Adv. Hort. Sci., 2018 32(3): 353-362 DOI: 10.13128/ahs-21927

AHS
Advances in Horticultural Science

Influence of soil and soilless agricultural growing system on postharvest quality of three ready-to-use multi-leaf lettuce cultivars

B. Pace [1, 2], I. Capotorto [1, 2], M. Gonnella[1], F. Baruzzi [1], M. Cefola [1, 2 (*)]

[1] *Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche (CNR), Via G. Amendola, 122/O, 70126 Bari, Italy.*

[2] *Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche (CNR), URT c/o CS-DAT, Via M. Protano, 71121 Foggia, Italy.*

Key words: ammonium, *Lactuca sativa* L., nitrate, microbial quality, postharvest storage, soilless cultivation.

Abstract: In this study the influence of soil and soilless growing systems (substrate 3:1 v/v perlite:peat) on quality and microbial traits of three multi-leaf lettuce cultivars (two green, 'Eztoril' and 'Ezabel', and one red, 'Ezra') was evaluated at harvest and after 7 and 13 days of storage at 8°C. At harvest, 'Ezra' showed a respiration activity and a total phenol content respectively 2-fold and 25% significantly higher than the green cultivars. Soil lettuces resulted more stressed than those grown in soilless, as indicated by their initial content in antioxidants. As for nitrate content, soilless grown lettuces at harvest showed an average concentration higher than soil-grown ones, although values are generally lower than limits imposed by the EU Regulation (No. 1258/2011). During storage, soilless lettuces showed no ammonium accumulation, differently from those cultivated in soil. In addition, lettuce cultivars grown in soilless condition showed unchanged content in the antioxidant activity and total phenols, and lower microbial counts than soil lettuces. Results of the present study showed that soilless growing system can positively affect qualitative and microbiological parameter of lettuces studied, and it can be considered a good soilless growing technique in order to obtain high quality multi-leaf lettuces for ready-to-use industry.

(*) **Corresponding author:**
maria.cefola@ispa.cnr.it

Citation:
PACE B., CAPOTORTO I., GONNELLA M., BARUZZI F., CEFOLA M., 2018 - *Influence of soil and soilless agricultural growing system on postharvest quality of three ready-to-use multi-leaf lettuce cultivars*. - Adv. Hort. Sci., 32(3): 353-362

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

Competing Interests:
The authors declare no competing interests.

Received for publication 10 October 2017
Accepted for publication 12 January 2018

1. Introduction

The consumer's demand of ready-to-use fruits and vegetables, and in particular that for minimally processed leafy vegetables, is continuously growing. Although iceberg lettuce is still the main lettuce used in the ready-to-use industry, consumers are requesting other types of lettuce with attractive colours and shapes combining the best quality characteristics from all varieties (Rijk Zwaan, 2009). The new baby-sized leaves,

baby- and multi-leaf have been developed recently as high quality lettuce varieties for the ready-to-use market. Some benefits of baby-sized lettuce, when compared with whole-head lettuce, include: i) greater efficiency with higher percentage of usable product; ii) easier and faster processing; iii) more attractive colour and shapes, and iv) minimal oxidation due to smaller stem diameter (Martínez-Sánchez *et al.,* 2012). Moreover, for both multi- and baby-leaf lettuces, no physical wounding was undertaken, except that of the harvesting, avoiding the physical damage that occurs during preparation of fresh-cut lettuce that causes an increase in respiration activity, biochemical changes and microbial spoilage, which may result in degradation of colour, texture and flavour of the ready-to-use produce (Cantwell, 2004). Likewise, cultivar selection is of great importance in the ready-to-use industry since quality characteristics (such as leaf colour, shape, freshness, texture and browning potential) can change largely depending on the genotype (Nicola *et al.,* 2009). The quality and shelf-life of ready-to-use leaves depend on genotypic traits of raw material and on several aspects from preharvest to postharvest processing (Clarkson *et al.,* 2003; Cantwell, 2004). Some physical and chemical indicators can be used for objective assessment of visual quality (Barrett *et al.*, 2010; Salinas-Hernández *et al.*, 2015). Among these, ammonium (NH_4^+), produced during storage as a consequence of senescence in various vegetables (Cefola *et al.*, 2010; Pace *et al.*, 2014), might be used as predictors of shelf-life (Cefola *et al.*, 2017). In general, preharvest factors should be aimed to optimize their impact on postharvest quality (Crisosto and Mitchel, 2002). From this point of view, soilless system is becoming of high interest since it can improve both, preharvest and postharvest quality of vegetables (Rodríguez-Hidalgo *et al.,* 2010). In particular, soilless agricultural growing system allows to set optimal conditions and nutrient concentration for plant growth (Silberbush and Ben-Asher, 2001; Selma *et al.,* 2012) with the following advantages: higher yields (Lopez-Medina *et al.,* 2004; Recamales *et al.,* 2007), better quality vegetables (Recamales *et al.,* 2007; Cefola *et al.,* 2011) and higher earliness (Recamales *et al*., 2007; Valenzano *et al*., 2008), compared to soil cultivation. The success of lettuce production depends to a great extent on the maintenance of a continuous growth rate by the optimal management of nutrients (Luna *et al.,* 2013). In addition, especially for leafy vegetables, the use of soilless system can avoid soil contaminants and improve the sanitary quality respect to traditional soil cultivation, leading benefits on raw materials for postharvest industry (Selma *et al.,* 2012). Starting from these findings, the aim of this work was to evaluate the influence of two growing system (soil and soilless) on postharvest quality of three multi-leaf lettuce genotypes, including two green and one red, stored under refrigeration for 13 days.

2. Materials and Methods

Reagents

Extraction solvents (MeOH, EtOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and all standards used in the experiments were obtained from Sigma-Aldrich (St. Louis, Mo., USA). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany).

Plant material and growing system

Three types of Dutch multi-leaf lettuces (*Lactuca sativa* L.), two green (cv. Eztoril and Ezabel) and one red (cv. Ezra) (Enza Zaden, Enkhuizen, Netherland) were cultivated in an unheated plastic tunnel under soil (S) or soilless (SL) agricultural growing system in the same greenhouse at the experimental farm "La Noria" of the Institute of Sciences of Food Production (CNR-ISPA) located in the South of Italy (Mola di Bari). A split-plot design with three replications was applied, randomizing the growing systems (GS) in the main plots and cultivars in the subplots. Main plots were of 3.6 m^2 (0.9 m wide and 4 m long). The SL system consisted of three single benches (4 m long x 0.3 m wide x 0.1 m high, with a slope of 2%) each plot containing a 3:1 (v:v) perlite:peat mixture as substrate. The nutrient solution was supplied to the SL system without recirculation and had the elemental composition given in Table 1, where the soil characteristics are reported too. The irrigation water had the following composition (expressed in mmol L^{-1}): 0.3 $N\text{-}NO_3$, 0.23 K, 0 P, 1.73 Mg, 1.82 Ca, 7.39 Cl, 4.05 Na. Nutrient solution and water were supplied to SL and S units based on a timer controlled schedule, using minimum substrate water content values, monitored by tensiometers. Furthermore, as additional reference control taking into account two different threshold levels of -5 and -25 kPa to start the irrigation supply in SL and S, respectively. For SL system a nutrient supply level criterion was additionally

Table 1 - Soil and soilless nutrient solution composition. Soil classified as clay soil (USDA textural soil classification, 1987). Values of nutrient solution are expressed in mmol L^{-1}. Micronutrients were supplied according to Johnson *et al*. (1957)

Mineral composition	Soil composition	Soilless nutrient solution
Sand	24.30%	-
Silt	31.90%	-
Clay	43.80%	-
pH	7.6	6.5
EC (dS m^{-1})	2.5	2.3
Cl^-	-	7.39
Mg^{2+}	-	1.73
Na^+	-	4.05
K^+	-	5.12
Ca^{2+}	-	4.74
NH_4+	-	0.5
NO_3^-	-	9.43
P-H2PO^{4-}	-	1.61
S-SO4^{2-}	-	0.81
CEC (cmol kg^{-1} dw)	31.8	-
Organic matter (g kg^{-1} dw)	14	-
Total N (g kg^{-1} dw)	0.95	-
Available P (g kg^{-1} dw)	110	-
Available K (g kg^{-1} dw)	244	-
$CaCO_3$ (g kg^{-1} dw)	0.11	-

applied. At transplant soil plots were fertilized with ammonium nitrate and monopotassium phosphate giving the equivalent of 50, 80, 50 kg ha^{-1} of N, P_2O_5 and K_2O, respectively, and after a month a 30 kg ha^{-1} integration of N from ammonium nitrate was applied. Seedlings were produced in greenhouse in polystyrene trays on peat and were transplanted 25 days after sowing on February 22. Harvest was performed 55 days after transplanting (on April 18) for the SL and after 73 days (on May 6) for the S system. Greenhouse ventilation temperature was 20°C. In figure 1 the climatic parameters measured in the greenhouse are reported. Daily air temperature was on average 21°C, and minimum and maximum air temperature ranged from - 0.2 to 17.5 and from 17.5 to 46.0°C, respectively (Fig. 1A). Air relative humidity was on average 50.5%; daily minimum and maximum relative humidity ranged from 6 to 56% and from 45 to 85% (Fig. 1B). The average photosynthetically active radiation was 282 µmol m^{-2} s^{-1}; its mean and maximum values changed from 80 to 420 and from 400 to 2,080 µmol m^{-2} s^{-1}, respectively (Fig. 1C). After harvest, lettuces were immediately transported under refrigerated condition in polystyrene boxes to the CNR ISPA- postharvest laboratory.

Processing and storage

After harvest, for each multi-leaf lettuce cultivar ('Ezra', 'Eztoril' and 'Ezabel'), and for each agricultural growing system (S or SL), leafs were selected in order to avoid damaged samples, and no washing or pre-treatment were applied. For each cultivar and GS about 600 g of leaves were used for quality evaluation at harvest, whereas about 1.2 Kg were used for the quality evaluations during storage. Thus, leaves were put in open polyethylene bags (about 200 g each bag), and stored at 8°C in dark conditions. For each cultivar, 12 bags (3 replicates × 2 GS, S or SL, × 2 storage periods, 7 and 13 days) were prepared.

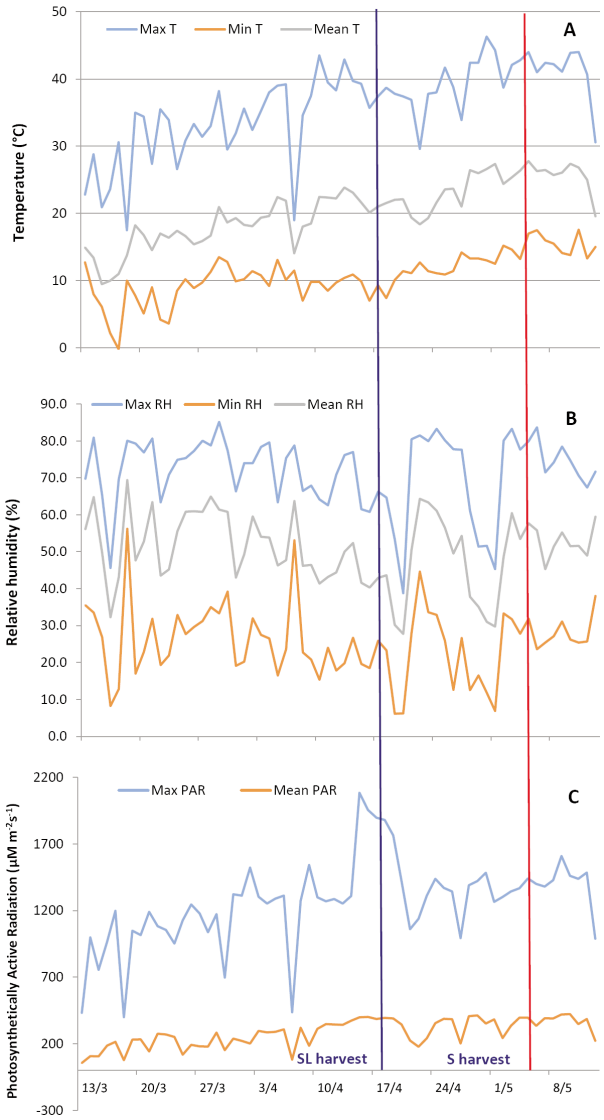

Fig. 1 - Climatic parameters (A= Temperature; B= air Relative humidity; C= Photosynthetically Active Radiation) measured in the greenhouse during the experiment, from seedling transplantation to harvest days. The blue and red perpendicular lines indicate the harvest day for lettuce cultivated in soilless and soil condition, respectively.

Respiration activity and the analysis of nitrate content were performed at harvest. In addition, antioxidant activity, total phenols, ammonium content and microbiological analysis were evaluated at harvest and after 7 and 13 days of storage.

Respiration activity

The respiration activity of each cultivar was measured using a closed system as reported by Kader (2002). About 100 g of leaves for each replicate were put into 6 L sealed plastic jars (one jar for replicate) where CO_2 was allowed to accumulate until the value of 0.1%. The time needed to reach this value was calculated, making CO_2 measurement at regular time intervals. For the CO_2 analysis, 1 mL gas sample was taken from the head space of the plastic jars through a rubber septum and injected into the gas chromatograph (p200 micro GC, Agilent, Santa Clara, CA) equipped with dual columns and thermal conductivity detector. CO_2 was analyzed with a retention time of 16 s and total run time of 120 s on a 10 m porous polymer (PPU) column at a constant temperature of 70°C. Respiration activity was expressed as mL $CO_2 \cdot kg^{-1} \cdot h^{-1}$.

Antioxidant activity, total phenol, ammonium and nitrate content

To determine both antioxidant activity and total phenols content, the extraction procedure reported by Cefola *et al.* (2012), was followed. In detail, 5 g samples were homogenized (Ultraturrax T-25, IKA Staufen, Germany) in a MeOH: water (80:20) solution for 1 min, and then centrifuged at 5°C at 6440 x *g* for 5 min. The supernatant was therefore used for the assays. The antioxidant activity assay was performed following the procedure described by Brand-Williams *et al.* (1995) with minor modifications. Briefly, the supernatant, proper diluted, was pipetted into 0.95 mL of DPPH solution to start the reaction. The absorbance was read after about 30 min at 515 nm. Trolox was used as a standard and the antioxidant activity was expressed in g of Trolox equivalents per kg of fresh weight (g TEAC kg^{-1} fw). The total phenol content was determined according to the method of Singleton and Rossi (1965). Each extract (100 µL), proper diluted, was mixed with 1.58 mL water, 100 µL of Folin-Ciocalteu reagent and 300 µL of sodium carbonate solution (200 g L^{-1}). The absorbance was read after 2 h at 765 nm. Total phenol content was calculated on the basis of the calibration curve of gallic acid and expressed as g of gallic acid per kg of fresh weight (g GA kg^{-1} fw).

For ammonium content the method reported by Weatherburn (1967) was used. In detail, 5 grams of chopped sample were homogenized (Ultraturrax T-25, IKA Staufen, Germany) with 20 mL distilled water for 2 min, centrifuged at 6440 x *g* for 5 min, and 0.5 mL extract was used for the analysis. Color development, caused by the reaction with a phenol nitroprusside reagent and alkaline hypochlorite solution, was determined after an incubation of 20 min at 37°C, by reading the absorbance at 635 nm (UV-1800, Shimadzu, Kyoto, Japan). Ammonium content was expressed as µmole NH_4^+ per kg of fresh weight (µmole NH_4^+ kg^{-1} fw).

As for nitrate content, samples (about 100 g for replicates) were dried in the oven (65°C until constant weight) and were ground to fine powder. The powder (0.5 g for each replicate) were extracted on orbital shaker for 20 minutes with 50 mL of a solution containing 3.5 mmol L^{-1} of sodium carbonate and 1 mmol L^{-1} sodium bicarbonate. Analysis were carried out using a ion exchange chromatography (Dionex DX 200, Dionex Corp, Sunyvale, CA, USA) with a conductivity detector, using an IonPac AG14 precolumn and an IonPac AS4A separation column (Dionex Corporation). Results were expressed in mg of nitrate per kg of fresh weight (mg NO_3^- kg^{-1} fw).

Microbiological analysis

Samples (30 g for replicates) were homogenized for 1 min in 0.1% sterile buffered peptone water (Difco Laboratories, Detroit, MI, USA) (1:5 dilution) using a stomacher (Seward, London, UK). Total aerobic mesophilic bacteria count was evaluated using plate count agar (Difco) incubated at 30°C for 48 h. Yeasts and moulds were counted on Sabouraud Dextrose Agar (Difco) supplemented with chloramphenicol and chlortetracycline (both 0.05 g L^{-1}) and incubated at 25°C for 5-7 days. Total counts of *Enterobacteriaceae* were obtained by pour-plating dilutions (1 mL) in Violet Red Bile Glucose agar (Difco) and plates were incubated at 37°C for 24 h. Microbiological counts were expressed as log CFU g^{-1} of fresh weight (log CFU g^{-1} fw).

Statistical analysis

In order to study the effect of GS (S or SL), cultivars, CV ('Ezra', 'Eztoril' and 'Ezabel') and their interaction (GS x CV) on quality parameters at harvest, and the effect of GS, CV, storage (0-7-13 days) and their interaction (GS x CV x storage) on quality parameters, two multifactor ANOVA were performed (Statistica Software). When significant effect of factors were detected, the Student Newman Keuls (SNK) test was applied to separate means. For a visual

analysis of the data, principal component analysis (PCA) (PRINCOMP procedure, SAS software, Cary, NC, USA; biplot by XLStat, Addinsoft, Paris, France) was performed on mean centered and standardized (unit variance scaled) data prior to analysis. The data matrix submitted to PCA was made up of 18 observations - 3 cultivars ('Ezra', 'Eztoril' and 'Ezabel') x 2 growing system (S and SL) x 3 storage times (0-7-13 days) and 6 quality parameters (antioxidant activity, total phenols, ammonium, mesophilic bacteria, yeasts and moulds, *Enterobacteriaceae*).

3. Results

Effect of growing systems and cultivars on lettuces quality traits at harvest

Yield response was influenced by genotypes more than GS (S or SL), since a lower fresh weight was produced by the red lettuce compared to the other two cultivars (2.7 *vs* 3.6 kg m^{-2}) and only in cv. *Eztoril* there was a higher yield in S compared to SL system (4.3 *vs* 3.3 kg m^{-2}).

The effect of GS, multi-leaf lettuce CV ('Ezra', 'Eztoril' and 'Ezabel') and their interaction on the quality parameters measured at harvest was investigated (Table 2). Ammonium content, antioxidant activity, total phenols and nitrate content were significantly affected by GS and CV, while respiration activity was affected only by CV. The interaction GS x CV was statistically significant only for nitrate content (Table 2). The respiration activity of cv. Ezra was two-fold higher than the green cultivars ('Eztoril' and 'Ezabel'). Regarding ammonium content, the values found for lettuces cultivated in SL were statistically higher respect to S and, between cultivars, red multi-leaf lettuce had mean values statistically higher than green cultivars (Table 2). Growing system affects significantly the antioxidant activity and total phenols: plants cultivated in S showed significantly higher mean content than SL samples (Table 2). Regarding CV, there were no differences between green multi-leaf lettuces in antioxidant activity and total phenols, while the red cultivar Ezra had lower values of antioxidants and higher values of total phenols respect to the green cultivars (Table 2). As for nitrate content was almost double in SL lettuces than S grown ones (838 *vs* 432 mg NO_3^- kg^{-1} fw). The red multi-leaf lettuce (cv. Ezra) had mean values of nitrate statistically higher than cv. Eztoril but not different from cv. Ezabel (Table 2).

Effect of growing systems and cultivars on lettuces' quality traits and microbial parameters during cold storage

The results of Multifactor Anova on antioxidant activity, total phenols and ammonium content as affected by GS, CV, storage time (0, 7 and 13 days) and their interaction were reported in Table 3. Growing system affected antioxidant activity and total phenols, CV affected total phenols and ammonium, while storage time affected antioxidant activity

Table 2 - Effect of growing system (soil and soilless) and cultivar (Ezra, Eztoril and Ezabel) on quality parameters measured at harvest

	Respiration activity (mL CO_2 kg^{-1} h^{-1})	Ammonium content (µmole NH_4^+ kg^{-1}fw)	Antioxidant activity (g TEAC kg^{-1} fw)	Total phenols (g GA kg^{-1} fw)	Nitrate content (mg NO_3^- kg^{-1} fw)
Growing system (GS)					
Soilless	47.44	66.7	2.33	1.32	838.12
Soil	45.73	49.6	4.82	1.84	431.96
Cultivar					
Ezra	72.67 a	72.80 a	3.07 b	1.89 a	765.71 a
Eztoril	35.82 b	48.10 b	3.82 a	1.41 b	524.32 b
Ezabel	31.26 b	53.60 b	3.84 a	1.44 b	615.09 ab
GS	NS	**	***	**	***
Cultivar	***	**	**	*	*
GS x cultivar	NS	NS	NS	NS	*

When interaction among factors was not significant, the results of the mean separation test (SNK test) are reported. Different letters indicate statistical difference within cultivars, respectively, for P≤0.05. NS, not significant; * P≤0.05; ** P≤0.01; *** P≤0.001.

and ammonium (Table 3). Considering the interaction among factors, antioxidant activity was affected by GS x Storage and CV x Storage; total phenols were influenced by GS x CV and by GS x CV x Storage, and ammonium was affected only by GS x Storage (Table 3).

Table 3 - Multifactor Anova of antioxidant activity total phenols and ammonium content as affected by growing system (soil or soilless), cultivar ('Ezra', 'Eztoril' and 'Ezabel') and storage time (0, 7 and 13 days)

	Antioxidant activity (g TEAC kg^{-1} fw)	Total phenols (g GA kg^{-1} fw)	Ammonium content (µmole $NH_4^+ kg^{-1}$fw)
Growing system (GS)	***	**	NS
Cultivar (CV)	NS	**	**
Storage time	***	NS	*
GS x CV	NS	**	NS
GS x Storage	*	NS	*
CV x Storage	***	NS	NS
GS x CV x Storage	NS	*	NS

NS= not significant; * P≤0.05; ** P≤0.01; *** P≤0.001.

In figure 2, changes in antioxidant activity (A), total phenols (B) and ammonium content (C) during storage of the three multi-leaf lettuce cultivars, cultivated in S and SL conditions, are reported. At harvest, lettuces cultivated in S showed values of antioxidant activity significantly higher than SL lettuce. However, during storage, antioxidant activity of lettuces cultivated in S decreased rapidly, reaching approximately the same values of samples cultivated in SL after 7 day of storage at 8°C; after it remained unchanged for cv. Ezabel (about 2 g TEAC kg^{-1} fw) and slightly increased for cv. Eztoril (about 3 g TEAC kg^{-1} fw). The cultivar Ezra cultivated in S showed a content in antioxidant activity almost constant during storage, with a 30% reduction at the end of storage (Fig. 2A). Whereas, lettuce cultivated in SL showed unchanged values of antioxidant activity during time, with a slight reduction at the end of storage for cv. Ezabel (about 1.3 g TEAC kg^{-1} fw) (Fig. 2A). Regarding the content of total phenols (Fig. 2B), green multi-leaf lettuces cultivated in S showed a slight decrease during the first week of storage, after then values rise again until the end of storage, reaching values of about 1.6 and 1.7 g GA kg^{-1} fw for cv. Eztoril and Ezabel, respectively (Fig. 2B). A specular trend for green multi-leaf lettuce cultivated in SL was observed (Fig. 2B). The cv. Ezra cultivated in SL showed the same behavior of green lettuces; whereas 'Ezra' cultivated in S showed an initial total phenol content of about 2.3 g GA kg^{-1} fw, which increased during the first week of storage, reducing to initial values until the end of the storage (Fig. 2B).

As regard data of ammonium content (Fig. 2C) lettuces cultivated in SL showed unchanged values during postharvest storage, starting from the initial mean values of about 79.9±7.5, 57.0±9.3 and 63.2±9.6 µmole NH_4^+ kg^{-1} fw in cv. Ezra, Eztoril and Ezabel, respectively. Whereas, lettuces cultivated in S showed an increase in ammonium content during storage, which doubled for all cultivars, starting from initial mean values of 65.5±15.7, 39.2±3.5 and 44.0±7.0 µmole NH_4^+ kg^{-1} in cv. Ezra, Eztoril and Ezabel, respectively.

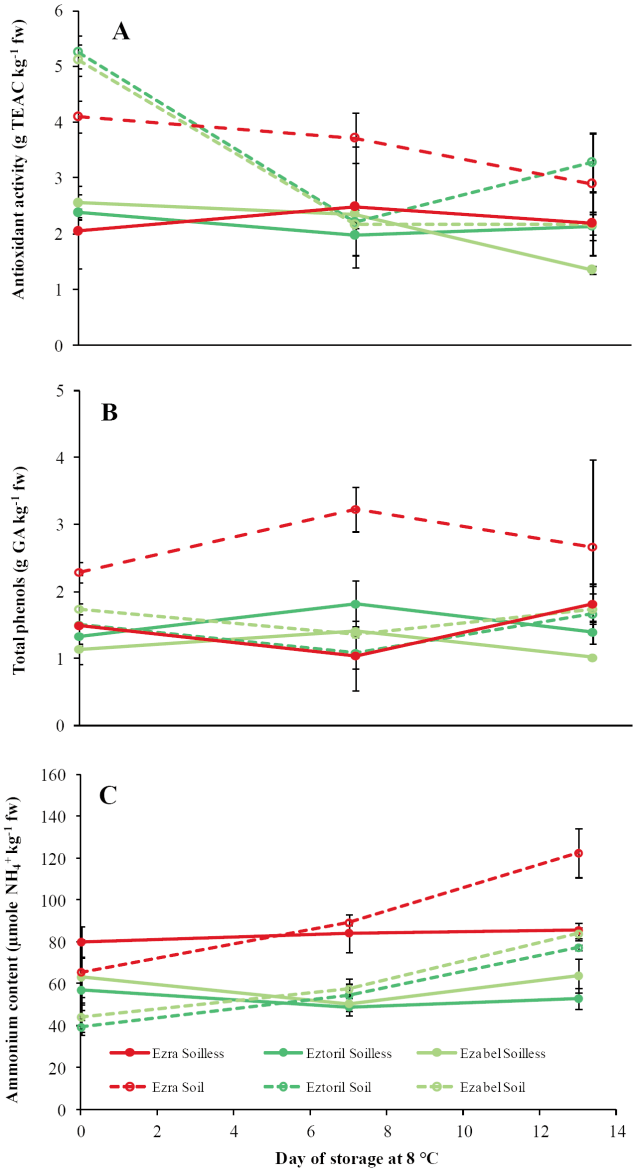

Fig. 2 - Changes in antioxidant activity (A), total phenols (B) and ammonium content (C) of three multi-leaf lettuce cultivars ('Ezra', 'Eztoril' and 'Ezabel'), cultivated in soil or soilless condition, during storage at 8°C. Mean ± SD.

As concerns microbial populations evaluated during the trial, results of multifactor Anova statistical analysis of mesophilic bacteria, yeasts and moulds and *Enterobacteriaceae* as affected by growing system (GS), cultivars (CV) and storage time are reported in Table 4. All factors and their interactions affected significantly the microbial populations evaluated, with the exception of GS x CV interaction for yeasts and moulds. As concerns total mesophilic bacteria, lettuces cultivated in SL showed an increase of about 1.5 log unit during storage, starting from initial mean values of 3.7±0.05 log CFU g^{-1} (cv. Eztoril), 4.9±0.22 log CFU g^{-1} (cv. Ezabel) and 6.1±0.29 log CFU g^{-1} (cv. Ezra), whereas lettuces cultivated in S growing condition showed an higher significant increase in mesophilic population during storage, of about 3 log unit, starting from a mean initial count of about 4.67±0.68 log CFU g^{-1} (Fig. 3A). Yeast and mould loads from S and SL were not found to be significantly different. However, during 13 days of cold storage their amount increased significanly for all cultivars, resulting statistically higher in multi-leaf lettuces cultivated in S respect to the SL ones (Fig. 3B). Also in the case of *Enterobacteriaceae*, lettuces cultivated in S showed a significant increase of about 4 log unit at the end of the storage, starting from initial values of 3.4±0.9, 2.4±0.26 and 4.10±0.10 log CFU g^{-1} for cv. Ezra, Eztoril and Ezabel, respectively. In lettuces cultivated in SL conditions, the increase of about one magnitude order was found for this microbial population (Fig. 3C).

Table 4 - Multifactor Anova of mesophilic bacteria, yeasts and moulds and *Enterobacteriaceae* as affected by growing system (soil or soilless), cultivar ('Ezra', 'Eztoril' and 'Ezabel') and storage time (0, 7 and 13 days)

	Mesophilic bacteria	Yeasts and moulds (log CFU g^{-1} fw)	*Entero-bacteriaceae*
Growing system (GS)	**	*	*
Cultivar (CV)	***	*	***
Storage time	***	***	***
GS x CV	***	NS	***
GS x Storage	***	***	***
CV x Storage	***	**	***
GS x CV x Storage	***	*	***

NS= not significant; * P≤0.05; ** P≤0.01; *** P≤0.001.

Principal component analysis

Principal component analysis revealed that almost 84% of the total variability of data was explained by the first two principal components. PC1 resulted mainly and positively correlated to ammonium and the counts of the three microorganisms groups. Each of them contributed to PC1 for 20-25% of the total variability (Fig. 4). On the other hand, antioxidant activity and total phenols contributed to PC2 for more than 45% each. Among observations, all S growing system samples after 13d storage showed a stronger and positive correlations with PC1, ammonium and the microbiological counts. At a proximate position collocated samples of the red cultivar Ezra collected from SL and stored for 7 and 13 days. On the contrary, all cultivars grown on the S system, at

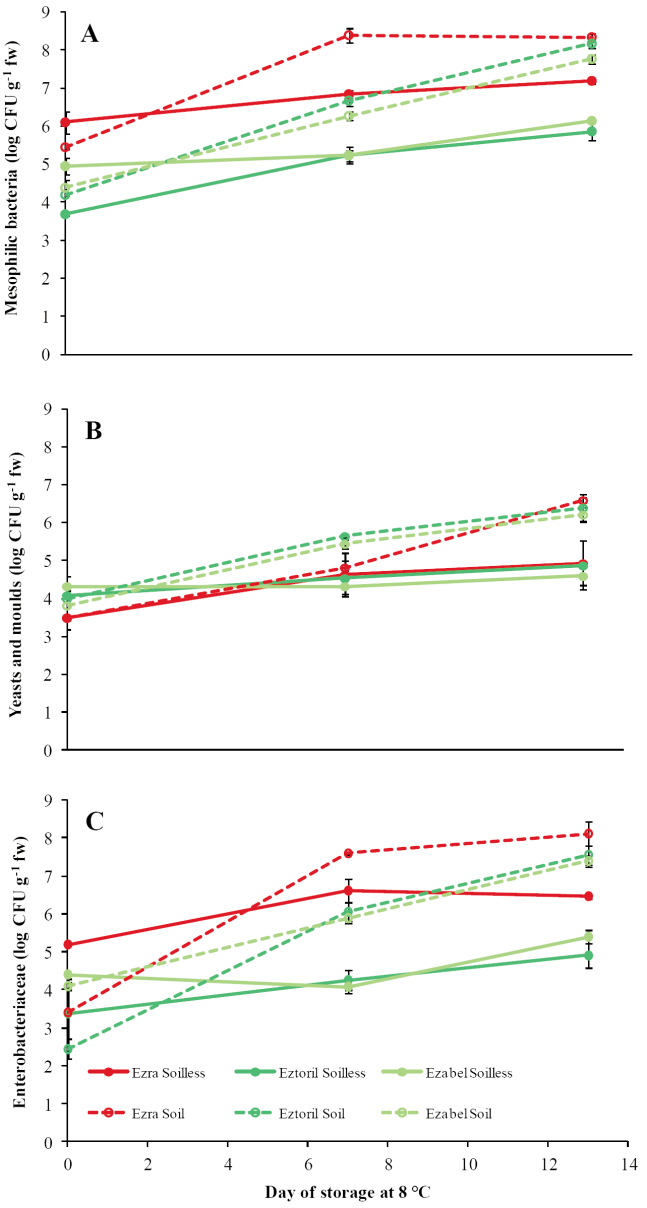

Fig. 3 - Changes in total mesophilic bacteria (A), yeasts and moulds (B) and *Enterobacteriaceae* (C) of three multi-leaf lettuce cultivars ('Ezra', 'Eztoril' and 'Ezabel'), cultivated in soil or soilless condition, during storage at 8°C. Mean ± SD.

harvest, were negatively correlated to PC1, on the opposite site of ammonium and microbial parameters. It seems that no observations were strongly correlated to antioxidant activity and total phenols, with the exception of soil-grown Ezra leaves sampled at harvest and at 7 days storage, followed by the other two cultivars coming from S at harvest and soil-grown Ezra at 13 days storage (Fig. 4). Among observations negatively correlated to PC2, one result noteworthy, the SL-grown 'Ezabel' sampled at 13 days after storage, since it showed a sharp decrease in the antioxidant activity at the end of the storage, as described in figure 2A.

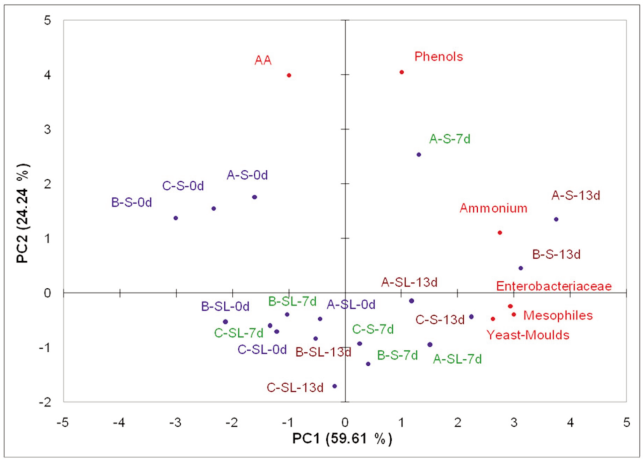

Fig. 4 - PCA biplot (PC1 *vs* PC2) describing the spatial distribution of quality and microbiological parameters of three multi-leaf lettuce cultivars ('Ezra', 'Eztoril' and 'Ezabel') grown in soil (S) and soilless (SL) system during storage; A= Ezra; B= Eztoril; C= Ezabel. AA= antioxidant activity; Phenols= total phenols; 0d – 7d – 13d: 0-7-13 days of storage.

4. Discussion and Conclusions

The three multi-leaves lettuces studied in this research showed different qualities at harvest. In particular, the green cultivars resulted very similar and suitable for postharvest processing, on the basis of respiration activity and ammonium content. At contrary, the red cultivar was considered more perishable than the green ones, due to the high respiration activity (Kader, 2002) even though it showed an higher content in polyphenols than the green cultivars, as previously reported by other authors (Martínez-Sánchez *et al.,* 2012; Selma *et al.,* 2012). As regards antioxidant activity and total phenols, the higher contents measured in S cultivated lettuces could be a plant response to applied stress treatments (Oh *et al*., 2009). Compared to the SL growing system, S irrigation management implies necessarily different water conditions, keeping S plants in not optimal and constant water availability all-day. As a consequence, S plants may have occasionally experienced a water stress combined to heat stress during the highest temperature hours under greenhouse, in the few days preceding harvest (Fig. 1). Even a time-limited stress can activate the antioxidant synthesis in the plant metabolism (Oh *et al*., 2009). On the other hand, the more constant availability of water and nutrients in SL grown plants allowed a higher nitrogen uptake, partially accumulated as nitrate in the vacuoles at higher rate than in S cultivated lettuces. However, the nitrate content measured at harvest was generally low in both GS compared to the limits imposed by the EU Regulation No. 1258/2011. In compliance with the current regulation, nitrate accumulation in lettuce grown in the spring-summer period under greenhouse should not exceed 4,000 mg kg^{-1} fw. This limit is in agreement with the potentially high nitrate accumulating capacity of lettuce. However, at our latitude, the optimal light conditions found by plants during the spring months allow an efficient and fast assimilation of the up-taken nitrate. During cold storage, the SL growing system resulted able to preserve the quality of lettuces since no increase in ammonium content (senescence indicator) was registered whereas multi-leaf lettuces cultivated in S that resulted more senescence-prone. Ammonium accumulates in leafy vegetables during storage, as consequence of protein catabolism. Thus, ammonium was used as indicator of quality and shelf-life of green vegetable. (Chandra *et al*., 2006; Pace *et al.*, 2014; Cefola *et al.*, 2015; Cefola and Pace, 2015; Cefola *et al.*, 2017). Data from ammonium confirms that SL could be considered a suitable growing system to preserve postharvest quality of the cultivars analysed, although genotyping characteristics of each cultivar need also to be taken into account (Selma *et al*., 2012). During storage nitrate measurements were not carried out, since in preliminary trials performed on the same lettuce genotype (cv. Ezra) no nitrate changes after storage at 8°C for 10 days were detected. In particular nitrate remained unchanged at 1550 and 1800 mg kg^{-1} fw, in soilless and soil lettuce, respectively (data not shown). This was supported by several contributes in literature, referring about no modification of nitrate concentration in lettuce and other species during storage at temperature in a range from 1 to 10°C (Siomos *et al*., 2002; Chung *et al*., 2004; Konstantopoulou *et al*., 2010). The SL cultivation showed a positive effect

also on microbiological quality of the green cultivars during storage. Similarly, results were reported by other authors on table grape (Cefola *et al.,* 2011) and on soilless growing systems (Scuderi *et al*., 2011; Selma *et al*., 2012).

In conclusion, the three multi-leaves lettuces studied in this research showed different qualities at harvest. In particular, green cultivars resulted very similar and suitable to postharvest processing, whereas, the red one was considered more perishable. At harvest, lettuces grown in soil showed the higher content in antioxidant activity and total phenols and the lower in nitrate than soilless samples. However, the nitrate content measured at harvest was generally low in both growing systems compared to the limits imposed by the EU Regulation No. 1258/2011. Regarding the postharvest storage, ready-to-use lettuces cultivated in soilless showed microbiological and qualitative performance better than those grown in soil. In particular, soilless growing system improved the storability of lettuces and allowed to the production of clean raw material, particularly suited for ready to use industry. It is interesting to note, as soilless system resulted able to limit ammonium accumulation (senescence indicator), also in red cultivar, which for genotipic traits resulted more senescence prone than green lettuces.

Acknowledgements

This research was financed by MIUR Research Projects: "High-Convenience Fruits and Vegetables: New Technologies for Quality and New Products," PON01_01435.

References

BARRETT D.M., BEAULIEU J.C., SHEWFELT R., 2010 - *Color, flavor, texture, and nutritional quality of fresh cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing.* - Cr. Rev. Food Sci., 50: 369-389.

BRAND-WILLIAMS W., CUVELIER M.E., BERSET C., 1995 - *Use of a free radical method to evaluate antioxidant activity*. - LWT - Food Sci. Technol., 28: 25-30.

CANTWELL M., 2004 - *Evaluating the quality of fresh-cut products*. - In: CANTWELL M. (ed.) *Fresh-cut products: Maintaining quality and safety*. Postharvest Horticulture Series No. 10. University of California, Davis, CA.

CEFOLA M., AMODIO M.L., RINALDI R., VANADIA S., COLELLI G., 2010 - *Exposure to 1-methylcyclopropene (1-MCP) delays the effects of ethylene on fresh-cut broccoli raab (*Brassica rapa *L.).* - Postharvest Biol. Technol., 58: 29-35.

CEFOLA M., D'ANTUONO I., PACE B., CALABRESE N., CARITO A., LINSALATA V., CARDINALI A., 2012 - *Biochemical relationships and browning index for assessing the storage suitability of artichoke genotypes*. - Food Res. Int., 48: 397-403.

CEFOLA M., DE BONIS M.V., PACE B., 2017 - *Preliminary modeling of the visual quality of broccoli along the cold chain.* - Eng. Agric. Environ. Food, 10(2): 109-114.

CEFOLA M., PACE B., 2015 - *Application of oxalic acid to preserve the overall quality of rocket and baby spinach leaves during storage*. - J. Food Process. Pres., 39: 2523-2532.

CEFOLA M., PACE B., BUTTARO D., SANTAMARIA P., SERIO F., 2011 - *Postharvest evaluation of soilless-grown table grape during storage in modified atmosphere*. - J. Sci. Food Agr., 91: 2153-2159.

CEFOLA M., PACE B., COLELLI G., CANTWELL M., 2015 - *Compositional and marketable quality of fresh-cut florets of four specialty brassicas in relation to controlled atmosphere storage*. - Acta Horticulturae, 1071: 455-462.

CHANDRA D., MATSUI T., SUZUKI H., KOSUGI Y., 2006 - *Postharvest changes in some physiological traits and activities of ammonia-assimilating enzymes in lettuce during storage*. - Asian J. Plant Sci., 5: 378-384.

CHUNG J., CHOU S., HWANG D., 2004 - *Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures*. - Food Additive Contam., 21(4): 317-322.

CLARKSON G.J.J., O'BYRNE E.E., ROTHWELL S.D., TAYLOR G., 2003 - *Identifying traits to improve postharvest processability in baby leaf salad*. - Postharvest Biol. Technol., 30: 287-298.

CRISOSTO C.H., MITCHELL J.P., 2002 - *Preharvest factors affecting fruit and vegetable quality*, pp. 49-54. - In: KADER A.A. (ed.) *Postharvest technology of horticultural crops*. Division of Agriculture and Natural Resources, University of California, CA, USA.

JOHNSON C.M., STOUT P.R., BROYER T.C, CARLTON A.B., 1957 - *Comparative chlorine requirements of different plant species*. - Plant and Soil, 8: 337-353.

KADER A.A., 2002 - *Postharvest biology and technology: an overview*, pp. 39-47. - In: KADER A.A. (ed.) *Postharvest Technology of Horticultural Crops*. - Division of Agriculture and Natural Resources, University of California.

KONSTANTOPOULOU E., KAPOTIS G., SALACHAS G., PETROPOULOS S.A., KARAPANOS I.C., PASSAM H.C., 2010 - *Nutritional quality of greenhouse lettuce at harvest and after storage in relation to N application and cultivation season*. - Sci. Hortic., 125(2): 93-95.

LOPEZ-MEDINA J., PERALBO A., FERNANDEZ M.A., HERNANDEZ D., TOSCANO G., HERNANDEZ M.C., FLORES F.,

2004 - *Substrate system for production of strawberry fruit in Spain and Mediterranean climates*. - Proc. Fifth Int. Conf. on Alternatives to Methyl Bromide, Lisbon, Portugal, pp. 47-51.

LUNA M.C., MARTÍNEZ-SÁNCHEZ A., SELMA M.V., TUDELA J.A., BAIXAULIB C., GIL M.I., 2013 - *Influence of nutrient solutions in an open-field soilless system on the quality characteristics and shelf life of fresh-cut red and green lettuces (*Lactuca sativa *L.) in different seasons*. - J Sci. Food Agric., 93: 415-421.

MARTÍNEZ-SÁNCHEZ A., LUNA M.C., SELMA M.V., TUDELA J.A., ABAD J., GIL M.I., 2012 - *Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry*. - Postharvest Biol. Technol., 63: 1-10.

NICOLA S., TIBALDI G., FONTANA E., 2009 - *Fresh-cut produce quality: implications for a systems approach*, pp. 247-275. - In: FLORKOWSKI W.J., R.L. SHEWFELT, B. BRUECKNER, and S.E. PRUSSIA (eds.) *Postharvest handling: A systems approach*. Second edition, Academic Press, NY, USA.

OH M.M., CAREY E.E., RAJASHEKAR C.B., 2009 - *Environmental stresses induce health-promoting phytochemicals in lettuce*. - Plant Physiol. Biochem., 47: 578-583.

PACE B., CARDINALI A., D'ANTUONO I., SERIO F., CEFOLA M., 2014 - *Relationship between quality parameters and the overall appearance in lettuce during storage.* - J. Food Process. Technol., 1: 18-26.

RECAMALES A.F., LOPEZ-MEDINA J., HERNANZ D., 2007 - *Physicochemical characteristics and mineral content of strawberries grown in soil and soilless system*. - J. Food Quality, 30: 837-853.

RIJK ZWAAN, 2009 - *Convenience catalogue, Vegetable varieties for the fresh cut industry.* www.rijkzwaan.com/convenience.

RODRÍGUEZ-HIDALGO S., ARTÉS-HERNÁNDEZ F., GÓMEZ P., ARTÉS F., 2010 - *Quality changes on minimally processed purslane baby leaves growth under floating trays system*. - Acta Horticulturae, 877: 641-648.

SALINAS-HERNÁNDEZ R.M., GONZÁLEZ-AGUILAR G.A., TIZNADO-HERNÁNDEZ M.E., 2015 - *Utilization of physicochemical variables developed from changes in sensory attributes and consumer acceptability to predict the shelf life of fresh-cut mango fruit*. - J. Food Sci. Technol., 52:63-77.

SCUDERI D., RESTUCCIA C., CHISARI M., BARBAGALLO R.N., CAGGIA C., GIUFFRIDA F., 2011 - *Salinity of nutrient solution influences the shelf-life of fresh-cut lettuce grown in floating system*. - Postharvest Biol. Technol., 59: 132-137.

SELMA M.V., LUNA M.C., MARTÍNEZ-SÁNCHEZ A., TUDELA J.A., BELTRÁN D., BAIXAULI C., GIL M.I., 2012 - *Sensory quality, bioactive constituents and microbiological quality of green and red fresh-cut lettuces (*Lactuca sativa *L.) are influenced by soil and soilless agricultural production systems*. - Postharvest Biol. Technol., 63: 16-24.

SILBERBUSH M., BEN-ASHER J., 2001 - *Simulation study of nutrients by plants from soilless cultures as affected by salinity build up and transpiration*. - Plant and Soil., 233: 59-69.

SINGLETON V.L., ROSSI J.A., 1965 - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents*. - Am. J. Enol. Viticult., 16: 144-158.

SIOMOS S.A, PAPADOPOULOU P.P., NIKLIS D.N, DOGRAS C.C., 2002 - *Quality of romaine and leaf lettuce at harvest and during storage*. - Acta Horticulturae, 579: 641-646.

VALENZANO V., PARENTE A., SERIO F., SANTAMARIA P., 2008 - *Effect of growing system and cultivar on yield and water-use efficiency of greenhouse-grown tomato*. - J Hortic. Sci. Biotechnol., 83: 71-75.

WEATHERBURN M.W., 1967 - *Phenol-hypochlorite reaction for determination of ammonium*. - Anal. Chem., 39: 971-974.