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Proceeding Paper

Physicochemical and Functional Value of Lettuce: Effect of Mulching Technique Used During Production on Postharvest Storage †

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Abstract: Lettuce is one of the most important vegetable crops cultivated worldwide. Mulch or mulching is used to protect the soil surface, create a physical barrier, and provide a more suitable environment for crops. The aim of our work was to evaluate the effect of different mulching techniques and test their effect on the morpho-physiological performance and nutritive value of Iceberg lettuce plants, and their changes during postharvest storage. Mulching soil treatments had a significant impact on biometric measurements such as yield, physicochemical values, and functional value in lettuce heads. Organic mulch had the best result, improving the bioactive compounds in lettuce.

Keywords: *Lactuca sativa* L.; mulching; yield; functional food; bioactive compounds

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1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the most important vegetable crops grown and the most popular green vegetable worldwide [1,2]. Lettuce quality can be affected by different factors, such as preharvest factors, postharvest processing, storage time, and environmental condition such as temperature, relative humidity, light and composition of atmosphere [3]. In addition, these factors can also cause variability in the physicochemical, biochemical, nutritional, and microbiological indices within the plant [4].

In lettuce production, there are several techniques to increase yield and improve the product quality that could be used, as well as reducing the environmental effects on cropping. The use of soil covers is one of the most important [5]. Mulch or mulching is an ancient horticultural technique, of which the objective is to protect the soil surface, creating a physical barrier and providing a more suitable environment for the crop [1]. In general, mulches are applied for the retention of soil moisture, reduction in water losses through evaporation, regulation of soil temperature, weed control, facilitating harvesting, improving postharvest quality, and boosting the commercialization [1,2,6]. In addition, mulches have been reported to improve yield and metabolite accumulation [7]. The soil covering materials can be permeable or impermeable, organic-originated (crop residues, bark, bagasse, straw, hay, etc.) or synthetic (polyester and plastics) [5]. Organic mulches reduce soil temperature and maintain higher soil moisture levels compared to black plastic mulch [2]. However, the advent of plastic has led to this technique being used on a large

scale worldwide due to its several advantages, and based on their color (black, clear or white), they absorb and/or reflect sunlight, differently varying soil temperature, affecting crop growth and productivity [8].

In spite of the importance of mulching for lettuce, there is a lack of research to support the potential exploitation of this technology and little information is available concerning the behavior of nutritional components, particularly antioxidant constituents, in Iceberg lettuce during storage and senescence. The aim of our work was to evaluate the effect of different mulching techniques, and test their effect on the morpho-physiological performance and nutritive value of Iceberg lettuce plants, and their changes during postharvest storage.

2. Materials and Methods

2.1. Experimental Design, Growth Conditions, and Sampling

The experiment was carried out during the autumn–winter season using a complete randomized design on a deep sandy loam soil (EEA La Consulta-INTA, Mendoza, Argentina, 33°44′ S, 69°70′ W). Raider cultivar lettuce seeds (Iceberg type) germinated on a commercial substrate and grown for 8 days, and after three true leaves had grown, they were transplanted into the field. The mulching soil treatments consisted of two mulching films (M): dry alfalfa added on top of the grown bed (DA) and bare ground as the control (BG) (Figure 1). The mulching films evaluated were one white biodegradable film (WPF) and one traditional black low-density polyethylene plastic film (BPF).

Figure 1. Mulching soil treatment. A White plastic foil. B Dry alfalfa. C Black plastic foil.

The harvest was carried out manually by cutting the plants in the crown area when the commercial weight was reached and the lettuce heads were stored in cold storage at 4 °C for 8 days. For chemical analysis, leaf scraps from different lettuce heads were used to obtain freeze-dried material. In summary, fresh leaves were frozen at −80 °C and freeze-dried for 72 h in a vacuum system. The resulting lyophilized material was ground into powder with a mortar and stored at room temperature until analysis.

2.2. Biometric Measurements

Each lettuce plant was first weighed as a whole in order to determine the total yield (as total fresh mass, MFT), while the commercial yield was estimated after the separation and weighing of leaves with yellowish coloration and/or burnt edges (commercial fresh mass, MFC). In addition, the weight and compactness of the head during storage were determined.

Eight measurements of the color parameters L*, a*, and b* of the six lettuce heads and stems were obtained. Color was measured using a colorimeter equipped with a D65 illuminant in the reflectance mode and on the CIE L* a* b* color scale. In detail, L* indicates the lightness from black (0 value) to white (100 value), a* the redness (+) or greenness

(−), and b* the yellowness (+) or blueness (−). The chroma (*C*) and hue angle (h°) were calculated as follows:

$$C = \sqrt{a^2 + b^2};\ h^\circ = \arctan\frac{b}{a}$$

2.3. Chlorophylls Pigments and Carotenoids

The concentration of photosynthetic pigments was analyzed by Lichtenhaler and Buschmann, 2001 [9], with slight modifications. The freeze-dried material (0.015 g) was homogenized in 5.5 mL of cold ethanol (95% *v*/*v*). Subsequently, it was homogenized in a vortex for 5 s and sonicated in an ultrasonic bath for 2 min. After that, the extracted mixture was centrifuged for 10 min at 14,000 rpm. Absorbance was measured at three different wavelengths: 649, 664, and 470 nm through a spectrophotometer. The values are expressed as mg%g^{-1} fresh weight. The calculations were made on the basis of the following equations:

$$\begin{gathered} \textit{Chlorophyll a,Chla} = 13.36\ A_{664} - 5.19\ A_{649} \\ \textit{Chlorophyll b, Chlb} = 27.43\ A_{649} - 8.12\ A_{664} \\ \textit{Total carotenoids, } C(x+c) = \frac{1000A_{470} - 2.13Chl_{\ a} - 97.64Chl_b}{209} \end{gathered}$$

2.4. Extraction and Quantification of Individuals and Total Phenolic Compounds

The freeze-dried material (0.025 g) was extracted in 2 mL of methanol:acid water with hydrochloric acid to pH = 2 (70:30 *v*/*v*). The extraction tubes were vortexed for 30 s and sonicated for 30 min at 25 °C. The supernatants were transferred to a microfuge tube and centrifuged at 14,000 rpm for 10 min at 4 °C. The extracts were filtered through a 0.22 µm nylon membrane.

The total phenolic compounds were quantified using the Folin–Ciocalteu method following the procedure previously described by Lemos et al., 2024 [10]. For this, 0.05 mL of the sample extract was mixed with 2.45 mL of distilled water, 0.25 mL Folin solution, and 0.75 mL of Na_2CO_3 (10% *w*/*v*). After 3 min, a constant volume of 2.5 mL of distilled water was added and incubated for 30 min under dark conditions. The absorbance was measured at 765 nm. Each sample was measured against a blank of reagents, containing distilled water instead of the extract. The total phenolic content was determined with a linear calibration curve equation and was expressed as a mean of mg of gallic acid equivalents (mg GAE%g^{-1} fresh weight).

The chromatographic separation of the individual's phenolic compounds was carried out using the mobile phases consisting of phase A: acidified water with 0.1% formic acid; and phase B: acetonitrile, run at 0.2 mL min^{-1}. Phenolic compounds were separated using a gradient elution system according to [10]. The flow rate was 0.2 mL min^{-1} and the injection volume was 0.5 µL. The column oven temperature was set at 35 °C. Phenolic compounds were identified on the basis of their UV spectra and retention time, and were quantified using the standard curves of authentic compounds. All data are expressed as mg%g^{-1} of fresh weight.

2.5. Chromatographic Determination of Vitamin C

Vitamin C extraction was carried out following the procedure described by Medina-Lozano et al. [11]. Briefly, 5 mL of the extraction solution (8% acetic acid (*v*/*v*), 1% MPA (*w*/*v*) and 1 mM EDTA) was added to 0.050 g of the lyophilized sample. The mixture was shaken in a vortex for 5 s, sonicated for 10 min at room temperature and centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was filtered through a 0.22 µm nylon membrane and recovered in an amber vial. The obtained filtrate was directly used to quantify vitamin C as ascorbic acid.

2.6. Statistical Analysis

Values are expressed as the means ± standard deviation. Data were analyzed by an analysis of variance (ANOVA) to test the significant differences. The means were compared

by Tukey's test using InfoStat-Statistical Software. The results were considered significant at $p \leq 0.05$, unless specified otherwise.

3. Results and Discussion

Mulching soil treatments had a significant impact on biometric measurements such as yield, physicochemical values, and functional value in lettuce heads.

The highest values of MFT and MFC were obtained with a black plastic film, while the lowest value was obtained with the dry alfalfa treatment (Table 1). The type of mulch can influence the surface radiation balance, and thus the plant microclimate. Additionally, it is well known that plastic mulching films increase soil temperature compared to bare ground. It can be deduced that variances in MFT could also be associated with differences in soil temperatures when temperature is a limiting factor. On the other hand, our results on MFC are generally in agreement with previous studies which state that soil temperature with different mulching materials had a pronounced effect on marketable lettuce yield [8,12,13]. In addition, BPF may have prevented water evaporation and preserved soil moisture [6].

Table 1. The total fresh mass (MFT) and commercial fresh mass (MFC) of lettuce grown under different mulching types.

Mulching Type	MFT (g/Plant)	MFC (g/Plant)
BG	265.87 ± 60.76 a [1]	216.98 ± 34.47 a
DA	284.57 ± 44.80 a	247.70 ± 42.08 a
BPF	454.47 ± 124.05 a	382.62 ± 122.81 ab
WPF	306.55 ± 86.48 b	262.00 ± 87.32 b
CV (%)	33	36

[1] Values represent the mean ± standard deviation of three determinations. Within a column, values not sharing a common letter are significantly different at $p < 0.05$.

In relation to plant weight, the highest values were reached with BPF in comparison with bare ground. The head compactness, and the Chroma of the head and stalk were not affected by mulch treatments ($p > 0.05$, Table 2). On the other hand, the ANOVA test indicates that there were significant responses to mulching types and storage time for huge angle of the head and stalk (Table 2).

Table 2. Plant weight, head compactness, Chroma of the head and stalk, and huge angle of the head and stalk of lettuce grown under different mulching types.

Mulching Type	Plant Weight (g)	Compactness	C Head [1]	h° Head	C Stalk [2]	h° Stalk
BG	262.27 ± 52.43 a [3]	2.44 ± 0.46 a	117.61 ± 0.32 a	34.31 ± 0.41 b	77.14 ± 19.89 a	26.24 ± 6.53 c
DA	281.25 ± 6.91 a	2.33 ± 0.35 a	118.06 ± 0.65 a	34.55 ± 0.92 b	75.76 ± 19.78 a	22.65 ± 3.91 b
BPF	446.70 ± 106.14 b	2.89 ± 1.02 a	117.68 ± 0.68 a	31.68 ± 1.31 a	72.01 ± 15.88 a	17.87 ± 5.10 a
WPF	302.62 ± 51.19 a	2.50 ± 0.43 a	117.44 ± 0.52 a	33.66 ± 1.12 b	76.80 ± 19.17 a	21.77 ± 5.19 b

[1] C head: Chroma of lettuce head. [2] C stalk: Chroma of lettuce stalk. [3] Values represent the mean ± standard deviation of three determinations. Within a column, values not sharing a common letter are significantly different at $p < 0.05$.

The interaction of mulch treatment x storage time was significant ($p < 0.05$) for chlorophyllous pigments and carotenoids, individual and total phenolic compounds, and vitamin C.

The highest levels of pigments were recorded 4 days after storage with a black plastic foil (Figure 2), while the highest levels of individual phenolic compounds, total phenolic compounds, and vitamin C levels were reached on the 4th and 8th days of storage with dry alfalfa added to the treatment of the grown bed (Figure 3). Vitamin C and phenolic compounds are important antioxidant agents involved in the removal of free radicals. These bioactive compounds play an important role in human diet and prevent several diseases [10,11]. The influence of mulches on phenolic compounds and vitamin C has

been outlined in other studies, but most of them focused on plastic film and some on organic mulches. Regarding the vitamin C content, we denoted, as previously reported by Tosic et al. (2019) [14] and Shah Jahan et al. (2018) [15], that the level of vitamin C depended on the soil mulching. Meanwhile, contradictory results have been reported for phenolic compounds, with increases [16] and decreases [17]. The fact that high levels of vitamin C and phenolic compounds were reached with dry alfalfa might be due to its large amounts of nutrients such as nitrogen and phosphorous, as well as significant amounts of sulfur, calcium, magnesium, iron, and other micronutrients [18]. Furthermore, dry alfalfa is completely degraded by soil microorganisms, providing more favorable conditions for plant development and resulting in the accumulation of bioactive compounds [19]. The present study indicates that dry alfalfa as mulch could be used to enhance plant metabolism and improve the antioxidant properties of lettuce.

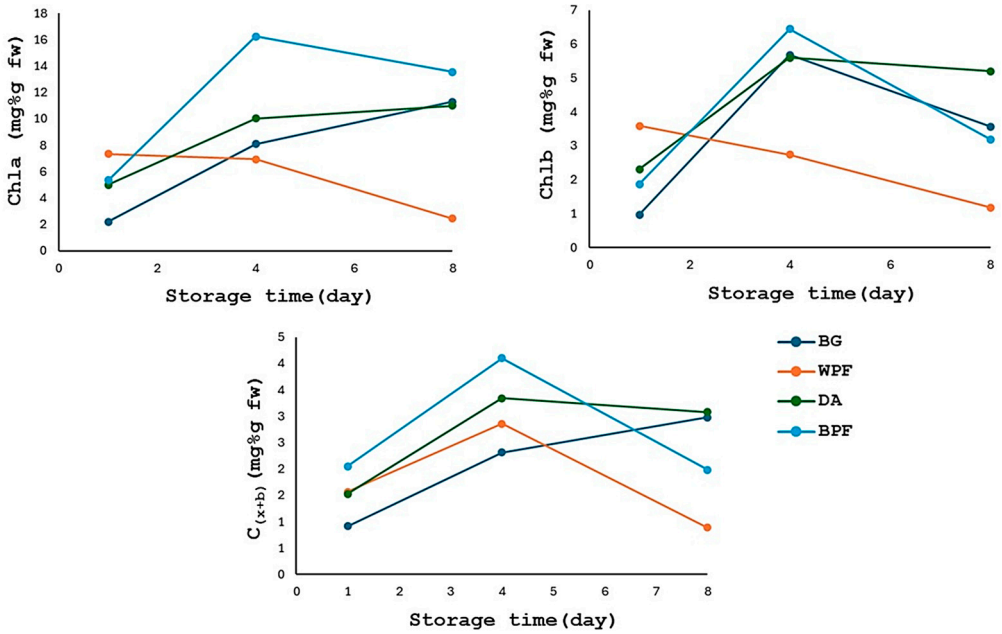

Figure 2. Time-course variation for the mean pigment and carotenoid content during cold storage at 4 °C. Chla: chlorophyll a; Chlb: chlorophyll b; $C_{(x+b)}$: carotenoid; BG: bare ground; WPF: white plastic foil; DA: dry alfalfa; BPF: black plastic foil.

The total phenolic content appeared to have an accumulation phenomenon during storage (Figure 3), and the main phenolic compounds found were chlorogenic and chicoric acid (Table S1). Similar studies have been conducted by Pernice et al. (2007) [20] and Patil et al. (2017) [21], who reported that the use of mulch during the growing period affected the total polyphenol content, but in contrast to these authors, we found changes in the qualitative pattern of polyphenols formed and vitamin C levels during postharvest storage. The highest levels of phenolic compounds and vitamin C, reached on the 4th and 8th days of storage with dry alfalfa added to the treatment of grown bed, could be due to photosynthetic activity and absence of wounds, which could have decreased the respiration rate and ethylene formation, as reported Patil et al. (2017) [21].

The relationship between the variables studied was analyzed using principal component analysis (PCA). On the PCA biplot, the first main component explained 44% of the total variance and the other 22% (Figure 4). The first axis was related to the content of total phenols, the total individual phenolic content, chlorogenic acid, chicoric acid, vitamin C, and biometric measurements such as the huge angle of the head and stalk, while the second axis was related to the weight of the plant, compactness and the content of chlorophylls and carotenoids. Based on this biplot, it can be seen that BPF had a significant impact on plant weight and compactness, WPF had a negative influence on the chroma of the lettuce

head and stalk, while BG had a negative effect on vitamin C and huge angle of the head and stalk of lettuce. In addition, dry alfalfa mulch was noted to have a positive and strong influence on bioactive compounds.

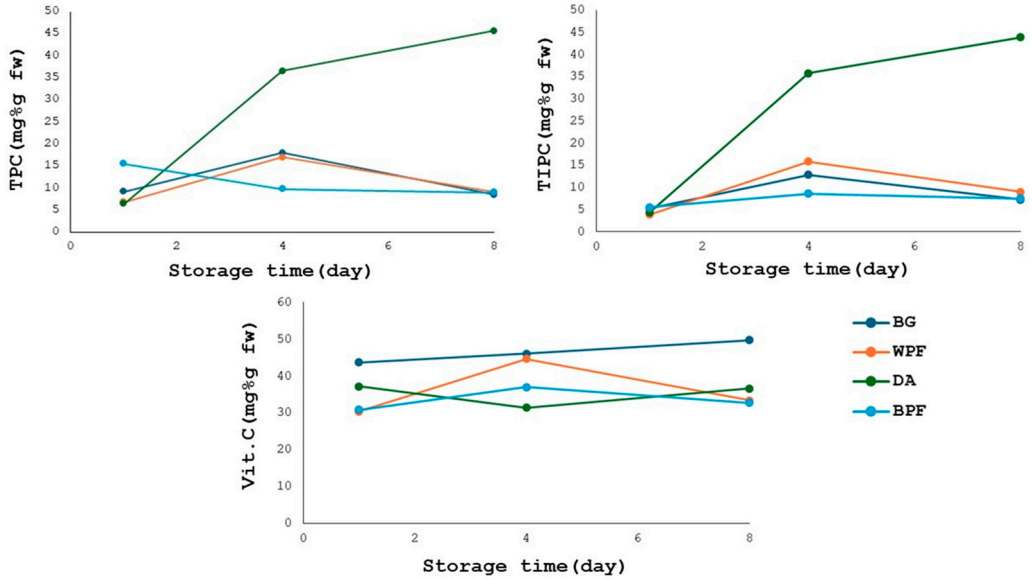

Figure 3. Time-course variation for the mean bioactive compound content during cold storage at 4 °C. TPC: total phenolic content; TIPC: total individual phenolic content; Vit. C: vitamin C; BG: bare ground; WPF: white plastic foil; DA: dry alfalfa; BPF: black plastic foil.

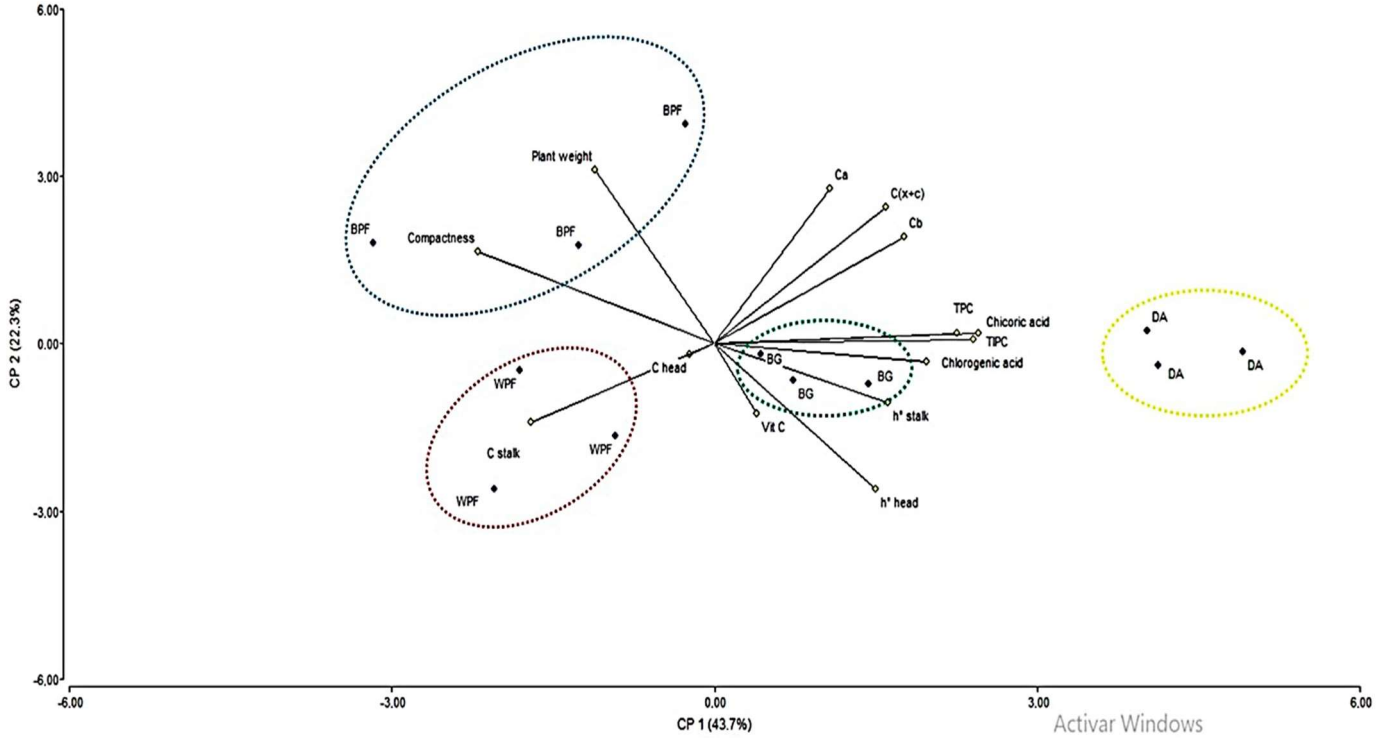

Figure 4. Biplot for mulching types and storage time. TPC: total phenolic content; TIPC: total individual phenolic content; Ca: chlorophyll a; Cb: chlorophyll b; $C_{(x+b)}$: carotenoid; Vit. C: vitamin C; C head: Chroma of the lettuce head; C stalk: Chroma of the lettuce stalk; BG: bare ground; WPF: white plastic foil; DA: dry alfalfa; BPF: black plastic foil.

4. Conclusions

Mulching soil treatments had a significant impact on biometric measurements such as yield, physicochemical values, and functional value in lettuce heads. Organic mulch had the best results, improving the bioactive compounds in the Iceberg lettuce studied, particularly in the antioxidant constituents, during storage and senescence, reaching, in general, the highest levels of bioactive compounds on the 4th day of storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/blsf2024040012/s1, Table S1: Individual phenolic content in lettuce for each soil treatment.

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