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Influence of Pre-Harvest Factors on Postharvest Quality of Fresh-Cut and Baby Leafy Vegetables

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Abstract: Shelf life of horticultural commodities is dependent on postharvest handling but also on a wide range of pre-harvest factors, which include genetic and environmental parameters. This study was conducted to explore the influence of cultivar, leaf position, and piece position on the leaf on visual quality of fresh-cut butterhead lettuce as well as the effect of a wide range of cultivation seasons on the postharvest quality and shelf life of baby leaves (spinach and 'wild' rocket). Six butterhead lettuce cultivars were used (cultivated soilless in an unheated plastic greenhouse) while the effect of leaf position on the plant (outer and inner leaves) and the piece position on the leaf (piece one close to the leaf base and piece four close to the top) were also evaluated. Baby leaves were cultivated under an unheated plastic greenhouse for winter production and under a nethouse for the rest of the growing season, with a total of five and seven sampling dates for spinach and 'wild' rocket respectively. The cultivar of butterhead lettuce had a significant effect on postharvest quality of fresh-cut product but more important was the piece position on the leaf. When this was closer to the base of the leaf, there was more browning on cut edges and limited shelf life for the fresh-cut lettuce. The result was associated in one tested cultivar with PAL activity, which was higher by 106% for piece one compared to piece four as an average for the whole storage period. The growing season of baby leaves had a great impact on their shelf life, with the season of mild environmental conditions achieving the highest marketability.

Keywords: lettuce; spinach; rocket; shelf life; color; PAL activity; browning; yellowing

1. Introduction

Consumers value the presence of fresh vegetables in their daily diet, because their consumption has been established to be beneficial for human health [1,2]. Leafy vegetables are important and popular because they offer, apart from a high nutritional value, a diversity of crops with different characteristics (color, taste, and texture). Recently, most leafy vegetables have become more popular in the market as fresh-cut or baby leaves than as intact, fresh commodities [3,4]. The most important advantages for these products are their convenience, the variety of mixed combinations with attractive presentation, their freshness, and their contents of bioactive compounds [3,5].

It is well established that the appearance of the horticultural products is the primary criterion for consumer purchasing [4,6]. Previous studies show that both fresh-cut as well as baby leafy vegetables are more perishable than intact products [7,8]. As a result, the shelf life of these products is shorter because of changes in their sensory quality and not the microbial spoilage [9,10].

Lettuce (*Lactuca sativa* L.) is an important leafy vegetable crop with the great advantage of availability all over the world for 12 months per year [11]. Although lettuce is one of the major fresh-cut vegetables, it is difficult to process because of its high perishability [11–13]. The major disorder of fresh-cut lettuce is the browning of cut surfaces, which significantly affects the visual quality and limits the shelf life of the product [14–16]. Therefore, it is important to control the browning of cut surfaces [13,17]. Even though most of the research has focused on browning control with different postharvest treatments [11,14,15,18], some selection can be done to choose appropriate plant material which, as a fresh-cut product, could exhibit less browning in order to prolong its shelf life with the above treatments. In the case of fresh-cut lettuce, different pre-harvest factors were found that could affect postharvest quality such as lettuce type, cultivar, and climatic and environmental conditions [15,16,19,20]. Apart from cultivar, the position of the leaf on the plant as well as the position of the fresh-cut leaf area could have an effect on the quality of the fresh-cut product, since the whole plant is used for processing. However, there is limited literature on the above factors since Fukumoto et al. [21] found different browning potential in photosynthetic and vascular tissues from the inner and outer leaves of iceberg lettuce.

Spinach (*Spinacia oleracea* L.) is a leafy, cool-season vegetable popular worldwide with an extensive cultivation area [22]. Baby spinach is characterized as a leafy vegetable with a very high perishable rate, which results in a marketability of only seven days when it is stored at 7 °C [7,23]. In the literature, the influence of different pre-harvest factors on the quality and shelf life of baby spinach, such as cultivar, physiological age, harvesting date, and growing season is well documented [24–26]. Although the effect of growing season has been previously studied, the range of the tested period focused mainly on the winter season, where slight changes were found, probably due to differences in environmental factors [24]. On the other hand, in recent years spinach cultivation has been successful during the warm season under nethouses [27]. However, no information is currently available on the effect of a wide range of cultivation seasons on the postharvest quality and shelf life of baby spinach.

'Rocket', or 'arugula', represents a worldwide variety of the *Brassicaceae* family species, that is well known for its spicy flavor and has a continuously increasing demand [28,29]. Especially, baby rocket leaves are a major economic product [30], as they are a basic ingredient of ready-to-eat or ready-to-use salads in combination with other leafy vegetables, such as lettuce and spinach. Among the rocket species, the most common are 'cultivated' or 'garden' rocket (*Eruca sativa* Mill.) and 'wild' rocket (*Diplotaxis tenuifolia* L.), which are considered cool-season crops because of wilting during long days with high temperatures. An important quality characteristic of rocket is the green color of the leaves [31]. Although for other leafy vegetables there are studies in the literature about the effect of pre-harvest factors on postharvest quality, for rocket there are only a few data regarding genotype, soilless cultivation systems, and typical growing seasons (autumn–winter and winter–spring) [32].

Therefore, the aim of this work was to investigate the effect of cultivar, leaf position, and piece position on the leaf on the sensory quality of fresh-cut butterhead lettuce as well as the effect of a wide range of growing seasons on the postharvest quality and shelf life of baby spinach and rocket.

2. Materials and Methods

2.1. Fresh-Cut Lettuce: Material and Handling

Six butterhead lettuce cultivars were cultivated soilless in an unheated plastic greenhouse at the experimental farm of Aristotle University of Thessaloniki, Central Macedonia, Greece under usual cultivation practices. The tested cultivars were 'Ercole', 'd'America rossastra', 'Morges', 'Agades', 'Reggina di magio' (butterhead), and 'Plenty'. Ten plants per cultivar were used and randomly placed in the greenhouse. Immediately after harvest, lettuce heads were washed with tap water and transferred to the laboratory within 10 min. To study the effect of leaf position on the plant on browning, leaves were divided into outer and inner and were cut with a sharp, stainless steel knife into 4 numbered pieces 4 × 2 cm, with number 1 representing the first piece from the leaf base. Then, pieces were kept in the dark at 10 °C with high humidity (>95%) for 7 days. For each treatment three replications were used, with 2 inner and outer leaves from 6 different plants per replication. The experiment was conducted twice, with very similar results, and thus the results from the second experiment are presented.

2.2. Fresh-Cut Lettuce: Determinations

The degree of browning was evaluated subjectively at the 2nd, 4th, and 7th day of storage and scored using a scale of 1 to 5, where 1 = none, 3 = moderate, and 5 = severe browning. When the pieces reached a score of 3, shelf life ended. General regression equations (normally polynomial) of the browning during storage were used to calculate the shelf life of fresh-cut lettuce. For pieces 1 and 4 of both the outer and inner leaves of the 'd'America rossastra' cultivar the activity of the enzyme phenylalanine ammonia lyase (PAL; EC 4.3.1.5) was assayed during storage [33].

2.3. Baby Leaves (Spinach and Rocket): Material and Handling

Baby leaves of spinach and 'wild' rocket were produced under unheated plastic greenhouse conditions for winter production and under nethouse conditions for the rest of the growing season, under commercial cultivation practices at Vezyroglou Farm, Alexandria Imathia, Central Macedonia, Greece. For both species the harvesting index was the outer leaf length, maximum 9–10 cm. The production conditions as well as corresponding dates of harvest are presented in Table 1. After harvest, all leaves were kept at 4 °C for 24 h before being mechanically packaged in packages of 100 g according to the commercial procedures. Immediately after harvest packages were transferred to the laboratory within 45 min, where they were stored at 4 °C for 12 days. For each treatment, three replications were used with one package per replication.

Table 1. Sampling dates for baby leaves as well as the average temperature, percentage of sunshine days, and the type of greenhouse that was used during their cultivation.

Sampling	Spinach	Rocket	Greenhouse	Average T (°C) Spinach/Rocket	Days with Sunshine (%) Spinach/Rocket
1st – 18/3/19	√	√	Plastic	9.7 / 11.6	76.9 / 69.2
2nd – 2/5/19	√	√	Plastic	14.4 / 15.9	45.5 / 85.7
3rd – 3/6/19	√	√	Shade	20.1 / 19.8	66.7 / 66.7
4th – 22/7/19	-	√	Shade	- / 25.7	- / 88.9
5th – 6/9/19	-	√	Shade	- / 26.9	- / 100
6th – 28/9/19	√	√	Shade	24.0 / 23.7	95.5 / 91.3
7th – 1/11/19	√	√	Shade	19.3 / 19.5	90.0 / 87.5

2.4. Baby Leaves (Spinach and Rocket): Determinations

The visual quality (appearance) of each package was subjectively evaluated using a scale of 9 to 1, where 9 = excellent, 7 = very good, 5 = good—limit of marketability, 3 = fair—limit of usability, and 1 = poor—inedible. General regression equations (normally polynomial) of the appearance score during storage were used to calculate the end of shelf life of the baby leaves (score = 5). Additionally, at each evaluation the color of the leaf surface was measured with a chromameter (CR-400 Chroma Meter, Konica Minolta Inc., Tokyo, Japan) using the colorimetric coordinates of lightness (L*), hue (h°), and chroma (C*) [34].

2.5. Statistical Analysis

Statistical analysis was conducted using IBM SPSS software (SPSS 23.0, IBM Corp., New York, NY, USA). For fresh-cut lettuce, an ANOVA was performed considering the cultivar, leaf position, and piece position on the leaf and their interactions as variation factors in order to find out which factor greatly affects the shelf life of fresh-cut lettuce. For baby leaves, data were analyzed by applying one-way analysis of variance (ANOVA). Comparisons of the means were performed using a Tukey test at $\alpha < 0.05$.

3. Results and Discussion

3.1. Fresh-Cut Lettuce

All the factors as well as their interactions had a significant impact on the shelf life of fresh-cut product (Table 2). However, most of the total variance was accounted for by differences between pieces on the leaf (65.9%). The next most important factor was the interaction of cultivar and piece on the leaf, with 11.1% variance. Cultivar, although significant, had a very small contribution to the total variance with only 8%. A positive coefficient (R = 0.994, $p < 0.00649$) indicates a strong direct connection between the shelf life of fresh-cut butterhead lettuce with piece position on the leaf. A significantly lower shelf life (5.3 days) was observed for the pieces from the bottom of the leaf (piece 1). The next categories of pieces (pieces two to four) exhibited an increase in shelf life by 24.2%, 41.8%, and 56.6% respectively (Figure 1). Only pieces three and four did not significantly differ regarding their shelf life.

Table 2. Analysis of variance for shelf life of fresh-cut butterhead lettuce, during storage at 10 °C for 7 days.

	DF ^{z}	MS ^{y}		%TV ^{x}
Cultivar (A)	5	7.24	***	7.97
Leaf Position (B)	1	2.75	***	3.03
Piece on the Leaf (C)	3	59.79	***	65.88
A x B	5	10.07	***	11.09
A x C	15	1.40	***	1.55
B x C	3	2.00	***	2.20
A x B x C	15	7.07	***	7.79
Error	96	0.44		

^{z} Degree of Freedom, ^{y} Mean Squares, ^{x} % of Total Variance. *, **, *** Significant effect at the 0.05, 0.01 and 0.001 level, respectively.

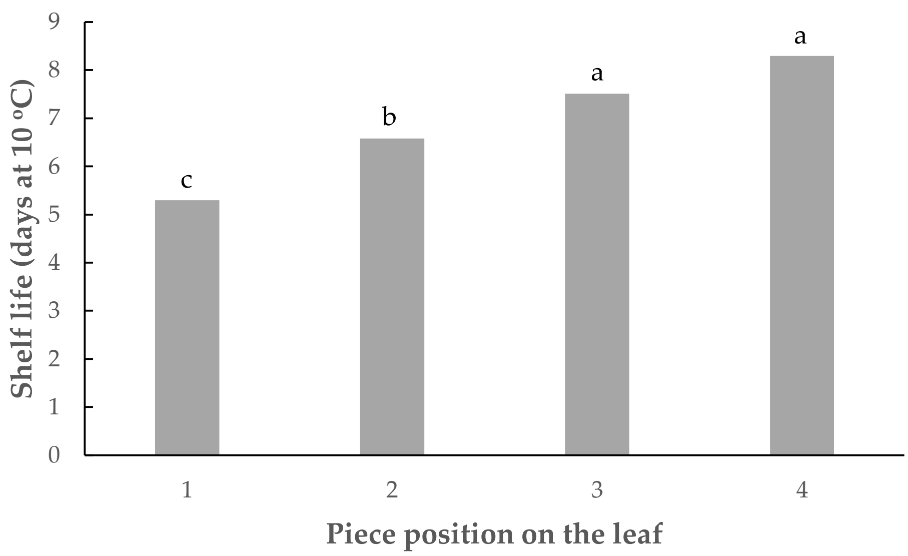

Figure 1. Shelf life of fresh-cut butterhead lettuce as affected by piece position on the leaf (piece one close to the leaf base and piece four close to the top), as an average of 6 cultivars and 2 leaf positions. Columns with different letters are significantly different ($a < 0.05$).

Many studies confirm that not all cultivars of a particular vegetable are suitable for use as fresh-cut product [35]. Cantos et al. [36] observed differences on the browning of fresh-cut product among six lettuce cultivars, with iceberg cultivar 'Mikonos' and romaine cultivar 'Cazorla' to be the most and the least susceptible, respectively. Also, Allende et al. [37] reported that fresh-cut lettuce from 'Lollo Rosso' had less browning than from 'Salinas' cultivar. A significant effect of lettuce type or cultivar on the browning of fresh-cut product was observed during storage in air or a controlled atmosphere [15,38]. Our study confirms the previous results regarding the effect of cultivar, but also indicates the importance of other pre-harvest factors, mainly the piece position on the leaf.

Based on the previous results, we further analyzed the results for one of the tested cultivars (d'America rossastra) regarding the browning during storage as well as PAL activity. It is well known that during the preparation of fresh-cut lettuce, phenylpropanoid metabolism is induced and results in the accumulation of phenolics compounds and tissue browning [17], with PAL being a key enzyme in this process [39].

Analysis of variance (ANOVA) was performed for each storage day with variation factors of the leaf position (inner or outer) and piece position on the leaf (piece one or piece four) as well as their interaction. For the second day of storage no significant effect was observed on the browning of fresh-cut lettuce (Table 3). On the other hand, analysis of variance for the fourth and seventh day of storage showed that only piece position on the leaf had a significant effect, since most of the total variance was accounted for by differences between piece positions on the leaf (64.0% and 81.8% on the fourth and the seventh day respectively). Significantly higher browning was recorded for piece one than piece four (Figure 2). Similar results have been reported in iceberg lettuce with a significantly higher browning score for vascular tissue than photosynthetic tissue for both outer and inner leaves from the fourth and seventh day, respectively, during 10 days' storage at 5 °C [21]. On the other hand, the effect of leaf position (outer or inner) was significant from the fourth day until the end of storage for vascular tissue, while for photosynthetic tissue the difference was significant only from the seventh day of storage [21].

Table 3. Analysis of variance for the browning of fresh-cut 'd'America rossastra' butterhead lettuce, during storage at 10 °C for 7 days.

Source of Variance	DF ^{z}	0 Day		2nd Day		4th Day		7th Day	
		MS ^{y}	%TV ^{x}	MS ^{y}	%TV ^{x}	MS ^{y}	%TV ^{x}	MS ^{y}	%TV ^{x}
Leaf Position (A)	1	0.0117 ***	84.8	0.0032	4.6	0.0216	28.0	0.0002	0.8
Piece Position (B)	1	0.0016 *	11.6	0.0461 *	65.8	0.0448 *	58.0	0.0233 **	90.0
A x B	1	0.0002	1.5	0.0162	23.1	0.0032	4.1	0.0008	3.1
Error	8	0.0003	-	0.0046	-	0.0076	-	0.0016	-

^{z} Degree of Freedom, ^{y} Mean Squares, ^{x} % of Total Variance; *,**,*** Significant effect at the 0.05, 0.01 and 0.001 level respectively.

Just after the processing, the ANOVA for PAL activity showed that both the independent factors (leaf position on the plant and piece position on the leaf) had a significant effect (Table 4). However, most of the total variance was accounted for by differences between leaf positions (85.8%). On the contrary, during storage, the only factor that significantly affected PAL activity was piece position on the leaf (Table 4). For the whole experiment both pieces showed a similar pattern of PAL activity, which was gradually increasing, reaching the maximum values on the fourth day of storage and then decreasing on the last day of storage (Figure 3). However, for all sampling dates (0, 2, 4, and 7 days) the PAL activity of piece one was significantly higher than that of piece four, by 36%, 160%, 69%, and 499% respectively. Similarly, Fukumoto et al. [21] reported higher PAL activity for vascular than photosynthetic tissues.

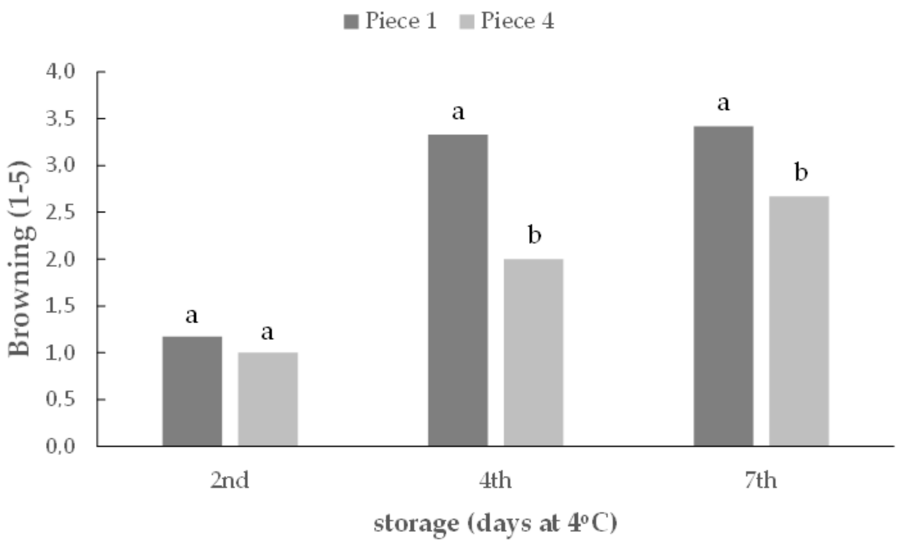

Figure 2. Browning of fresh-cut 'd'America rossastra' butterhead lettuce during storage at 10 °C, as affected by piece position on the leaf. Comparisons of the means were separately performed for each storage day. Columns with different letters are significantly different ($a < 0.05$).

Table 4. Analysis of variance for PAL activity of fresh-cut 'd'America rossastra' butterhead lettuce, during storage at 10 °C for 7 days.

Source of Variance	DF ^{z}	2nd Day		4th Day		7th Day	
		MS ^{y}	%TV ^{x}	MS ^{y}	%TV ^{x}	MS ^{y}	%TV ^{x}
Leaf Position (A)	1	0.083	25.0	1.333	16.0	0.021	1.0
Piece Position (B)	1	0.083	25.0	5.333 **	64.0	1.688 *	81.8
A x B	1	0.083	25.0	1.333	16.0	0.188	9.1
Error	8	0.083	-	0.333	-	0.167	-

^{z} Degree of Freedom, ^{y} Mean Squares, ^{x} % of Total Variance; *,**,*** Significant effect at the 0.05, 0.01 and 0.001 level respectively.

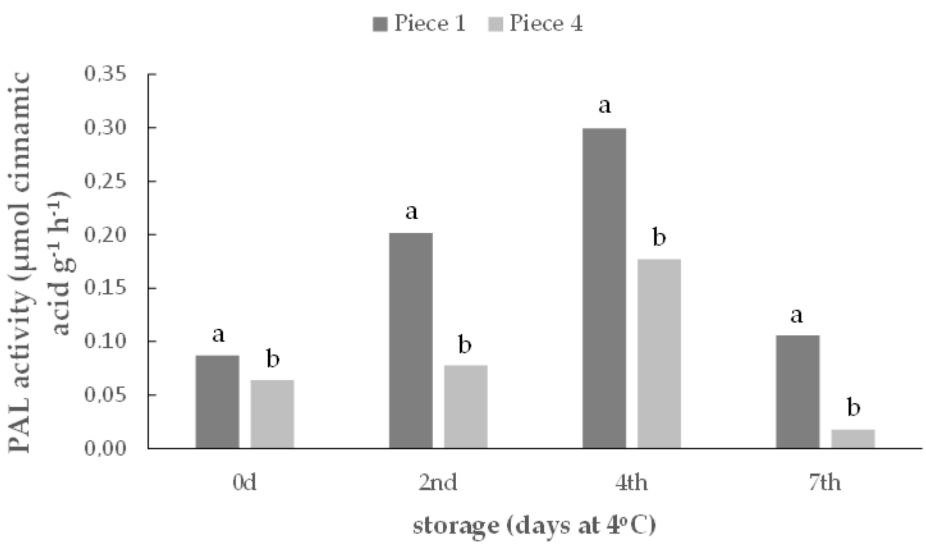

Figure 3. PAL activity of fresh-cut 'd'America rossastra' butterhead lettuce during storage at 10 °C as affected by piece position on the leaf. Comparisons of the means were separately performed for each storage day. Columns with different letters are significantly different ($a < 0.05$).

3.2. Baby Leaves (Spinach and Rocket)

At harvest, the color of the baby spinach leaves was significantly different between growing seasons (Table 5). The highest (125.1 h°) and the lowest (122.1 h°) hue values were observed during the winter and summer cultivation seasons respectively. Mid values were recorded for the samples from the mid-season growing seasons. Similarly, for baby rocket leaves the two summer sampling dates had significantly lower hue values than all the other growing season samples, except the sixth (Table 6). During the winter growing season the baby rocket leaves had the highest values of color parameter hue. Table 5 shows the changes in hue values of stored baby spinach leaves, where for each storage day the leaves of the samples with the lower initial values continue to record more or less the significant lower values. Similarly, sampling of baby spinach from January to March grown in Mediterranean conditions showed color variation due to the environmental conditions of the growing season during harvest, while color parameters remained unaffected by the storage conditions [24]. In Table 6 the hue values for baby rocket leaves are presented, where, until the tenth day of storage the trend was similar with that of the spinach (lower initial values were accompanied by lower values per storage sampling date). However, on the last day of storage only the color of leaves of the first (mainly winter growing season) sample had significantly higher values than all the other growing seasons. During storage, a higher color parameter L* was observed for the winter–spring cultivation of baby rocket leaf that was affected by different growing seasons (autumn–winter or winter–spring) [32]. Probably the increasing temperature and sunlight during the rocket cultivation resulted in a higher alteration of color during storage, as has been previously found in spinach [24].

Table 5. Hue color parameter of baby spinach leaf during storage at 4 °C for 12 days as affected by growing season. Comparisons of the means were separately performed for each storage day. Means with different letters are significantly different ($a < 0.05$).

Sampling	Storage (Days at 4 °C)				
	0	4	7	10	12
1st	124.2 b	124.1 ab	124.3 a	124.5 a	124.0 a
2nd	124.1 b	123.6 b	123.7 ab	123.4 ab	122.3 b
3rd	122.1 c	122.3 c	122.9 bc	122.3 bc	119.8 c
6th	123.6 b	123.9 ab	122.5 c	121.6 c	122.6 b
7th	125.1 a	124.4 a	123.7 ab	123.7 a	123.2 ab

Table 6. Hue color parameter of baby rocket leaf during storage at 4 °C for 12 days as affected by growing season. Comparisons of the means were separately performed for each storage day. Means with different letters are significantly different ($a < 0.05$).

Sampling	Storage (Days at 4 °C)				
	0	4	7	10	12
1st	125.9 a	125.8 a	125.4 a	124.5 a	124.4 a
2nd	124.8 b	124.1 b	124.0 b	123.8 ab	122.2 b
3rd	124.7 b	123.3 bc	124.0 b	122.3 c	121.8 b
4th	123.8 c	123.2 c	123.0 cd	122.8 c	121.8 b
5th	123.8 c	123.7 bc	123.5 bcd	122.5 c	122.0 b
6th	124.2 bc	123.5 bc	122.9 d	122.4 c	122.4 b
7th	124.8 b	123.7 bc	123.8 bc	123.0 bc	122.0 b

The results show a significant effect of the growing season on the shelf life of baby leaves of both species. In particularly, for spinach the shorter shelf life was observed for samples harvested at the end of the two growing seasons with the lower and higher average temperatures during the growing season (Figure 4). The highest shelf life was 16.1 days for the third sampling date (3/6), which was higher by 58% than the shelf life for the sixth sampling date (28/9). Similarly, for baby rocket, the shelf life of leaves could be clearly distinguished in the different categories by the significant differences among them. In the first group were the sampling dates from the mild seasons (3/6 and 6/9), in the second group were the samples grown under mild to extreme environmental conditions (autumn and spring), and finally the samples grown during summer (Figure 5). Moreover, the highest shelf life for baby rocket leaves was 17.7 days (sample 6/9) which was higher by 168% than the lowest shelf life of just the previous sampling date (22/7).

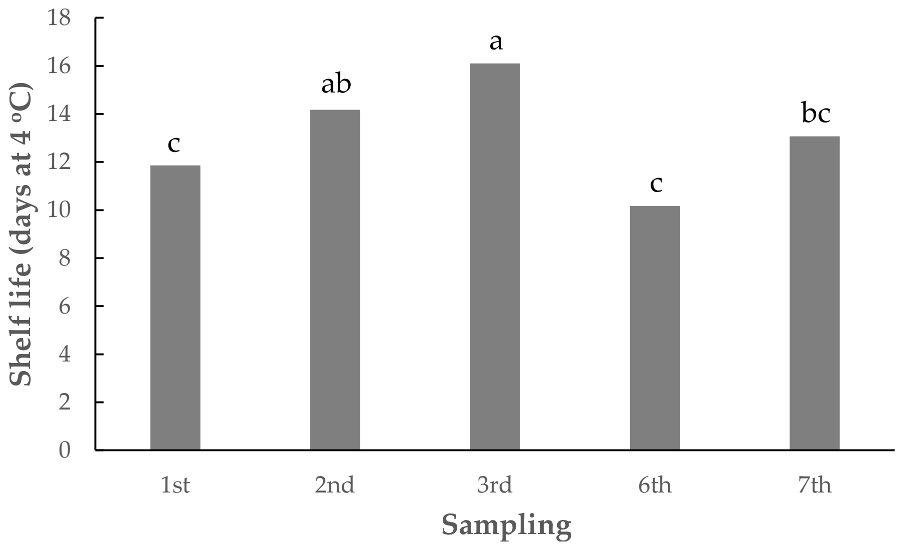

Figure 4. Shelf life of baby spinach leaf stored at 4 °C as affected by growing season. Columns with different letters are significantly different (*a* < 0.05).

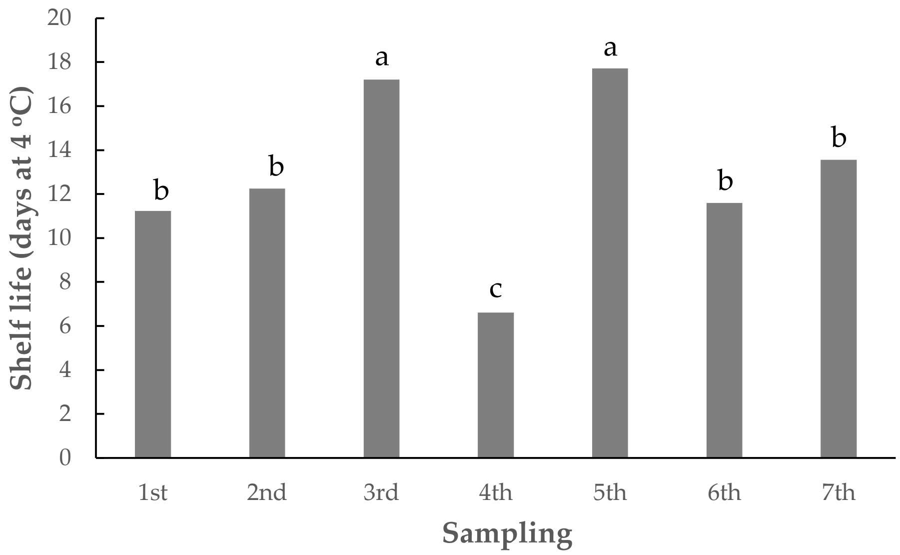

Figure 5. Shelf life of baby rocket leaf stored at 4 °C, as affected by growing season. Columns with different letters are significantly different (*a* < 0.05).

Environmental conditions during plant growth have been well recognized as an important factor for postharvest quality and marketability of many baby leaves or fresh-cut vegetables such as rocket, spinach and lettuce [24,30,40]. In our study, both baby leaves in growing seasons of optimum environmental conditions with mild stress (higher day length for third sampling date or higher temperature for fifth sampling date) presented the higher shelf life. In many cases a moderate abiotic stress potentially improves postharvest behavior through up-regulating genes and pathways [41]. On the contrary, fresh-cut wild rocket produced by a floating system in an unheated Mediterranean greenhouse shows better postharvest behavior when cultivated in the autumn–winter cycle compared to the winter–spring cycle [32]. The above difference could be explained, as suggested by the authors, by oxygen concentration in the nutrient solution which may reach limiting levels for root respiration. In our study, because of the soil cultivation the oxygen concentration in the soil should not be such a limiting factor. Finally, our findings for baby rocket leaves' shelf life is in agreement with the study from Hall et al. [30], which reported great differences in the shelf life of both cultivated and wild rocket year round.

4. Conclusions

Pre harvest factors clearly had a significant impact on quality and postharvest shelf life of both fresh-cut lettuce and baby leaves (spinach and rocket). The influence of cultivar was more or less an expected result for fresh-cut lettuce, however the effect of piece position on the leaf was even more significant. The above results denote the importance of the selection of a suitable cultivar for processing, such as 'Agades'. They also offer a helpful tool for the industry by suggesting the removal of the lower part of the lettuce leaves. The year-round growing season for the production of baby leaves provides raw material with a wide range of shelf life attribute, from 10.1 to 16.1 days and 6.6 to 17.7 days for baby spinach and baby rocket respectively. The above results indicate the need for different postharvest handling depending on the growing season, with the application of suitable factors such as anti-yellowing agent to achieve an increase in the marketable period of the products.

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