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Effects of Lactoperoxidase System Activation-Oregano Essential Oilor Chlorine on the Quality of Modified Atmosphere Packaged Fresh-Cut Iceberg Lettuce

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Abstract

The present work focused on the effects of the Modified Atmosphere Packaging (MAP) 1 (5% O_2 and 10% CO_2) or 2 (2% O_2 and 5% CO_2) and the previous addition of Lactoperoxidase System (LPS) and Oregano essential oil or chlorine washing on the quality of fresh-cut lettuce during refrigerated storage at +4˚C. Our results showed the significant effect of this combined treatment on quality improvement during storage. Thus, mesophilic bacteria was reduced in treated samples compared to those untreated with number which not exceeded the critical of 5×10^7 UFC·g^{-1} ($p < 0.05$). In addition, the elevated O_2 and CO_2 levels created by both atmosphere were not significantly different between the two treatments ($p > 0.05$). Brightness of lettuce samples was significantly reduced during storage. Thereafter, the PCA data showed the effect of combined treatment on the preservation of hygienic, physico-chemical and sensory quality up to the 7th day of refrigerated storage of these treated samples. The results obtained draw attention to modified atmosphere packaging lettuce and the addition of bio-preservatives which could be an alternative of choice to replace chlorine to preserve the sanitary quality of green products.

Keywords

Fresh-Cut Lettuce, Modified Atmosphere, Lactoperoxidase System, Oregano, Quality

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1. Introduction

Fresh-cut or minimally-processed (MP) vegetables are vegetables that have been cut in small pieces, are packaged and stored under refrigerated conditions [1]. Shelf life of leafy green vegetables such as lettuce is limited at a few days resulting from metabolism in the product, at the same time, from growth of spoilage and pathogenic micro-organisms [2].

Disinfection using chlorine is often applied to fresh vegetables to enhance safety and shelf-life profiles. This process has presented some limitations and disadvantages, such as reduced antimicrobial efficacy. Indeed, studies have shown that treatment with water containing 50 - 200 ppm of chlorine resulted in a reduction of bacterial populations of less than 2 log CFU g^{-1} on fruits and vegetables [3]. In addition to such limited efficacy, the formation of carcinogenic chlorinated compounds (chloramines and trihalomethanes) in water is possible [4]. Besides, taste and odor defect in treated products can be caused even when chlorine is used at low concentration. Due to these problems, there is a need to find alternatives for preservation of fresh-cut vegetables in order to improve the efficacy of washing treatments [5].

For this reason, the demand for natural alternatives has increased. In this context, Lactoperoxidase System (LPS) is attracting interest for this potential as natural food preservative and displays a wide spectrum of antimicrobial activity, with potential for control of foodborne pathogens and spoilage bacteria associated with minimally-processed vegetables [6]. The lactopero-xidasethiocyanate-hydrogen-peroxide (LPO) system points out the dairy origin. The antimicrobial action of this system was exerted through short-lived oxidation products, mainly hypothiocyanite ($OSCN^-$) and HOSCN, which produce microbicidal or microbiostatic effects by the oxidation of thiol groups (–SH) of cytoplasmic enzymes [7] and damage to other cellular elements such as the outer membrane, cell wall or cytoplasmic membrane, transport systems, glycolytic enzymes and nucleic acids [8].

The development and application of more natural sanitizers with a broad spectrum antimicrobial activity and no toxicity for human in MP providing enhanced sensory quality and extended shelf-life are of interest to catering industry and consumers [9]. In this context, this trend prompts a particular increased interest in the use of essential oils as antimicrobial compounds to be applied in MPF.

With the objective to improve quality and safety of MPV products and reduce preservatives, the industry is seeking novel and alternative technologies with the objective of improving quality and safety of food products.

With increased concern about efficacy and toxicological safety of chemicals and synthetic preservatives, the demand for natural alternatives has increased. In this context, plant essential oils (EOs) are attracting interest for their potential as natural food preservatives as they have Generally Recognised As Safe (GRAS) status and many of them display a wide spectrum of antimicrobial activity, with potential for control of foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables [10]. Oregano (*Origanum vulgare L.*) and thyme (*Thymus vulgaris L.*) oils, whose main components arespices and herbs have been used for preventing food spoilage, deterioration and also for extending shelf-life of food since ancient times. But, the recent enhancement of interest in green products has led to a renewal of scientific interest in essential oils and their components [11]. However the evaluation of the antimicrobial properties of essential oils when they are applied during vegetable production as a preharvest sanitizing treatment constitutes a novel research area. In this sense, essential oils have the advantage of being an ecological product which generates no toxic waste and are compatible with organic production methods [12]. In particular, the *Origanum* is safe and able to protect human health against genotoxic agents [13]. This essential oil has been reported one of the most effective to have antimicrobial activity against a variety of Gram-positive, Gram-negative bacteria and yeasts [14].

Moreover, to reduce the effects of microbiological, chemical and physical events, it is possible to act on processing or, more usually, on packaging. Modified atmosphere packaging (MAP) has been successfully used to maintain the quality of fresh-cut fruit and vegetables [15]. It is commonly used to extend the shelf-life of fresh-cut lettuce by providing a sufficiently low oxygen partial pressure and enhancing concentration of carbon dioxide to retard oxidative browning. This preservation technique is also intended to inhibit or reduce the growth of some pathogenic micro-organisms particularly due to the low O_2 concentration [16].

Therefore, this study was undertaken to examine the influence of the addition of LPS and oregano or chlorine in combination with modified atmosphere packaging 1or 2 on ther efrigerated shelf-life of fresh-cut lettuce.

2. Material and Methods

2.1. Product Preparation and Storage

Biological iceberg lettuce (*Lactuca sativa L.*)were harvested at optimum maturity from fields in three regions in Tunisia, each of which is characterized by particular climatic conditions (Cap Bon, Bizerte and Gafsa). Samples were then transferred to the laboratory (Research Unit "Bio-conservation and Valorization of Agro-Food Products" (UR13AGR02, ESIAT), Tunis, Tunisia)and stored up to 24 h at 4˚C before treatment. Three external leaves were removed by hand (using sterile gloves) and the other parts of the lettuce were shredded in pieces of approximately 2 cm^2, using a sterile blade, having the same size used for "salad packs". The fresh-cut lettuce was washed with distilled water to remove soil residues. Subsequently, the excess water was removed by centrifugation in a manual salad spinner.After that, the fresh-cut lettuce was treated with a distilled water containing the activated lactoperoxidase system (5 UI, 30˚C, 4 h) and the oregano essential oil (75 ppm, 20˚C, 15 min) or chlorine (80 ppm, 4˚C, 20 min)

[6]. Then, the samples were stored were weighted (15 ± 1 g) in oriented polypropylene film bags (12 cm × 20 cm) (Oxygen transmission rate: 1500 $mL \cdot mm^{-2} \cdot day^{-1}$) under modified atmosphere 1 (5% O_2 and 10% CO_2) or 2 (2% O_2 and 5% CO_2). Fresh cut lettuce samples were stored at +4˚C for 7 and 9 days, respectively for unpackaged samples and those packaged under modified atmosphere 1 or 2.Samples were taken at the following stages: 0 (initial stage immediately after treatment), 1^{st}, 2^{nd}, 3^{rd}, 5^{th}, 7^{th} and 9^{th} day of refrigerated storage at +4˚C.

2.2. Microbiological Analyses

Each 10 g of lettuce was mixed with 90 mL of sterile peptone water with shaking. The mixture was diluted decimally in peptone water. One milliliter of the diluted samples was transferred on plate count agar (Merck, Darmstadt, Germany) to enumerate mesophilic aerobic bacteria and incubated at 30˚C for 48 h. Psychrotrophic bacteria was performed in the same medium but incubated at 6˚C for 5 - 7 days. Total coli forms in Desoxycholate 1‰ agar (Merck, Darmstadt, Germany) incubatedat 37˚C for 24 h. Lactic acid bacteria in Man Rogosa and Sharpeagar (MRS) (Merck) incubated at 37˚C for 48 h. Molds and yeast in SabouraudChloramphenicol agar (Merck, Darmstadt, Germany) incubated at 25˚C for 5 days.

2.3. Physico-Chimical Parameters

2.3.1. pH

Ten grams of prepared samples were mixed for 2 min with 20 mL of distilled water. pH of the slurry was determined at room temperature using a pH-meter (Hach Company Electrochemical Meters, USA) [17].

2.3.2. Weight Loss

Samples were weighed immediately after treatment (IW) and then, after removal from refrigerated storage (FW). Weight loss of each sample was calculated as follows:

$$\mathrm{WL}(\%) = (1 - \mathrm{FW}/\mathrm{IW}) \times 100$$

where "WL" was expressed as percentage of weight loss with respect to initial mass.

2.3.3. Water Content

The water content (WC) of fresh-cut lettuce was determined according to Roura *et al.* [18]. Ten grams were taken and weighed to obtain the fresh mass (FM). They were then placed in a conventional oven (Memmert, UL 60, Germany) at 80˚C for 24 h and then weighed again to obtain the dry mass (DM). The water content, expressed in percent of the fresh material, was calculated as follows:

$$\mathrm{WC}(\%) = \left[(\mathrm{FM} - \mathrm{DM})/\mathrm{FM}\right] \times 100$$

2.3.4. Chlorophyll Content

The total chlorophyll content (TC) of fresh-cut lettuce was determined according to the methodology described by Moreira *et al.* [19]. A one gram of sample was taken and then homogenized in the presence of 19 mL of a cold solution composed of propanone (18 mL) and 1 mL of ammonium hydroxide (0.1 mol·L^{-1}). The mixture was then filtered and the water was removed from the filtrate using anhydrous sodium sulfate. The absorbance of the filtrate at 660 and 642.5 nm was measured using a UV-visible spectrophotometer (Shimadzu Corporation, Japan). The chlorophyll content, reported in mg chlorophyll/g dry weight, was calculated as follows:

$$TC = 7.12 \times A_{660} + 16.8 \times A_{642.5}$$

2.3.5. Colorimetric Measurements

Lettuce color measurements were performed through a colorimeter (Minolta CR-300, Japan) in order to evaluate the visual color changes. The color space co-ordinates were expressed as L*, a* and b* (lightness, red value and yellow value, respectively, on the Hunter scale) and allow to evaluate the intensity of enzymatic browning [20].

2.3.6. Head Space Gas Composition

O_2 and CO_2 contents present in modified atmosphere packaged fresh-cut lettuce were measured during storage using a gas analyzer (Multivac, Germany). The volume taken from the package headspace for gas analysis was about 10 cm^3 using a syringe. The concentration was expressed as a percentage of CO_2 and O_2 [20].

2.4. Sensory Evaluation

Sensory analysis made on the basis of questionnaire results by a trained tasting panel of 15 qualified persons. The questionnaires had a five-grade scale for color, fresh appearance, odor and general acceptability. There were also questions to evaluate the overall appearance color and impression of the panellists.

2.5. Statistical Analysis

The trials were performed at least in triplicate and repeated twice. The software, used to calculate means, standard deviations, and 95% t confidence intervals, was SPSS version 20.0. The Student's test was also used and the threshold differences ($P < 0.05$) were considered statistically significant. Also, the principal component analysis (PCA) was performed for all the data measured during refrigerated storage of modified atmosphere packaged fresh-cut lettuce.

3. Results and Discussion

3.1. Microbiological Analyses

The analysis of the results relating to the total mesophyllic bacteria, total psychrophilic bacteria, total coliforms, total lactic acidbacteria count and yeast and mold counts present in fresh-cut lettuce samples treated and controls and conditioned under modified atmosphere 1 or 2 has demonstrated a significant antibacterial effect of the combined treatment on reducing the number of contamination flora during samples storage (Table 1).

Table 1. Comparative effect of the addition of bio-protective agents or chlorine on the evolution of microflora during refrigerated storage at +4˚C of fresh-cut lettuce and packaged under modified atmosphere 1 or 2.

Storage (days)	Micro-flora (log_{10} CFU g^{-1})	Treatment					
		Control 1	*LPS-Origano* 1	*Cl* 1	*Control* 2	*LPS-Origano* 2	*Cl* 2
0	MES	$6.6\ 0.5^{a}$	$3.1 \pm 0.2b$	3.6 ± 0.4^{b}	6.7 ± 0.5^{a}	3.1 ± 0.2^{b}	3.6 ± 0.2^{b}
	PSC	5.3 ± 0.2^{a}	ab^{b}	ab^{b}	5.8 ± 0.2^{c}	ab^{b}	ab^{b}
	TC	3.9 ± 0.0^{a}	ab^{b}	ab^{b}	3.8 ± 0.1^{c}	ab^{b}	ab^{b}
	LAB	5.4 ± 0.7^{a}	ab^{b}	1.4 ± 0.4^{c}	5.0 ± 0.3^{a}	ab^{b}	$1.2 \pm 0.2b^{c}$
	YM	4.9 ± 0.2^{a}	ab^{b}	1.5 ± 0.0^{c}	4.9 ± 0.1^{a}	ab^{b}	1.5 ± 0.4^{c}
1	MES	6.8 ± 0.2^{a}	3.1 ± 0.2^{b}	$3.8 \pm 0.5b^{d}$	7.0 ± 0.0^{c}	$3.7 \pm 0.5b^{d}$	4.0 ± 0.3^{d}
	PSC	5.7 ± 0.3^{a}	ab^{b}	1.5 ± 0.0^{b}	6.0 ± 0.2^{c}	ab^{b}	1.8 ± 0.0^{d}
	TC	3.9 ± 3.5^{a}	ab^{b}	ab^{b}	3.9 ± 3.2^{a}	ab^{b}	ab^{b}
	LAB	5.7 ± 0.5^{a}	ab^{b}	1.4 ± 0.4^{b}	5.9 ± 0.1^{a}	ab^{b}	1.4 ± 0.4^{b}
	YM	5.8 ± 0.6^{a}	ab^{b}	1.6 ± 0.2^{b}	5.5 ± 0.6^{a}	ab^{b}	1.7 ± 0.3^{c}
2	MES	7.6 ± 0.3^{a}	3.4 ± 0.2^{b}	4.0 ± 0.1^{b}	7.8 ± 0.1^{a}	3.8 ± 0.1^{b}	4.5 ± 0.2^{b}
	PSC	5.6 ± 0.1^{a}	ab^{b}	1.9 ± 0.2^{c}	5.9 ± 0.3^{d}	ab^{b}	2.3 ± 0.1^{e}
	TC	4.6 ± 0.2^{a}	ab^{b}	ab^{b}	5.0 ± 0.0^{a}	ab^{b}	ab^{b}
	LAB	6.5 ± 0.2^{a}	1.4 ± 0.1^{b}	$3.3 \pm 0.3b^{c}$	6.7 ± 0.5^{a}	1.4 ± 0.3^{b}	3.6 ± 0.4^{c}
	YM	6.7 ± 0.6^{a}	1.5 ± 0.4^{b}	4.2 ± 0.1^{c}	6.4 ± 0.5^{a}	1.0 ± 0.0^{b}	4.1 ± 0.6^{c}
3	MES	8.8 ± 0.3^{a}	3.9 ± 0.1^{b}	4.1 ± 0.1^{b}	8.9 ± 0.2^{a}	4.3 ± 0.9^{b}	4.5 ± 0.2^{b}
	PSC	6.7 ± 0.1^{a}	1.3 ± 0.2^{b}	2.1 ± 0.1^{b}	7.6 ± 0.4^{c}	2.0 ± 0.0^{b}	2.8 ± 0.1^{d}
	TC	4.8 ± 0.5^{a}	ab^{b}	ab^{b}	5.7 ± 0.5^{c}	ab^{b}	ab^{b}
	LAB	7.5 ± 0.6^{a}	1.9 ± 0.0^{b}	4.6 ± 0.2^{b}	6.5 ± 0.7^{a}	2.0 ± 0.0^{b}	4.8 ± 0.2^{c}
	YM	6.8 ± 0.6^{a}	2.0 ± 0.1^{b}	4.5 ± 0.1^{b}	6.6 ± 0.6^{a}	2.2 ± 0.2^{b}	4.7 ± 0.2^{d}
5	MES	9.8 ± 0.7^{a}	5.0 ± 0.0^{b}	5.4 ± 0.1^{b}	10.8 ± 0.7^{c}	5.4 ± 0.1^{b}	5.7 ± 0.1^{d}
	PSC	8.6 ± 0.0^{a}	2.7 ± 0.2^{b}	4.4 ± 0.3^{c}	9.0 ± 0.8^{d}	3.3 ± 0.4^{b}	4.3 ± 0.0^{c}
	TC	5.7 ± 0.5^{a}	1.0 ± 0.0^{b}	1.3 ± 0.2^{b}	6.6 ± 0.5^{c}	1.3 ± 0.2^{b}	2.2 ± 0.2^{d}
	LAB	7.6 ± 0.1^{a}	2.7 ± 0.2^{b}	4.5 ± 0.2^{c}	6.9 ± 0.1^{d}	2.4 ± 0.4^{b}	5.1 ± 0.1^{e}
	YM	8.8 ± 0.1^{a}	3.5 ± 0.3^{b}	4.7 ± 0.1^{c}	8.9 ± 0.2^{a}	4.2 ± 0.4^{b}	4.6 ± 0.1^{c}
7	MES	11.3 ± 0.8^{a}	6.3 ± 0.2^{b}	6.7 ± 0.1^{c}	12.3 ± 0.7^{a}	6.5 ± 0.0^{b}	6.9 ± 0.1^{d}
	PSC	8.9 ± 0.2^{a}	1.7 ± 0.2^{b}	5.7 ± 0.2^{c}	9.6 ± 0.6^{a}	3.8 ± 0.2^{b}	5.9 ± 0.1^{d}
	TC	6.7 ± 0.6^{a}	1.7 ± 0.2^{b}	2.5 ± 0.1^{c}	8.2 ± 0.6^{d}	2.5 ± 0.2^{c}	2.6 ± 0.1^{c}
	LAB	10.6 ± 0.4^{a}	3.3 ± 0.2^{b}	5.6 ± 0.1^{b}	9.6 ± 0.4^{c}	3.2 ± 0.3^{b}	6.6 ± 0.1^{d}
	YM	8.8 ± 0.2^{a}	4.4 ± 0.2^{b}	5.2 ± 0.3^{c}	9.3 ± 0.4^{a}	4.4 ± 0.2^{b}	$5.0 \pm 0.0b^{c}$
9	MES	13.5 ± 0.5^{a}	8.6 ± 0.5^{b}	9.6 ± 0.6^{a}	14.4 ± 0.4^{c}	8.8 ± 0.5^{b}	9.5 ± 0.6^{a}
	PSC	13.3 ± 0.3^{a}	7.3 ± 0.9^{b}	8.5 ± 0.4^{b}	13.7 ± 0.5^{c}	7.4 ± 0.8^{b}	8.9 ± 0.1^{b}
	TC	8.7 ± 0.5^{a}	3.5 ± 0.2^{b}	4.0 ± 0.1^{b}	11.7 ± 0.7^{c}	4.9 ± 0.2^{b}	5.7 ± 0.5^{d}
	LAB	14.5 ± 0.6^{a}	7.5 ± 0.6^{a}	8.5 ± 0.5^{a}	14.4 ± 1.1^{a}	7.3 ± 0.8^{a}	9.5 ± 0.9^{a}
	YM	12.8 ± 0.8^{a}	6.4 ± 0.2^{b}	7.7 ± 0.3^{c}	13.6 ± 0.1^{d}	6.9 ± 0.2^{b}	8.8 ± 0.6^{e}

LPS-Origano 1: Fresh-cut lettuce samples processed with Lactoperoxidase-Oregano and packaged under modified atmosphere 1; *LPS-Origano* 2: Fresh-cut lettuce samples processed with Lactoperoxidase-Oregano and packaged under modified atmosphere 2; *Cl* 1: Chlorine treated fresh-cut lettuce samples, conditioned under modified atmosphere 1; *Cl* 2: Chlorine treated fresh-cut lettuce samples, conditioned under modified atmosphere 2; *Control* 1*:* Untreated Fresh-cut lettuce samples and packaged under modified atmosphere 1; *Control* 2: Untreated Fresh-cut lettuce samples and packaged under modified atmosphere 2 ; ab: absent. Means followed by different letters are significantly different ($p < 0.05$).

Thus, regardless of the refrigerated storage and the modified atmosphere packaging, the numbers of the different contamination germs, present in the cut lettuce treated with LPS and the oregano essential oil, remain significantly lower ($p < 0.05$) than those measured on fresh lettuce samples treated with chlorine or controls.

In addition, the total germs present in fresh-cut lettuce samples treated with chlorine or LPS and oregano essential oil remain below the recommended value for the consumption of vegetables, and this is on the 7th day of refrigerated storage (<7.7 $\log_{10}$ CFU g^{-1}) [21]. Thus, the treatment combining the modified atmospheres with bio-preservatives or the chlorine applied to the lettuce samples ensured the stability and the safety of this food until the 7th day of storage, with a more pronounced effect on lettuce conditioned under a modified atmosphere 1. These results are similar to those obtained by Arvanitoyannis *et al.* [22], on lettuce and rocket, pointing out that the high CO_2 levels created by the same modified atmospheres inhibited psychrotrophic and mesophilic total aerobic floras growth after 10 days of refrigerated storage. Similarly, the work of Ahn *et al.* [23] on Chinese cabbage showed that a modified atmosphere rich in CO_2 effectively reduced the total germs and total coliforms counts during storage.

3.2. Physico-Chemical Parameters

3.2.1. Evolution of Package Atmospheres

These experimental conditions show that lettuce samples are continuously exposed to lower concentrations of O_2 and higher CO_2, which is generally recommended for the products packaging [24] in order to decelerate the respiratory phenomena during their conservation.

The oxygen levels in all packaged chopped and processed fresh lettuce samples decreased significantly during storage ($p < 0.05$), while those of CO_2 levels increased regularly for both modified atmospheres (**Table 2**).

Oxygen consumption and CO_2 release remained significantly more pronounced ($p < 0.05$) during the refrigerated storage of control lettuce samples compared to those measured on lettuce treated with LPS and oregano essential oil or chlorine, suggesting that the addition of bio-preservatives or chlorine can similarly alleviate the respiratory phenomena of processed lettuce. It should be noted that the rate of oxygen consumption is a measure of fresh lettuce alteration, linked to respiration, while the total amount of oxygen consumed by the product is directly related to the speed of metabolic activities (level of senescence) associated with respiration [25].

3.2.2. pH

The pH evolution of frresh-cut lettuce and control samples shows a progressive decrease, particularly, because of the proliferation of acidifying lactic acid bacteria throughout their storage (**Table 2**). However, the decrease of pH remains significantly lower ($p < 0.05$) for chopped fresh lettuce treated with chlorine or LPS and oregano essential oil compared to that observed on the

Table 2. Comparative effect of the addition of bio-protective agents or chlorine on the evolution of physico-chemical parameters during refrigerated storage at +4˚C of fresh-cut lettuce and packaged under modified atmosphere 1 or 2.

Storage(days)	Physico-chemical parameter	Treatment					
		Control 1	*LPS-Origano* 1	*Cl* 1	*Control* 2	*LPS-Origano* 2	*Cl* 2
0	pH	6.53 ± 0.06^{A}	6.36 ± 0.02^{B}	6.25 ± 0.04^{C}	6.57 ± 0.06^{A}	6.35 ± 0.05^{B}	6.22 ± 0.02^{C}
	O_2 (%)	5.00 ± 0.00^{A}	5.00 ± 0.00^{A}	5.00 ± 0.00^{A}	2.00 ± 0.00^{B}	2.00 ± 0.00^{B}	2.00 ± 0.00^{B}
	CO_2 (%)	10.00 ± 0.00^{A}	10.00 ± 0.00^{A}	10.00 ± 0.00^{A}	5.00 ± 0.00^{B}	5.00 ± 0.00^{B}	5.00 ± 0.00^{B}
	WL (%)	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
1	pH	6.32 ± 0.07^{AB}	6.37 ± 0.03^{A}	6.19 ± 0.04^{C}	6.27 ± 0.02^{B}	6.30 ± 0.01^{AB}	6.21 ± 0.02^{C}
	O_2 (%)	4.60 ± 0.10^{A}	4.91 ± 0.01^{B}	4.92 ± 0.02^{B}	1.60 ± 0.01^{C}	1.91 ± 0.10^{D}	1.85 ± 0.06^{D}
	CO_2 (%)	11.47 ± 0.06^{A}	10.93 ± 0.15^{B}	10.93 ± 0.23^{B}	6.77 ± 0.25^{C}	6.03 ± 0.25^{D}	6.07 ± 0.12^{D}
	WL (%)	5.12 ± 0.16^{A}	0.58 ± 0.03^{B}	0.80 ± 0.13^{B}	4.75 ± 0.58^{A}	0.58 ± 0.06^{B}	0.63 ± 0.07^{B}
2	pH	6.10 ± 0.06^{A}	6.33 ± 0.01^{B}	6.12 ± 0.03^{A}	6.14 ± 0.04^{A}	6.31 ± 0.01^{B}	6.09 ± 0.03^{A}
	O_2 (%)	3.90 ± 0.10^{A}	4.82 ± 0.02^{B}	4.83 ± 0.04^{B}	1.38 ± 0.07^{C}	1.80 ± 0.02^{D}	1.75 ± 0.04^{D}
	CO_2 (%)	14.47 ± 0.40^{A}	11.90 ± 0.10^{B}	11.83 ± 0.06^{B}	11.47 ± 0.29^{C}	7.73 ± 0.25^{D}	8.07 ± 0.32^{D}
	WL (%)	10.89 ± 0.39^{A}	2.58 ± 0.05^{B}	3.13 ± 0.03^{C}	10.39 ± 0.47^{A}	2.51 ± 0.88^{B}	3.07 ± 0.03^{C}
3	pH	5.88 ± 0.02^{A}	6.30 ± 0.00^{B}	6.08 ± 0.03^{C}	5.83 ± 0.05^{A}	6.30 ± 0.00^{B}	6.03 ± 0.03^{C}
	O_2 (%)	3.30 ± 0.26^{A}	4.00 ± 0.10^{B}	3.90 ± 0.10^{B}	0.80 ± 0.07^{C}	1.11 ± 0.08^{D}	0.98 ± 0.03^{CD}
	CO_2 (%)	15.37 ± 0.25^{A}	13.57 ± 0.12^{B}	13.30 ± 0.17^{B}	11.97 ± 0.30^{C}	9.50 ± 0.30^{D}	9.73 ± 0.15^{D}
	WL (%)	13.29 ± 0.74^{A}	4.65 ± 0.18^{B}	4.68 ± 0.57^{B}	12.45 ± 0.71^{A}	4.27 ± 0.03^{B}	4.97 ± 0.10^{B}
5	pH	5.35 ± 0.17^{A}	6.26 ± 0.02^{B}	6.02 ± 0.02^{C}	5.38 ± 0.09^{A}	6.26 ± 0.03^{B}	5.99 ± 0.04^{C}
	O_2 (%)	2.00 ± 0.10^{A}	2.45 ± 0.05^{B}	2.42 ± 0.03^{B}	0.50 ± 0.10^{C}	0.95 ± 0.05^{D}	0.87 ± 0.06^{D}
	CO_2 (%)	15.37 ± 0.47^{A}	14.03 ± 0.15^{B}	14.07 ± 0.15^{B}	11.47 ± 0.31^{C}	10.23 ± 0.15^{D}	10.63 ± 0.23^{D}
	WL (%)	15.93 ± 0.86^{A}	8.79 ± 0.11^{BC}	9.38 ± 0.36^{BD}	15.49 ± 0.60^{A}	8.09 ± 0.08^{C}	9.95 ± 0.10^{D}
7	pH	5.08 ± 0.03^{A}	6.20 ± 0.01^{B}	5.98 ± 0.04^{C}	5.11 ± 0.01^{A}	6.17 ± 0.02^{B}	5.94 ± 0.04^{C}
	O_2 (%)	1.40 ± 0.06^{A}	2.13 ± 0.06^{B}	2.00 ± 0.10^{C}	0.12 ± 0.05^{D}	0.35 ± 0.05^{E}	0.25 ± 0.06^{E}
	CO_2 (%)	16.27 ± 0.38^{A}	14.93 ± 0.21^{B}	15.07 ± 0.06^{B}	11.97 ± 0.12^{C}	10.47 ± 0.23^{D}	10.97 ± 0.21^{E}
	WL (%)	16.16 ± 0.13^{A}	9.36 ± 0.25^{B}	10.13 ± 0.04^{C}	16.26 ± 0.13^{D}	9.76 ± 0.49^{B}	11.23 ± 0.13^{C}
9	pH	4.70 ± 0.14^{A}	5.89 ± 0.09^{B}	5.51 ± 0.10^{C}	4.77 ± 0.06^{A}	5.88 ± 0.09^{B}	5.62 ± 0.11^{C}
	O_2 (%)	0.00 ± 0.00^{A}	1.08 ± 0.08^{B}	1.01 ± 0.12^{B}	0.00 ± 0.00^{A}	0.08 ± 0.02^{A}	0.08 ± 0.02^{A}
	CO_2 (%)	19.03 ± 0.15^{A}	18.20 ± 0.10^{B}	18.17 ± 0.31^{B}	15.23 ± 0.42^{C}	13.13 ± 0.06^{D}	12.83 ± 0.15^{D}
	WL (%)	17.77 ± 0.46^{A}	10.91 ± 0.32^{B}	12.94 ± 0.81^{C}	17.59 ± 1.23^{D}	11.49 ± 0.50^{B}	14.11 ± 0.76^{C}

control. Thus, the overall relative decreases in pH, measured during storage, are around 10.7%, 7.5% and 27.7% respectively, and this for the both modified atmospheres.

3.2.3. Weight Loss

For both modified atmospheres used, weight losses increased significantly ($p < 0.05$) during storage of processed or untreated cut lettuce (Table 2). Weight loss values increased and this is related to changes in preserved lettuce samples quality [26].

Regardless of the modified atmosphere tested, the overall relative weight losses, recorded throughout storage, are around 11.2%, 13.5%, and 17.7%, respectively, for treated samples with LPS-oregano or chlorine and untreated, indicating that the lettuce samples treated by these two biological agents remain of higher quality compared to that measured on samples treated with chlorine or controls.

3.2.4. Color

Color is an important sensory attribute and is a critical factor affecting the quality of food product. Table 3 illustrated the variations of the different color parameters (L, a and b) during refrigerated storage of the different lettuce samples.

Table 3. Comparative effect of the addition of bio-protective agents or chlorine on the evolution of color parameters during refrigerated storage at +4˚C of fresh-cut lettuce and packaged under modified atmosphere 1 or 2.

Storage (days)	Color parameter	Treatment					
		Control 1	*LPS-Origano* 1	*Cl* 1	*Control* 2	*LPS-Origano* 2	*Cl* 2
0	L	65.76 ± 0.80^{a}	61.28 ± 0.95^{b}	61.72 ± 0.52^{b}	64.76 ± 1.30^{ac}	61.13 ± 0.62^{b}	63.18 ± 1.75^{bc}
	a	−17.45 ± 1.30^{ab}	−17.95 ± 0.63^{a}	−16.11 ± 0.43^{b}	−17.42 ± 0.41^{ab}	−16.26 ± 0.96^{ab}	−16.72 ± 1.08^{ab}
	b	29.77 ± 0.76^{a}	30.12 ± 0.50^{a}	27.91 ± 0.30^{b}	29.77 ± 1.61^{a}	29.65 ± 0.36^{a}	27.53 ± 1.02^{b}
1	L	60.16 ± 1.29^{ab}	60.76 ± 0.88^{bc}	61.95 ± 0.83^{c}	60.73 ± 0.85^{bc}	58.65 ± 0.66^{a}	61.70 ± 0.49^{bc}
	a	−15.89 ± 0.40^{ab}	−16.57 ± 0.35^{a}	−15.04 ± 0.30^{bc}	−15.98 ± 0.15^{ab}	−16.03 ± 0.55^{ab}	−14.60 ± 1.44^{c}
	b	29.26 ± 0.32^{ab}	29.58 ± 0.91^{a}	28.00 ± 0.76^{b}	29.12 ± 0.64^{ab}	28.63 ± 1.07^{ab}	26.50 ± 0.25^{c}
2	L	53.61 ± 0.64^{ab}	60.92 ± 0.19^{c}	60.03 ± 0.15^{c}	51.96 ± 0.84^{a}	57.97 ± 0.68^{bc}	59.57 ± 1.80^{bc}
	a	−15.19 ± 0.59^{a}	−15.17 ± 0.92^{a}	−13.47 ± 0.47^{b}	−13.75 ± 0.59^{ab}	−14.83 ± 0.53^{a}	−11.93 ± 0.72^{c}
	b	27.69 ± 0.53^{a}	29.46 ± 0.57^{b}	26.82 ± 0.42^{ac}	26.36 ± 0.56^{c}	27.72 ± 0.59^{a}	24.52 ± 0.59^{d}
3	L	50.25 ± 0.56^{a}	59.78 ± 0.31^{b}	58.04 ± 1.23^{c}	49.85 ± 1.02^{a}	57.42 ± 0.68^{c}	58.08 ± 1.26^{c}
	a	−12.92 ± 0.76^{a}	−12.75 ± 0.64^{ab}	−11.97 ± 0.16^{bc}	−12.15 ± 0.06^{abc}	−12.03 ± 0.55^{abc}	−11.50 ± 0.06^{c}
	b	25.70 ± 0.57^{a}	29.16 ± 0.16^{b}	25.41 ± 0.52^{ac}	24.60 ± 0.74^{c}	26.76 ± 0.60^{d}	22.23 ± 0.66^{e}
5	L	45.30 ± 0.66^{a}	57.46 ± 1.11^{b}	56.19 ± 1.42^{bc}	42.84 ± 0.86^{d}	55.74 ± 0.84^{bc}	55.33 ± 0.33^{c}
	a	−9.65 ± 1.10^{a}	−12.75 ± 0.51^{b}	−10.85 ± 0.38^{cd}	−9.68 ± 0.49^{a}	−11.59 ± 0.36^{c}	−10.22 ± 0.28^{ad}
	b	22.21 ± 1.03^{a}	27.16 ± 0.66^{b}	24.23 ± 0.48^{c}	20.38 ± 0.75^{d}	25.16 ± 0.35^{c}	21.24 ± 1.12^{ad}
7	L	40.46 ± 0.56^{a}	54.15 ± 0.17^{b}	52.05 ± 0.93^{b}	37.79 ± 1.34^{a}	53.27 ± 0.24^{b}	50.39 ± 1.45^{b}
	a	−9.05 ± 0.85^{ab}	−11.75 ± 0.77^{c}	−10.17 ± 0.16^{d}	−9.05 ± 0.60^{a}	−10.03 ± 0.26^{bd}	−9.19 ± 0.19^{abd}
	b	19.10 ± 0.90^{a}	25.63 ± 0.84^{b}	22.05 ± 0.09^{c}	15.05 ± 0.98^{a}	23.73 ± 1.00^{e}	19.44 ± 1.01^{a}
9	L	36.08 ± 0.98^{a}	49.55 ± 1.14^{b}	46.07 ± 1.05^{c}	35.76 ± 1.59^{a}	49.68 ± 1.39^{b}	44.47 ± 0.42^{c}
	a	−4.80 ± 0.55^{a}	−7.77 ± 0.80^{b}	−6.83 ± 0.49^{bc}	−4.37 ± 0.97^{a}	−7.37 ± 0.29^{b}	−6.17 ± 0.97^{c}
	b	14.87 ± 0.75^{a}	22.44 ± 0.98^{b}	19.59 ± 0.57^{c}	14.35 ± 0.45^{a}	20.49 ± 0.91^{c}	17.79 ± 0.85^{d}

The average values of the brightness (L) decreased significantly ($p < 0.05$) during storage processed fresh-cut lettuce or control and conditioned under the two modified atmospheres 1 and 2. Thus, the average values pass initially from 61.2, 62.5, 65.3, reaching, at the end of storage, the values of 49.6, 45.3, 35.9, respectively for the samples treated with the LPS-oregano or chlorine and untreated samples. Decreases observed for (L) values are correlated with significant increases in parameter (a), indicating a gradual change of greenery to redness, observed for all lettuce samples.

At the same time, we noted that the color parameter (b) decreases during refrigerated storage of all lettuce samples studied. In addition, the evolution of this parameter remains significantly more marked ($p < 0.05$) during refrigerated storage of all packaged samples under modified atmosphere 1 than that measured on packaged samples under modified atmosphere 2. It also appears that the addition of LPS-oregano or chlorine to modified atmospheres packaging significantly prevented fresh lettuce yellowing throughout storage, due to the deceleration of enzymatic browning affecting this very perishable commodity [22]. Similarly, Martinez-Ferrer *et al.* [27] reported that a modified atmosphere of 4% O_2 and 10% CO_2 maintained the L and b values of fresh cut mangoes constant after 25 days of storage at +5˚C.

3.3. Sensory Profiles

Color, odor, fresh appearance and general acceptability are key aspects used in sensory analysis to assess the overall quality of product. This is especially true for lettuce where browning is a critical factor in the apparent loss of quality. The sensory profile were better maintained in treated samples of both atmospheres supplemented with LPS-oregano compared to controls (**Table 4**).

Thus, the tasting panel found significant differences ($p < 0.05$) in color, odor, fresh appearance and general acceptability between treated and untreated samples, regardless of modified atmosphere and storage. Significantly, similar scores ($p > 0.05$) were recorded for each of the sensory parameters of lettuce treated with chlorine or with LPS -oregano essential

oil. Similar results were obtained for iceberg lettuce leaves washed with sodium hypochlorite and aqueous chlorine dioxide and stored under active MAP where the product remained acceptable from the sensory point of view even after 10 days of storage [28].

3.4. Principal Component Analysis (PCA)

A principal component statistical analysis (PCA) was performed on all analytical data obtained during refrigerated storage of fresh-fresh lettuce samples processed and conditioned under two different modified atmospheres (**Figure 1**).

The factorial map 1 - 2 is defined by the first two principal components (CP1 and CP2) or (factor 1 and factor 2), explaining 82.51% of the total variance,

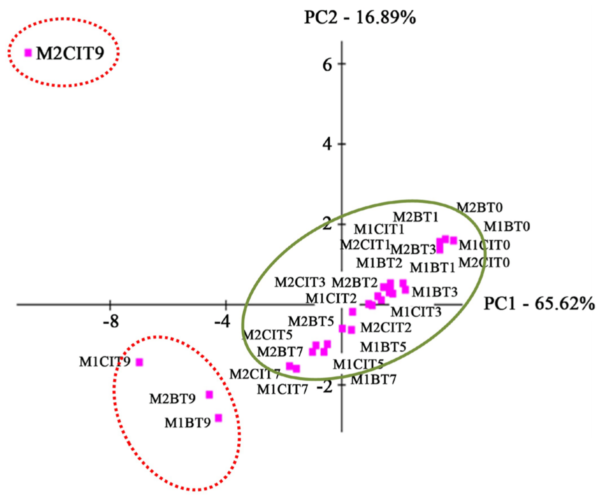

Figure 1. Principal component analysis performed on the microbiological, physico-chemical and organoleptic parameters measured during refrigerated storage of fresh-cut lettuce samples treated with chlorine or LPS-oregano and packaged under modified atmosphere 1 or 2. M1B0→M1B9: Fresh-cut lettuce samples treated with LPS-Oregano, packaged under modified atmosphere 1, and stored for 0, 1, 2, 3, 5,7 and 9 days at +4˚C; M2B0 →M2B9: Fresh-cut lettuce samples treated with LPS-Oregano, packaged under modified atmosphere 2, and stored for 0, 1, 2, 3, 5,7 and 9 days at +4˚C; M1Cl0→M1Cl9: Chlorine treated fresh-cut lettuce samples, packaged under modified atmosphere 1, and stored for 0, 1, 2, 3, 5.7 and 9 days at +4˚C; M2Cl0 →M2Cl9: Chlorine treated fresh-cut lettuce samples, packaged under modified atmosphere 2, and stored for 0, 1, 2, 3, 5.7 and 9 days at +4˚C.

Table 4. Comparative effect of the addition of bio-protective agents or chlorine on sensory quality during refrigerated storage at +4˚C of fresh-cut lettuce and packaged under modified atmosphere 1 or 2.

Storage (days)	Parameter	Treatment					
		Control 1	*LPS-Origano* 1	*Cl* 1	*Control* 2	*LPS-Origano* 2	*Cl* 2
0	Color	0.00 ± 0.00^a	6.67 ± 0.72^a	6.60 ± 0.80^a	0.00 ± 0.00^a	6.60 ± 0.74^a	6.60 ± 0.74^a
	Odor	0.00 ± 0.00^a	7.20 ± 0.68^b	7.33 ± 0.72^b	0.00 ± 0.00^a	7.13 ± 0.83^b	7.27 ± 0.70^b
	Freshappearance	0.00 ± 0.00^a	7.53 ± 0.52^b	7.47 ± 0.52^b	0.00 ± 0.00^a	7.47 ± 0.52^b	7.47 ± 0.52^b
	Generalacceptability	0.00 ± 0.00^a	7.47 ± 0.52^b	7.67 ± 0.49^b	0.00 ± 0.00^a	7.53 ± 0.52^b	7.47 ± 0.52^b
3	Color	0.00 ± 0.00^a	5.40 ± 0.51^a	5.40 ± 0.51^a	0.00 ± 0.00^a	5.40 ± 0.51^a	5.40 ± 0.51^a
	Odor	0.00 ± 0.00^a	7.13 ± 0.83^b	7.07 ± 0.80^b	0.00 ± 0.00^a	6.93 ± 0.88^b	7.00 ± 0.85^b
	Freshappearance	0.00 ± 0.00^a	7.13 ± 0.35^b	7.00 ± 0.00^b	0.00 ± 0.00^a	7.13 ± 0.35^b	7.00 ± 0.00^b
	Generalacceptability	0.00 ± 0.00^a	7.07 ± 0.46^b	7.00 ± 0.53^b	0.00 ± 0.00^a	6.87 ± 0.64^b	7.47 ± 0.70^b
7	Color	0.00 ± 0.00^a	3.60 ± 0.51^a	3.60 ± 0.51^a	0.00 ± 0.00^a	3.53 ± 0.52^a	3.60 ± 0.51^a
	Odor	0.00 ± 0.00^a	5.47 ± 0.64^b	5.47 ± 0.64^b	0.00 ± 0.00^a	5.33 ± 0.82^b	5.57 ± 0.64^b
	Freshappearance	0.00 ± 0.00^a	4.00 ± 0.93^b	4.00 ± 1.13^b	0.00 ± 0.00^a	3.93 ± 1.03^b	3.93 ± 1.22^b
	Generalacceptability	0.00 ± 0.00^a	4.93 ± 0.80^b	4.87 ± 0.74^b	0.00 ± 0.00^a	4.80 ± 0.77^b	4.87 ± 1.27^b
9	Color	0.00 ± 0.00^a	2.40 ± 0.51^{bc}	2.27 ± 0.46^b	0.00 ± 0.00^a	2.13 ± 0.35^{bc}	2.47 ± 0.52^c
	Odor	0.00 ± 0.00^a	1.93 ± 0.59^b	1.87 ± 0.64^b	0.00 ± 0.00^a	2.00 ± 0.53^b	1.87 ± 0.64^b
	Freshappearance	0.00 ± 0.00^a	1.00 ± 0.00^a	1.00 ± 0.00^a	0.00 ± 0.00^a	1.00 ± 0.00^a	1.00 ± 0.00^a
	Generalacceptability	0.00 ± 0.00^a	1.13 ± 0.35^{bc}	1.27 ± 0.41^{bc}	0.00 ± 0.00^a	1.20 ± 0.41^b	1.00 ± 0.00^c

which is very satisfactory for an interpretation of the results. The dispersion of these data on the factorial map 1 explains nearly 65.62% of the total inertia.

Therefore, the aim is to analyze the relationships between physico-chemical (pH, weight loss, O_2, CO_2, L, a, b, color, odor, fresh appearance and general acceptability) and hygienic (total mesophilic aerobic flora, total psychrotrophic aerobic flora, total coliforms, lactic flora and yeasts and molds) paramaters and identify the proximities between lettuce samples at all stages of storage.

Taking into account the position of processed and packaged lettuce samples under the two modified atmospheres with respect to CP1, we can easily discriminate samples treated with chlorine or LPS and oregano stored during 9 days of those stored for 7 days at +4˚C, underlining that the hygienic, physico-chemical and sensory quality of the lettuce treated with chlorine or LPS-oregano was considered satisfactory until the 7th day of their conservation, as previously discussed [6].

Also, the PCA analysis predicts that modified atmosphere packaged lettuce samples 1 or 2 have similar characteristics for the same treatment, regardless of the refrigerated storage. In addition, this analysis showed the existence of strong positive correlations within a first group including the variables color, odor, fresh appearance, general acceptability, O_2, L and b and pH. Similarly, positive correlations were also noted between the variables of a second group consisting of total mesophilic aerobic flora, total psychrotrophic aerobic flora, total coliforms, lactic flora, yeasts and molds, weight loss and CO_2. Moreover, the variables of these two groups are negatively correlated with each other.

Thus, we noted that pH and weight loss are the two main parameters that significantly ($p < 0.05$) influence the evolution of color parameters (L, a and b) and organoleptic characteristics during storage, because of their involvement in the enzymatic browning of fresh-cut lettuce. It follows that cutting the lettuce leaves into pieces would cause lysis of the cell membranes and contact the oxidation enzymes and phenolic substrates naturally present in the lettuce, which would be the cause of the enzymatic browning.

4. Conclusion

It emerges from this study that the treatment combining modified atmosphere packaging, having a lower O_2 content compared to that of CO_2, and the addition of bio-preservatives agents to fresh-cut lettuce, such as essential oils and activated LPS could be an alternative of choice to replace chlorine, a disinfectant commonly used to maintain the safety of products, but its antimicrobial action could be associated with sensory changes perceived in these treated products and possible formation of carcinogenic chlorinated compounds, since this combined treatment has preserved its quality until the 7th day of storage.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

MP: Minimally-Processed
LPS: Lactoperoxidase System
LPO: lactoperoxidase–thiocyanate–hydrogen-peroxide
EOs: Essential Oils
GRAS: Generally Recognised As Safe
MAP: Modified atmosphere packaging