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Effect of Agronomic Practices on Yield and Quality of Borage at Harvest and During Storage as Minimally-Processed Produce

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Abstract: Borage (*Borago officinalis* L.) is a hairy pubescent herb known throughout the world for its folk medicinal uses, as well as for many culinary uses. There is still little information on the cultivation needs of this species, especially for its use as vegetable crop and as fresh-cut produce. Hence, the aim of the research was to study the effects of agronomic practices on yield and quality of borage and on the storability as minimally-processed product. Two experiments were carried out in two consecutive years in order to evaluate the effect of plant density and plastic mulching on yield and quality of two borage accessions at harvest and during storage as minimally-processed produce for 14 days at 4 °C. The highest plant density (8 plants m^2) determined the highest yield of plants and minimally-processed leaves with good quality retention during storage. Mulching had a positive effect on earliness, yield, and shelf life of minimally-processed leaves but also increased nitrate accumulation and reduced ascorbic acid content. Borage plants with lower spacing grown on mulched soil showed the best yield of plants and minimally-processed leaves irrespective of the borage accession tested. Borage plants can be used to produce minimally-processed entire leaves with good quality characteristics.

Keywords: *Borago officinalis*; plant density; mulching; leafy vegetables; minimal processing; nitrate accumulation; ascorbic acid; shelf life

1. Introduction

Borage (*Borago officinalis* L.) is a hairy pubescent herb of the *Boraginaceae* family. It is considered native of both the Mediterranean area and Asia, widely present in Mediterranean countries and naturalized in many other regions. This plant is known throughout the world for its folk medicinal uses [1], as well as for preparing beverages and salads and for other culinary uses [2]. The borage mood-elevating properties were known since the first century A.D. [3]. The plant is considered as diuretic, antihypertensive, antipyretic, antispasmodic, aphrodisiac, demulcent, and is also used to treat kidney ailments, cramps, diarrhea, asthma, bronchitis, and palpitations [4,5]. Many studies have revealed the presence of various phytochemicals (tannins, resins, ascorbic acid, beta-carotene, niacin, riboflavin, thiamine, silicic acid, choline arabinose, unsaturated pyrrolizidines alkaloids, and polyphenolics) [5–11]. The seeds are rich of gamma-linolenic acid (more than 20% of GLA) that confers to the borage oil many potential medical uses: as antithrombotic, to lower blood pressure and inhibiting cholesterol formation, for treating atopic eczema [12], inflammatory disorders, Alzheimer's disease, asthma, and gastrointestinal disorders [11] and for reducing side effects of diabetes, such as vascular damage, altered platelet function, and arteriosclerosis [5]. The leaves and the flowers of borage are traditionally used as diuretic, demulcent, emollient, expectorant, nerve and cardiac tonic, home remedy for blood purification, swelling and inflammation, coughs, and other respiratory complaints [13,14]. The plants of *Boraginaceae* family are also known to be effective against some human pathogens [15–17].

The culinary uses of borage are very different according to the different Country and traditions. Regional recipes and traditional local gastronomy make use of raw or cooked leaves, stems, and flowers. In Italy, the basal leaves and the aerial parts are eaten fried, boiled, stewed or used to prepare green pasta or as stuffing for pies, ravioli, and tortelli [18]. In other countries (i.e., Spain), only the stems are consumed for culinary purposes. The flowers are used by adding them to salads, to which confer a light cucumber flavor, or to decorate beverages and desserts as they are one of the few truly blue-colored edible substances.

Although borage has been traditionally cultivated for food and medicinal uses, nowadays, the main aim of cultivation is the production of seeds for oil extraction and the cultivation for vegetable production is rather limited even in those regions where it is commonly used for traditional preparations [18]. The demand for borage plants is largely satisfied by harvesting the plants that grow wild in various environments, but the morpho-physiological and agronomical characteristics of this species make it suitable for cultivation as a vegetable. The introduction of borage among specialized cultivations would allow to diversify horticultural crops and to widen the supply of leafy vegetables. Consumers' need to vary their vegetable consumption could ensure an easy placement of borage production in the market as fresh or minimally-processed leafy vegetable [19]. Moreover, the increasing demand for vegetable convenience foods could promote the spread of this species in the market of ready-to-eat (RTE) products. Although *B. officinalis* seeds have been the objective of various studies as a potential source of GLA, there is still little information on the cultivation needs of this species (planting time and density, water and mineral nutrition, crop management, etc.), especially for its use as vegetable crop and as fresh-cut produce. Spacing and mulching are very important to achieve optimum production in many vegetables. The choice of plant density is one of the most important factors that affect yield and quality. Proper plant spacing can allow to reach optimum yield while too low or too high plant density could result in reduced yield and quality [20]. Mulching has various effects on both soil and plants. It contributes to limit soil temperature fluctuation, control weed and the leaching of fertilizers, retain soil moisture and enhance irrigation efficiency [20,21]. The management of plant spacing and mulching should be optimized for each vegetable species, hence, the aim of the research was to study the effects of these agronomic practices on yield and quality of borage and on the storability as minimally-processed product.

2. Materials and Methods

Two experiments were carried out in the experimental farm of the Department of Agricultural, Food and Forest Sciences (SAAF—University of Palermo, Italy) (Istituto Agrario Castelnuovo, 38°9′ 23′′ N 13°19′ 58′′ E; altitude 38 m) in two consecutive years (2016–2018). The first experiment was aimed at evaluating the effect of plant density while in the second trial the best plant density was adopted for assessing the effect of plastic mulching on yield and quality of borage (*Borago officinalis* L.).

During the first trial (November 2016—February 2017), the average minimum and maximum temperatures were 11.0 °C and 17.4 °C, respectively and ranged between 6.0 °C and 24.8 °C. The total amount of rainfall during the cultivation period was 279.6 mm. During the second year (November 2017—February 2018), the average minimum and maximum temperatures were 11.5 °C and 17.9 °C, respectively and ranged between 6.0 °C and 26.6 °C. The trend of minimum and maximum temperatures during the second trial was similar to those recorded in the first year, while the total amount of rainfall during the cultivation period was higher and recorded 323.8 mm (+44.2 mm).

Seeds of two borage accessions (D4 and H) of a wild germplasm collection from different Sicilian locations [22] maintained at the vegetable laboratory of the Department of Agricultural, Food, and Forest Sciences (SAAF—University of Palermo, Italy) (Table 1), were sown in polystyrene trays (84 holes) filled with a commercial substrate (SER CA-V7 Special semine, Vigorplant Italia srl, Fombio, Italy). These accessions both have good vigor and green-grayish leaves with grayish spot, are blue flowered and very spiny. They differ in plant height (small D4 and tall H), in leaf size and shape and in flowering time (late for D4 and early for H) (Figure 1). Seedlings were grown in a cold greenhouse for 30 days from sowing until they had 3–4 true leaves and were ready for transplant.

Table 1. Germplasm passport information of borage accessions subjected to different agronomic practices and minimal processing.

Multi-Crop Passport Descriptors	Borage Accessions	
Accession name	D4	H
Accession number	BoPA005	BoPA001
Holding institute	Vegetable laboratory—Department of Agricultural, Food, and Forest Sciences (SAAF—University of Palermo, Italy)	
Genus	*Borago*	
Species	*officinalis*	
Common crop name	Borage	
Country of origin	ITA	ITA
Location of collecting site	Monte Pellegrino-Palermo (PA)	Piana dei colli-Palermo (PA)
Latitude of collecting site	381031 N	380925 N
Longitude of collecting site	0132136 E	0131957 E
Elevation of collecting site	438 m	35 m
Collecting date	201102–	201103–
Biological status of accession	Wild	Wild
Collecting/acquisition source	Woodland	Fallow land
Type of germplasm storage	Seed collection	Seed collection

Figure 1. Borage accessions D4 (**a**) and H (**b**).

At the beginning of November 2016 (first experiment), seedlings of D4 and H were transplanted, open-air, in bare soil (alfisols "Red Mediterranean soils") adopting two plant density: 4 (0.50 × 0.50 m) and 8 (0.25 × 0.50 m) plants m^{-2} (Figure 2). The treatments were arranged in a randomized complete block design with three replicates (10 m^2 each).

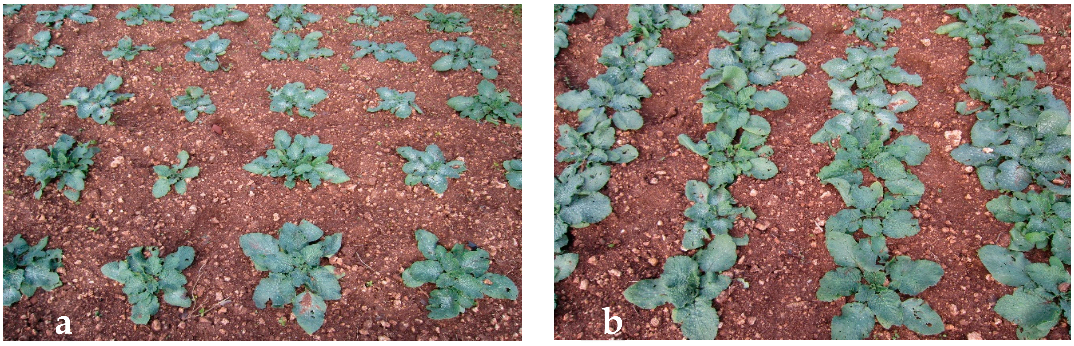

Figure 2. Borage plants grown at 4 (**a**) and 8 (**b**) plants m^{-2}.

Before transplanting, fertilizers were broadcast (70 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5 and 70 kg ha^{-1} of K_2O) and incorporated into the soil. Water supply was based only on rainfall. During plant growth weeds were controlled by conventional cultivation until plants covered the soil.

For the second experiment, the seedlings of D4 and H accessions were transplanted, open-air, at the beginning of November 2017 in bare soil or in soil mulched with a 20 µm black PE film (8 plants m^{-2}) (Figure 3). Treatments were arranged in a split-plot design with three replicates (10 m^2 each sub-plot), with mulching as the main factor. Mineral nutrients were supplied (70 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5 and 70 kg ha^{-1} of K_2O) before seedling transplant. During growth, mulched and unmulched plants were watered according to seasonal needs through a drip irrigation system and weeds grown in the unmulched plots were controlled by conventional cultivation until plants covered the soil.

Figure 3. Borage plants grown in bare soil (**a**) or in soil mulched with black PE film (**b**).

Borage was harvested, in both experiments, at the beginning of axillary bud shooting (39th BBCH growth stage for leafy vegetables not forming heads [23]), by cutting the whole plant at the base. Yield and average plant weight were calculated after eliminating the decayed external leaves. Ten plants, randomly selected for each replicate, were separated into leaves and stalks, weighed and then oven dried at 85 °C to constant weight for fresh and dry biomass determination.

In both experiments, after harvesting, plants were directly transported to the vegetable laboratory of the Department of Agricultural, Food, and Forest Sciences (SAAF—University of Palermo, Italy) and stored at 4 °C. Plants were processed for fresh-cut production within 24 h from harvesting; leaves were detached with the entire petioles and those with defects such as yellowing, decay, cuts, and bruising were removed. Then they were repeatedly washed (2 or 3 times according to the amount of soil particles withheld by leaves) with cold tap water until soil particles were fully removed, immersed in chlorinated water (50 ppm) for 5 min, rinsed to lower the free chlorine, drained and finally centrifuged for 1 min using a handheld salad spinner to remove excess water. At the end of processing (Figure 4b), the yield of minimally-processed product was calculated.

Figure 4. Borage leaves at harvest (**a**) and after minimal processing (**b**).

Samples of 200 g of each treatment were immediately placed in multilayer low-density polyethylene (0.023 mm thick), heat sealed and stored at 4 °C for 14 days. Immediately after packaging and after 7 and 14 days of storage, three samples of each treatment were randomly taken to evaluate the effect of agronomic practice and cold storage on the physicochemical characteristics and the overall quality of fresh-cut borage. Weight loss was evaluated by weighing samples soon after processing and at each sampling date. Before performing the destructive analysis, the overall sensory quality (OQ) was evaluated by an informal panel made of ten people (6 men and 4 women, aged 25–50) using scores from 1 to 5, with 5 = excellent—with freshly harvested appearance and full sensory quality (e.g., no browning or yellowing, with no defects or decay), 3 = fair/acceptable and still marketable (e.g., minor defects or moderated color alteration), and 1 = poor/unmarketable—major defects, great color alteration, or decay symptoms.

Leaf color changes were measured at two points of photosynthetic tissue on the upper side of ten, randomly selected, leaves for each sample of every treatments using a colorimeter (Chroma-meter CR-400, Minolta corporation, Ltd., Osaka, Japan) that recorded chromaticity coordinates of the CIELAB scale: L* (lightness), a* (positive values for reddish colors and negative values for greenish colors), and b* (positive values for yellowish colors and negative values for bluish colors). Hue angle (h°) and Chroma (C*) were calculated as $h° = 180° + \arctan(b^*/a^*)$ [24] and $C^* = (a^{*2} + b^{*2})^{1/2}$.

A water extract was then obtained homogenizing 30 g of each sample with H_2O (1:1 *w*/*v*); the water extracts were centrifuged at 3500 rpm for 10 min and the supernatants were used for chemical determinations.

The chlorophyll content (first experiment) was determined following the methodology described by Moreira et al. [25]. Borage water extract (3 g) were homogenized with 19 mL of a cold solution 18:1 propanone: NH_4OH (0.1 M). This homogenate was filtered through sintered glass and water was removed from the filtrate with anhydrous sodium sulfate. Absorbance of the filtrate at 660.0 and 642.5 nm was measured with a UV-vis spectrophotometer (Beckman DU 640 Spectrophotometer; Beckman Coulter, Brea, CA, USA). Chlorophyll content was calculated applying the formula $TC = 7.12\ A_{660} + 16.8\ A_{642.5}$, in which TC is the total chlorophyll concentration (mg L^{-1}) and A_{660} and $A_{642.5}$ are the absorbance at the corresponding wavelengths. Chlorophyll content is reported as mg of chlorophyll for 100 g of fresh weight (mg 100 g^{-1}).

Total soluble solids (TSS expressed as °Brix) were determined using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co., Ltd., Taiwan, China). Titratable acidity (TA) was determined (second experiment) by potentiometric titration of 10 mL of extract with 0.1 M NaOH up to pH 8.1 and expressed as mg of citric acid for 100 g of fresh weight. Nitrate (first and second experiment) and ascorbic acid contents (second experiment) were determined using a Reflectometer RQflex10 Reflectoquant and the Reflectoquant nitrate and ascorbic acid test strips (Merck, Germany) [26,27] (procedures described in art. 1.16971.0001 and 1.16981.0001 by Merck [28]).

To determine the effect of agronomic practices and borage accessions, a two-way analysis of variance (ANOVA) was carried out. Mean values were compared by the least significant difference (LSD) test at $p = 0.05$, in order to identify significant differences among treatments.

A completely randomized design with three replicates per treatment was performed for minimal processing trials. To determine the effect of storage time, agronomic practices and borage accessions, a three-way ANOVA was carried out. Mean values were compared by the LSD test at $p = 0.05$ to identify significant differences among treatments and significant interactions between factors.

3. Results and Discussion

3.1. Effect of Plant Density on Yield, Minimal Processing, and Cold Storage

Plant growth was mostly influenced by borage accessions, while no effect due to plant density was recorded on plant development. The plants of accession H were ready for harvesting after 104 days from transplant, 9 days earlier than accession D4. The yield of borage was significantly influenced only by plant density (Table 2). At harvest, the plants of both borage accessions had similar development and plant weight (on average 369.7 g plant^{-1} and 41.7 g plant^{-1} of fresh and dry weight, respectively). The increase of plant density determined an increase of total yield from 1683.8 g m^{-2} to 2547.0 g m^{-2} (+51.3%) on average, even if the average fresh weight of plants dropped by 24.3% and the average dry weight dropped by 19.2% (Table 2). The dry matter percentage of the plants was influenced neither by borage accessions nor by plant density and was on average 11.4% (Table 2). Similarly, plant spacing has a significant influence on different yield attributes of other leafy vegetables. The rise of plant density in lettuce, cabbage, mustard, and spinach cultivations determines a significant reduction of plant fresh and dry weight caused by the increased competition for nutrients, water and light among the plants. As found in this experiment for borage, the other leafy vegetables reached the highest yield at closer spacing due to the increase in the number of plants per unit area [20,29–31].

Table 2. Effect of accession and plant density on plant characteristics, total yield and minimally-processed leaves.

Source of Variance		Plant			Total Yield (g m^{-2})	Minimally Processed Leaves		
		Fresh Weight (g plant^{-1})	Dry Weight (g plant^{-1})	Dry Matter (%)		Yield (%)	Yield (g m^{-2})	Dry Matter (%)
Accession								
D4		[z] 370.9	40.0	10.9	2152.5	38.5	806.3 a	8.3
H		368.4	43.4	11.9	2078.3	32.3	673.3 b	8.1
Plant density								
4 (plant m^{-2})		421.0 a	46.1 a	11.0	1683.8 b	37.0	620.1 b	8.3
8 (plant m^{-2})		318.4 b	37.3 b	11.8	2547.0 a	33.7	859.5 a	8.1
Accession × Density								
D4	4	407.4	42.5	10.5	1629.5	42.5 a	693.3	8.3
	8	334.4	37.5	11.3	2675.6	34.4 b	919.2	8.3
H	4	434.5	49.7	11.4	1738.1	31.5 b	546.8	8.3
	8	302.3	37.1	12.3	2418.4	33.1 b	799.8	7.8
Significance [x]								
Accession		ns	ns	ns	ns	**	*	ns
Density		***	**	ns	***	*	**	ns
Accession × Density		ns	ns	ns	ns	*	ns	ns

[z] Each value is the mean of three replicated plots of 10 m^2 each. For each factor, values in a column followed by the same letter are not significantly different, according to LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

After harvesting, the plants were processed in order to obtain a ready to eat (RTE) product. Leaves with no physical or biotic alteration were picked off stems and washed several times until soil particles were completely removed. Three rinses were necessary to completely clean the leaves as they withheld a high amount of soil particles due to their wrinkled and almost prickly surface and to the prostate habit of the plants (Figure 4a). After processing, the yield of fresh-cut produce was calculated. The percentage of minimally-processed leaves obtained from borage plants (Table 2) was on average 32.3% for accession H, with no differences due to plant density, while the percentage yield of accession D4 decreased as increasing plant density and ranged from 42.5% (4 plants m^{-2}) to 34.4% (8 plants m^{-2}). Nonetheless, the yield of fresh-cut borage leaves resulted higher in both accessions when planted at the higher plant density (919.2 and 799.8 g m^{-2} for accession D4 and H, respectively) (Table 2).

Minimally processed leafy vegetables may undergo significant weight loss during storage that could determine appearance alteration and quality degradation resulting in loss of commercial value [32,33]. Fresh-cut borage retained a high water content until the end of cold storage. No significant weight loss was related to plant density. Accession D4 had a higher water retention than accession H during the storage period. The weight losses of H samples after 7 and 14 days of storage were on average 1.41 and 2.38 g 100 g^{-1} fresh weight (fw) and resulted respectively 65.7% and 108.2% higher than D4 samples (Table 3 and Figure 5).

Weight losses may compromise marketability of leafy vegetables when they are higher than 4–6% [34]. Accession D4 and H remained well below this threshold during the 14 d of storage at 4 °C.

Total soluble solids were on average 5.9 °Brix at day 0 and remained almost constant until the end of cold storage (5.5 °Brix on average at day 14) (Table 3). As reported by others authors for various leafy vegetables [26,35–39], minimally-processed vegetables packed in sealed plastic bags characterized by low permeability to water vapor do not suffer dehydration due to the very high relative humidity inside the sealed packages (near to 100%). Moreover, fresh-cut leafy vegetables cold stored in sealed plastic films usually have a low respiration rate [36–38], as confirmed by the small variation of TSS recorded for the borage samples irrespective of accession and plant density (Table 3).

The nitrate content should be monitored in leafy vegetables as it can have serious effects on human health. Nitrate is not toxic when its uptake is below certain levels; nevertheless, it is accumulated in edible plant tissues in amounts that overcome the maximum admitted levels and can be reduced to nitrite and become dangerous. When nitrite reacts with amines and amides can form N-nitroso compounds which can be carcinogenic [40,41]. The nitrate content of borage leaves was low in both accessions (228.3 mg kg^{-1} fw on average). The increase of plant density had no effect on the accumulation of nitrates. This could indicate that the plants did not compete for the N in the soil and that they were not close enough to shade each other; self-shading could reduce light interception and thus limit the nitrate reductase efficiency [42,43]. Cold storage did not affect the nitrate content which remained almost constant without significant changes for 14 days (Table 3).

The changes in chlorophyll content of leafy vegetables may determine changes of leaf appearance that are closely related with quality characteristics and marketability. The chlorophyll content was slightly higher in the accession D4 and was not significantly influenced by plant density. Moreover, no significant change was recorded during cold storage; borage leaves were not affected by chlorophyll degradation when stored in the dark at low temperature (4 °C) and high relative humidity, as already found for other leafy vegetables [26,44–47].

Table 3. Effect of accession, plant density and time of storage on weight loss, chlorophyll content, total soluble solids (TSS) and nitrates of minimally-processed borage leaves.

Source of Variance			Weight Loss (g 100 g^{-1})	TSS (°Brix)	N-NO_3 (mg kg^{-1})	Chlorophyll (mg 100 g^{-1})
Accession						
D4			[z] 0.67 a	6.0	227.8	50.2 a
H			1.26 b	5.5	249.5	35.7 b
Plant density						
4 (plant m^{-2})			0.91	5.7	227.8	44.6
8 (plant m^{-2})			1.02	5.8	249.5	41.3
Storage (d at 4 °C)						
0			0.00 c	5.9	228.3 b	39.5
7			1.13 b	5.9	226.7 b	43.8
14			1.76 a	5.5	260.9 a	45.5
Accession × Density × Storage						
D4	4	0	0.00	5.5	233.3	46.4
		7	0.94	6.0	193.3	50.7
		14	1.27	5.4	223.3	58.8
	8	0	0.00	6.5	210.0	42.9
		7	0.77	6.3	230.0	46.1
		14	1.01	6.1	276.7	56.2
H	4	0	0.00	5.9	233.3	40.4
		7	1.34	5.9	206.7	36.5
		14	1.92	5.5	276.7	34.6
	8	0	0.00	5.5	236.7	28.3
		7	1.48	5.3	276.7	42.0
		14	2.84	5.1	266.7	32.4
Significance [x]						
Accession			***	ns	ns	*
Plant density			ns	ns	ns	ns
Storage			***	ns	*	ns
Accession × density			*	**	ns	ns
Accession × Storage			***	ns	ns	ns
Density × Storage			ns	ns	ns	ns
Accession × Density × Storage			ns	ns	ns	ns

[z] Each value is the mean of three replicated samples of 200 g each. For each factor, values in a column followed by the same letter are not significantly different, according to LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

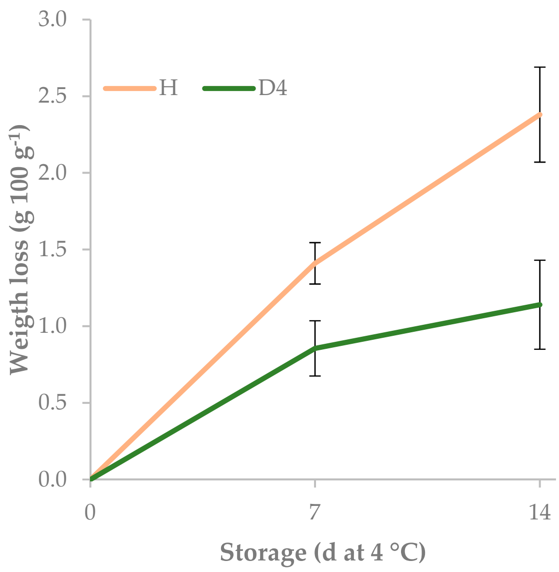

Figure 5. Influence of accession (D4 and H) and time of storage on weight loss of minimally-processed borage leaves.

Consumer's perception of vegetable quality is mainly influenced by their color and appearance; thus, color modifications and overall appearance are the primary parameters for the quality evaluation of vegetables, especially those minimally-processed [44,48]. These modifications may occur due to both pre-harvest [48–52] or post-harvest [17,19,35–37,53,54] factors. The color variability of borage leaves was mainly due to the difference between the tested borage accessions as already reported for chlorophyll content; accession H had leaves more yellowish (higher a* values) and greenish (higher b* values), resulting in a more vivid color (chroma) than accession D4 (Table 4). A significant increase of chroma was determined also by the increase of plant density. Minimally processed borage leaves showed a good color stability during cold storage. In fact, no significant changes in color parameters was recorded during 14 d of storage except for the increase of lightness (Figure 6a) and the reduction of hue angle on day 14 in the leaves of accession H grown at the lowest plant density (Figure 6b) as also found for minimally-processed borage stored at 6 °C [19].

Table 4. Effect of accession, plant density and time of storage on leaf color and appearance of minimally-processed borage leaves.

Source of Variance			L*	a*	b*	Chroma	Hue Angle	Overall Quality
Accession								
D4			[z] 38.6	−13.1 a	17.4 b	21.8 b	127.2	4.1
H			38.8	−13.9 b	19.4 a	23.9 a	126.0	3.9
Plant density								
4 (plant m^{-2})			38.4	−13.1 a	17.7 b	22.0 b	126.9	4.1
8 (plant m^{-2})			39.0	−13.9 b	19.1 a	23.6 a	126.3	3.9
Storage (d at 4 °C)								
0			37.6	−13.7	18.3	22.9	127.0	5.0
7			38.4	−13.3	18.0	22.4	126.6	3.8
14			40.0	−13.5	18.8	23.2	126.2	3.1
Accession × Density × Storage								
D4	4	0	37.0	−13.1	17.0	21.5	127.8	5.0
		7	38.0	−12.4	16.1	20.4	127.7	3.8
		14	38.8	−12.7	16.4	20.8	128.2	3.8
	8	0	38.5	−13.3	18.0	22.4	126.5	5.0
		7	38.9	−13.4	18.2	22.6	126.6	3.7
		14	40.4	−13.4	18.5	22.9	126.2	3.0
H	4	0	37.6	−13.8	18.4	23.0	127.2	5.0
		7	38.6	−13.3	18.5	22.8	125.9	3.8
		14	40.7	−13.3	19.7	23.8	124.6	3.0
	8	0	37.3	−14.5	20.0	24.7	126.3	5.0
		7	38.3	−14.1	19.2	23.8	126.5	3.7
		14	40.3	−14.5	20.6	25.3	125.7	2.7
Significance [x]								
Accession			ns	***	***	***	***	ns
Plant density			ns	***	***	***	*	*
Storage			***	ns	ns	ns	*	***
Accession × Density			**	ns	ns	ns	***	ns
Accession × Storage			*	ns	ns	ns	*	*
Density × Storage			ns	ns	ns	ns	ns	ns
Accession × Density × Storage			ns	ns	ns	ns	ns	ns

[z] Each value is the mean of three replicates (sixty measures for color determinations; samples of 200 g each for Overall Quality evaluation). Values in a column followed by the same letter are not significantly different. According to LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

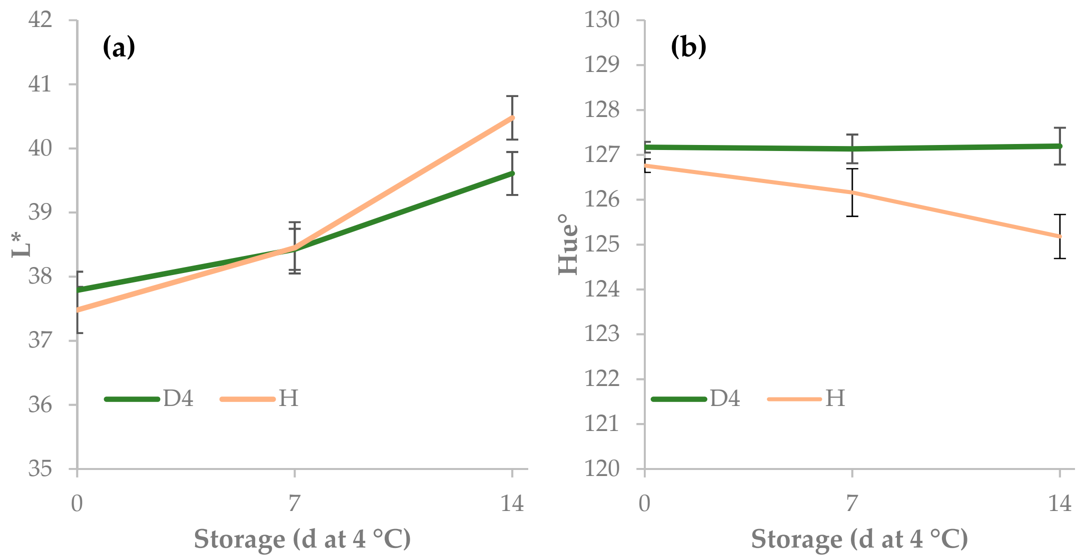

Figure 6. Influence of accession (D4 and H) and time of storage on color lightness (L*) (**a**) and hue angle (**b**) of minimally-processed borage leaves.

Even though the color variations were small, the perceived visual quality showed a reduction of the scores during storage. The score of the overall quality dropped significantly after 7 d of storage as function of accessions and plant densities; accession D4 planted at the lowest density (4 plants m^{-2}) maintained its score up to the end of the trial, while the other samples were at the threshold of marketability (D4—8 plants m^{-2} and H—4 plants m^{-2}) or below it (H—8 plants m^{-2}) (Figure 7).

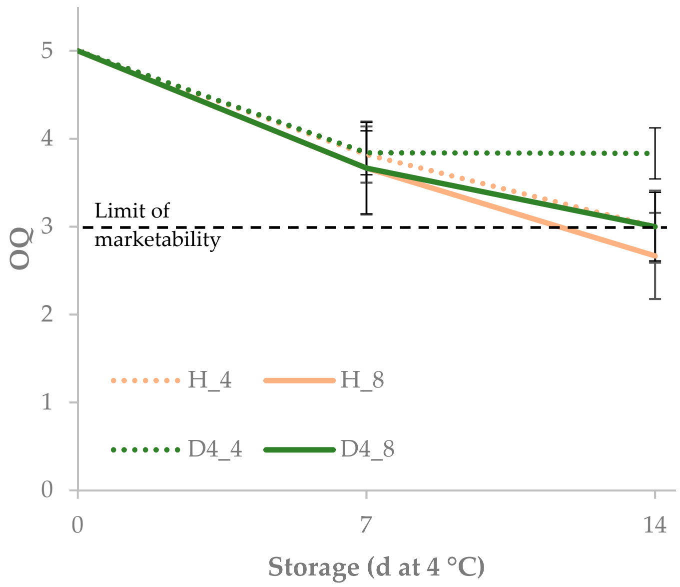

Figure 7. Influence of accession, plant density and time of storage on overall sensory quality (OQ) of minimally-processed borage leaves (5: excellent or having a fresh appearance; 3: average—limit of marketability; 1: unmarketable).

3.2. Effect of Mulching on Yield, Minimal Processing and Cold Storage

The accessions D4 and H were grown during the second experiment on bare soil or on soil mulched with black PE adopting the plant density that during the first year determined the highest yield and a good quality retention during cold storage of minimally-processed leaves (8 plants m^{-2}).

Plastic mulching positively influenced the growth of borage plants that were more vigorous and had a faster growth than those grown on bare soil. Mulched plants were ready for harvesting after 92 d after transplant, while the plants grown on bare soil were harvested after 12 and 18 d respectively for borage H and D4, thus, having a similar growth period than the plants of the first year of cultivation.

The fresh biomass of borage plants was significantly influenced by mulching. The average fresh weight of unmulched plants was 379.1 g, while mulching increased the plant fresh weight by 29.5% (Table 5). The dry weight of borage plant was similar in both borage accessions, as found in the first experiment (43.3 g plant^{-1} on average) and was significantly lower in the plants grown on mulched soil (42.0 g plant^{-1}, −6.1% compared to the bare soil plants) (Table 5). The use of plastic mulching reduced also the dry matter percentage of the plants from 11.8% to 8.6% (−27.5%). The yield of plants grown on bare soil was on average 3027.4 g m^{-2} with no significant difference between borage accessions (Table 5). Mulching was highly effective in increasing plant yield by 29% and 30.8% for D4 and H respectively. The effects of plastic mulching on plant vigor and earliness have been studied on many vegetable crops [55–60]. Borage plants benefited of mulching in terms of higher vigor, earliness, fresh biomass production and water retention. Similar effects of plastic mulching have been reported for other leafy vegetables [20,21,61–64] and can be ascribed to increased soil moisture and temperature, improved soil structure and microbial activity, and to reduced water evaporation and fertilizer leaching [63,65,66].

Table 5. Effect of accession and mulching with black PE on plant characteristics, total yield and minimally-processed leaves.

Source of Variance		Plant			Total Yield (g m^{-2})	Minimally Processed Leaves		
		fresh Weight (g plant^{-1})	Dry Weight (g plant^{-1})	Dry Matter (%)		Yield (%)	Yield (g m^{-2})	Dry Matter (%)
Accession								
D4		^{z} 426.4	43.7	10.5	3434.3	27.1	974.0	7.8 b
H		443.5	42.9	9.9	3519.3	28.9	1107.5	8.2 a
Soil treatment								
bare soil		379.1 b	44.7 a	11.8 a	3027.4 b	22.7 b	798.7 b	9.6 a
mulched		490.8 a	42.0 b	8.6 b	3926.1 a	33.3 a	1282.8 a	6.5 b
Accession × Soil treatment								
D4	bare soil	369.5	45.8	12.4	3001.9	20.9	707.4	9.3
	mulched	483.3	41.6	8.6	3866.7	33.4	1240.6	6.3
H	bare soil	388.7	43.5	11.2	3053.0	24.5	889.9	9.8
	mulched	498.2	42.3	8.5	3985.6	33.3	1325.1	6.6
Significance ^{x}								
Accession		ns	ns	ns	ns	ns	ns	*
Soil treatment		**	**	***	***	***	***	***
Accession × Soil treatment		ns	ns	ns	ns	ns	ns	ns

^{z} Each value is the mean of three replicated plots of 10 m^{2} each. For each factor, values in a column followed by the same letter are not significantly different, according to LSD test. ^{x} Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

The plants of the second experiment were processed as done in the first experiment. The leaves were picked off stems and washed until soil particles were completely removed. The leaves of the plants grown on mulched soil were cleaner than the plants grown on bare soil that withheld a higher amount of soil particles and dirty. The leaves of mulched plants were completely clean after two rinses while the leaves of the plants grown on bare soil needed one more rinse to be suitable for packaging and storage. The use of plastic mulch can give cleaner and higher quality vegetables as it separates the soil from the plants and eliminate soil splashing on leaves or fruits, especially for leafy vegetables that produce at soil level [65,67,68]

The yield of fresh-cut produce was positively affected by mulching. Mulched plants supplied 33.3% of minimally-processed leaves (+46.9%) and yielded 1282.8 g of leaves m^{-2}, with an increase of 484.2 g m^{-2} compared to the non-mulched plants (+60.6%). Minimally processed leaves differed also for dry matter percentage that was significantly higher in borage H and in non-mulched plants (Table 5).

After processing, the leaves of mulched and non-mulched plants were stored for 14 days at 4 °C. The samples of non-mulched H borage recorded the highest weight losses and at the end of the trial reached 3.27 g 100 g^{-1} fw. Borage D4 grown on mulched soil had the lowest weight loss during the first week of storage and at the end of storage both D4 and H borage grown on mulched soil recorded the lowest weight loss (1.30 g 100 g^{-1} fw on average) (Table 6 and Figure 8).

Table 6. Effect of accession, soil treatment and time of storage on weight loss, titratable acidity (TA), total soluble solids (TSS), nitrates and ascorbic acid content of minimally-processed borage leaves.

Source of Variance			Weight Loss (g 100 g^{-1})	TA [y] (mg 100 g^{-1})	TSS (°Brix)	N-NO_3 (mg kg^{-1})	Ascorbic Acid (mg 100 g^{-1})
Accession							
D4			[z] 0.88	169.6	4.6 a	1168.4	60.9
H			1.23	181.4	5.0 b	551.6	69.6
Soil treatment							
bare soil			1.40	182.0	5.9 a	160.0	87.9
mulched			0.72	169.0	3.8 b	1560.0	42.5
Storage (d at 4 °C)							
0			0.00	101.6 c	4.7	913.3	55.3
7			1.25	166.5 b	4.9	860.7	70.9
14			1.93	258.4 a	4.9	806.0	69.5
Accession × Soil treatment × Storage							
D4	bare soil	0	0.00 e	88.8	5.9	176.7	84.0
		7	1.48 bc	203.2	5.8	133.3	98.4
		14	1.84 bc	225.4	5.3	154.0	82.4
	mulched	0	0.00 e	100.8	3.4	2290.0	24.0
		7	0.67 d	140.9	3.4	2200.0	41.6
		14	1.31 c	258.3	3.8	2056.7	35.0
H	bare soil	0	0.00 e	100.8	5.6	197.3	74.3
		7	1.79 b	192.1	6.5	197.3	93.9
		14	3.27 a	281.8	6.1	101.3	94.7
	mulched	0	0.00 e	116.1	4.0	989.3	38.9
		7	1.05 c	129.8	3.8	912.0	49.9
		14	1.29 c	267.9	4.2	912.0	65.9
Significance [x]							
Accession			***	ns	**	***	ns
Soil treatment			***	ns	***	***	***
Storage			***	***	ns	ns	***
Accession × Soil treatment			***	ns	ns	***	ns
Accession × Storage			***	ns	ns	ns	*
Soil treatment × Storage			***	ns	ns	ns	ns
Accession × Soil treatment × Storage			***	ns	ns	ns	ns

[z] Each value is the mean of three replicated samples of 200 g each. For each factor, values in a column followed by the same letter are not significantly different, according to LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$. [y] Titratable acidity expressed as citric acid.

The weight losses recorded in the minimally-processed leaves of non-mulched plants were similar to those recorded in the first experiment, hence, even in the second experiment, they did not reach the level that may compromise marketability [34]. Mulching reduced significantly the weight loss in both borage accessions. Weight loss occurring during cold storage could be linked to water loss or to mass loss caused by the respiration that degrades carbohydrate reserves. Mulching can improve the water content of leafy vegetable [69], thus determining a higher water retention during storage. Moreover, mulching affects nutrient availability by reducing nutrient leaching and by increasing soil temperature and microbial activity [65,66]. The increased N availability has been related to a reduction in weight loss during cold storage of butterhead lettuce [70], and a similar effect could have been determined on borage by the increased N availability in the mulched soil.

The TSS was slightly higher in borage H and in the leaves of the plants grown on bare soil, but, as found in the first trial, it remained almost constant during the storage period (Table 6).

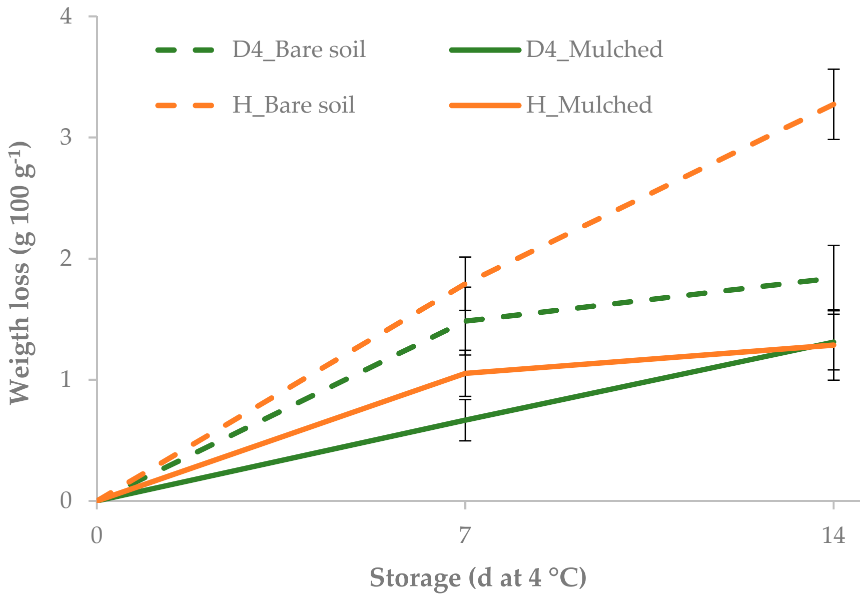

Figure 8. Influence of accession, soil treatment and time of storage on weight loss of minimally-processed borage leaves.

Borage plants of both accessions had a small content of nitrate (187.0 mg kg^{-1} fw on average) when grown on bare soil, confirming the results of the first year of experiments. Mulching significantly changed the amount of nitrates in the leaves, albeit in varying degrees for the two accessions. In fact, borage D4 grown on mulched soil overcame 2000 mg kg^{-1} fw of N-NO_3^- and was significantly higher than borage H that had about half of the nitrate content (989.3 mg kg^{-1} fw at harvest) (Table 6 and Figure 9). A number of factors can influence nitrate accumulation in vegetables. The nitrate content can show significant differences among the cultivars of a species [42,71,72] or it can be affected by the agronomic practices. Among these, mulching may influence water and nutrient distribution and consequently affects nutrient dynamics in the soil, thus affecting also plant absorption of water and nutrients [73]. Borage plants grown on mulched soil had higher nitrate accumulation as found for other leafy vegetables, probably because mulching has minimized nitrogen leaching caused by seasonal rainfall and has increased water availability [74–77]. Nevertheless, the nitrate content of borage leaves from mulched soil did not exceed the maximum level imposed by the European Commission (EC Reg. No. 1258/2011) for fresh spinach (3500 mg kg^{-1} fw) or for lettuce (4000 mg kg^{-1} fw) grown in the open air and harvested from October 1st to March 31th [78], as previously demonstrated for several baby leaf vegetables [79].

Titratable acidity of borage leaves soon after processing showed no significant differences due to accessions or mulching and was on average 101.6 mg 100 g^{-1} fw of citric acid equivalent. This parameter increased significantly during storage in all the tested samples up to 258.4 mg 100 g^{-1} fw of citric acid equivalent on average after 14 d at 4 °C. Similar results has been found for minimally-processed borage, cauliflower, red chicory, and escarole that showed increases of titratable acidity during cold storage [19,35–37,80].

The antioxidant and nutritional value of vegetables is often related with their content of ascorbic acid, as over 90% of vitamin C in the human diet comes from fresh vegetables. Mulching negatively affected the ascorbic acid content of minimally-processed borage leaves, with a reduction of 47.6% and 71.4% for borage H and D4 respectively (Table 6). This result agrees with those of other authors that found a negative effect of black plastic mulching on the ascorbic acid content of lettuce, celery, pepper, and potato [74,81–84]. The accessions of borage showed a significant interaction with storage time

(Table 6 and Figure 10); borage D4 had no significant change of ascorbic acid content during storage while it significantly increased in borage H after 7 d of storage at 4 °C.

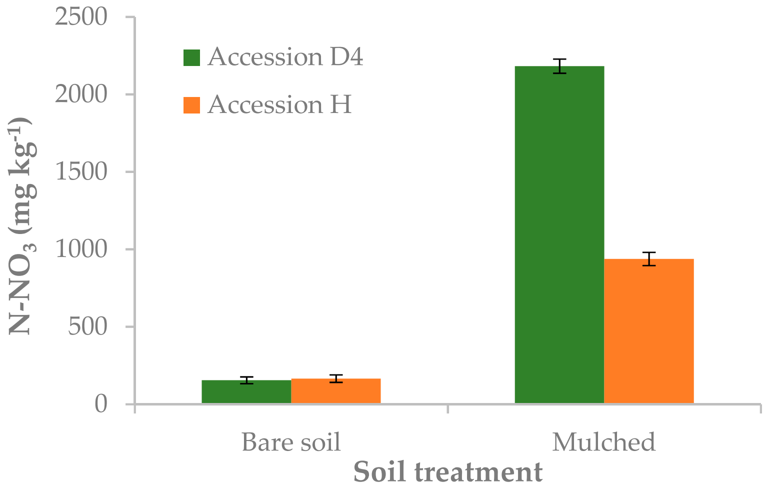

Figure 9. Influence of accession and soil treatment on nitrate content of minimally-processed borage leaves.

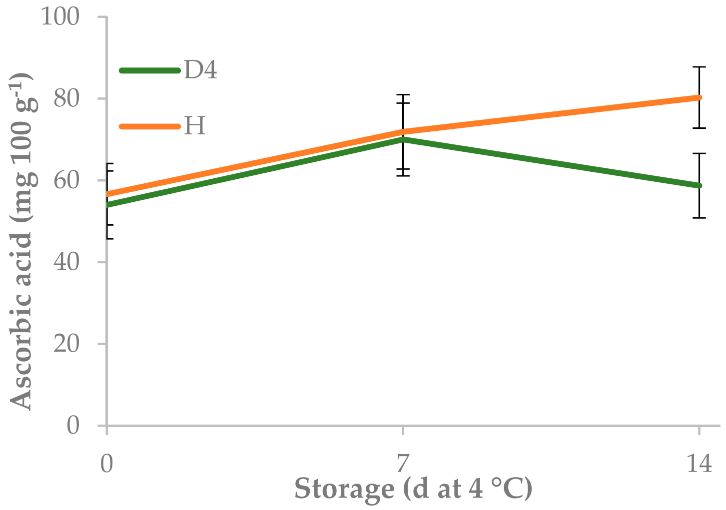

Figure 10. Influence of accession and time of storage on ascorbic acid content of minimally-processed borage leaves.

This difference confirms that the borage accessions tested differs in post-harvest metabolism as well as in pre-harvest metabolism as already reported for nitrate accumulation. Ascorbic acid is considered a very labile compound that can quickly degrade during storage at ambient temperature. Nonetheless, cold storage of vegetables packed in sealed bags showed to strongly slow down its deterioration or even increase its content as found for borage, broccoli, green asparagus, carrots, Swiss chard, and rocket [19,26,49,53,85–87]. These increases have been attributed to physicochemical changes or to unidentified enzymes that regenerate ascorbic acid [86].

The color of borage leaves at the beginning of the storage period had only minimal differences due to accessions or soil treatments (Table 7). Borage D4 grown on mulched soil had leaves with a less vivid color but with a higher hue angle than when it was grown on bare soil. During storage, the leaves increased their L* values corresponding to a lighter and less intense color. The increase of L* was greater in the samples grown on bare soil, that had also a higher chroma and a lower hue angle at the end of the storage period, thus indicating a yellowing trend.

Table 7. Effect of accession, soil treatment and time of storage on leaf color and appearance of minimally-processed borage leaves.

Source of Variance			L*	a*	b*	Chroma	Hue Angle	Overall Quality
Accession								
D4			[z] 39.4	−14.7	20.8	25.6	126.2	3.6
H			41.0	−15.1	22.2	26.9	125.1	3.4
Soil treatment								
bare soil			41.4	−15.3	23.6	28.2	124.0	3.1
mulched			39.0	−14.5	19.4	24.3	127.4	3.9
Storage (d at 4 °C)								
0			36.2	−13.5	17.3	22.0	128.2	5.0
7			39.9	−14.9	20.6	25.5	126.5	3.4
14			44.6	−16.3	26.5	31.2	122.3	2.1
Accession × Soil treatment × Storage								
D4	bare soil	0	37.3	−14.6	19.5 cd	24.4 c	127.0 b	5.0 a
		7	38.6	−14.6	20.2 c	25.0 c	126.1 bc	3.3 c
		14	45.0	−16.1	28.5 ab	32.9 ab	120.6 d	1.7 ef
	mulched	0	35.3	−12.8	15.3 d	20.0 d	130.2 a	5.0 a
		7	38.7	−14.5	18.1 cd	23.2 cd	128.8 ab	3.8 b
		14	41.6	−15.6	23.0 bc	27.9 bc	124.5 c	2.8 e
H	bare soil	0	36.6	−13.6	18.1 cd	22.6 cd	127.1 b	5.0 a
		7	42.9	−15.6	25.4 b	29.9 b	122.2 cd	2.7 d
		14	48.1	−17.3	29.7 a	34.4 a	120.8 d	1.2 f
	mulched	0	35.6	−13.1	16.5 d	21.0 d	128.5 ab	5.0 a
		7	39.4	−14.9	18.7 dc	23.9 cd	128.8 ab	3.9 b
		14	43.6	−16.2	24.9 b	29.8 b	123.5 c	2.8 e
Significance [x]								
Accession			***	*	**	**	**	*
Soil treatment			***	***	***	***	***	***
Storage			***	***	***	***	***	***
Accession × Soil treatment			ns	ns	ns	ns	ns	*
Accession × Storage			**	**	*	*	ns	ns
Soil treatment × Storage			*	ns	ns	ns	*	**
Accession × Soil treatment × Storage			ns	ns	**	**	**	ns

[z] Each value is the mean of three replicates (sixty measures for color determinations; samples of 200 g each for Overall Quality evaluation). Values in a column followed by the same letter are not significantly different. According to LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

The color variations were of paramount importance in influencing the overall sensory quality perceived by the judges. The scores of the samples of minimally-processed leaves obtained from non-mulched plants rapidly decreased during the first week of storage, especially those of borage H (2.7), and dropped down to 2 at the end of storage. The leaves produced on mulched soil retained a good overall quality during the first week of cold storage (3.9 on average) but reached the limit of marketability at the end of the trial (Table 7; Figure 11).

The scores for overall visual quality of borage accessions grown on bare soil resulted lower than those of the first experiment. This could be due to the higher precipitation amount recorded in the second year, that has probably increased the soil splashing on the leaves [88] and, consequently, the contamination of microorganisms responsible for leaf decay [65,67,68]. On the contrary, mulching may significantly reduce soil content on the leaf after harvest [88] and modify the microbial population throughout the cold storage [89,90], resulting in overall visual quality scores higher than those of bare soil samples [90] as found in this experiment.

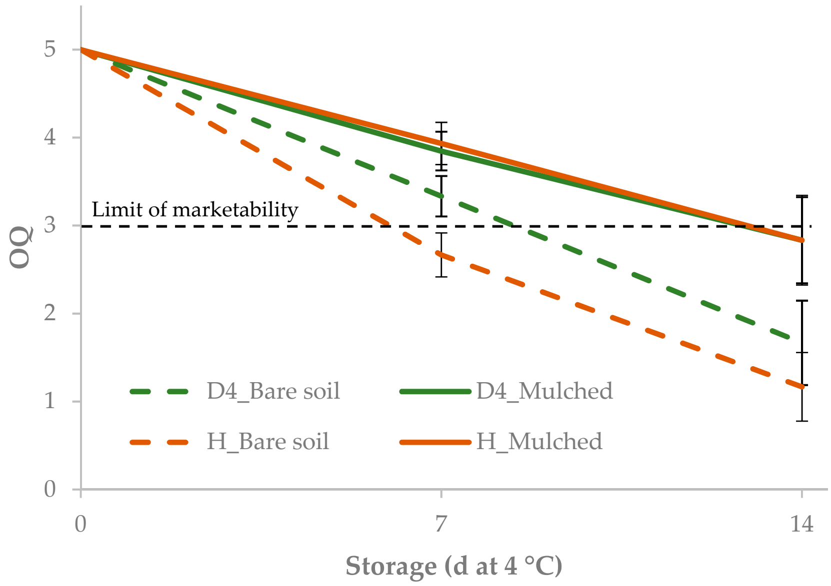

Figure 11. Influence of accession, soil treatment and time of storage on overall sensory quality (OQ) of minimally-processed borage leaves (5: excellent or having a fresh appearance; 3: average—limit of marketability; 1: unmarketable).

4. Conclusions

The borage accessions tested showed to be suitable for vegetable production and to have low cultivation needs as regard water and nutrients during the autumn–winter season in the Mediterranean area. These characteristics could allow to introduce borage among specialized cultivations or low environmental impact rotations, and to diversify the vegetable crops and enlarge the offer of leafy vegetables. The introduction of new vegetables as ready-to-eat products needs the investigation of suitable varieties and the formulation of sustainable cultivation practices to improve the yield and quality as well as to reduce the negative effects of minimal processing on shelf-life. Borage plants with lower spacing grown on mulched soil showed the best yield of plants and minimally-processed leaves irrespective of the borage accession tested. Borage plants can be used to produce minimally-processed entire leaves with good quality characteristics, that could satisfy the request of consumers to diversify the vegetables in their diet. The pre-harvest factors tested in this work affected the quality of borage at harvest and during cold storage of minimally-processed leaves. The accession D4 showed lower weight loss and higher color retention during cold storage than accession H. Mulching affected the yield of minimally-processed leaves and reduced weight loss but determined also a reduction in ascorbic acid content and an increase of nitrate accumulation especially in accession D4. Nonetheless, these variations did not affect the quality of borage leaves that maintained their marketability for at least 10 days when produced from plants grown at 8 plants m^2 on mulched soil. Shelf-life of minimally-processed borage leaves could be further prolonged by improving crop management and minimal processing techniques.

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