefano.brizzolara@santannapisa.it

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Abstract: Dynamic Controlled Atmosphere (DCA) is beneficial in maintaining specific quality parameters but, due to the extreme oxygen levels applied, can cause adverse effects on the fruit by inducing excessive anaerobic metabolism and the production of off-flavors. The metabolic adaptation and responses of apples (*Malus domestica* Borkh.) cv. Red Delicious to static or dynamic oxygen concentrations (0.3 and 0.8%, with sequential shifts) during cold storage for 7 months were studied by monitoring quality parameters and the expression of genes involved in sugar, fermentative metabolism, and ethylene physiology. Ethanol content reached the highest levels (around 400 mg/kg FW) under 0.3% oxygen concentration and fruit firmness appeared to be reduced in samples accumulating the highest levels of ethanol. The oxygen switch was effective in reducing the ethanol concentrations with timing-dependent variable effects. The expression of fermentative (*alcohol dehydrogenase, lactate dehydrogenase, pyruvate decarboxylase)* and sugar metabolism *(β-amylase; phosphofructokinase*; *sucrose synthase)* genes resulted to be differently affected by the hypoxic conditions imposed, in particular during the early stages of storage. *Sucrose synthase* expression appeared to be highly sensitive to changes in low oxygen concentration. Ethylene biosynthesis (*ACC synthase* and *oxidase*) genes showed marked differences in their expression in relation to the static and dynamic protocols and the hypoxic conditions, as well as six Ethylene Responsive Factors (ERF) genes, some of them possibly involved in the oxygen sensing mechanism operating in fruit tissues.

1. Introduction

Dynamic Controlled Atmosphere (DCA) represents one of the latest technical innovations for the long storage of apples (and few other fruit crops) (Tonutti, 2015). With this technology, fruits are kept under extremely low oxygen concentrations (0.4 kPa or lower) that are beneficial for maintaining specific quality parameters (e.g., flesh firmness, acidity). However, by activating anaerobic metabolism, an accumulation of ethanol takes place. Low concentrations of ethanol are desirable in terms of improving organoleptic traits, reducing the incidence of chilling injuries (e.g., superficial scald) and limiting ethylene biosynthesis (Dixon and Hewett, 2000; Scott *et al*., 2000; Weber *et al*., 2020). Yet, the accumulation of excessive ethanol results in the appearance of off-flavors and physiological disorders (Pedreschi *et al*., 2009). Thus, based on different stress indicators (chlorophyll fluorescence -CF-, respiratory quotient -RQ-, and ethanol concentration), oxygen must be promptly adjusted (increased) to reach "safe" concentrations. The imposed extreme hypoxic conditions induce selective responses of apple tissues starting from the modulation of gene expression involved in particular in primary metabolism and hormone (mainly ethylene) physiology (Cukrov *et al*., 2019). In Granny Smith, one of the apple cultivar most frequently stored in DCA, differential expression of *sucrose synthase (SuSy), alcohol dehydrogenase (ADH)* and pyruvate-related metabolism (*lactate dehydrogenase, LDH, pyruvate decarboxylase, PDC,* and *alanine aminotransferase, AlaAT*) genes was detected when comparing 0.4 with 0.8 kPa oxygen concentration (Cukrov *et al*., 2016). When, according to the DCA protocol, oxygen level is increased from the lowest applied concentrations, molecular and metabolic rearrangements rapidly occur, with changes in both primary and secondary metabolism (Brizzolara *et al.*, 2019), indicating that highly reactive mechanisms and oxygen sensors are present in apple cortex. The expression of genes involved in fermentative metabolisms (e.g., *ADH*), in secondary metabolism (e.g., phenylpropanoid pathway), hormonal responses and regulatory mechanisms (ethylene biosynthesis, ERFs) resulted to be affected by the oxygen switch.

The duration of the storage and the oxygen concentrations applied obviously play a key role in determining the fruit metabolic responses and the dynamics of fermentative metabolite accumulation. In addition, apple varieties react differently to extremely low oxygen conditions during storage, in particular in terms of fermentation, ethanol production and accumulation (Thewes *et al.*, 2019; Brizzolara *et al*., 2020; Thewes *et al.*, 2021 a; Park *et al*., 2022). Zanella and Stürz (2015) showed that, differently from eight other varieties, 'Red Delicious' apples react significantly and accumulate higher ethanol levels under hypoxia, and in a specific comparison between Granny Smith and Red delicious (Brizzolara *et al*., 2017), it was reported that the latter considerably accumulated ethanol under both ULO (0.9 kPa oxygen) and DCA (0.2-0.55 kPa oxygen) conditions, as also observed by Lumpkin *et al*. (2014).

Among important commercial apple varieties, the responses of Red Delicious to controlled atmosphere (CA) and DCA still need to be compared and clarified, which makes this cultivar a genotype of interest in terms of both applied aspects and physiological studies related to DCA conditions and different oxygen regimes and concentration adjustment protocols.

2. Materials and Methods

Experimental design and sampling

Organic apple (*Malus domestica* Borkh., cv. Red delicious) fruit were harvested in correspondence of an average TSS value of 10.8°Brix. Fruit were selected for their uniformity and absence of physical defects/decay and then kept for 3 days of acclimation at low temperature (0°C).

Control atmosphere storage was applied by dividing the fruit into two groups in two different cold chambers. The first group (about 300 fruit) was initially stored under oxygen concentration of 0.3% (0.3ox) while the second group (60 fruit) was stored under the safer oxygen concentration of 0.8% (0.8ox) for a total period of 218 DIA (days in atmosphere). Samples were collected at harvest and T0 sampling was carried out after 24h in atmosphere (1 DIA). To simulate the dynamic changes in oxygen concentrations applied during a DCA storage, at T1 (10 DIA) 60 fruit originally stored under 0.3ox were shifted to 0.8% oxygen level and kept under these conditions for the whole period of storage (218 DIA). These samples were called Shift 1 and were sampled successively at the following time points. At T2 (20 DIA), another 60 fruit were moved from 0.3% to 0.8% oxygen and were called Shift 2. Shift 3 was performed at T3 (31 DIA) and Shift 4 took place at T4 (110 DIA). A

schematic diagram of the experimental design is reported in figure 1. At each sampling point, three biological replicates taken from three different fruit for each treatment were considered. Samples of cortex tissue were collected, immediately frozen in liquid nitrogen, and stored at -80°C.

Flesh firmness, total soluble sugars content and ethanol quantification

Flesh firmness was measured at harvest (T0) and at the end of the storage at T5 (218 DIA). Measurements were taken at the two opposite sides of the equatorial part using a fruit penetrometer (mod. FT 327, 3-27 Lbs) with a large plunger tip (11 mm-diameter) after removing 1 mm of the peel.

Total soluble solid content (TSS, °Brix) was determined in the flesh juice of apples using a portable refractometer (Sinergica Soluzioni, Pescara, Italy); measurements were performed at harvest and the end of the storage at T5 (218 DIA) on pulp juice samples taken from the opposite sides of the fruit.
Ethanol content was measured by using a TectroniK (Tectronik, Padova, Italy) Senzytec analyser, following the instructions of the manufacturer and using 100µL of juice obtained by collectively pressing portions (approximately 1/3) of the cortex of three apples representing the biological replicate.

RNA extraction and cDNA synthesis

Total RNA was isolated from cortex tissue using 'SIGMA -Aldrich' RNA extraction kit following the manufacturer instructions. Total RNA was quantified (ng/µL) using UV spectrophotometry calibrated with RNase-free water. RNA purity was assessed by evaluating the absorbance ratio at 260/280 and 260/230 nm. Ribosomal RNA bands integrity was verified using GelRed™-stained 1% agarose gel (Aranda *et al.*, 2012).

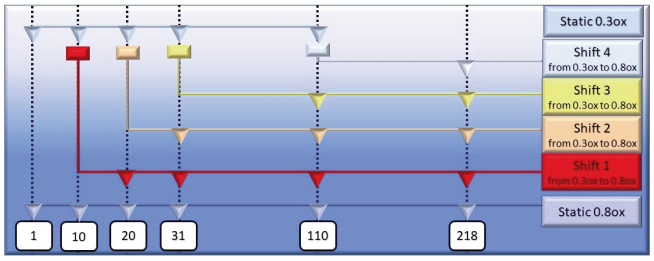

Fig. 1 - Red delicious DCA storage experimental design. The numbers indicate the days in atmosphere (DIA), ▽ Indicates the sampling with color coded for different samples, 0.3ox (Blue), 0.8ox (Purple), Sh1 (Red), Sh2 (Orange), Sh3 (Yellow) and Sh4 (Light blue).

RNA was reverse-transcribed into first-strand cDNA using 4 µL ReadyScript™ (Sigma, RDRT-500RxN), starting from 400 ng of total RNA in a final volume of 20 µL using DEPC treated water. The reaction was incubated at 25°C for 5 minutes, then at 46°C for 20 min and then heated at 95°C for 1 min and finally at 4°C for 15 min. The synthetized cDNA was diluted 1:5 by adding sterile water.

Gene expression analysis by real-time PCR

Quantitative Real-time PCR was performed using three biological replicates and two technical replicates for each sample. Based on the paper by Cukrov *et al.* (2016), primer pairs of genes related to sucrose/starch metabolism (*β-amylase, MdBAM; phosphofructokinase, MdPFK*; *sucrose synthase, MdSuSy*), the fermentative/pyruvic acid metabolism (*alcohol dehydrogenase, MdADH; lactate dehydrogenase, MdLDH; pyruvate decarboxylase, MdPDC; alanine aminotransferase, MdAlaAT*), ethylene biosynthesis (*ACC synthase, MdACS; ACC oxidase, MdACO*), and 6 *Ethylene Responsive Factors* (ERFs) were used (Table 1). *Actin* was used as a housekeeping gene.

Reaction mixtures were prepared, under sterile conditions, for the target and reference genes, containing each 5 µL of 2XSYBR® Green qPCR ReadyMix™ (SIGMA), 1 µL of each primer (Forward and Reverse) (10 µM), 2 µL RNase-free water and 1 µL of cDNA. The automated thermal cycler was programmed according to the following conditions: initial denaturation of 95°C for 30 sec followed by 40 cycles of: denaturation at 95°C for 10 sec, primer annealing (according to primer Tm) for 30 sec and extension at 72°C for 30 sec. Finally, melt-curve stage at 65°C for 0.5 sec followed by 95°C for 0.5 sec.

The Ct-values generated were used to evaluate the results of the gene expression levels comparing the expression of each target gene to the housekeeping gene (*Actin*). For samples under static 0.8ox, data were expressed with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and normalized to the corresponding sample at T1 (1 DIA). Data of samples under 0.3ox and the shifts were expressed as fold change of the expression level using the formula: $FC=Log_2(2^{-\Delta\Delta Ct})$ normalized to the corresponding sample of 0.8ox at each time point starting from 10 DIA.

Statistical analysis

For gene expression, data analysis was performed on six replicates (3 biological x 2 technical replicates). RStudio was used with external package "pcr" to calculate the relative expression and fold change. For

Table 1 - Primers pairs used in the RT-qPCR analysis

Genes	Primer sequence 5'-3'	Length (bp)	GC content (%)	Tm (°C)
ACC synthase MD15G1302200	F AAGTGGCGAACTGGAGTCGA	20	55	67.2
	R GGTTTGATGGGTTCGTGACC	20	55	66.4
ACC oxidase MD10G1328100	F CAGTCGGATGGGACCAGAA	19	57.8	66.3
	R GCTTGGAATTTCAGGCCAGA	20	50	66.3
Pyruvate decarboxylase MD04G1160100	F CAAGGCAGTAAAGCCGGTTA	20	50	63.9
	R AAATCGGTCCAGCAAACAAG	20	45	63.9
Alcohol dehydrogenase MD10G1014200	F GGAAGCACTGAAGCCATGAT	20	50	64.2
	R CTCCACGACAGAGGGAATGT	20	55	64.2
Lactate dehydrogenase MDP0000143956	F CATAAAACTCCTTCAGGCTCCA	22	45.4	64.1
	R GTGSGGTCTTGGGTGAGGAT	22	55	63.9
Beta-amylase 6 MD09G1103800	F CTATGTGCCGATCTTCGTGA	20	50	63.8
	R ACTGCTTGAAACACGCTCCT	20	50	63.9
Sucrose synthase MD15G1223500	F TCCGTGTTCACTGCTACGAG	20	55	64.1
	R GCCTCAAGAAGGTCCAACAG	20	55	63.8
Phosphofructokinase MD04G1042400	F AGTCGTGGAGTGGTGGAATC	20	55	64.1
	R TAGAGGGTGAGGGCTTCAGA	20	55	63.9
Alanine aminotransferease MD09G1173000	F TGCTGTCCGAGGTGAAATCGTC	22	54.5	70.6
	R AGCCCGGATTCGCCTTTAACTC	22	54.5	69.9
Ethylene response factor MD11G1306500 variant	F CGGTGGTGCTATAATCTCCG	20	55	64.2
	R GGAATTGAGTCGGTGTGAGTAGTT	24	45.8	64.3
Ethylene response factor MD11G1306500	F CTCCCTTCGCCAAGTTCG	18	61.1	65.9
	R TTGAGTCGGTGCGATTAACC	20	50	64.9
Ethylene response factor MD16G1162900	F CCAGAAGCCCAAACCATCAG	20	55	66.7
	R TTCCTCGGCGGTGTTGTA	18	55.5	65.4
Ethylene response factor MD13G1163300	F GGTGGGGAAATGTATGCTAAGA	22	45.4	63.7
	R GTCATCCAGCATCCACAGG	19	57.8	64.4
Ethylene response factor MD17G1152400	F CTTCTGCAAAGCGTTCTGTG	20	50	63.7
	R GGCAGGATCGGATGGAG	17	64.7	64.5
Ethylene response factor MD09G1174400	F TTCTGCAAAGCGTTCCATC	19	47.3	64
	R TTCATTGGCAGGGAAGGTG	19	52.6	66
Actin MD04G1127400	F TGACCGAATGAGCAAGGAAATTA	25	40	67.4
	R TACTCAGCTTTGGCAATCCACATC	24	45.8	68

The table reports the genes nomenclature according to https://iris.angers.inra.fr/gddh13/, the primers sequence, length, GC content and melting temperature (Tm)

fruit firmness, TSS and ethanol content 3 biological replicates were used. Internal statistical functions and external package "agricolae" were used to analyze the data (Kronthaler and Zoellner, 2021). All data were analyzed using *t-test* for samples at 1 DIA to compare 0.3 to 0.8ox samples. One-way ANOVA and mean comparison with Least significant difference (LSD) post-hoc test ($p \leq 0.05$) was used to compare different samples of shifts to the corresponding 0.8ox at each time point starting from 10 DIA. A Kruskal-Wallis test was applied to non-parametric data ($p \leq 0.05$).

3. Results

Flesh firmness, TSS and ethanol production

At the end of the storage period and after five days of shelf life at room temperature no physiological disorders or external/internal defects were observed in all analysed samples collected from the different protocols.

Concerning technological parameters, Table 2 reports apple firmness and TSS values for samples taken at harvest (T0) and at the end of the trial at 218 DIA. Results showed that samples stored for 30

Table 2 - Firmness and total soluble solids (TSS). Mean values (±SE) of apple samples at harvest (T0) and after 218 DIA of static (0.8ox) and dynamic atmosphere storage (Shift 1-4) are reported in the table

Samples	DIA	Firmness (N)	TSS (°Brix)
At harvest	0	75.10 ± 0.96 a	10.85 ± 0.45
Static 0.8ox	218	67.67 ± 2.85 ab	11.40 ± 0.06
Shift 1	218	62.52 ± 3.51 ab	11.76 ± 0.32
Shift 2	218	67.91 ± 0.73 ab	11.53 ± 0.24
Shift 3	218	58.10 ± 6.76 b	10.86 ± 0.13
Shift 4	218	61.30 ± 5.83 b	11.40 ± 0.06

Different letters indicate significant differences among samples (ANOVA, LSD post-hoc test (p≤0.05).

(Sh3) and 110 (Sh4) days under 0.3% oxygen before being shifted to 0.8% oxygen showed the lowest values of firmness at the end of the trial. While 0.8ox, Sh1 and Sh2 samples maintained firmness values not significantly different from those detected in T0 samples.

No significant difference (*p=0.414, alpha level ≤ 0.05)* was recorded for TSS levels over time or between the different applied protocols.

Ethanol levels have been monitored along the entire experimental period to assess the activation of fermentative metabolism under static and dynamic CA storage (Fig. 2). 0.3ox samples already showed higher levels than those of the 0.8ox sample at 10 DIA, and a further increase from 10 to 20 DIA with values around 400 mg/kg FW up to 110 DIA (last sampling time for this specific treatment). On the other hand, 0.8ox samples showed a similar trend in

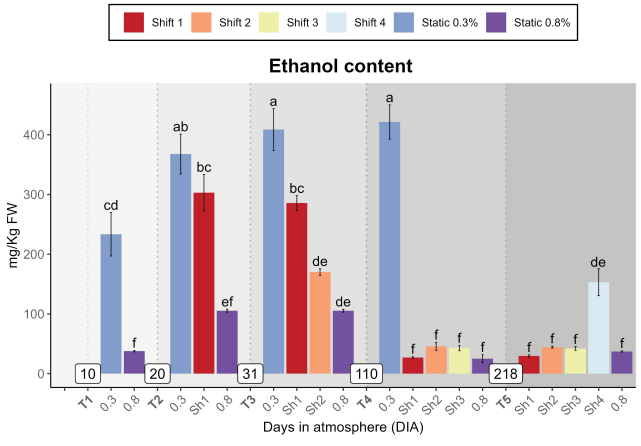

Fig. 2 - Ethanol content (mg/Kg FW). Samples stored in 0.3ox (Blue), 0.8ox (Purple), Sh1 (Red), Sh2 (Orange), Sh3 (Yellow) and Sh4 (Light blue) analysed from 10 to 218 days in atmosphere (DIA). Different letters indicate significant differences among samples (ANOVA, LSD post-hoc test (p ≤ 0.05).

terms of ethanol accumulation but with significantly lower amounts compared to 0.3ox samples and a decreasing trend after the highest concentrations detected at 20 and 31 DIA. Samples subjected to partial re-oxygenation at different time points (Sh1, Sh2, Sh3 and Sh4) showed a different behaviour: Sh1 did not show significant difference from 0.3ox at 20 DIA , but displayed a reduced amount at 31 and at 110 DIA as also observed for Sh2.

Interestingly, at 110 DIA ethanol levels were similar in all shifted and in 0.8ox samples. This was also observed at 218 DIA, except for Sh4 apples that displayed still significant higher levels (Fig. 2).

Effect of different protocols on sugar metabolism- and fermentation-related gene expression

For gene expression data analysis, 0.8ox samples, kept under a static concentration throughout the experiment (from 1 to 218 DIA), were considered as a reference for the other storage protocols (0.3ox, Sh1, Sh2, Sh3 and Sh4). Consequently, the gene expression levels of these latter samples were expressed as fold change in relation to 0.8ox.

Considering sugar metabolism, the expression levels of three genes were monitored throughout storage (Fig. 3). In 0.8ox samples, *MdBAM* gene revealed a significant up regulation, compared to T0, at 31 and 110 DIA. A significantly lower expression level was recorded in 0.3ox samples at 10 and 110 DIA. Sh1, Sh2, Sh3 and Sh4 samples had significantly lower levels of expression at 110 DIA. At the last sampling time, 218 DIA, all shifted samples, except Sh3, revealed significantly higher expression values compared to 0.8ox. *MdPFK* gene expression showed a steady state in samples stored at 0.8ox. A lower expression level of this gene was detected at 10 DIA in 0.3ox samples. Sh1 samples showed higher expression at 31 DIA, Sh2 had lower expression level at 31 DIA and higher at 110 and 218 DIA, while Sh3 samples showed higher expression levels at 218 DIA. Considering *MdSuSY, in* 0.8ox apples the expression showed a significant induction at 10 and 20 DIA, followed by a basal expression level at all the other sampling points. Samples stored under 0.3ox revealed two marked and significant peaks of induction, at 10 and 110 DIA. Interestingly, Sh1 showed significantly reduced levels of expression at 20 DIA, while Sh2 and Sh4 revealed significantly higher expression at 218 DIA.

Concerning the gene related to the fermentative/pyruvic acid metabolism (Fig. 4), *MdLDH* expression showed, in 0.8ox samples, a significant increase

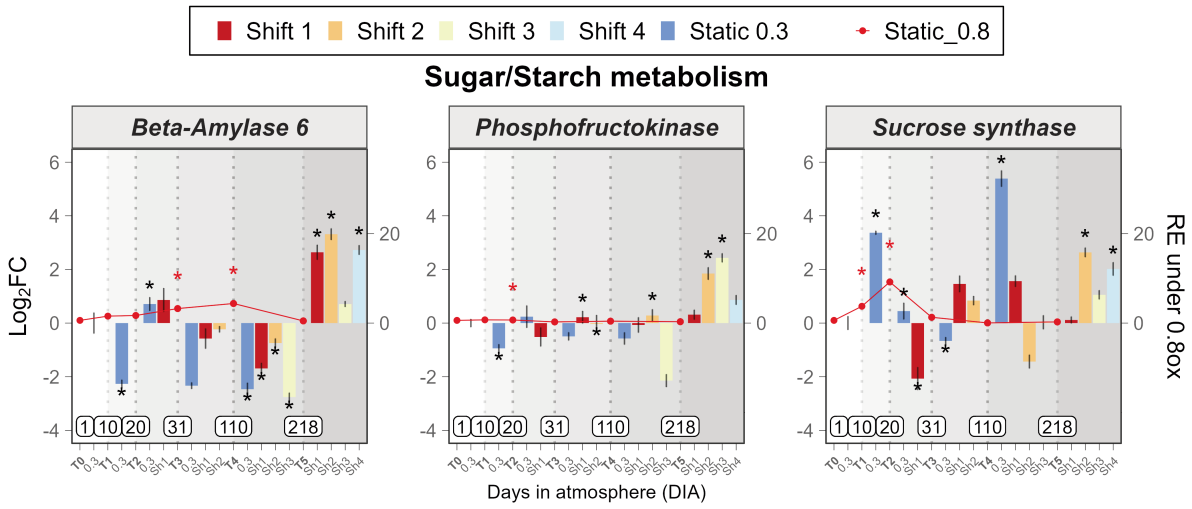

Fig. 3 - Relative expression of genes related to sugar metabolism and energy production β-amylase, phosphofructokinase, and sucrose synthase. For samples 0.3ox (Blue), Sh1 (Red), Sh2 (Orange), Sh3 (Yellow) and Sh4 (Light blue) the expression level is reported from 1 to 218 days in atmosphere (DIA) as *log$_2$ FC* normalized on 0.8ox expression level at each time point. The red line represents gene relative expression in 0.8ox samples. Black asterisks indicate significant differences (ANOVA, LSD post-hoc test (p≤0.05) comparing each sample to 0.8ox level at the same sampling time. Red asterisks indicate significant differences (ANOVA, LSD post-hoc test (p≤0.05) between 0.8ox samples at the specific time points and 0.8ox apples at 1 DIA.

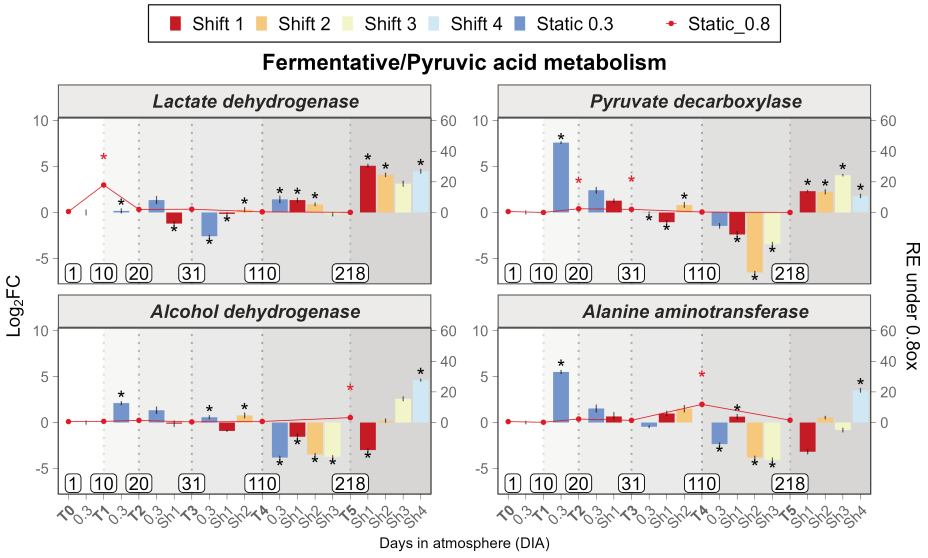

Fig. 4 - Relative expression of genes related to fermentative/pyruvic acid metabolism, lactate dehydrogenase, pyruvate decarboxylase, alcohol dehydrogenase and alanine aminotransferase. For samples 0.3ox (Blue), Sh1 (Red), Sh2 (Orange), Sh3 (Yellow) and Sh4 (Light blue) the expression level is reported from 1 to 218 days in atmosphere (DIA) as *log$_2$ FC* normalized on 0.8ox expression level at each time point. The red line represents gene relative expression in 0.8ox samples. Black asterisks indicate significant differences (ANOVA, LSD post-hoc test (p≤0.05) comparing each sample to 0.8ox level at the same sampling time. Red asterisks indicate significant differences (ANOVA, LSD post-hoc test (p ≤ 0.05) between 0.8ox samples at the specific time points and 0.8ox apples at 1 DIA.

of expression only at 10 DIA. Compared to 0.8ox samples, 0.3ox treatment induced higher expression at 10 and 110 DIA, and a general up-regulation in shifted samples at 218 DIA. *MdPDC*, involved in the production of acetaldehyde, increased its expression at 20 and 31 DIA in 0.8ox samples. 0.3ox apples revealed an earlier increase in the expression of *MdPDC* gene, significant at 10 DIA. Among samples that underwent partial re-oxygenation, a general lower expression, always compared to 0.8ox, was observed at 110 DIA, followed by increased levels at 218 DIA. Acetaldehyde can be further converted to

ethanol under low oxygen levels by the enzyme coded by *MdADH* genes, and this is a crucial reaction in apple tissue under hypoxia. Compared to T0 samples, the selected *MdADH* gene showed a significant increase only at 218 DIA in 0.8ox sample. The application of 0.3% oxygen resulted in general higher levels of expression until 31 DIA, significant at 10 and 31 DIA. At 110 DIA, a significant decrease in *MdADH* expression was recorded for 0.3ox apples as well as for all shifted samples.

MdAlaAT is involved in the reversible transfer of an amino group from glutamate to pyruvate, which in turn forms 2-oxoglutarate and alanine. In 0.8ox samples, this gene showed a peak of expression at 110 DIA. The expression in 0.3ox samples is highly induced at 10 DIA, while it strongly decreased at 110 DIA, similarly to Sh2 and Sh3. Higher expression of this gene was detected at 218 in Sh4 sample (Fig. 4).

Ethylene biosynthesis and ERFs gene expression

The expression of two genes involved in ethylene biosynthesis, namely *MdACS* and *MdACO*, has been analysed (Fig. 5). These two genes appeared to be highly affected during storage under 0.8% oxygen concentration. The expression of both genes increased with time, constantly for *MdACO,* which also reached the highest recorded levels of expression, while, in the case of *MdACS,* a peak at 110 DIA was detected. Regarding *MdACS* gene expression, 0.3ox samples showed increased values (compared to 0.8ox) at 10 DIA, but lower levels at 20, 31 and 110 DIA, when also Sh2 and 3 showed low expression levels.

MdACO gene revealed marked higher levels in 0.3 ox samples at 10 DIA and in Sh1 samples at 20, 31, and 110 DIA. Lower expression levels were detected in 0.3ox apples at 20, 31 and 110 DIA. Interestingly, Sh1 samples showed higher levels of expression, compared to 0.8ox, at 20, 31, and 110 DIA. All shifted samples had lower expression level than 0.8ox apples at 218 DIA.

The ethylene signalling and response pathway includes *Ethylene Response Factors* (ERFs), which belong to the transcription factor family APETALA2/ERF that plays important roles in stress-related responses. The effect of the different applied storage protocols on the expression level of six ERF genes has been investigated throughout the experiment (Fig. 6). In general, samples showed relatively similar responses in terms of ERF expression. Overall, considering 0.8ox samples a general increase of ERF genes expression was observed up to 110 DIA, which was significant at different time points for the different analysed ERFs (*MD09G1174400* gene at 110 DIA; *MD13G1163300* gene at 10, 20 and 31 DIA; *MD11G1306500* gene at 10, 20 and 31 DIA; *MD16G1162900* gene at 10, 31 and 110 DIA; *MD11G1306500 VARIANT* gene at 10, 20 and 31 DIA;

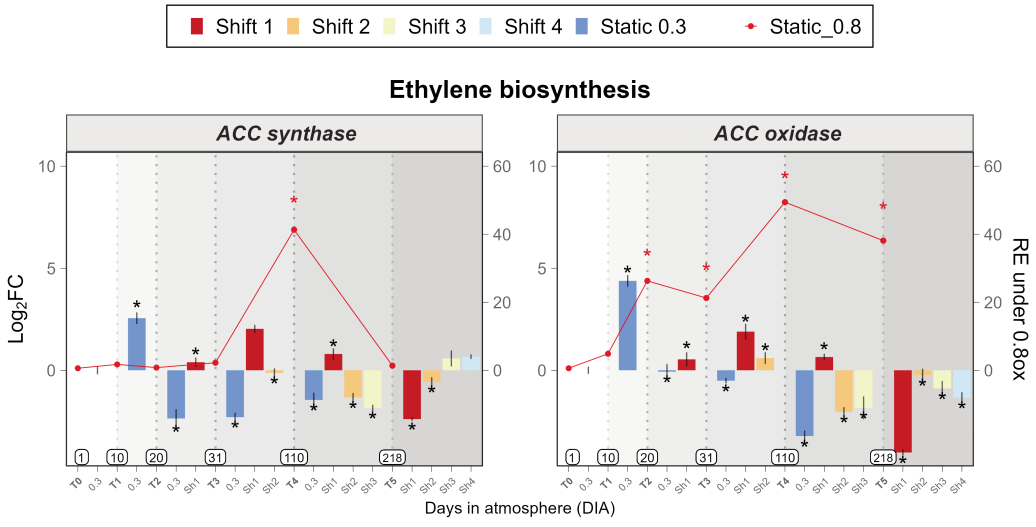

Fig. 5 - Relative expression of ethylene biosynthesis genes ACC-synthase and ACC-oxidase. For samples 0.3ox (Blue), Sh1 (Red), Sh2 (Orange), Sh3 (Yellow) and Sh4 (Light blue) the expression level is reported from 1 to 218 days in atmosphere (DIA) as *log2 FC* normalized on 0.8ox expression level at each time point. The red line represents gene relative expression in 0.8ox samples. Black asterisks indicate significant differences (ANOVA, LSD post-hoc test ($p \leq 0.05$) comparing each sample to 0.8ox level at the same sampling time. Red asterisks indicate significant differences (ANOVA, LSD post-hoc test ($p \leq 0.05$) between 0.8ox samples at the specific time points and 0.8ox apples at 1 DIA.

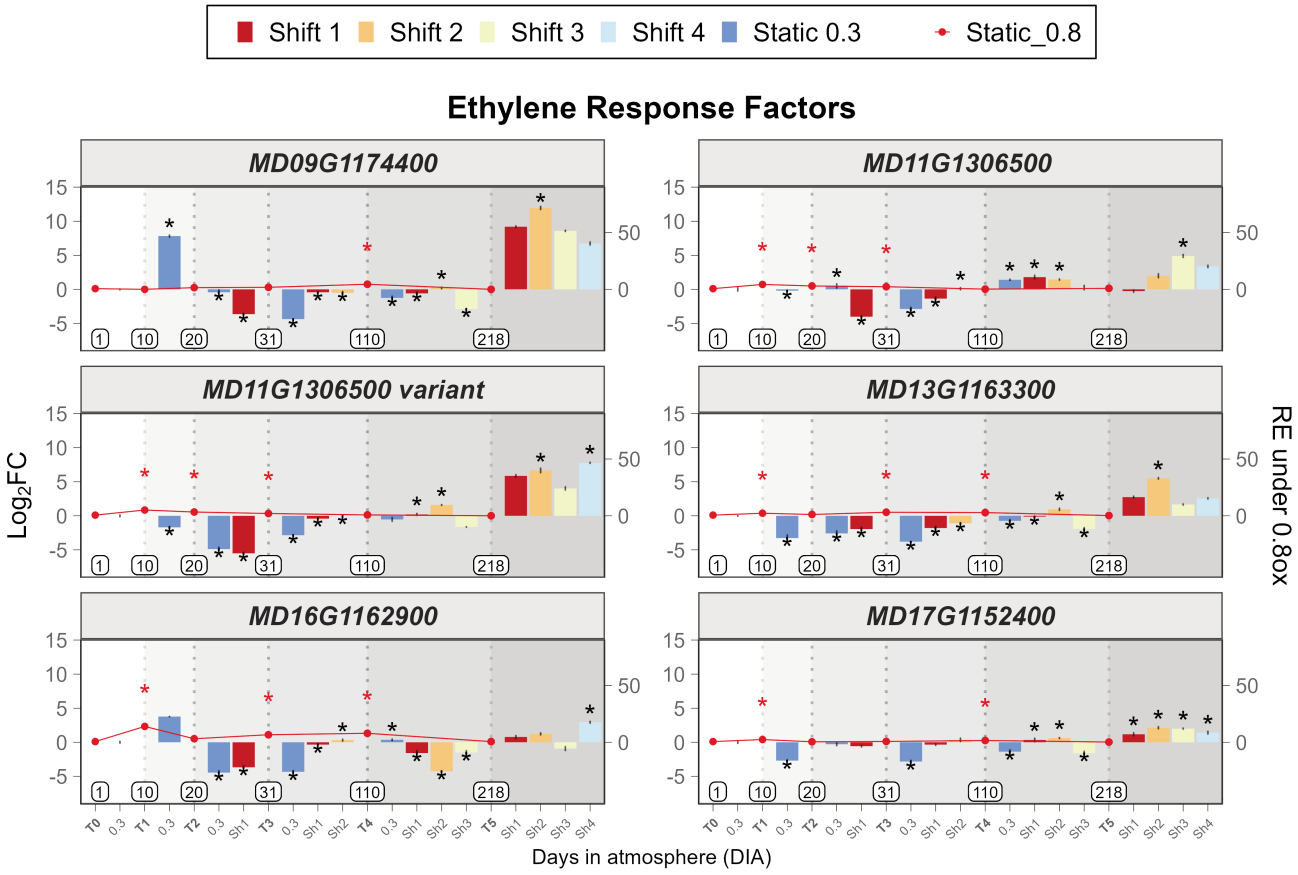

Fig. 6 - Relative expression of genes belonging to Ethylene Response Factor (ERF) gene family. For samples 0.3ox (Blue), Sh1 (Red), Sh2 (Orange), Sh3 (Yellow) and Sh4 (Light blue) the expression level is reported from 1 to 218 days in atmosphere (DIA) as *log2 FC* normalized on 0.8ox expression level at each time point. The red line represents gene relative expression in 0.8ox samples. Black asterisks indicate significant differences (ANOVA, LSD post-hoc test ($p \leq 0.05$) comparing each sample to 0.8%ox level at the same sampling time. Red asterisks indicate significant differences (ANOVA, LSD post-hoc test ($p \leq 0.05$) between 0.8ox samples at the specific time points and 0.8ox apples at 1 DIA.

MD17G1152400 gene at 10 and 110 DIA).

The expression of ERF genes in 0.3ox samples was instead characterized by significant lower levels at the different time points with only two exceptions: *MD09G1174400* gene at 10 DIA and *MD11G1306500* gene at 20 DIA, when significantly higher levels of expression than those of 0.8ox apples were recorded. On the other hand, considering apples subjected to partial re-oxygenation samplings, lower levels of expression were generally detected up to 31 DIA, with only two exceptions (MD*11G1306500* and *MD16G1162900*) concerning Sh2 samples at 31 DIA, when a significantly higher level of expression was observed.

After 110 DIA, the different samples revealed variable patterns. Concerning *MD09G1174400* and *MD13G1163300* genes, Sh1 and Sh3 showed significantly lower levels of expression, while Sh2 had significantly higher levels. For these two genes at 218 DIA only Sh2 revealed significantly higher levels of expression compared to 0.8ox samples, despite a general increasing trend in all apples subjected to partial re-oxygenation.

As far as the *MD11G1306500* gene is concerned, significant higher expression was detected for both Sh1 and Sh2 at 110 DIA, while only Sh3 had significantly higher levels at 218 DIA. *MD16G1162900* gene expression in shifted samples revealed significant lower levels at 110 DIA, while only Sh4 had significantly higher levels at 218 DIA. In the case of *MD11G1306500 VARIANT* gene Sh1 and Sh2 had higher expression levels at 110 DIA, while only Sh2 and Sh4 showed significantly higher levels at 218 DIA. Lastly, *MD17G1152400* gene had a significantly higher level of expression in samples of Sh1 and Sh2 at 110 DIA, while Sh3 apples had significantly lower

expression for this gene. On the other hand, all shifted samples showed significantly higher expression of this gene at 218 DIA compared to 0.8ox apples. A general trend was identified for all samples subjected to partial re-oxygenation: in general the expression level of the ERF gene was induced at 218 DIA.

4. Discussion and Conclusions

An in-depth understanding of low oxygen responses in fruits is of fundamental importance for the optimisation of storage approaches and for the development of protocols aimed at maintaining optimal quality while preventing the occurrence of physiological disorders associated with long term storage. Metabolic adaptation responses to hypoxic conditions have only recently started to be clarified in apple fruits and are gaining increasing interest since apples are routinely stored for very long periods of time thanks to the adoption of low oxygen (0.8 kPa oxygen, Ultra Low Oxygen, ULO) or dynamic controlled atmosphere (DCA, 0.4 or lower kPa oxygen) protocols. Primary metabolism and ethylene physiology are markedly affected by hypoxia with differences depending on the oxygen concentration and modulation, and the genotype. In this study we characterized the ethanol accumulation and the expression pattern of sugar/fermentative metabolism- and ethylene physiology-related genes of Red delicious apples in CA/DCA storage. Our goal was that of better understanding the behaviour and the responses (also in terms of specific quality parameters) of this apple variety in relation to two levels of low oxygen concentration and the variable (in terms of timing) switch from 0.3 to 0.8% oxygen levels.

The storage under 0.3% oxygen resulted in an early significant accumulation of ethanol already at 10 DIA and that further increased at 20 DIA. This level remained rather stable as long as the fruit were kept at 0.3% oxygen atmosphere until 110 DIA. Although to a lesser extent, ethanol content also increased in 0.8ox samples, confirming what observed by Brizzolara *et al*. (2017). In these apples, ethanol content levelled off after three months of storage. The metabolization of ethanol seemed to be more sensitive to re-oxygenation when apples had experienced a shorter period of DCA. In fact, the longest storage under 0.3% oxygen resulted in more stable ethanol levels in the cortex at the end of the storage period (218 DIA, Sh4 in Fig. 2). Considering one of the main parameters dictating the commercial life of apples, the samples Sh3 and Sh4 showed the lowest values of flesh firmness at the end of the trial. This behaviour could be associated to the highest levels of ethanol accumulated in these samples. In Braeburn apples ethanol production exceeding 472 $\mu L \cdot L^{-1}$ and the overproduction of anaerobic metabolites in Royal Gala resulted in a decrease of flesh firmness (Weber *et al*., 2020; Thewes *et al*., 2021 b). In persimmon, it has been observed that accelerated loss of flesh firmness during storage was induced by ethanol treatments applied to reduce astringency (Vilhena *et al*., 2022). These authors observed that this event is closely related to greater parenchyma degradation during storage caused by ethanol treatment. If this cellular event also occurs in apple fruit accumulating high ethanol levels following hypoxic storage conditions remains to be elucidated.

It is interesting to note that even in the samples with the highest ethanol content (0.3ox) no internal physiological disorders (e.g., flesh breakdown) were detected.

The gene expression data confirmed that the molecular regulation of hypoxic responses is overall conserved among apple varieties: the up-regulation of *MdPDC*, *MdADH* and *MdAlaAT* in response to extreme levels of hypoxia (0.3 oxygen concentration) is readily activated and peaks at 10 DIA, after which is promptly and progressively levelled down until 110 DIA, when a general low level of expression (compared to 0.8ox samples) is present in 0.3ox and shifted samples. This possibly suggests a negative feedback exerted by ethanol on its own synthesis. In agreement with these findings, one of the genes encoding group VII ethylene response factors (*MD09G1174400*), with similarity to RAP2 proteins involved in low oxygen signalling in model systems (Licausi *et al.*, 2011; Gibbs *et al*., 2011), displayed an expression pattern overlapping with that of the fermentative metabolic genes with a transient up-regulation at 10 DIA and low expression levels at 110 DIA.

It is well known that under energy shortage conditions, such as those induced by low oxygen conditions, plant tissues and organs (including fruit) instead of using invertases and hexokinases to produce hexose-phosphates to form sucrose, an ATP consuming process, can use sucrose synthase as alternative energy saving pathway (Mustroph *et al*., 2014). The activation under hypoxic conditions of sucrose synthase was already reported by Cukrov *et al.* (2016) in Granny Smith apples, and our expression

data (showing a high induction at 10 DIA in 0.3ox and a prompt reduction of expression level in Sh1 apples at 31 DIA) confirm that this gene can be considered highly sensitive to oxygen levels also in cv. Red Delicious. A cultivar-specific behaviour is, instead, observed regarding beta-amylase and phosphofructokinase. In fact, these genes in Granny Smith apples follow a similar expression pattern compared to *MdSuSY* (Cukrov *et al*., 2016), not observed in the present trials on Red Delicious.

As far as ethylene biosynthesis is concerned, the expression pattern of ACC oxidase detected in 0.8ox Red Delicious samples mirrors that observed in Granny Smith (Cukrov *et al.*, 2016), while the transient higher expression levels observed for both ACC synthase and oxidase at 10 DIA under 0.3% oxygen appear to be a specific response of cv. Red Delicious apple.

Interestingly, the transcription of both genes appeared to be re-activated exclusively in the first and second shift to 0.8ox (Sh1 and Sh2), performed after 10 and 20 DIA, respectively, and showed a peak at 31 DIA followed by lower expression levels. However, the increase of *MdACS* and *MdACO* transcript following the oxygen resupply reached a level significantly lower than that reached by apples that had been constantly kept at 0.8ox. It could be hypothesised that this may be due to the higher levels of ethanol accumulated in the pulp of DCA stored apples, which might exert a suppressive action on ethylene biosynthesis as previously shown by some authors in different apple varieties (Pesis *et al*., 2005; Thewes *et al*., 2019; Weber *et al*., 2020; Thewes *et al*., 2021 a).

Concluding, the recovery from anoxia in apple fruits is dependent on the length of exposure to the anoxic stress (Wood *et al*., 2022). Our data on ethanol accumulation and ethylene-related gene expression are in line with these findings, showing that longer periods of exposure to 0.3% oxygen result in the maintenance of higher levels of ethanol and on the prevention of transcription of ethylene biosynthetic genes. The effect of low oxygen storage of Red Delicious apples on transcript abundance of several important genes related to hypoxic stress response in apple fruit revealed both similarity with Granny Smith apples stored under the same CA protocols, and specific responses of cv. Red Delicious. In both Red Delicious and Granny Smith apples two phases can be recognized in relation to fermentative metabolism: a first phase characterised by the activation of fermentative pathways, and a second phase (from two months onward) in which a generalized de-activation of fermentative metabolism is observed.

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Application of antiperspirants to improve the condition of ornamental plants subject to medium- and long-distance transport in refrigerated container

L. Ghiselli, D. Bonetti, D. Prisa, S. Nin, G. Burchi (*)
Council for Agricultural Research and Economics, Research Centre for Vegetables and Ornamental Crops, Via dei Fiori, 8, 51012 Pescia (PT), Italy.

Key words: Cold storage, nursery industry, post-harvest physiology, potted shrubs.

(*) **Corresponding author:**
gianluca.burchi@crea.gov.it

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: The ornamental nursery sector sells and delivers its products not only within the European Union but throughout the world, thus shipping for long distances has become commonplace in the industry. Extended transport times may result in loss of quality and reduced longevity. Consequently, an effective logistics strategy is of competitive importance for nursery production. This research was carried out with the aim of improving long-distance transport conditions (up to 6 weeks) of ornamental plants produced in the nurseries of the Pistoia District. Phenotipic and physiological parameters of plants during transport were studied, testing three biodegradable antiperspirants and a biodegradable microfilm to protect plants on five important pot ornamental species: maple (*Acer palmatum*), cypress (*Cupressocypari leylandii*), privet (*Ligustrum texanum*), nandina (*Nandina domestica*) and viburnum (*Viburnum tinus*). Plant tolerance to storage conditions in refrigerated cell or container (T° = 8-12°C) varied considerably according to the considered species, with cypress resulting extremely tolerant and maple and nandina very sensitive. Treatments with antiperspirants did not exhibit particularly evident effects on the tested species. The use of biodegradable film was inadequate to protect plant quality during long-distance shipments. Even in cases of total or partial loss of leaves by species such as maple and nandina, an optimal recovery of vegetative development was highlighted once these species were relocated in outdoor cultivation. Among physiological parameters, MDA and phenols contents were the most stress-related variables, being negatively correlated to the quality decay of plants transported in dark refrigerated cells for 2-6 weeks.

1. Introduction

The Nursery District of Pistoia (Tuscany) is the heart of Italian ornamental production and leader in Europe. This activity covers over 5200 ha, with about ha 1000 of pottery, 1500 companies, over 5500 direct employees (in addition to the related industries) and a Gross Saleable

Production over 300 M €, of which 160 M € are exported. The distribution of cultivation is as follows: evergreen trees and shrubs 1600 ha, conifers 1350 ha, ornamental deciduous trees 1420 ha, deciduous shrubs 350 ha, creepers and other shrubs 380 ha, roses 100 ha (Marzialetti, 2015).

The ornamental nursery sector sells and delivers its products not only within the European Union but throughout the world, thus shipping, often for long distances, has become commonplace in the industry. Plant transfer, mainly carried by road, rail or sometimes by sea, and in crowded, stifling hot or refrigerated truck, can also be very long. The extended shipping and/or storage times may result in loss of quality. Consequently, an effective logistics strategy is of competitive importance for companies to make sure plants are delivered on time and in the best conditions. Plants travelling long distances are frequently negatively affected by the critical environmental conditions during transport, such as exclusion from light in closed containers, exposure to harmful gases and temperature extremes, poor air ventilation, high relative humidity (RH) and vibration. These conditions can lead to deterioration of even the highest quality plants. Further, the environmental and physical stresses imposed upon plants during transfer are worsened if plants are improperly produced, incorrectly packaged and/or mishandled during shipping or upon receipt. Thus, keeping the quality of potted ornamental plants is an essential condition for their commercial success and for promoting trust in customer relationships.

The main quality parameters for leafy pot plants are the size and the green colour of the leaves (Wang *et al*., 2005). Biotic and abiotic stresses during shipping lead to several physiological disorders with numerous negative effects, such as: leaf yellowing due to decreased photosynthesis (Starman *et al*., 2007), leaf and flower abscission with slowed growth and uptake of water and nutrients, color loss of flowers and leaves, damage to cell membrane phospholipids with increased lipid peroxidase (Mittler, 2002). Phenolic compounds including flavonoids play a role in plant defense against various oxidative stresses, with antioxidant and free radical scavenging activity thus improving plant tolerance to stresses (Trchounian *et al*., 2016; Tohidi *et al*., 2017). The accumulation of these various secondary metabolites has been shown to be influenced by interactions between plant genotype (species, and variety within species) and environmental factors, including cultivation technique, season, abiotic and biotic stress, and nutrient status (Dixon and Paiva, 1995; Vyn *et al*., 2002; Downey *et al*., 2006; Ksouri *et al*., 2007).

This research was carried out within the In.Tra.Viva Project, funded by the Tuscany Region, with the aim of improving long-distance transport conditions (up to 6 weeks) of ornamental plants produced in the nurseries of the Pistoia District and to reduce the die-off of potted plants during transport (up to 30%), mainly caused by the fall of the leaves and the inability to recover the vitality that the same plants had on departure. The commitment of CREA-OF Pescia to the Project includes the following research activities: i) monitoring the phenotypic and physiological behaviour of plants during transport; ii) testing new biodegradable antiperspirant products to increase the resistance duration of plants; iii) testing a biodegradable microfilm to protect plants during transport.

2. Materials and Methods

Five popular pot plant species, commonly grown for outdoor use, were tested for their tolerance to long distance shipping: maple (*Acer palmatum*), cypress (*Cupressocypari leylandii*), privet (*Ligustrum texanum*), nandina (*Nandina domestica*) and viburnum (*Viburnum tinus*). Thirty plants of each species were provided by Giorgio Tesi Group, Pistoia, at the end of March: the plants were 4 years old, grown in 9 L pots Ø 24 cm (maple and viburnum) or 3 L pots Ø 18 cm (cypress, privet and nandina).

Medium and long-distance transport simulation tests were carried out in refrigerated cells (T° = 10°C) at the experimental farm and laboratories of CREA Research Centre for Vegetable and Ornamental Crops (Pescia, PT) during Spring 2021. The spring season is the most important and critical season for the farmers, both from an economic and a physiological point of view since the plants are in full vegetation or yet at the beginning of the flowering stage. Five plants of each species were placed in the nursery, in open air (OA), thus acting as an untreated control not stored in a refrigerated cell. The remaining 25 plants of each species were transferred in the laboratories on April 2nd, measured, treated with antiperspirants and then placed in a refrigerated cell simulating a medium-distance transport (T1 = 2 weeks, from April 14th to April 28th) and a long-distance transport (T2 = 6 weeks, from April 14th to May 26th) at T° = 10°C. The

plants were subjected to the following treatments: i) spraying with 'Barzaghi-A 10%' (A) and ii) with 'Barzaghi-B 10%' (B), two experimental and biodegradable antiperspirants based on carboxymethylcellulose; iii) spraying with Vapor Gard® 5% (V), a commercial antiperspirant based on pinolene 96% (di-L-para-menthene); iv) wrapping in a hermetically sealed experimental biodegradable film (P) provided by LaMPo (Department of Chemistry, University of Milan); v) spraying with tap water (C), considered as the stored control treatment. Antiperspirants (A) and (B) were provided by Barzaghi Speciality Chemicals srl, Arluno (MI). The percentages of the active ingredients are not disclosed to the public as these ingredients are protected by patent (N.1428533/15.5.2017). The manufacturer claims about the effectiveness of these products based on specific private research. This study could demonstrate the efficacy of this biodegradable antiperspirant and the manufacturer could use this information to market the product to consumers.

Phenotypical data of all plants (height and diameter) were measured at the start of the trial (April 2nd to 14th), at T1 (April 28th) and T2 (May 26th). Leaf physiological measurements (chlorophyll A, chlorophyll B, phenols, carotenoids and malondialdehyde content) were carried out on leaf samples collected from all plants of the three evergreen species cypress, privet and viburnum at T1 and T2. On the other hand, semi-evergreen nandina and deciduous maple shrubs lost all their leaves during their stay in the cell, hence pigment analysis and estimates of malondialdehyde levels were not performed on these species.

At the end of the cold storage experiment (end of May), all plants were moved to the nursery in open air and placed together with the non-stored control plants (OA).

Malondialdehyde content (MDA), the final product of the lipid peroxidation process, is a widely used marker of oxidative lipid injury caused by environmental stress (Kong *et al*., 2016). MDA content was measured by 2-thiobarbituric acid (TBA) reaction as reported by Li *et al.* (2010). The absorbance of the aqueous phase was detected at 450, 532 and 600 nm. MDA content was calculated based on the following formula:

$$C\ (\mu mol/g\ weight) = (6.45 \times (A532 - A600) - 0.56 \times A450) / W\ (sample\ weight\ g)$$

Leaf chlorophyll, carotenoid and phenol contents were analyzed following the method reported by Lichtenthaler and Buschmann (2001) on fresh frozen (-80°C) leaf discs obtained by excising 5-6 fully expanded leaves collected from the middle portion of the plants grown in container at T1 and T2. The absorbances of chlorophyll a and b were assessed spectrophotometrically (Thermo Evolution 300 UV-Visible Spectrophotometer) at 665.2 nm, 652.4 nm, and 470 nm, respectively, while carotenoid and phenol absorbances were read at 260 nm and 530 nm, respectively.

Collected data were subjected to the analysis of variance (ANOVA) to determine the significance level of the different sources of variation: treatment (Tr = 5 levels = A, B, V, C, OA), storage time (ST = 2 levels = T1 and T2) and Tr x ST interaction. Differences between means were tested using Duncan's multiple comparison test with a confidence level of 95%. The statistical analysis packages used for processing were IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY).

3. Results and Discussion

No perceptible increments in plant growth (height and diameter) were detected in all refrigerated plant species during the time interval from T0 to T1 and T2, regardless of the type of antiperspirant treatment and biodegradable film used. Conversely, control plants kept in open air (OA) grew and developed as expected (data not reported).

Plant protection with the biodegradable parafilm proved to be ineffective for the purpose. At T1 the biodegradable film resulted perforated in several points by the twigs of the plants, piled up into the refrigerated cell (Fig. 1), while at T2 the film was rotten due to the contact with the humidity of the leaves because of their transpiration. Thus, the parafilm treatment was not considered in the statistical analysis.

After fifteen days of refrigeration, nandina and maple plants lost part of the leaves, whereas at the end of the refrigerated storage, regardless of the treatment, all the leaves had fallen or rotted on the plant (Fig. 2). All nandina plants, when moved outdoors at the end of May, resumed their vegetative activity, reaching development rates comparable to control plants at the end of October (Fig. 3 a). On the contrary, the plants of maple not treated with antiperspirants (C, P and OA) started regularly to veg-

Fig. 1 - The biodegradable parafilm proved to be ineffective for the purpose: at long storage time (T2 = 6 weeks), , relative humidity levels were too high and there was lack of air circulation; plant transpiration caused the humidity around the leaves to be saturated with water vapor and the pellicle to rot.

Fig. 2 - After 6 weeks of refrigerated storage, all the leaves of nandina (2 a) and maple (2 b) had fallen or rotted on the plant.

etate again in the nursery but all those treated with antiperspirants (A, B and V) died (Fig. 3 b). This phenomenon has to be further investigated but it is possible that the antiperspirants reduced transpiration

Fig. 3 - All nandina plants resumed their vegetative activity once they were relocated in the field reaching development rates comparable to control plants after 5 months of cultivation in open air (3 a). Maple plants that were not treated with antiperspirants started regularly to vegetate again in the nursery (3 b, on the left), while those treated with antiperspirants died (3b, on the right).

of maple and thus the plants suffered a too high level of humidity in the pot substrate. It is very important to avoid excessive wetting of pot substrate before loading the plants into the container and to irrigate the plants a couple of days prior to scheduled shipment, so that the excess water can drain completely. In this trial, maybe the humidity level of the soil substrate resulted correct for the control plants but too high for the plants treated with antiperspirants.

No phenotypical differences were observed among cypress plants stored for 15 days and 6 weeks in the refrigerated cells and control plants maintained in the field (Fig. 4) (data not reported). Once moved outdoors in the field, cypresses began to

Fig. 4 - Regardless of the type of spraying treatment used, the cypress plants that were kept in refrigerated cells for 6 weeks did not exhibit any damage.

sprout into new vegetation as the control plants.

Concerning physiological responses of cypress plants during refrigerated storage, ST had a significant effect on all considered parameters, except for carotenoid content, while Tr x ST interaction significantly influenced all parameters of cypresses except for MDA content (Table 1). Only MDA was significantly affected by plant treatment with antiperspirants. More specifically, plants sprayed with tap water only (C) showed significantly higher level of MDA (190.60 µmol/g DW) than those treated with Barzaghi biodegradable antiperspirants (A and B) and Vapor Gard® (V), evidencing a higher level of stress of the untreated plants. MDA values were lowest in B and V treated plants (114.97 and 109.76 µmol/g DW, respectively), highlighting some protective action of these antiperspirants on plants subjected to transport stress, even if the phenotypic analyses did not show significant differences among treatments.

In general, cypress plants kept in open air (OA) showed the highest values of phenols (Fig. 5), carotenoids, chlorophyll a and b compared to stored plants. These plants suffered a late spring frost in mid-April (-0.9°C to -3.3°C from h 4:00 am to h 9:00 am on April 8, 2021). Since plants exposed to various abiotic stress conditions produce many secondary metabolites, including phenolic compounds and carotenoids, in higher concentrations (Yeshi *et al*., 2022), it can be hypothesized that in our experiment plants may have produced high amounts of phenols and carotenoids in response of spring frost hazard. Moreover, chlorophyll a and b decreased significantly from T1 to T2, indicating a reduction in plant photosynthetic activity during transport over a long period. Indeed, it is well known that stressed plants reduce plant metabolism, especially photosynthetic activity, in order to resist adverse conditions (Starman *et al*., 2007).

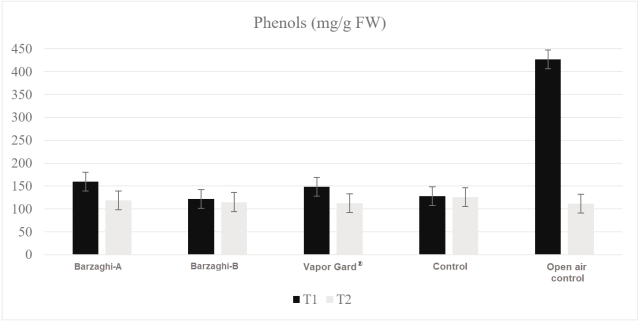

Fig. 5 - Effect of treatment x storage time interaction on phenols content of potted cypress. Error bars indicate the standard error of the mean. T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

In privet (Fig. 6 a) and in viburnum (Fig. 6 b), no apparent differences were observed in the growth of plants stored in the refrigerated cell and of control plants maintained in the field (data not reported). About plant development, it was noted that all plants treated with antiperspirants (A, B, V) were characterized by new shoot sprouting, which was absent in all plants not sprayed with antiperspirants (C, P, OA) (Fig. 6 a, b), the meaning of this phenomenon should be furtherly analysed. Moreover, as it was noted also on cypress, all plants of privet and viburnum treated with Vapor Gard® had shinier and brighter green leaves: this was due to the oily matrix of the product which creates this pleasant optical effect. All cold

Table 1 - Effect of storage time (ST) and treatment (Tr) on oxidative stress (MDA), phenols, carotenoids, and chlorophyll contents of potted cypress

Source of variation	MDA µmol/g DW	Phenols mg/g FW	Carotenoids µg/g FW	Chlorophyll a µg/g FW	Chloropyll b µg/g FW
Storage time (ST)	**	**	NS	**	*
T1 = 2 weeks	162.59 a	196.83 a	0.048	0.277 a	0.195 a
T2 = 6 weeks	119.27 b	116.77 b	0.03	0.169 b	0.122 b
Treatment (Tr)	**	NS	NS	NS	NS
Barzaghi-A	141.79 b	139.14	0.025	0.191	0.152
Barzaghi-B	114.97 c	118.5	0.023	0.148	0.114
Vapor Gard ®	109.76 c	130.68	0.026	0.199	0.158
Control	190.60 a	126.64	0.031	0.186	0.123
Open air control	151.31 b	269.05	0.081	0.391	0.248
ST x Tr	NS	**	**	**	*

** significant at p≤001; * significant at p≤005; NS= not significant. Mean values within each column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Fig. 6 - In privet (6 a: left, Vapor Gard®; right, Control) and in viburnum (6 b: left, Vapor Gard®; right, Control), it was noted that all plants treated with antiperspirants were characterized by new shoot sprouting; moreover, all plants treated with Vapor Gard® had shinier and brighter green leaves.

stored plants recovered after being transferred to the open field at the end of May, resulting in final growth developmental patterns like control plants at the end of October (data not reported).

In privet, A, B and V antiperspirant treated plants showed a significantly lower phenol content, while storage time significantly affected carotenoids content (Table 2). In addition, a statistically significant interaction between these factors was found for phenols and chlorophyll a. The untreated OA and C plants showed the highest phenol values: privet outdoor plants experienced spring frost disturbance in mid-April, as described for cypress, while the higher phenols content in C plants suggests that untreated plants get stressed by transport conditions more than plants treated with antiperspirants (A, B and V) (Fig. 7). Carotenoid content raised in cold stored privet plants from T1 to T2, indicating that plant stress

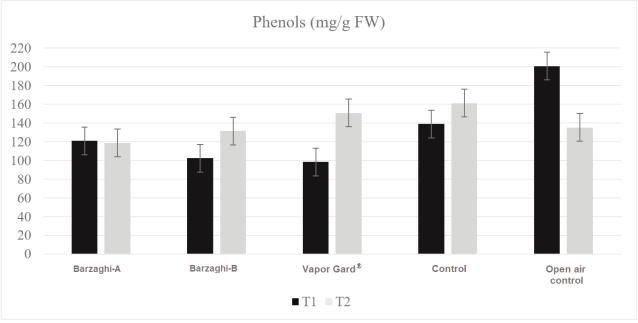

Fig. 7 - Effect of treatment x storage time interaction on phenols content of potted privet. Error bars indicate the standard error of the mean.T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

Table 2 - Effect of storage time (ST) and treatment (Tr) on phenols, carotenoids, and chlorophyll contents of potted privet

Source of variation	Phenols mg/g FW	Carotenoids µg/g FW	Chlorophyll a µg/g FW	Chloropyll b µg/g FW
Storage time (ST)	NS	**	NS	NS
T1 = 2 weeks	132.3	0.104 b	0.518	0.324
T2 = 6 weeks	139.5	0.166 a	0.619	0.276
Treatment (Tr)	**	NS	NS	NS
Barzaghi-A	119.78 bc	0.142	0.577	0.282
Barzaghi-B	116.90 c	0.125	0.514	0.275
Vapor Gard ®	124.61 bc	0.11	0.479	0.256
Control	150.17 ab	0.171	0.717	0.366
Open air control	168.06 a	0.128	0.555	0.322
ST x Tr	**	NS	*	NS

** significant at p≤001; * significant at p≤005; NS = not significant. Mean values within each column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

increased during transport with increasing ST (Table 2). On the contrary, phenols content in OA plants reached the highest value in April (T1) due to late spring frost damages, but thereafter levels were cut down to a normal range within 6 weeks (Fig. 7). Chlorophyll pigment molecules play a key role in photosynthesis; plants use chlorophyll to absorb light and convert it into chemical energy (Bollivar, 2006). In privet plants, chlorophyll content increased from T1 to T2 in both treated (A, B, and V) and untreated (C) plants maintained inside the cold container, while the untreated open-air (OA) plants showed an opposite trend (Fig. 8). In this context, it is probably realistic to assume that the increase in chlorophyll content might be related to water loss occurring in leaves during prolonged storage or transportation rather than to an actual increase in photosynthetic activity (Ferrante *et al*., 2015). The data regarding MDA analysis were not considered for privet. In fact, the method used to assess MDA was the thiobarbituric acid (TBA) reactive substance assay. This analysis is simple and quick, but it was found to be ineffective for privet species as pointed out by Wang *et al*. (2013). Indeed, it seems that there are substances present in the leaves of this species that interfere with the TBA reagent.

In viburnum species, ST significantly affected carotenoids and chlorophylls contents (Table 3), with highest values (more shiny leaves) found in plants at T1, contrary to what was recorded for privet. MDA levels were influenced by both antiperspirant treatment and Tr x ST interaction. Indeed, MDA values were highest in the control and in plants treated with antiperspirants A and B after 6 weeks of cold storage (T2), indicating that viburnum shrubs get more stress with increasing storage time (Fig. 9). On the other hand, Vapor Gard® (V), seemed to exert some protective action on viburnum plants over time. As expected, plants maintained in open air showed no

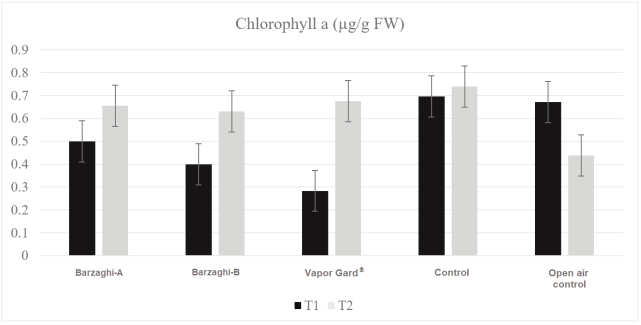

Fig. 8 - Effect of treatment x storage time interaction on chlorophyll a content of potted privet. Error bars indicate the standard error of the mean.T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

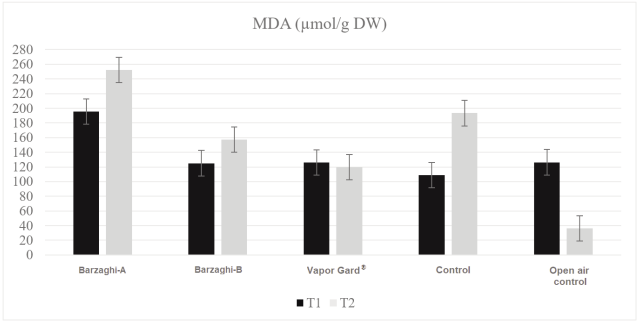

Fig. 9 - Effect of treatment x storage time interaction on malondialdehyde content of potted viburnum. Error bars indicate the standard error of the mean.T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

Table 3 - Effect of storage time (ST) and treatment (Tr) on oxidative stress (MDA), phenols, carotenoids, and chlorophyll contents of viburnum privet

Source of variation	MDA µmol/g DW	Carotenoids µg/g FW	Chlorophyll a µg/g FW	Chloropyll b µg/g FW
Storage time (ST)	NS	**	**	**
T1 = 2 weeks	136.22	0.233 a	0.918 a	0.438 a
T2 = 6 weeks	151.77	0.043 b	0.226 b	0.117 b
Treatment (Tr)	**	NS	NS	NS
Barzaghi-A	223.97 a	0.15	0.555	0.261
Barzaghi-B	141.15 b	0.124	0.513	0.242
Vapor Gard ®	122.73 b	0.101	0.501	0.286
Control	150.78 b	0.145	0.626	0.302
Open air control	81.34 c	0.169	0.665	0.296
ST x Tr	**	NS	NS	NS

** significant at p≤001; * significant at p≤005; NS = not significant. Mean values within each column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

signs of stress over the long term.

It is likely that dark conditions and lack of water over a 6-week period are not limiting factors for the tested species once the optimal conditions of temperature and humidity are met into the container, as it was during our trials. Potted plants must be adequately prepared and carefully handled before long-distance transport to overcome problems of this transitory phase by reducing both the plant's metabolism and normal physiological processes.

In general, even if phenotypical data did not show evident differences for plant growth between long term stored and not stored plants, the physiological analyses on cypressus, privet and viburnum showed interesting significant differences for MDA, phenols and carotenoids: these parameters seem to be correlated to abiotic storage stress of plants and thus could be useful in further studies to monitor the quality of plants before, during and after storage for short, mid and long times in refrigerated cells. This could also help various sectors of the post-harvest ornamentals supply chain to: i) assess the potential quality of plants before shipment; ii) improve plant transport conditions; iii) monitor plant quality throughout the various stages of shipping "from farm to buyer", through the various steps with other components of the supply chain (transporters, wholesalers, markets); iv) understand whether any deterioration in the quality of the plants at the end of the travel was perhaps due to non-maintenance of the optimal conditions envisaged during transport, due to negligence by the operators.

4. Conclusions

Plant tolerance to storage conditions in refrigerated cell or container (T° = 8-12°C) varied considerably according to the considered species. Cypress proved to be extremely tolerant to storage conditions over long periods. Maple and nandina, on the contrary, resulted the most sensitive species to medium- and long-distance transport with a high percentage of fallen or rotten leaves occurring during spring storage in refrigerated cells. Treatments with antiperspirants did not exhibit particularly evident effect on quality value (plant growth and aesthetic appearance of the leaves) in plants kept in the dark in a cold room or container. Only the antiperspirant Vapor Gard® seemed to improve the aesthetic appearance of cypress, viburnum, and privet with shinier and brighter green leaves, probably due to the oily matrix of the product. Furthermore, even in cases of total or partial loss of leaves by species such as maple and nandina, an optimal recovery of vegetative development was highlighted once these species were relocated in outdoor cultivation. The use of the tested biodegradable film was inadequate to protect plant quality during long-distance shipments, thus, further research is needed to improve microfilm performances by changing its thickness and composition. Among physiological parameters, MDA, phenols, and carotenoids contents were the most stress-related variables, being negatively correlated to the quality decay of plants transported in dark refrigerated cells for 2-6 weeks. It is a preliminary study and some uncertainty and/or not complete discussion are due to the lack of some measurement (i.e. leaf colour)., however these parameters could be useful in further studies to monitor the quality of plants before, during and after storage for short-, mid- and long-term transport in refrigerated containers.

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Application of computer vision systems for assessing bergamot fruit external features

S. Benalia (*), V. Calogero, M. Anello, G. Zimbalatti, B. Bernardi
Dipartimento di Agraria, Università degli Studi Mediterranea di Reggio Calabria, Località Feo di Vito, snc, 89122 Reggio di Calabria (RC), Italy.

Key words: Aspect ratio, *Citrus x bergamia* Risso & Poiteau, citrus colour index (CCI), dimensions, HunterLab, imaging, RGB.

(*) **Corresponding author:**
soraya.benalia@unirc.it

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Bergamot *Citrus x bergamia* Risso & Poiteau is an emblematic Citrus species of Reggio Calabria province (Southern Italy) where more than 90% of the global production thrives. The present work deals with the use of a non-destructive technique based on a computer vision system to evaluate bergamot fruit peel colour, as well as dimensional features. To this purpose, experimental trials considered three bergamot cultivars, namely 'Femminello', 'Castagnaro' and 'Fantastico'. Bergamot fruit RGB images were taken using a laboratory inspection chamber equipped with a lighting system and a digital camera Nikon D5200 directly connected to a personal computer, to enable remote image acquisition. First, images were pre-processed according to a previously created colour profile. After that, bergamot fruit colour was analysed and expressed in terms of Hunter *L*, *a*, and *b* coordinates, which were used to calculate Standard Citrus Colour Index (CCI). In addition, dimensional features and shape descriptors were measured for each cultivar. Statistical data analysis, by applying the Kruskal-Wallis test at $p<0.05$ on CCI data highlighted significant differences between the assessed cultivars, and discriminant analysis (LDA) applied on CCI and dimensional features enabled a classification rate of 78.86% between cultivars, proving the reliability of computer vision techniques in assessing bergamot external features.

1. Introduction

Bergamot *Citrus x bergamia* Risso & Poiteau (Pellegrino *et al.*, 2015) is an emblematic Citrus species of Reggio Calabria province (Southern Italy), where more than 90% of the global production is located. Until recently, the production has been exclusively destinated to peel essential oil extraction and subsequent use in perfumery, cosmetic and pharmaceutic preparations, food flavouring and confectionery (Navarra *et al.*, 2015; Gioffrè *et al.*, 2020). However, the rising interest toward bergamot-based food and beverage for their high content in functional bioactive compounds is directing the production toward other market channels. This trend is also accompanied by the necessity of agrifood industries to devel-

op particularly from a technological point of view, to keep up and adequately address global market requirements and standards. In fact, great attention has been paid, in the last years, to the development and implementation of non-destructive techniques based on artificial intelligence in industrial processes including agrifood sector, for their reliability, accuracy, and timeliness in determining qualitative parameters of the product, especially when employed in automated post-harvest processes. Several studies dealt with the use of computer vision systems for citrus fruit quality assessment or grading (Cubero *et al*., 2014; Lorente *et al.*, 2015; Zhang *et al.*, 2020; Riccioli *et al.*, 2021; Zhang *et al.*, 2022), but up to now, no one regarded bergamot fruits. In this context, the present work aims at assessing fruit peel colour, expressed in terms of Standard Citrus Colour Index (CCI) as well as dimensional features and shape descriptors, including fruit area, fruit perimeter, major axis, minor axis, circularity, roundness, aspect ratio and solidity of three cultivars of bergamot fruit, by means of computer vision systems.

2. Materials and Methods

Bergamot samples

Experimental trials considered three bergamot cultivars, namely: *Citrus x bergamia* Risso & Poiteau, 'Femminello', 'Castagnaro' and 'Fantastico'. Fruits were collected at the end of their ripening stage (since mid-January to the beginning of February) in private farms located in Reggio Calabria province. A total of 248 fruits were used.

Methodology set-up and RGB image acquisition

Bergamot RGB image acquisition was performed using a laboratory squared inspection chamber equipped with a digital camera Nikon D5200 directly connected to a personal computer, to enable remote image acquisition, and a lighting system including 4 fluorescent linear lamps (BIOLUX T8- 18W/965, 6500 K) placed on the top of the inspection chamber in a 0°/45° configuration as shown in figure 1. The used camera was equipped with a CMOS 23.5 x 15.6 mm sensor, allowing getting a resolution of 24.1 million pixels; and a Nikkor f/2.8G lens with a 1:1 reproduction ratio.

Prior to image acquisition, several configurations of operational parameters were tested until getting the most appropriate one, which considered the following parameters: ISO: 100; exposition time: 1/125; diaphragm opening f: 5.6; 4 lamps (instead of 8), without polarizing filter.

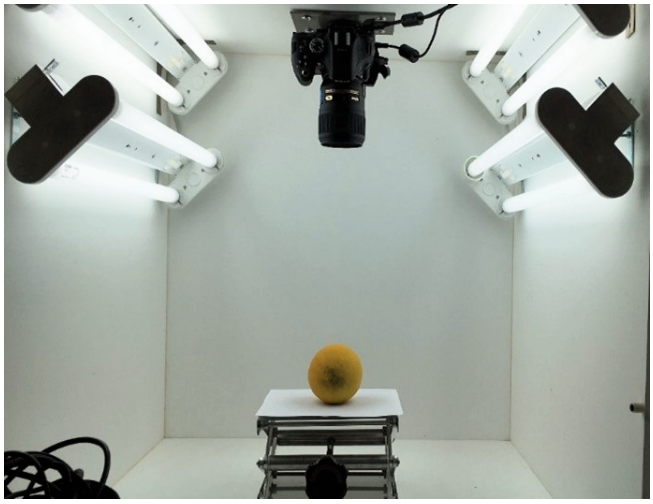

Fig. 1 - Bergamot RGB image acquisition using a laboratory inspection chamber equipped with a computer vision system.

Image analysis and bergamot colour determination

Bergamot images were calibrated, first, by performing the white balance, and subsequently the chromatic correction according to a previously created profile with the ColorChecker Classic target (X-Rite Inc., USA), through the Colorchecker Passport Software as performed in Benalia *et al.* (2017).

Bergamot RGB images were then analysed as perfomed by Benalia *et al.* (2016) using a specific application (Fig. 2) that enables to convert mean R, G, B values of all pixels contained in a selected Region Of Interest (ROI) into *L*, *a*, *b* coordinates considering the CIE illuminant D65 and the 10° observer standard (Cubero *et al.*, 2018).

Besides and for comparison purpose, bergamot

Fig. 2 - Bergamot colour determination through RGB image analysis.

fruit colour was determined by means of a portable Konica Minolta CM-700D spectrophotometer being the only widely standardized instrument used for instantaneous food colour evaluation in agrifood industries. To this end, six measurements were carried out randomly on each fruit.

Determination of dimensional features and shape descriptors

Dimensional features and shape descriptors, including fruit area in the image, fruit perimeter, major axis, minor axis, circularity, roundness, aspect ratio and solidity were determined using the software ImageJ 1.50i using Java 1.8.0.77 (Fig. 3), according to a previously set scale.

Data analysis

Hunter *L, a* and *b* values were used to calculate the Standard Citrus Colour Index (CCI) according to the following formula (Eq. 1) and to compare whether there is a difference between the examined cultivars.

$$CCI = 1000\, a/(L \cdot b) \quad \text{(Eq. 1)}$$

With *L* value indicating lightness (0 = darkness -> 100 = lightness), *a* value representing red (positive value) or green (negative value); and *b* representing yellow (positive value) or blue (negative value).

CCI data were statistically analysed using the open-source application R (version 3.3.1). Hence, data normal distribution was checked by applying the Shapiro-Wilk normality test, and according to the results, the most appropriate test was applied to compare the examined cultivars in terms of colour features.

Furthermore, CCI data as well as dimensional features and shape descriptors retrieved from bergamot RGB image analyses were used to build an exhaustive dataset and multivariate image analyses (MIA), particularly, Principal Component Analysis (PCA) and Discriminant Analysis (LDA) were applied using Past 4.12b (Hammer *et al.*, 2001).

3. Results and Discussion

Citrus Colour Index (CCI) values calculated from bergamot image analysis were compared with those obtained using the portable spectrophotometer for each cultivar. The obtained results are shown in the following figure 4. Particularly, the coefficient of

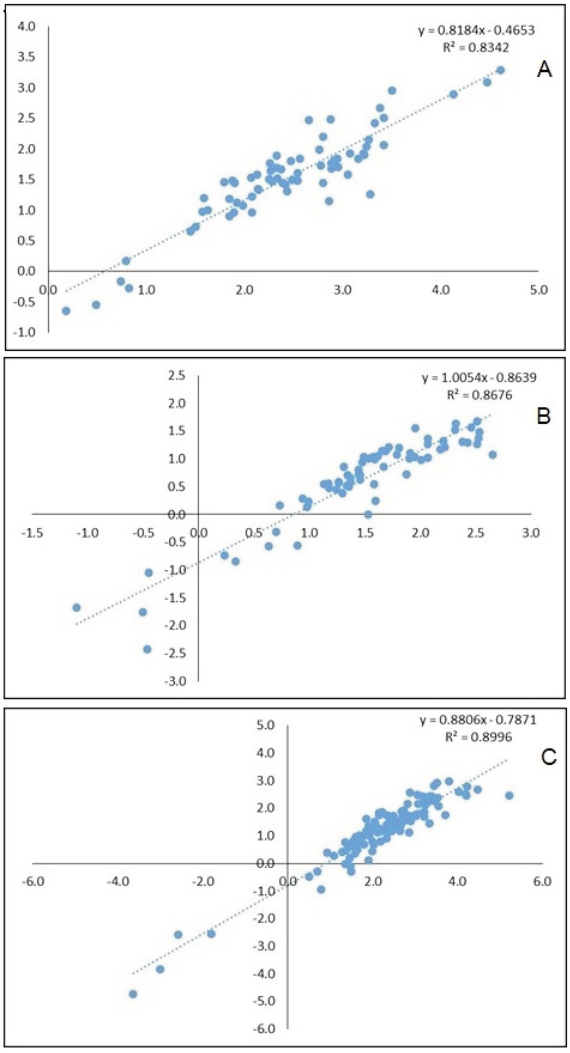

Fig. 4 - Standard Citrus Colour Index (CCI) from Image analysis Vs portable spectrophotometer. A: 'Castagnaro', B: 'Fantastico and C: 'Femminello'.

Fig. 3 - Bergamot dimensional feature and shape descriptor calculation through RGB image analysis using ImageJ.

determination R^2 was equal to 0.83, 0.87 and 0.90 respectively for the cultivars Castagnaro, Fantastico and Femminello, making it possible to substitute colour analysis using conventional instruments with that based on image analysis. Indeed, diversely from the portable spectrophotometer, which offers the possibility to only analyse a small area of the fruit surface, image analysis allows assessing fruit colour of the whole surface of each fruit.

The obtained R^2 values in this study are, however, lower than those reported by Vidal *et al.* (2013), who tested two algorithms for the estimation of oranges cv. Navelina CCI based on computer vision. This difference could mainly be attributed to the difference in the used hardware and operation parameters. Indeed, up to now, a standardized methodology concerning the use of CVS is still missing.

The results of Shapiro-Wilk test demonstrated that CCI data do not follow a normal distribution. Therefore, the non-parametric test Kruskal-Wallis was applied.

The outputs highlighted a significant difference between the cultivars in terms of CCI at $p<0.05$ (Table 1). CCI mean and median values are reported in Table 2. CCI mean values were equal to 3.01±1.51 for cv. Castagnaro and 2.36±2.26 for cv. Femminello, indicating a yellowish colour, while it was much lower for cv. Fantastico with a value of 1.21±1.52 corresponding to a greenish colour yet (Table 2).

As mentioned above, multivariate image analysis, particularly, Principal Component Analysis (PCA) and Discriminant Analysis (LDA) were applied considering CCI values as well as dimensional features and shape descriptors. Hence, the three cultivars were compared according to 9 variables. PCA highlighted that most of the variability was expressed by the first two components, with respectively 46.86% for PC1 and 21.38% for PC2. The first component PC1 is mostly and positively influenced by fruit area, fruit perimeter as well as by major and minor axis. To this regard, Giuffrè (2019) and Giuffrè and Nobile (2020) found significant differences between the bergamot cultivars when comparing both major and minor axes. However, diversely from our method they used conventional measuring method. The first component PC1 is also negatively influenced by roundness. The second component PC2 is, however, positively, and mainly influenced by roundness and subsequently solidity, while it is negatively affected by CCI and aspect ratio (Fig. 5).

Table 1 - Results of Kruskal-Wallis test for CCI according to the cultivar

Test	χ^2	*df*	*p*
Kruskal-Wallis: (CCI by cultivar)	46.55	2	$7.793e^{-11}$

Table 2 - Mean and median values of CCI according to the cultivar

Cultivars	Mean values ± St dev.	Median values
Castagnaro	3.01 ± 1.51	3.05
Fantastico	1.21 ± 1.52	1.57
Femminello	2.36 ± 2.26	2.61

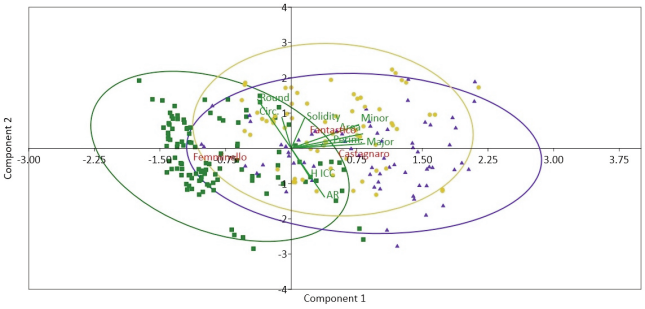

Fig. 5 - PCA score plot. Green filled square: 'Femminello', purple filled triangle: Castagnaro, yellow dots: Fantastico.

A better discrimination between the cultivars was obtained when LDA was applied (Fig. 6), and 80.49% of correctly classified fruit according to cultivar was reached. This rate decreases to 78.86% when Jackknif cross-validation method is applied (Table 3).

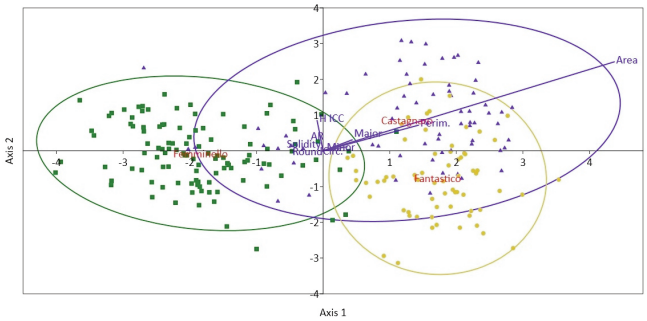

Fig. 6 - LDA plot. Green filled square: Femminello, purple filled triangle: Castagnaro, yellow dots: Fantastico.

This study focuses on the use of computer vision system to retrieve colour and dimensional features with the aim of distinguishing between three cultivars grown in the province of Reggio Calabria. As previously stated, no work dealing with this theme have

been performed so far. To our knowledge the only one, which reports the characterization of bergamot physical properties using CVS is that of Rafiee *et al.* (2007), who measured fruit size, mass, projected area, fruit density, solid density, bulk density, bulk porosity, packing coefficient, density ratio, geometric diameter, sphericity and surface area of *Citrus medica*, which characteristics are different from *Citrus x bergamia* Risso & Poiteau.

Table 3 - Jackknife cross validation results resulting from discriminant analysis (LDA)

Given groups	Predicted groups			Total
	Femminello	Castagnaro	Fantastico	
Femminello	99	8	3	110
Castagnaro	13	42	14	69
Fantastico	0	14	53	67

4. Conclusions

The obtained results in this study confirm the reliability of computer vision systems in characterizing bergamot fruit external properties, considering particularly, colour and dimensional features. Although preliminary, the outputs clearly demonstrate the efficiency and accuracy of these techniques in distinguishing the examined cultivars, promoting therefore their implementation in bergamot post-harvest processes. Indeed, both features, i.e., colour and dimensions are important to be considered for the development of new technologies as they represent important qualitative indicators.

Further research activities should be performed to validate findings obtained in this study, and to investigate other aspects using CVS such as ripening progress and essential oil peel content.

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AHS
Advances in Horticultural Science

Sanitization system in horticultural sector

L. Buglia
Fruit Control Equipments Srl, via R. Luxemburg, 55, 20085 Locate di Triulzi (MI), Italy.

Key words: fruit preservation, ionization, sanitation, sanitization, storage.

Abstract: The food industry has recognized the importance of environmental sanitation, and fruit control, a renowned leader in controlled atmosphere, has invested in sanitation through the use of ionizers to eliminate microorganisms in agri-food environments. This report presents results of tests conducted on various products, both in experimental and real scenarios in fruit and vegetable distribution platforms, to evaluate the effectiveness of ionization on vegetable products. The report covers three different situations: the first two focused on the effects of ionization on radish and table grape cells, while the last test verified the impact of ionization on a distribution platform that processes and markets various types of fruits and vegetables, such as pepper, tropical fruits, blueberry, apples, pears, table grape, chicory, and more.

(*) **Corresponding author:**
buglia@fruitcontrol.it

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

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1. Introduction

The preservation of fruit and vegetables has always gone hand in hand with the need to ensure the healthiness of the stored products. In fact, it is necessary that the products are stored in the best conditions, from the organoleptic but also from the health point of view (Gnanasekharan *et al*., 1992; Karabulut *et al*., 2004; Del-Valle *et al*., 2005). Lately this aspect has become increasingly important, especially for greater protection for consumers using physical means such as irradiation (γradiation), ultraviolet-C light, sub-atmospheric pressure, high hydrostatic pressure and ionized gases (gas plasma) (Romanazzi *et al.*, 2001; Moreau *et al*., 2008; Tappi *et al*., 2014; Papoutsis *et al*., 2019).

Ionization consists in the generation of one or more ions due to the removal or addition of electrons from a neutral molecular entity which can be caused by collisions between particles or by absorption of radiation (Lin and Lin, 2017; Baggio *et al*., 2020; Tanaka, 2022) The atoms or molecules that have a number of electrons lower than the atomic number remain positively charged and are called “cations”; those that have a number of electrons greater than the atomic number, remain negatively charged and are called “anions” (Forney *et al*., 2001; Fan *et al*., 2002; Lin and Lin, 2017; Tanaka, 2022).

Many tests and researches have been done in collaboration with research institutes and universities on many fruit and vegetables (i.e. mango, melon, lettuce, apple, kiwifruit) taking into consideration multiple parameters (acidity, hardness, color, etc.) (Perni *et al*., 2008; Tamaki and Uyama, 2008; Bernardinelli *et al*., 2012; Tappi *et al*., 2014; Ramazzina *et al*., 2015; Tappi *et al*., 2016, Woo *et al*., 2017).
This report is a demonstration of some of the tests done in last years, especially at industrial level, and they take in consideration the results relating to the phytosanitary aspects, the subject of this symposium. The tests were conducted with the equipment of our production called "IONNY". IONNY is produced in different versions and sizes depending on the volume of the cells in which it is installed. There are also cabinet versions intended for research institutes.

The tests carried out and reported are essentially aimed at verifying the elimination of micro-organisms during the conservation and processing of fruit and vegetables, with the aim of verifying whether ionization can actually be considered a valid system for preventing rotting and sanitizing such products. The machines used were sized according to the volumes of the cells and the environments in which they were installed.

2. Materials and Methods

Plants material and experimental setup

Three separate tests were conducted:

First test done in cold room containing radish (*Cicorium intybus*) using a Ionny 400;

Second test done in cold room containing table grape (*Vitis vinifera,* cv. Italia) with Ionny 400;

Third test done in a cold warehouse (CE.DI.OR, Zelo Buon Persico, Lodi, Italy). Inside this cold warehouse three different areas were tested (cold rooms, processing, and shipping rooms) using ionizers mod DUCT as compared to control (without ionizer).

Radish test

The test was carried out at GEOFUR, Gallese Verona, Italy, in a storage warehouse where radishes were stored in a refrigerated cell with a volume of 400 m^3. The ionization process was performed using an IONNY 400 and the storage room was maintained at a temperature of -1°C and a relative humidity of 95%. The radishes were stored in bins measuring 110 cm x 11 cm x 56 cm. The IONNY 400 ionizer was capable of covering a volume of up to 400 m^3, making it suitable for the test.

Table grape test

To ionize table grapes cv Italia that were stored in a refrigerated cell measuring 400 m^3, an IONNY 400 (mod. Industrial, NOACOOP - Noicattaro BA - Italy) was used. The ionizer effectively covered the entire volume of the cell. The grapes were harvested from the same orchard and stored in wooden boxes weighing 15 kg each. The storage warehouse had a volume of 300 m^3 and was maintained at a temperature of 4°C and a relative humidity of 95%.

Warehouse platform test

The experiment was carried out in a storage warehouse located in Zelo Buon Persico LO, Italy, called CE.DI.OR. The warehouse stored and processed various types of fruits and vegetables under real conditions. To ensure the ionizator machines had the appropriate capacity for each area, one or more units (model DUCT) were installed in each room without any construction work needed. The machines were fixed in the upper part of the rooms, near the evaporator positioned in the suction area. The products were stored in different types of packaging, such as bins, wooden boxes, and cardboard boxes of varying sizes. The storage temperature ranged between 0-10°C. Specifically, during the experiment, the temperature was between 1-4°C in the cold storage and 10-15°C in the processing and shipping areas of the logistic structure CE.DI.OR. The humidity level was between 85-90% in the cold rooms and 75-89% in the processing and shipping areas. Additionally, during the experiment, the amount of ozone generated (tested only for this experiment with a manual analyzer) did not exceed 0.06 ppm inside the room, even after 8 hours of operation.

Petri dishes containing Tryptic Soy Contact Agar + LT + ICR were positioned in the storage room and other areas at ground level, specifically in the corner of the cold room store (Fig. 1): cold room (which was totally full) for test 1 and 2; cold room, processing area, and shipping room for test 3. These Petri dishes were placed at a height of approximately 70 cm. The air sampling procedure and incubation conditions of the plates were carried out as described below.

Microbial analysis during storage

For each of the tests described above, Petri dishes (plates) (90 mm diameter) containing Tryptic soy contact agar + LT + ICR (Merck, Darmstadt, Germany)

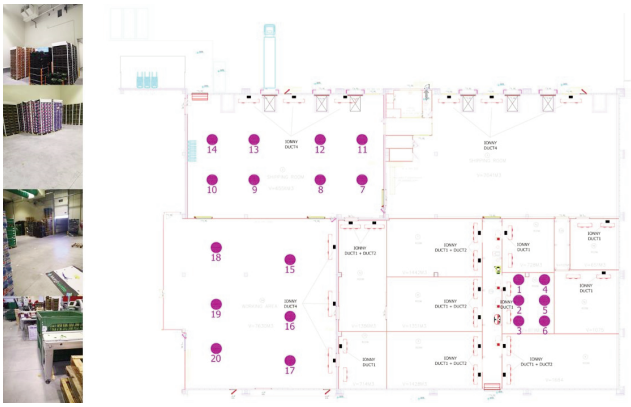

Fig. 1 - Cedior logistic platform, position of Petri dishes (violet) and model of IONNY used.

were positioned by hand (with the use of gloves in order to ensure absolute non-contamination by the operator) in the area of interest according to the test performed in each case investigated. Air samples were taken before the use and after the operation of the ionizers used in each case. During air sampling, plates remained open for 1 min and then closed again. Incubation of the plates took place at 18-22°C for 7 days and the colonies developed in each Petri dish were counted, after dividing them into fungi (black-grey), bacteria (yellow) and yeasts (white). For the determination of total yeast and mold counts, plates were incubated at 20-25°C for 5-7 days (for all the test done). Results were calculated as colony forming units (CFU) per m^3 using the following equation proposed by Omelyansky (1940), $N(CFU\ m^{-3})=5a \times 10^4 (bt)^{-1}$, where: a is the number of colonies counted per Petri dish, b the Petri dish area (in cm^2), and t the exposure time (in min) and further expressed as CFU m^{-3} x 10^3 (Viani *et al*., 2020).

Ion cluster generator and its principle

When a voltage is applied to electrode plate with the air between them, if the applied voltage exceed a certain level, the air layer is ionized to flow the current between electrode plates, creating, so-called, an arc discharge. If electrode plates are separated by 1 cm, when at 30.000 volts is applied, a discharge occurs (in the air, if the intensity of electric field is more than 30 kv/cm, a discharge occurs). At this time, ions are created, they are bonded with surrounding water molecules to produce, so-called, ions. There is no other fluid used except air. Ventilation is due to evaporator ventilation (ducted model) or internal fan (in case of industrial model).

3. Results and Discussion

The effects of IONNY operation in a cold room for radish storage and in a general purpose fruit and vegetable distribution platform (industrial test) are presented in figure 2. Not using IONNY in a cold room containing radishes, bacterial counts ranged between 7.07 and 20.43 CFU m^{-3} x 10^3, while fungi populations reached up to 2.36 CFU m^{-3} x 10^3. The use of IONNY resulted in a dramatic decrease of both bacteria and fungi, with numbers found at levels of 0.79 CFU m^{-3} x 10^3 for both. Overall, the reduction of air microorganisms (fungi and bacteria) inside the cold rooms containing radishes with the use of IONNY is clearly indicated in figure 2. This microbial reduction might be attributed to the formation of hydroxyl radical ($OH^{\cdot}$) during air ionization. This radical can compromise the function of the microbial membranes due to oxidation of the unsaturated fatty acids in the membranes (Zhang *et al*., 2019). In addition, the different structure of the bacterial and fungal cell wall could also influence the susceptibility to ionization, as it has been reported that chitin the main component of

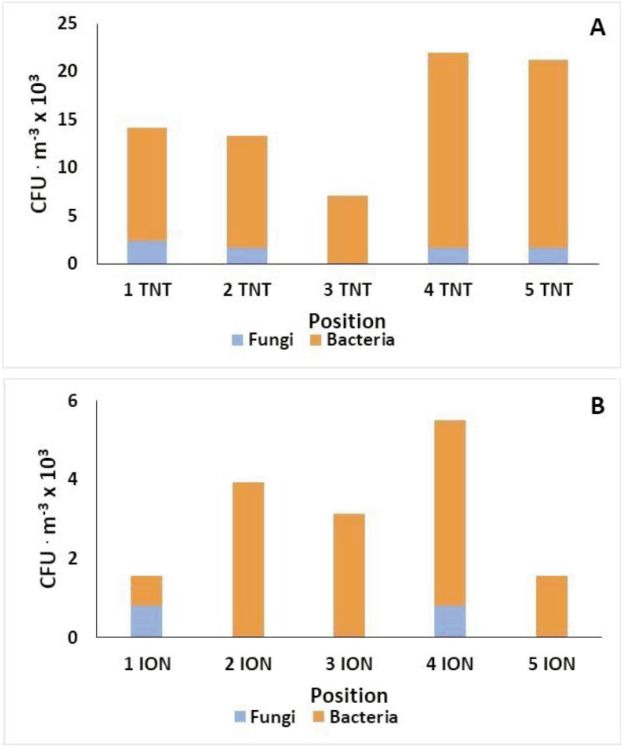

Fig. 2 - Microorganism inside a cold room during storage of radish without (A) and with (B) IONNY use after 48h from switching on of ionizator. TNT: not treated commodity; ION: IONNY use; 1-5: positions inside the cold room where the Petri dishes were placed.

fungal cell wall is a more rigid material compared to peptidoglycan (Liang *et al*., 2012).

A diversity among bacteria, fungi and yeasts populations was observed in different areas of a cold storage in a distribution platform (industrial test) when the IONNY was not used (for 48 h) (Fig. 3A). However, the use of IONNY for 48 h resulted in significant decrease of the tested microorganisms. In this case, the total effectiveness of IONNY was equal to approx. 70% and the effect of this reduction was found similar to all the investigated microorganisms (fungi, bacteria and yeasts). These findings are not in accordance with a previous study were cold atmospheric plasma treatment (created by dielectric barrier discharge-DBD) on ginseng seed surface presented great bactericidal and fungicidal activity, with fungi found to be more susceptible than bacteria (Lee *et al*., 2021). This difference might be attributed to the time/duration of application, the type of ionizer used and the microorganisms exposed; among other factors (Arnold *et al*., 2004; Tappi *et al*., 2014; Zhang *et al*., 2019).

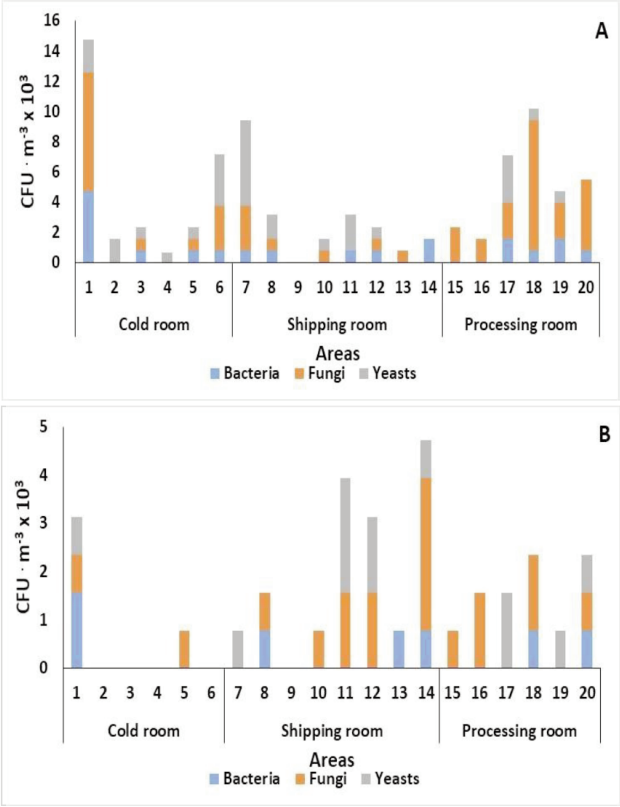

Fig. 3 - Bacteria, fungus and yeasts inside a cold room without (A) and with (B) IONNY use for a period of 48 h inside different areas of a commercial distribution warehouse platform. 1-20: positions inside the warehouse where the Petri dishes were placed.

The effectiveness of IONNY on the different areas examined (cold room, processing room and shipping room) was more or less similar. Moreover, fungi were absent and/or in low populations after the treatment with IONNY (except in some cases) (Fig. 3B). These findings suggest that the use of IONNY can lower the risk of fresh fruit and vegetables contamination in an industrial level. A previous study showed a reduction of airborne and surface *Salmonella* Enteritidis artificially (generated aerosol) ranging from 72 up to 98% when exposed to negative air ions (Seo *et al*., 2001). The differences between the microorganisms examined inside the three investigated areas in the distribution warehouse especially prior the use of IONNY might be attributed mainly to the different actions that take place there. For instance, more movement and increased air exchange might be observed more frequently in the processing and shipping room compared to the cold room. Also, the different temperatures of the areas might also affect the air's microbial load i.e. cold room temperature: 1-4°C, whereas processing and shipping room temperature: 10-15°C.

The use of IONNY during cold storage of table grapes for 21 days, showed a decrease in molds populations inside the room after 10 and 21 days, compared to non-treated room (Fig. 4A). This phenomenon was more evident on the 21st day of storage. When high voltage atmospheric cold plasma was applied against *Aspergillus flavus* spores, inactivation of spore forms was observed as well as degradation of fungal culture and its mycotoxin (deoxynivalenol) (Ott *et al*., 2021). Moreover, in the present work yeasts population was found to decrease in a room used for cold storage of table grapes when IONNY was applied, 10 and 21 day after application (Fig. 4B). The numbers of yeasts were decreased during the 21 days of storage for both treated and un-treated with IONNY samples, with greater decrease been seen on the 21st day with the IONNY use (1.02 and 1.57 CFU m^{-3} x 10^3, respectively).

Kikuchi *et al*. (2020) showed that dielectric barrier dischargers presented great antifungal activity against an airborne fungi (*Penicillium italicum*) (up to 2.5 log decrease), whilst spores adhered to the ionizers were not affected. The antimicrobial effects of ionization on molds and yeasts might be attributed to the ionization of hydroxyl groups, atomic oxygen and nitrogen and the subsequent production of reactive oxygen and nitrogen species (ROS and RNS) i.e. ozone (O_3), nitrogen dioxide (NO_2), nitrate (NO_3), dini-

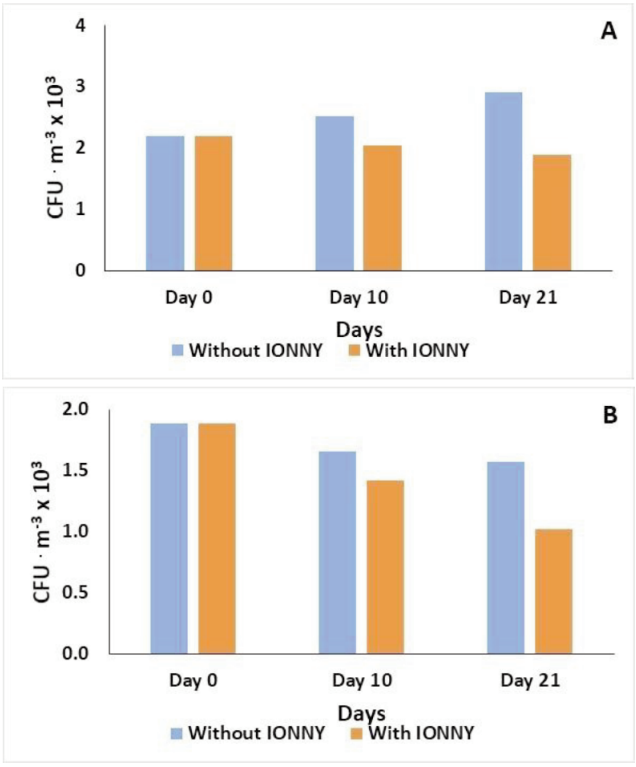

Fig. 4 - Populations of molds (A) and yeasts (B) inside a table grapes room without and with IONNY use for a period of 21 days.

trogen tetroxide (N_2O_4), and dinitrogen pentoxide(N_2O_5) (Ott *et al*., 2021). These oxidizing forms have been previously mentioned to interfere and decrease *Cordyceps pruinosa* spore viability, by damaging the fungal cell wall (deformation and deterioration), increasing permeability and cell components leakage (Kim *et al*., 2016). In table 1, different IONNY sanitization system models are available for different uses are shown.

Table 1 - Different IONNY sanitization system models are available for different uses

Model	Area coverage	Fluid used
IONNY 150 HOME AND OFFICE	up to 150 m^3	air
IONNY 300 HOME AND OFFICE	up to 300 m^3	air
IONNY 400	up to 400 m^3	air
IONNY EVO 1000	up to 800 m^3	air
IONNY EVO 2000	up to 1600 m^3	air
IONNY DUCTED 1	up to 1200 m^3	air
IONNY DUCTED 2	up to 2400 m^3	air
IONNY DUCTED 4	up to 4800 m^3	air
IONNY DUCTED 6	up to 7200 m^3	air
IONNY DUCTED 8	up to 9600 m^3	air

4. Conclusions

Ionization is a valid alternative sanitizing, physical method for the reduction of microorganisms (bacteria, fungi and yeasts), which can replace and eliminate the use chemical and other sanitizing systems in the food sector. This method does not cause harm to human health; neither does damage metallic surfaces. These benefits among others suggest that ionization can be used in a continuously manner without endangering the environment. In fact, if the ion source is properly sized relatively to the application volume, recommendations and limits issued by the USA Agency for Safety and Health at Work (OSHA) 0.06 ppm (50 ppb) for a continuous 8 h/5 days exposure (MAC 8 hrs) 0.3 ppm (300 ppb) for a 15 min exposure (MAC15min), can be completely respected ensuring human health and protection.

Ionny reduces ethylene, molds, fungi, virus, yeast and air born bacteria, extending extends the fruit shelf life, preserving value of the produce and lowering costs with economic benefits for the producer(Buglia *et al*., 2013; Fadanelli *et al*., 2017).

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Advances in Horticultural Science

Sustaining low-impact practices in horticulture through non-destructive approach to provide more information on fresh produce history and quality: the SUS&LOW project

M. Amodio [1], G. Attolico [2], L. Bonelli [3], M. Cefola [4], H. Fazayeli [1], F.F. Montesano [3,5], B. Pace [4], M. Palumbo [1,4], F. Serio [3], A. Stasi [1], G. Colelli [1(*)]

[1] *Department of Science of Agriculture, Food and Environment, University of Foggia, Via Napoli, 25, 71122 Foggia, Italy.*

[2] *Institute on Intelligent Industrial Systems and Technologies for Advanced Manufacturing, National Research Council (CNR), Via G. Amendola, 122/O, 70126 Bari, Italy.*

[3] *Institute of Sciences of Food Production, National Research Council (CNR), Via Giovanni Amendola, 122/O, 70125 Bari, Italy.*

[4] *Institute of Sciences of Food Production, National Research Council of Italy (CNR), c/o CS-DAT, Via Michele Protano, 71121 Foggia, Italy.*

[5] *Department of Soil, Plant and Food Sciences, University of Bari, Via Aldo Moro, 70126 Bari, Italy.*

Key words: Marketing strategies sustainability, non-destructive assessment, quality, shelf-life.

Abstract: The general aim of the project SUS&LOW is to increase the sustainability of fresh produce by testing and implementing low-input agricultural practices (LIP) with positive impact on product quality with the support of non-destructive (ND) tools for real-time quality assessment and for product discrimination. Additionally, new marketing strategies are generated to better support the added value of the products and to satisfy the final consumers' preferences. The SUS&LOW project consists of three work packages (WP) and the adopted methodology used two model crops: rocket salad and tomato. The WP1, focused on the reduction of agricultural inputs, showed that sensor-based fertigation management might improve sustainability of soilless cultivation. Results coming from WP2, aimed to the evaluation of ND techniques, outlined the high potentiality of hyperspectral imaging (HSI) and Fourier transformed-near infrared (FT-NIR) techniques for the authentication of sustainable growing methods. Moreover, project activities' proved computer vision system (CVS) as an effective tool for evaluating the product quality also through the bag. The WP3, dealing with marketing strategies, indicated a positive approach of consumers compared to LIP products certified through a visual storytelling platform.

(*) **Corresponding author:**
giancarlo.colelli@unifg.it

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1. Introduction

Production of vegetable crops under controlled environments (i.e. greenhouses) has expanded considerably over recent decades in

Mediterranean areas (FAO, 2013). Initially, research efforts and the related introduction of technical innovations focused on high-quality, healthy products. However, concern with environmentally-sustainable production has risen in the last decade as industrial greenhouse crops are usually seen as entailing high environmental impact (Torrellas *et al.*, 2012). On the other hand, there is also plenty of evidence that greenhouse vegetable production may decrease the environmental impact compared to the field cultivation (Stanghellini, 2014).

The efficient use of resources (water and fertilizers), in irrigated greenhouse agriculture, is a promising and increasingly adopted strategy to achieve better crop performance, improved nutritional and sensorial quality (Montesano *et al.*, 2015; Montesano *et al.*, 2018). With respect to traditional systems, soilless cultivation and, particularly, closed-cycle with recycling of nutrient solution (NS) produce a number of benefits, including the possibility to standardize the production process, to improve plant growth and yield, and to obtain higher efficiency in water and nutrients use. In addition, it is also possible to modulate the regulation of the secondary metabolism of plants through an optimal control of the nutrient solution composition, or by imposing controlled stresses, or through biofortification treatments, generally leading to an improvement in the nutritional value of products (Rouphael and Kyriacon, 2018; Renna *et al.*, 2022). Innovative technologies based on the use of sensor networks for fertigation management may considerably reduce water and fertilizers consumption and increase the overall use efficiency of those inputs, and may lead to qualitative and quantitative improvements while preventing both under- and over-irrigation.

The most used instrumental techniques to measure quality attributes of fruits and vegetables are destructive and involve a considerable amount of manual work, primarily due to sample preparation. In addition, most of these analytical techniques are time consuming and sometimes may require sophisticated equipment. Finally, they can be performed only on a limited number of specimens (samples) and therefore their statistical relevance may be limited (Amodio *et al.*, 2017 a). Research has been focused on developing non-contact, rapid, environmental-friendly, and accurate methods for non-invasive evaluation of quality in fruits and vegetables. Nowadays, there are a few emerging non-destructive analytical instruments and approaches for this task, including spectroscopy, hyperspectral imaging, and computer vision (Liu *et al.*, 2017).

Near infrared spectroscopy has gained wide attention in the food sector due to its capacity of providing fingerprints of different products on the base of the interaction between their molecular structure and the incident light (Workman and Shenk, 2004) which is the result of different pre-harvest factors that also affect the final composition and quality. The feasibility of NIRS-based analysis to evaluate quality attributes of fresh fruits for commercial application have been reported by numerous authors (Arendse *et al.*, 2018; Palumbo *et al.*, 2022 a).

Hyperspectral imaging (HSI) combines the principles of spectroscopy and conventional imaging or computer vision. It is mainly used for internal bruise and defect detection in fruits and vegetables (Ariana and Lu, 2010; Babellahi *et al.*, 2020; Tsouvaltzis *et al.*, 2020) but also to predict the internal composition (Piazzolla *et al.*, 2013; Yang *et al.*, 2015; Liu *et al.*, 2017; Piazzolla *et al.*, 2017). Amodio *et al.* (2017 a) showed the potentiality of hyperspectral imaging in the Vis-NIR spectral range to predict internal content of soluble solids, phenols, and antioxidant activity of fennel heads. In addition, this technique provided important information about the maturity of fennel heads which may be used to determine the optimal harvest time. Some studies successfully applied these methods for the discrimination of production origin and agricultural practices, as revised in Amodio *et al.* (2020). NIR and HIS were in fact used for the classification of apples (Guo *et al.*, 2013), persimmon (Khanmohammadi *et al.*, 2014), and arabica coffee (Bona *et al.,* 2017) from different origins. As for production systems (Sánchez *et al.*, 2013) investigated the potentiality of NIRS technologies to discriminate green asparagus grown under organic and conventional methods. More recently, Amodio *et al.* (2017 b) successfully discriminated conventionally and organically grown strawberries, being also able to identify two different types of organic production systems applied to the same genetic material on the same site, soil, unheated tunnel.

All these studies have suggested multispectral and hyperspectral systems as valid tools to evaluate quality of different agricultural products and, more interestingly, as tools for product authentication.

Finally, Computer Vision Systems (CVS) may be applied to extend quality prediction and discrimination along the whole supply chain from harvesting up to consumers. CVS combine mechanics, optical

instrumentation, electromagnetic sensing, and digital image processing technology (Patel *et al.*, 2012). Recently, CVSs have been used to assess quality and marketability of tomatoes (Arias *et al.,* 2000), artichokes (Amodio *et al.,* 2011), fresh-cut nectarines (Pace *et al.,* 2011), fresh-cut lettuce (Pace *et al.,* 2014), fresh-cut radicchio (Pace *et al.,* 2015), and rocket leaves (Cavallo *et al.,* 2017). Moreover, they have been applied for the prediction of internal quality of colored carrots (Pace *et al.,* 2013). Even more interesting is the application of these systems during the post-packaging phase and along the whole distribution chain. Despite the relevance of quality evaluation of packaged products, few investigations were reported in literature. Multi-spectral reflective image analysis has been applied to monitor the evolution and spoilage of leafy spinach covered by plastic materials (Lara *et al.,* 2013); more recently, Cavallo *et al.* (2018) have proposed an application of image analysis by CVS for non-destructive and contactless evaluation of quality of packaged fresh-cut lettuce. Therefore, the interest of investigating the application of CVS to detect quality and shelf-life of packaged products.

Finally, the possibility of using non-destructive technique for increasing the information on product history (e.g. growing location and agricultural practices) may be considered as baseline to develop marketing tools to promote the diffusion of sustainable production system. Cost barrier is an obstacle for choosing low input products instead of the conventional, even if environment is mentioned as a strong commitment (Krystallis and Chryssohoidis, 2005). Therefore, the knowledge about consumer preferences for the adoption of LIP is still matter of debate.

The general aim of the project is to increase the amount of sustainably-produced food by testing and implementing low-input agricultural practices with positive impact on product quality with the support of non-destructive tools for real-time quality assessment and product discrimination, which may inspire new marketing strategies to better support the added value of the products and increase incomes of potential users.

2. Project activities and main results

The SUS&LOW project structure consists of three work packages (WP). WP1 focused on research activities aimed to reduce agricultural inputs (water and fertilizers) in greenhouse cultivation, chosen as a strategic high-value sector for Mediterranean agriculture. This WP was also in charge of making available to the project team vegetables products (rocket and tomato) different for the level of sustainability characterizing the cropping system adopted, to be used in other WPs for the related investigations. Then, WP2 was aimed to the quality assessment and to the implementation of new tools to acquire information about quality and history of fresh produce obtained with LIP (WP1). Non-destructive methods (including NIR, hyperspectral imaging and image analysis by CVS) have been used for food authentication, showing interesting and promising results. Finally, WP3 realized ad hoc survey to analyze the consumer behaviour with respect to the possibility of purchasing fruit and vegetables LIP certified (WP1) and identified by ND technologies (WP2) with the aim to implement adequate marketing strategies. In this section, an overview of the research strategies and approaches adopted in the three WPs is provided. The main results are reported and discussed.

WP1: quality crops through low-impact practices

Based on the overall project structure, this WP was focused on soilless cultivation, since it has the potentiality to achieve extremely high water and fertilizers use efficiency, beside high yield and quality, in intensive cropping systems. However, the adoption of free-drain open cycle with empiric fertigation schedule management operated by timers (the predominant case in Mediterranean area), may compromise the sustainability of soilless culture. Therefore, the adoption of strategies aimed to rational use of water and fertilizers and excess leaching prevention is a key-factor for increased sustainability and reduced environmental impact of soilless culture (Massa *et al.*, 2020). In this context, substrate moisture/EC (electrical conductivity) sensor-based irrigation is a promising and increasingly adopted strategy to reduce water and fertilizers consumption and losses, and to improve the overall crop performance, product quality and production process sustainability in soilless greenhouse cultivation (Palumbo *et al.*, 2021 a).

Several experiments were carried out at the Experimental Farm La Noria (Mola di Bari, BA) of the CNR-ISPA (Bari), with the common approach to compare treatments providing traditionally adopted empirical fertigation management techniques with treatments in which advanced sensor-based fertiga-

tion management was implemented. The main results of selected experiments carried out during the project are reported hereafter.

The research activities focused on two model species [rocket salad (*Diplotaxis tenuifolia* L.) and tomato (*Solanum lycopersicum* L.) selected for their relevance in Mediterranean greenhouse vegetable production. In particular, rocket is reported as an emerging leaf vegetable which cultivation is widespread and in further expansion (Schiattone *et al.*, 2017), while tomato is the most important greenhouse crop grown in soilless cultivation systems (Montesano *et al.*, 2015).

A study was carried out to test two irrigation scheduling approaches (timer- or sensor-based) and two fertilization levels (high or low, with reference to the standard dosage range recommended for the specific fertilizers used) of open-cycle soilless rocket in Mediterranean autumn-winter unheated greenhouse conditions (Montesano *et al.*, 2021). Rocket plants (cv. Dallas, Isi Sementi) were grown in a peat:perlite (3:1) mixture in 4.5 L plastic pots. Four treatments were compared: timer with high or low fertilization (T-HF, T-LF), and sensor-based with high or low fertilization (S-HF, S-LF). In timer-based treatments, irrigation schedule was periodically adjusted based on leaching fraction measurements (≈35% was set as a target, according to common practice). In sensor-based treatments, on-demand irrigation was operated based on substrate EC/temperature/moisture sensors (GS3, Decagon Devices). These were connected to a CR1000 datalogger programmed to automatically open irrigation valves and supply water enough to constantly maintain volumetric water content to a pre-defined set-point (0.35 m^3 m^{-3}, close to maximum water holding capacity), with no leaching. Slow-release fertilizers (Osmocote Exact and CalMag, ICL) were mixed with the substrate at high (3.75 and 1 g L^{-1}, respectively) or low dosage (2.25 and 0.6 g L^{-1}). Yield, quality, water use and substrate parameters trends were evaluated. Sensors improved water use efficiency compared to timer (34.4 vs 21.4 g FW L^{-1}, on average) matching water supply with plant needs, and preventing leaching (Fig. 1) (no interactive effects of fertilization treatments were observed on those parameters). Sensor-based irrigation also provided the best plant growth conditions, with interesting interactive effects with fertilization rate. In particular, the highest and the lowest cumulative (three harvests) yield values were obtained in S-HF and T-LF respectively (144.8 and 102.2 g FW pot^{-1}), while similar values were observed in S-LF and T-HF (131.4 g FW pot^{-1}, on average) (Fig. 1). The partial fertilizer factor productivity (g product fresh weight / g fertilizers applied) was higher at low dosage, and, with the same dosage, when the sensors were used (Fig. 1). After each harvest time the fresh-cut rocket leaves were immediately transported in refrigerate conditions to the postharvest laboratory (see WP2 section below) (Palumbo *et al.*, 2021 b).

In another set of studies, we aimed to apply approaches for the sustainable fertigation management of soilless tomato (semi-closed cycle recirculation; sensor-based nutrient solution supply management) in comparison with a traditional open cycle free-drain nutrient solution management providing the use timer for fertigation schedule. Experiments were conducted with different tomato types (cherry - cv. Carminio, Seminis-Bayer, and intermediate type - cv. Mose, Syngenta), and in different environmental conditions typical of Mediterranean areas (including the use of brackish water for nutrient solution preparation). In general, both approaches (semiclosed-cycle cultivation and open cycle with sensor-based fertigation management) reduced the environmental impact of the production process (reduced water/fer-

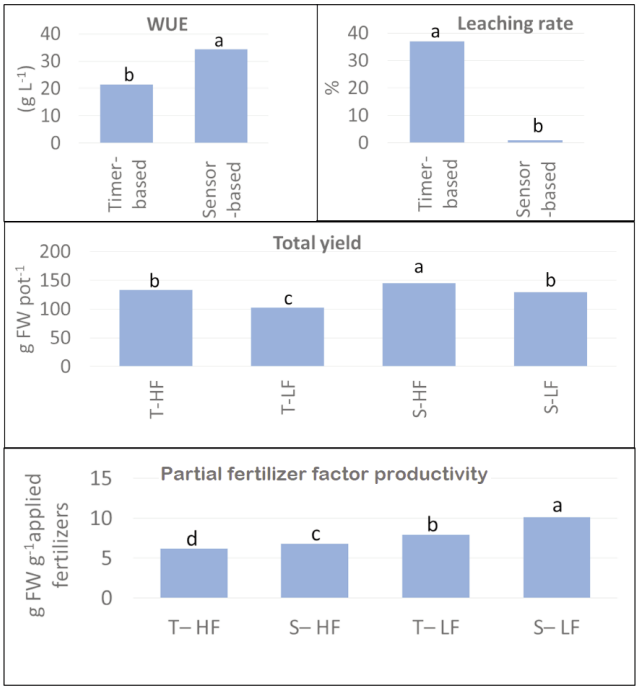

Fig. 1 - Water use efficiency (WUE), leaching rate, total yield, and partial fertilizer factor productivity of rocket (*Diplotaxis tenuifolia*) grown in open free drain soilless system with timer- (T) or sensor-based (S) irrigation management, and subjected to high (HF) or low (LF) fertilization rate.

tilizers usage; less nutrient solution released into the environment, increased water use efficiency) and positively affected tomato quality traits, compared to empirically management open-free drain cultivation.

WP2: non-destructive discrimination for low-impact practices and non-destructive quality assessment

NIR spectroscopy and Hiperspectral imaging. In this WP, the objective of the tasks was to assess the potentiality of Fourier transformed-near infrared (FT-NIR) spectrometry and hyperspectral imaging (HSI) to discriminate tomatoes and rocket leaves produced with different level of input as described in WP1, taking also into account the degree of efficiency in water and fertilizers used efficiency (WUE and FUE indexes). A hyperspectral line-scan scanner (Version 1.4, DV srl, Padova, Italy) equipped with two spectrographs, one in the Vis-NIR range, and the second in the NIR range, was used to obtain the HS images. The Vis-NIR spectrograph (400-1000 nm) has a spatial resolution of 1000 × 2000 pixels with a spectral resolution of 5 nm and was connected to a CCD camera. As for the NIR spectrograph (900-1700 nm), the spatial resolution was 600 × 320 pixels with a spectral resolution of 5 nm; and a CMOS (Specim Spectral Imaging Ltd., Oulu, Finland) with 50 frames per second equipped with C-mount lenses was used. As for FT-NIR spectrometry an MPA Multi-Purpose (FT-NIR Analyzer, Bruker Optics, Ettlingen, Germany), was used during spectral acquisition over the range of 800-2777 nm (sphere macrosample re-solution 1.71 nm, scanner velocity 10 kHz, sample scan time 64 scans, background scan time 64 scans). After image processing and spectra extraction for the HSI, all spectra belonging to HSI and FT-NIR were tested in discrimination using the agronomic treatments as discriminant classes and Partial Least Squares-Discriminant Analysis (PLS-DA) as classification technique. As for rocket leaves, PLS-DA was conducted with the 4 classes (T-HF; T-LF; S-HF, S-LF) described in the paragraph related to WP1, using 70 percent of samples for calibration purpose and the remaining 30% for the external validation. The model performance was evaluated based on the accuracy, which is an average of the sensitivity calculated over the various classes, and gives an overall idea of the goodness of the classification. Results indicated HSI as a promising technique for the discrimination of rocket produced with different cultural techniques, with an accuracy of classification in the prediction phase of 97.2% in Vis-NIR and 99.5% in NIR range. In figure 2, the results of the discrimination

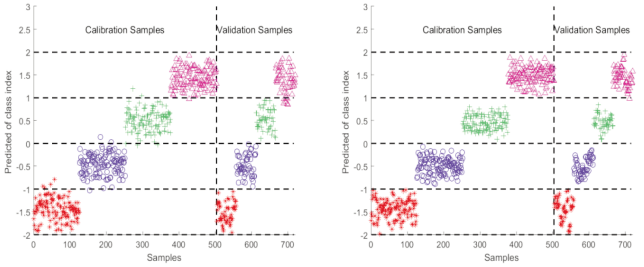

Fig. 2 - Estimated class index values in the calibration and in the prediction process for the classification based on PLS-DA modes shown on table 2 in a) VNIR range (left) b) NIR range (right).

models can be observed.

Regarding tomato, where 2 experiments with 2 different varieties were conducted (WP1), for each trial a first PLS-DA was aimed to discriminate the three treatments of cultivation and a second discrimination was performed for different levels of WUE and FUE. According to the efficiency of use of water and fertilizers we could individuate 2 levels (high and low) in each experiment and 3 levels (High, medium, and low) merging the data of both experiments. Therefore, a PLS-DA with 3 levels of WUE (and FUE) was also generated with the full dataset. Among the different non-destructive techniques, FT-NIR and HIS in the VIS-NIR range gave comparable performances in discriminating tomato according to cultural practices and different use of sources. Discrimination for WUE for each variety improved the classification results, respect to the individual treatments, but the highest accuracy was obtained when the discrimination was based on 3 levels of WUE merging the 2 datasets, reaching 92.1%. In literature there are no studies aimed to discriminate crops for WUE or FUE, while we may find the application of HSI for the classification of water-stressed plants, as for the case of tomatoes (Rinaldi *et al.*, 2015). In comparison to this study, reporting a mean accuracy of around 77% for discrimination of the two differently irrigated areas, our findings showed higher accuracy, exploring new area of the application for these techniques.

Application of CVS for non-destructive quality evaluation on packaged products

A research activity was carried out to develop and validate an innovative CVS integrating a Random Forest model for classification: this model automatically selects from the image the most relevant colour features for the task of interest. The developed CVS was applied to digital images of fresh-cut rocket

leaves cultivated with LIP (WP1) to objectively estimate the evolution of their quality levels (QL) during storage and to discriminate the cultivation approach applied on field. At harvest, rocket leaves were stored at 10°C in open polypropylene (PP) bags for a number of days required to reach the lowest QL, according to the rating scale from 5 (very good) to 1 (very poor), as reported in figure 3.

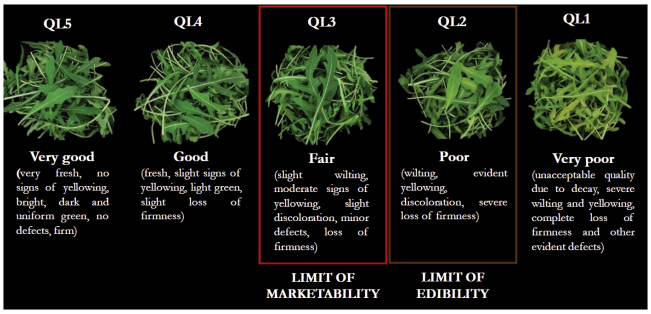

Fig. 3 - Changes in the sensory quality level (QL) of fresh-cut rocket leaves during the storage at 10°C according to the 5 to 1 rating scale reported by Palumbo *et al*. (2021 b). In detail, QL5= very good; QL4= good; QL3= fair; QL2= poor; QL1= very poor.

At each QL, all the samples were subjected to postharvest quality evaluation, detecting colour parameters by a traditional colorimeter (CR400, Konica Minolta, Osaka, Japan) and physical and chemical parameters, in detail respiration rate (Kader, 2002), electrolyte leakage (Kim *et al.*, 2005) and total chlorophyll content (Cefola and Pace, 2015). Then, images of the same samples were acquired by the CVS for non-destructive quality assessment and for recognizing traits related to the sustainability of the cultivation management used on the field, with specific reference to water and nutrients use (WP1). Image pre-processing was applied: to separate the product from the background; to identify the colour-chart placed in the scene to estimate the effects of lights and of the sensors and to correct colours to minimize these effects. Three colour correction methods (white balance, linear correction, and polynomial correction) with increasing level of complexity were evaluated and compared in terms of consistency of colour measurements and of classification performance. Linear colour correction proved to be the best trade-off between efficacy and efficiency providing a slightly lower performance than polynomial correction with significantly simpler computation. Finally, a Random Forest model was used to train classifiers to assess the QL of rocket leaves and to identify the treatments used during the cultivation.

All the postharvest quality parameters measured by traditional destructive methods were significant in QL assessment of fresh-cut rocket leaves. The proposed classifier based on the Random Forest model was able to identify and select the most relevant colour traits for both the tasks (QL assessment and treatment identification) without human intervention. The accuracy achieved in evaluating QLs of rocket leaves during storage was high (about 95%), while the performance in discriminating the cultivation approach was lower and not sufficient for practical applications (about 65-70%). Indeed, the different cultivation approaches did not significantly affect the visual characteristics of the product and the destructive measures: this task needs further investigations.
Another research activity was carried out to develop and validate the capability of the non-destructive and contactless CVS to assess the visual quality changes during the cold storage of fresh-cut rocket leaves coming from soil and soilless growing systems (WP1) and to estimate some internal quality attributes (chlorophyll and ammonia content) also through the packaging material. Evaluating quality through the package is critical to identify the regions of the bag where the product is visible without shadows or highlights created by illumination: this is mandatory to measure colour properties in a reliable and meaningful way. At harvest, rocket leaves, cultivated on soil or soilless system (WP1), were packed in open PP bags and stored at 10°C for about 18 d. During storage, all samples were observed to attribute the QL according to the rating scale reported in figure 3 and the postharvest quality traits were evaluated by destructive conventional methods [colour parameters, chlorophyll content, ammonia content (Fadda *et al.,* 2016) and electrolyte leakage]. Then, images of unpackaged and packaged samples were acquired by the CVS. During image acquisition, no constraints were imposed on the position of the product in the bag, on the position of the bag in the scene or on the highlights created by the illumination on the surface of the bag: this was necessary to demonstrate the applicability of this technology into a real industrial line. Colour correction was performed by the linear model, identified as the best trade-off between effectiveness and computational complexity in the previous research activity. Packed and unpacked products were processed using exactly the same phases apart from the artefacts' elimination step applied to the images of packaged products to select the regions where the colour information was meaningful, without interference from light artefacts and reflections. At last, the Random Forest model was used to solve

both the classification problem (assessment of the QLs) and the regression problems (estimation of quality marker parameters such as chlorophyll and ammonia contents). The same architecture was used for all the tasks, by simply changing the training data. The histogram of the image, evaluated in the a-b plane of the CIELab colour space, was used as the set of features. The Random Forest model was able to automatically select the subset of values more suitable for solving each task.

All the postharvest quality parameters detected by conventional analysis during the storage of fresh-cut rocket leaves were significant in the QL assessment and, among them, chlorophyll and ammonia contents proved to be useful marker parameters for the objective separation of each QL considered, both on soil and soilless cultivation approach.

The CVS was able to operate without relevant differences on unpackaged and packaged products. The test was done joining all the samples, regardless of the cultivation approach: the results showed a not significant performance loss on packaged leaves (Pearson's linear correlation coefficient of 0.84 for chlorophyll and 0.91 for ammonia) with respect to unpackaged ones (0.86 for chlorophyll and 0.92 for ammonia) (Fig. 4).

Finally, three Partial Least Square (PLS) models were performed to predict the QL using as predictors chlorophyll and ammonia contents obtained by destructive methods (Model I), by CVS on packaged products (Model II) and by CVS on unpackaged ones (Model III) (Table 1).

The results showed high performances in terms of R^2 and the model obtained by predictors estimated non-destructively by the CVS (Model II and III) provided better performances in the QL prediction than one obtained by destructive analysis, in both calibration and validation.

WP3: marketing strategies to support the added value of the products LIP and ND certified

Implementing a marketing strategy, based on often intangible characteristics to consumers such as

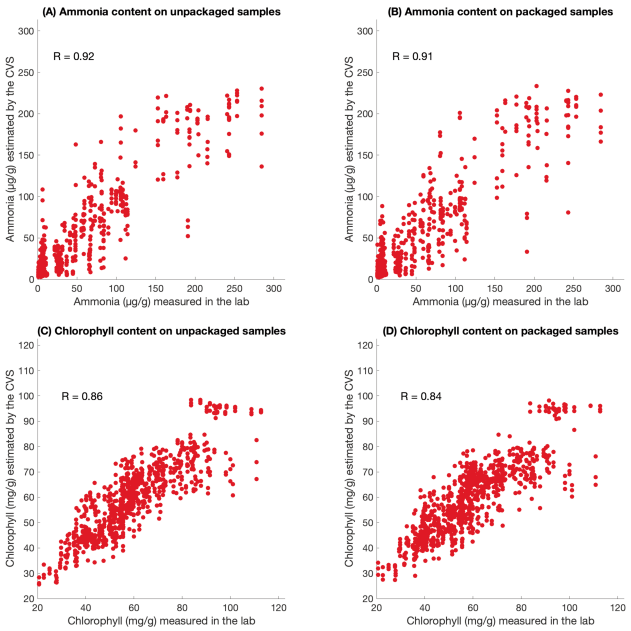

Fig. 4 - Values estimated by the CVS (abscissa) vs. values measured in the laboratory (ordinate) for ammonia content on unpackaged (A) and packaged (B) rocket leaves and for total chlorophyll content on unpackaged (C) and packaged (D) samples (Palumbo *et al.*, 2022 b).

LIP and ND, it is not an easy task. Low impact practices do not have a highly distinctive impact on product characteristics nor determine unique taste, flavour, or look elements to consumers. However, certifications could be used to signal quality through the application of standards of quality and practices. Whether certifications could be effective in terms of marketing in the case of products LIP and ND, or for signalling quality in general is matter of discussion. Vecchio and Annunziata (2011), for instance, in their work question the possibility of effective understanding of certification by consumers. At this purpose the research team of WP3 decided to implement a different strategy and test it on the market. Visual storytelling certifying LIP and ND has been then hypothesized to better communicate the importance and the impact of those practices on food.

The research activity, therefore, has been organized in three steps: identifying the communication

Table 1 - Root Mean Square Error (RMSE) and the coefficient of determination (R2) in calibration (c) or validation (v) of the Partial Least Square (PLS) Models predicting visual quality of rocket leaves (Palumbo *et al.*, 2022 b)

PLS Models	Predictors	$RMSE_c$	R^2_c	$RMSE_v$	R^2_v
I	Total chlorophyll and ammonia obtained by destructive methods	0.45	0.9	0.86	0.70
II	Total chlorophyll and ammonia obtained by CVS on packaged rocket leaves	0.46	0.89	0.75	0.77
III	Total chlorophyll and ammonia obtained by CVS on unpackaged rocket leaves	0.46	0.89	0.7	0.8

strategy and set-up; testing through focus-groups the opportunity conditions for farms and companies; testing though a survey and an econometric analysis the consumers' preference and their willingness to pay for products with LIP and ND. Therefore, a draft platform has been developed containing basic communication rules in order to highlight sustainability attributes of products through storytelling. Workflow has been established and a simulation has been conducted (Fig. 5). Focus group with producers has allowed verifying the general appreciation for the marketing approach and allowed a better set-up of the strategy. Finally, a picture-based simulation has been produced for the final test and the survey to consumers (Fig. 6).

As last activity, a questionnaire based survey has been prepared and administered to 467 consumers and an econometric model to estimate willingness to pay and consumers orientation has been set up and then estimated. The whole set of activties within the research project allowed understanding how important is a correct communication of products and how different could be the perception of a product based on how you certify or narrate the production method. Result allow understanding that older conumsers are more aware of sustainability and are more willing to pay for LIP products. Psicological profile such as traditionalism and benevolence identify the consumer that, more than other profiles, would be willing to pay a higher price.

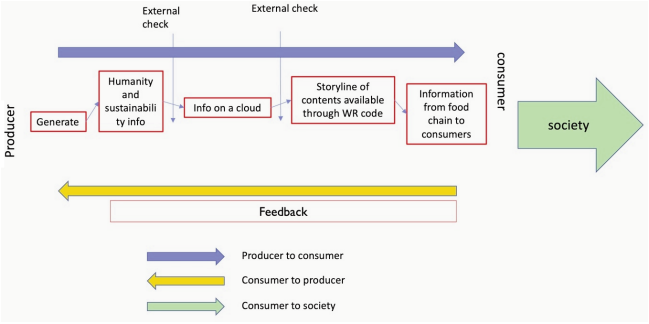

Fig. 5 - Workflow for products LIP and ND certified platform.

Fig. 6 - Picture based simulation of visual storytelling certification for LIP producs and ND.

3. Conclusions

Sensor-based fertigation management applied to rocket leaves and tomato confirmed to be a feasible approach to improve sustainability of soilless cultivation, also in cases where the complete and rapid switch to closed cycle recirculation systems is still impaired by economic, social, and environmental factors such as in Mediterranean area.

The results of this project related to non-destructive discrimination of tomatoes and rocket leaves, according to cultural practices using different levels of inputs (water and fertilizers), indicated the high potentiality of HSI and FT-NIR techniques for the authentication of sustainable growing methods. Moreover, project activities' proved CVS as an effective tool for evaluating the product quality also through the bag, even working only on the regions of the image that provide meaningful colour information about the product's surface. The integration of machine learning modules inside the CVS confirmed to be useful to simplify the design and tuning, done mostly automatically without human intervention. Moreover, the flexibility introduced by machine learning makes the resulting architecture more flexible in adapting to different products and applications.

As regards the marketing approach, consumers resulted willing to pay a higher price for LIP products certified through a visual storytelling platform. In the next future, there could be a good chance that sustainability-oriented practices coupled with a visual storytelling certification style could gain shares on food markets.

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Short note

AHS
Advances in Horticultural Science

Nanosponges and CPPU: a scoping review and a pre-test to assess the potentiality for shelf-life prolongation of cut carnations

L. Battisti [1] (*), F. Caldera [2], G. Hoti [2], F. Trotta [2], M. Devecchi [3]

[1] *Department of Cultures, Politics and Society, University of Turin, Lungo Dora Siena, 100/A, 10153 Torino, Italy.*

[2] *Department of Chemistry, University of Turin, Via P. Giuria, 9, 10125 Torino, Italy.*

[3] *Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini, 2, 10095 Grugliasco (TO), Italy.*

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(*) **Corresponding author:**
luca.battisti@unito.it

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Abstract: Nanosponges can favour the gradual release of molecules over a prolonged time, increasing the bioavailability and action of preservatives and phytoregulators, reducing the concentrations usually adopted. In floriculture, they have previously been proposed for the delivery of anti-ethylene compounds to improve the shelf-life of cut flowers. However, the potential of nanosponges is not only limited to these compounds. The present scoping review evaluated the effects of β-cyclodextrin-based nanosponges and growth regulators on the post-harvest longevity of cut flowers of ornamental species. One novelty was the use of Forchlorfenuron (CPPU), a growth regulator belonging to the group of cytokinins predominantly used in fruit cultivation, to evaluate its potential to increase the shelf-life of cut carnations (*Dianthus caryophyllus L*). In particular, an in-depth analysis of a pre-test involving the use of nanosponges and CPPU is proposed. Specifically, as far as post-harvest longevity is concerned, the treatments involved the use of: deionised water; nanosponges and deionised water; nanosponges loaded with CPPU; nanosponges loaded with a classic solution for cut flowers, composed of sucrose, aluminium sulphate and 8-hydroxyquinoline sulphate. Preliminary results show that the nanosponge and deionised water complex and the nanosponge and classical solution complex prolonged the longevity of the cut flower by up to 20 days, compared to the control (17 days). In contrast, the CPPU-nanosponge complex showed similar results to the control. Replication of the research is necessary to validate the results.

1. Introduction

Cyclodextrin Nanosponges (CDNSs) are cage-like network polymers prepared by connecting cyclodextrin molecules with various kinds of bi- or polyfunctional linking agents. The term “nanosponge” was first intro-

duced by Ma and Li, in a paper focusing on the sequestering capacity of polyurethane crosslinked CD polymers, to highlight the nanometric porosity of this class of materials (Ma and Li, 1999). In addition to the central lipophilic cavity of CD molecules, CDNSs exhibit a second type of pores, which are the empty spaces among CD molecules, whose polarity and size can be modulated by varying the chemical structure of the linking agent and its concentration. Such features make CDNSs highly versatile materials with potential applications as nanostructured containers and delivery systems for a broad set of active principles. Furthermore, CDNSs are low-cost, easy to prepare, safe and sustainable materials, as they are mainly composed of starch derivatives and, in some cases, even the linker comes from renewable sources (e.g., citric acid) (Caldera *et al.*, 2017).

The application of CDNSs in the pharmacological field has been widely explored. Several classes of drugs have been successfully encapsulated, stabilized and released with controlled kinetics, including anticancer, anti-inflammatory, antiviral, antibacterial, antifungal drugs and many others. Recently, the functionalization of CDNSs with specific moieties has led to the development of a new generation of stimuli-responsive CDNSs, capable of releasing drugs on-demand and highly selective CDNSs for targeted drug delivery.

In the environmental field, CDNSs have been used as absorbing agents for the removal of organic pollutants as well as heavy metal cations for water decontamination (Krabicova *et al.*, 2020).

Although CDNSs have been extensively studied for biomedical and environmental applications, their full potential in the horticultural field remains largely unknown. To date, only a few studies have explored the use of CDNSs for the encapsulation and release of the herbicide ailanthone and some anti-ethylene molecules (Demasi *et al.*, 2021).

Forchlorfenuron [N-(2-chloro-4-pyridyl)-N'-phenylurea], whose acronym is CPPU, is a plant growth regulator (synthetic cytokinin) that promotes the periclinal cell division and is often used in agriculture to increase the berry size, the quality and the yield of grapes and kiwifruit (Peppi and Fidelibus, 2008; Cruz-Castillo *et al.*, 2014).

Although several studies have evaluated the efficacy of CPPU in postharvest in fruits (Zhang *et al.*, 2017; Chang, 2021), few studies have focused on cut foliage and flowers, in contrast to a similar molecule, Thidiazuron, which has been used for years (Ferrante *et al.*, 2002; Chamani *et al.*, 2006).

The aim of this scoping review is to highlight the application potential of nanosponges in the postharvest context. In addition, an in-depth study is proposed on the production and testing of nanosponges and CPPU in order to assess their effectiveness in prolonging the *shelf-life* of cut carnations. More specifically, we intend to describe the pre-test phase of the two products listed above, which is also useful from an economic point of view in order to produce a quantity of nanosponges in the laboratory suitable for performing an experimental test with replications, as well as to proceed with the purchase of CPPU in larger quantities.

2. Materials and Methods

In the coming sections, the following will be reported: a scoping review on the use of nanosponges in floriculture; a first trial of the use of CPPU and nanosponges on cut flower carnation.

Synthesis of a carbonate cyclodextrin nanosponge

A carbonate cyclodextrin nanosponge was synthesized by heating β-cyclodextrin (β-CD, 6.500 g, 5.73 mmol) and 1,1'-carbonyldiimidazole (CDI, 3.715 g, 22.92 mmol) in 39 mL of N,N-dimethylformamide at 90°C for 4 h. In the end, the solid gel formed during the crosslinking reaction was crushed and washed with approximately 2 L of deionized water via Buchner filtration. The nanosponge was further purified in a Soxhlet extractor with ethanol for 20 h and, once dry, finely ground in a planetary ball mill.

CPPU loading in the nanosponge

The loading of CPPU into the nanosponges was performed subsequent to the synthesis and purification of the nanosponge. Briefly, 450 mg of nanosponge was added to a solution of 50 mg of CPPU dissolved in 25 mL ethanol. Then, the dispersion was stirred at room temperature for 24 h. Finally, the CPPU-loaded nanosponge was collected by removing the entire volume of ethanol in a rotary evaporator at 50°C under vacuum. The complex was stored in a desiccator at room temperature until use.

Pre-test setting

The pre-test was conducted in March and April 2022, in the laboratories of the Department of Chemistry and Department of Agricultural, Forest and Food Sciences, University of Turin.

The carnations were collected, packaged and pur-

chased at the flower market in Sanremo (Italy) on 27 March at 6 p.m. and were transported to Turin and kept in the transported condition until 3 p.m. on 28 March, when the pre-test began. The four packages, purchased from 'Cooperativa Tre Ponti' (intermediary), contained 20 flowers each.

The first phase consisted of eliminating flowers that visually showed diseases, lesions and flowers at a different stage of flowering from the others. A total of 70 flowers were selected and the stems were cut into the water leaving a length of 30 cm.

The pre-test comprised seven different treatments (solutions with deionized water), each applied to 10 flowers. Each flower was placed in a glass tube containing 50 ml of solution. On day 8 and 16, 10 ml of the specific solution was added to all tubes. Measurements were conducted daily, and data were acquired for the following parameters: cut carnation weight (g) and flower diameter (cm). The pre-test lasted 20 days, with the following environmental conditions: 12 hours of light and 12 hours of darkness; temperature 19±2°C. The treatments are shown in Table 1.

3. Results and Discussions

Pre-test results

Preliminary results (Fig. 1) show that the treatment A and the treatment C prolonged the longevity of the cut flower by up to 20 days, compared to the Control (17 days). In contrast, the treatment E complex showed similar results to the control. There was no inhomogeneity in the diameters of the flowers within the various treatments; however, the largest diameters (9.5 cm) were more frequently reached by the treatment F. Lateral shoot development was a common side effect of CPPU and commercial solution treatments.

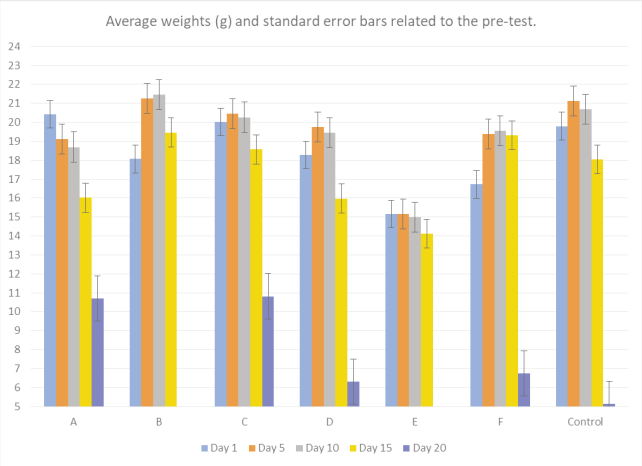

Fig. 1 - Average values of weights (g) and standard error bars of cut carnations during the pre-test.

The pre-test yielded interesting results that allow planning the production of nanosponges and the purchase of CPPU in order to set up a scientific trial. Of particular interest could be the application in solution and spray of nanosponges and CPPU on cut foliage.

Final discussions and next steps

The application of CDNSs in floriculture, and more specifically post-harvest preservation, is an emerging line of research still widely unexplored. To date, only few scientific papers focusing on the encapsulation of anti-ethylene compounds in CDNSs have appeared in the literature. Of these ethylene inhibitors, 1-methylcyclopropene (1-MCP) is one of the most studied, especially in combination with CDs and CD-derivatives.

In 1994, Serek *et al.* described for the first time the ability of 1-MCP to prolong the post-harvest shelf-life of plant material (Serek *et al.*, 1994). Since then, 1-MCP has been extensively studied as a non-toxic anti-ethylene agent to inhibit the senescence

Table 1 - Pre-test treatments

Code	Treatments
A	Nanosponges (5 g/l)
B	Sucrose 30 g/l + aluminium sulphate (200 mg/Kg) + 8-hydroxyquinoline sulphate (200 mg/l)
C	Sucrose 30 g/l + aluminium sulphate (200 mg/Kg) + Nanosponges (5g/l)
D	CPPU (200 mg/l)
E	CPPU in Nanosponges (200 mg/l)
F	Commercial solution - Chrisal prof. 2 (10 ml/l)
Control	Deionised water

process of cut flowers, vegetables and fruits at the receptor level (Blankenship *et al.*, 2003; Nasiri *et al.*, 2020). In 2000, 1-MCP appeared on the market in two different formulations commercialized by Floralife, Inc. (Walterboro, SC) and AgroFresh, Inc. (Spring House, PA). The Floralife product is named EthylBloc® and it is meant to be applied on ornamentals only. While, Agrofresh provides a formulation, named SmartFresh®, that can be used with edible crops. However, both products are based on the inclusion complex of 1-MCP gas in α-CD (Gehla *et al.*, 2003).

The efficacy of these formulations is time-limited, as most of 1-MCP is released within 20-30 minutes from application, under normal temperature and pressure conditions (Blankenship *et al.*, 2003). To overcome such limitations, new materials such as CDNSs have been tested for the encapsulation and prolonged release of 1-MCP.

In a first attempt, 1-MCP, 2,5-norbornadiene and silver nitrate were encapsulated in a carbonate CDNS and added to two flower species, specifically *Dianthus caryophyllus* 'Idra di Muraglia' and *Ranunculus asiaticus* 'Elegance'. While the formulation containing 1-MCP allowed to extend the vase life of *Dianthus caryophyllus* up to 23 days, the other formulations did not show significant effects and none of the formulations was able to improve the longevity of ranunculus (Devecchi *et al.*, 2009).

With the aim of understanding the role played by the central cavity of CD molecules in the encapsulation and controlled release of 1-MCP, carbonate NSs based on α-CD (6 glucose units, diameter of the cavity: 4.7-5.3 Å) were used in comparison with the analogous carbonate NSs prepared with β-CD (7 glucose units, diameter of the cavity 6.0-6.5 Å). The tests were performed on *Dianthus caryophyllus* 'Idra di Muraglia'. Results demonstrated that β-CD is remarkably more effective than α-CD in extending the postharvest life of the *Dianthus* flowers, as no evidence of deterioration was observed up to nearly 11 days. Whilst, the 1-MCP-loaded NSs based on α-CD did not show any significant anti-ethylene activity (Sceglie *et al.*, 2011 a).

The next step was to determine the influence of the NS's degree of crosslinking on the encapsulation and slow release of 1-MCP. A series of samples of β-CDNS were synthesized with different carbonate to CD molar ratio (i.e., 2, 4 and 8), resulting in different degree of crosslinking. After loading with 1-MCP, the NSs were used to extend the vase life of carnation cut flowers. All the NS formulations exhibited anti-ethylene activity. However, the NS with the highest degree of crosslinking (i.e., linker/β-CD molar ratio of 8), and therefore the most densely crosslinked polymer structure, showed the highest efficacy for prolonged time, even at low concentration (Sceglie *et al.*, 2011 b).

The protective effect of the 1-MCP-loaded β-CDNS with monomer ratio 1:8 against infection by Botrytis cinerea on *Dianthus caryophyllus* L. 'Idra di Muraglia' was studied as well. After eleven days of treatment, the NS at low dosage reduced the spreading of the grey mould by approximately 60 %. At higher dosage, the NS formulation outperformed the commercial 1-MCP product, used as a reference, reaching a value above 90%, while the commercial formulation stopped at 76% (Sceglie *et al.*, 2012).

As a final step, the effectiveness of the 1-MCP/ β-CDNS(1:8) complex in delaying the senescence process of cut flowers was evaluated in different floral species. Specifically, *Anemone coronaria L. multicolor*, *Paeonia lactiflora L.* 'Sarah Bernhardt', *Helianthus annuus L.* 'Sunrich Orange', *Ranunculus asiaticus L.* 'Minou Abrown', *Papaver nudicaule L. multicolor* and *Rosa hybrida L.* 'Jupiter' were treated with the NS formulation in the presence of exogenous ethylene. Although the mechanism of action was different, depending on the flower species, the CDNS increased the anti-ethylene activity of 1-MCP in all the tested samples (Sceglie *et al.*, 2013).

Despite the positive results described above, the use of 1-MCP has some disadvantages, including its high cost, difficult handling and volatility. Moreover, EthylBloc® and SmartFresh® are not available in some countries. Therefore, the development of new formulations based on easily manageable active principles, such as salicylic acid (Cocetta and Ferrante, 2018), synergistic formulations of cumin essential oil and 8-hydroxyquinoline sulfate (Mirjalili *et al.*, 2018), CPPU and others with prolonged release kinetics and thus long-term efficacy, are of special interest.

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