Effect of alternative sanitizers on functional parameters of watercress leaves (*Nasturtium officinale*) stored under modified atmosphere

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Abstract

Currently in the industry of fresh salads, watercress is shown as an alternative supply of gourmet products, being recognized for its high content of health promoting compounds such as antioxidants and phenolic compounds. The aim of this study was to evaluate different sanitizing agents such as chlorine dioxide (CD, 10 mg L^{-1}), acidified sodium chlorite (ASC, 500 mg L^{-1}) and peroxyacetic acid (PAA 90 mg L^{-1}) as an alternative to sodium hypochlorite (SH, 100 mg L^{-1}). The treated samples were stored under modified atmosphere packaging (MAP) for 13 days at 5°C and 95% RH. Watercress treated with SH in a perforated bag (air atmosphere conditions) was used as a control. The evolution of the psychrophilic bacteria counts, during 13 days of refrigerated storage, was determined in Plate Count Agar, and incubated at 5°C for 7 days. Determinations were performed in triplicate. Sensory analyses were carried out to determine the effect of all treatments on the watercress quality. All treatments in MAP showed a gradual decrease in O_2 levels by 37% and an increase in CO_2 levels by 316% on day 13, relative values on day 0. During the storage period, MAP maintained the antioxidant levels of around 2 mg g^{-1} of Trolox activity, while watercress stored in perforated bags showed a decrease to 1 mg g^{-1} Trolox activity on day 13. The total phenolic contents showed a similar trend as the antioxidant levels, where MAP and perforated bags reached 3 and 3.9 mg g^{-1} gallic acid equivalents (GAE). Initial counts of psychrophiles in raw material reached 6.4 log CFU g^{-1}. However, after one day of storage all treatments reduced to counts between 1 and 2 log units. The modified atmosphere combined with all sanitizers turned out to be a useful tool to maintain bioactive compounds levels as well as extend the shelf-life of watercress stored at 5°C.

Keywords: antioxidant capacity, phenolic compounds, microbial quality, leafy vegetables

INTRODUCTION

Several studies have shown that a diet rich in fruits and vegetables contributes to better health and prevention of degenerative diseases such as cancer (Martínez, 2008). The development of these diseases and even accelerated aging have been related with the harmful effects of oxygen radicals and other highly reactive oxygen species. Phenolic compounds and other antioxidants are secondary metabolites of plants which act as functional compounds protecting the organism from oxidation. Vegetables should be a sufficient source of functional compounds for humans. However, there is insufficient information on the content of phenolic and antioxidant compounds of some vegetables, such as watercress (Stratil et al., 2006).

The processing of vegetables causes physiological changes and tissue damage, decreasing the shelf life and quality of the product due to the high susceptibility of the cutting surfaces and the active metabolism of the tissues. Modified atmosphere packaging (MAP) protects the product from mechanical, microbiological and biological deteriorations (Aguayo, 2003).

The aim of this study was to extend the shelf life of fresh-cut watercress using different sanitizing agents such as chlorine dioxide (CD), acidified sodium chlorite (ASC) and peroxyacetic acid (PAA) as alternatives to sodium hypochlorite (SH). Treated samples were stored under modified atmosphere packaging (MAP) at 5°C.

MATERIALS AND METHODS

Watercress (*Nasturtium officinale* R. Br.) leaves from a hydroponic culture were obtained from a commercial grower (Hidrohuerta Tango Ltda., Santiago, Chile). The leaves were prewashed with tap water at 5°C for 5 min. Treatments were carried out by immersing leaves for 3 min in the sanitizer solutions: 10 mg L^{-1} chlorine dioxide (Winzaclor 5, Winkler, Santiago, Chile), 500 mg L^{-1} acidified sodium chlorite (SigmaAldrich, St. Louis, MO, USA) which was acidified with anhydrous citric acid (RZBC, Rizhao, China), and 90 mg L^{-1} peroxyacetic acid (Tsunami100, Ecolab, St Paul, MN, USA). The efficiency of the sanitizers was compared with that of sodium hypochlorite 100 mg L^{-1}. Leaves were packed (50 g) in plastic bags (BB4 Cryovac, Sealed Air Corporation Chile, Santiago, Chile) having dimensions of 20×17 cm, with permeability values of: 3000 mL m^{-2} d^{-1} O_2 and 21,000 mL m^{-2} d^{-1} CO_2 at 23°C and 1 atm. Bags were heat sealed generating a passive modified atmosphere and stored at 5°C for 13 days. One extra sample treated with SH was prepared, in order to simulate a humidified air condition (around 18% O_2, less than 1% CO_2 and ≥95% RH). It was packaged and sealed in a plastic bag with seven perforations made with a 0.7 mm diameter needle. Three replicates were performed for each treatment. The evolution of O_2 and CO_2 inside the plastic bags was monitored periodically by taking gas samples with a 10 mL syringe and injected into a gas chromatograph (Hewlett Packard 5890 Series II, Palo Alto, CA, USA) equipped with a thermal conductivity detector.

The evolution of psychrophic bacteria counts during cold storage was monitored periodically placing the samples (10 g) in sterile bags with peptone water 0.1% (90 mL) and agitated in a stomacher. Serial dilutions were plated onto Plate Count Agar, (Merck, Darmstadt, Germany) and incubated (5°C, 7 days). Three samples per treatment were analyzed.

The total phenolic content was estimated using the Folin-Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965) using Gallic acid as a standard phenolic compound. The extract was prepared from 1 g of fresh leaves homogenized with 9 mL 80% Methanol. The extracts (500 µL) were mixed with 5 mL of sodium tartratecarbonate buffer and 2.5 mL of 0.2 N Folin-Ciocalteu reagent. After 1 h of incubation at room temperature, the absorption of the reaction mixture was measured at 660 nm. The calibration curve was obtained with methanolic solutions of gallic acid in the range of 0.425-0.085 mg mL^{-1}.

Antioxidant capacity was determined using FRAP methodology (Benzie and Strain, 1999). The antioxidant capacity of the samples was measured against a Trolox standard and was expressed as equivalents of Trolox g^{-1} of fresh weight (fw). The leaves were briefly frozen with liquid nitrogen, and extracted with 9 mL 80% ethanol. Aliquots (20 µL) of diluted extract were mixed with 900 µL of FRAP reagent. The FRAP reagent contained 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl plus 20 mM $FeCl_3$ and 0.3M acetate buffer. The absorbance of the reaction mixture was measured at 595 nm. The calibration curve was obtained with ethanolic solutions of Trolox in the range of 0.012-0.225 mg mL^{-1}.

RESULTS AND DISCUSSION

Modified atmosphere packaging (MAP)

The gas evolution inside the plastic bags generated by the passive modified atmosphere is presented in Figure 1. The figure also shows the gas profile in the perforated bag (PB), which represents the external atmosphere conditions with more than 18% O_2 and less than 1% CO_2. Oxygen levels decreased and CO_2 levels increased in all MAP packages during storage at 5°C due to watercress respiration and gas permeability of the plastic bag. The oxygen level was reduced to a range of 4.3-6.4% after 13 days of storage (Figure 1). Carbon dioxide levels promptly increased to more than 10% in the first 5 days. The concentration of CO_2 kept increasing during the 13 days of storage, reaching values from 11 to 16%. Differences in CO_2 levels were observed by different washings after 5 days of storage. Treatments with ASC turned to be the highest producers of CO_2, which correlates with the highest O_2 consumption, implicating that the highest respiration rates are associated with

accelerated senescence. These results were in agreement with another study that assessed the effect of seven commercial sanitizers on fresh-cut escarole and lettuce. Those authors showed similar composition of gas headspace within bags treated with ASC, PAA, CD, and SH (Allende et al., 2008).

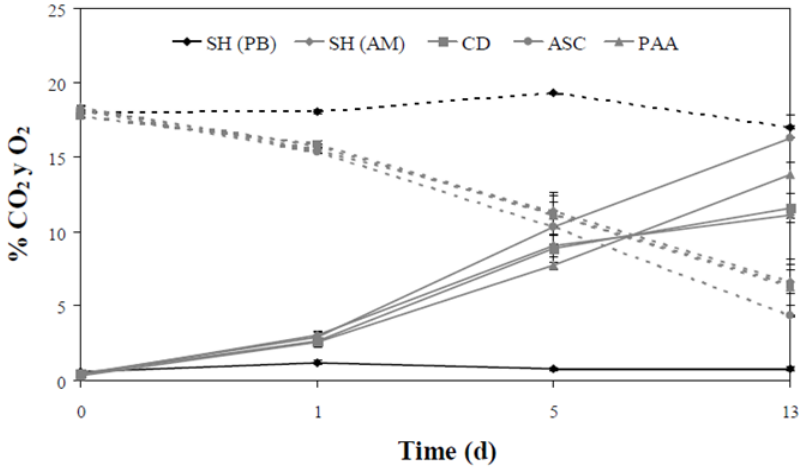

Figure 1. Changes in O_2 and CO_2 levels (%) in watercress treated by different sanitizers and kept under MAP during 13 days at 5°C: (—) CO_2; (---) O_2.

Growth of the psychrotrophic bacteria is represented in Figure 2. The microbial load in raw material was 6.4±0.1 log CFU g^{-1}. Initial microbial reduction was observed for most sanitizers tested. ASC and SH (MAP) had the greatest effect (with 2.3 and 1.5 log units reductions, respectively). The rest of the sanitizers reduced about 1 log CFU g^{-1} of psychrotrophic bacteria compared with the initial load. In spite of this, all the sanitizers maintained counts at 7 log CFU g^{-1} on day 13. The gas levels reached by MAP added a slight inhibitory effect on psychrotrophic bacteria proliferation, as shown by lower counts obtained from SH treatment packed under MAP compared to the same sanitizer packed in a perforated bag (Figure 2). This behaviour may have been caused by the reduced levels of oxygen available for aerobic microorganisms.

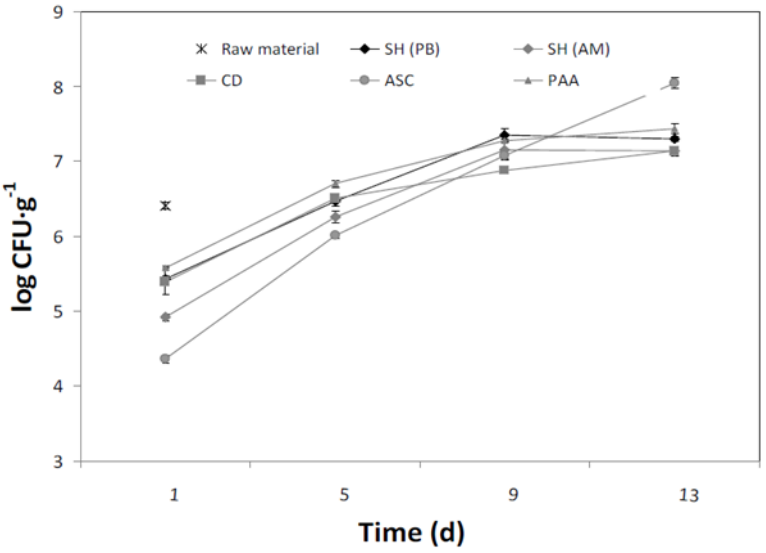

Figure 2. Evolution of psychrotrophic bacteria in watercress treated by different sanitizers and kept under MAP during 13 days at 5°C.

Functional compounds

The total phenolic content evolution during cold storage is shown in Table 1. Raw material contained 3.4 mg GAE g^{-1} fw. The phenolic content did not change with any of the sanitizer treatments immediately after washing. Total phenolics were retained during 13 days of storage. A slight increment (0.6 mg GAE g^{-1} fw) was observed in watercress packaged in perforated bags.

Table 1. Functional compounds in watercress treated by different sanitizers and kept under MAP during 13 days at 5°C.

	Functional compounds				
	Total phenolic compounds (mg GAE)			Antioxidant capacity (mg Trolox equiv)	
	Days				
	1	7	13	1	13
Raw material	3.4±0.061			1.47±0.16	
SH (BP)	3.2±0.138	3.3±0.226	3.8±0.122	2.21±0.12	1.18±0.37
SH (AM)	3.4±0.024	3.1±0.170	3.4±0.270	2.51±0.10	2.43±0.06
CD	3.4±0.112	2.5±0.217	3.2±0.367	2.09±0.54	2.00±0.19
ASC	3.3±0.271	2.8±0.166	3.2±0.342	1.87±0.26	1.74±0.12
PAA	3.4±0.308	2.6±0.089	3.2±0.128	2.21±0.05	1.84±0.08

The antioxidant activity was measured at the beginning and at the end of storage (Table 1). Raw material presented an antioxidant activity of 1.4 mg g^{-1} of Trolox equivalents. All treatments showed an increased antioxidant capacity during the first day of storage. Apparently, the fresh-cut process increases the initial antioxidant capacity of watercress for all treatments. A slight delay was observed after 13 days of storage. MAP gas levels showed a protective effect on the antioxidant activity because a large decrease in values for leaves packed in the perforated bags.

CONCLUSIONS

All tested sanitizers reduced the initial microbial load and could be used as an alternative to SH. The greatest effect was observed for ASC treatment. The combination of sanitizing treatment and modified atmosphere packaging turned out to be a useful tool to keep the bioactive compounds levels (antioxidant capacity and phenolic activity) as well as extend the shelf-life of watercress stored at 5°C.

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