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The individual and combined effect of ozone and UV-C on mass loss, respiration, texture and colour changes of fresh-cut lettuce

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Abstract

The aim of this study was to investigate the effect of UV-C and ozonation as individual and combined treatments on the quality characteristics (mass loss, colour, texture and respiration rate) of fresh-cut lettuce, employing three different UV-C fluences and one O_3 concentration that have been reported in the literature to have a positive effect on disinfection of fresh-cut lettuce. Fresh-cut 'Romaine' lettuce (*Lactuca sativa L. var. longifolia*) was exposed to three fluences 0.20, 0.40 and 0.80 kJ/m^2 of ultraviolet-C (254 nm) radiation, to gaseous O_3 concentration of 5 mg/L for 5 min, and their combination. The produce was stored unpackaged for 5 days at 5.7^{o}C and 91.95%. The applications of O_3, UV-C and their combinations (UV-C+O_3) gave good results in terms of quality characteristics preservation. Individual or combined treatments prevented mass loss throughout the cold storage compared to control samples. The combined treatment of UV-C with O_3 achieved more than 25% less mass loss (maximum values) compared to the other tested treatments and control samples. The respiration rate was increased in individual and combined treatments, especially in the UV-C treatment. The UV-C fluence < 0.80 kJ/m^2 and O_3 concentration 5 mg/L for 5 mins applications achieved improved texture quality in terms of positive peaks (*crispness*). The *crispness* of the treated samples was increased by 35% compared to control samples, especially for the UV-C+O_3 combination. Limited colour degradation was noted since the UV-C fluence and O_3 concentration and dosage were kept below the limits of UV-C fluence < 5 kJ/m^2 and O_3 concentration < 5 mg/L for which quality degradation has been reported in the literature.

1. Introduction

Vegetables are a significant chapter of the human diet due to their nutritional value and as that, occupy a considerable part of the food market. Vegetables are rich in vitamins, minerals, dietary fibres, complex carbohydrates, and non-nutrient substances including plant sterols, flavonols, anthocyanins, and phenolic acids. Vegetables are appreciated due to their attractive sensorial qualities such as taste, aroma, texture, colour, gloss, shape, size and of course the absence of defects and decay (Sarron *et al.*, 2021). It is well known that the short shelf-life of vegetables has been associated with a series of foodborne illness outbreaks that have been attributed to their consumption (Denis *et al.*, 2016). From the latter, it is understood the need of applying decontamination treatments as an integrative part of vegetable processing lines. Therefore, vegetables' shelf-life can be extended by employing conventional chemical treatments, described as antimicrobial solutions such as chlorine, electrolyzed water and hydrogen peroxide. Chlorine has been extensively used in the food industry as sodium hypochlorite in aqueous formulations and different operations such as washing and spraying, acting effectively on foodborne pathogens and maintaining the overall quality of treated product's shelf-life (Sarron *et al.*, 2021). In the past decades, consumer awareness, regarding health and food safety issues initiated by the use of food synthetic additives, has extensively increased. The increasing demand for natural food additives as a substitute for chemical disinfectants has driven the industry towards the development and application of more sustainable technologies for preserving vegetable safety and quality (Miller *et al.*, 2013).

The non-thermal technologies of ultraviolet-C (UV-C) radiation and ozonation have been studied as

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replacements for surface decontamination of fruits and vegetables, and are considered highly efficient, non-toxic, and environment-friendly. The UV-C radiation is generated at wavelengths of 250-280 nm and has been reported to disrupt the functionality and integrity of microorganisms' DNA along with the generation of reactive oxygen species that regulate physiological processes to induce secondary metabolite production (Allende and Artes, 2003; Allende *et al.*, 2006). On the other hand, ozone (O_3) is a powerful sanitizer that may meet the consumers' acceptance, the manufacturers' expectations and the regulatory agencies approval. Since 1997, ozone has gained the Generally Recognized as Safe (GRAS) status for direct contact with foods. In June 2001, gaseous and aqueous O_3 was approved by the U.S. Food and Drug Administration (USFDA) as an antimicrobial additive for direct contact with foods (Code of Federal Regulations, 2001). In the European Union, the application of O_3 in food processing commenced in the early 1900s after its first use for water treatment. The European Council of Ministers has adopted the proposal of permitting the O_3 treatment of natural mineral water. In 2019, the French Food Safety Authority (Agence Nationale de Sécurité Sanitaire (ANSES), 2019) recommended the use of O_3 in water, as a disinfectant in the washing of ready-to-use salads. Since then, research and commercial applications have taken place to investigate the potential use of O_3 as a substitute for traditional sanitizing agents and the benefits that may have in extending the shelf-life of various vegetables. In vegetable handling lines, O_3 can be applied in gaseous form, added continuously or intermittently and in an aqueous form, added immediately after vegetables harvest or during their washing by spraying, rinsing, or dipping.

Chinchkar *et al.* (2022) reviewed many of the sanitizers and disinfectants of fresh fruits and vegetables, presenting their working principles, applications, effectiveness, cons and pros and the related legislation issued by the U.S. Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA) regarding the physical and chemical disinfection technologies (chlorine, chlorine dioxide, O_3, peroxyacetic acid, hydrogen peroxide, trisodium phosphate, organic acids such as lactic acid, ascorbic, acetic acid, citric acid and tartaric acid, electrolyzed water, ionizing radiation such as gamma rays, x-rays, e-beams. and ultrasound) and their effectiveness for the shelf-life extension of fresh produce. Chemical disinfection technologies (chlorine dioxide, O_3, electrolyzed water, organic acid) are promising substitutes, dealing with the rigorous food safety and shelf-life demands (Chinchkar *et al.,* 2022). The chlorine application has been banned in some countries due to human health and environmental raised issues. Chinchkar *et al.* (2022) reported that market preference has been shifted to chlorine dioxide and other chemicals and added that the novel chemicals in combination with reliable regulatory policies could successfully decline the rate of contamination in fruits and vegetables. Physical technologies such as ultrasound and ionizing radiation are referred to, as gentle pasteurization modes that are effective in microbial load reduction with minimal effects on sensory and nutritional profiles. Concluding, Chinchkar *et al.* (2022) highlighted the need for further research regarding the combined preservation techniques that might lead to promising approaches for microbiological safety.

The efficacy of O_3 as a disinfectant is affected by a number of intrinsic factors (*food product*: fruit and vegetable type, volume/mass, surface characteristics, available water; *microbial load*: microbial strains characteristics, the physical state of bacterial strains, natural microflora, artificially inoculated microorganisms, population size) and extrinsic factors (*water quality*: pH, organic matter, pressure, and temperature, *air quality*: relative humidity and *ozone treatment*: concentration and dosages). The benefits of O_3 application in the gaseous phase to sustain the quality of stored lettuce are based on O_3 molecules that have a longer half-life in the air than in an aqueous solution and a higher diffusion rate (Sarron *et al.*, 2021). Although research regarding the UV-C and ozonation as disinfection treatments can be found in literature, insufficient information is provided on what degree and in which way the main quality characteristics (mass loss, colour, texture and respiration rate) of fresh-cut lettuce are affected especially if the variable combination of them is employed, to extend the beneficial effects on fresh and fresh-cut vegetables such as lettuce.

This study aimed to investigate the effect of UV-C and ozonation as individual and combined treatments on the quality characteristics (mass loss, colour, texture and respiration rate) of fresh-cut lettuce, employing three different UV-C fluences and one O_3 concentration that have been reported in the literature to have a positive effect on disinfection of fresh-cut lettuce.

2. Materials and methods

2.1 Raw materials

'*Romaine*' lettuce (*Lactuca sativa* L. *var. longifolia*) heads were obtained from a local grower (Attica, Greece) at commercial maturity. The heads were transported to the laboratory of Farm Machine Systems (Agricultural University of Athens, Greece) where the outer three or four leaves from each lettuce head and

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core were discarded. The remaining leaves were rinsed with tap water for 1 min. Intact and unwilted leaves were sliced using a sterile knife into 3 cm thick pieces and the formed pieces were washed again with deionised water at 5°C for 2 mins and the excess water was removed by a salad spinner. Upon washing completion, samples of 60 g each, were formed and used in the tested treatments.

2.2 Individual and combined treatments by UV-C and gaseous ozone

Sarron *et al.* (2021) reported that low temperatures affect positively the biocidal efficiency of O_3, increasing its solubility, stability, and availability in the medium. On the contrary, as temperature increases, O_3 becomes less soluble and less stable. The effectiveness of gaseous O_3 is beneficial for fruits and vegetables disinfection when relative humidity is ≥ 80% (Sarron *et al.*, 2021). Based on the previous discussion, the treatments were carried out in environments of temperatures 10-15°C and relative humidity ≥ 80%, while cold-storage was taken place at temperatures 5.7±0.3°C and relative humidity 91.95±3.7%. The fresh-cut lettuce samples were treated initially by UV-C radiation. For this cause, a portable refrigerator of 40 L volume was covered internally by an aluminium foil to maximize the reflectance and its top cover was equipped internally with one UV-C lamp (LADVANCE, MLS Co., Ltd, PRC) T8 of nominal wattage 15 W (55 V) G13, diameter 25.5 mm and length 436 mm (Figure 1). The radiated power of the lamp at 254 nm is 5.1 W. The fresh-cut lettuce samples were placed on the bottom of the refrigerator so that the distance between the UV-C lamp level and the samples' layer was 22.5 cm. The samples' fluence (radiant energy received from a surface) was calculated as Keitz (1971) suggested as 0.20 kJ/m^2 for 30 s application, 0.40 kJ/m^2 for 60 s application and 0.80 kJ/m^2 for 120 s application. Experiments of UV-C and O_3 as well as their combinations were carried out at 5°C in a walk-in cold room. The UV-C treatments took place at three-time levels of 30 s, 60 s and 120 s, while ozonation was applied for 5 mins.

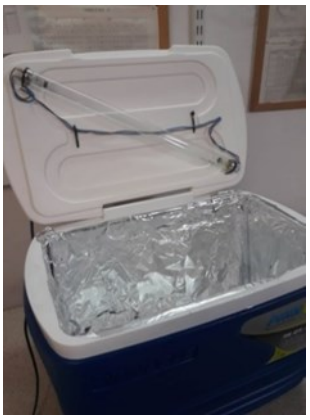

Figure 1. Experimental setup for UV-C and O_3 treatments.

Ozone gas was generated using an O_3 generator QLA-400 (Guangzhou Qili Environmental Equipment Co., Ltd., Guangzhou, PRC) with a production rate of 400 mg/h. The O_3 concentration was measured using a portable O_3 detector, D-16 PortaSens III Gas Detector-Ozone 0-20 mg/L (GasSensing, Inwood, USA). The concentration of O_3 in the air was kept at the level of 5 mg/L for 5 mins throughout the treatments. The combined effect of UV-C and O_3 gas was also investigated employing three-time levels as follows, UV (30 s) + O_3 (5 mg/L), UV (60 s) + O_3 (5 mg/L) and UV (120 s) + O_3 (5 mg/L).

2.3 Experimental procedure

2.3.1 Mass loss estimation

In total, nine samples of 60 g each were used, in each of the eight tested treatments, control, O_3 (5 mg/mL), UV (30 s), UV (60 s), UV (120 s), UV (30 s) + O_3 (5 mg/L), UV (60 s) + O_3 (5 mg/L), UV (120 s) + O_3 (5 mg/L). The UV-C doses were varied based on the exposure time while keeping the working distance between the lamp and tested samples constant. The tested samples upon treatment were stored unpackaged in a walk-in cold room at a temperature of 5.7±0.3°C and relative humidity of 91.95±3.7% for 5 days. On each storage day, the samples were weighted to calculate their mass loss, and colour change was evaluated in three points of each sample in a random way. The texture of the samples was evaluated at the beginning and end of their storage. Mass loss was calculated daily, throughout the storage period and expressed as percentage of the initial mass of each sample using an electronic scale of ±0.01 g accuracy.

2.3.2 Texture assessment

Firmness was measured by a Texture Analyser TA-XT2i (Stable Micro Systems Ltd., Godalming, UK) employing a 5-bladed *Kramer* shear-cell for testing non-uniform samples or samples with variable geometry. The shear cell's five parallel steel blades were driven down through guide slots into a rectangular container with corresponding slots in the base. The contained sample is sheared, compressed, and extruded through the bottom openings. A *Kramer* shear test was conducted on three lettuce samples at the beginning (t = 1st day) and end (t = 5th day) of the experiment at a deformation rate of 2 mm/s. From the force-distance curves, values of maximum force (FKMF), work (FKW) and positive peak forces (FKPP) required to shear the tested food samples, were measured, and presented in Figure 2. In particular, the Texture Analyser TA-XT2i was programmed to start the *Kramer* shear tests when the shear-cell was in contact with the sample (Anchor 1 in Figure 2) and to end the test when the shear-cell had fully sheared the sample (Anchor 2 in Figure 2). As Bruns and Bourne (1975)

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discussed, materials having a *crispy* nature, exhibit a characteristic force-deformation curve, as seen in Figure 2, that is identified by the linear slope of the curve's initial section, that continues up to a sharp peak (maximum force), and then a steep drop in force after sample's fracture. Bruns and Bourne (1975) also suggested that this initial positive slope of the force-deformation curve is a good indicator of the sample's *crispness*. Nath and Chattopadhyay (2007) and Cruzy Celis *et al.* (1996) suggested that the *crispness* evaluation should be considered in terms of positive peaks. Therefore, for *crispness* measurement, a macro was created to count the number of positive peaks during the initial positive slope of the force-deformation curve of the shear-compression test. Álvarez *et al.* (2020) reported that each food sample has a distinctive shape of force-distance curve which depends on its structural characteristics such as the variability of its *fracturability*. According to ISO 5492 (2008), a *crispy* food is characterised by a high level of *fracturability* which is related to *cohesiveness* and *hardness* and to force needed to break down a foodstuff into smaller pieces (ISO, 2008). Lettuce, crackers, celery, and potato chips are characterised as *crispy* foods, snapping easily when deformed, emitting a crunchy/crackly sound. Szczesniak (1988) concluded that by definition, potato chips are *crispy*, ice is *crunchy*, and fresh celery, which snaps cleanly and has a series of fractures when chewed, is both *crispy* and *crunchy*. In the conducted experiments regarding the textural changes of the lettuce samples, three replications were performed per treatment (nine samples in total) at the beginning (t = 1st day) and at the end (t = 5th day) of the cold storage.

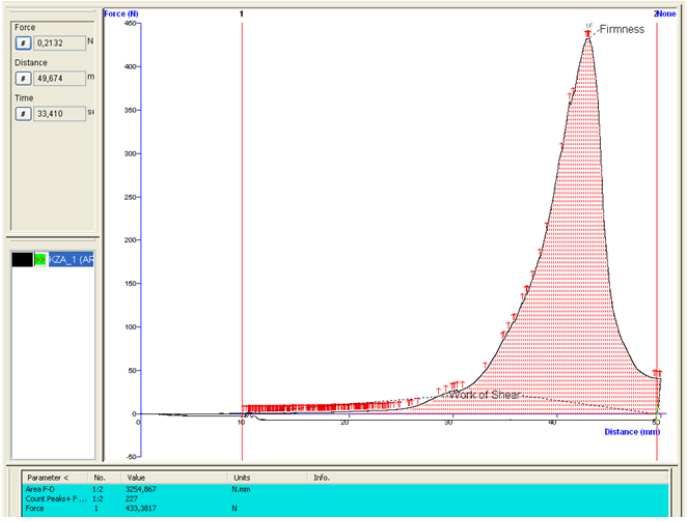

Figure 2. Force-distance curve presenting the maximum force -firmness (FKMF), work of shear (FKW) and positive peaks-red arrows (FKPP) during *Kramer shear test* of fresh-cut lettuce samples.

2.3.3 Respiration rate estimation

The respiration rate in terms of CO_2 production was estimated by employing the patented portable experimental setup (RICKLOS) (Mitropoulos *et al.*, 2000). The RICKLOS is a closed-static respiration system in which the accumulated CO_2 is measured by a portable CO_2 gas monitor RI-411A (Riken Keiki Co. Ltd., Tokyo, Japan) having accuracy ±2% and resolution 25 mg/L. The measurement of the respiration rate took place at three samples (60 g each) per treatment in the respiration cells. Three replications were performed per treatment (nine samples in total). The samples were taken out of the containers where they were stored at predefined times and placed in the respiration cells, connected via a network of plastic tubes to the Riken Keiki RI-411A. The average measurement time was two hours, to ensure that a sufficient amount of CO_2 had been accumulated in the respiration cells according to the measurement sensitivity of the Riken Keiki RI-411A instrument.

2.3.4 Colour parameters estimation

The colour of the lettuce samples was measured by a Lovibond RT300 (The Tintometer Ltd, UK) portable spectrophotometer in CIE L*a*b* tristimulus chromatic space. The colour measurements were taken on three different points of each lettuce sample (3 points×3 samples×3 replicates = 27 sampling points) aiming to cover most of the exposed surface of the fresh-cut samples. The L* index ranges between 0 (black) to 100 (white) and is a useful indicator of darkening or lightening either from oxidative browning or pigments' changes. The scale of a* index extends from red (+a*) to green colour (-a*) and b* scale from yellow (+b*) to blue (-b*) colour. The values of a* and b* indices were used to calculate the Chroma, $C^* = (a^{*2}+b^{*2})^{0.5}$ values and the Hue angle degrees, $h^* = \arctan^{-1}(b^*/a^*)$; if h*>90°, this corresponds to a more intense green colour whilst h* values closer to 90° to yellowish colour. Chroma defines the colour intensity or purity of the hue angle where values close to 0 correspond to neutral colours (ex. grey colour), and close to 60 to bright colours (McGuire, 1992). Pathare *et al.* (2013) reported that colour changes can be also evaluated by the modulus of the distance vector between the initial colour values and the colour coordinates at time t, as $\Delta E^* = (\Delta a^{*2}+\Delta b^{*2}+\Delta L^{*2})^{0.5}$. Depending on the sampling strategy and the desired accuracy, the L*, a*, b*, a*/b*, h*, C* or ΔE* values can provide an accurate colour description. Pathare *et al.* (2013) reported that Chroma (C*), is considered a quantitative colour property and the higher the C* values, the higher the colour intensity perceived by humans. On the other hand, hue angle (h*), is considered a qualitative colour property according to which colours are defined as reddish, or greenish (Pathare *et al.*, 2013).

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2.4 Statistical analysis and Weibull distribution

All the samples used for organoleptic evaluation per tested treatment were prepared in triplicate and each test was replicated three times, making a total of nine samples analysed for each tested parameter as previously discussed. In the present study, the experiments were conducted according to a full factorial design, considering two factors (UV-C and O_3) at four levels for UV-C (0, 30, 60, 120s), two levels for O_3 (0, 5 mg/L) and also the UV-C×O_3 interactions. In total, eight treatments were tested, control [O_3 (0 mg/mL), UV (0 s)], O_3 (5 mg/mL), UV (30 s), UV (30 s)+O_3 (5 mg/L), UV (60 s), UV (120 s), UV (60 s) + O_3 (5 mg/L), UV (120 s) + O_3 (5 mg/L). The collected data were analysed using *Fisher's Least Significant Difference* test (LSD) employing Statgraphics 19 (StatPoint Technologies Inc., VA, USA) to determine significant differences ($P \leq 0.05$) among the mean values of the quality properties. The LSD test is liberal with respect to the comparison-wise error rate or type I errors, compared to other multiple comparison tests such as *Duncan's, Newman-Keul's, Bonferroni's, Tukey's* and *Scheffe's*, but is powerful to detect true differences among mean values (Mason *et al*., 2003).

The three-parameter *Weibull* distribution is a well-known probability distribution function used to describe the behaviour of systems or events that have some degree of temporal variability. *Weibull* distribution was developed to analyse materials' strength and the consequent failure with time under stress conditions but later, was used to describe also quality control, biology, enzymatic and chemical degradation kinetics. The probability density function of the *Weibull* distribution is described as follows (Hahn and Shapiro, 1967; Rinne, 2009).

$$f(x)=\begin{cases}\frac{\beta}{\alpha}\left(\frac{x-\gamma}{\alpha}\right)^{\beta-1}\exp\left[-\left(\frac{x-\gamma}{\alpha}\right)^{\beta}\right], x>0\\ 0, \textit{elsewhere}\end{cases} \quad (1)$$

with $\alpha > 0$ and $\beta > 0$, where α is the *scale parameter* as a reaction rate constant, β is the *shape parameter* as a behaviour index and γ is the location parameter which locates the distribution along the abscissa. Changing the value of γ has the effect of "sliding" the distribution and its associated function either to the right (if $\gamma > 0$) or to the left (if $\gamma < 0$). The *Weibull* distribution is reduced to a first-order decay/growth kinetics when $\beta = 1$. As discussed by Hahn and Shapiro (1967) and Gacula and Kubala (1975), the failure rate for the *Weibull* model is an increasing function of time for $\beta > 1$ (concave downward) and a decreasing of time for $\beta < 1$ (concave upward). When $\beta = 1$, the distribution becomes exponential and its failure rate becomes constant. The *shape parameter* (dimensionless) determines the shape of the curve (upward or downward curvature), while the *scale parameter* is a rate constant. Therefore, if $\beta < 1$ the failure rate decreases over time, if $\beta = 1$ the failure rate is constant over time (random external events are causing degradation) and if $\beta > 1$ the failure rate increases with time (an *"aging process"* is taking place).

3. Results and discussion

3.1 Mass loss and respiration rate estimation

The mass loss of the fresh-cut lettuce was expressed as $(m_i-m_t)/m_i$ where m_i is the initial sample mass (g) and m_t the sample mass (g) at time t (h). The analysis of variance in Table 1 showed that the interaction of storage time with the eight treatments affected significantly the mass loss ($P \leq 0.05$). The applied treatments caused variable degree of mass losses (Figure 3) despite the fact that the samples were stored unpackaged under the same cold storage conditions (temperature 5.7±0.3°C and relative humidity 91.95±3.7%). In Figure 3, it can be seen that the maximum values of the mass loss per treatment (last day of the experiment) ranged between 6.5-10.6%; in the control samples, the maximum mass loss was 10.6% while in the other treatments, the maximum values were O_3 (5 mg/mL) = 8.2%, UV (30 s) = 8.5%, UV (30 s) + O_3 (5 mg/L) = 7.6%, UV (60 s) = 10.5%, UV (120 s) = 9.7%, UV (60 s) + O_3 (5 mg/L) = 8.2%, UV (120 s)+O_3 (5 mg/L) = 6.5%. The mean values (mean±SD) of the eight tested treatments were calculated as control = 5.78±3.1%, O_3 (5 mg/mL) = 4.01±2.9%, UV (30 s) = 4.25±3.0%, UV (30 s)+O_3 (5 mg/L) = 3.50±2.6%, UV (60 s) = 4.66±3.6%, UV (120 s) = 4.49±3.3%, UV (60 s) + O_3 (5 mg/L) = 3.90±2.8%, UV (120 s) + O_3 (5 mg/L) = 3.23±2.3%. Most vegetables become unmarketable when mass loss ranges between 5-

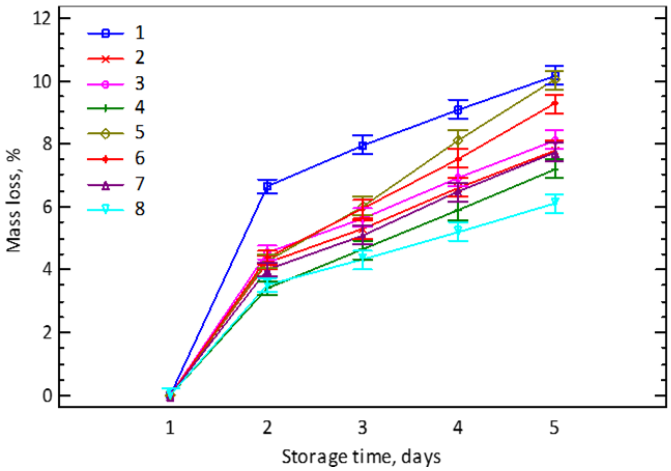

Figure 3. Mass loss of fresh-cut lettuce with storage time. Treatments: 1 = control, 2 = O_3 (5 mg/mL), 3 = UV (30 s), 4 = UV (30 s) + O_3 (5 mg/L), 5 = UV (60 s), 6 = UV (120 s), 7 = UV (60 s) + O_3 (5 mg/L), 8 = UV (120 s) + O_3 (5 mg/L). Mean error bars are the standard deviation of mean values. Each point is the average of n = 9 samples.

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Table 1. Analysis of variance of mass loss (%) and respiration rate (mL_{CO2}/100 g h) in respect to the experimental factors of storage time and treatments.

Source	df	Mass loss		Respiration Rate	
		F-ratio	P-value	F-ratio	P-value
Main effects					
A: storage time (days)	4	2688.8	≤ 0.001*	116.1	≤ 0.001*
B: treatment	7	114.57	≤ 0.001*	42.18	≤ 0.001*
Interactions					
A×B	28	13.4	≤ 0.001*	3.25	≤ 0.001*

*significant at P ≤ 0.05, df: degree of freedom.
All F-ratios are based on the residual mean square error.

10% of their initial weight (Robinson *et al.*, 1975). It is interesting that the treatments regarding the combined UV and O_3 applications exhibited the least maximum mass losses (7.47-9.71%) compared to the rest of the tested treatments which exhibited higher mass losses ranging between 10.55-11.68% although the same storage conditions were applied.

Allende and Artes (2003) conducting UV-C radiation experiments with *Lollo Rosso* lettuce at different fluences (0.4, 0.81, 2.44, 4.07 and 8.14 kJ/m^2) observed an increase in lettuce crispness which was attributed to possible lignification-like processes induced by the lettuce tissue to protect itself against the UV-C stress. This process was also observed in this study and will be discussed in the *Texture Analysis* section. Dean (2018) reported that lignin (recalcitrant polymer of phenylpropanoid) units are linked in a complex and irregular network which varies from species to species, tissue to tissue, and cell to cell, utilizing this network, to line their conductive vascular tissues as a barrier to water loss. Therefore, it is possible that the tested combined UV and O_3 applications, UV (30 s) + O_3(5 mg/L), UV (60 s) + O_3(5 mg/L), UV (120 s) + O_3(5 mg/L) induced a lignification-like process as it is discussed in the *Texture Analysis* section, which in turn reduced the mass loss compared to the other treatments. The previous discussion is based on a theoretical analysis and further experimental investigation is needed regarding the lignin quantification per treatment. Dean (2018) explained the difficulties in lignin calculation reporting that there are no absolute methods for lignin quantification and concluded that lignin should be measured using two or more assays based on independent physical or chemical properties; the results should be regarded as relative values which may or may not be appropriate for comparison with those obtained from other tissues using other techniques. The previous uncertainty in lignin quantification is attributed to three fundamental factors. First, the total lignin in any given plant is a heterogeneous collection of polymers with different structures and chemical properties. Second, the vast array of interfering compounds contains aromatic or phenolic constituents, produced by plants. Removal of these contaminants causes some lignin removal, but when left in place, the contaminants lead to overestimates of lignin content. Third, any manipulation of the plant material, from milling to water extraction, will alter or remove lignin, and the amount removed, will be dependent on the structure and composition of the lignin in that particular tissue. Since these changes are also dependent on the duration of treatment and the environment (solvent, temperature, oxygen levels) in which it is performed, they can add significant variability to subsequent analyses (Dean, 2018).

The analysis of variance in Table 1 showed that the interaction of storage time with the eight treatments affected significantly the respiration rates (P ≤ 0.05). The mean respiration rates (mean±stdev) are within the range reported by Saltveit (1997) for lettuce such as 12-17.5 mL_{CO2}/kg h. In particular, control = 17.4±6.2 mL_{CO2}/kg h, O_3(5 mg/mL) = 20.2±7.1 mL_{CO2}/kg h, UV (30 s) = 14.2±4.8 mL_{CO2}/kg h, UV (30 s) + O_3(5 mg/L) = 19.1±8.7 mL_{CO2}/kg h, UV (60 s) = 19.6±4.4 mL_{CO2}/kg h, UV (120 s) = 29.5±7.4 mL_{CO2}/kg h, UV (60 s) + O_3(5 mg/L) = 22.2±8.7 mL_{CO2}/kg h, UV (120 s) + O_3(5 mg/L) = 15.2±8.4 mL_{CO2}/kg h. Allende and Artés (2003) evaluating the respiration rate of fresh processed "*Lollo Rosso*" lettuce treated by UV-C radiation, reported an increase in the respiration rate from 21.1 mL_{CO2}/kg h for control samples to 31.8 mL_{CO2}/kg h in samples treated by 2.44 kJ/m^2 UV-C fluence, to 38.9 mL_{CO2}/kg h in samples treated by 4.06 kJ/m^2 UV-C fluence and to 41.8 mL_{CO2}/kgh in samples treated by 8.14 kJ/m^2 UV-C fluence. In this study, the calculated respiration rates were below those of Allende and Artes (2003) since the highest used UV-C fluence was 0.80 kJ/m^2. In particular, the selected O_3 dose and UV fluences resulted in mean respiration rates that were close to the control value of 17.4 mL_{CO2}/kg h and ranging between 14.2-22.2 mL_{CO2}/kg h having only one value significantly higher that the other values corresponding to the respiration rate of UV(120 s)- 0.80 kJ/m^2 treatment which had mean value of 29.5 mL_{CO2}/kg h. Based on the latter findings and Allende and Artes (2003) reported values, can be concluded that there is a

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limit in UV-C fluence application, beyond which, the respiration rate increases significantly, resulting in a lowering of the fresh product's' storability.

3.2 Texture analysis (positive peaks, F_{max}, work)

Figure 2 presents a typical force-distance curve of fresh-cut lettuce in which the combined processes of compression and shearing are triggered by the 5-bladed *Kramer* shear-cell. Pressing the five blades on lettuce samples causes compression of the microstructures as the force load of the texture analyser increases, and then the 5-blades shears through the samples before passing through the slotted plate. This test causes lettuce to shredding into small pieces in a fragmentation degree similar to what occurs during the chewing process. The ability of solid foods to resist compression and shearing was expressed by the maximum force (FKMF), the absorbed energy or work (FKW) as the red highlighted area under the curve (Figure 2) and the positive peaks (FKPP) as red arrows, seen in the same force-distance curve as Nath and Chattopadhyay (2007) suggested in their study.

The analysis of variance of the three textural properties (FKMF, FKW and FKPP) regarding the storage time (days) and the eight treatments is presented in Table 2 where the maximum force was significantly affected by the storage time and the employed treatments but not by their interaction; work was significantly affected by the interaction of the storage time (days) and the eight treatments (storage time × treatments) and finally, the positive peaks were significantly affected by the interaction of the storage time (days) and the eight treatments (storage time × treatments).

Allende and Artes (2003) conducting UV-C experiments with *Lollo Rosso* lettuce at different fluences (0.4, 0.81, 2.44, 4.07 and 8.14 kJ/m^2) reported an increase in lettuce crispness which was attributed to possible lignification-like processes induced by the lettuce tissue to protect itself against the UV-C stress. Allende *et al.* (2006) treated fresh-cut "*Red Oak leaf*" lettuce with three UV-C fluences of 2.36, 4.74, and 14.22 kJ/m^2 and reported limited, or no effects, on sugar or organic acid content of treated lettuce. The highest fluence (14.22 kJ/m^2) induced texture loss and browning after 7 days of storage at 5 ^{o}C while UV-C fluence below 4.74 kJ/m^2 caused no damage to the lettuce tissue.

From the statistical analysis seen in Table 2 is concluded that the applied treatments significantly affected the three texture properties (FKMF, FKW and FKPP) but to a variable degree as can be seen in Figure 4. Interestingly, the positive peaks regarding the O_3 application, UV-C application [UV (30 s), UV (60 s), UV (120 s)] and combinations of UV-C+O_3 [UV (30 s) + O_3 (5 mg/L), UV (60 s) + O_3 (5 mg/L), UV (120 s) + O_3 (5 mg/L)] increased at the end of the unpackaged cold storage (t = 5th day) in a range from 35% up to 112% of their initial (t = 1st day) FKPP number. The aforementioned response seen in Figure 4 is systematic in the treatments of the UV-C+O_3 combinations. In particular, the FKPP and FKW presented for the treatments UV (30 s) + O_3 (5 mg/L), UV (60 s) + O_3 (5 mg/L) and UV (120 s) + O_3 (5 mg/L) respectively 50.6%, 43.5%, 35.6% and 12.7%, 9.0%, 1.9% increase between the initial (t = 1st day) and the final day (t = 5th day). This textural change showed that the mass loss in terms of water loss during cold storage, although it was carried out under the same conditions of air temperature and relative humidity, exhibited different responses regarding the different tested treatments. This fact can be interpreted from the different effects that treatments had on the fresh-cut lettuce and in particular the combination of UV-C and O_3 on the final product. This point deserves further investigation in terms of electron microscopy analysis to identify possible lignin accumulation as was previously discussed that could affect the crispness of the fresh-cut lettuce samples. Glowacz *et al.* (2015) concluded that the use of O_3 as a disinfectant is a simple and feasible solution in the fresh produce industry, reducing microbial contamination, but it is also essential to establish appropriate concentrations, to balance between the beneficial reduction of microbial contamination and quality safety, in terms of weight loss, texture, visual quality and nutrient content. Thus, based

Table 2. Analysis of variance of FKMF(N), FKW(J) and FKPP in respect to the experimental factors of storage time (days) and treatments.

Source	df	FKMF		FKW			
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Main effects							
A: storage time (days)	1	4.30	0.0464*	1.54	0.2230^{NS}	14.75	0.0005*
B: treatment	7	16.51	≤ 0.001*	11.70	≤ 0.001*	3.33	0.0089*
Interactions							
A×B	7	1.24	0.3106^{NS}	2.81	0.0212*	2.80	0.0216*

*significant at P ≤ 0.05, ^{NS}not significant, df: degree of freedom.
All F-ratios are based on the residual mean square error.

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on the previous discussion and Figure 4, can be concluded that overall, the crispness of fresh-cut lettuce in terms of FKPP was improved compared to control samples in which the FKPP value was reduced by 33.3% due to significant mass loss (Figure 3). This response agrees with other published studies (Robinson *et al.*, 1975; Miller *et al.*, 2013; Glowacz *et al.*, 2015; Sarron *et al.*, 2021) where proper UV-C fluence and O_3 concentrations combined with proper application times, were found to reduce mass loss and improve texture quality. In the present study, this response was found to be more pronounced in the combined UV-C and O_3 treatments.

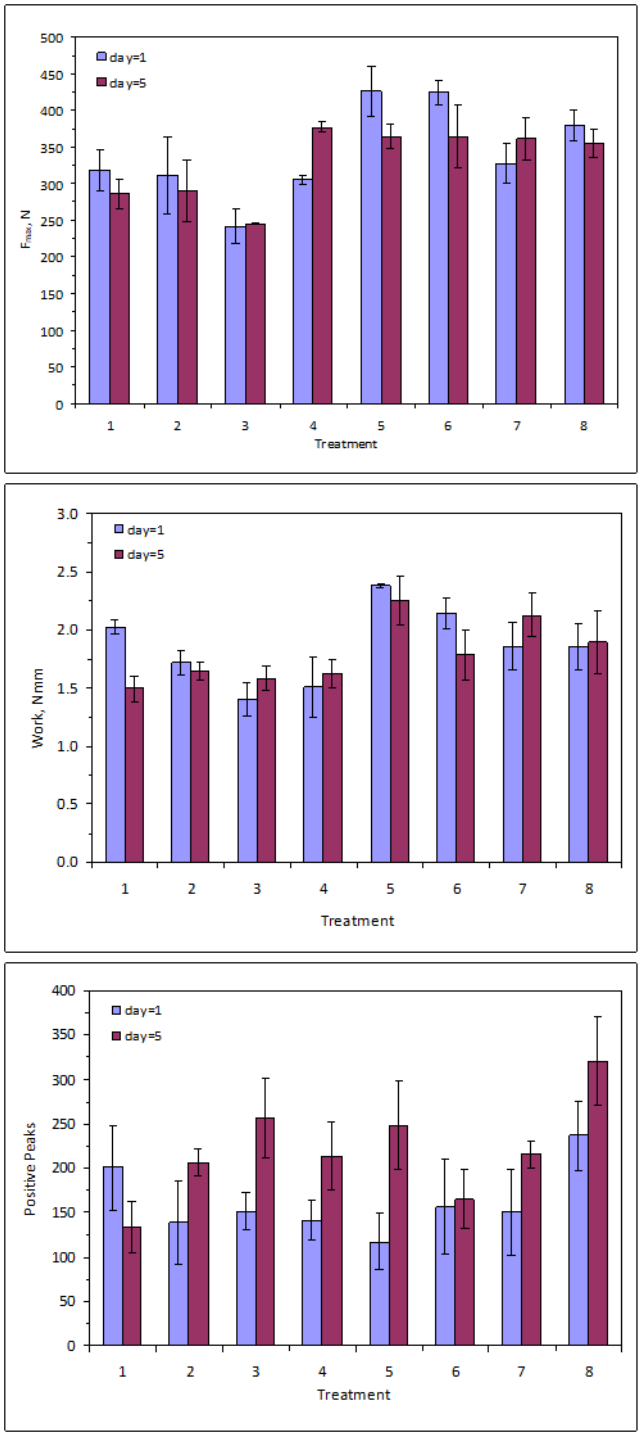

Figure 4. FKMF, FKW and FKPP values of the treatments [1 = control, 2 = O_3 (5 mg/mL), 3 = UV (30 s), 4 = UV (30 s) + O_3 (5 mg/L), 5 = UV (60 s), 6 = UV (120 s), 7 = UV (60 s) + O_3 (5 mg/L), 8 = UV (120 s) + O_3 (5 mg/L)] for the initial and the final day of storage. Each point is the average of n = 9 samples. Mean error bars are the standard deviation of mean values.

3.3 Colour analysis

The analysis of variance of the colour indices (L*, a*, b*, a*/b*, C*, h* and ΔE*) regarding the storage time (days) and the eight treatments is presented in Table 3. From this table, it is seen that the L* index was significantly affected by the storage time and the tested treatments but not by their interaction (storage time × treatments). The a* index was significantly affected by the storage time and the tested treatments as well as by their interaction (storage time × treatments). The b* index was significantly affected only by the treatments. The a*/b* index was significantly affected by the storage time and the treatments as well as by their interaction (storage time × treatments). The C* was significantly affected by the treatments as well as by their interaction (storage time × treatments). The h* was significantly affected only by the interaction (storage time × treatments) of the storage time and the treatments and finally the ΔE* index was significantly affected only by the storage time. During the cold storage of unpackaged fresh-cut lettuce, the degradation of the leaves' green colour takes place, which is caused by chlorophyll loss, a process that is preceded of green colour loss (Xanthopoulos *et al.*, 2016). This colour degradation is expressed as the turning of the cut leaves' green colour to yellow. In this context, the main colour indices that have been proven to be more representative of this colour turning (from greenish to yellowish) are Chroma and hue angle. Pathare *et al.* (2013) suggested that for colour assessment can be employed the Chroma (C*) property as a quantitative attribute and the hue angle (h*) as a qualitative attribute. This approach was adopted in this study since the conducted colour analysis was focused on the qualitative colour changes of the fresh-cut lettuce during its unpackaged cold storage.

Table 4 presents the mean values of the colour indices (L*, a*, b*, a*/b*, C*, h* and ΔE*) along with the standard deviation and the *Fisher's least significant differences* (LSD). The mean L* values regarding the UV-C treatments ranged between 41.07±8.9 up to 44.35±7.3 and the respective values for the combined UV-C+O_3 treatments were between 41.26±8.5 and 42.25±9.7. In all the tested treatments the mean L* values were significantly increased compared to the control samples (in a range of 7.4-16.0%) where the mean L* values were 38.24±7.7 (Table 4) revealing that treated lettuce samples lost part of their dark green colour. The small variations in the hue angle (Table 4)

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Table 3. Analysis of variance of L*, a*, b*, a*/b*, C*, h* and ΔE* in respect to the experimental factors of storage time and treatments.

Source	df	*L**		*a**											
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Main effects															
A: storage time (days)	4	2.55	0.038*	6.28	0.001*	1.01	0.3994^{NS}	9.93	≤ 0.001*	0.89	0.4722^{NS}	6.17	0.0001*	7.67	≤0.001*
B: treatment	7	5.76	≤ 0.001*	5.30	≤ 0.001*	11.90	≤ 0.001*	5.66	≤ 0.001*	11.12	≤ 0.001*	3.32	0.0017*	1.17	0.3181^{NS}
Interactions															
A×B	28	0.85	0.6959^{NS}	2.02	0.0013*	1.39	0.0844^{NS}	1.59	0.0027*	1.50	0.0453*	1.78	0.2774^{NS}	0.72	0.7634^{NS}

*significant at P ≤ 0.05, ^{NS}not significant, df: degree of freedom.
All F-ratios are based on the residual mean square error.

Table 4. Colour parameters and functions of fresh-cut lettuce subjected to the tested treatments.

Treatments	Colour parameters						
	*L**	*a**	*b**	*a*/b**	*Chroma (C*)*	*hue (h*)*	*ΔE**
Control	38.24±7.7^{a}	-8.54±1.9^{b}	24.32±5.1^{ab}	-0.35±0.08^{b}	25.76±5.3^{ab}	178.78±0.23^{ab}	6.31±5.7^{ab}
O_3 (5 mg/L)	41.76±8.1^{b}	-8.96±1.9^{ab}	26.65±4.8^{de}	-0.34±0.08^{b}	28.14±4.9^{de}	178.75±0.06^{b}	5.64±4.6^{ab}
UV (30 s)	42.94±9.3^{bc}	-8.05±2.4^{c}	25.27±6.7^{bc}	-0.32±0.11^{d}	26.47±7.0^{abc}	178.73±0.23^{a}	7.59±5.7^{b}
UV (30 s) + O_3 (5 mg/L)	41.93±8.1^{b}	-8.61±1.7^{b}	25.15±4.7^{bc}	-0.34±0.05^{ab}	26.62±4.8^{bc}	178.76±0.05^{b}	6.34±5.4^{ab}
UV (60 s)	41.07±8.9^{b}	-8.71±1.8^{b}	23.80±4.3^{a}	-0.37±0.05^{a}	25.37±4.6^{a}	178.80±0.2^{b}	5.20±4.4^{a}
UV (120 s)	44.35±7.3^{c}	-9.32±1.7^{a}	28.02±4.3^{f}	-0.33±0.05^{bc}	29.56±4.4^{f}	178.75±0.05^{b}	5.20±4.5^{a}
UV (60 s) + O_3 (5 mg/L)	42.25±9.7^{b}	-8.99±1.9^{ab}	25.67±4.7^{cd}	-0.35±0.06^{ab}	27.24±4.8^{cd}	178.77±0.05^{b}	5.73±4.5^{ab}
UV (120 s) + O_3 (5 mg/L)	41.26±8.5^{b}	-8.86±2.2^{ab}	27.53±5.3^{ef}	-0.32±0.06^{c}	28.96±5.6^{ef}	178.74±0.05^{b}	5.95±4.4^{ab}
± LSD	±1.01	±0.24	±0.60	±0.01	±0.63	±0.02	±1.03

Values are presented as mean±SD (n = 3 points × 3 samples × 3 replicates × 5 storage days = 135 sampling points) and represent the colour parameters throughout the storage: UV-C fluence: 0.41-1.65 kJ/m2, O3 concentration 5 mg/L and their combinations. Values with different superscripts within the same column are statistically significantly different (P ≤ 0.05), Fisher's Least Significant Difference test.

were mainly due to the short storage period (5 days in total) since the aim of this study was to investigate the effect of the specific treatments on the quality of fresh-cut lettuce stored unpackaged in a walk-in cold room rather the well-known behaviour during the prolonged cold storage of green vegetables (Xanthopoulos *et al*., 2016). The statistical analysis concluded that the hue angle of the control samples was significantly different from the other treatments (Table 4) apart from the UV (30 s) treatment which was the lowest used UV fluence (0.20 kJ/m^2). Also, the a*/b* index gave interesting results since in all the treatments, the estimated ratios were negative, suggesting that yellowing of the samples took place since -a* corresponds to greenish colour and b* to yellowish. The Chroma values in Table 4 showed no systematic responses. This behaviour can be attributed to low O_3 doses or/and UV fluences used in the experiments.

The hue angle was further analysed, fitting the calculated h* values (3 points×3 samples×3 replicates = 27 sampling points per storage day), during the 5 days storage period to the 3-parameter *Weibull* distribution and then the derived temporal *shape* factor (β) was associated with the colour degradation during the cold storage of fresh-cut lettuce. In all the tested treatments regarding the UV-C and the combined UV-C and O_3 application, the goodness-of-fit tests for h* values exhibited that the smallest P-value amongst the performed tests was ≤ 0.05, therefore could not be rejected the assumption that h* value comes from a *Weibull* distribution with 95% confidence. In Figure 5 are presented the histograms of h* value, regarding the UV (30 s) + O_3 (5 mg/L) treatment, fitted to the *Weibull* distribution (Equation 1) at the beginning (t = 1st day) and at the end (t = 5th day) of the cold storage. Similar hue angle histograms have been developed for the other tested treatments.

In Figures 6 and 7 are presented the temporal variation of the *shape factor* (β) for the treatments where UV-C was used as a standalone and in combination with O_3. In all the treatments, the *shape factor* was greater than one (β >> 1) indicating that the colour degradation rate increased with time. In general, the higher the β values, the faster the colour degradation, a fact which is more pronounced in the combined UV-C and O_3 treatments. Further analysis of Figures 6 and 7 revealed that the *shape factor* increased between the 2nd and 3rd day of the cold storage, a response that is more obvious in the UV-C treatments and to a lesser degree in the combined UV-C and O_3 treatments. The *shape factor* increase, towards the 3rd day of storage, is driven by the ongoing colour degradation of stored fresh-cut lettuce. This specific response was more pronounced in the UV (60 s)+O_3 treatment where the β value was two to three orders of magnitude greater than the other treatments, a fact that deserves further investigation. Beyond the 3rd day of cold storage, the β value was decreased probably due to the deceleration of colour degradation. On the last day (t = 5th day) in the combined UV-C and O_3 treatments, the β value was increased again, probably due to the physiological degradation which was favoured by the combined effect of UV-C and O_3 treatment.

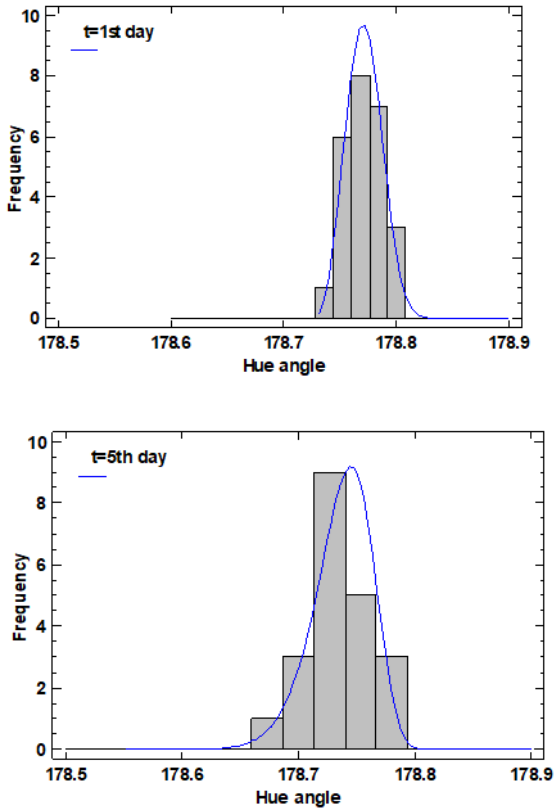

Figure 5. Histograms of estimated h* values regarding UV (120 s) + O_3 (5 mg/L) treatment of fresh-cut lettuce on the 1st day and 5th day of the cold storage. Each histogram contains n = 3 points × 3 samples × 3 replicates = 27 sampling points.

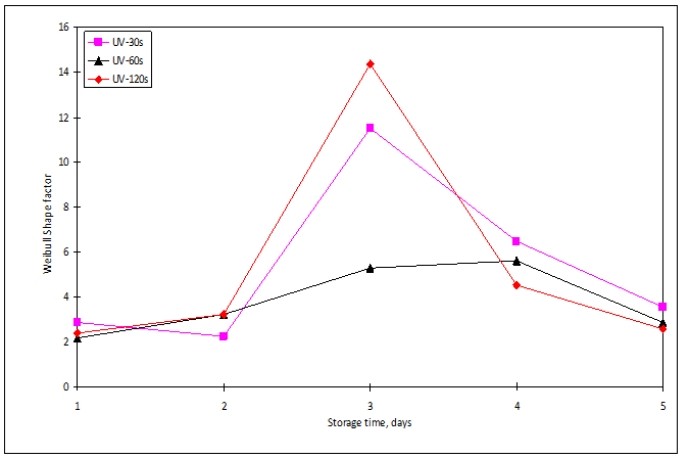

Figure 6. Temporal variation of the *Weibull shape factor (β)* regarding the hue angle of fresh-cut lettuce subjected in UV (30 s), UV (60 s) and UV (120 s) treatments. Each point (β) has been derived by fitting all sampling points in the *Weibull* distribution (3 points × 3 samples × 3 replicates = 27 sampling points per storage day).

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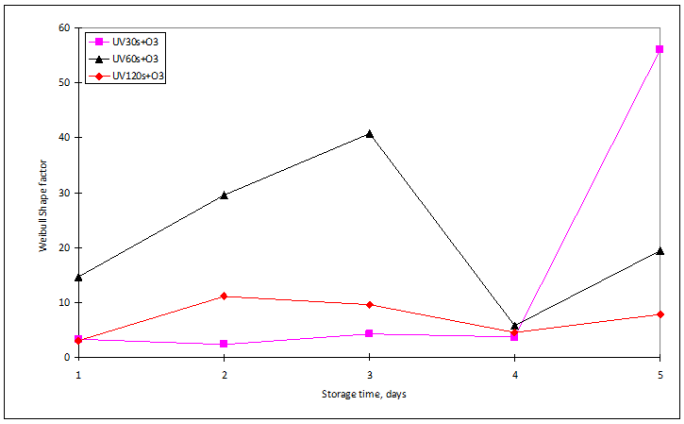

Figure 7. Temporal variation of the *Weibull shape factor (β)* regarding the hue angle of fresh-cut lettuce subjected in UV (30 s) + O_3, UV (60 s) + O_3, UV (120 s) + O_3 treatments. Each point (β) has been derived by fitting all sampling points in the *Weibull* distribution (3 points × 3 samples × 3 replicates = 27 sampling points per storage day).

Bermúdez-Aguirre and Barbosa-Cánovas (2013) reported that some changes in the produce's colour can be controlled if the exposure time and/or concentration of the disinfection agent are kept as low as possible to inactivate the microorganism without compromising its overall quality. In this point, Bermúdez-Aguirre and Barbosa-Cánovas (2013) and Birmpa *et al.* (2013) conducted UV-C irradiation and O_3 experiments with fresh-cut lettuce employing variable UV-C fluences (up to 60 mins) and O_3 concentration of 5 mg/L up to 15 mins, reported that the ΔE*and C* ranged between 4.86-13.45 and 18.23-22.81 respectively. In the present work, the values of ΔE* and C* in Table 4, had a similar range which was 5.20-7.59 and 25.37-29.56 respectively. Bermudez-Aguirre and Barbosa-Cánovas (2013) and Birmpa *et al.* (2013) pointed out that higher UV-C fluence and O_3 concentration and dosages are maybe more effective in disinfection but cause significant visual degradation. Ölmez and Akbas (2009) treating lettuce with O_3 concentration > 2.5 mg/L observed a decrease in the overall visual quality, however, when the samples were treated with 4 mg/L and stored for 5 days, they discovered that some leafy parts developed a translucent appearance. Ölmez and Akbas (2009) attributed this response to the high oxidant power of O_3 on lettuce tissue which initiates the enzymatic activity of phenylalanine ammonia lyase (PAL). Similar findings were noted when Iceberg lettuce was treated with O_3 between 3 to 10 mg/L (Koseki and Isobe, 2006). Thus, the aforementioned lightness and whiteness index increase was expected in lettuce leaves which become translucent later on, since it had been treated with a concentration of gaseous O_3 5 mg/L for 15 mins according to Bermudez-Aguirre and Barbosa-Cánovas (2013). The same response was noted in the present study regarding the L* values as was previously analysed. The latter authors reported that the applied UV-C treatment for less than 45 mins, did not significantly affect the general appearance. Cordero *et al.* (2019) reported that UV-C treatment of fresh-cut Iceberg lettuce at fluences higher than 0.5 kJ/m^2 (60-300 s) initiated oxidative discolouration and increased respiration rate due to physiological stress in plant tissues. Esua *et al.* (2020) reviewing studies concerning UV-C treatments by low fluence 0.00176-1.0 kJ/m^2 and their effect on the quality of fruits and vegetables concluded that the UV-C fluence affects in variable degrees the bioactive compound production, enzymatic activity, products shelf-life, and of course the degradative action on the biological processes regulating proteins, responsible for the deteriorative changes taking place during postharvest life.

4. Conclusion

The present study showed that the applications of O_3, UV-C and their combinations gave good results in terms of quality characteristics employing UV-C fluence ≤ 0.80 kJ/m^2 and O_3 concentration (5 mg/L for 5 mins). In general, individual or combined treatments presented less mass loss throughout the 5 days of cold storage compared to control (untreated) samples. In particular, the combined treatment of UV-C with O_3 exhibited more than 25% less mass loss (maximum values) compared to the other treatments including the control samples. The respiration rate increase was initiated by the individual or combined treatments. The tested UV-C fluence ≤ 0.80 kJ/m^2 and O_3 concentration (5 mg/L for 5 mins) gave good texture quality in terms of the positive peaks (crispness) and energy (work) consumption. The crispness of the treated samples was found to increase by > 35% compared to control samples, especially in the combined UV-C and O_3 treatment. Limited colour degradation was noted since the UV-C fluence and O_3 concentration and dosage were kept lower than the limits (for UV-C < 5 kJ/m^2; O_3 < 5 mg/L) published in literature beyond which, has been reported that significant colour degradation is observed. It would be very interesting to further evaluate and analyse the effect of the applied treatments on the texture of green vegetables used as ingredients in ready-to-eat salads, in terms of lignin accumulation since this affect positively not only the final product's texture but also reduces the resulted mass loss.

Conflicts of interest

The authors declare no conflict of interest.

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