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Application of ozone in fresh-cut iceberg lettuce refrigeration

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Abstract: Recently, technological innovations have been geared to supporting environmental sustainability, also in the fruit and vegetable industry. The application of ozone in the cold storage of fruits and vegetables is a sustainable technology used to improve product quality and its antimicrobial effect, simple use, and characteristic of not leaving any residue, makes this treatment suitable for many applications in this field. The aim of this work was to evaluate the effect of refrigeration at 4°C, associated with ozonization treatment at a concentration of 0.2 ppm on the shelf life of fresh-cut lettuce, compared to a lettuce control stored only at 4°C. Lettuce quality throughout the storage period (7 days) was determined by means of color and microbiological indexes, such as total bacterial count, total coliforms, *Escherichia coli, Pseudomonadaceae,* yeasts and molds. The lettuce used in the experiment was found to have a low microbiological load. Microbiological results obtained at different storage times have shown that the use of ozone is effective in containing microbial growth during chilling storage of the raw material compared to the refrigerated control. In particular, the positive effects of ozonation were appreciable after the third day of storage. Furthermore, the ozone treatment did not affect the color of the product.

1. Introduction

It is well known that disinfection represents one of the most critical processing steps influencing quality, safety and shelf-life of fresh-cut fruits and vegetables. In the fresh-cut industry, chlorine is generally used as a product sanitizer; however, there is a trend in eliminating this substance due to the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (Gil *et al*., 2009).

Ozonation represents a sustainable technology able to improve the quality of the product; ozone is useful in diminishing the microbial load and the level of toxic organic compounds. Furthermore, this gas has a stronger and more rapid antimicrobial action against spores, faecal and pathogenic microorganisms and viruses with respect to chlorine (Artés and Allende, 2005). The antimicrobial effect of ozone and its relatively simple application, in addition to its characteristic of not leaving any residue or forming carcinogenic trihalomethanes (such as chlorination), make this treatment suitable for various applications in the food industry (Gil *et al*., 2009; Alexopoulos *et al*., 2013). Moreover, products treated with ozone do not modify their sensory characteristics. After it gained GRAS (Generally Recognized As Safe) status in 1997, the use of this gas has been approved as a disinfectant or sanitizer in foods and food processing in Europe and in the US (Ölmez and Kretzschmar, 2009).

The primary systems for ozone application include gaseous phase storage and ozonated dips (Artés *et al*., 2009). When ozone is used as a gas, the exposure time is longer (1-4 h) than ozone dissolved in water (1-10 min) (Carletti *et al*., 2013).

In recent years the use of this gas as a pretreatment to preserve fruit and vegetable quality has received great attention for its antimicrobial activity against bacteria, fungi, viruses, and bacterial and fungal spores. Several studies have focused on the effect of ozone treatment on the safety and quality of iceberg lettuce (Rico *et al*., 2006; Yuk *et al*., 2006; Hassenberg *et al*., 2007). In addition, various applications have been proposed concerned the sanitizing effect of ozone dissolved in water used for washing on the quality and safety of minimally processed fruits and vegetables (Seydim *et al*., 2004; Zhang *et al*., 2004; Selma *et al*., 2008; Artes *et al*., 2009; Ölmez and Kretzschmar, 2009), but there is scant literature about ozone applied to fresh-cut iceberg lettuce (Beltran *et al*., 2005; Ölmez and Akbas, 2009).

The aim of this work was to evaluate the effect of refrigeration at 4°C, associated with ozonization treatment

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at a concentration of 0.2 ppm on the shelf life of fresh-cut lettuce, compared to a lettuce control stored only for 7 days at 4°C.

2. Materials and Methods

Sampling and ozone treatment

Iceberg lettuce (*Lactuca sativa L.*) was obtained from Società Agricola "Rago" (Battipaglia, SA, Italy) and, immediately after harvesting, it was stored at 4°C and submitted to a continuous ozone exposure of 0.2 ppm. A control sample was stored at 4°C without ozone treatment. Ozone gas was generated using a corona discharge ozone generator (Model OXY MET TWIN, MET, San Lazzaro di Savena, BO, Italy) from purified oxygen gas. Functioning of this equipment takes place through sensors placed inside the storage compartment/chamber that reveal the exact concentration present in the refrigeration cell and it is reported on the display of the instrument. The tests lasted up to 7 days and the ozonated and non-ozonated product was sampled at 1, 2, 3, 5 and 7 days for analyses.

Colorimetric analysis

Color was measured on the lettuce surface using a Minolta Chroma meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan), with a D 65 illuminant, using CIELAB L*, a*, b* values. The data reported are the average of a set of four determinations carried out on the lower and upper surface of two leaves of lettuce.

Microbiological analysis

At each treatment time, 9 g of ozonated or control product were placed in a stomacher bag containing 81 ml of Tryptone Water (Liofilchem, Teramo, Italia) and homogenized for 2 min. Samples were then serially diluted in tripton water and surface plated in duplicate on Compact-Dry EC (PBI International, Milano, Italy) for the recovery of surviving total coliforms and *Escherichia coli*, on Compact-Dry YM (PBI International, Milano, Italy) for yeasts and moulds and on Cetrimide Agar (Liofilchem, Teramo, Italy) for the enumeration of *Pseudomonas aeruginosa* and *Pseudomonas* spp. Total mesophilic bacteria were enumerated on Plate Count Agar (Liofilchem, Teramo, Italy) using the pour plate technique. All the inoculated media were incubated at 30°C for 48 h.

Statistical analysis

In order to study the effect of ozone treatment and storage time on fresh-cut iceberg lettuce quality characteristics, data were processed by two-way analysis of variance (ANOVA). Moreover, a Student test was performed to compare the mean values between the samples treated with ozone and control samples for each time of storage. All statistical procedures were computed using the statistical package SYSTAT for Windows (ver. 10, 2003) (Systat Software, Chicago, USA).

3. Results and Discussion

Ozone can be used to disinfect fruit and vegetables by dissolving it in the water used for washing (Gil *et al.*, 2009), or by keeping a constant ozone concentration, through the use of an appropriate generator, during storage of the product in a cooling room at 4°C.

In this paper we evaluated the effect of cold storage at 4°C, accompanied by ozonization at a concentration of 0.2 ppm on the shelf life of the fresh-cut lettuce, compared to a control subjected only to cold storage. Preliminary tests showed that the use of ozone at low concentrations, such as 0.2 ppm, did not determine sensory alterations; for this reason, the effect of treatment on both perceptible sensory parameters, such as color stability, and microbiological parameters, for safety of the product, has been evaluated. A two-way analysis (time, treatment) showed a significant effect of both factors on the colorimetric parameters ($p= 0.000$). In particular, total bacterial count and coliforms increased throughout the storage period, being significantly lower for ozonated samples than control samples. Therefore, the microbiological results obtained at different storage times show that the use of ozone is effective in containing microbial growth during storage in a cooling room of raw material compared to the untreated control. In particular, the positive effects of ozonation were appreciable after the third day of storage, as shown by the trend of the total bacterial count and total coliforms of the chilled and ozonized product compared to the refrigerated only product (Fig. 1 and 2). In particular, ozone was able to inhibit the proliferation of total coliforms, which were not detectable even after 7 days of storage in the ozonized samples, while in control samples these microorganisms reached 1.6×10^3 cfu/g at the same storage time. The growth of colonies on the plates inoculated for the detection of other microorganisms was sporadic and not significant for both the experimental theses considered. In the literature a reduction of 3-4 log units of *Escherichia coli* O157: H7 inoculated in lettuce and carrots, by sequential washes with aqueous solutions of Cl_2O/ozonated water, has been reported (Singh *et al.*, 2002). Various experiments carried out by treating vegetables with ozonated water have shown the ability of ozone to reduce the growth of different microorganisms, including pathogens, and also to reduce enzymatic activities, including enzymatic browning (Artes *et al.*, 2009). Alexopoulos *et al.* (2013) have highlighted the importance of keeping the concentration of ozone constant during treatment by continuous generation. In fact, the immersion of vegetables in water presaturated with ozone determined the reduction of 0.5 log of CBT after 15 min, whereas the immersion for the same time in continuously ozonated water involved the reduction of 2 log of CBT, showing in this latter case a greater effectiveness of ozonization with respect to chlorination. In fact, the reduction from 1.5 to 2.5 log units of the microflora of the lettuce was obtained with ozonated water with 1.5-3 ppm; this effect was comparable to the use of 100 ppm of chlorine

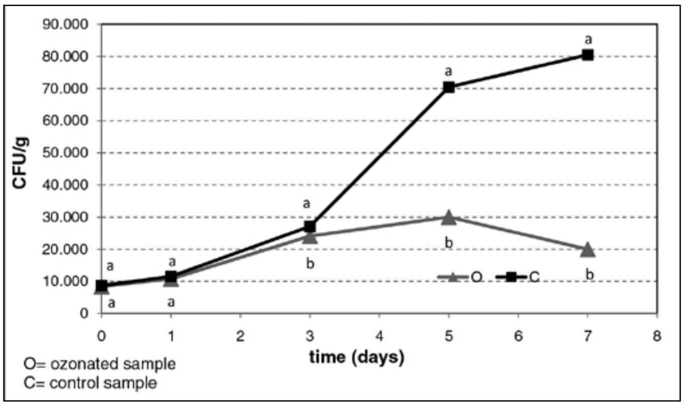

Fig. 1 - Evolution of total mesophilic bacteria during storage at 4°C. For each storage time, data followed by different letters are significantly different (t- Student test at p≤0.05).

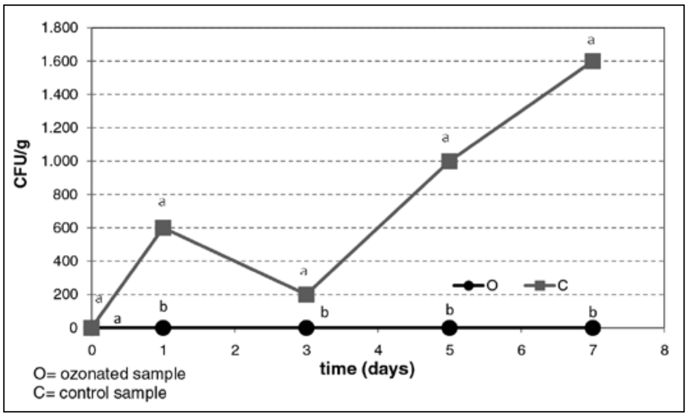

Fig. 2 - Evolution of total coliforms during storage at 4°C. For each storage time, data followed by different letters are significantly different (t- Student test at p≤0.05).

(Ölmez and Kretzschma, 2009).

Furthermore, ozone treatment did not adversely affect product color (Fig. 3 and 4), as also confirmed by two-way analysis of variance; in fact, a non significant effect of ozone treatment and storage time was recorded for the colorimetric indexes. It is well known that ozone is a strong oxidizing agent, which can induce damage to the structure and may influence the color of the products depending on the time of contact, the concentrations applied, and the type of product (Horvitz and Cantalejo, 2014; Olmez *et al.*, 2009). However, in the present study, at concentrations of 0.2 ppm of gaseous ozone on lettuce stored at 4°C, no difference was detected relative to colorimetric parameters L *, a *, and b * between the control and the product treated with ozone has (Fig. 5 and 6).

Fig. 3 - Lettuce not treated with ozone after 3 days of storage at 4°C.

Fig. 4 - Lettuce treated with ozone after 3 days of storage at 4°C.

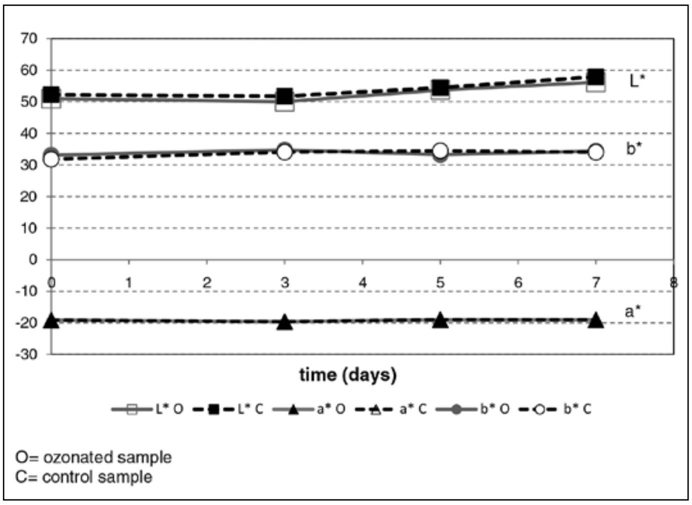

Fig. 5 - L*, a* and b* values determined on the upper leaf surface.

4. Conclusions

The microbiological results obtained at different storage times have shown that the use of ozone is effective in containing microbial growth during storage of the raw material compared to the untreated control. In particular, the positive effects of ozonation were appreciable after the third day of storage. Moreover, ozone treatment did not affect the color of the product. In order to better assess the treatment effect of ozonation on lettuce, studies considering material with a higher degree of microbial contamination, or inoculated with microorganisms that have not been detected even in the thesis used as a control in this experiment, should be undertaken.

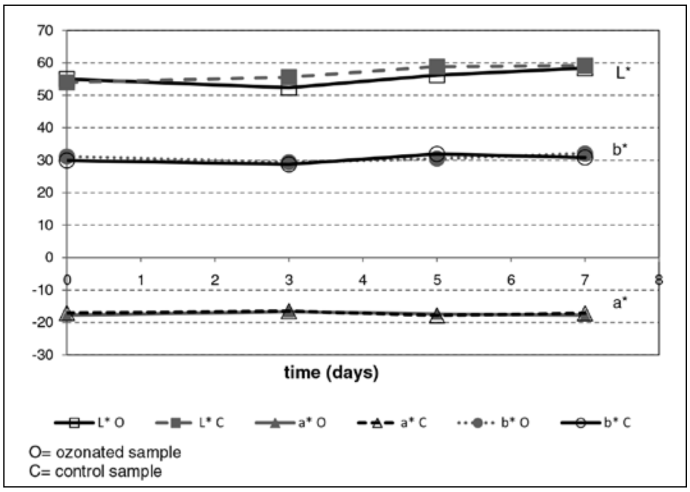

Fig. 6 - L*, a* and b* values determined on the lower leaf surface.

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References

ALEXOPOULOS A., PLESSAS S., CECIU S., LAZAR, V., MANTZOURANII., VOIDAROU C., STAVROPOULOU E., BEZIRTZOGLOU E, 2013 - *Evaluation of ozone efficacy on the reduction of microbial population of fresh-cut lettuce (*Lactuca sativa*) and green bell pepper (*Capsicum annuum*).* - Food Control, 30: 491-496.

ARTÉS F., ALLENDE A., 2005 - *Processing lines and alternative preservation techniques to prolong the shelf-life of minimally fresh processed leafy vegetables.* - Europ. J. Hort. Sci., 70: 1-15.

ARTÉS F., GÓMEZ P., AGUAYO E., ESCALONA V., ARTÉS-HERNÁNDEZ F., 2009 - *Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities.* - Postharv. Biol. Technol., 51: 287-296.

BELTRAN D., SELMA M.V., MARIN A., GIL M.I., 2005 - *Ozonated water extends the shelf-life of fresh-cut lettuce.* - J. Agric. Food Chem. 53(14): 5654-5663.

CARLETTI L., BOTONDI R., MOSCETTI R., STELLA E., MONARCA D., CECCHINI M., MASSANTINI R., 2013 - *Use of ozone in sanitation and storage of fresh fruits and vegetables.* - J. Food Agric. Environm., 11: 585-589.

GIL M.I., SELMA M.V., LÓPEZ-GÁLVEZ F., ALLENDE A. 2009 - *Fresh-cut product sanitation and wash disinfection: problems and solutions*. - Int. J. Food Microbiol., 134: 37-45.

HASSENBERG K., IDLER C., MOLLOY E., GEYER M., PLÖCHL M., BARNES J., 2007 - *Use of ozone in a lettuce-washing process: an industrial trial*. - J. Sci. Food Agric., 87: 914-919.

HORVITZ S., CANTALEJO M.J., 2014 - *Application of ozone for the postharvest treatment of fruits and vegetables.* - Crit. Rev. Food Sci. Technol., 54: 312-339.

ÖLMEZ H., AKBAS M.Y., 2009 - *Optimization of ozone treatment of fresh-cut green leaf lettuce.* - J. Food. Eng., 90(4): 487-494.

ÖLMEZ H., KRETZSCHMAR U., 2009 - *Potential alternative methods for organic fresh-cut industry for minimizing water consumption and environmental impact.* - LWT Food Sci. Technol., 42: 686-693.

RICO D., MARTIN-DIANA A.B., FRIAS J.M., HENEHAN G.T.M., BARRY-RYAN C., 2006 - *Effects of ozone and calcium lactate treatments on browning and texture properties of fresh-cut lettuce.* - J. Sci. Food Agric., 86: 2179-2188.

SELMA M., ALLENDE A., LÓPEZ-GÁLVEZ F., CONESA M.A., GIL M.I., 2008 - *Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetables industry*. - Food Microbiol., 25: 809-814.

SEYDIM Z.G., BEVER P.I., GREENE A.K., 2004 - *Efficacy of ozone to reduce bacterial populations in the presence of food components*. - Food Microbiol., 21: 475-479.

SINGH N., SINGH R.K., BHUNIA A.H., STROSHINE R.L., 2002- *Efficacy of chlorine dioxide, ozone, and thyme essential or a sequential washing in killing Escherichia coli O157:H7 on lettuce and baby carrots.* - Lebensm. Wiss. U. Technol., 35: 720-729.

YUK H.G., YOO M.Y., YOON J.W., MOON K.D., MARSHALL D.L., OH D.H., 2006 - *Effect of combined ozone and organic acid treatment for control of* Escherichia coli *O157: H7 and* Listeria monocytogenes *on lettuce.* - J. Food Sci., 71(3): 83-87.

ZHANG L., ZHAOXIN L., ZHIFANG Y., XIANG G., 2004 - *Preservation of fresh-cut celery by treatment of ozonated water*. - Food Control, 16: 279-283.