

Does the use of ozonized water influence the chemical characteristics of organic cabbage (*Brassica oleracea* var. *capitata*)?

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Revised: 13 March 2015 / Accepted: 18 March 2015 / Published online: 29 March 2015
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Abstract The aim of this study was to assess the influence of different cultivation procedures (conventional and organic), different sanitizers (ozone and chlorine) during the post-harvest storage of cabbages hybrid Fuyutoyo (*Brassica oleracea* var. *capitata*). The cabbage plants were purchased directly from producers. At the end of the cropping cycle, which occurred 120 days after sowing, crop harvesting was carried out and the plants were immediately sanitized with water, chlorine and ozone. After cleansing, the cabbage plants were stored in a cooling chamber at 5 °C between 12 and 20 days. To predict the effect of commercialization, the cabbage head were removed between 12 and 20 days from the cooling chamber, one part was analyzed and the rest maintained in a local temperature (22±2 °C) for 4 days. The biochemical analysis of the following were determined: total phenols, total flavonoids, vitamin C, total chlorophyll, nitrate, polyamines and antioxidant activity after 0, 12 and 20 days storage and 4 days at room temperature (12+4 and 20+4 days

of storage), for market analysis. The biochemical analyses showed no statistical differences between conventional and organic cabbages. Thus, the consumption of organic or conventional cabbage provide the same content of antioxidant compounds analyzed and the sanitizing procedure (ozonated water) did not modify the antioxidant capacity of the plant.

Keywords Sanitation · Cultivation method · Ozone · Antioxidant · Polyamine

Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is the fifth vegetable most produced in Brazil and also consumed by worldwide people due to its nutritional composition, its versatility in fresh consumption and industrial processing. It is rich in a number of biologically active metabolites such as vitamins, phenolic acids, flavonoids and isothiocyanates, which are associated with antioxidant, antibacterial and anticancer properties and contribute to health promotion (Jaiswal and Abu-Ghannam 2013).

In some countries, such as in Germany, Belgium and Holland, chlorine is prohibited as sanitizing agent. In other European countries, its application as a sanitizer is restricted, since chlorinated wastes or by products must be absent by law in the final consumer product. In the U.S., sodium hypochlorite can be used, provided its removal, as much as possible, by washing in water to eliminate chlorinated wastes. In addition, worldwide consumers of organic products reject the use of chlorinated water for sanitization (Baur et al. 2004).

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The ozonated water can be used for cleaning post-harvest of fruits and vegetables to control the pathogens infections (Mahapatra et al. 2005) and ozone can destroy pesticides and chemical waste and convert organic materials biodegradable forms of non-biodegradable (Rodgers et al. 2004). In addition, due to its rapid decomposition to oxygen and the fact that it does not leave residues in the treated products, its application in food processing is permitted by organic certification (Selma et al. 2008a). Ozone presents advantages, and it is widely used for food hygiene, for waste water treatment for the elimination of tastes and odors, for the treatment of effluents, for the elimination of trihalomethanes and the removal of soluble iron and manganese. These effects including the disinfection from *Salmonella typhimurium*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Listeria monocytogenes* and *E. coli* O157:H7 (Selma et al. 2008b).

The ozone can also cause some damage to cells due to its oxidizing potential. The use of ozone may promote the production of numerous free radical species, such as hydroperoxyl ($\text{H}_2\text{O}^\bullet$), hydroxyl ($\cdot\text{OH}$), and superoxide ($\cdot\text{O}_2^-$) radical (Hoigné and Bader 1983), and in response the byproducts of ozone decomposition, the cells as a form of protection against oxidative damage, may form antioxidant compounds, aimed at protection and scavenging of free radicals.

To extend the life after harvest the low temperature soon after the harvest is the most used technique to prolong the preservation of fruits and vegetables. Temperature reduction causes the slowing down of enzyme reactions, especially those associated with respiration and senescence. This decrease in respiratory activity is the main post-harvest physiological process, and provides lower losses of physical and physicochemical quality attributes of fruits and vegetables, such as aroma, flavor, texture and color (Bron and Jacomino 2009).

The aim of the present study was to evaluate the influence of different cultivation systems and sanitation procedures on cabbage, evaluating some antioxidants compounds, such as phenolics, vitamin C, and polyamines, in order to explore the possibility to implement an alternative and safe sanitizer (ozone) to replace chlorine.

Materials and methods

Materials

Cabbages (*Brassica oleracea* var. *capitata*, hybrid Fuyutoyo) were grown on two farms, one in the area of conventional cultivation and the other was in an organic area, in the region of Botucatu, São Paulo State, Brazil, 22°53'09" S latitude and 48°26'42" W longitude and at 804 m altitude. Cabbages heads were collected early in the morning, selected and transported

immediately to the laboratory, and were subjected to sanitation treatments.

Sanitizing treatments

Cabbages grown under organic and conventional producing systems were subjected to immersion in sanitation solution. Subsequently, they were stored according to the “minimum temperature of security” at 5 °C and RH>90 %, for 0, 12, and 20 days. At 12 and 20 days, cabbages were removed from the cold chamber for analyses, and kept at room temperature for 4 days, to simulate commercialization.

The cabbages carefully selected (size, without damage or blemishes) were submitted at three sanitation treatments, chlorine (100 mg L⁻¹ sodium hypochlorite for 10 min) and ozone (two exposure times), 10 and 20 min and the control (immersion on distilled water). The equipment (Degradatox/OZ Engenharia, Indústria de Equipamentos Geradores de Ozônio-LTDA, Rio Grande do Sul, Brazil), was coupled in a tank of 180 L capacity, generates approximately 1 g of ozone per liter per second. The same tank was used for immersion in distilled water and in chlorinated water.

Phenol content

The determination of total phenol was performed (Singleton and Rossi 1965) using 100 mg sample fresh material and powered by manual grinding in liquid nitrogen. The tubes with the sample and 50 % acetone in water were incubated in an ultrasonic bath for 20 min and centrifuged at 6000×g (HettichZentrifugen, Mikro220R) for 10 min. The supernatants were re-extracted and combined. Folin–Ciocalteu reagent was added; after 3 min at 25 °C, a saturated solution of Na₂CO₃ (Merck) was added, and the reaction mixture was then incubated for 1 h. The absorbance was measured at 760 nm (Pharmacia Biotech, Ultrospec 2000), and the results were expressed on a 100 g⁻¹ fresh weight basis as mg gallic acid (GAE) (Sigma Aldrich, Brazil) equivalents.

Flavonoid content

The extraction of total flavonoid (Popova et al. 2004) was prepared with adjustments. Briefly, fresh material samples were powdered by manual grinding in liquid nitrogen, weighed and mixed with 10 % (w/v) acidified methanol (Sigma Aldrich, Brazil). The samples were subsequently placed in an ultrasonic bath for 30 min, and a 5 % aluminum chloride solution was added. The samples were then centrifuged for 20 min at 10,000×g (Jouan MR 12). Finally, the samples were filtered, and the absorbance was measured at 505 nm (Pharmacia Biotech, Ultrospec 2000). The results were expressed as mg flavonoids 100 g⁻¹ fresh weight and as rutin (Sigma Aldrich, Brazil) equivalents.

Vitamin C

The determination of ascorbic acid was performed according to the Tillmans method by titrimetry, based on the reduction of the 2,6-dichlorophenol indophenols (DCPIP) dye by ascorbic acid (AOAC 1998). The samples (5 g) were powdered by manual grinding in liquid nitrogen and homogenized with 50 mL metaphosphoric acid (2 % w/v). After centrifugation at 3000 g for 10 min, the supernatant was used for determination of vitamin C by titration. The vitamin C content was expressed as mg 100 g⁻¹ fresh weight.

Total chlorophyll content

The analysis was carried out on fresh samples according to the method validated (Sims and Gamon 2002), which was based on the molar absorption coefficient pigments (chlorophyll and anthocyanins in buffered acetone). After centrifugation, sample absorbance were determined by spectrophotometry at 663 nm for chlorophyll A, 647 nm for chlorophyll B, and 537 nm for anthocyanins, using Pharmacia Biotech, Ultrospec 2000. The absorbance values were converted in concentrations using the equations proposed (Sims and Gamon 2002). The total chlorophyll content was found as the sum of chlorophyll (chlorophyll A and chlorophyll B) and was expressed in µg 100 g⁻¹ fresh weight.

Nitrate

The nitrate content was determined from pure extract of the cabbages using a “compact Ion meter” Model C-141 from Horiba (Japan). Calculations were based on the direct reading in ppm (µg mL⁻¹), comparing with a calibration curve, using solutions of known concentrations (Coelho et al. 2014), taking into account sample weight and dilution. Analyses were carried out in triplicate.

Polyamines

Samples of cabbage of each treatment were analyzed according to the method using TLC (Thin Layer Chromatography) (Lima et al. 2009), as follows. The fresh material was homogenized for 1 min in 5 % (v/v) cold perchloric acid (Merck), using a food homogenizer. After centrifugation for 20 min at 4 °C, dansyl chloride (Sigma, 95 %) and saturated sodium carbonate were added to the supernatant. Proline (Sigma, min. 99 %) was added after 1 h at 60 °C, and the mixture was maintained in the dark for 30 min, at room temperature. Toluene was used to extract the dansylated polyamines, and aliquots were applied onto thin-layer chromatography plates [glass plates coated with 60G silica Gel – Merck (20×20 cm)] and were separated in laboratory bowls containing chloroform:triethylamine (Merck) (10:1). Putrescine (Sigma,

min. 98 %), spermidine (Sigma, min. 98 %), and spermine (Sigma, min 95 %) standards were submitted to the same process. The entire procedure was monitored with UV light (254 nm). The polyamines were quantified by comparison against the standards, which were also applied onto the plates, by fluorescence emission spectroscopy (excitation at 350 nm and emission measurement at 495 nm), in a Video Documentation System, using the Image Master version 2.0 software program by Amersham Pharmacia Biotech 1995, 1996. The free polyamine contents were expressed as µg g⁻¹ fresh matter.

Antioxidant activity (DPPH)

The extraction of the antioxidants was carried out in absolute ethanol (Brand-Williams et al. 1995). Samples were diluted to a final concentration of about 1 mg/mL. Then they were desiccated by drying in a water bath at 100 °C. The dry extract was reconstituted with the same volume of absolute ethanol. Antioxidant activity was determined according the DPPH assay in a wide range of sample concentrations (10, 50, 100, 125 and 250 µg mL⁻¹), on at least 5 repetitions. The reaction started with the addition of 1 mL of 0.3 mM DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) (Sigma-Aldrich-Brazil) in ethanol. The incubation process was carried out at room temperature for 60 min, in the dark. The negative control was prepared by substituting samples with absolute ethanol. The absorbance readings at 518 nm (Pharmacia Biotech, Ultrospec 2000), were used to calculate the antioxidant activity (AA), according to the following eq:

$$\text{AA}(\%) = 100 - [(A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}}] \times 100.$$

Microbiological analyses

Conventional and organic vegetables, after dipping in water, chlorine and ozone, for sanitation, were subjected to microbiological analyses according to the recommendations of the National Agency for Sanitary Vigilance, RDC n° 12, 2001. According to this resolution, the final vegetal product must be free of *Salmonella*, and it must present thermotolerant coliforms below 10² CFU/g, and *Staphylococcus aureus* and *Escherichia coli* below 10³ CFU g⁻¹. Samples were analyzed using the most probable number (MPN) for total and fecal coliforms (Kornacki and Johnson 2001), the presence of *Salmonella* and coagulase-positive *Staphylococcus* (Lancette and Bennett 2001).

Cabbages were pulverized in liquid nitrogen and resuspended in 0.1 M sodium phosphate buffer, pH 7.0, previously sterilized at 121 °C, 1 atm, for 20 min, and inoculated into the culture media for checking the presence of bacteria and fungi, according Lima et al. (2014). All tests were performed in

triplicate. In particular, culture medium 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 (CFU 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).

Residues

The determination of carbamates, organochlorides, organophosphates and herbicides was performed immediately after the biochemical characterizations and sanitizing treatments by thin layer chromatography (AOAC 1998), where samples (5 g) were added to distilled water (5 mL), containing formic acid (0.5 mL) and 30 % sodium sulphate (2 mL). Then, acetone (30 mL) was added and samples were stirred for 10 min, followed by a centrifugation at 2000 g for 10 min. The supernatants were placed in separatory funnels, and extracted two times with petroleum ether (20 mL). The water-acetone phase was discarded. The petroleum ether phase was filtered over anhydrous sodium sulfate and dried. Then, it was resuspended in petroleum ether (2 mL) and deposited on silica gel plate for chromatography (20×20 cm plate; 0.25 µm; SiO₂ 60 G Merck-Brazil), using rhodamine as developer, as regards the characteristic chemical groups (carbamates, organochlorides,

organophosphates and herbicides). Diazinon, Malation, Chlorpiryfos, Carbofuran, Aldica were used as standards.

Statistical analysis

The experiment was arranged in a completely randomized design with four sanitation treatments (water, chlorine and ozonized water for 10 and 20 min) and two cultivation systems (organic and conventional). All vegetable material from the field was subjected to four sanitation treatments and stored in cold room. The obtained data were subjected to variance analysis (F Test) and the averages were compared by the Tukey test (*P<0.01), by the SigmaStat 2.0 program.

Results and discussion

A clear trend showing a higher content of total phenols was observed in conventional cabbages after 12 days of cold storage (Fig. 1). This level remained constant until the 20th day, for all the sanitation treatments. After sample removal from the cold chamber (market simulation), we noted that cabbages produced under conventional cultivation, at 12+4 days, showed a higher content of phenols, in comparison with organic samples. At 20+4 days, there was a reduction in the phenol content in conventional cabbages, except for the ones treated with ozonized water. The higher content of total phenolic compounds in conventional vegetables was independent on the sanitizing process used.

Generally, sanitizers may induce modifications in cells, leading to the generation of reactive oxygen species (ROS) and inducing the activity of enzymes involved in protection from oxidative damage, such as phenylalanine ammonialiase (PAL, EC 4.2.1.5), the key enzyme in the synthesis of phenolic compounds (Alothman et al. 2010). Among used sanitizers (Fig. 1), in all the days analyzed, except the end of the storage period (20+4) using ozone 20 min, sanitizer seems not to have had influence on the content of phenols.

In this study, the higher content of total phenols in conventional cabbage was not followed by a subsequent accumulation of flavonoids. Higher levels of total flavonoids (Fig. 2) were observed just after the sanitation of organic cabbages, with all the treatments. However, this trend was not observed in prolonged storage periods. On the 12th day, a higher total flavonoid content was observed in conventional cabbages sanitized with chlorine. On the 20th day, organic samples treated with all sanitizers, as well as in conventional ones sanitized with ozone for 10 min, showed the highest levels of these compounds.

Some reports claimed that in some plants the increasing of phenolic compounds, including flavonoids, might be caused by changes in cell walls during exposure to ozone, and these modifications increase the extractability and release of cell

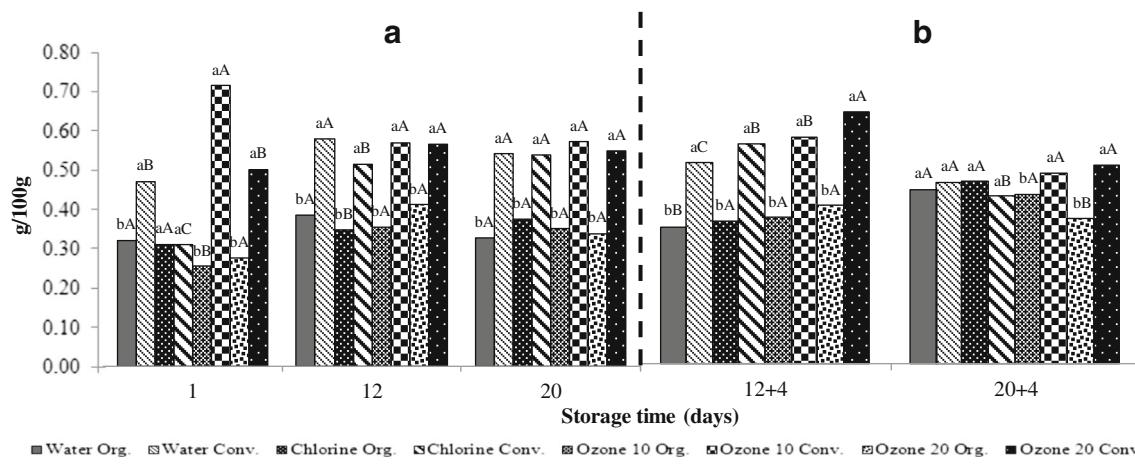


Fig. 1 Total phenol content in organic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within each

cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)

wall phenolics (Alothman et al. 2010). Thus, plants treated with ozone tend to present higher levels of phenolic compounds, including flavonoids. However, these results were not observed in cabbages treated with ozonized water. Considering the content of flavonoids, the higher content was only observed in conventional cabbages treated with ozonized water for 20 min, and in organic samples.

Furthermore, from the analyses of organic or conventional cabbages stored at room temperature (market simulation), and subjected to different sanitation treatments, we can assert that the sanitizers used did not influence the content of flavonoids in cabbages. The higher content on total flavonoids occur in organic cabbages treated with water and in conventional cabbages treated with chlorine and ozone 10 min., at 12+4 days, while at the second withdrawal (20+4 days), a significant increase was observed in organic samples treated with water and ozone for 20 min, and in conventional cabbages treated with chlorine. No changes in the levels of flavonoids in rocket

(*Eruca sativa*), treated with ozonized water were observed (Sanchez-Martínez et al. 2006).

Phenolic compounds, including flavonoids, are reported to occur at higher levels in organic vegetables (Lima and Vianello 2011), being influenced by culture, growing conditions and time of harvesting. In the present study, total phenols were higher in organic farming, and the sanitization treatment had minor influence on the level of these substances. Differences between organic and conventional production systems, especially in the management of soil fertility, can affect the nutritional composition of plants, including plant secondary metabolites (Vallverdú-Queralt et al. 2012).

Some researchers suggested that, among all of the nutrients used for quality measurements, phytochemicals have the highest potential to differentiate organic from conventional samples (Woese et al. 1997). Among phytochemicals, phenolic compounds deserve attention because of they are related to antioxidant activity, supposed to promote human health.

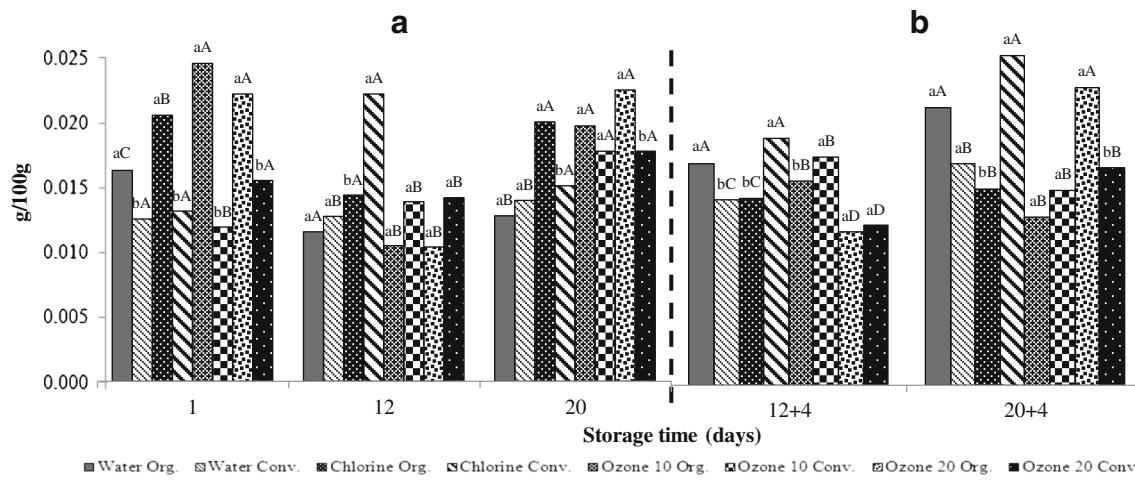


Fig. 2 Flavonoids content in organic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within each

cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)

Phenolic compounds, despite not having direct nutritional importance, have received attention due to their biological activity. One hypothesis suggests that foods containing plant secondary metabolite compounds have beneficial health effects, including anti-inflammatory and antioxidant (Lima and Vianello 2011). Several studies have demonstrated the antioxidant activity exerted by flavonoids (a phenolic compound), especially in the scavenging of several oxidizing species, such as superoxide anion, hydroxyl radical and peroxy radical. Besides the antioxidant potential, flavonoids have other biological activities, such as antimutagenic activity, reducing the risk of cardiovascular diseases, anti-proliferative action on tumors, arteriosclerosis protection, radioprotecting action, hair tonic, replenishing natural hormones in menopausal women and antimicrobial properties (Yao et al. 2004). Thus, it is expected that organic cabbages presented higher levels of these compounds. However, in our research, only total phenols were in the highest amount in organic samples. For flavonoids, no clear differences were observed, differently from other works showing, for example, that organically grown cabbages contained higher levels of flavonoids.

The vitamin C content was determined in all treated samples (Fig. 3). Once again, it can be seen that the treatment with ozone did not affect its content. Our results lead us to suppose that the cultivation procedure was more influencing on ascorbic acid level than the sanitization treatment. In the metabolism of both animals and plants, the biological function of L-ascorbic acid is focused on its antioxidant properties. Several studies have shown the protective effect of this substance on oxidative stress in plants and in several chronic diseases in mammals, which are caused by oxidative stress (Davey et al. 2000), and many studies have reported that organically grown vegetables tend to have higher levels of vitamin C (Lima and Vianello 2011). The vitamin C contents found in the present study were very similar to those described in the literature. A study by on different genotypes of cabbages (Singh et al.

2007), reported on concentrations between 5.9 and 12.9 mg of vitamin C per 100 g, and authors attributed differences to genetic variations, environmental influence, harvesting time, as well as to the analytical method. Variations in vitamin C content can also be influenced by vegetable storage and processing, on minimally processed cabbages, show initial values of 25 mg 100 g⁻¹, and after 15 days of refrigerated storage, the levels of vitamin C reached approximately 10 mg 100 g⁻¹ (Rinaldi et al. 2005).

In the present study, sanitizing treatments had a little influence on the content of vitamin C. After 12 days of storage the content of vitamin C in organic cabbages was higher when compared with conventional cabbages. This same trend tends to occur after the withdrawal of cabbages from the chamber (12+4 days). At 20+4 days there are not more significant differences between the types of crops or even between sanitizers. Vitamin C is integral part of the antioxidant system, and it should increase in the case of injuries (Keutgen and Pawelzik 2008). In organic farming, probably, as plants grow without the protection of pesticides, a higher level of vitamin C and other antioxidant substances is expected. As ozone is capable of promoting oxidative stress, its treatment could cause an alteration in the content of this vitamin, but don't occur in this study. According to some reports, the generation of ROS induces a loss of vitamin C, for cell defense. However, this effect was not observed in the present study, which could contribute to the hypothesis that ozone treatment did not induce oxidative stress in plants, as deduced from the levels of antioxidants, such as phenolic compounds and vitamin C. Other studies have also found that the use of ozone or chlorine did not affect the content of vitamin C, as reported in minimally processed lettuce (Beltrán et al. 2005).

Higher chlorophyll levels (Fig. 4) were observed immediately after sanitation in organically grown cabbages, except for ozone treated ones for 20 min. The highest total chlorophyll content was in cabbages washed only with water. On the

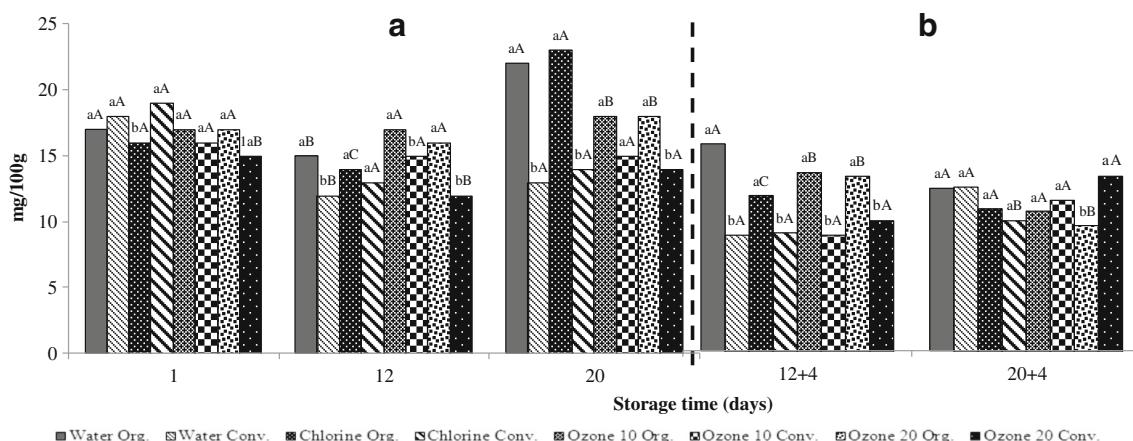


Fig. 3 Vitamin C content in organic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within each

cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)

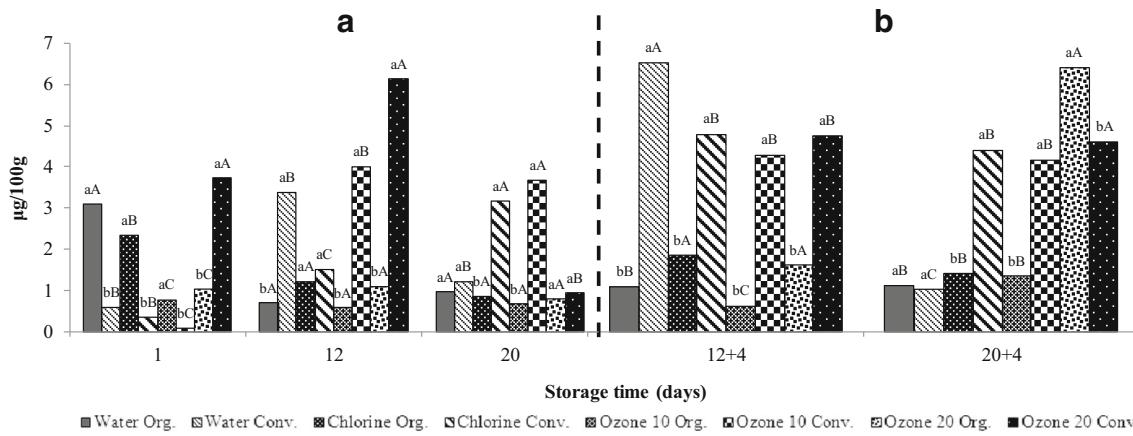


Fig. 4 Total chlorophyll content in organic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within

each cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)

12th day, a higher level of pigment was observed in conventional cabbages, except those treated with chlorine, and no variations between cropping systems and ozone treatment for 20 min were observed and organic vegetables showed no significant variations among sanitization treatments.

For cabbages stored at room temperature (market simulation) at 12+4 days, conventional cabbages showed a higher level of total chlorophyll (chlorophyll *a* + *b*). The same effect was not observed at 20+4 days. In these days, variations were observed between cultivation systems, which prevent us to assert a definite answer. However, it should be noted that the use of ozonized water for 20 min induced a higher levels of total chlorophyll in both the cultivation systems.

The sanitizers used did not show a definite trend in inducing alterations of chlorophyll levels in cabbages, whether organic or conventional. Some studies observed that the use of ozone can promote the destruction of chlorophyll and/or carotenoids (Saitanis et al. 2001). In our study on cabbages, this effect was not observed (Fig. 4), except in the first day, after

washing organic cabbages with sanitizers and at the 12th day in conventional cabbages.

Higher nitrate content was observed at the beginning of the experiment in conventional cabbages treated with ozone for 10 min (Fig. 5). This trend was also found on 20th day of cold storage and market simulation for 4 days, in samples treated with ozonized water for 20 min. In the 12th and 20th day, higher nitrate content in conventional cabbages was found, except at treatment with water (control). Others studies reported that organically grown vegetables tend to have higher nitrate content (Lima and Vianello 2011).

Putrescine contents were higher immediately after harvesting, in conventional cabbages (Fig. 6). Furthermore, sanitizers used had no influence on the content of putrescine in these samples. On the 12th day, higher levels of putrescine were observed in organic cabbages, regardless the sanitation treatment, and, as well, the nature of the sanitizer did not influence the content of putrescine. On the 20th day, the highest level this diamine occurred in conventional cabbages. The

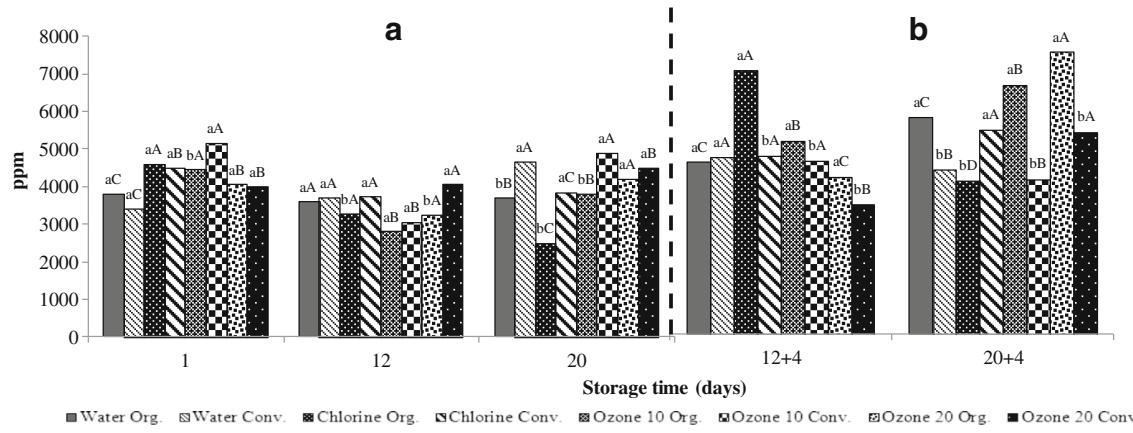
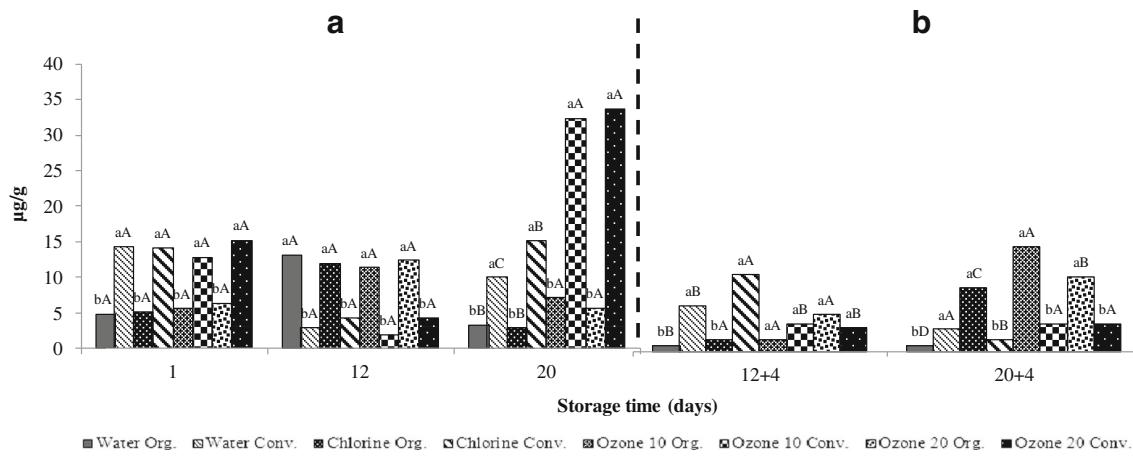


Fig. 5 Nitrate content in organic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within

each cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)



treatment with ozonized water for 10 and 20 min led to higher levels of putrescine in conventional and organic cabbages. After 16 days (12+4 at room temperature) conventional cabbages treated with water and chlorine exhibited the highest putrescine content, while at 24 days (20 day refrigerated and 4 at room temperature) cabbages from organic farming tended to show higher levels of putrescine, especially for those treated with ozonized water.

The levels of spermidine (Fig. 7) in conventional cabbages treated with chlorine, at the first day of analysis, showed no significant differences when compared with organics ones, even if this sanitizer is not allowed for organic vegetables. At 12 days, all conventional cabbages, independently of the sanitation treatment, showed lower levels of spermidine. When they were kept for other 4 days at room temperature, this result was inverted, that is, a conventional vegetable contains a higher content of this triamine. This same trend was observed at 20 days under refrigeration and 4 at room temperature.

The use of ozonized water for 20 min and chlorine induced higher levels of spermine (Fig. 8) on the first day of analysis. At 12 days, results showed values well below the remaining days and even in vegetables exposed at room temperature (12+4 days). At 20 days of cold storage, the content of spermine in organic cabbages treated with ozonized water was found above the overall average, and this value was not maintained after the removal from the cold chamber and the storage for 4 days at room temperature. The highest values were observed in conventional cabbages.

Cultivar, storage conditions (humidity, temperature, treatment with growth regulators), and time after harvest, have been described as the main factors affecting the quality and/or the amount of polyamines (Lima et al. 2009). It was noted that organic cabbages, sanitized or not, showed lower levels of putrescine and spermidine. In our study, this diamine showed lower levels when compared to spermidine and spermine. Generally, the accumulation of putrescine occurs when there is not the conversion to spermidine and/or spermine, due to

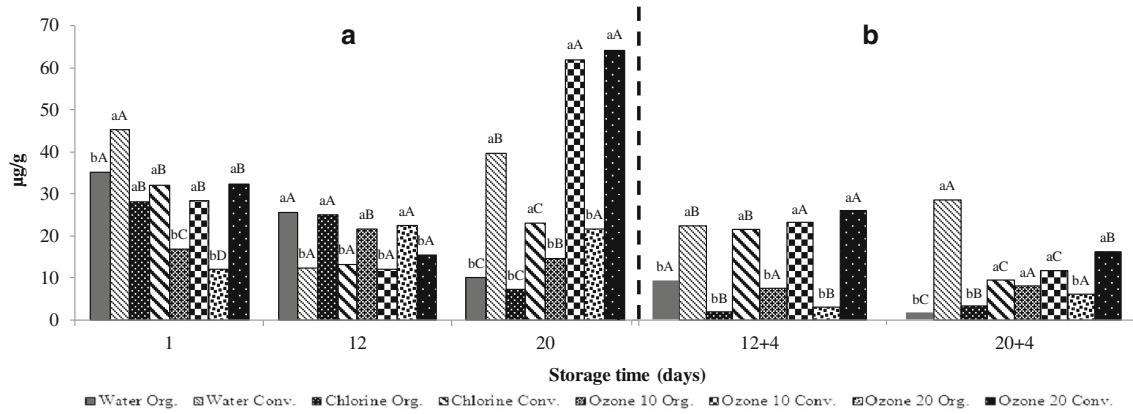
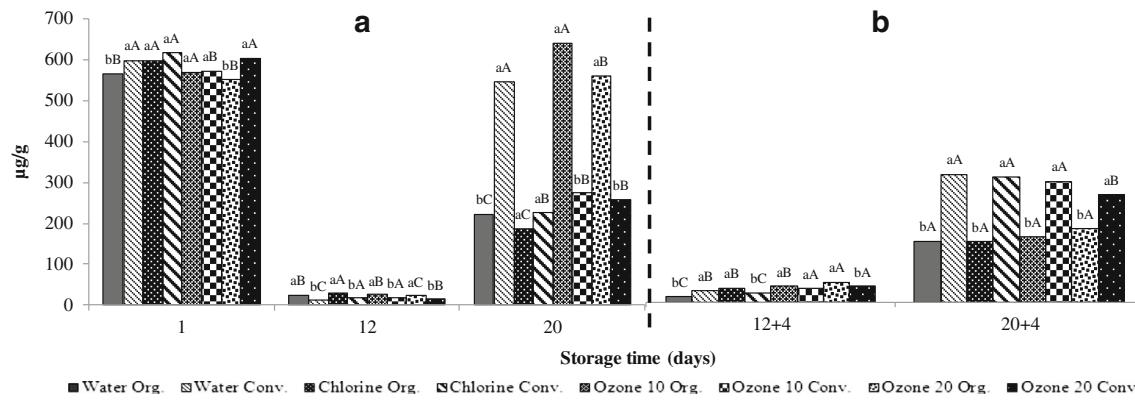


Fig. 6 Putrescine content inorganic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within each

cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)



alteration in the enzyme SAMDC (S-adenosylmethionine decarboxylase), inducing the synthesis of ethylene (Bouchereau et al. 1999). Thus, the levels found in organic cabbages may show a greater preservation of vegetables during the postharvest phase.

The use of ozonized water as sanitizer influenced the levels of polyamine, mainly at 20th day of cold storage and during the room temperature period. Ozone presents an oxidative effect, which may cause damage to cell membranes, as described by different authors (Bors et al. 1989). As polyamines are described as antioxidants, an increase in levels of these substances levels observed after sanitization with ozonized water could be related to the reduction of cell deterioration promoted by free radicals formed during the sanitation process. By observing Fig. 9 (regarding the total antioxidant potential), it should be noted that sanitation treatments led to an increase of antioxidant capacity in organic cabbages. As for flavonoids and vitamin C, polyamines could participate at the protection against free radicals, by helping to increase the antioxidant potential of the system.

Polyamines exhibit antioxidant activity protecting from nucleic acids oxidation, enzymatic denaturation and preventing lipid peroxidation (Bouchereau et al. 1999). Polyamines act as metal chelators, reducing metal-catalyzed oxidations within the cells. In plants, polyamines are involved in flowering, fruit development, stress response and in the inhibition of ethylene production and senescence. Endogenous polyamines are essential for life and assume important roles in cell proliferation, regulating the function of nucleic acids, protein synthesis, brain development, organism growth and regeneration of various cells (Kalac and Krausová 2005). Nevertheless, low levels of biogenic amines in foods are not considered harmful for health. However, when consumed in excessive amounts, they can have pharmacological, physiological and toxic effects. It has been reported a relationship between high levels of food biogenic amines and colon cancer (Paproski et al. 2002).

Immediately after harvesting and washing with different sanitizers, antioxidant activity of cabbages was measured on

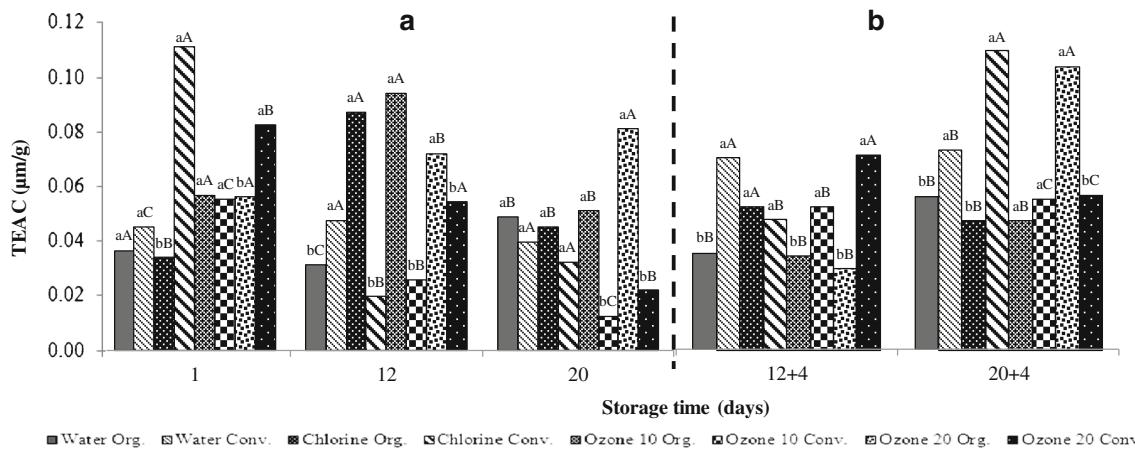


Fig. 9 Total antioxidant capacity of organic and conventional cabbages treated with different sanitation methods, stored in a cold chamber (**a**) and room temperature (**b**). Lowercase letters compare treatment means within each cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)

each cultivar (organic or conventional). Capital letters compare means between sanitizers for each crop. Means followed by the same letter do not differ significantly ($P < 0.01$)

Table 1 Effect of water-washings, chlorine and ozonized water washings on microorganisms of organic and conventional cabbage

Treatment	<i>Salmonella</i> (CFU g ⁻¹)	Coliforms MPN g ⁻¹	Thermotolerant coliforms MPN g ⁻¹	<i>Staphylococcus aureus</i> (CFU g ⁻¹)	<i>Escherichia coli</i> (CFU g ⁻¹)	Fungi
Org. Water	–	+	–	–	–	–
Org. Chlorine	–	–	–	–	–	–
Org. Ozone 10	–	–	–	–	–	–
Org. Ozone 20	–	–	–	–	–	–
Conv. Water	+	–	–	–	–	–
Conv. Chlorine	–	–	–	–	–	–
Conv. Ozone10	–	+	+	–	–	–
Conv. Ozone 20	–	+	+	–	–	–

Org. Organic cabbage; Conv. Conventional cabbage; (–) not detected, (+) detected

the first day of analysis by the DPPH assay (Fig. 9). Results showed that antioxidant capacity of organically grown cabbages was affected by the treatment with chlorine and ozonized water for 20 min. After 12 and 20 days of cold storage, organic vegetables showed a higher antioxidant activity when washed with ozonized water, regardless the time of exposure. However, this result varied after 12 days cold storage and 4 at room temperature, the total antioxidant activity, after ozone treatment, was higher in conventional cabbages, whereas at 20 days cold storage and 4 market simulation, this result was repeated only in cabbages washed with ozonized water for 20 min. From the results obtained on total antioxidant activity, it is not possible state that organic cabbages were better than conventional ones, as several studies pointed out (Jin et al. 2011).

The sanitizing treatment used did not show a definite trend on antioxidant activity of cabbages, whether organic or conventional. Generally, ozone is used to extend the life after the harvest of fruits and vegetables. A study on melons showed that the use of ozone induced an increase in antioxidant activity, measured by the DPPH assay (Silveira et al. 2010).

Finally, we analyzed all cabbage samples for pesticide residues and microorganisms. We did not detect the presence of pesticides in organic and conventional samples of cabbages, differently from many other authors finding higher pesticide contamination in conventionally grown plants, when compared to organic ones (Baker et al. 2002). As well, the microorganisms analyzed (Table 1) *Staphylococcus aureus* and *Escherichia coli* were not found in samples from organic and conventional cabbages after the use of ozonated water. The thermotolerant coliforms and *E. coli* observed in cabbage head after the treatments applied remained within the standards of acceptability for human consumption, according to ANVISA (Brasil 2015) in relation to vegetable quality. Our results show that the ozonated water can be used as a good sanitizer for organic cabbages in the elimination of fungi. The treatments with ozonated water showed no fungal growth in

tested samples and the elimination of fungi occurred short after the processing.

Conclusion

Results reported in the present paper showed that organically grown cabbages did not show any superiority over conventional ones. The sanitizing treatment with ozone was not effective in reduce the microbiological load of cabbage heads conventional (coliforms and thermotolerant coliforms).

Acknowledgments This study was supported by funding from São Paulo Research Foundation (FAPESP) (2011/16291-9), National Council for Scientific and Technological Development (CNPq) (478375/2010-7, 304095/2009-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (2057/2009).

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