academicJournals

Vol. 12(18), pp. 2456-2463, 1 May, 2013
DOI: 10.5897/AJB2012.2997
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Influence of sanitation on the physico-chemical and microbiological quality of organic and conventional broccoli

Giuseppina Pace Pereira Lima[1]*, Tatiana Marquini Machado[1], Natalia Reis Furtado[1], Luciana Francisco Fleuri[1], Marizete Cavalcante de Souza Vieira[1], Luciana Manoel Oliveira[2] and Fabio Vianello[3]

[1]Department of Chemistry and Biochemistry, Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil.
[2]Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), Campus Avaré, São Paulo, Brazil.
[3]Department of Comparative Biomedicine and Food Science, University of Padova (UNIPD), Padova, Italy.

Accepted 12 April, 2013

The present study was carried out in order to evaluate the effect of chlorinated and ozonized water on the physico-chemical characteristics of broccoli, produced under organic and conventional cultivation procedures. Organic and conventional broccolis were subjected to two sanitation treatments, using chlorine and ozone, and were kept under cold storage for seven days. Analyses of pH, titrable acidity, soluble solids and weight loss were performed and the content of Cu, Mn and Zn was determined. In addition, the presence of pesticides was verified. The results show no influence of the cultivation method or the sanitation treatment on sample weight loss. Cultivation methods and sanitizing treatments affected broccoli pH, titratable acidity, and soluble solids during the evaluation period. No differences regarding the metal content on organic and conventional broccoli were observed. Furthermore, the presence of organochlorine compounds, nor other pesticides, was not detected both in organic and conventional vegetables.

Key words: *Brassica oleracea* var. *Italica,* pesticide residues, titrable acidity, soluble solids, pH, metals, ozone, sanitation treatment.

INTRODUCTION

The consumption of organically grown vegetables has increased in recent years due to several reasons, such as consumer wish for foods free from pesticides, with higher levels of some nutrients and with increased content of compounds with antimutagenic potential. Several studies have been conducted on broccoli grown according to organic and conventional manure, which show that, when grown organically, this vegetable has a higher level of some phytochemicals (Naguib et al., 2012). Organic agriculture is an alternative cultivation model, involving the practice of vegetable production without chemical additives or even growth hormones (Seufert et al., 2012). As all other vegetables, when broccoli are subjected to uncontrolled storage conditions, physiological changes can quickly lead to senescence, making tissues more susceptible to attack by

*Corresponding authors: gpplima@ibb.unesp.br

micro-organisms. Thus, the control of physiological changes and the maintenance of post-harvest quality can increase the lifetime of these vegetables (Pinheiro et al., 2013).

The quality of vegetables is related to their appearance, soluble solids content, acidity, pH, texture and flavor. Various techniques can increase fruit and vegetable lifetime and, among these, the use of sanitizers has been proven effective, since it acts in the control of micro-organism growth, which induces deterioration. Chlorine is by far the most commonly used sanitizer at 100 to 200 µL L^{-1}, due to its low cost. However, it can generate carcinogens, such as trihalomethanes (THMs), which are formed when chlorinated water is in contact with humic and fulvic acids, products of organic matter decomposition (Gallard and von Gunten, 2002). The amount of THM clearly reflects water quality and the adopted practice of chlorinated water treatment, which is different in different countries. In Germany, as an example, where water contains low concentrations of total reduced organic carbon (2 mg L^{-1}) and chlorine dosage is limited below 1 mg L^{-1}, the concentrations of resulting THM are lower than the ones found in United States (Meyer, 1994). Thus, it is important that chlorine containing water be free from organic matter and have a pH between 6.0 and 7.0. Total chlorine up to 200 µL L^{-1} is allowed by law, although 50 to 100 µL L^{-1} is usually sufficient for a proper sanitation, if water pH is maintained between 6.0 and 7.0 (Gorny and Zagory, 2002). Therefore, it should not be used in food products from organic farming. Other options are acetic acid, iodine, hydrogen peroxide and ozone (Klaiber et al., 2005). The germicidal effect of ozone differs from that of other sanitizers, by rapidly and directly affecting the cell wall of pathogens, causing cell rupture and death. Depending on the type of micro-organism, ozone can act up to 3 thousand times faster than chlorine in inactivating cells (Chiattone et al., 2008). The concentration of ozone used as a sanitizer can vary from 0.02 (Chiattone et al., 2008) to 4.5 ppm (Ölmez and Akbaş, 2009). Due to the increased consumption of organic foods and to the impossibility to use chlorine sanitation, it becomes necessary to search for other sanitizers, which may be used in organic, as well as in conventional, vegetable and fruit production.

One of the guidelines of organic production is the offer of foods characterized by quality attributes, such as the absence of chemical residues and synthetic additives, leading to high quality products, free of chemical, physical or biological contaminants (Seufert et al., 2012), and ozone may be an option for these purposes.

MATERIALS AND METHODS

Broccoli (*Brassica oleracea L.* cv *Italica*), produced by organic and conventional cultivation, were purchased from local producers in the city of Botucatu, (SP) Brazil. The approximate geographical coordinates are 22°44'50'' latitude South and 48°34'00'' longitude west (Greenwich), with an altitude of around 765 m. The climate type is mesothermal, humid subtropical, rainy during the winter period. The total average annual rainfall is of 1,534 mm, presenting an average for the wettest month (January) of 242 and 38 mm for the driest months (July and August). The average annual temperature is 21°C.

Plants of the same cultivar and of the same physiological age were purchased, in order to ensure the comparison. Plants were harvested early in the morning and selected. Broccolis, after being selected, were washed to remove large impurities, and were subjected to sanitation treatments with chlorine or ozone. Ozone treatment was carried out twice. Control experiments were carried out with tap water in order to simulate home or industry practices.

The sanitation treatment with chlorine consisted of the immersion of broccoli in water containing 0.1% sodium hypochlorite for 5 min, while the treatment with ozone was performed by plant immersion in 186 L container, coupled to an ozone generator for 5 and 10 min (0.5 ppm per minute). Controls consisted of the immersion of broccoli in tap water (pH 6.5) for 5 min. Then, broccoli were selected again, gathered in bundles and stored in cold room at 6±1°C for 7 days, at a relative humidity of 93±1% (Extech RH 520A thermohigrometer). Samples were collected at the time of arrival from the field (time 0), after washing with water to remove larger impurities, after sanitation (time 1) and after 4 and 7 days of cold storage.

The experimental design was completely randomized, consisting of eight treatments: organic or conventional broccoli immersed in water for 5 min, immersed in 0.1% chlorinated water for 5 min, or soaked in ozonized water for 5 and 10 min. Each treatment consisted of three replicates of two lots of commercial broccoli. The experimental parcels (treatments) were constituted of nine plants, performing three repetitions, each with three broccoli plants.

The weight loss percentage was calculated from Equation 1, where WL = weight loss (%) W_i = initial weight of the vegetable (g) and W_j = weight of the vegetable in a subsequent period (g).

$$WL (\%) = [(W_i - W_j) / W_i] \times 100 \quad (1)$$

For the analysis of pH (model HI 4221, Hanna Instruments Brazil), samples (10 g) were weighed and homogenized with 10 ml of distilled water. The pH value was determined by potentiometry, using pH buffer solution (4.01 and 7.01). Instrument calibration was carried out daily.

The soluble solids (SS) were determined by direct reading on a digital refractometer (Schmidit/Haensch, model DHR-60) and the results were expressed in °Brix.

The titratable acidity (TA) was determined according to the methodology by IAL (2005). After homogenization of broccoli (10 g in 100 mL of distilled water), the suspension was titrated with a sodium hydroxide solution (0.1 N NaOH), using phenolphthalein as indicator. The SS/TA ratio was determined according to the method of Tressler and Joslyn (1961).

The determination of copper, manganese and zinc was performed by atomic absorption spectrometry, using a graphite furnace, after sample mineralization. Measurements were carried out with a Shimadzu model AA-6800 instrument, equipped with background absorption correction by a deuterium lamp system, a pyrolytic graphite tube with integrated platform and an ASC-6100 autosampler. Hollow cathode lamps (Shimadzu) operated at 10 mA for copper, manganese and zinc, were used. The wavelength used was 324.7 nm for copper, 279.5 nm for manganese and 213.8 nm for zinc, with a spectral resolution of 0.5 nm. Argon was used as inert gas during the heating program, at a constant flow of 1 mL min^{-1}, except in the atomization step, in which the gas flow was stopped. The absorbance peak area signals were determined (Neves et al., 2009).

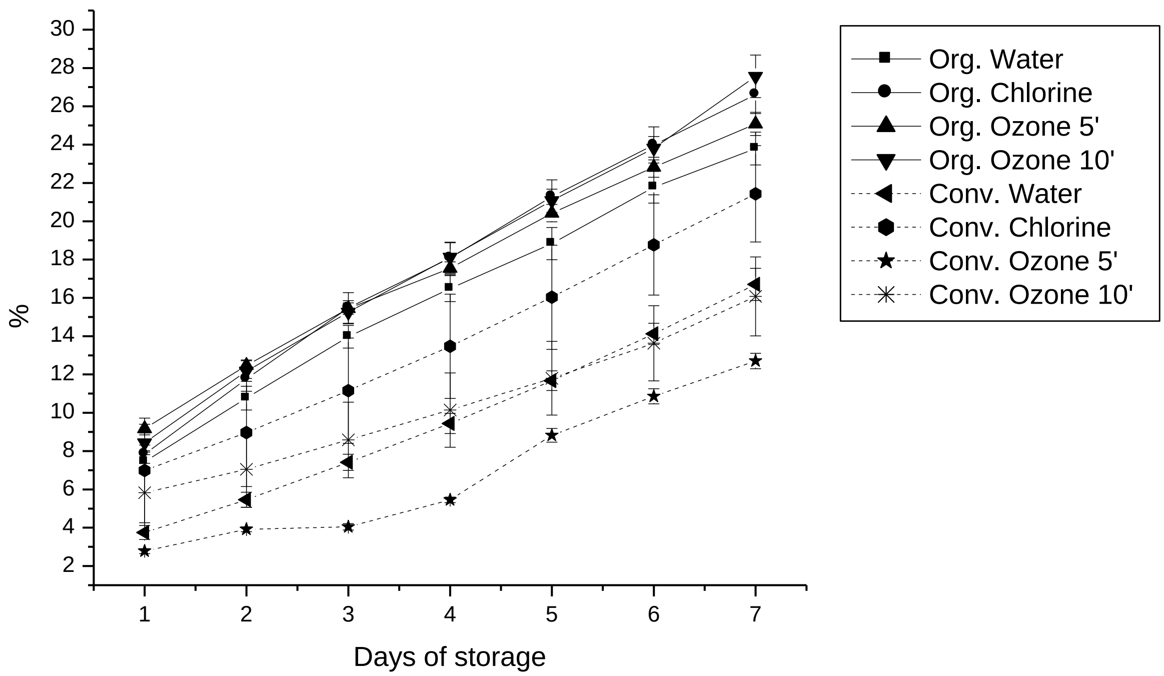

Figure 1. Weight loss (%) in organic and conventional broccoli sanitized with water, chlorine or ozone.

Pesticide residues were qualitatively analyzed by grinding plant samples (5 g) and extracting with n-hexane (20 mL). The identification was performed by thin layer chromatography, according to the methodology described by Moraes et al. (1991). The results were compared with Diazinon, Malathion, Clhorpiryfos, Carbofuran and Aldica standards.

For microbiological analysis, vegetables were pulverized in liquid nitrogen and an aliquot of 25 g was resuspended in 225 mL of sterile saline peptone solution (0.1%). The vegetable suspension were diluted in 0.1 M sodium phosphate buffer, pH 7.0, previously sterilized at 121°C, 1 atm, for 20 min and inoculated into culture media for the verification of the presence of fungi and bacteria. All tests were performed in triplicate as described by Manafi (2000) and APHA (2001). For fungi detection, suspension aliquots (100 µL) of the plant extract were inoculated on the surface of fungal agar medium (Himedia) in Petri dishes, previously sterilized and spread with a Drigalski handle. Plates containing the culture medium and the inoculum were incubated at 30°C for 120 h. For the detection of *Staphylococcus aureus*, *Salmonella sp.* and *Escherichia coli*, plant extract aliquots (100 µL) were inoculated in Petri dishes containing, *Staphylococcus* agar no110 (Himedia), Salmonella Agar Shigella M108 (Himedia) and agar Hicoliforme Rapido (Himedia), respectively. The plates containing the culture medium and the inoculum were incubated at 37°C for 24 h. The culture media used were chromogenic and allowed the identification of the bacteria, according the specific staining after growth.

For the detection of fungi, *Salmonella* and *E. coli* was verified by presence (+) or absence (-) of colonies and the total numbers (UFC g^{-1}) of *S. aureus* was estimates by a direct-counting technique. For the detection of Total Coliforms and Fecal Coliform, the technique of the most probable number (MPN) method was used. The presumptive test was performed by distributing, in triplicate, extract aliquots (100 µL) in inverted Durham tubes, containing sodium lauryl sulphate broth (LST), which were incubated at 35°C for 48 h. The tubes showing turbidity and gas evolution were considered as positive for the presence of total coliforms. Then, aliquots of these positive cultures (100 µL) were seeded into inverted Durham tubes containing *E. coli* broth (EC medium) (5 mL) and incubated for 24 h in water bath at 44.5°C. Turbidity and gas production within the medium were considered for test positivity. All the results of these readings were expressed as most probable number of bacteria per gram of analyzed material (MPN g^{-1}).

For metal and pesticide analyses, samples from day 1 (after treatments) were used. The analysis of weight loss was performed daily, for seven days. Microbiological analyses were performed at 0, 1, 4 and 7 days for fungi, *Salmonella, E. coli* and *S. aureus,* and at 0, 1 and 7 for different types of coliforms. The data were subjected to analysis of variance (ANOVA) and means were compared by the Scott-Knott test ($p < 0.05$), using the Assistat version 7.6 program (Silva and Azevedo, 2009).

RESULTS AND DISCUSSION

A tendency, although not statistically significant, showing higher weight loss values in organic broccoli, for all the sanitization treatments during cold storage, than in conventional samples (Figure 1), was observed. This weight loss gradually increased during the experimental period. The initial value was 6.52 and 21.25% at the last day of evaluation. Generally, weight loss is attributed to water loss, and transpiration is the most important cause of deterioration in post-harvest vegetables (Brackmann et al., 2005), resulting in quantitative and qualitative appearance, and losses in textural qualities and nutritional properties (Reis et al., 2006).

Both chlorine and ozone treatments also induced a higher water loss in organic broccoli, compared with conventional plants and with samples simply washed with

Table 1. Effect of water-washings, chlorine and ozonized water washings on pH and titratable acidity (TA) of organic and conventional broccoli.

Treatment	Days of analysis							
	pH				Titratable acidity (TA)			
	0	1	4	7	0	1	4	7
Org/Water	5.35^{bC}	6.70^{aA}	6.35^{aB}	6.39^{aB}	0.59^{aA}	0.17^{dC}	0.28^{aB}	0.29^{aB}
Org/Chlorine	5.35^{bB}	6.53^{aA}	6.47^{aA}	6.39^{aA}	0.59^{aA}	0.27^{cC}	0.23^{aC}	0.34^{aB}
Org/Ozone 5’	5.35^{bB}	5.33^{cB}	6.43^{aA}	6.47^{aA}	0.59^{aA}	0.23^{cC}	0.20^{bC}	0.29^{aB}
Org/Ozone 10’	5.35^{bB}	6.48^{aA}	6.51^{aA}	6.45^{aA}	0.59^{aA}	0.25^{cC}	0.17^{bD}	0.34^{aB}
Conv/ Water	6.42^{aA}	6.29^{bA}	6.37^{aA}	6.37^{aA}	0.59^{aA}	0.44^{bB}	0.17^{bC}	0.17^{bC}
Conv/Chlorine	6.42^{aA}	6.39^{bA}	6.53^{aA}	6.36^{aA}	0.59^{aA}	0.40^{bB}	0.17^{bC}	0.16^{bC}
Conv/Ozone 5’	6.42^{aB}	6.21^{bB}	6.62^{aA}	6.36^{aB}	0.59^{aA}	0.57^{aA}	0.15^{bB}	0.15^{bC}
Conv/Ozone10’	6.42^{aA}	6.33^{bA}	6.35^{aA}	6.45^{aA}	0.59^{aA}	0.52^{aB}	0.15^{bC}	0.16^{bC}
C.V.	1.99				11.66			

The CV (coefficient of variation) at $p < 0.05$ is indicated. Different letters within the same line (capital) and column (lowercase) differ significantly (n=3) by Scott-Knott test. Cv, conventional cultive; Org, organic cultive; C, cultive; S, sanitation.

tap water. Several studies showed that weight loss does not depend on the cultivation practice, even if some authors found differences in vegetables of organic origin. According to Schuphan (1974), collenchyma of organic plants is thicker than in conventional ones, leading to a reduction of water loss. In the present work, we noted that organic broccoli showed a higher weight loss, even if without significant differences, with respect to conventional ones, and with different values from those reported in literature (Santos et al., 2001), independently of the sanitation treatment used.

Furthermore, in the sample characterization at day 0, the pH value of organic broccoli was lower than that of plants cultivated in a conventional manner (Table 1). Considering sanitizing treatments, only organic broccoli treated with tap water showed a decrease of pH during storage. No significant changes of pH values were observed during the evaluation period in conventional broccoli, upon washing in tap and chlorinated water while, when conventional broccoli were subjected to ozonization treatment for 5 min, sample pH increased in the fourth day of analysis, similar to that observed at 10 days of storage.

Lower pH values were observed in organic broccoli at plant arrival from the field. This result was reversed during storage, and no significant differences were found at the final days of storage. The values of pH found in the present study were consistent with those reported by Artés et al. (2001), reporting on variations from pH 6.50 to 6.72.

In organic broccoli washed with tap water, titrable acidity decreased immediately after washing (1 day), regardless the sanitizer used, while it increased at the seventh days of storage. In conventional broccoli, all considered sanitation treatments induced a decrease in titrable acidity during storage (Table 1). In organically grown broccoli, higher values of TA occurred at the seventh day of analysis, regardless of the treatment used for sanitizing.

Probably, the increase of pH values over time, observed with all treatments, corresponds to the observed decrease in TA (Table 1), both in organic and in conventional broccoli. Titrable acidity indicates the presence of organic acids in plants. With few exceptions, a low acidity in vegetables increases the susceptibility of attacks by bacteria and fungi, thus reducing the plant shelf-life (Pitt and Rocking, 2009). This is an important factor in the choice of the sanitizer, which is supposed to maintain the lowest contamination by micro-organisms, as well as to preserve the other quality parameters.

Soluble solid content in organic broccoli decreased after immersion in tap water or sanitized with chlorine and ozone for 10 min; interestingly, it increased from the fourth day of storage (Table 2).

A decrease in soluble solid content was observed in conventional broccoli after all the sanitation treatments. Conversely, when immersed in tap water, soluble solids showed a considerable increase at the last day of analysis, reaching a value of 9.75 °Brix.

Conventional and organic production systems differ in the amount of irrigation water, in the amounts of nutrients used in fertilizers and in organic matter applied to soil, especially regarding nitrogen, which, along with other factors, may influence the synthesis of soluble solids and other substances (Vallverdú-Queralt et al., 2012). Generally, organic manure is richer in nitrogen containing compounds. Therefore, it would be expected to reduce the overall soluble solids in organic broccoli. However, this effect was not observed in the present study. Py et al. (1984) reported that an increase of nitrogen manuring, which generally causes a decrease of plant acidity, may or may not reduce soluble solids (SS). Generally, SS increase with postharvest elapsing time by the progress of biosynthetic processes or by degradation of the cell

Table 2. Effect of water-washings, chlorine and ozonized water washings on Soluble Solids (SS) (°Brix) and SS/TA ratio on organic and conventional broccolis.

Treatment	Days of analysis							
	Soluble solid (SS)				SS/TA Ratio			
	0	1	4	7	0	1	4	7
Org/Water	8.95^{aB}	5.87^{cD}	8.30^{bC}	9.63^{bA}	15.17^{B}	34.53^{aA}	29.64^{dA}	33.21^{dA}
Org/ Chlorine	8.95^{aB}	7.27^{bC}	7.47^{cC}	9.37^{bA}	15.17^{aC}	26.93^{bB}	32.48^{dA}	27.56^{eB}
Org/ Ozone 5’	8.95^{aC}	8.67^{aC}	9.23^{aB}	11.35^{aA}	15.17^{aC}	37.70^{aB}	46.15^{bA}	39.13^{cB}
Org/Ozone 10’	8.95^{aA}	6.80^{cB}	9.03^{aA}	8.73^{cA}	15.17^{aC}	27.20^{bB}	53.11^{aA}	25.68^{eB}
Conv/ Water	8.67^{aB}	6.47^{cC}	6.43^{dC}	9.75^{bA}	15.17^{aC}	14.70^{cC}	37.82^{bB}	57.35^{cA}
Conv/Chlorine	8.67^{aA}	4.74^{eC}	7.37^{cB}	8.60^{cA}	15.17^{aC}	11.85^{cC}	39.92^{cB}	53.75^{bA}
Conv/Ozone 5’	8.67^{aA}	6.83^{cD}	7.53^{cC}	8.20^{dB}	15.17^{aB}	11.19^{cB}	49.00^{bA}	54.67^{bA}
Conv/Ozon10’	8.67^{aB}	7.43^{bC}	7.70^{cC}	9.40^{bA}	15.17^{aC}	14.28^{cC}	51.33^{bB}	58.75^{aA}
C.V.	3.03				9.89			

The CV (coefficient of variation) at $p < 0.05$ is indicated. Different letters within the same line (capital) and column (lowercase) differ significantly (n=3) by Scott-Knott test. Cv, conventional cultive; Org, organic cultive; C, cultive; S, sanitation.

wall polysaccharides (Puerta-Gomez and Cisneros-Zevallos, 2011), which may explain the variations observed and not only the effect of the sanitizers.

The germicidal effect of chlorine is not selective, while ozone possesses two modes of action, a selective and a non-selective one. The use of both these sanitizers could affect SS content because their action may damage plant cells, contributing to the change of cytoplasmic substances (Chiattone et al., 2008), which would influence SS content in broccoli. However, this effect was not clearly observed in the present study.

As a consequence, an increase of the SS/TA ratio was observed in organic broccoli immediately after washing and regardless of the type of sanitizer used. The SS/TA ratio remained constant until the 4th day of storage (Table 2). At day 0, no significant differences between the modes of broccoli cultivation were found. Soon after sanitation, the highest SS/TA ratio values were observed in organic broccoli, with values higher than in control. At day 4, organic broccoli treated with ozonized water for 10 min showed the highest SS/TA ratio values, whereas the highest values on the seventh day of evaluation was found in conventional broccoli after sanitization with ozonized water for 10 min. It appears that this treatment induced an increase in the SS/TA ratio, either in organic or conventional farming.

In the present work, our results showthat the use of ozone did not affect the SS/TA ratio, thereby no detrimental effect on this quality parameter was observed. Interestingly, the SS/TA ratio is one of the parameters used to evaluate fruit and vegetable flavor, that is the higher the values for the SS/TA ratio, the better the taste.

The analyses of metals (Cu, Mn and Zn) were performed immediately after sanitation, showing no effect of cultivation procedure or sanitizer treatment. Organic broccoli showed a copper content of 5.2 mg kg^{-1}; 26 mg kg^{-1} of manganese and 40.2 mg kg^{-1} of zinc; whereas in conventional broccoli, 4.8 mg kg^{-1} of copper; 21.8 mg kg^{-1} of manganese and 46.2 mg kg^{-1} of zinc, were detected.

In a review on the differences between organic and conventional vegetables, Worthington (2001) reported on 41 studies on plant mineral content. Results indicate that regarding copper and zinc, in around 10% of reviewed papers, the highest metal content was found in organic food, differently from the data obtained in the present study. Regarding manganese, the same author found that around 50% of the considered studies showed higher Mn levels in organic vegetables than in conventional ones. Also in this case, our findings did not corroborate this data. Other works reported no significant differences in plant manganese and zinc content, regarding cultivation method, as in the study on leaves and stems of *Vitis vinifera* L. grown in the two cultivation modes (Nikolaidou et al., 2010). Similar results were obtained by Warman and Havard (1997) on potatoes and corn produced in the two ways for three consecutive years, where showed no significant differences in Cu, Mn and Zn content in tubers and leaves were found.

As regards organochlorine and other pesticide residues, no differences between the two cultivation modes, organic and conventional, were found in the studied broccoli samples, since analyzed pesticides were not detectable. Other studies reported on the presence of contaminants in conventionally grown vegetables, such as in organic and conventional beets, in which organochlorines were found in leaves and peels. The absence of pesticides, as qualitatively determined, was observed both in organic and conventional vegetables. Accordingly, the absence of pesticides in organic vegetables, by qualitative analysis, was already shown by Lima et al. (2012). This finding is important in order to

Table 3. Effect of water-washings, chlorine and ozonized water washings on microorganisms of organic and conventional broccoli during of storage in cold chamber.

Treatment	Microrganism																					
	Fungi				*Salmonella* sp.				*E. coli*				*S. aureus* (CFU g^{-1})				Coliforms (MPN g^{-1})			Thermotolerant coliforms (MPN g^{-1})		
	0	1	4	7	0	1	4	7	0	1	4	7	0	1	4	7	0	1	7	0	1	7
Org/Water	+	+	+	+	-	-	-	-	-	-	-	-	$2.2x10^{4a}$	$<10^{b}$	$<10^{b}$	$<10^{b}$	$9.1x10^{8a}$	$2.4x10^{5c}$	$4.6x10^{7b}$	$4.6x10^{6a}$	93^{b}	$2.3x10^{6a}$
Org/Chlorine	+	-	+	+	-	-	-	-	-	-	-	-	$2.2x10^{4a}$	$1.7x10^{2b}$	$2.0x10^{3b}$	$3.7x10^{2b}$	$9.1x10^{8a}$	$2.4x10^{5b}$	$2.1x10^{5b}$	$4.6x10^{6a}$	36^{c}	$1.1x10^{4b}$
Org/ Ozone 5’	+	-	-	-	-	-	-	-	-	-	-	-	$2.2x10^{4a}$	$<10^{b}$	$<10^{b}$	$<10^{b}$	$9.1x10^{8a}$	$2.4x10^{5c}$	$2.3x10^{7b}$	$4.6x10^{6a}$	43^{c}	$1.1x10^{5b}$
Org/Ozon10’	+	-	-	-	-	-	-	-	-	-	-	-	$2.2x10^{4a}$	$<10^{b}$	$<10^{b}$	$<10^{b}$	$9.1x10^{8a}$	$2.4x10^{5c}$	$2.4x10^{6b}$	$4.6x10^{6a}$	36^{c}	$9.1x10^{4b}$
Conv/ Water	+	+	+	+	-	-	-	-	-	-	-	-	$1.0x10^{2b}$	$7.5x10^{3a}$	$7.6x10^{3a}$	$5.7x10^{3a}$	$1.5x10^{6b}$	$2.4x10^{5c}$	$2.3x10^{7a}$	$9.1x10^{4b}$	-	$4.6x10^{6a}$
Conv/Chlorine	+	+	-	-	-	-	-	-	-	-	-	-	$1.0x10^{2a}$	$<10^{b}$	$<10^{b}$	$<10^{b}$	$1.5x10^{6b}$	$2.4x10^{5c}$	$1.5x10^{7a}$	$9.1x10^{4b}$	-	$2.4x10^{6a}$
Conv/Ozone 5’	+	-	-	-	-	-	-	-	-	-	-	-	$1.0x10^{2a}$	$<10^{b}$	$<10^{b}$	$<10^{b}$	$1.5x10^{6b}$	$1.1x10^{5c}$	$1.1x10^{7a}$	$9.1x10^{4b}$	-	$4.6x10^{6a}$
Conv/Ozon10’	+	-	-	-	-	-	-	-	-	-	-	-	$1.0x10^{2a}$	$<10^{b}$	$<10^{b}$	$<10^{b}$	$1.5x10^{6b}$	$1.1x10^{5c}$	$1.1x10^{7a}$	$9.1x10^{4b}$	-	$4.6x10^{6a}$

The bars represent standard deviations. Values with different characters (lowercase) in line for the same treatment time differ significantly ($p < 0.05$). +, Presence ; -, absence. CFU, colony-forming units; MPN, most probable number.

characterize organic cultivation mode, and it is important, as well, for consumers of conventional broccoli. Certainly, pesticides used by conventional farmers had already been degraded, or it may be that the washing procedure on broccoli was effective in residue elimination. Thus, in the present work, residue content does not give any additional information about the differences between organic and conventional broccoli.

Fungi were detected in conventional broccoli, immersed in tap water and treated with chlorinated water (Table 3). The treatments with ozone 5’ and 10’ showed no fungal growth in tested samples and the elimination of fungi occurred short after the processing. Our results shows the efficiency of ozone in the elimination of fungi, even if other studies reported that, even at longer ozone exposure times, the appearance of fungi, coliforms and *S. aureus* (Najafi and Khodaparast, 2009) was determined. During the post-harvest period, the presence of micro-organisms can compromise vegetable life-time and the use of ozone for eliminating fungi can be recommended for broccoli.

In conventional broccoli, the treatment with chlorine and ozonized water for 5 and 10 min was effective in reducing microbial load as *S. aureus* (Table 3). The efficiency of ozone can also be observed in organic broccoli.

The culture media chromogenic and fluorogenic are some specific bacterial serotypes. The medium Salmonella agar Shigella M108 (Himedia) allows to detect serotypes typhimurium (14028), typhi (6539) and enteritidis (13076). The medium agar Hicoliforme Rapido (Himedia) used the enzyme β-glucoronidase for detection of *E. coli*, however the main characteristics that distinguish E. coli O157: H7 (Enterohaemorrhagic *E. coli*, EHEC) from other strains are poor or no growth at 44°C and the inability of using the sorbitol and produce the enzyme β -glucoronidase. These differences were not detected in analyzes of fecal coliforms by the most probable number method (MPN) which utilizes the fermentation of lactose to 44.5°C and neither in direct analysis of *E. coli* using substrates for this enzyme.

It was found that considered samples studied did not show the presence of *Salmonella sp.*. The sanitary quality of organic and conventional broccoli remained within the standards of acceptability for human consumption, in relation to the absence of contamination by *Salmonella* (FDA, 2002). In mature and immature “cantalupo” melons (*Cucumis melo*) exposed to ozone gas (10,000 ppm for 30 min under reduced atmosphere) the *Salmonella* microbial load decreased, with a maximum reduction to 4.2 and 2.8 log CFU (Selma et al., 2.008), respectively.

As regards total and fecal coliforms (Table 3), sanitation treatments significantly decreased the number of microorganisms in conventional and organic broccoli after one day storage. However, after seven days, microbial load increased in both organic and conventional broccoli.

In relation to contamination by thermotolerant coliforms and *E. coli*, organic and conventional broccoli, stored for seven days, remained within the standards of acceptability for human consum-ption, according to FDA (2002) in relation to vegetable quality.

Other studies also confirmed the efficiency of ozone action as sanitizer. Yuk et al. (2007) studied the effect of ozone and citric acid on mushroom quality. They observed that the combination of ozone and citric acid was more effective in eliminating the presence of *E. coli* than the simple treatment with ozone. In lettuce, sanitized with ozone and chlorine, the treatment with ozone and chlorine induced a reduction of coliforms, compared with washing with tap water (Beltrán et al., 2005). Alexandre et al. (2011) studied the action of ozone and other sanitizers on the cold storage of strawberry, and they found that samples pre-treated with ozone showed a reduction of microbial load, in comparison to samples treated with ultrasound and UV radiation.

Conclusion

In the present study, broccoli, coming from organic and conventional cultivation practice, was analyzed. The different cultivation mode, organic or conventional, coupled with washing treatments, significantly influenced TA and SS. No influence of cultivation methods on Cu, Zn and Mn content was observed. Furthermore, the use of ozone did not alter the physico-chemical properties of broccoli, regardless the method of cultivation, suggesting that it may be a promising alternative as sanitizer. Finally, ozone was effective in reducing microbial load, as regards fungal and bacterial contamination, in broccoli and it can be successfully used to replace other sanitizers.

REFERENCES

Alexandre EMC, Santos-Pedro DM, Brandão TRS, Silva CLM (2011). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. J. Food Eng. 105:277-282.

APHA. American Public Health Association (2001). Compendium of methods for the microbiological examination of foods. Washington, DC: APHA.

Artés F, Vallejo F, Martínez JA (2001). Quality of broccoli as influenced by film wrapping during shipment. Eur. Food Res. Technol. 213:480-483.

Beltrán D, Selma MV, Marín A, Gil IM (2005). Ozonated water extends the shelf life of fresh-cut lettuce. J. Agric. Food Chem. 53:5654-5663.

Brackmann A, Trevisan JN, Martins GAK, Freitas ST, Mello AM (2005). Postharvest quality of 'Teresópolis gigante' cauliflower treated with ethylene, ethylene absorbent and 1-methylcyclopropene. Ciênc. Rural 35:1444-1447.

Chiattone PV, Torres LM, Zambiazi RC (2008). Aplication of ozone in industry of food. Alim. Nutr. 19:341-349.

FDA. U.S. Food and Drug Administration, 2002. Bacteriological Analytical Manual. Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070080.htm (Chapter 4a).

Gallard H, von Gunten U (2002). Chlorination of natural organic matter: kinetics of chlorination and of THM formation. Water Res. 36:65-74.

Gorny JR, Zagory D (2002). Food safety. http://globalripening.com/ne-postharvest.com/hb66/023foodsafety.pdf. acessed in 04.08.2013.

IAL. Instituto Aldolf Lutz (1985). Normas analíticas: métodos físicos e químicos para análise de alimentos. 3ed. Instituto Adolf Lutz, São Paulo. 533 p.

Klaiber RG, Baur S, Magel L, Hammes WP, Carle R (2004). Quality of shredded, packaged carrots as affected by different washing treatments. J. Food Sci. 69:SNQ161-SNQ166.

Lima GPP, Teixeira da Silva JA, Bernhard AB, Pirozzi DCZ, Fleuri LF, Vianello F (2012). Organic and conventional fertilisation procedures on the nitrate, antioxidants and pesticide content in parts of vegetables. Food Add. Contam.: Part B: Surveillance 5:188-193.

Manafi, M. (2000). New developments in chromogenic and fluorogenic culture media. Inter. J. Food Microb. 60:205-218.

Meyer SR (1994). Chlorine use in water disinfection, trihalomethane formation, and potential risks to public health. Cad. Saúde Públ. 10:99-110.

Moraes ECF (1991). Manual de toxicologia analítica. Roca, São Paulo. pp. 98-99.

Naguib AE-M, El-Baz FK, Salama ZA, Hanaa HAEB, Ali HF, Gaafar AA (2012). Enhancement of phenolics, flavonoids and glucosinolates of broccoli (*Brassica oleracea*, var. *Italica*) as antioxidants in response to organic and bio-organic fertilizers. J. Saudi Soc. Agric. Sci. 11:135-142.

Najarif MB, Khodaparast MHH (2009). Efficacy of ozone to reduce microbial populations in date fruits. Food Control 20:27-30.

Neves RCF, Moraes PM, Saleh MAD, Loureiro VR, Barros MM, Padilha CCF, Alves Jorge SM, Padilha PM (2009). FAAS determination of metal nutrients in fish feed after ultrasound extraction. Food Chem. 113:679-683.

Nikolaidou AE, Pavlatou-Ve AK, Kostopoulou SK, Mamolos AP, Kalburtji KL (2010). Litter quality and decomposition of *Vitis vinifera* L. residues under organic and conventional farming systems. European J. Soil Biol. 46:208-217.

Ölmez H, Akbas MY (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. J. Food Eng. 90:487-494.

Pinheiro J, Alegria C, Abreu M, Gonçalves EM, Silva CLM (2013). Kinetics of changes in the physical quality parameters of fresh tomato fruits (*Solanum lycopersicum*, cv. 'Zinac') during storage. J. Food Eng. 114:338-345.

Pitt JI, Rocking AD (2009). Fungi and food spoilage. In: Fresh and Perishable Foods. Springer Dordrecht Heidelberg London. pp 383-400.

Puerta-Gomez AF, Cisneros-Zevallos L (2011). Postharvest studies beyond fresh market eating quality: Phytochemical antioxidant changes in peach and plum fruit during ripening and advanced senescence. Postharvest Biol. Techn. 60:220-224.

Py C, Lacoeuilhe JJ, Teisson C (1984). L'ananas: sa culture, sés produits. Maisonneuve, Paris. 563 p.

Reis KC, Elias HHS, Lima LCO, Silva JDS, Pereira J. (2006). Japanese cucumber (*Cucumis sativus* L.) submitted of the treatment with cassava starch film. Ciênc. Agrotec. 30:487-493.

Santos RHS, Silva F, Casali VWD, Condé RA (2001). Post harvest conservation of lettuce cultivated with organic compost. Pesq. Agropec. Bras. 36:521-525.

Schuphan W (1974). Nutritional value of crops as influenced by organic and inorganic fertilizer treatments. Results of 12 years' experiments with vegetables (1960-1972). QuaI. Plant - P1. Fds. Hum. Nutr. XXIII 4:333-358.

Selma M.V., Ibáñez AM, Allende A, Cantwell M, Suslow T (2008). Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of Escherichia coli O157:H7 during cutting. Food Microb. 25:162-168.

Seufert V, Ramankutty N, Foley J. (2012). Comparing the yields of organic and conventional agriculture. Nature, 485:229-232.

Silva FASE, Azevedo CAV (2009). Principal components analysis in the software Assistat-Statistical Attendance. In: World Congress on computers in agriculture, 7, American Society of Agricultural and Biological Engineers, Reno-NV-USA.

Tressler DJ, Joslyn MA (1961). Fruits and vegetable juice processing. Westort: AVI, 1028 p.

Vallverdú-Queralt A, Medina-Remón A, Casals-Ribes I, Lamuela-Raventos RM (2012). Is there any difference between the phenolic content of organic and conventional tomato juices? Food Chem. 130:222-227.

Warman PR, Havard DA (1997). Yield, vitamin and mineral contents of organically and conventionally grown potatoes and sweet corn. Agric. Eco. Environ. 68:207-216.

Worthington, V. (2001). Nutritional quality of organic versus conventional fruits, vegetables, and grains. J. Altern. Compl. Med. 7:161-173.

Yuk HG, Yoo MY, Yoon JW, Marshall DL (2007). Effect of combined ozone and organic acid treatment for control of Escherichia coli O157:H7 and Listeria monocytogenes on enoki mushroom. Food Control 18:548-553.