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Evaluation of the Impact of Nitrous oxide use on Quality and Shelf life of Packaged Fresh-cut 'Iceberg' Lettuce and Wild Rocket

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Shelf life of fresh-cut salad is limited by deterioration of quality characteristics during storage. The objective of this study was to assess the potential of the use of non conventional nitrous oxide gas in maintaining the quality of fresh-cut 'Iceberg' lettuce (*Lactuca sativa* L.) and Wild rocket (*Diplotaxis tenuifolia*) under modified atmosphere packaging (MAP) in comparison to nitrogen gas. Leaves of rocket and cut 'Iceberg' lettuce were washed, dried and packaged fresh in two different non-micro perforated plastic film bags (polypropylene +polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET). The headspace of each pair of two plastic films was filled and sealed with three gas mix treatment compositions: (97 % N_2O + 3 % O_2), (50 % N_2O + 47 % N_2 + 3 % O_2) and (97 % N_2 + 3 % O_2) for "Iceberg lettuce" and two gas mix treatments: (95% N_2O + 5 % O_2) and (95 % N_2 + 5 % O_2) for rocket leaves. Lettuce and rocket leaves were stored over a period of 9 days and 12 days respectively at 5 °C. Results showed that the two plastic films maintained the nitrous oxide concentration within the bags, with some slight differences in O_2 and CO_2 concentrations. No significant differences ($p<0.05$) were observed in texture, colour, and sensory quality (off-odor, off-flavor, texture and overall acceptability). All treatments delayed microbial growth (mesophiles, yeast and mould, psychrophiles and enterobacteria) and compared to control in air, reduced weight losses in both rocket and lettuce. MAP treatments maintained very good quality and freshness of 'Iceberg' lettuce for 4 days. Slight increase in off-odor and off-flavor observed during storage were below the moderately acceptable limit even after 9 days. As for rocket, although quality characteristics of the leaves were lessening over the storage period, treatment with nitrogen gas in PP+PA bags seemed to maintain them slightly better than treatment with nitrous oxide gas. The results suggested that nitrous oxide gas does not improve quality compared nitrogen modified atmosphere storage.

1. Introduction

Fresh cut fruits and vegetable industry has grown in the last years to meet the increasing consumer demand for fresh, convenient, nutritious and healthy foods. Cut "Iceberg" lettuce is among the most used fresh-cut product on the market; contributing to the total 80% lettuce required by consumers for over a decade now (Beltran et al., 2005). Though it is noted to have low vitamins, flavour and nutrients, it is characterised by high water content, fibre and crispy texture which makes it ideal for salads mixtures and side dishes. Rocket leaves have risen in importance among vegetables, spices and medicinal plants in many parts of Europe, the Middle East and the United States (Lamy et al., 2008). They are rich in vitamins, minerals, fibre and antioxidants. Rocket is typically sold refrigerated, in modified atmosphere packages or films to minimise microbial contamination, physical damage, wilting, loss of colour and dehydration; it has a usual shelf life of 8-12days at 0 °C and 100 % relative humidity (Char et al. 2012) which is below commercial storage temperature of ~5 °C. As for cut lettuce, the shelf life is usually compromised by microbial contamination, colour changes and development of off-flavours and odours. Hole (2003) stated that the major factors affecting shelf life are temperature, moisture content and packaging. Well designed modified atmosphere packaging (MAP) at low temperature, with low O_2 and high CO_2 has been used to reduce respiration and hence moisture content and to extend storage life of cut vegetables. Advances in this technology has led to the use super atmospheric oxygen as well as novel gases like nitrous oxide, argon and helium in passive and active storage of fruits and vegetables with benefits in reducing microbial growth, off-flavour and browning (Artes et al., 2009). Research is still on-going towards commercialisation and validation of the use of these gases. However, nitrous oxide has been accepted by the European Union for food use (Day 1996). In this study, the effectiveness of nitrous oxide compared to nitrogen gas in maintaining shelf life was tested for fresh-cut 'Iceberg' lettuce and rocket leaves under modified atmosphere packaging (MAP) conditions.

2. Materials and Methods

2.1 Raw material and Preparation

'Iceberg' Lettuce (*Lactuca Sativa* L.) and Wild Rocket (*Diplotaxis tenuifolia*) used for the experiment were purchased from a local processor in the South of Italy. Rocket leaves were already washed, sanitised and dried by the company, while lettuce heads were processed in the laboratory. After sorting, lettuces were sanitized in a 100ppm sodium hypochlorite solution and then centrifuged for 90 s in a hand salad spinner; 80 g of samples were placed in bags of non-micro perforated polypropylene +polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET and sealed in three different gas mixtures; (97 % Nitrous oxide + 3 % Oxygen), (50 % Nitrous oxide + 47 % Nitrogen + 3 % Oxygen) and (97 % Nitrogen + 3 % Oxygen). Rocket leaves were packaged in the same material but using only two different gas mixtures; (95 % Nitrous oxide + 5 % Oxygen) and (95 % Nitrogen + 5 % Oxygen). A control in air was added by leaving the samples in unsealed bags. The bags were then stored in a cold chamber at 5 ºC in triplicates. Quality evaluations were conducted on 2, 4 and 9 days in the case of lettuce and 3, 7 and12 days for rocket leaves.

2.2 Headspace analysis

Levels of oxygen O_2 and carbon dioxide CO_2 gases in film bags were determined each sampling time during storage. To measure the O_2 and CO_2 concentration in the headspace of bags, dioxide gas analyzer (Dan sensor, Model Checkmate 3, Denmark) was used in sampling the gas through a needle, using a built-in pump. Nitrous oxide gas concentration was determined by gas chromatography (Shimadzu, model 17A, Kyoto, Japan) according to the methods of Benkeblia et al. (2001) with slight modifications.

2.3 Weight loss

The weight loss was calculated as percentage of the initial weight.

2.4 Texture analysis

Texture was determined using the Instron Universal Testing Machine (model 3343, Canton, MA, US) equipped with a Kramer Shear press of five blades. Five grams of leaves were placed in the Kramer shear cell and extruded with the crosshead at a speed of 50 mm/min. Firmness was measured as the maximum peak recorded force on the chart and expressed in Newton (N).

2.5 Total vitamin C

Five grams of fresh leaves were homogenized with 10 mL of MeOH: H_2O (5:95) plus citric acid (21 g L^{-1}) with EDTA (0.5 g L^{-1}) and NaF (0.168 g L^{-1}). Ascorbic acid (AA) and dehydro-ascorbic acid (DHAA) contents were determined with an Agilent 1200 Series HPLC (Waldbronn, Germany) as described by Zapata and Dufour (1992). Ascorbic acid (AA), dehydroascorbic acid (DHAA) and vitamin C (AA + DHAA) were expressed in mg 100 g^{-1} of fresh weight (FW)

2.6 Sensory evaluation

The sensory characteristics; "colour", "off-odor", "texture", "overall acceptability" at each sampling time were assessed by an expert panel of three people. Overall acceptability, color and texture were evaluated and scored on a 5-1 scale, where 5= excellent, 3=fair (limit of marketability), and 1=very poor. Off-odor and off-flavor was scored on 1-5 scale where 1= no symptoms 3 = moderate and 5= severe.

2.7 Microbiology analysis

Microbial analyses were performed on duplicate samples for each of the gas treatments on each sampling day. Ten grams of samples were homogenized in 90 ml sterile physiological saline solution (0.85 % NaCl) in a stomacher (Bag mixer interscience 78860 St Nom France) for 60 s. After decimal dilutions, colony forming unit (CFU) of aerobic mesophils (30 ºC for 2 days) and psychrophilics (5 ºC for 7 days); yeast and moulds (30 ºC for 2 days) and enterobacteriaceae (30 ºC for 2days) were counted in Plate Count Agar, Potato Dextrose Agar and Violet Red Bile Glucose Agar (VRBGA) respectively. Microbial counts were expressed as log CFU/g.

2.8 Statistical analysis

The experiment was a 3-factorial design combining plastic material, with gas mixtures and storage times. Due to the 3 way significant interaction, data for each storage time were analyzed as a 2- way factorial. Least Significant difference (LSD) was used to separate the means of treatment samples. Genstat version 9.2 Statistical Software (Genstat, 2007) was used to carry out the analysis.

3. Results

Bags maintained N_2O gas and yielded safe oxygen levels for modified atmosphere storage of fresh products resulting in about 1% at the end of storage (Gorny, 1997). Inversely packages containing N_2 gas had almost no oxygen in its headspaces by the end of the storage period (Figure 1A). Increased CO_2 levels (Figure 1B) in all packages corresponded to the rate of decrease in O_2 for the various gas treatments in both lettuce (Figure 1) and rocket (not shown).

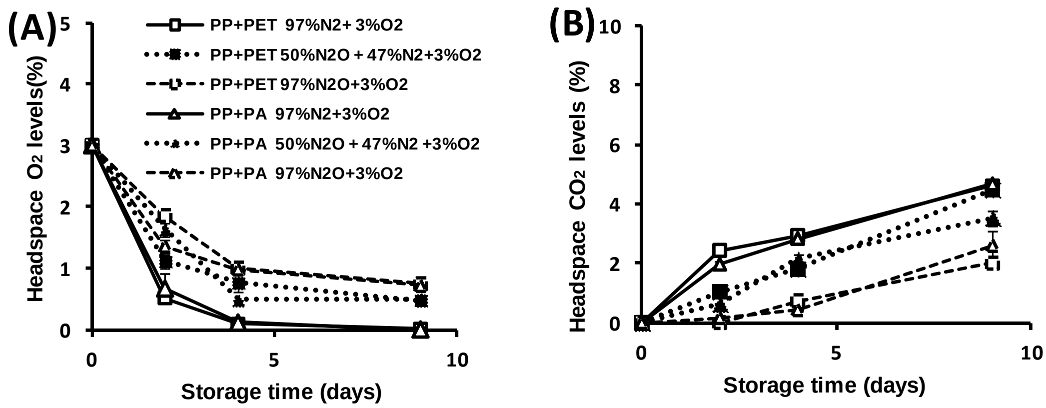


Figure 1: Percentage of (A)O_2 and (B)CO_2 gas levels inside 97 % N_2O + 3 % O_2 (---), 50 % N_2O + 47 % N_2 + 3 % O_2 (….) and 97 % N_2 + 3 % O_2 (—) gas sealed packages (non-micro perforated polypropylene + polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET) containing "iceberg" lettuce stored at 5 °C for 9 days. Error bars signifies the standard error of triplicate sample means (n=3)

Colour of cut "Iceberg" lettuce was lost over the storage period (Figure 2A), with no significant differences among treatments. Cut lettuce stored in PP+PA and PP+PET sealed packages containing 97 % N_2O + 3 % O_2, 50 % N_2O + 47 % N_2 + 3 % O_2 maintained marketable colour with slight loss of fresh appearance, whereas samples packaged in 97 % N_2 + 3 % O_2 visibly lost the appealing fresh colour, which limited its marketability by the end of 9 day storage. The control sample (in air) showed a significant ($p<0.05$) decrease in colour with storage time, leading to pronounced discoloration which limited the edibility of samples. A slight loss of crispness due to transpiration was detected in MAP packaged samples (Figure 2B), in contrast with the control (samples in air) which showed sensible softening reducing its marketability. The observed texture results could be due to the ability of the MAP storage conditions to preserve moisture content (Harker et al., 1997). MAP samples maintained the initial weight compared to the control treatment (in air) which lost 4 %, 9 % and 15 % of the weight after 2, 4 and 9 days respectively. Weight retention in gas sealed lettuce may have resulted from the steam barrier properties of the PP+PA and PP+PET packaging materials (Carvalho and Clemente, 2004).

Until day 9 of storage, no off-flavours (Figure 2C) and off-odours were observed in both control and treated lettuce samples. All treatments resulted in moderately acceptable overall characteristics (Figure 2D) but for the control (in air) and the samples packaged in PP+PA with 50 % N_2O + 47 % N_2 + 3 % O_2 which were judged to be lower than 3 (limit of edibility) at 9 days of storage.

Similar to the sensory evaluation, instrumental measurement showed that the texture did not change significantly over the storage period in all treatments as observed by Foley et al. (2002). Nonetheless instrumental measurements did not show significant differences between treatment samples and the control, depicting slight inconsistencies between instrumental measurement and sensory evaluation (Baur et al. 2004).

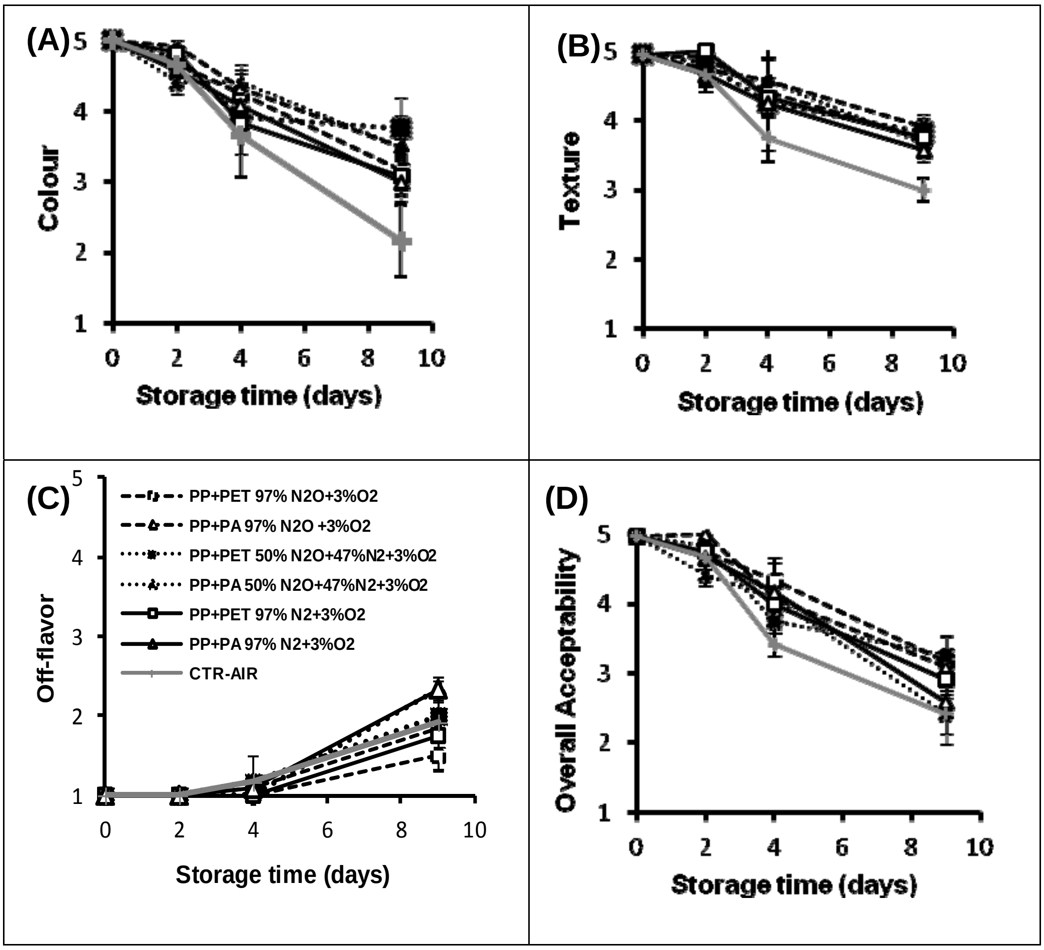
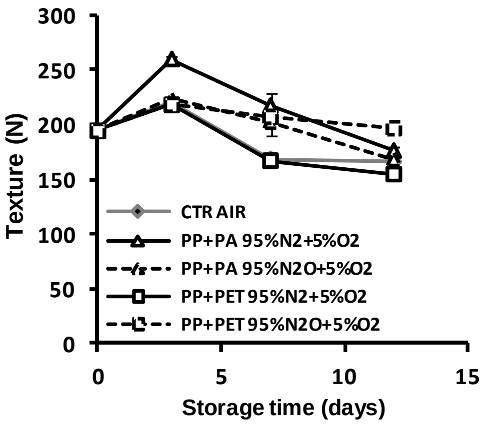


Figure 2: Sensory evaluation of (A) colour, (B) texture, (C) off-flavour and (D). Overall acceptability of fresh-cut "Iceberg" lettuce stored for 9 days at 5 °C in 97 % N_2O + 3 % O_2 (---), 50 % N_2O + 47 % N_2 + 3 % O_2 (….) and 97 % N_2 + 3 % O_2 (—) gas sealed packages (non-micro perforated polypropylene + polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET). Error bars signifies the standard error of means

Colour and off-odour characteristics of rocket leaves did not change significantly on day 3 of storage, but evaluation of overall acceptability resulted in significant difference between the control and the different atmosphere package combinations ($p<0.05$) (data not shown). All treatments showed a decrease in overall quality by day 12. However in all cases PP+PET N_2O bags produced the worst result for storing rocket leaves even on day 7 when colour, off-odour and overall quality were barely acceptable (Silveira et al. 2014).

The texture of rocket leaves in N_2O and N_2 gas filled PP+PA and PP+PET bags were similar in most cases though slight reductions in firmness were observed as reported by Guevara et al. (2001) in passive modified atmosphere storage. Throughout the storage period N_2O gas mixture in PP+PET bag ($p<0.05$) provided the best conditions for maintaining the textural characteristics of rocket leaves (Figure 3). PP+PA bags maintained better the vitamin C content of rocket leaves, with the highest value of 64.9 mg/100 g fw in 95 % N_2 + 5 %O_2 gas at the end of storage (versus an initial content of 82.3mg/100 g fw). Total vitamin C (AA+DHA) content reduced with storage time in all treatments (Zenoozian 2011) to as low as 19.4 mg/100 g fw in nitrous oxide gas sealed in PP+PET bag (Figure 4).



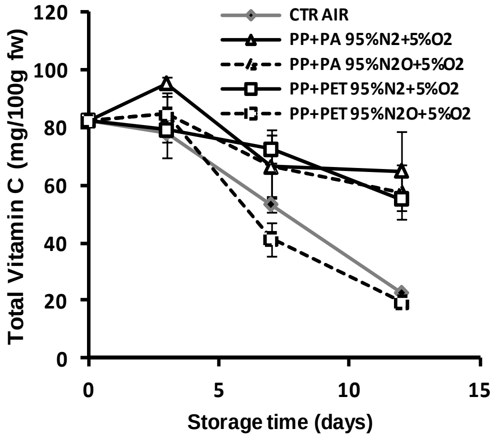


Figure 3: Texture of rocket leaves in 95 % N_2O + 5 % O_2 (---) and 95 % N_2 + 5 % O_2 (—) gas sealed packages (non-micro perforated polypropylene + polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET) stored at 5 ^oC 12 days

Figure 4: Vitamin C content in rocket leaves stored in 95 % N_2O + 5 % O_2 (---) and 95 % N_2 + 5 % O_2 (—) gas sealed packages (non-micro perforated polypropylene + polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET) stored at 5 ^oC for 12 days

Modified atmosphere was able to delay the growth of microbial groups of psychrophilics, yeast and moulds, mesophilics and enterobacteriaceae below 7 log CFU/g on 'Iceberg' lettuce (Figure 5) and 8 log CFU/g for Wild rocket (not shown) for the whole storage period. In stored rocket leaves, yeast and moulds reduced by 0.5 log cfu/g; mesophilics and enterobacteriaceae were almost stable as observed by Tomas-Callejas et al. (2011). Slight reductions of 0.30 log cfu/g were observed in enterobacteriaceae for PP+PA 95%N_2+5%O_2 treatment in rocket leaves. Interestingly, the control samples in air also recorded slow growth rate of microbes, attributable to low temperature of 5ºC, Beuchat and Brackett (1990); regardless of storage conditions or hypochlorite wash solution used on products prior to storage. Hence the effect of the gas mixtures in modified atmosphere packaging on the microbial growth of stored "Iceberg" lettuce and Wild rocket may be negligible (Molin, 2000)

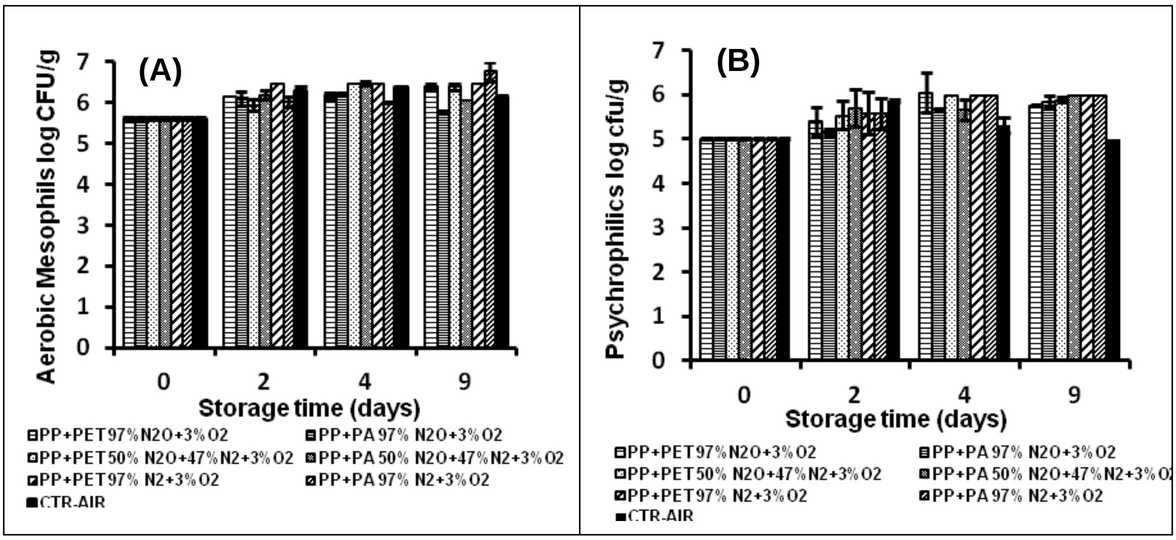


Figure 5: Microbial population of (A) Aerobic Mesophils, (B) Psychrophilics on fresh-cut "iceberg" lettuce stored in 97 % N_2O + 3 % O_2, 50 % N_2O + 47 % N_2 + 3 % O_2 and 97 % N_2 + 3% O_2 gas sealed packages

4. Conclusions

Results showed that there were no significant differences between the N_2O and N_2 gas mixtures used in modified atmosphere packaging, despite what has been reported in some literature. Non-micro perforated polypropylene + polyamide, PP+PA and polypropylene + polyethylene terephthalate, PP+PET possess good barrier properties for keeping gas mixtures (and particularly high levels of nitrous oxide); except that for effective use, oxygen levels should be carefully monitored to ensure that they are within the limits required for aerobic respiration.

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