APPLICATION OF ASCORBIC ACID AND MILD HEAT SHOCK TO IMPROVE SHELF LIFE AND ENSURE MICROBIAL SAFETY OF SLICED RADISH (*RAPHANUS SATIVUS* L.)

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ABSTRACT

Effects of hurdle technologies (ascorbic acid 2%, 5 min + mild thermal shock 50C, 1.5 min) on physicochemical, nutritional and antioxidant parameters of sliced radishes during 7 days of storage were investigated. At day 7, enzyme activities for treated samples were much lower than for control; whereas the browning parameters increased. For treated samples, b^* value remained constant until day 4, and a significant increase was observed at day 7. The hurdles reduced the initial microbial populations on radish slices, and maintained that for 4 days under refrigerated storage. Treated samples had higher content of flavonoids compared to control samples during all storage. Oxygen radical absorbance capacity assay results were positively correlated to total phenolic content (TPC) and total flavonoids content ($r^2 = 0.712$ and $r^2 = 0.750$, respectively). In addition, a high correlation was observed for TPC and 1,1-diphenyl-2-picrylhydrazyl ($r^2 = 0.996$). More investigations are needed to characterize bioactive compounds of radish to include them in nutraceutical formulations.

PRACTICAL APPLICATIONS

Although radish composition presents highly medicinal and nutritional values, reports about its quality parameters (physicochemical, nutritional and antioxidant) during minimal processing and refrigerated storage are still scarce. Therefore, and in response to consumer preference, low cost and safer preservation hurdles, application of ascorbic acid and mild heat shocks were evaluated in this investigation. Polyphenol oxidase activities, color, vitamin C, total phenolic compounds, antioxidant capacity, browning potential as well as microbiological aspects were assessed during 7 days of storage. Based on results, the application of this combination of treatments improved relevant sample parameters compared to control samples, which are related to functional properties of these roots in an attempt to maintain consumer's health and prevent different diseases.

INTRODUCTION

Consumption of diets rich in vegetables has extensively been demonstrated to be important for attaining and maintaining a good health. Their consumption is highly recommended by many organizations, like World Health Organization, Food and Agriculture Organization, United States Department of Agriculture (USDA) and European Food Safety Authority (Ramos *et al.* 2013).

Cruciferae (Brassicaceae) are globally popular vegetables and their intake reduce the risk of many types of cancer (Pocasap *et al.* 2013). In particular, radish (*Raphanus sativus*

L.) is a root vegetable belonging to this family; it is a red-colored edible bulb with a spicy flavor. It is cultivated worldwide and used by people with different gastrointestinal, biliary, hepatic, urinary and respiratory disorders, and in cardiovascular diseases such as hypertension (Blažević and Mastelić 2009; Ayub and Spinardi 2013; Lukatkin *et al.* 2014). Radish is consumed in salads with a minimum processing, e.g., as a sliced product. Therefore, hurdle technologies are applied as strategies to inhibit or inactivate the factors responsible for the radish spoilage, avoiding the use of more severe single treatments. The most common hurdles used are storage temperature, water activity, pH, redox potential, modified atmosphere and addition of preservatives. The main goal of these preservation techniques is to prolong storage stability with minimal detrimental effects on the quality attributes of the product (Rico *et al.* 2007; Ramos *et al.* 2013). In the last years, as a response to consumers' requirements to reduce or eliminate chemically synthesized additives, fresh-like products gained more attention and numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth in fresh-cut vegetables (Corbo *et al.* 2010; Ramos *et al.* 2013). Among numerous natural treatments, ascorbic acid was frequently proposed to reduce microbial populations (Corbo *et al.* 2010). Ascorbic acid (L-ascorbic acid) and its various neutral salts act as antioxidants for fruits and vegetables preventing browning and other oxidative reactions. Ascorbic acid also acts as an oxygen scavenger, removing molecular oxygen in polyphenol oxidase (PPO) reactions (Rico *et al.* 2007). Moreover, thermal methods are extensively used for the preservation and preparation of foods. However, these treatments lead to undesirable changes such as loss of vitamins and minerals, formation of thermal reaction components of biopolymers and, in minimal processing terms, loss of fresh appearance, flavor and texture (Rico *et al.* 2007). Mild heat shock is a method which usually implies a washing step at a temperature ranging from 45 to 70C for a few minutes, usually less than 5 min. Moreover, this treatment appears to be very useful as a quality preservation agent for fresh-cut produce by preventing quality deterioration and helping to maintain texture and color qualities longer (Corbo *et al.* 2010).

In addition, product shelf life must exceed the minimum distribution time required from the processor to the consumer to allow a reasonable period for home storage and use (Piagentini and Güemes 2002). This shelf life is defined as the time period during which products retain market-acceptable quality while meeting legal and safety requirements (Escobedo-Avellaneda *et al.* 2012).

The aim of this work was to evaluate the effect of the combination of ascorbic acid and mild heat shock treatments on preserving quality and extending shelf life of sliced radish stored under refrigeration. PPO activities, color, vitamin C, total phenolic compounds, antioxidant capacity, browning potential (BP) as well as microbiological aspects were analyzed.

MATERIALS AND METHODS

Plant Material and Treatment Application

Field grown radishes were purchased from a local market in Mar del Plata, Argentina. They were kept at 5 ± 1C in darkness prior to processing. Radish roots were separated from leaves. The roots were washed in tap water to eliminate any surface contamination and cut with a vegetable cutter (HLC-300, Dynam-h, SYSTEL S.A., Buenos Aires, Argentine) into slices of 4 mm. Then, they were washed again in tap water using a sliced radish to water ratio of 1:10 (w : v). The samples were divided into two lots: one for control samples (CO) and the other for treatment application (TT-AA). According to previous work, optimal conditions for treatment samples were immersion in water for 1.5 min at 50C followed by immersion on 2% of ascorbic acid solution. Thermal treatment was carried out in a thermostatically controlled water bath with recirculation (Lauda E300, Berlin, Germany). Thereafter, samples were removed from the bath and cooled immediately in cold water at 0–4C for 3 min. Then, immersion on ascorbic acid solution was carried out for 5 min. Finally, the slices were dried by a manual centrifuge and samples of 50 g were packaged in polyethylene bags (25 cm × 30 cm) of 25.4 μm thickness (with an O_2 transmission rate of 600 cm^3/m^2d, CO_2 transmission rate of 4,000 cm^3/m^2d, and water vapor transmission rate of 4 cm^3/m^2d; $P = 101{,}325$ Pa, T = 25C) using a manual impulse sealer (HL, FS-300, Buenos Aires Argentina). Three bags for each treatment were analyzed at 0, 2, 4 and 7 days of storage at 5 ± 1C.

Shelf Life Parameters

Analysis of physicochemical (moisture, A_w, pH, polyphenoloxidase activity, BP, color changes and sensory analysis), microbial (mesophilic bacteria and molds and yeast) and nutritional (ascorbic acid, total phenolic compounds and antioxidant capacity) quality markers was monitored throughout the entire storage (0, 2, 4 and 7 days) in sliced radishes stored at 5 ± 1C. All measurements were done in triplicate.

Physicochemical Parameters: pH, Water Activity and Moisture Content

Ten grams of vegetable tissue was blended for 2 min in 20 mL of deionized water. The pH of the slurry was mea-

sured at room temperature using a pH-meter (HANNA pH 201) between 18 and 20C. Water activity (A_w) was measured at 25C using a water activity meter (Testo 650, Testo Argentina). Moisture content was determined by the AOAC Official Method No. 934.06 (AOAC 1990).

Enzyme Activity

PPO activity was measured by the colorimetric method according to Goyeneche *et al.* (2013). Ten grams of radishes was homogenized with a commercial mixer at 1:2 ratio with 0.5 mol/L phosphate buffer (pH = 7.0) in the presence of 50 g/L polyvinylpyrrolidone (ICN Biomedicals, Inc., OH) and centrifuged at 12,700 × g for 30 min. The supernatant was used as the enzyme source (PPO crude vegetable extract). Crude extract was maintained at 0C until use. Gallic acid 4 mmol/L on phosphate buffer pH = 7 was used as the substrate solution. The reaction cuvette contained 2.9 mL of substrate solution and 0.1 mL of PPO crude vegetable extract, and the reference cuvette contained substrate solution. The enzyme activity was defined as a 0.001 change in absorbance at 350 nm between 0 and 60 s under the assay conditions. Enzyme activity was measured and residual activity PPO_R was expressed as

$$PPO_R = PPO/PPO_0 \quad (1)$$

where PPO is the enzyme activity after the treatment and PPO_0 is the initial enzyme activity of fresh samples.

BP

Overall BP was measured according to Pereyra *et al.* (2005). One gram of radish tissue was homogenized with 20 mL of distilled water using a tissue homogenizer (Braun, Kronberg, Germany) with a speed of 3,500–7,000 × g. The homogenate was filtered through Whatman No. 42 filter paper. The cloudy supernatant was centrifuged at 10,000 × g for 15 min. The absorbance of a supernatant aliquot was measured with a spectrophotometer (UV-1601 PC, Shimadzu, Kyoto, Japan) at 320 nm.

Color

The color development was measured on the sliced surfaces by a colorimeter (Lovibond, RT Series, London, England). The colorimeter had been standardized against a white tile ($L^\star = 97.63$, $a^\star = 0.3133$, $b^\star = 0.3192$). The measurements were made in triplicate over each surface sample on three samples per treatment and evaluation date. Color was recorded using a CIE – $L^\star a^\star b^\star$ uniform color space (Lab), where $L^\star$ indicates lightness (whiteness or brightness/darkness), $a^\star$ indicates chromaticity on a green (–) to red (+) axis, and $b^\star$ chromaticity on a blue (–) to yellow (+) axis (CIE 1978).

Sensory Evaluation

Sensory acceptability of radish slices was assessed by a trained sensory panel of five members using a descriptive test, as described by Goyeneche *et al.* (2014b). Color (two scales, brown scale to observe browning emergence – ColorM- and violet scale to observe pigment diffusion – ColorV-) and the texture were also evaluated on three samples according to the treatment and the evaluation date. For each index (color and texture), the scale used rated from 0 (best quality) to 5 (worst) and scores above 3 indicated the rejection of the product.

Microbiological Parameters

The enumeration of mesophilic aerobic bacteria microorganisms was performed on plate count agar incubated at 35C for 24–48 h (ICMSF 1983). Molds and yeast were counted in yeast–glucose–chloramphenicol medium incubated at 25C for 5 days (ICMSF 1983). All culture mediums were from Britania, Buenos Aires, Argentina.

Ascorbic Acid Content

Ascorbic acid content was determined by the titrimetric assay described by Moreira *et al.* (2003). Twenty grams of radish slices was homogenized with 40 mL of 2% oxalic acid solution. This mixture was vacuum filtered through a glass fiber. Five milliliter aliquots of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid contents are calculated as mg of reduced ascorbic acid/100 g of sample on a wet basis and relative content $VITC_R$ was expressed as

$$VITC_R = VITC/VITC_0 \quad (2)$$

where $VITC_0$ and VITC are the initial ascorbic acid content and after the treatment, respectively.

Phenolics, Antioxidant Capacity and Flavonoids

Preparation of Radish Extract Solutions. Extract solution was performed by a shaker holding 0.5 g lyophilized tissue in 10 mL 80% methanol for 63 min at 250 rpm, according to the method of Lopez-Martinez *et al.* (2009) with some modifications. After centrifugation at 5,000 rpm for 3 min, the supernatant was removed and extraction was repeated one more time in a similar way for 30 min by a shaker. The combined extracts were evaporated at 37C and

redissolved in 10 mL MeOH–formic acid (99:1). The extracts were used for the estimation of total phenolics, antioxidant capacity and flavonoid determination.

Total Phenolic Content. Total phenolic content (TPC) was determined colorimetrically by the Folin–Ciocalteu method according to Vega-Gálvez *et al.* (2014). Up to 0.5 mL aliquot of the radish extract solution was transferred to a glass tube, 0.5 mL of Folin–Ciocalteu reagent and 2 mL of 20% Na_2CO_3 solution were added and mixed well in a vortex. After 15 min of incubation at room temperature, 10 mL of ultra pure water was added and the precipitate formed was removed by centrifugation during 5 min at 4,000 × g. Finally, the absorbance was measured in a spectrophotometer (Spectronic 20 Genesys, IL) at 725 nm and compared to a gallic acid equivalent (GAE) calibration curve. Results were expressed as mg GAE/100 g of dry matter (d.m.). All reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany).

Total Flavonoid Content. Total flavonoid content of the radish extracts is performed following the protocol described by Kim *et al.* (2003) slightly modified. An aliquot of methanolic extract (0.1 mL) was mixed with 2.4 mL deionized water in a 5 mL microcentrifuge tube, added 0.15 mL $NaNO_2$ (50 mg/mL), and allowed to react for 5 min. Following this, 0.15 mL $AlCl_3$ (100 mg/mL) was added and the mixture allowed standing for further 6 min. Finally, 1.0 mL 1 mol/L NaOH and 1.2 mL deionized water were added to the reaction mixture and the absorbance at 510 nm was obtained against a blank by replacing the extract with deionized water. Total flavonoid content was calculated from a calibration curve using catechin (CE) as standard, and expressed as mg catechin/100 g d.m.

Antioxidant Capacity. The antioxidant capacity was determined by the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and oxygen radical absorbance capacity (ORAC) assay. DPPH followed the procedure described by Turkmen *et al.* (2005) with some modifications. An aliquot (3.9 mL) of 0.15 mmol/L DPPH radical in methanol was added to a test tube with 0.1 mL of the sample extract. The reaction mixture was vortex-mixed for 30 s and left to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (Spectronic 20 Genesys). Eighty percent (v/v) methanol was used as blank solution. Calibration curves were made for each assay using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as mmol TE (Trolox equivalent)/100 g d.m.

The ORAC assay followed the procedure described by Zhang *et al.* (2013) with some modifications. Up to 0.5 g of lyophilized tissue was centrifuged 30 min at 5000 × g with 25 mL of phosphate buffer (75 mmol/L, pH 7.4). A fluorescein stock solution (100 mol/L) in phosphate buffer was prepared and kept at 4C in the dark. Fresh working fluorescein solution (100 mmol/L) was prepared daily by diluting the stock solution in phosphate buffer. Next, 200 μL of the working fluorescein solution was added to each 40 μL of mixture sample or Trolox standard prepared in phosphate buffer in a black 96-well plate and incubated for 20 min at 37C. The assay was initiated by adding the peroxyl radical generator prepared in phosphate buffer. Specifically, 35 μL of 2,2′-azobis-2-amidinopropane (AAPH, 0.36 mol/L) was added and the fluorescence was measured (ex = 485 nm and em = 535 nm) every minute using a multimode plate reader Victor TMX3 (Perkin-Elmer, Turku, Finland) maintained at 37C until the lecture had declined to less than 5% of the initial reading. Standards and samples were run in triplicate. Results for ORAC were determined using a regression equation relating Trolox concentrations and the net area under the kinetic fluorescein decay curve. The ORAC value of radish extracts was expressed in mmol TE/100 g d.m.

Statistical Analysis

Results reported in this research are LS mean values (least square mean, means estimators by the method of least squares) together with their standard deviations. Experimental data were analyzed using SAS software (version 9.0, SAS Institute Inc., Cary, NC, 2002). The general linear model procedure (PROC GLM) was used to carry out the analysis of variance (ANOVA), with confidence limits of 95%.

A statistical model was used to evaluate the effects of mild heat shock treatment with ascorbic acid immersion respect control on different parameters during storage. Thus, a two-way ANOVA was applied using the following factors as variation sources: storage time (ST: 0, 2, 4 and 7 days), treatment (T: CO, TT-AA) and the interaction between them (T*ST). Differences between treatments and days of storage were determined by the Tukey–Kramer multiple comparison test ($P < 0.05$). For all models, PROC UNIVARIATE was applied to validate ANOVA assumptions (Kuehl and Kuehl 2000).

RESULTS AND DISCUSSION

The ANOVA analyses of regression models indicated that the models were highly significant ($P < 0.05$), exhibiting no significant lack of fit ($P > 0.05$). The coefficients of determination (R^2), explaining the variability of experimental data, were satisfactory. Based on all these tests, the prediction models were accepted.

Shelf Life Parameters

Moisture Content (MC) and Water Activity. Based on statistical analysis, moisture content did not show significant interaction between factors under consideration, and both storage time (ST) and temperature (T) resulted not significant ($P > 0.05$) for this parameter. According to ANOVA analysis, no significant differences were obtained between CO and TT-AA samples during storage for MC. This implies that neither treatment application nor storage time affected moisture content of the slices of radish. MC was kept constant at a value of 95.12 ± 0.40 g/100 of fresh weight (FW). This means that the saturation atmosphere in the film bag prevented water loss in radish slices.

Water activity has become one of the most important intrinsic properties in predicting the survival of microorganisms in foods due to its direct influence on product quality and stability (Tapia *et al.* 2008). Table 1 shows water activity (A_w) for control (CO) and treated samples (TT-AA) during refrigerated storage. Based on statistical analysis, A_w did not show significant interaction between factors under consideration, but both ST and T resulted significant ($P < 0.05$). According to ANOVA analysis, significant differences were obtained between CO and TT-AA samples during storage. CO samples remained constant during ST, and always higher than TT-AA. Only A_w measured at 2 and 4 days presented significant difference respect to ST ($P < 0.05$).

pH

The pH level is indicative of the flavor of vegetables; processing and storage conditions can affect the pH of the samples (Ayub and Spinardi 2013). A factor linking pH and browning which is a main quality marker is the activity of PPO. PPO most effectively catalyzes cut surface discoloration at a neutral pH of approximately 7 (Rico *et al.* 2007).

Table 1 shows pH values for control (CO) and treated samples (TT-AA) during refrigerated storage. The statistical model used to evaluate the effects of treatment together with storage time (ST) on pH of radish slices yielded a significant T*ST interaction ($P < 0.05$). This result indicated that the behavior of pH during storage was dependent on treatment application. Initially, TT-AA caused a significant reduction in the pH value respect to CO. Organic acids, whether naturally present in foods due to fermentation or intentionally added during processing, have been used for many years in food preservation. Some organic acids behave primarily as fungicides or fungistats, whereas others tend to be more effective when inhibiting bacterial growth. The mode of action of organic acids is related to the pH reduction of the substrate, acidification of internal components of cell membranes by ionization of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability. The undissociated portion of the acid molecule is primarily responsible for antimicrobial activity; therefore, effectiveness depends upon the dissociation constants of the acid (Barbosa-Cánovas 2003).

Decreases in the pH were not observed during all the storage for CO and for TT-AA samples, which might be associated with good quality maintenance of the fresh-cut products (Martin-Diana *et al.* 2006).

Enzyme Activity and BP

The statistical model used to evaluate the effect of treatment (T) together with storage time (ST) showed a significant interaction ($P < 0.05$) for PPO relative activity, indicating that the behavior of this index during storage was dependent on the treatment application (Fig. 1). Significant differences ($P < 0.05$) were observed between control and treated samples at all storage times. Relative enzyme activity values for TT-AA samples were much lower than for CO immediately after treatment application and during all the refrigerated storage, as shown in Fig. 1. This behavior might indicate an inhibitory effect of treatment applied on the PPO activity. Differences in PPO values were reflected in the sensory analysis data where the panel could find differences in visual browning between samples. Maghoumi *et al.* (2013) reported similar results. They found significant inhibition on pomegranate arils PPO with heat treatment at 55C during 30 s.

TABLE 1. WATER ACTIVITY AND pH FOR CONTROL AND TREATED SAMPLES DURING STORAGE

Storage time (days)	A_w		pH	
	CO	TT-AA	CO	TT-AA
0	$0.917^{aA} \pm 0.003$	$0.900^{bA} \pm 0.001$	$7.03^{aA} \pm 0.07$	$5.81^{bB} \pm 0.09$
2	$0.898^{aA} \pm 0.004$	$0.890^{cB} \pm 0.000$	$6.98^{aA} \pm 0.06$	$6.71^{aA} \pm 0.06$
4	$0.903^{aA} \pm 0.006$	$0.885^{cB} \pm 0.001$	$7.12^{aA} \pm 0.03$	$6.77^{aB} \pm 0.01$
7	$0.917^{aA} \pm 0.004$	$0.912^{aA} \pm 0.004$	$7.06^{aA} \pm 0.08$	$6.87^{aA} \pm 0.03$

Note: Values are mean ± SD, $n = 3$. Values with the same lowercase letter in the same column are not significantly different ($P < 0.05$); values with the same capital letter in the same row are not significantly different ($P < 0.05$), $n = 3$.

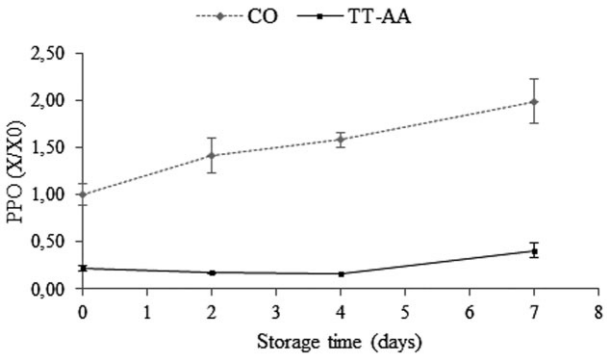

FIG. 1. EVOLUTION OF RELATIVE ENZYME ACTIVITY FOR CONTROL AND TREATED SAMPLES DURING REFRIGERATED STORAGE
Values are mean ± SD. Values with the same letter are not significantly different ($P < 0.05$), $n = 3$.

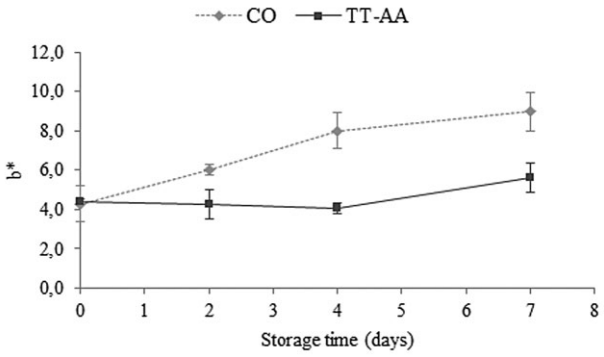

FIG. 3. EVOLUTION OF *B** PARAMETER FOR CONTROL AND TREATED SAMPLES DURING REFRIGERATED STORAGE
Values are mean ± SD. Values with the same letter are not significantly different ($P < 0.05$), $n = 3$.

Activity of PPO results in the production of orthoquinones, which are reduced by ascorbic acid. PPO inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed o-quinones to their precursor diphenols (Rico *et al.* 2007). With the inhibition of PPO by treatment application, presumably there is lesser demand on ascorbic acid and hence better food preservation (Pushkala *et al.* 2012).

Meanwhile, for BP no interaction between factors was obtained ($P < 0.05$). Organic acids have shown high capacity to prevent browning of fresh-cut tissues by the inhibition of PPO. Several other studies have confirmed the effects of organic acids in controlling browning of plant tissues (Ansorena *et al.* 2014). As shown in Fig. 2, for TT-AA samples, until day 2, BP remains constant, following a significant decrease at day 4 ($P < 0.05$). After 7 days of refrigerated storage, a significant increase in BP was observed for TT-AA samples. For CO slices, BP remains higher than TT-AA samples. Furthermore, BP for these samples remains constant nearly all the time of storage.

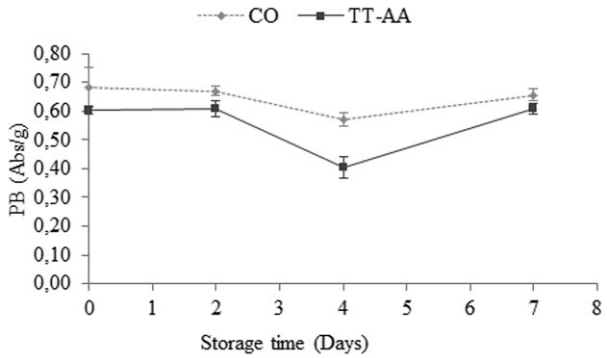

FIG. 2. EVOLUTION OF BROWNING POTENTIAL FOR CONTROL AND TREATED SAMPLES DURING REFRIGERATED STORAGE
Values are mean ± SD. Values with the same letter are not significantly different ($P < 0.05$), $n = 3$.

Color and Sensory Evaluation

Color change is a factor limiting the shelf life of fresh vegetables and is caused mainly by the degradation of pigments and the incidence of enzymatic browning (Curutchet *et al.* 2014). In the present work, changes of surface color were evaluated by measuring the *b** parameter. The statistical model used to evaluate the effect of T together with ST showed significant interaction ($P < 0.05$) for this parameter, indicating that the behavior of them during storage was dependent on the treatment application.

As shown in Fig. 3, *b** value increased in CO samples in the first 4 days of storage, and remained constant until day 7. Similar behavior was reported previously for the same raw material (Goyeneche *et al.* 2014a). For TT-AA samples, *b** value remained constant until day 4, and significant increase ($P < 0.05$) was observed at day 7. Nevertheless, the final *b** value obtained for control samples was nearly twice the value of the treated samples. Thus, the recommended temperature (5C) and the treatment applied in this work maintained the initial color of fresh-cut radish for 4 days. Regarding differences between CO and TT-AA samples at each time, only at zero time no significant differences were obtained, which indicates that the treatment does not affect the value of this parameter. During storage, *b** value for CO was always higher than TT-AA. It was observed that the white part of the CO slices turned yellowish brown with increasing ST. These changes in *b** values could be related to browning reactions observed in the nontreated samples due to endogenous enzymes (e.g., polyphenoloxidase, PPO). The quinones formed by means of PPO reactions could take part in secondary reactions bringing about the formation of

dark secondary products (Rocha and Morais 2003). Comparable results were reported by del Aguila *et al.* (2008) working with shredded radish and Goyeneche *et al.* (2014a) with sliced radish.

Regarding sensorial analysis, at 0, 2 and 4 days of refrigerated storage, all samples, both control and treated, were acceptable (all score parameters below 3). Only at day 7 CO samples were rejected because Color M is out of acceptance limit (ColorM > 3). This indicates that sensory panel rejects these samples because of browning occurrence. Furthermore, in addition to sensory analysis, judges were asked if they consumed the product. At day zero, 100% of panelists would consume both control and treated samples. Regarding CO samples, at second day of storage 70% of respondents would consume the product, while at days 4 and 7 only 20 and 15% of respondents would consume respectively. Respect to treated samples, at day 2 and day 4, up to 90% of the panelists would consume radish slices. At the end of storage, 35% of respondents still consume the slices.

Mesophilic Counts, Molds and Yeast

For the three populations evaluated, the statistical model used to evaluate the effect of T together with ST showed significant interaction ($P < 0.05$) for this parameter, indicating that the behavior of them during storage was dependent on the treatment application. For both CO and TT-AA samples, during storage mesophilic counts are increasing (Table 2). Immediately after treatment application, this microbiological index results in a reduction of 2 log respect CO samples. At first 2 days of storage, microbial counts of treated samples were significantly lower than control. After 7 days of storage, treated samples had counts 2 log higher respect controls. Thermal treatment would disrupt membrane physical barriers liberating nutrients from the cells which facilitated access to nutrients leading for the grater proliferation of microorganisms (Moreira *et al.* 2003). Thermal shock combined with ascorbic acid could help reduce the initial microbial populations on radish slices, and maintain that for 2 days of refrigerated storage, but would not prevent their growth during refrigerated storage. Actually, they could accelerate their growth. In fresh-cut lettuce, mesophilic counts increased during storage for samples washed with whey permeate and chlorine. This increase was more obvious between days 3 and 7 (Martin-Diana *et al.* 2006).

For molds and yeast, initially, a reduction of 3 log was obtained on TT-AA respect to CO samples (Table 2). After 2 days of refrigerated storage, TT-AA remains 1.5 log under CO samples. As for the case of mesophilic count, after 4 days of storage no significant differences ($P > 0.05$) were observed between samples, and at 7 days mold and yeast counts were significantly higher respect CO samples.

Despite these results, sensory and nutritional properties of TT-AA samples far exceed those for CO samples.

Ascorbic Acid Content

Among natural antioxidants, vitamin C is considered as an indicator of the quality of food processing due to its high degree of water solubility and low stability during heat treatment (Podsędek 2007). Vitamin C is a bioactive compound sensitive to degradation upon processing and storage, like when bruising, trimming and cutting occur (del Aguila *et al.* 2006). The changes in the ascorbic acid content of the radish slices upon storage are depicted in Fig. 4 for control and treated samples. The statistical model used to evaluate the effect of T together with ST showed significant interaction ($P < 0.05$) for VITC, indicating that the behavior of this index during storage was dependent on the treatment application. In the present study, continuous degradation of VITC was observed during storage. This could be attributed to the stress induced during slicing operation causing an increase in activity of the enzymes ascorbate oxidase and PPO (Pushkala *et al.* 2012).

Initially, there was a statistically significant increase in the ascorbic acid content of the slices, induced by the addition of exogenous ascorbic acid during the dipping treatment, compared with control samples. This pattern remained throughout the storage period. Increments in the ascorbic acid amount were also observed shortly after treatments in other experiments with minimally processed radishes (del

TABLE 2. MESOPHILIC COUNTS AND MOLDS AND YEAST FOR CONTROL AND TREATED SAMPLES DURING REFRIGERATED STORAGE

Storage time (days)	Mesophilic counts (log CFU/g)		Molds and yeast (log CFU/g)	
	CO	TT-AA	CO	TT-AA
0	$5.01^{dA} \pm 0.41$	$3.34^{dB} \pm 0.95$	$5.10^{cA} \pm 0.36$	$2.00^{dB} \pm 0.00$
2	$5.83^{cA} \pm 0.27$	$4.58^{cB} \pm 0.51$	$5.50^{bcA} \pm 0.09$	$3.74^{cB} \pm 1.51$
4	$6.67^{bA} \pm 0.10$	$6.70^{bA} \pm 0.31$	$5.71^{abA} \pm 0.72$	$5.81^{bA} \pm 0.36$
7	$7.88^{aB} \pm 0.36$	$9.38^{aA} \pm 0.18$	$6.65^{aB} \pm 0.33$	$10.12^{aA} \pm 0.70$

Note: Values are mean ± SD, $n = 3$. Values with the same lowercase letter in the same column are not significantly different ($P < 0.05$); values with the same capital letter in the same row are not significantly different ($P < 0.05$), $n = 3$.

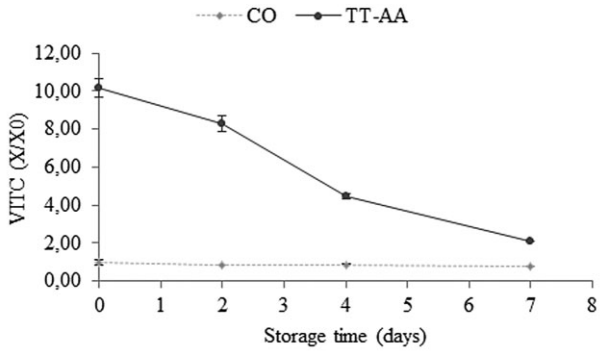

FIG. 4. EVOLUTION OF RELATIVE VITAMIN C CONTENT FOR CONTROL AND TREATED SAMPLES DURING REFRIGERATED STORAGE
Values are mean ± SD. Values with the same letter are not significantly different ($P < 0.05$), $n = 3$.

Aguila *et al.* 2008) and with kiwifruit (Carvalho and Lima 2002). The high level of ascorbic acid may indicate a superior antioxidant capacity (Han *et al.* 2014).

Regarding the evolution of ascorbic acid content during storage of fresh-cut products, results reported in the literature vary with the product (Curutchet *et al.* 2014). For example, working with minimally processed heat-treated broccoli, Ansorena *et al.* (2011) indicated that the reduced ascorbic acid content of samples decreased with the storage time at 5C, regardless of the use of edible coating.

TPC and Total Flavonoids Content

Phenolic compounds are a large group of the secondary metabolites widespread in plant kingdom. They are categorized into classes depending on their structures. The most widespread and diverse group of the polyphenols are the flavonoids (Podsędek 2007).

The statistical model used to evaluate the effect of treatment (T) together with storage time (ST) showed significant interaction ($P < 0.05$) for TPC, indicating that the behavior of this index during storage was dependent on the treatment application. Significant differences ($P < 0.05$) were observed between CO and TT-AA at all storage times. For TT-AA samples, until day 2, TPC remained constant, followed by a significant decrease by day 4 and a slight increase at day 7 (1435.3 mg GAE/100 g d.m.) ($P < 0.05$). Although the value of TPC decreased during storage compared to the correspondent at day 0, the value of TPC at day 7 remained higher than the TPC reported for fresh blueberry, a fruit known for its antioxidant capacity (López *et al.* 2010).

The intake of flavonoids is dominated by flavan-3-ols, followed by flavanones, flavonols, anthocyanidins, flavones and isoflavones. Epidemiological studies suggested an inverse relationship between the consumption of flavonoid-rich diets and development of many aging-associated diseases and neurodegenerative disorders (Babu *et al.* 2013). High concentrations of flavonols (e.g., quercetin, kaempferol, isorhamnetin and myricetin) were detected in capers, radishes and onions (USDA 2003). Table 3 shows the content of total flavonoids for CO and TT-AA samples during refrigerated storage. The statistical model used to evaluate the effect of treatment (T) together with storage time (ST) showed significant interaction ($P < 0.05$) for total flavonoids content (TFC), indicating that the behavior of this index during storage was dependent on the treatment application. Significant differences ($P < 0.05$) were observed between CO and TT-AA at all storage times. Treated samples had higher content of flavonoids regarding the control sample during all storage. For example, at day 7 the TFC was 174.0 mg CE/100 g d.m. compared to 134.3 mg CE/100 g d.m. at day 0.

Antioxidant Capacity

Table 3 shows the antioxidant capacity determined by means of DPPH and ORAC assays for CO and TT-AA samples during refrigerated storage. The statistical model used to evaluate the effect of treatment (T) together with storage time (ST) showed significant interaction ($P < 0.05$) for DPPH and ORAC, indicating that the behavior of these indices during storage was dependent on the treatment application. Significant differences ($P < 0.05$) were observed between CO and TT-AA at all storage times. TT-AA always maintained higher antioxidant capacity than CO.

TABLE 3. FLAVONOIDS CONTENT, TOTAL PHENOLICS AND ANTIOXIDANT CAPACITY FOR CONTROL AND TREATED SAMPLES DURING STORAGE

Storage time	TFC (mg CE/100 g)		TPC (GAE/100 g)		DPPH (mg TE/100 g)		ORAC (mmol TE/100 g)	
(days)	CO	TT-AA	CO	TT-AA	CO	TT-AA	CO	TT-AA
0	$105.8^{aB} \pm 5.2$	$134.0^{bA} \pm 4.3$	$411.2^{bB} \pm 16.4$	$1864.4^{aA} \pm 42.1$	$1027.8^{aB} \pm 48.8$	$7745.0^{aA} \pm 40.3$	1997.4 ± 209.3	52376.8 ± 4676.1
2	$96.3^{aB} \pm 4.6$	$133.9^{bA} \pm 2.3$	$419.3^{bB} \pm 3.4$	$1824.0^{aA} \pm 16.0$	$867.3^{bB} \pm 44.2$	$7531.9^{aA} \pm 123.5$	2552.1 ± 151.3	45112.12 ± 3620.4
4	$122.5^{aB} \pm 12.5$	$160.2^{aA} \pm 3.4$	$510.6^{aB} \pm 5.2$	$1179.4^{cA} \pm 42.7$	$1022.6^{aB} \pm 35.1$	$3516.1^{cA} \pm 192.5$	2585.8 ± 96.3	60029.6 ± 4746.7
7	$112.1^{aB} \pm 7.0$	$174.0^{aA} \pm 7.7$	$444.2^{bB} \pm 6.0$	$1435.3^{bA} \pm 23.1$	$1023.0^{aB} \pm 44.1$	$5364.2^{bA} \pm 68.8$	3686.5 ± 234.0	59701.8 ± 5512.5

Note: Values are mean ± SD, $n = 3$. Values with the same lowercase letter in the same column are not significantly different ($P < 0.05$); values with the same capital letter in the same row are not significantly different ($P < 0.05$), $n = 3$.

The antioxidant capacity of the samples showed similar trends to those observed with the bioactive compounds, mainly vitamin C and polyphenols, thereby suggesting that these compounds are likely to be the main contributors to antioxidant activity in radish slices. Evidence for this has also been provided in other studies for shredded carrots (Pushkala *et al.* 2012) and commonly consumed polyphenol-rich beverages in the United States (Seeram *et al.* 2008).

To study the influence of vitamin C, TPC and TFC on antioxidant capacity in sliced radish, correlations between the antioxidant capacity (ORAC and DPPH) and these parameters were determined. Results of the ORAC and DPPH assay were positive but weakly correlated to the vitamin C ($r^2 = 0.576$ and $r^2 = 0.601$, respectively). ORAC results were positively correlated to TPC and TFC ($r^2 = 0.712$ and $r^2 = 0.750$, respectively). In addition, a high correlation was observed for TPC and DPPH ($r^2 = 0.996$), indicating that phenolic acids might be responsible for the antioxidant capacity exerted by radishes. Additional investigations are needed to characterize active compounds and biological activities of these radish extracts in order to include them in nutraceutical formulations.

CONCLUSIONS

Visual appearance, nutritional contents, and microbiological as well as antioxidant capacity are the most important quality parameters used to estimate shelf life of sliced radishes by applying hurdle technologies. Acid ascorbic immersion and mild heat shock application were successful hurdles to preserve the radish slices during 7 days of refrigerated storage. Total phenolic and flavonoid contents, together with the antioxidant capacity, were significantly increased by the application of the combination of thermal and ascorbic acid treatments at the end of the storage. In addition, microbiological counts indicated that until day 4 samples are under permissible values for mesophilic counts. Therefore, the application of this combination of treatments increased relevant parameters which are related to functional properties of these roots.

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