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Shelf-life extension of minimally processed carrots by gaseous chlorine dioxide

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Abstract

Chlorine dioxide (ClO_2) gas is a strong oxidizing and sanitizing agent that has a broad and high biocidal effectiveness and big penetration ability; its efficacy to prolong the shelf-life of a minimally processed (MP) vegetable, grated carrots (*Daucus carota* L.), was tested in this study. Carrots were sorted, their ends removed, hand peeled, cut, washed, spin dried and separated in 2 portions, one to be treated with ClO_2 gas and the other to remain untreated for comparisons. MP carrots were decontaminated in a cabinet at 91% relative humidity and 28 °C for up to 6 min, including 30 s of ClO_2 injection to the cabinet, then stored under equilibrium modified atmosphere (4.5% O_2, 8.9% CO_2, 86.6% N_2) at 7 °C for shelf-life studies. ClO_2 concentration in the cabinet rose to 1.33 mg/l after 30 s of treatment, and then fell to nil before 6 min. The shelf-life study included: O_2 and CO_2 headspace concentrations, microbiological quality (mesophilic aerobic bacteria, psychrotrophs, lactic acid bacteria, and yeasts), sensory quality (odour, flavour, texture, overall visual quality, and white blushing), and pH. ClO_2 did not affect respiration rate of MP carrots significantly ($\alpha \leq 0.05$), and lowered the pH significantly ($\alpha \leq 0.05$). The applied packaging configuration kept O_2 headspace concentrations in treated samples in equilibrium and prevented CO_2 accumulation. After ClO_2 treatment, the decontamination levels (log CFU/g) achieved were 1.88, 1.71, 2.60, and 0.66 for mesophilic aerobic bacteria, psychrotrophs, and yeasts respectively. The initial sensory quality of MP carrots was not impaired significantly ($\alpha \leq 0.05$). A lag phase of at least 2 days was observed for mesophilic aerobic bacteria, psychrotrophs, and lactic acid bacteria in treated samples, while mesophilic aerobic bacteria and psychrotrophs increased parallelly. Odour was the only important attribute in sensory deterioration, but it reached an unacceptable score when samples were already rejected from the microbiological point of view. The shelf-life extension was limited to one day due to the restricted effect of the ClO_2 treatment on yeast counts. Nevertheless, ClO_2 seems to be a promising alternative to prolong the shelf-life of grated carrots.

Keywords: Chlorine dioxide; Minimal processing; Fresh-cut; Decontamination; Shelf-life; Carrots

1. Introduction

Chlorine dioxide (ClO_2) has received attention as a decontaminant for vegetables, largely because its efficacy is less affected by pH and organic matter and it does not react with ammonia to form chloramines, as do liquid chlorine and hypochlorites (Beuchat, 1998). It is a strong oxidizing and sanitizing agent that has a broad and high biocidal effectiveness. Because gas has greater penetration ability than liquid, ClO_2 gas may be more effective for surface sanitation than aqueous ClO_2 (Han et al., 2001b) or other aqueous sanitizers.

Different factors can influence the lethality of a ClO_2 gas treatment. Han et al. (2001a) reported in a study about the inactivation of *Escherichia coli* O157:H7 on green peppers, that the order of significance of the factors from the most important to the least was ClO_2 gas concentration, time, relative humidity, and temperature; moreover a synergistic effect was found between gas concentration and relative humidity.

Studies on the efficacy of ClO_2 to inactivate microorganisms inoculated onto fruit and vegetable surfaces have focused on pathogens such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella Typhimurium* (Han et al., 2000, 2004; Singh et al., 2002; Du et al., 2002, 2003; Lee et al., 2004).

Comparatively few studies have been devoted to the effect of ClO_2 gas on the spoilage microflora or sensory properties of the

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treated fruit and vegetables. Singh et al. (2002) reported the decolourisation of lettuce leaves after treatment, which may have been due to oxidation of chlorophyll. Sy et al. (2005a) reported reductions in populations of yeasts and moulds of 2.5, 4.2 and 3.0 log CFU/g on blueberries, strawberries and raspberries respectively. A treatment with 4.1 mg/l of ClO_2 did not markedly affect the sensory quality of fruits stored for up to 10 days at 8 °C. In a companion article, Sy et al. (2005b) reported reductions in populations of yeasts and moulds of 1.68 log CFU/apple and 2.65 log CFU/peach, but no significant reduction was found for those microorganisms on tomato and onion. Sensory qualities of peaches or fresh-cut cabbage, carrot and lettuce were impaired by treatment with ClO_2 but apples, tomatoes and onions were not markedly affected.

Minimally processed carrots (MP carrots) constitute one of the major minimally processed vegetables (MPV). The main problems that limit their shelf-life are white blush discolouration caused by tissue dehydration, and microbial spoilage (Emmambux and Minnaar, 2003). Therefore, a treatment that could inactivate their natural microflora keeping the tissue hydrated seems appropriate to prolong their shelf-life. Hence ClO_2 might be a good alternative when used under conditions that achieve decontamination without impairing sensory attributes.

Consequently, the present study was designed to evaluate the effect of a treatment with gaseous ClO_2 on the shelf-life of MP carrots. This is the first report about prolonging the shelf-life of a MPV by ClO_2 either in gas or in liquid phase.

2. Materials and methods

2.1. Experimental lay-out

In the first experiment, the respiration rate of treated MP carrots was assessed compared to untreated MP carrots in order to determine possible differences in physiological activity and to be able to design the appropriate packaging configuration (Jacxsens et al., 1999a). In a second experiment, those packages were used during the shelf-life study.

2.2. Carrot processing

Carrots (*Daucus carota* L.) were purchased in a local produce market and stored at 4 °C overnight before use. To prepare the sticks, carrots were sorted for any cracks and defects, their ends removed using a sharp knife, hand peeled, cut in 4 cm portions using a sharp knife and grated in 0.3 × 0.3 × 4 cm sticks using a Compacto Kitchen Cutter (Philips, Eindhoven, The Netherlands). They were immersed in tap water at ambient temperature for 1 min and dried for 1 min by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland). For shelf-life studies, 4 kg of MP carrots were produced, the batch was then divided in two, one to be treated with ClO_2 and the other to remain untreated.

2.3. ClO_2 gas treatment

Two kilograms of grated carrots were placed in a 48 l closed cabinet with a glass window (covered by aluminium foil). Then the relative humidity of the cabinet was adjusted to 91% by a flow of hot wet air (4 l/h). A thermohygrometer (Digitron 2020R, Devon, England) was used to measure relative humidity and temperature. A 1000 mg/l solution of ClO_2 was prepared by diluting a stock solution (Vernagene, UK) whose concentration had been previously determined by the method indicated below (Section 2.5). ClO_2 was stripped from the diluted solution, previously warmed up to 48 °C, by air bubbling (4 l/h) and led by the same air stream to the cabinet where perforated plastic pipes together with cabinet tumbling procured a homogeneous contact between the sanitizer gas and the carrot pieces. Treatment was performed at 28 °C and took 6 min, including 30 s of stripping. ClO_2 concentration inside the cabinet was monitored by taking gas samples at regular intervals, the highest ClO_2 concentration was 1.33 mg/l measured immediately after the end of the stripping time. When the pre-set treatment time was completed, the cabinet was opened and samples were taken for the respiration rate measurement (Section 2.6) or the shelf-life study (Section 2.8).

2.4. Determination of ClO_2 in air

This determination was performed by an iodometric method. For each sample, air was sucked out of the cabinet by means of an air sampling pump (Gylair 3, Sensydine, England) for up to 15 s. Two impingers were serially placed between the cabinet and the pump, so that the sample was scrubbed through a buffered 7% (w/v) KI (Sigma-Aldrich, Steinheim, Germany) solution with pH of 7.0. The content of the impingers was quantitatively transferred to a recipient, where 3 ml of 6 N HCl (Merck, Darmstadt, Germany) was added. The mixture was titrated with sodium thiosulfate solution 0.01024 N (Aldrich, WI) to a clear colourless endpoint, using soluble starch (Difco, Becton Dickinson, Meylan, France) as indicator.

2.5. Determination of ClO_2 in solution

To determine the concentration of ClO_2 in solution, 5 ml of that solution was scrubbed through 5 ml of buffered 7% (w/v) KI solution with pH of 7.0 in a recipient. The mixture was titrated with sodium thiosulfate solution 0.01024 N to a clear colourless endpoint, using soluble starch as indicator.

2.6. Respiration rate measurements

To measure the respiration rate of untreated and treated MP carrots, 100 g of MPV were placed in airtight glass jars (635 ± 11 ml); five replicates were used. The jars were closed under a gas mixture of 13% O_2, 1% CO_2 and 86% N_2 as initial gas atmosphere by means of a gas packaging unit (gas mixer, WITT M618–3MSO, Gasetechnik, Germany; gas packaging, Multivac A300/42 Hagenmüller KG, Wolfertschwenden, Germany). Air products (Vilvoorde, Belgium) supplied the gases. Jars were stored at 7 °C and a gas sample was taken periodically through an airtight septum and analysed by gas chromatography (MicroGC M200, columns: molecular sieve 5A PLOT at 35 °C and Paraplot Q at 45 °C (Agilent, DE, USA)) and

helium as gas carrier (Air Liquide, Liege, Belgium). Data were processed according to Jacxsens et al. (1999a) to estimate O_2 consumption at 7 °C and 3% O_2. Subsequently, an appropriate packaging film could be selected.

2.7. Packaging of the MP carrots

Samples were packed under equilibrium modified atmosphere packaging (EMAP) where, by matching the film permeability, fill weight and bag dimensions with the respiration rate of the produce at a specific temperature, a constant gas composition is maintained inside the bags during the whole shelf-life. The applied package (WA7805–1, Hyplast N.V., Hoogstraten, Belgium) was an experimental film with a permeability for O_2 at 7 °C and 90% relative humidity of 3529 ml O_2/kg h, and was selected based on its oxygen permeability. The packaging configuration was designed by using the method validated by Jacxsens et al. (1999a). One hundred grams of MP carrots were packed in bags with dimensions of 20 cm × 10.5 cm. Then, a gas mixture of 4.5% O_2, 8.9% CO_2 and 86.6% N_2 was injected into the bags as initial gas atmosphere by the gas packaging unit described in Section 2.6.

2.8. Shelf-life study

Untreated and treated MP carrots were packed in the designed bags and stored at 7 °C. During the shelf-life study, three bags per condition (treated and untreated) were taken at several days and independently analysed for O_2 and CO_2 headspace concentrations in the bags, microbiological and sensory quality, and pH. Additionally, samples for microbiological analysis were taken immediately after grating. The end of the shelf-life arrived when the population of a group of microorganisms reached an unacceptable level or when the sensory panel rejected the samples.

2.8.1. Headspace O_2 and CO_2 monitoring

O_2 and CO_2 concentrations in the headspace of the bags were determined by a Servomex gas analyser (Servomex 1450, Crowborough, England) before opening bags to take samples for the rest of the analyses. The gas analyser determines O_2 concentration by paramagnetism and CO_2 concentration by the infrared single beam–single wavelength technique.

2.8.2. Microbiological analysis of spoilage microorganisms

The following media and incubation conditions were used for microorganism enumeration: Plate Count Agar (Oxoid, CM325, Basingstoke, Hampshire, England) for mesophilic aerobic bacteria, pour plated and incubated at 30 °C for 3 days, and also for total aerobic psychrotrophic count, pour plated and incubated at 22 °C for 5 days; de Man–Rogosa–Sharpe medium (Oxoid, CM361), with addition of 0.14% sorbic acid (Sigma, S-1626) to suppress yeast growth, for lactic acid bacteria (LAB), pour plated, overlaid with the same medium and incubated aerobically at 30 °C for 3 days; a prepared medium composed of 15 g agar (Agar No 1, Oxoid, LP0011), 5 g yeast extract (Oxoid, L21), and 20 g dextrose (Sigma-Aldrich, Steinheim, Germany) per litre with 50 mg/l (Tournas et al., 1998) chlortetracycline (Difco, 233331) to enumerate yeasts, spread plated and incubated at 30 °C for 3 days. Microbiological counts were done in triplicate (3 packages/analysis day for both treated and untreated MP carrots) by taking 30 g of sample and mixing it with 270 ml peptone saline solution (8.5 g/l NaCl (VWR, Fontenay sous Bois, France) and 1 g/l peptone (Oxoid, L34)) in a sterile Stomacher bag, and homogenisation for 60 s with a Colworth Stomacher (Steward Laboratory, London, UK). Tenfold dilution series were made in peptone saline solution for plating. The specifications proposed by Debevere (1996) were used to determine the end of the shelf-life from the microbiological point of view, which are: 8 log CFU/g for mesophilic aerobic bacteria and psychrotrophs, 7 log CFU/g (plus sensory analysis) for LAB or 5 log CFU/g for yeasts.

2.8.3. Evaluation of sensory quality

After taking samples for microbiological analyses and pH, samples were transferred to closed plastic recipients coded with random numbers. Samples taken from six bags were evaluated, three with treated samples and three with controls. The appraisal was performed by a semi-trained panel of six persons in a special room with individual booths. The first part of the evaluation (odour, flavour and texture) was judged under red light; under daylight the overall visual quality and white blushing were evaluated. The end of the shelf-life from the sensory point of view was reached when at least one of the mean scores was above the middle point of the respective scale, which ranged from 1 (fresh) to 9 (spoiled) for all attributes except for white blushing, for which the scale ranged from 1 (none) to 5 (severe) (Botta, 1995).

2.8.4. pH measurement

A sample of 10 g of MP carrots was homogenised by using a mixer (Commercial blender 8010, Waring, Connecticut, USA) with 50 ml of demineralised water. The pH was measured by using an electrode (PH 915600, Orion, Boston, USA) and measure unit (model 525A, Orion, Boston, USA).

2.9. Statistical analysis

Data were analysed for mean differences between treated and untreated MP carrots with the *t*-test for independent samples and the Mann–Whitney test by using the statistics program SPSS 12.0 (SPSS Inc., Chicago, USA), with $P \leq 0.05$.

3. Results and discussion

3.1. Respiration rates

In order to design appropriate packaging configurations to reach an equilibrated modified atmosphere it is necessary to know the respiration rate of the produce. Since ClO_2 is an oxidant, the metabolism of the carrot tissue could have resulted as altered during treatment, leading to a respiration rate different from that of the untreated produce. However, no statistical differences ($\alpha \leq 0.05$) were found between the respiration rates

of untreated and treated samples, which were respectively 10.69± 3.63 ml O_2/kg h and 9.98±3.47 ml O_2/kg h. The average of both was taken to calculate the packaging configuration with regard to the shelf-life study. The values for the respiration rate of the untreated MP carrots found in this study agreed with those reported by Jacxsens et al. (1999a), who worked under the same conditions used in this study.

3.2. ClO_2 degradation

The highest ClO_2 concentration was 1.33 mg/l, measured after finishing the stripping. However, because of the experimental set-up, where the sample remains in contact with ClO_2 for some time after the end of the stripping, which results in degradation of ClO_2 by organic matter, it is not possible to report a single value of ClO_2 concentration but the actual changing concentration during the treatment time as it is shown in Fig. 1. It can be observed that the concentration of this gas increased during the stripping time and then fell to nil before 6 min. Complete consumption of ClO_2 is desirable, otherwise it is necessary to evacuate and destroy it. It is known that ClO_2 is unstable and is also absorbed by plastic and glass. Tests ran with empty treatment cabinet showed that these effects were negligible for up to 10 min. Therefore the observed decrease can be only accounted to degradation by the MPV.

When comparing studies on decontamination with ClO_2, it is important to consider the actual meaning of the reported ClO_2 concentrations. In the present work, the ClO_2 concentration during treatment increased, reached a peak and then decreased as it is explained above. This is different from the set-ups used by Lee et al. (2004) and Sy et al. (2005a,b) where the ClO_2 concentration increased during the whole treatment time; moreover it is also different from the set-ups of Du et al. (2002, 2003) and Han et al. (2000, 2001a,b) where the dynamics of ClO_2 concentration were not followed, maybe because of the use of a large excess of cabinet volume with respect to the sample size, resulting in a ClO_2 concentration that could have been considered constant. Therefore, comparisons between different studies should be taken with precaution because of the possible changes in ClO_2 concentration during the treatment.

3.3. Headspace O_2 and CO_2 concentrations

The goal of an EMAP is to establish constant O_2 and CO_2 concentrations during the entire shelf-life study. To check the appropriateness of the selected packaging configurations, a periodic monitoring of the concentration of these gases was carried out. The mean O_2 concentration through the shelf-life was about 4%, close to the desired 3%, for both treated and untreated samples. However, a high variability was observed, and some packages of untreated samples were at hypoxic level (<0.5%) and had to be discarded. But for treated samples, the designed bags were able to maintain an acceptable O_2 level for the whole period of the shelf-life (5 days). The mean concentration of CO_2 through the shelf-life was around 6%, CO_2 percentage dropped from their initial values, then stabilized or increased but without accumulating enough to reach the initial levels. Therefore, for ClO_2 treated samples, the designed EMAP was able to keep O_2 and CO_2 concentrations at the desired levels (Jacxsens et al., 1999a) enough to retard respiration without causing fermentation (Beaudry, 1999).

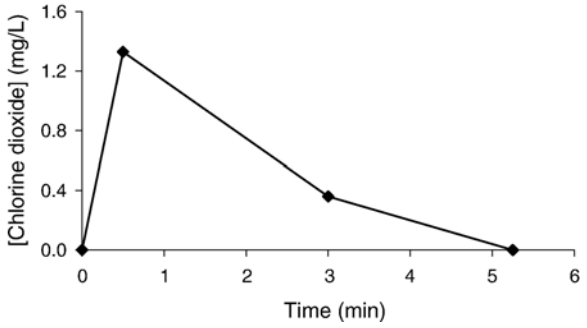

Fig. 1. Concentration of ClO_2 gas during the treatment of MP carrots.

Table 1
Counts (log CFU/g) of different groups of microorganisms in minimally processed carrots and respective reduction after treatment with ClO_2 gas

	Mesophilic aerobic bacteria	Psychrotrophs	Lactic acid bacteria	Yeasts
Initial	4.72±0.17 [a]	4.82±0.14 [a]	2.22±0.18 [b]	2.23±0.29 [a]
Untreated	5.07±0.23 [a]	5.06±0.19 [a]	2.93±0.17 [a]	2.44±0.09 [a]
Treated	3.19±0.16 [b]	3.35±0.08 [b]	0.33±0.58 [c]	1.78±0.08 [b]
Reduction [c]	1.88	1.71	2.60	0.66

[ab] Within the same column, means sharing the same superscript are statistically equal ($\alpha \leq 0.05$), for mesophilic aerobic bacteria and psychrotrophs according to the *t*-test, and for lactic acid bacteria and yeasts according to the Mann–Whitney test. Results are mean±standard deviation.
[c] Count of untreated–count of treated.

3.4. Decontamination

When compared with the counts found for the initial (after grating) microbial load, the counts of LAB increased after the washing–drying (Table 1), likely due to contamination acquired in the spin dryer (centrifugation). Increment of contamination during the preparation of MPV has also been reported by Garg et al. (1990) for shredders used to prepare chopped lettuce and coleslaw, and by Allende et al. (2004) for the draining, rinsing, centrifugation and packaging steps required to prepare an MP Lollo Rosso lettuce.

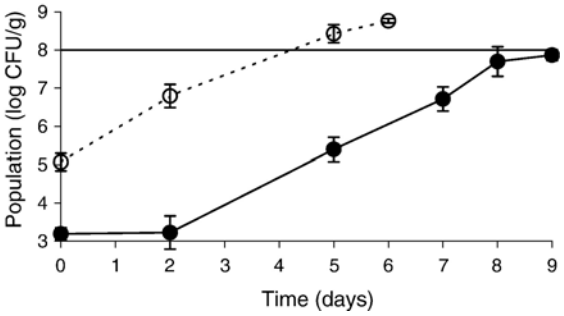

Fig. 2. Mesophilic aerobic bacteria counts of untreated (—○—) and ClO_2 gas treated (—●—) minimally processed carrots stored at 7 °C under modified atmosphere packaging. Horizontal line indicates the limit for shelf-life. Error bars are mean±standard deviation.

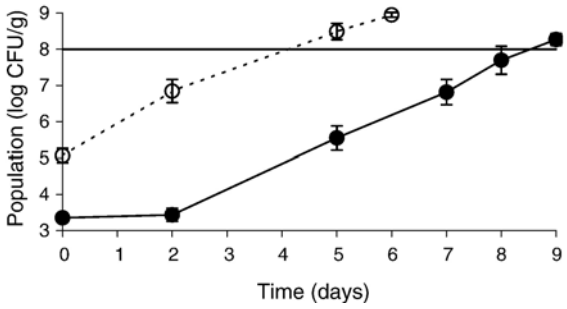

Fig. 3. Psychrotrophs count of untreated (—○—) and ClO_2 gas treated (—●—) minimally processed carrots stored at 7 °C under modified atmosphere packaging. Horizontal line indicates the limit for shelf-life. Error bars are mean ± standard deviation.

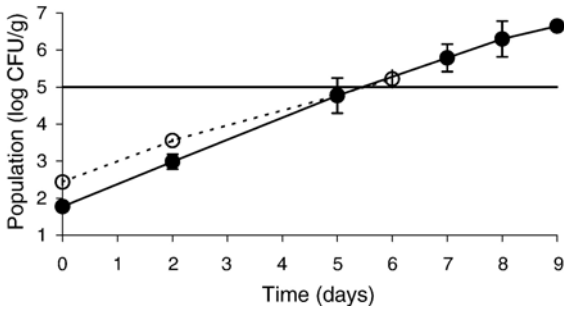

Fig. 5. Yeasts count of untreated (—○—) and ClO_2 gas treated (—●—) minimally processed carrots stored at 7 °C under modified atmosphere packaging. Horizontal line indicates the limit for shelf-life. Error bars are mean ± standard deviation.

The levels of reduction for specific groups of microorganisms are reported in Table 1. As it is shown below, these reductions, all statistically significant ($\alpha \leq 0.05$), were enough to prolong the shelf-life of MP carrots for one day (see Section 3.5). The susceptibility of the different groups of spoilage microorganisms to ClO_2 has not been reported yet. It is known that yeasts are more resistant than bacteria to ozone, another powerful oxidant and biocidal agent (Russell, 2003).

3.5. Microbial analysis during shelf-life

The changes in microbial populations during the shelf-life study can be observed in Figs. 2–5. A lag phase of at least 2 days was observed for mesophilic aerobic bacteria (Fig. 2), psychrotrophs (Fig. 3) and LAB (Fig. 4), which is not common in these kinds of studies and reveals a sublethal damage occasioned by ClO_2 to these groups of microorganisms. However, this effect was not observed to occur in yeasts (Fig. 5). After the lag phase, LAB (Fig. 4) on treated samples started to grow but neither them nor those on untreated samples reached high counts during shelf-life. Also after the lag phase, mesophilic (Fig. 2) and psychrotrophic (Fig. 3) microorganisms on treated samples grew as fast as in untreated samples. The similitude observed in the growth curves of both suggests that the same microorganisms grew at both incubation temperatures. The psychrotrophic counts reached unacceptable levels after 4 days on untreated samples but only after 8 days on the treated ones. This could have resulted in a shelf-life extension of 4 days, from the microbiological point of view, accounted to the sublethal effect of ClO_2 causing a lag phase together with the decontamination achieved. However, the yeast counts (Fig. 5) of the treated samples, initially only minimally reduced with respect to the untreated ones, became the same at the fifth day and reached unacceptable levels ($>10^5$ CFU/g) almost on that day. When analysed together with the other results, it can be concluded that, from the microbiological point of view, only one extra day in shelf-life was achieved by applying gaseous ClO_2 to MP carrots, with yeasts as the determinant for treated samples, and psychrotrophs for the untreated ones.

It is possible to speculate that the use of higher ClO_2 concentrations could have led to a longer shelf-life by reducing more the levels of the microbial populations. However, higher decontamination levels could increase the risk of pathogen proliferation, i.e. illness transmission. For example, it has been demonstrated that a treatment able to reduce the initial population of aerobic microorganisms of iceberg lettuce by 1.73–1.96 log CFU/g (Li et al., 2001) also enhances growth of *L. monocytogenes* during subsequent storage, which may result from reduction in numbers of competitive background microflora (Li et al., 2002). However the specific case of MP carrots deserves a special consideration. Studies summarized by Jacxsens et al. (1999b) and Sy et al. (2005b) have revealed a lack of growth and even a reduction in population of pathogens inoculated onto fresh-cut carrots. In spite of the possible presence in carrots of natural

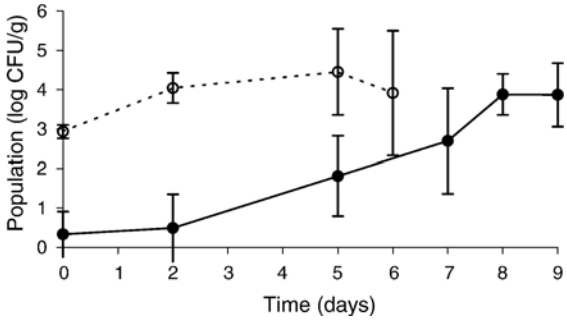

Fig. 4. Lactic acid bacteria count of untreated (—○—) and ClO_2 gas treated (—●—) minimally processed carrots stored at 7 °C under modified atmosphere packaging. Error bars are mean ± standard deviation.

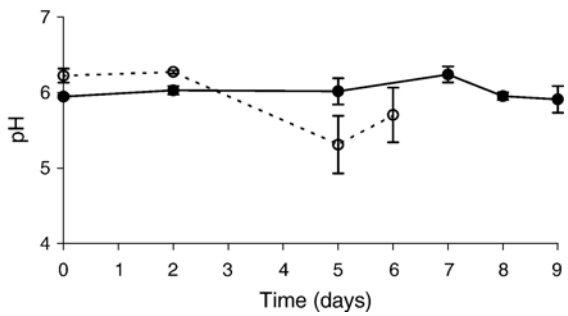

Fig. 6. Changes in pH of untreated (—○—) and ClO_2 gas treated (—●—) minimally processed carrots stored at 7 °C under modified atmosphere packaging. Error bars are mean ± standard deviation.

Table 2
Sensory evaluation of untreated and treated with ClO_2 minimally processed carrots stored at 7 °C under modified atmosphere packaging

Quality attributes		Time (days)					
		0	2	5	6	7	8
Odour [a]	Untreated	2.7±0.6 [d]	3.7±0.7	4.5±1.5	4.7±1.9	– [c]	–
	Treated	2.3±0.5 [d]	3.8±0.3	4.1±0.3	–	**5.1**±0.2	**5.8**±0.2
Flavour [a]	Untreated	1.3±0.1 [e]	3.6±0.5	4.5±0.2	4.3±1.0	–	–
	Treated	1.4±0.3 [e]	2.7±0.7	3.6±0.3	–	3.2±0.3	4.7±0.1
OVQ [a]	Untreated	1.2±0.2	3.1±0.5	3.6±0.2	3.9±0.3	–	–
	Treated	1.2±0.0	2.8±0.5	3.6±0.3	–	0.7±4.1	0.2±4.4
Texture [a]	Untreated	1.1±0.2	2.9±0.4	3.5±0.4	3.9±1.1	–	–
	Treated	1.1±0.1	2.8±0.4	3.0±0.3	–	3.1±0.7	4.3±0.2
White blushing [b]	Untreated	1.6±0.2 [f]	2.4±0.3	2.9±0.2	2.9±0.3	–	–
	Treated	1.4±0.2 [f]	2.4±0.3	2.6±0.4	–	3.0±0.3	**3.1**±0.3

Mean±SD. Numbers in bold are scores above the acceptability limit.
[a] Odour, flavour, OVQ (overall visual quality) and texture scores: 1 = fresh, 9 = spoiled.
[b] White blushing: 1 = none, 5 = severe.
[c] –, not determined.
[d–f] Means with the same superscript are statistically equal ($\alpha \leq 0.05$) according to the *t*-test.

antimicrobial compounds, the safety of any MPV highly deprived from its natural microflora needs to be evaluated before looking for higher decontamination levels.

It has been generally considered that the shelf-life of MP carrots is limited by LAB and yeast growth (Nguyen-the and Carlin, 1994). However LAB counts were far from determining the shelf-life in this study, LAB growth in untreated samples stopped after 5 days and in the treated ones after 8 days (Fig. 4), in both cases when the LAB population reached 4 log CFU/g. The same days for APC and psychrotrophic counts reached respectively unacceptable levels (Figs. 2–3). This suggests that competition by non-LAB bacteria suppressed LAB growth. Also, Sinigaglia et al. (1999) and Klaiber et al. (2005) have found that LAB did not determine the shelf-life of this product.

It is worth mentioning that the method used to determine the shelf-life does not take into account sample variability. A better approach could be that proposed by Corbo et al. (2007), which allows to determine confidence intervals for the shelf-life value by using a re-parameterized modified Gompertz equation. However, only four measuring points are available in this study for the growth curves from the untreated samples, which precludes modelling.

A slight but significant ($\alpha \leq 0.05$) decrease of pH (Fig. 6) was detected after treatment, possibly due to formation of acid by the reaction between ClO_2 and organic matter. According to the review by Fukayama et al. (1986), ClO_2 can react with carbohydrates to form carboxylic acids and the progressive reduction of ClO_2 can also form acid. The decrease of pH during storage is probably due to the high psychrotrophic counts ($>10^8$ CFU/g) reached after 5 days in untreated samples and after 8 days in treated samples. At those high levels micro-organisms can produce acids.

3.6. Sensory analysis during shelf-life

Treatment did not significantly impair the sensory attributes of MP carrots (Table 2) ($\alpha \leq 0.05$). This finding contrasts with the results reported by Sy et al. (2005b) for Julienne-style cut carrots, which showed a slight whitening after treatment with 1.4 mg/l of ClO_2 for 6.4 to 10.5 min at 79% to 84% relative humidity. There are two possibilities for carrot whitening, the so called "white blushing" or the carotene bleaching. White blushing is a white translucent appearance of cut carrots that has been attributed to tissue dehydration (Tatsumi et al., 1991) and lignification (Bolin and Huxsoll, 1991). It can not be assured that the whitening reported by Sy et al. (2005b) was either white blushing or carotene bleaching. When comparing their results with ours, the higher relative humidity used during treatment in this study avoided dehydration, and consequently white blushing, and no bleaching effect was observed. Nevertheless, it is not possible to completely rule out a role of ClO_2 in carrot whitening under other treatment conditions. In this regard Sy et al. (2005b) also reported that higher ClO_2 concentrations (2.7 and 4.1 mg/l) caused more whitening, once again these ClO_2 levels were also associated with relative humidity that is lower than 90% for even longer exposure times. Moreover, Sy et al. (2005b) concluded that adverse effects on sensory quality render the application of ClO_2 to carrots on a commercial scale questionable, our results point towards a different conclusion.

In this study, the scores given by the panel to the different attributes were similar between untreated and treated samples along the shelf-life study. The only important attribute for the shelf-life from the sensory point of view was odour. After 6 days, untreated samples remained acceptable, the treated ones were unacceptable on the seventh day; however, since from the microbiological point of view both samples were not acceptable at earlier dates, no sensory parameter was important for determining shelf-life.

4. Conclusions

Gaseous ClO_2 is a promising alternative to prolong the shelf-life of MP carrots. Under the conditions used in this work, a treatment with gaseous ClO_2 does not affect the respiration rate nor the sensory attributes of MP carrots, decontaminate them

and prolong their shelf-life for 1 day. Yeast growth limited the shelf-life of treated samples.

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