




Article

# Suitability of *Borago officinalis* for Minimal Processing as Fresh-Cut Produce

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**Abstract:** Borage (*Borago officinalis* L.) is a wild vegetable appreciated as a folk medicine and for culinary preparations. The introduction of borage as a specialized cultivation would allow for the diversification of vegetable crops and would widen the offerings of raw and minimally processed leafy vegetables. Thus, the aim of the research was to evaluate the quality and shelf-life of fresh-cut borage stored at different temperatures. Borage plants were grown during the autumn–winter season and immediately minimally processed after harvest. Fresh-cut borage leaves packed in sealed bags were stored at 2 or 6 °C for 21 d. Weight loss, total soluble solids (TSS), titratable acidity (TA), ascorbic acid, nitrates, leaf color characteristics and overall quality were determined through the storage period. Borage plants were deemed suitable for minimal processing. Storage temperature significantly influenced the rate of quality loss. Borage leaves had an initial nitrate content of 329.3 mg kg<sup>-1</sup> FW that was not affected by temperature or storage. TSS and TA were higher in leaves stored at 6 °C. TSS, TA and ascorbic acid content increased during storage. Minimally processed borage leaves stored at 2 °C had lower weight loss and leaf color modifications during storage and a longer shelf life than those stored at 6 °C, so were still marketable after 21 d of storage.

**Keywords:** borage; leafy vegetables; wild vegetables; postharvest; minimal processing; storage temperature; ascorbic acid; nitrates; shelf-life

## 1. Introduction

Borage (*Borago officinalis* L.) is a hairy annual herb known from ancient times throughout the Mediterranean region, Europe and Northern Asia. It is actually widespread all over the world and is appreciated by folk medicine as a diuretic, demulcent, emollient, expectorant, nerves and cardiac tonic, home remedy for blood purification, for the treatment of swelling and inflammation, coughs and other respiratory complaints, and as a culinary preparation [1–3]. Many studies have revealed the presence of various phytochemicals in borage plants (tannins, resins, ascorbic acid, beta-carotene, niacin, riboflavin, thiamine, silicic acid, choline arabinose, unsaturated pyrrolizidine alkaloids and polyphenolics [4–7]). The plants of *Boraginaceae* family have also shown antibacterial activity against some human pathogens [8–10]. Borage is eaten raw in salads or cooked as part of several typical regional preparations. Recently, agricultural interest in borage has increased for the potential market of gamma linolenic acid (all-cis-6,9,12-octadecatrienoic) extracted from the seed; it is an essential and unusual fatty acid that is an intermediate of indispensable compounds in the human body [11], and has many potential medical uses: as an antithrombotic, to lower blood pressure and cholesterol formation,

for treating atopic eczema [12] and for limiting the side effects of diabetes, such as vascular damage, altered platelet function and arteriosclerosis [5]. For those reasons, borage is mainly cultivated for seed production while cultivation for fresh vegetable production is rather circumscribed, even in those regions where it is commonly used for traditional preparations [13]. The demand for borage plants is satisfied to a large extent by harvesting the plants that grow wild in various environments, but the morpho-physiological characteristics of this species make it suitable for cultivation as a vegetable. The introduction of borage for specialized cultivation would allow diversification of vegetable crops and widening of the offerings of leafy vegetables. Consumers' needs for varying their vegetable consumption could ensure the easy placement of borage in the market. Moreover, the increasing demand for convenient vegetable foods could promote the spread of this species in the market of ready-to-eat (RTE) products. These are mainly obtained from leafy vegetables which undergo minimal processing, packaging and storage under refrigeration. Minimally processed vegetables have a faster decay than raw vegetables due to wounding and mechanical stresses associated with processing, which might determine physical and physiological changes affecting produce shelf life and quality. These impacts may affect sensory qualities, including appearance, texture and flavor (taste and aroma) [14] and make RTE vegetables highly perishable. The shelf life of leafy vegetables under cold storage conditions is usually no longer than 7–10 d [15,16]. Minimally processed vegetables are likely to support the growth of pathogenic micro-organisms or the formation of toxins, hence must not be held at temperatures that might result in a risk to health and the cold chain is not to be interrupted [17]. Leafy vegetables benefit from low temperatures during postharvest and storage; chilling injuries can appear only if susceptible vegetables are stored for long time near or below 0 °C. Minimally processed vegetables are usually stored, transported and sold at temperatures between 2 and 8 °C (usually 4–5 °C), because temperatures near 0 °C can be harmful to many products and the maximum storage temperature must be below 8 °C according to Italian Regulation number 77/2011 and Italian Ministerial Decree number 3746/2014 [18]. The expiration date of RTE vegetable products or salads usually established by manufacturers falls between 7 and 14 d from processing, depending on the type of fresh produce [19].

There is still little information on the suitability of borage for minimal processing as a ready to eat vegetable and on its response to cold storage. Therefore, the aim of this research was to evaluate the quality and shelf-life of fresh-cut borage during storage at different cold temperatures.

## 2. Materials and Methods

The research was 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 48 m) from October 2016 to March 2017. Seeds of borage accessions from a wild germplasm collection from different Sicilian locations were sown in polystyrene trays (84 holes) filled with a commercial substrate (SER CA-V7 Special semine, Vigorplant Italia srl, Fombio, Italy) and plantlets were grown in an unheated greenhouse until they had 3 or 4 true leaves and were ready for transplant. On 5 November 2016, seedlings were transplanted into open-air field plots in bare soil (8 plants m<sup>-2</sup>). Before transplanting, fertilizer was applied (70 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) and incorporated into the soil. Irrigation water was supplied only by rainfall. During plant growth, weeds were controlled by conventional cultivation until borage plants covered the soil. When axillary buds initiated shoots (20 February 2017), plants were harvested by cutting the entire aboveground part of the plant at the base. After harvest, plants were directly transported to the laboratory and immediately processed. Leaves were separated from the stalks with their entire petiole and those with defects such as yellowing, bruising, or decay were discarded. Then, they were washed several times with cold tap water until soil particles were completely removed, immersed in chlorinated water (50 ppm) for 5 min, rinsed to lower the free chlorine, and finally centrifuged for 1 min using a handheld salad spinner to remove excess water. Then, they were packed as described below. At the end of processing, the yield of minimally-processed product was calculated.

Samples of 200 g were packed in sealed bags of multilayer low-density polyethylene (0.023 mm thick) and stored up to 21 d at 2 °C (12 samples) or 6 °C (12 samples). Soon after packaging and after 7, 14 and 21 d of cold storage, 3 randomly selected samples from each storage temperature were evaluated to test the effects of cold storage on the physico-chemical characteristics (weight loss, leaf color, total soluble solids, titratable acidity, nitrate and ascorbic acid content) and on the overall quality of minimally processed borage leaves. All the samples were weighed soon after processing and at each sampling date in order to evaluate the weight loss ( $\text{g } 100 \text{ g}^{-1}$  of initial fresh weight). Leaf color was determined with a colorimeter (Chroma Meter CR-400C, Minolta corporation, Ltd., Osaka, Japan). The CIELAB parameters  $L^*$ ,  $a^*$  and  $b^*$  were measured on two points of photosynthetic tissue of the upper side of ten, randomly selected, leaves for each sample at each storage temperature. Chroma ( $C^*$ ) and hue angle ( $h^\circ$ ) were calculated as  $C^* = (a^{*2} + b^{*2})^{1/2}$  and  $h^\circ = 180^\circ + \arctan(b^*/a^*)$  [20]. Total color difference ( $\Delta E$ ) was also calculated at each sampling date as  $\Delta E = [(L^* - L^*_0) + (a^* - a^*_0) + (b^* - b^*_0)]^{1/2}$ , where  $L^*_0$ ,  $a^*_0$  and  $b^*_0$  were the control values at the beginning of storage ( $T_0$ ). The overall sensory quality (OQ) was evaluated by an informal panel made of 9 people (5 men and 4 women, aged 25–55). The panel was trained to identify sensory properties of borage leaves and used a 1 to 5 scale, with 5 = excellent or having a freshly harvested appearance and full sensory acceptability (e.g., no yellowing or browning, free from any defects and decay, with no undesired odors or softening), 3 = fair/still acceptable and marketable (e.g., presence of minor defects or moderated color alteration, very limited softening or off odors), and 1 = poor/unmarketable, with great color alteration, and major defects (off odors) or decay symptoms (softening or tissue breakup). Samples of 30 g were then homogenized with  $\text{H}_2\text{O}$  (1:1 w/v); the water extracts were centrifuged at 3500 rpm for 10 min and the supernatants were used to determine total soluble solids (TSS), titratable acidity (TA), nitrate and ascorbic acid content. Ascorbic acid ( $\text{mg } 100 \text{ g}^{-1}$  fresh weight (FW)) and nitrate contents ( $\text{mg kg}^{-1}$  FW) were determined reflectometrically using a Reflectometer RQflex10 Reflectoquant and the Reflectoquant ascorbic acid and nitrate test strips (Merck, Germany) (procedures described in article 1.16981.0001 and 1.16971.0001 by Merck (<http://www.merckmillipore.com/chemicals/>)). TSS ( $^\circ\text{Brix}$ ) were determined using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd., Taiwan). Titratable acidity (TA) was determined by titration of 10 mL of water extract with 0.1 M NaOH up to pH 8.1 and expressed as mg of citric acid for 100 g of fresh weight (FW).

A completely randomized design with three replicates per treatment was performed. To determine the effect of storage time and storage temperature, a two-way ANOVA was performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) statistical software. Mean values were compared by the least significant differences test at  $P = 0.05$  to identify significant differences among treatments and significant interactions between factors.

### 3. Results and Discussion

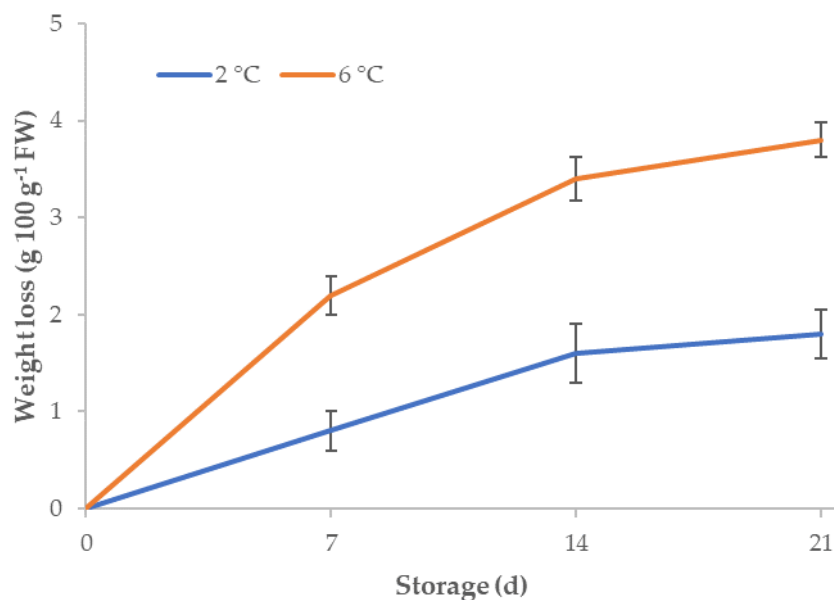
Borage leaves with no defects were separated from the stalks, washed, centrifuged and packed; at the end of this production process, the yield of minimally processed product was about 40% of the processed plants and corresponded to  $1.01 \text{ kg m}^{-2}$  of fresh-cut borage leaves ( $126.3 \text{ g plant}^{-1}$ ).

After harvest, vegetables undergo a natural weight loss due to transpiration and evaporation from cut zones, especially in minimally processed leafy vegetables that have a high surface area/volume ratio [21,22]. Water loss during storage could determine sensory and appearance alteration and quality degradation, resulting in a loss of commercial value or even marketability [23]. Minimally processed borage samples stored at 2 and 6 °C showed different trends in weight loss during storage (Table 1; Figure 1).

**Table 1.** Effect of temperature and time of storage on weight loss, total soluble solids (TSS), titratable acidity (TA), and ascorbic acid and nitrate content of minimally processed borage leaves.

Source of Variance	Weight Loss	TSS	TA <sup>z</sup>	N-NO <sub>3</sub>	Ascorbic Acid
	(g 100 g <sup>-1</sup> )	(°Brix)	(mg 100 g <sup>-1</sup> FW)	(mg kg <sup>-1</sup> FW)	(mg 100 g <sup>-1</sup> FW)
Temperature					
2 °C	1.4 <sup>y</sup>	4.6b <sup>y</sup>	213.1b	343.3	93.7
6 °C	3.1	4.8a	229.6a	337.4	97.5
Storage time					
0 d		4.2b	183.2c	329.3	59.5c
7 d	1.5	4.6ab	209.0b	329.5	98.8b
14 d	2.5	4.9a	253.6a	326.0	100.3b
21 d	2.8	5.0a	239.5a	376.5	123.8a
Temperature x Storage					
2 °C					
0 d		4.2	183.2	329.3	59.5
7 d	0.8c	4.5	197.3	340.7	98.3
14 d	1.6b	4.7	251.6	308.0	99.0
21 d	1.8b	4.8	258.4	395.0	117.8
6 °C					
0 d		4.2	183.2	329.3	59.5
7 d	2.2b	4.7	220.7	318.3	99.2
14 d	3.4a	5.1	253.6	344.0	101.6
21 d	3.8a	5.2	260.7	358.0	129.8
Significance					
Temperature	*** x	*	*	ns	ns
Storage	***	**	***	ns	**
Temperature x Storage	*	ns	ns	ns	ns

<sup>z</sup> Titratable acidity expressed as citric acid. <sup>y</sup> 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 at  $P = 0.05$ . \* Significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; \*\*\* = significant at  $P < 0.001$ .

**Figure 1.** Influence of temperature and time of storage on weight loss of minimally processed borage leaves.

Weight loss increased significantly until 14 d of storage at both temperatures, with no further significant loss until the end of the experiment (Figure 1). The weight loss after 21 d at the highest temperature of storage (6 °C) was almost twice that of samples stored at 2 °C (3.8 g and 1.8 100 g<sup>-1</sup>, respectively). Maximum acceptable weight losses that do not compromise marketability may vary among vegetables; lettuce and other leafy vegetables become unmarketable when weight loss is higher

than 4–6% [24]. Borage leaves did not exceed this threshold during the experiment and remained well below it when stored at 2 °C.

Weight loss of leafy vegetables can be attributed to evaporation of a moisture layer that persists on the leaf surface after washing, as well as to leakage of cellular fluids from damaged tissues and to an increase of permeability of cell membranes enhanced by ethylene [25,26]. The weight loss recorded during storage could be not directly linked with water loss, but can be due also to respiration that degrades carbohydrate reserves. The mechanical damages that leaves may suffer during minimal processing can increase respiratory activity and stimulate ethylene production. In turn, food reserves (sugars) are transformed to organic acids and to other simple carbon compounds. Thus, weight loss during storage in high RH conditions could be due also to consumption of constituents through metabolic processes [25,26].

Increased metabolic activity of borage leaves during cold storage was evident from the increase in TSS and TA (Table 1). The increase of TSS was greater at 6 °C and could be ascribed to hydrolysis of starch and other reserve sugars. Titratable acidity also increased during storage (+41,7% after 21 d) and was significantly higher in borage leaves stored at 6 °C. Other authors have found similar increases in TA during cold storage of minimally processed cauliflower, red chicory and escarole [27–30]. Among the organic acids in borage leaves, ascorbic acid also increased significantly during cold storage from 59.5 mg 100 g<sup>-1</sup> fresh weight (FW) (0 d) to 123.8 mg 100 g<sup>-1</sup> FW (21 d) (Table 1). The content of ascorbic acid in vegetables is often related to their antioxidant and nutritional value, as over 90% of vitamin C in the human diet comes from vegetables. This organic acid is considered a very labile compound that can give a sensitive indication of product degradation when its content during storage is monitored. From our results, the content of ascorbic acid in minimally processed borage leaves was positively influenced by cold storage and was only minimally affected by weight loss. Ascorbic acid can quickly deteriorate during the postharvest and storage of vegetables at ambient temperature, but its losses can be strongly reduced, and its content can even increase in cold-stored vegetables packed in sealed bags. Increases of ascorbic acid content during the initial days of cold storage were also reported for broccoli [31,32], green asparagus [33], carrots [34], Swiss chard [35] and rocket [36], and could be ascribed to a physicochemical change or to an unidentified enzyme that regenerates ascorbic acid [32].

The presence of great amounts of nitrates in leafy vegetables can be harmful for human health, thus their levels affect the nutritional quality of the product [37]. Minimally processed borage leaves showed an average initial content of 329.3 mg kg<sup>-1</sup> FW of nitrates; hence, it can be classified among vegetables with low contents of nitrates (200–500 mg kg<sup>-1</sup> FW) [38]. Nitrate content was not affected by storage, with an average content at the end of the trial of 376.5 mg kg<sup>-1</sup> FW on average (Table 1).

Color is of paramount importance for assessing the quality of a ready to eat product, as it can strongly affect the visual appearance of the product, has a direct effect on attractiveness to consumers [39], and may also influence the consumer's perception of the sensory quality. Color changes may be determined by preharvest [34,40,41] or postharvest [27–29,36,42] factors. Leafy vegetables may suffer color variations mainly due to chlorophyll degradation and browning caused by processing or storage conditions. These modifications may determine the loss of marketability even before physico-chemical and microbiological decay occurs [34]. Color was measured by means of a colorimeter that recorded L\* (brightness), a\* (blue/yellow) and b\* (red/green) values, which were used to calculate Chroma, hue angle and total color difference ( $\Delta E$ ) (Table 2).

**Table 2.** Effect of temperature and time of storage on leaf appearance of minimally processed borage leaves.

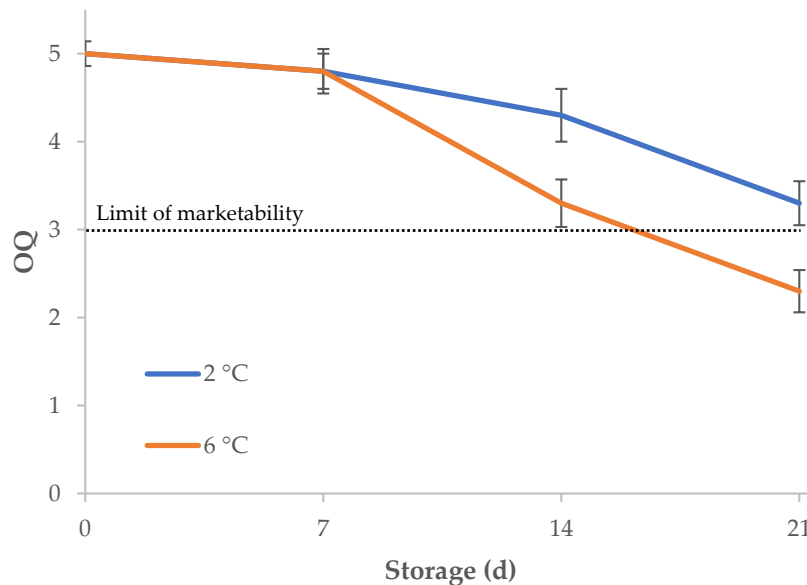
Source of Variance	L*	a*	b*	Chroma	Hue°	ΔE	OQ
Temperature							
2 °C	38.9 <sup>z</sup>	−14.3	18.3	23.3	128.4	5.5	4.4
6 °C	40.3	−15.0	20.1	25.1	127.3	7.7	3.9
Storage time							
0 d		−14.3	18.0	23.0	128.6		5.0
7 d	39.0	−13.9	16.9	21.9	129.6	4.9	4.8
14 d	41.0	−14.9	19.9	24.9	127.2	6.5	3.8
21 d	42.6	−15.5	22.1	27.1	126.0	8.5	2.8
Temperature x Storage							
2 °C							
0 d	36.0d	−14.3ab	18.0cd	23.0cd	128.6ab		5.0a
7 d	38.5c	−13.4a	16.3d	21.1d	129.7a	4.8c	4.8ab
14 d	39.7c	−14.6b	18.7c	23.7c	128.1b	5.2c	4.3b
21 d	41.5b	−14.9b	20.3bc	25.3bc	127.2c	6.6bc	3.3c
6 °C							
0 d	36.0d	−14.3ab	18.0cd	23.0cd	128.6ab		5.0a
7 d	39.4c	−14.3ab	17.5cd	22.6cd	129.5ab	5.1c	4.8ab
14 d	42.2ab	−15.2b	21.0b	26.0b	126.3bc	7.7b	3.3c
21 d	43.7a	−16.1c	23.9a	28.9a	124.7d	10.3a	2.3d
Significance <sup>x</sup>							
Temperature	***	***	***	***	***	***	***
Storage	***	***	***	***	***	***	***
Temperature x Storage	**	*	**	**	**	***	***

<sup>z</sup> Each value is the mean of three replicates (sixty measures for color determinations; samples of 200 g each for overall sensory quality (OQ) evaluation). Values in a column followed by the same letter are not significantly different, according to LSD tests at  $P = 0.05$ . <sup>x</sup> Significance: ns = not significant; \* = significant at  $P < 0.05$ ; \*\* = significant at  $P < 0.01$ ; \*\*\* = significant at  $P < 0.001$ .

The parameter L\* increased during storage even though it differed depending on the storage temperature. The rise of the L\* values occurred earlier and was greater in the leaves stored at 6 °C, especially after 14 d of storage. During the storage period, a\*, b\* and chroma were almost constant in the leaves stored at 2 °C, whereas those stored at 6 °C showed a significant reduction of a\* values and an increase of b\* values after 14 and 21 d of storage compared to the initial value and to the a\* values recorded at 2 °C. Color saturation (chroma) also increased significantly after 14 d in the leaves stored at 6 °C, indicating a trend towards a more vivid color, hence a greater variation compared to the initial color. This trend was also found in the hue angle which showed a decrease after 14 d of storage, but to a greater extent in the leaves stored at 6 °C (−1.4 and −3.9 after 21 d for 2 °C and 6 °C, respectively; Table 2). The variations recorded at 6 °C indicated that the color of the leaves became a light yellowish green, probably due to chlorophyll degradation that occurs faster at higher storage temperatures, as found for other leafy vegetables [43–45]. The changes in chlorophyll content and color parameters during storage can limit shelf-life and are a major problem for marketing minimally processed leafy vegetables. Hence, color changes and overall quality should be considered primary parameters for the quality assessment of minimally processed vegetables [40]. The analysis of color variation against the color of leaves at day 0 showed a significant effect of storage temperature and storage time on total color difference (ΔE). ΔE values of borage samples were very distinct (ΔE > 3) [46] for both storage temperatures after 7 d of storage (4.9 on average) (Table 2). The samples stored at 6 °C exhibited significant changes in ΔE after 14 and 21 d of storage, as they increased by 50.7% (7.7) and 101.3% (10.3), respectively. The overall sensory quality (OQ) of minimally processed borage was nearly unchanged during the first week of cold storage, irrespective of storage temperature. Afterwards, OQ decreased significantly in all borage samples, but those stored at 6 °C were close to the limit of



marketability after 14 d of storage (3.3) and had lost marketability at the end of the experiment (2.3) (Figure 2). Minimally processed borage leaves stored at 2 °C had an OQ score higher than four after 14 d and were still marketable after 21 d of cold storage.



**Figure 2.** Influence of temperature 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).

Minimally processed borage leaves exhibited a shelf life longer than the expiration date usually adopted for fresh-cut vegetables (7–10 d), especially when stored continuously at a low temperature, as was also found for other minimally processed leafy vegetables packed in plastic bags [40,43,47,48].

The perceived quality was negatively correlated to color difference, as it linearly decreased as  $\Delta E$  increased ( $R^2 = 0.994$  and  $R^2 = 0.987$  for 2 °C and 6 °C, respectively). This correlation confirmed that total color difference ( $\Delta E$ ) can be considered a sensitive parameter for the evaluation of the perceived quality.

#### 4. Conclusions

These results suggest that borage plants can be minimally processed to produce detached whole leaves with good quality characteristics that could satisfy consumer demand for varying their vegetable consumption. Storage temperature can significantly influence the rate of quality loss. Minimally processed borage leaves stored at 2 °C showed lower color and chemical modifications, and a longer shelf life, than those stored at 6 °C, and were still marketable after 21 d of storage.

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