

## Mixing fresh-cut baby green and red leaf lettuce from soilless cultivation preserves phytochemical content and safety

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The objective of this study was to evaluate the impact of different mixtures of two fresh-cut baby lettuce (*Lactuca sativa* L. var. *crispa* cv. Lollo Bionda [LB] and cv. Lollo Rossa [LR]) cultivars on lettuce phytochemical composition during postharvest. Lettuces were grown in a soilless culture system with continuous flotation (FL) in a greenhouse, mixed at harvest and packaged in polypropylene bags and stored at 4 °C for 9 days (d9). Mixes were made of 100, 75, 50, 25 and 0% of LB, respectively. The results showed that the phytochemicals were preserved during storage. In specific, 25LB had the highest pigment content on d1, while 50LB and 25LB had the highest inherent quality on d1. FL led to a reduced microbial contamination, thus, limiting its growth during storage. The results have revealed that high quality and microbiologically safe baby leaf vegetables (BLV), can be obtained by means of FL. The adopting a mix of lettuce cultivars could represent a positive postharvest practice to preserve the phytochemicals of BLV throughout their shelf life.

**Key words:** floating system, minimally processed food, shelf life, microbial contamination, 'Lollo' lettuce, baby leaf vegetables

### Introduction

Consumption of green leafy vegetables has been growing in response to the emergent number of degenerative diseases (Johnson et al. 2019). Increasing evidence has shown that ingestion of fresh products can decrease the risk of cancer and cardiovascular disorders (FAO 2019). Vegetables are important, since they supply bioactive compounds such as dietary fiber, vitamins, minerals, and numerous health-related benefits (Silva 2012).

The quality of raw material at harvest and its suitability to be processed are of fundamental importance to maintain high-quality fresh cut products during their shelf life (Ansah et al. 2018). Lettuce (*Lactuca sativa* L.) is one of the primary fresh-cut leafy vegetables and the major constituent of fresh salads (Selma et al. 2012). In the past, whole-head and multi-leaf lettuces were predominantly used to prepare fresh-cut salad mixes, but recently other types of lettuces, such as baby leaves, have increasingly been used. Baby leaf vegetables (BLV) are coreless, completely edible, and can be easily and quickly processed (Conesa et al. 2015). The market for minimally processed ready-to-eat baby-leaf vegetables is constantly increasing, and is offering to consumers the products rich in bioactive compounds. These vegetables are collected at a very early stage of development and prepared with minimal processing methods such as cutting, washing, rinsing, drying and packaging.

BLV have a low microbial contamination at harvest and have only a small section, the petiole, exposed to oxidation, thus, suggesting a relative increase in the postharvest shelf life compared to whole-head and multi-leaf lettuces (Rodríguez-Hidalgo et al. 2010). In recent years, the use of red leaf lettuce in fresh cut mixes has increased because the consumers consider the pigmentation a positive characteristic (Martínez Sánchez et al. 2012). The intensive red color of the leaves has been attributed to the phenolic compounds and in part to carotenoids (Sarker et al. 2018).

Phenolic compounds have been attracting attention due to their importance in preserving human health (Bettaieb et al. 2011). The pharmacological effects of different phenolic compounds comprise anti-inflammatory, antimicrobial, cardiac and gastrointestinal protection, and improved blood circulation properties (Alinian et al. 2016, Németh-Zámbori et al. 2016). Carotenoids are naturally occurring phytochemicals that exhibit various health benefits. Amongst the carotenoids,  $\beta$ - and  $\alpha$ -carotene possess provitamin-A activity and lutein and zeaxanthin are studied for their putative action in the prevention of macular degeneration (SanGiovanni et al. 2012). Limited information is available regarding the phytochemical pigment contents in BLV after minimal processing during postharvest period.

The growing systems, such as soilless culture systems (SCS), used in protected cultivations, have partially replaced traditional culture systems (TCS) in soil. Floating growing systems (FGS) can be implemented in SCS with continuous flotation (FL), that is, with trays floating continuously on a bed of water or on a hydroponic nutrient solution (HNS), or with ebb and-flow flotation system, in this way scheduling the drying period for species suffering from root hypoxia (Nicola et al. 2015, 2016). Tissue ions accumulation can easily be controlled with SCS by varying the N-ratio in the HNS or its composition.

Nitrate is considered a threat to human health, not so much due to its toxicity, which is low, but due to the conversion to nitrite in the organism. The toxicity of nitrate is due to its reduction to nitrite and conversion to nitrosamines and nitrosamides through reaction with amines and amides, whose carcinogenic action is well known (Demeyer et al. 2016).

The sanitation of the process and processing ambient, as well as of the raw materials used, are important aspects for the microbiological safety of products in fresh-cut vegetable production. As a result, the absence of an inactivation phase before consumption, fresh produce is subject to an augmented risk of contamination to different pathogens (Dar et al. 2020). Besides the avoidable microbial contamination and damaged tissue, fresh cut products must be attractive to consumers because general appearance is one of the most important attributes evaluated before they are bought (Bahram-Parvar and Lim 2018).

Although attention to the BLV that are used as fresh-cut products, either on their own or mixed with others, has increased, little information is available in literature on the postharvest behavior of mixed fresh-cut BLV. Therefore, the objective of this work was to investigate the influence of the phytochemical composition and safety of two baby leaf lettuces grown in an FGS, that is, FL.

To the best of the authors' knowledge, there is a lack of literature on the quality of fresh-cut leafy vegetables, resulting from the mix effect. Consequently, fresh-cut products were mixed in different ratios to depth the synergic effects of lettuce in postharvest period. To this aim, phytochemical compounds related to postharvest physiological processes of lettuce were evaluated.

## Materials and methods

### Plant material and growing conditions

The research was carried out at the DISAFA Tetti Frati Experimental Center (44°53'11.67"N; 7°41'7.00"E - 231 m a.s.l. Carmagnola [TO]), Italy) from June to July in an automatically-controlled temperature greenhouse. Two BLV were used: green lettuce seeds (*Lactuca sativa* L. var. *crispa* cv. Lollo Bionda, hereafter: LB) and red lettuce seeds (*Lactuca sativa* L. var. *crispa* cv. Lollo Rossa, hereafter: LR) (Furia Seed S.r.l., Monticelli Terme [PR], Italy), obtained from Oasi dell'Agricoltore di Cappa L. & C. S.n.c. (Moncalieri [TO], Italy).

The experiment consisted of growing plants in 60-cell Styrofoam trays (0.51 m × 0.30 m; with 0.044 m upper and 0.025 m lower diameter cells) in FL with an HNS. Sixty trays were filled with ≈ 50 cm<sup>3</sup> per cell of a specific commercial peat-based horticultural medium (Neuhaus Huminsubstrat N17; Klasmann-Deilmann® GmbH, Geeste, Niedersachsen, Germany) by means of an automatic filling machine on the farm Azienda Agricola Vivaistica Ricca Sebastiano (Carignano [TO], Italy). Fifty percent of the trays were sown with LB and the other 50% with LR. The trays were then placed inside a plastic greenhouse and overhead irrigated twice per day for 1 min.

Sowing thinning was performed after cotyledon expansion to reach a final plant density of 120 plants per tray (≈ 784 plants m<sup>-2</sup>). About three weeks after seeding, the trays were moved into a lab-scale pilot plant (LSPP) in the same Experimental Center, equipped with three benches and polypropylene basin beds filled with 10 l of a 40/60 N-NO<sub>3</sub><sup>-</sup>/N-NH<sub>4</sub><sup>+</sup> HNS composed of (all in mmol l<sup>-1</sup>): 6N–2P–6K, 2 Mg, and 2.5 Ca per tray. Microelements (Lysodin® Multimix; Intrachem Bio Deutschland GmbH, Bad Camberg, Hessen, Germany), obtained from Cerealceretto S.r.l. (Carignano [TO], Italy), were added to the HNS. The pH of the HNS was monitored weekly and kept close to 5.5. Harvesting took place after 34 d of cultivation, early in the morning, to avoid the hottest hours of the day. The tools used for harvesting were sanitized before use. At harvest, the raw material was immediately transferred to the postharvest laboratory, located in the same structure as the LSPP, to be processed.

In addition, samples were taken and frozen to perform all chemical analyses; fresh samples were taken to evaluate the microbial spoilage. For each analysis 3 replicates were performed.

### Biometric parameters at harvest

The leaf fresh production (LFP) per square meter and leaf fresh weight (LFW) per plant were measured at harvest. Dry matter (DM) was calculated after drying at 60 °C.

### Processing, packaging and storage conditions

Raw material was sorted in a cold temperature room, and any damaged or yellow leaves were discarded. Five mixes of LB and LR were prepared with different weight ratios: 100% LB (100LB); 75% LB (75LB); 50% LB (50LB); 25% LB (25LB); 0% LB (0LB). Samples of 125 g were packaged in 0.25 m × 0.35 m thermo-sealed bags that had previously been prepared with polypropylene film with the following characteristics: oxygen permeance 1990 cm<sup>3</sup>·m<sup>-2</sup>·d<sup>-1</sup>·bar<sup>-1</sup>; carbon dioxide permeance 7800 cm<sup>3</sup>·m<sup>-2</sup>·d<sup>-1</sup>·bar<sup>-1</sup>; water vapor permeance 5.8 g·m<sup>-2</sup>·d<sup>-1</sup>·bar<sup>-1</sup>; film thickness 20 μm; weight 18.2 g·m<sup>-2</sup> (Alvapack S.r.l., Bologna, Italy).

The packaged samples were stored at 4 °C for a 9-d shelf life in refrigerated chambers (MEDIKA 600; C.F. di Ciro Fiocchetti & C. S.n.c., Luzzara,[RE], Italy), without light in the display cabinet. The phytochemical content and microbial contamination were measured at harvest (day 0 = d0), after 1 day of storage (d1) and at the end of the shelf-life (d9) in the DISAFA analytical laboratories.

### Fresh-cut product fresh weight loss

Fresh-cut product fresh weight loss (FWL) was measured by weighing the bags daily during storage, and calculated on the basis of the comparison with the d0 value as an index of freshness decay. The results were expressed as percentages.

### Pigment content analysis

Chlorophyll *a*, chlorophyll *b*, and the carotenoid (Chl. *a*, Chl. *b*, and *Car.*, respectively) contents were determined according to the Lichtenthaler and Wellburn (1983) method, with some modifications (Dere et al. 1998, Zhan et al. 2009). The results were expressed as mg g<sup>-1</sup> fresh weight (FW) according to the Lichtenthaler and Wellburn (1983) formula.

### Antioxidant capacity

Antioxidant capacity (AC) was determined using the ferric reducing ability of plasma (FRAP) assay, based on the Benzie and Strain (1996) method, with some modifications (Llorach et al. 2008), as a measure of antioxidant capacity. The results were expressed as μmol Fe<sup>2+</sup> g<sup>-1</sup> FW, and the calibration curve was prepared with a solution of ammonium ferrous sulfate hexahydrate.

### Total phenolic content

Total phenolic (TP) content was determined using the Folin-Ciocalteu procedure, method, with some modifications (Zhan et al. 2009). The results were expressed as mg gallic acid g<sup>-1</sup> FW, and the calibration curve with a methanol solution of gallic acid.

### Browning potential and soluble o-quinone content

The browning potential and soluble o-quinone (BP and So-Q, respectively) content were determined according to the Couture et al. (1993) method, with some modifications (Tardelli et al. 2013). The results were expressed as raw absorbance units (Abs<sub>340</sub> and Abs<sub>437</sub> for BP and So-Q, respectively).

### Enzyme activity analysis

The peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase (POD, PPO, and PAL, respectively) activities were determined according to literature. POD activity was determined as described by Nickel and Cunningham (1969), with some modifications (Zhan et al. 2009). The results were expressed as ΔA<sub>470</sub> min<sup>-1</sup> g<sup>-1</sup> FW. PPO activity was determined as described by Degl'Innocenti et al. (2005), with some modifications (Zhan et al. 2009).

The results were expressed as PPO Units g<sup>-1</sup> FW, with the calibration curve prepared daily with the PBS pH 7.0 solution and tyrosinase. PAL activity was determined as described by Campos et al. (2004) and Degl'Innocenti et al. (2005), with some modifications (Zhan et al. 2009). The results were expressed as μmol cinnamic acid h<sup>-1</sup> g<sup>-1</sup> FW, with the calibration curve prepared daily with the PBS pH 8.0 solution and trans-cinnamic acid.

### Ascorbic acid and dehydroascorbic acid contents

Ascorbic acid and dehydroascorbic acid (AA and DHAA, respectively) contents were determined according to Kampfenkel et al. (1995) method, with some modifications (Zhan et al. 2009). The results were expressed as mg g<sup>-1</sup> FW, and the calibration curves were prepared daily with L-ascorbic acid and dehydroascorbic acid. The DHAA content was computed as the difference between the vitamin C and the AA contents.

### Tissue ion and salt contents

Nitrate, phosphate, and calcium carbonate (NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, and CaCO<sub>3</sub>, respectively) contents were determined using a refractometric kit (Merck Reflectoquant RQflex2<sup>®</sup>; Merck KGaA, Darmstadt, Hessen, Germany) following the manufacturer's instructions. An aliquot of frozen tissue (10.0 g) from each sample was stomached for 2 min at normal speed with 10.0 ml of distilled water and subsequently filtered. The results were expressed as mg g<sup>-1</sup> FW.

### Microbial analysis

The total bacterial (TB) count was determined using the Plate Count Agar substrate, while the yeast + mold (Y+M) count was determined using the Yeast Extract Glucose Chloramphenicol Agar substrate. An aliquot of fresh tissue (25.0 g) from each sample was stomached for 2 min at normal speed with 225.0 ml of Ringer's buffer, then diluted and subsequently transferred to petri dishes with the substrate. The TB and Y+M counts were performed on d0 and on d9, after incubation at 30 °C for 48 h. The results were expressed as colony-forming units (cfu g<sup>-1</sup>) FW (Abadias et al. 2008, ISO 21527-1:2008, ISO 4833:2003, Jacxsens et al. 2003).

### Sampling size and statistical analysis

The statistical experimental design was a randomized complete block design (RCBD). A single factorial experimental design (2 cultivars × 3 blocks) was adopted during the growing period. Ten Styrofoam trays were taken to represent the experimental unit per each block. Fifteen plants per cultivar and per block were used at harvest to measure the biometric parameters. The postharvest experiment consisted of 5 mixes of LB and LR × 3 blocks. Two bags per mix per block were used in the postharvest period, using one bag per mix per block at each sampling date during the shelf life. The harvest and postharvest data were submitted to the analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS Version 19.0; SPSS Inc., Chicago, IL, US). When ANOVA was significant, the cultivar effect was tested using the F test, and the mix effect was tested using Tukey's multiple range test.

## Results

### Biometric parameters at harvest

The LFP per square meter, LFW per plant, and DM were not significantly influenced by the cultivar (data not shown), thus, the raw material was considered homogeneous. The average yield reached 2054 g m<sup>-2</sup> and 2.62 g/plant, while the average DM was 6.50%.

### Fresh-cut product fresh weight loss

As expected, the fresh-cut product FWL increased over time, although this increase was slow, and it was influenced significantly by the mix treatment on d5 and d6 of the shelf life (Table 1). The lowest FWL on d5 and d6 was found in 100LB, which resulted to be statistically different from 25LB and 0LB, but not from 75LB or 50LB. Neither 75LB nor 50LB were statistically different from any of the other leafy mixes. FWL in general resulted to be limited on d9, less than 0.70% of the original weight.

Table 1. Fresh weight loss (FWL) in fresh-cut product (100% ‘Lollo Bionda’ lettuce [100LB], 75% LB [75LB], 50% LB [50LB], 25% LB [25LB], 0% LB [0LB]) during the 9-d shelf life

Mix	FWL (%)								
	d1	d2	d3	d4	d5	d6	d7	d8	d9
100LB	0.09 <sup>z</sup>	0.05	0.13	0.07	0.09 <sup>b</sup>	0.12 <sup>b</sup>	0.14	0.18	0.23
75LB	0.12	0.06	0.16	0.15	0.19 <sup>ab</sup>	0.22 <sup>ab</sup>	0.24	0.32	0.32
50LB	0.11	0.07	0.23	0.20	0.25 <sup>ab</sup>	0.29 <sup>ab</sup>	0.35	0.41	0.44
25LB	0.07	0.09	0.21	0.25	0.28 <sup>a</sup>	0.32 <sup>a</sup>	0.35	0.44	0.45
0LB	0.11	0.08	0.18	0.18	0.26 <sup>a</sup>	0.32 <sup>a</sup>	0.39	0.64	0.66
Mean	0.10	0.07	0.18	0.17	0.21	0.25	0.29	0.40	0.42
Significance	NS <sup>y</sup>	ns	ns	ns	*	*	ns	ns	NS

z = Mean separation (in columns) by Tukey’s multiple range test at  $p \leq 0.05$  (lowercase letters) or 0.01 (uppercase letters); y = NS, \*, \*\*, \*\*\*: nonsignificant, significant at  $p \leq 0.05$ , 0.01, or 0.001, respectively. The values are the means of three replicates.

### Pigment content analysis

The cultivar had no significant effect on pigments at harvest (data not shown), and average values of Chl. *a*, Chl. *b*, and *Car.* of 0.18, 0.03, and 0.06 mg g<sup>-1</sup> FW, respectively, were thus obtained. The mix treatment significantly influenced the pigment content on d1, but not on d9 (Table 2). The pigment content was not completely proportional to the amount of LB in the mixes. The highest contents of Chl. *a*, Chl. *b*, and *Car.* on d1 were found in 25LB, which resulted to be statistically different from all the other leafy mixes. The Chl. *a* and *Car.* contents were lower in 100LB, 50LB and 0LB. The Chl. *b* content was not statistically different for any of the mixes, apart from 25LB. The average contents of Chl. *a*, Chl. *b*, and *Car.* on d9 were 0.17, 0.06, and 0.07 mg g<sup>-1</sup> FW, respectively.

Table 2. Chlorophyll a (Chl. *a*), chlorophyll b (Chl. *b*) and carotenoid (*Car.*) contents in the fresh-cut product (100% ‘Lollo Bionda’ lettuce [100LB], 75% LB [75LB], 50% LB [50LB], 25% LB [25LB], 0% LB [0LB]) at d1 and d9 of shelf life. The results are expressed per fresh weight (FW).

Mix	Chl. <i>a</i> (mg g <sup>-1</sup> FW)		Chl. <i>b</i> (mg g <sup>-1</sup> FW)		<i>Car.</i> (mg g <sup>-1</sup> FW)	
	d1	d9	d1	d9	d1	d9
100LB	0.12 C <sup>z</sup>	0.25	0.02B	0.13	0.04C	0.10
75LB	0.24B	0.14	0.04B	0.04	0.09B	0.06
50LB	0.19BC	0.18	0.04B	0.05	0.07BC	0.08
25LB	0.35A	0.15	0.08A	0.04	0.14A	0.09
0LB	0.14C	0.13	0.01B	0.03	0.05BC	0.05
Mean	0.21	0.17	0.04	0.06	0.08	0.07
Significance	***y	ns	***	ns	***	ns

z = Mean separation (in columns) by Tukey’s multiple range test at  $p \leq 0.05$  (lowercase letters) or 0.01 (uppercase letters); y = NS, \*, \*\*, \*\*\*: nonsignificant, significant at  $p \leq 0.05$ , 0.01, or 0.001, respectively. The values are the means of three replicates.

### Antioxidant capacity

The cultivar had no significant effect on AC at harvest (data not shown), and showed an average value of 3.08 μmol Fe<sup>2+</sup>·g<sup>-1</sup> FW. The mix treatment significantly influenced the AC on d1 and d9 (Table 3).

The AC measured on d1 was partly related to the different ratios of LB and LR used in the mixes. It was found that 50LB and 25LB had the highest AC on d1, and they resulted to be statistically different from 100LB and 75LB, but not from 0LB. It was also found that 100LB and 75LB were not statistically different from each other and that 100LB was not statistically different from 0LB. The highest AC on d9 was found in 100LB, which was statistically different from all the other leafy mixes.

Table 3. Antioxidant capacity (AC), total phenolic (TP) content, browning potential (BP), soluble quinone (So-Q) content and phenylalanine ammonia lyase (PAL) activity in the fresh-cut product (100% ‘Lollo Bionda’ lettuce [100LB], 75% LB [75LB], 50% LB [50LB], 25% LB [25LB], 0% LB [0LB]) at d1 and d9 of shelf life. The results are expressed per fresh weight (FW).

Mix	AC ( $\mu\text{mol Fe}^{2+} \text{g}^{-1} \text{FW}$ )		TP (mg gallic acid $\text{g}^{-1} \text{FW}$ )		BP ( $\text{Abs}_{340}$ )		So-Q ( $\text{Abs}_{437}$ )		PAL ( $\mu\text{mol cinnamic acid h}^{-1} \text{g}^{-1} \text{FW}$ )	
	d1	d9	d1	d9	d1	d9	d1	d9	d1	d9
100LB	2.50 BC <sup>z</sup>	3.99 A	0.27 ab	0.27 A	0.43 A	0.39	0.25	0.13 b	1.75	1.79 ab
75LB	2.23 C	2.15 B	0.25 b	0.18 B	0.38 B	0.29	0.25	0.17 a	1.99	1.20 b
50LB	3.34 A	2.49 B	0.39 a	0.25 A	0.34 C	0.46	0.25	0.18 a	1.35	2.07 a
25LB	3.42 A	2.66 B	0.24 b	0.27 A	0.38 B	0.45	0.25	0.16 ab	1.65	1.90 ab
0LB	2.94 AB	2.12 B	0.34 ab	0.23 AB	0.40 AB	0.22	0.25	0.15 ab	2.17	1.38 ab
Mean	2.88	2.68	0.30	0.24	0.38	0.36	0.25	0.16	1.78	1.67
Significance	***y	***	*	***	***	NS	NS	*	NS	*

z = Mean separation (in columns) by Tukey’s multiple range test at  $p \leq 0.05$  (lowercase letters) or 0.01 (uppercase letters); y = NS, \*, \*\*, \*\*\*: nonsignificant, significant at  $p \leq 0.05, 0.01, \text{ or } 0.001$ , respectively. The values are the means of three replicates.

### Total phenolic content

The cultivar had no significant effect on the TP content at harvest (data not shown), which had an average value of 0.22 mg gallic acid  $\text{g}^{-1} \text{FW}$ . The mix treatment significantly influenced the TP content on d1 and d9 (Table 3). The TP content increased on d1 compared to d0, then generally decreased till d9. In the present study, 50LB had the highest TP content on d1, while 75LB and 25LB had the lowest. Both 100LB and 0LB were in between and were not statistically different from any of the other leafy mixes. The highest TP content on d9 was found for 100LB, 50LB, and 25LB, the lowest was found for 75LB, and 0LB was in between and not statistically different from any of the other leafy mixes.

### Browning potential and soluble o-quinone content

The cultivar had no significant effect on the BP or So-Q content at harvest (data not shown), which showed average values of 0.29  $\text{Abs}_{340}$  and 0.25  $\text{Abs}_{437}$ , respectively. The mix treatment significantly influenced the BP on d1 and the So-Q content on d9 (Table 3). Basically, the BP content increased from d0 to d1 and then remained constant. It was found that 100LB had the highest BP on d1, while 50LB had the lowest. 0LB was in between and not statistically different from 100 LB, 75LB, or 25LB. The average value of BP on d9 was 0.36  $\text{Abs}_{340}$ . So-Q did not change from d0 to d1, but then decreased from d1 to d9 in all the mixes. The average So-Q content on d1 was 0.25  $\text{Abs}_{437}$ . The highest So-Q content on d9 was found in 75LB and 50LB, which resulted to be statistically different from 100LB but not from 25LB or 0LB, which in turn were not statistically different from any of the other leafy mixes.

### Enzyme activity analysis

POD activity was below the detection limit at harvest and during storage (data not shown). The cultivar had no significant effect on PPO or PAL activities at harvest (data not shown), which had average values of 23.52 PPO Units  $\text{g}^{-1} \text{FW}$  and 1.20  $\mu\text{mol cinnamic acid h}^{-1} \text{g}^{-1} \text{FW}$ , respectively. The mix treatment did not influence PPO activity on d1 or on d9 (data not shown) or PAL on d1, while it significantly influenced the PAL activity on d9 (Table 3). The PPO and PAL activities were both higher on d1 than on d0, with average values of 24.70 PPO Units  $\text{g}^{-1} \text{FW}$  and 1.78  $\mu\text{mol cinnamic acid h}^{-1} \text{g}^{-1} \text{FW}$ , respectively. The PPO activity decreased on d9, with an average value of 18.21 PPO Units  $\text{g}^{-1} \text{FW}$ . The highest PAL activity on d9 was found in 50LB, which resulted to be statistically different from 75LB, but not from 100LB, 25LB, or 0LB, which in turn were not statistically different from 75LB.

### Ascorbic acid and dehydroascorbic acid contents

The cultivar showed no significant effect on the AA or DHAA contents at harvest (data not shown), which had average values of 0.23 and 0.02 mg  $\text{g}^{-1} \text{FW}$ , respectively. The mix treatment significantly influenced the AA and DHAA content on d1 and the AA content on d9 (Table 4). In general, AA, constant from d0 till d1, decreased until d9, while the DHAA content increased over time. It was found that 50LB had the highest AA content on d1, and that it resulted to be statistically different from 25LB and 0LB, but not from 100LB or 75LB, which in turn were not statistically different from 25LB or 0LB. 0LB had the highest DHAA content, and was statistically different from 50LB and 25LB, but not from 100LB or 75LB.

The latter two mixes were not statistically different from 25LB. The highest AA content was found in 50LB on d9, and it was statistically different from 75LB, but not from 100LB, 25LB or 0LB, which in turn were not statistically different from 75LB. The average content of DHAA was 0.07 mg g<sup>-1</sup> FW on d9.

Table 4. Ascorbic acid (AA), dehydroascorbic acid (DHAA) and nitrate (NO<sub>3</sub><sup>-</sup>) contents in the fresh-cut product (100% 'Lollo Bionda' lettuce [100LB], 75% LB [75LB], 50% LB [50LB], 25% LB [25LB], 0% LB [0LB]) at d1 and d9 of shelf life. The results are expressed per fresh weight (FW).

Mix	AA (mg g <sup>-1</sup> FW)		DHAA (mg g <sup>-1</sup> FW)		NO <sub>3</sub> <sup>-</sup> (mg g <sup>-1</sup> FW)	
	d1	d9	d1	d9	d1	d9
100LB	0.235 AB <sup>z</sup>	0.187 ab	0.036 AB	0.065	0.61	0.56 B
75LB	0.234 AB	0.168 b	0.034 AB	0.066	0.45	0.93 A
50LB	0.242 A	0.197 a	0.028 C	0.060	0.59	0.53 B
25LB	0.228 B	0.178 ab	0.033 B	0.074	0.81	0.51 B
0LB	0.226 B	0.173 ab	0.038 A	0.066	0.66	0.66 B
Mean	0.233	0.181	0.034	0.066	0.63	0.64
Significance	**y	*	***	NS	NS	***

z = Mean separation (in columns) by Tukey's multiple range test at  $p \leq 0.05$  (lowercase letters) or 0.01 (uppercase letters); y = NS, \*, \*\*, \*\*\*: nonsignificant, significant at  $p \leq 0.05$ , 0.01, or 0.001, respectively. The values are the means of three replicates.

### Tissue ion and salt contents

Although the same HNS was used to grow the plants, the cultivar had a significant effect on the NO<sub>3</sub><sup>-</sup> content at harvest, which was the highest in LR (0.80 mg g<sup>-1</sup> FW), that is, 67% more than in LB (0.48 mg g<sup>-1</sup> FW). No significant effect was found for PO<sub>4</sub><sup>3-</sup> or CaCO<sub>3</sub> content (data not shown). The average contents of PO<sub>4</sub><sup>3-</sup> and CaCO<sub>3</sub> were 0.12 and 0.59 mg g<sup>-1</sup> FW, respectively, but the limited literature available on PO<sub>4</sub><sup>3-</sup> and CaCO<sub>3</sub> contents does not allow a comparison of the results to be made. The mix treatment did not significantly influence the NO<sub>3</sub><sup>-</sup> content on d1, but it did on d9 (Table 4). Moreover, it did not influence PO<sub>4</sub><sup>3-</sup> or CaCO<sub>3</sub> on d1 or d9 (data not shown). In general, the NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> contents both remained constant during storage, while the CaCO<sub>3</sub> content increased. The average content of NO<sub>3</sub><sup>-</sup> on d1 was 0.63 mg g<sup>-1</sup> FW. The highest NO<sub>3</sub><sup>-</sup> content on d9 was found in 75LB, and resulted to be statistically different from all the other leafy mixes. The average PO<sub>4</sub><sup>3-</sup> and CaCO<sub>3</sub> contents on d1 were 0.12 and 0.57 mg g<sup>-1</sup> FW, respectively, while the average PO<sub>4</sub><sup>3-</sup> and CaCO<sub>3</sub> contents on d9 were 0.12 and 0.77 mg g<sup>-1</sup> FW, respectively.

### Microbial analysis

The cultivar had no significant effect on the TB count or Y+M count at harvest (data not shown), which had average values of 7.42×10<sup>3</sup> and 2.71×10<sup>1</sup> cfu g<sup>-1</sup> FW, respectively. The mix treatment had no significant effect on the TB count or Y+M count on d9 (data not shown), which showed average values of 6.11×10<sup>3</sup> and 4.75×10<sup>1</sup> cfu g<sup>-1</sup> FW, respectively. The TB and Y+M counts both remained constant from d0 to d9.

### Discussion

In this study, the baby green and red leaf lettuce grown in SCS show a low dry matter content, due to the constant water and nutrient solution supply. The continuous flotation was able to ensure short growing cycle of BLV (<35 d) and, at the same time, ensures high yields (Falovo et al. 2009). The positive results concerning the fresh weight loss can be attributed to the low-cut surface to volume ratio of BLV, which contributes to the reduction of water transpiration during their shelf life (Fontana and Nicola 2008).

In general, the content of total chlorophyll remains constant in all mixes during the post-harvest period. As the percentage of Lollo Rossa increased, the total chlorophyll content decrease, due to the higher concentration of carotenoids, phenols and flavonoids in LR respect to the chlorophylls. These pigments can be used as a useful parameter to evaluate the quality of vegetables during storage, since they are degraded gradually during post-harvest senescence (Mamatha et al. 2011). In this research, the mix treatment significantly influenced the chlorophyll content on d1 but in a no proportional manner of LB mix. The generally steady level over time was probably due to the decreasing amount of lipid peroxidation of lettuce during plant senescence (Conrad et al. 2018),

that can be explained by the presence of carotenoids. These compounds exert important physiological functions in plants, because they are essential components of the photosynthetic machinery, and play a critical role in preventing photooxidative damage (Howitt et al. 2006). The maintenance of the carotenoids content over time, observed in this study, was also reported by Spinardi et al. (2018) in lettuce, and by Bevly et al. (2016) in traditional leafy vegetables of Southern Africa.

The antioxidant capacity of lettuce, was related to the different ratios of LB and LR used in the mixes. Indeed, red-leafy lettuces, such as 'Lollo Rossa', have been reported to have a higher antioxidant capacity than green lettuces, cultivated in the same conditions (Martínez-Sánchez et al. 2012). AC of plants was related to the polyphenol contents (Heimler et al. 2009). These compounds are produced through the activity of enzyme phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) that catalyzes the first committed step by converting phenylalanine to trans-cinnamic acid and tyrosine to p-coumaric acid. The activity of PAL at harvest was higher than that observed by some authors in green and red cultivars of fresh-cut fully mature head lettuce (Degl'Innocenti et al. 2005). PAL activity of fresh-cut fully mature lettuce increased after harvest and reached a maximum value at the beginning of the refrigerated shelf life period as reported by López-Gálvez et al. (1996). Nevertheless, the factors regulating and controlling the quality and quantity of phenols in plant tissues still remain controversial. Our results showed that the difference in total phenols (TP) content may be due to the different varieties of lettuce. During storage, TP increase on d1 of 4 °C as reported by Zhan et al. (2009) in fresh-cut garden cress. The TP content along the shelf life is the results of the combination of the pre-existing phenolic amount, damage-induced biosynthesis and browning enzyme activity.

The reduced browning potential (BP) on d9 may be related to the decreased leaf damaged area and the dark storage conditions during the shelf life as reported by Martínez-Sánchez et al. (2011). Browning of plant tissue is generally due to enzymatic oxidation by polyphenol oxidase (PPO). The browning enzyme activity might be related to the soluble o-quinone (So-Q) which catalyzes the oxidation of phenols to quinones and to the ascorbic acid (AA) content, which inhibits PPO catalytic activity (Zhan et al. 2012). Degl'Innocenti and coauthors (2007) found that the soluble o-quinone (So-Q) content of fresh-cut fully mature butterhead lettuce was quite variable, with a decrease in shelf life at 4 °C on d3, as found on d9 in this study.

The ascorbic acid (AA+DHAA) content at harvest was up to 8-fold higher than that observed in fully mature green and red heads of different lettuce genotypes cultivated either in SCS or TCS (Selma et al. 2012). Ascorbic acid (AA) is one of the most important antioxidants in plants and its capacity to lose or donate electrons is the basis of biologically antioxidant capacity (Smirnoff 2000). A reduced time lag between harvest and processing, as in this research, would in general involve a zero-quality loss, as reported by Lee and Kader (2000), who indicated that delays between harvesting and cooling or processing accelerates AA losses. The higher amount of AA than DHAA was expected, because similar results have been reported for fully mature head Romaine lettuce heads (Zhan et al. 2013). Furthermore, the AA-to-DHAA ratio is also in line with other results in which DHAA represents less than 10% of the total AA+DHAA content in many horticultural crops. During storage, AA is oxidized to DHAA in leafy tissues by enzymes, such as ascorbate peroxidase and ascorbate oxidase, and this explains why the AA content tends to decrease, while the DHAA content increases over time in fresh-cut product (Zhan et al. 2012).

Even though DHAA increased more than 2-fold upon storage, as also reported for fresh-cut fully mature Romaine lettuce heads by Zhan et al. (2013), the AA-to-DHAA ratio in this study remained in favor of AA. In addition, the AA+DHAA content did not change during storage, although some authors have reported an AA+DHAA decrease during refrigerated shelf life (at 4 °C for 3 d and at 7 °C until d14) in fresh-cut baby green and red colored leaf lettuce (Martínez-Sánchez et al. 2012). These results may be due to the juvenal phenological stage of the leaves (Bergquist et al. 2006).

The nitrate content found in both LB and LR was very low and much below the limit established by Regulation (EU) No 1258/2011 (e.g., 4 mg g<sup>-1</sup> FW for spring-summer lettuce grown under cover). Optimizing plant nutrition using a low NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> ratio can reduce the accumulation of nitrate in leaves. In a study of Ferrante et al. (2003), the authors showed that soil-grown rocket accumulated a higher concentration of nitrate and gave lower yields than floating systems did. On the contrary, rocket grown in the floating system showed lower nitrate content (Fontana and Nicola 2009). Our results are also in line with those of Konstantopoulou et al. (2010) who reported the unchanged content of nitrate in fully mature Romaine lettuce heads stored at 5 °C for 10 d.

Postharvest microbial contamination of fresh produce is becoming more important in the public debate (Gil 2016). In this research, the total bacterial count (TB) at harvest was more than  $10^2$  cfu g<sup>-1</sup> lower than the previously reported for different varieties of fully mature lettuce heads (Abadias et al. 2008), while the Y+M count was  $\approx 10^3$  cfu g<sup>-1</sup> lower than that reported for different baby green and red leaf varieties, and fully mature lettuce heads (Selma et al. 2012). These results may be due to the limited presence of damaged and physiologically compromised tissues (Martínez-Sánchez et al. 2012) and to the SCS characteristics (Nicola et al. 2007). On the contrary, some authors observed a contamination growth, even at a refrigerated storage temperature (Martínez-Sánchez et al. 2012). The obtained results are all below the microbial tolerance values of  $10^5$ – $10^8$  cfu g<sup>-1</sup> FW suggested by various authors for fresh-cut vegetables as the maximum recommended limit for consumption, although exceeding the microbial limit does not always result in the occurrence of visual defects (Scuderi et al. 2011). A higher amount of TB than of Y+M can be expected in fresh and minimally processed vegetables (Jacxsens et al. 2003).

## Conclusions

The research has shown that the development of baby-sized species is recommended because of their health-promoting phytochemical contents and their excellent processing characteristics, which could positively affect the shelf life. There are no notable differences in the quality parameters of raw LB and LR material at harvest and the FL used favors the production of standardized raw material, by reducing the nitrate content and microbial contamination, and limiting its growth at the end of the shelf life. The preservation of pigment, antioxidant, phenolic, AA, and DHAA contents during storage would seem to suggest that storing fresh-cut baby leaf lettuce at 4 °C for 9 d could be recommended to guarantee freshness and high-quality characteristics. Mixing baby green and red leaf lettuce in the same salad bag is appealing to consumers, and the present research has highlighted that inherent and commercial quality characteristics are affected by this postharvest practice. The chosen mix treatment has been shown to influence the measured phytochemical parameters, on both d1 and d9 of the shelf life. In general, 25LB had the highest pigment content on d1, while 50LB and 25LB had the highest inherent quality on d1, as determined by the AC, TP, and AA contents. The phytochemical quality on d9 was affected differently by the mixes but, in general, the values measured were satisfactory.

Further studies will be needed to clarify the physiological mechanisms that lead to the preservation of phytochemicals in postharvest.

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