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Moderate and High Doses of Sodium Hypochlorite, Neutral Electrolyzed Oxidizing Water, Peroxyacetic Acid, and Gaseous Chlorine Dioxide Did Not Affect the Nutritional and Sensory Qualities of Fresh-Cut Iceberg Lettuce (*Lactuca sativa* Var. *capitata* L.) after Washing

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Besides the traditionally used sodium hypochlorite (20 and 200 mg L^{-1}), alternative sanitizers such as peroxyacetic acid (80 and 250 mg L^{-1}) and neutral electrolyzed oxidizing water (4.5 and 30 mg L^{-1} free chlorine) as well as chlorine dioxide gas (1.54 mg L^{-1}) were evaluated for their efficiency in reducing the microbial load of fresh-cut iceberg lettuce. An additional rinsing step with tap water and cooling of the sanitizing solutions, which are obvious for the fresh-cut industry, were not performed within the current study. The high doses of sodium hypochlorite and peroxyacetic acid tested within this study do not conform to the normally used concentrations within the fresh-cut industry. Neutral electrolyzed oxidizing water (30 mg L^{-1}), peroxyacetic acid (250 mg L^{-1}), and gaseous chlorine dioxide significantly reduced the total aerobic plate count of cut lettuce in comparison with water wash treatments alone. None of the treatments significantly affected the sensory quality of the lettuce, although small color changes were observed after colorimetric measurements. From a nutritional point of view water rinsing significantly decreased the vitamin C (maximum 35%) and phenol (maximum 17%) contents, but did not affect the carotenoid and α-tocopherol contents. Additional effects caused by adding a sanitizer to the wash water were not observed for vitamin C and phenols. Conversely, washing with 250 mg L^{-1} peroxyacetic acid reduced the β-carotene content by about 30%, whereas using 200 mg L^{-1} sodium hypochlorite reduced both the lactucaxanthin and the lutein contents by about 60%. Use of gaseous chlorine dioxide also had an impact on the lutein content (−18%). Furthermore, the α-tocopherol content was reduced by 19.7 and 15.4% when the two concentrations of neutral electrolyzed oxidizing water were used, respectively. These data represent the situation on day 0. In a next phase, shelf-life studies considering microbial and sensory quality and nutrient content should be conducted.

KEYWORDS: Sanitizers; nutritional value; quality; sensory evaluation; *Lactuca sativa* var. *capitata* L.; sodium hypochlorite; peroxyacetic acid; neutral electrolyzed oxidizing water; chlorine dioxide gas

INTRODUCTION

Today consumers are more aware of the relationship between nutrition and health. Food products typically perceived as healthy by the consumer are fruits and vegetables. Food processors as well as the retail business anticipated this market trend and offer a broad range of packaged fresh-cut vegetables and position these products as excellent alternatives to combine "health" and "convenience". In the meantime the sales figures of fresh-cut produce have increased tremendously. Fresh-cut produce sales in 2005 in the United States were estimated at $12 billion, which is an increase of 25% with respect to 2003. In 2004, the United Kingdom, France, and Italy were the predominant European markets for fresh-cut produce, representing 120,000, 77,000 and 42,000 tonnes, respectively (*1*). Although a whole spectrum of fresh-cut vegetables is present on the market, salads are still the principal product.

Although iceberg lettuce is difficult to process because of its high mechanical and physiological fragility, it is omnipresent in salads. Moreover, it is already heavily contaminated with microorganisms the moment it enters the processing chain. Furthermore, some processing procedures such as shredding can even increase the total microbial count (*2*). In a Spanish survey of

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minimally processed lettuce, including 29 samples, an average total aerobic plate count of 6.3 log cfu g^{-1} was retrieved (*3*). Although the major part of the retrieved micro-organisms on fresh-cut lettuce was responsible for spoilage, contamination with pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Salmonella* spp. occasionally occurred (*3*). Moreover, *L. monocytogenes* is able to grow by 2 log cfu g^{-1} in 14 days on fresh-cut lettuce even at refrigerated temperatures (5 °C) and under modified atmosphere packaging (*4*). Consequently, this type of product can represent a risk for the consumer when GAP and GMP measures are ignored, indicating the importance of good hygiene measures throughout the food chain from farm to table. One of the potential strategies to mitigate the presence of pathogens and/or to prolong the shelf life in fresh-cut produce is the implementation of a decontamination step during processing. Many publications have dealt with the use of decontamination agents to remove artificially inoculated pathogenic bacteria from fresh-cut iceberg lettuce (*5*−*8*), whereas the effect on the endogenous microflora is less studied (*9*).

In addition to achieving microbial reductions, the effect on other quality attributes such as sensory quality and nutrient content should also considered in the evaluation of the adequacy of a decontamination process. Sensory attributes in general and, more particularly, appearance is the most important attribute evaluated by the consumer in the decision to accept a packaged leafy vegetable (*10*). One of the most common quality problems associated with fresh-cut iceberg lettuce is browning of the cut edges. Altered phenolic metabolism is involved in lettuce tissue browning. Wounding during the preparation of fresh-cut lettuce induces the synthesis of enzymes of the phenylpropanoid metabolism, the synthesis and accumulation of phenolic compounds, and subsequent tissue browning (*11*). It was stated that decontamination agents such as chlorine dioxide, having oxidizing properties, can induce browning with the same pattern characteristic of enzymatic browning (*12*).

Fruits and vegetables in general and more particularly lettuce are associated with beneficial effects for health due to the presence of vitamins such as vitamins C and E and secondary metabolites such as carotenoids and phenolics (*13*). These health-promoting effects are mainly related to their capacity for direct electron donation, reactive oxygen quenching, enzymatic reduction, and/or metal chelation (*14*). Consequently, epidemiological studies established a positive correlation between the intake of fruits and vegetables and prevention of diseases such as atherosclerosis, cancer, diabetes, and arthritis (*15*).

With regard to the effect of decontamination on the nutrient content of minimally processed iceberg lettuce, Martín-Diana et al. (*16*) and Beltran et al. (*17*) indicated that the effect of ozonated water and chlorine on the vitamin C content of lettuce was more affected by storage time than by washing. Although some studies included the influence of decontamination on the nutritional value of lettuce, the majority focused on both the microbial and sensorial qualities. The objective of the current study was to evaluate, in addition to water washing, different decontamination agents (sodium hypochlorite, peroxyacetic acid, neutral electrolyzed oxidizing water, and gaseous chlorine dioxide) used in different concentration levels for their ability to reduce the microbial load of fresh-cut iceberg lettuce on day 0. These sanitation procedures deviate from the recommended steps in the fresh-cut produce industry because the washing solutions were not cooled and no supplementary rinsing step with tap water was included after the sanitation step. Furthermore, their impact on both the sensory quality and the nutrient content of fresh-cut iceberg lettuce on day 0 (vitamins C and E, carotenoids, phenols, antioxidant capacity) was quantified.

MATERIALS AND METHODS

Plant Material. Iceberg lettuce (*Lactuca sativa* L.) was purchased from a local wholesale business (Van Landschoot NV, Ghent, Belgium). The lettuce heads were transported to the laboratory within 30 min. On arrival, the lettuce was manually processed by removing the outer leaves and the inner core and cutting the lettuce in 1 cm shreds by means of a sharp knife. Because of practical limitations, different lettuce heads bought at different times were used to test the different decontamination agents. Consequently, to study the effect of a particular sanitizer on a specific parameter, each experiment included a control series (unwashed lettuce) and series with water-washed lettuce.

Reagents. Ethanol (pa), methanol (HPLC grade and pa), petroleum ether (HPLC grade), ethyl acetate (HPLC grade), acetonitrile (HPLC grade), chloroform (pa), sodium sulfate (anhydrous), sodium chloride (pa), sodium acetate.3aq (pa), ascorbic acid, and acetic acid (pa) were purchased from Chemlab (Zedelgem, Belgium). Magnesium carbonate, 2,6-di-*tert*-butyl-4-methylphenol (pa) (BHT), active charcoal (Norit SA2), and oxalic acid dehydrate (99%, pa) were obtained from Acros Organics (Geel, Belgium). β-Carotene (≥95%, HPLC), lutein, *trans*-β-apo-8′-carotenal, α-tocopherol (synthetic, ∼95%, HPLC), γ-tocopherol (≥96%, HPLC), δ-tocopherol, gallic acid, Folin−Ciocalteu's phenol reagent (2 N, with respect to acid), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ, ≥99.0%, TLC), iron(II) sulfate heptahydrate (pa, ACS reagent, ≥99.5%, RT), sodium hypochlorite (≥4%, reagent grade), and orthophenylenediamine dihydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Triethylamine, antifoam agent (Extran AP31), and isopropanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Hexane (pa and HPLC grade), sodium carbonate (pa), metaphosphoric acid (pa), NaCl, and hydrochloric acid (37%, pa) were obtained from VWR (Fontenay Sous Bois, France). Peroxyacetic acid (Chriox 5) was obtained from Christeyns NV (Ghent, Belgium). Chlorine dioxide stock solution was delivered by Vernagene (Bolton, U.K.).

Decontamination Treatments. A mass of 100 g of iceberg lettuce was treated in 1 L of disinfectant solution at 16−18 °C under continuous agitation (150 rpm) on an orbital shaker (Ika, Staufen, Germany) for 5 min. A washing step with tap water for 1 min was conducted before the chlorine dioxide gas treatment. The temperature of the washing solution was 16−18 °C. Afterward, the excess of surface water or disinfectant solution was removed during 1 min by means of a manual kitchen centrifuge (Zyliss, Bern, Zwitserland).

The produce was treated with different disinfectant solutions: sodium hypochlorite, neutral electrolyzed oxidizing water (EOW), commercially available peroxyacetic acid, and gaseous chlorine dioxide. The liquid agents were tested in two concentrations covering the concentrations used in the literature. The lowest concentration is more important from a practical point of view; the highest was used to evaluate if its impact on nutrient content is concentration-dependent. The control series corresponded with unwashed fresh-cut iceberg lettuce. Rinsing the cut lettuce with potable water (1 or 5 min) was used as reference treatment. Starting from an aqueous sodium hypochlorite solution and after performing an iodometric titration in acidic environment, solutions with the appropriate concentrations (20 and 200 mg of free chlorine L^{-1}) were made. Finally, the pH was adjusted to pH 6.00 with hydrochloric acid.

On a daily basis the concentration of the commercially available peroxyacetic acid stock solution was determined by an iodometric titration. Furthermore, appropriate dilutions were made to obtain concentrations of 80 and 250 mg L^{-1} peroxyacetic acid corresponding to pH values of 5.74 and 4.42, respectively.

Neutral EOW was generated using an Ecodis 0.20.2-4A/2 (Ecodis NV, Schoten, Belgium). It mainly contains a vessel with water or diluted NaCl solution, a pump (IWAKI, Tokyo, Japan), a flow meter (SK 50, Georg Fisher, Brussels, Belgium), a control unit, and an electrolytic cell with two anodes and one cathode without a separating membrane. By alternately varying the NaCl concentration in the starting solution, the flow rate, and the voltage or the current over the electrodes, EOW with different free chlorine concentrations was produced. The free chlorine concentration in EOW was determined according to the *N*,*N*′-diethyl-*p*-phenylenediamine (DPD) method by means of a single-ion portable photometer (HI 93711, Hanna Instruments, Woonsocket, RI). To produce EOW with 4.5 mg L^{-1} of free chlorine (pH 7.50) the electrolytic cell was fed with 15 L h^{-1} of

potable water and the current over the electrolytic cell was 0.7 A. A NaCl solution of 0.1% (w/v) combined with a flow rate of 20 L h^{-1} and a current of 1.3 A over the electrolytic cell was necessary to obtain water with 30 mg L^{-1} of free chlorine (pH 7.87).

The treatment of minimally processed iceberg lettuce with gaseous chlorine dioxide as well as the determination of the chlorine dioxide concentration in the liquid phase and in the gas phase was performed according to the procedure of Vandekinderen et al. (*18*). Briefly, 2 kg of fresh-cut iceberg lettuce was treated with gaseous chlorine dioxide in a specially designed treatment chamber. Starting from a 1000 mg L^{-1} solution of chlorine dioxide, chlorine dioxide gas was stripped from the solution by air bubbling during 30 s, and the gas was passed by tubing to the cabinet. Furthermore, the lettuce was kept in contact with the gas during 9.5 min at room temperature and a relative humidity of 90.5 ± 1.0%. At regular time intervals gas samples were taken from the cabinet to monitor the evolution of the chlorine dioxide gas concentration during treatment. When the treatment was finished, the cabinet was opened and samples were taken for analysis. Determinations of the chlorine dioxide gas concentrations were based on iodometric titrations.

Microbiological Analysis. A 30 g sample of the iceberg lettuce was aseptically taken and transferred into a sterile stomacher bag. A 10-fold dilution was made in Peptone Physiologic Salt solution [PPS; 8.5 g L^{-1} NaCl and 1 g L^{-1} neutralized bacteriological peptone (Oxoid, Hampshire, U.K.)], and the sample was homogenized during 60 s by means of a Stomacher Seward Laboratory Blender 400 (UAC House, London, U.K.). Subsequently, a decimal dilution series in PPS was made, and appropriate dilutions were brought on pouring plates of Plate Count Agar (PCA, Oxoid) to determine the total aerobic plate count. After an incubation period of 3 days at 30 °C, colony forming units (cfu) were counted.

Sensorial Analysis. Triangle tests were conducted to determine whether a difference in sensory quality existed between products due to the applied decontamination treatment. The samples were treated and transferred into plastic, closed recipients at 4 °C during 60 min until sensory evaluation. The sensory evaluation occurred in a tasting room with isolated booths. For each test three coded samples, of which one differed from the two other samples, were presented to 18 trained panelists, and equal numbers of the possible combinations were at random presented. Each panelist had to evaluate the samples for overall sensory quality and had to select the odd sample. Furthermore, the panelists were asked for the reason why they choose a particular sample.

Color Measurement. Color measurements were conducted as an objective measurement for sensory analysis. Color measurements were performed by means of a portable spectrophotometer (CM-2500d, Konica Minolta Sensing, Osaka, Japan) running on Spectra Magix NX (Color Data software CM-S100w, Konica Minolta Sensing) software and expressed in the CIE $L^*a^*b^*$ color space. A plastic 2 cm deep Petri dish was filled completely with iceberg lettuce to prevent color interference of the underlying tabletop. The dish was closed, and the measurement was performed by placing the instrument viewing port on the cover. To exclude variable cover surface conditions, the specular interference was included in the color measurement. Calibration of the instrument was performed by means of the measurement of a white tile (white calibration) and a zero calibration. The following settings were used: 100% UV; illuminant, D65; observer angle, 10°; measurement area, 8 mm. Ten measurements were randomly performed on one Petri dish.

Vitamin C. The determination of the vitamin C content [ascorbic acid (AA) and dehydroascorbic acid (DHA)] was performed according to the procedure described by Dodson et al. (*19*) with some minor modifications. All glasswork during the analysis was protected from daylight. After homogenization, 10 g of lettuce was analytically weighed in a beaker. After the addition of three drops of antifoam agent and 50 mL of extraction buffer [3% (w/v) metaphosphoric acid in acetic acid (8%, (v/v))], everything was mixed and quantitatively transferred to a 100 mL volumetric flask. Then, the contents of the flask were diluted with extraction buffer to 100 mL. After filtration over a folded filter (Schleicher & Schuell Microscience GmbH, Dassel, Germany), 10 mL of the filtrate was brought in a tube containing 200 mg of acid-washed active charcoal to convert the present AA into DHA. The tube was shaken vigorously during 30 s and centrifuged during 11 min at 6000*g*. Again, the supernatant was filtered, and 5 mL of the filtrate with 1.25 mL of sodium acetate.3aq (50%, w/v) and 14 mL of methanol was combined and diluted with distilled water to 25 mL. After filtration through a Whatman filter (no. 40, Whatman International, Maidstone, U.K.), 5 mL was transferred to a 10 mL flask. Subsequently, 1 mL of orthophenylenediamine (OPD, 2.5 mg mL^{-1}) was added and further diluted to the volume of the flask with mobile phase (methanol/water, 55:45, v/v). During the procedure external calibration was used. Starting from a stock solution of ascorbic acid (1 g L^{-1}) in extraction buffer, a decimal dilution of the latter solution was made. Then, 10 mL of the diluted solution was added to 200 mg of acid-washed active charcoal. Subsequently, the procedure was followed as previously described. To prepare the calibration curve, 0, 0.5, 1.0, 1.5, 2, and 2.5 mL of filtrate was combined with 1 mL of OPD solution and subsequently diluted with mobile phase to 10 mL. The standard curves were prepared daily to account for the day-to-day variation in the fluorescence response. After 60 min of incubation in the dark, the obtained fluorophore [3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one] was determined by means of HPLC with fluorescence detection. The injection volume was 20 μL. HPLC analyses were performed with a HPLC 1100 series (Agilent, Waldbronn, Germany) composed of an online degasser (G1379), a quaternary pump (G1311) pumping at 1.0 mL/min, and an autosampler (G1329, 4 °C) with an ALS Thermostat II (G1330, 22 °C) connected with a fluorescence detector with the excitation wavelength set on 350 nm and the emission wavelength on 430 nm. The HPLC system was running on ChemStation Rev. A. 10.02 software. The column used was a LiChrosorb RP18 (250 mm × 4.6 mm i.d. × 1.4 in., 10 μm, 60 Å, Varian, Palo Alto, CA). The recovery of ascorbic acid was 96 ± 4%. The results were expressed as milligrams of vitamin C per 100 g of fresh weight (fw).

Carotenoids. Stock solutions of 12.5 mg of β-carotene, 17.5 mg of *trans*-β-apo-8′-carotenal, and 1.0 mg of lutein in 100 mL of chloroform with 0.1% (w/v) BHT as antioxidant were made and were stored in amber bottles at −18 °C. Starting from the stock solutions, working standards were made to check weekly the concentration by measuring the absorbance at maximum wavelength. To prepare working solutions of β-carotene, *trans*-β-apo-8′-carotenal, and lutein, respectively, 400 μL, 400 μL, and 1 mL were taken and evaporated under N_2. Subsequently, the β-carotene residue was dissolved in hexane to obtain 0.50 ± 0.03 AU at 450 nm ($E_{1\%}$ = 2592). The lutein residue was dissolved in ethanol to obtain 0.1 ± 0.02 AU at 445 nm ($E_{1\%}$ = 2550) and the *trans*-β-apo-8′-carotenal residue in petroleum ether to obtain 0.76 ± 0.03 AU at 457 nm ($E_{1\%}$ = 2640).

The extraction procedure was performed according to the method of Taungbodhitham et al. (*20*) with some minor modifications. A saponification procedure was not included because the main carotenoid peaks and the internal standard peak were separated from the chlorophyll peaks. During the analysis samples were protected from daylight by covering the glassware with aluminum foil. The lettuce samples were mixed with a handblender (Miniprimer, MR5000M, Braun, Krönberg, Germany), and then 3 g of lettuce was analytically weighed in an Erlenmeyer flask. After the addition of *trans*-β-apo-8′-carotenal as internal standard and 35 mL of ethanol/hexane (4:3, v/v), the sample was vigorously mixed by means of an Ultraturrax (Zipperer, Staufen, Germany). Subsequently, the samples were extracted under continuous agitation (Edmund Bühler, KS-15, Hechingen, Germany) for 15 min under N_2 at room temperature. After a second mixing step, the mixture was filtered on a separation funnel. The folded filter was washed with 35 mL of ethanol/hexane mixture, twice with 12.5 mL of ethanol, and finally with 12.5 mL of hexane until a white residue remained on the filter. Then, the filtrate was successively washed with 10% NaCl (w/v, twice) and with water (three times). After every washing step, the aqueous layer was discarded. The organic layer containing the carotenoids was dried over anhydrous sodium sulfate in a round-bottom flask and evaporated under reduced pressure at 40 °C until an oleoresin was obtained. Then, the resin was evaporated until dryness under nitrogen. The residue was redissolved with 3 mL of acetonitrile, sonicated (Transsonic 460/H, Elma Hans Schmidbauer, Singen, Germany) for 15 s, and filtered through a 0.45 μm HPLC filter (13 mm, PolyPure II Syringe Filters, Alltech Associates, Lokeren, Belgium) in an amber storage vial. Finally, appropriate dilutions of the carotenoid extracts were made in mobile phase, and HPLC analyses were performed immediately.

HPLC analyses for individual carotenoids were carried out according to the procedure of Vandekinderen et al. (*18*). The different carotenoids were identified by their absorbance spectra (wavelengths of maximum absorption and the spectral shape) and their degree of polarity, which is linked to their elution time, and for lutein and β-carotene by comparison

with the retention time of standards. To quantify those carotenoids for which no commercial standard is available, the response factor of lutein was used for the xanthophylls, whereas the response factor of β-carotene was used for the carotenes. The recovery of the internal standard (*trans*-β-apo-8′-carotenal) in each lettuce sample was higher than 83%. The results are expressed in micrograms per 100 g of fresh weight.

α-Tocopherol. Stock solutions of α- and δ-tocopherol were made by adding 25 mg to 100 mL of hexane. The solutions were stored at −18 °C protected from light. A volume of 4 mL was taken and evaporated under N_2. The residue was redissolved with 10 mL of methanol. Subsequently, absorbance readings were taken for the α-tocopherol standards at 292 nm ($E_{1\%}$ = 76) and for the δ-tocopherol standards at 298 nm ($E_{1\%}$ = 87). The concentration of the tocopherols was checked weekly by measuring the absorbance at the appropriate emission wavelength. The samples were mixed, and 5 g of sample was analytically weighed in an Erlenmeyer flask of 100 mL. Subsequently, 50 mL of hexane/ethanol (4:1, v/v) with 0.05% BHT was added. δ-Tocopherol was used as internal standard as δ-tocopherol is not present in iceberg lettuce. The mixture was filtered on a separating funnel after a 15 min extraction at room temperature. After the filter had been washed with 25 mL of hexane, the filtrate was washed twice with 10 mL of water. The organic layer was dried over Na_2SO_4 and was evaporated under reduced pressure at 50 °C until an oleoresin was obtained. Furthermore, the oleoresin was evaporated until dryness under nitrogen. The residue was redissolved with hexane, filtered through a 0.45 μm HPLC filter (13 mm, Nylon, Syringe Filters, Alltech Associates, Lokeren, Belgium) in a vial, and injected into the HPLC system described for the procedure for vitamin C determination. The tocopherols (α- and δ-) were identified by comparing their retention times and fluorescence spectra with these of standards. The recovery of the internal standard (δ-tocopherol) was higher than 85% in each lettuce sample. The results are expressed in micrograms per 100 g of fresh weight.

Total Phenol Content. To determine the total phenol content the extraction procedure for total phenols described by Vinson et al. (*21*) was used. An analytically weighed portion (5 g) was diluted to 50 mL with 1.2 M HCl in 50% aqueous methanol, shaken for 2 h at 80 °C, and filtered. The extracts were performed in triplicates and stored for a maximum of 24 h at −20 °C. The total phenol content was determined with the Folin−Ciocalteu method. The procedure described by Waterman and Mole (*22*) was followed. An appropriate volume (5 mL) of the filtrate was added to 5 mL of Folin−Ciocalteu reagent (10 times diluted) in a volumetric flask of 100 mL. After 6 min ± 10 s, 15 mL of 20% Na_2CO_3 (w/v) was added. After dilution to the mark with distilled water and shaking, the mixture reacted during 2 h at room temperature in the dark. Absorbance readings were taken against the blank at 760 nm. The standard curve of total phenolics was made using gallic acid standard solutions (0−400 mg L^{-1}) under the same conditions as previously described for the samples, except that an aliquot of 1 mL was used. Total phenolics in iceberg lettuce were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh sample.

Antioxidant Capacity. The antioxidant capacity was determined by means of the ferric reducing antioxidant power (FRAP) technique (*23*). The extraction was based on the procedure of Kaur and Kapoor (*24*) with some minor modifications. About 5 g of homogenized iceberg lettuce was weighed analytically and extracted with 20 mL of ethanol/water (8:2, v/v) during 15 min under nitrogen. Subsequently, the mixture was centrifuged for 15 min at 5000*g* at 10 °C, and the supernatant was retained. The extraction procedure was repeated and finalized by centrifuging for 15 min at 12000*g* at 10 °C. Both supernatants were collected and adjusted to 50 mL with 95% ethanol. Finally, the supernatants were filtered and stored under nitrogen at −80 °C.

The FRAP was obtained by monitoring the absorbance change at 593 nm caused by the reduction of the Fe^{3+}−TPTZ complex to the ferrous form at pH 3.6. The FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ solution (10 mM) made in 40 mM HCl, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution (20 mM) and was stored at 37 °C before use. Briefly, 100 μL of the FRAP reagent or acetate buffer (blanks) was added to the samples or standards. Absorbance was measured every minute during 20 min at 37 °C. FRAP values were obtained by comparing the absorbance change in the samples with those obtained from increasing concentrations of Fe^{3+}.

All absorbance readings were performed by means of a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA) using Microplate Manager software 5.2.1 (Bio-Rad). Results were expressed as micromoles of Fe^{2+} per 100 g of fresh weight.

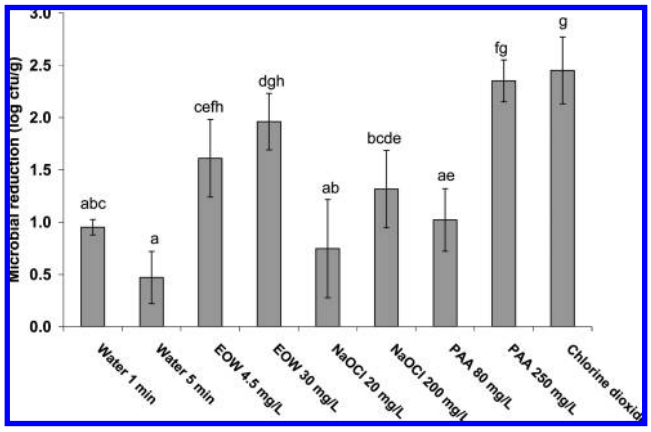

Figure 1. Efficiency of different sanitizers toward the initial total aerobic plate count of fresh-cut iceberg lettuce, n = 3. Error bars are standard deviations; a−g, bars with different letters show statistical significance (α = 0.05).

Statistical Analysis. Three replicates of each liquid treatment were conducted to study their effect on initial microbial quality, carotenoid and α-tocopherol contents, and FRAP antioxidant capacity, whereas two replicates were conducted to assess their impact on vitamin C and phenol contents. For ClO_2 gas treatments, three samples were randomly taken from one batch of treated vegetables. Microbial reductions, color measurements, and nutrient contents were analyzed for significant differences (P < 0.05) between the different series [control, water (reference treatment), low concentration of a disinfection agent, high concentration of a disinfection agent] by using analysis of variance (one-way ANOVA) in case three or more replicates were available and the conditions for normality and equal variances were fulfilled. In the case of significant differences, multiple comparison of means was established with the post hoc multiple-comparison Tukey test. In the case of non-normality or unequal variances, the nonparametric equivalents (Kruskal−Wallis test, Mann−Whitney test) were used. When only two repetitions were available, t tests were used. All statistical analyses were performed using the software SPlus 7.0 for Windows (Insightful Corp., Seattle, WA). The results from the triangle tests were analyzed according to a one-sided binomial test. In the case of 18 assessors, the critical number of correct responses to obtain a statistically significant (α = 0.05) difference is 10 (*25*).

RESULTS

Effect of a Decontamination Step on the Initial Microbial Load. The microbial reductions achieved after rinsing with water, neutral EOW containing 4.5 and 30 mg L^{-1} free chlorine, sodium hypochlorite (20 and 200 mg L^{-1}), and peroxyacetic acid (80 and 250 mg L^{-1}) as well as contact with 1.54 ± 0.07 mg L^{-1} chlorine dioxide gas are depicted in **Figure 1**. Generally, a minimum of a 1 log reduction is considered to be a practically important inactivation of the total microbial load (*18*). When compared with the unwashed lettuce, disinfection of lettuce with neutral EOW (4.5 and 30 mg L^{-1} free chlorine), 200 mg L^{-1} NaOCl, and peroxyacetic acid (80 and 250 mg L^{-1}) and contact with chlorine dioxide gas reduced the microbial load by 1.6, 2.0, 1.3, 1.0, 2.4, and 2.5 log cfu g^{-1}, respectively, and complied with the previously stated 1 log reduction requirement. However, only a part of the latter treatments, being neutral EOW containing 30 mg L^{-1} free chlorine, 250 mg L^{-1} peroxyacetic acid, and contact with chlorine dioxide gas, induced a microbial reduction that differed significantly from the reduction achieved after water washing.

The decline of the gaseous chlorine dioxide concentration in the empty treatment cabinet as well as during the treatment of 2 kg of iceberg lettuce is depicted in **Figure 2**. The decay of chlorine dioxide gas in the empty cabinet is limited within 9.5 min

after stopping gaseous chlorine dioxide supply. The initial chlorine dioxide concentration in the filled chamber (1.54 mg L^{-1}) showed an exponential decay. After 9.5 min, no chlorine dioxide gas could be retrieved. When the area between the curve describing the decay of chlorine dioxide in the empty chamber and the curve describing the exponential decay in the filled chamber is taken as a measure for the chlorine dioxide gas consumption by the iceberg lettuce, 80.5 ± 2.2% of the chlorine dioxide was consumed by the lettuce.

Effect of Decontamination Agents on the Sensory Quality. To evaluate whether or not the sensory quality of the chemically decontaminated fresh-cut iceberg lettuce differed from the sensory quality of water-washed lettuce or to assess the effect of the used concentration of a decontamination agent on the sensory quality, triangle tests were conducted. On the basis of these triangle tests it could be concluded that none of the treatments affected the sensory quality of fresh-cut iceberg lettuce when compared with water washing (**Figure 3**). Furthermore, when iceberg lettuce was washed with a liquid decontamination agent used in its low and respectively high concentration, no significant effect on the sensory quality was observed by the assessors. Apparently, the used concentration did not play a role in the effect on the overall initial sensory quality of iceberg lettuce.

To quantify the effect of the previously mentioned decontamination agents on the color of minimally processed iceberg lettuce, color measurements were performed. The corresponding L^*, a^*, and b^* values are presented in **Tables 1**−**4**. Similar to the results obtained after the triangle tests, water washing or using a sanitizing agent did not affect the color of fresh-cut iceberg lettuce or affected the color to only a limited extent.

Effect of Decontamination Agents on Nutrient Content. *Vitamin C*. Within the framework of this study the effect of different decontamination agents on the vitamin C content, which is the sum of ascorbic and dehydroascorbic acid, was determined (**Tables 1**−**4**). Vitamin C is an example of a hydrophilic nutrient sensitive for leaching and oxidation. Its hydrophilic character as well as its susceptibility for leaching was confirmed by the reduction of the vitamin C content ranging between 10 and 35% after a washing step with potable water. However, the latter effect was not always significant. None of the decontamination agents used in both the high and low concentrations caused a significant supplementary decrease of the vitamin C content of iceberg lettuce.

Carotenoids. The following carotenoids were retrieved in the untreated iceberg lettuce: *all-trans*-β-carotene, 9-*cis*-β-carotene, neoxanthin, lactucaxanthin, and lutein in concentrations of 66.4−160.2, 8.2−28.4, 3.3−13.7, 16.9−49.9, and 40.0−84.3 μg/100 g of fw, respectively. Washing with potable water during 5 min did not have an effect on the content of the individual carotenoids (**Tables 1**−**4**). Moreover, contact with gaseous chlorine dioxide and rinsing with neutral EOW did not reduce significantly the carotenoid content of fresh-cut iceberg lettuce with the exception of the lutein content, which was decreased by

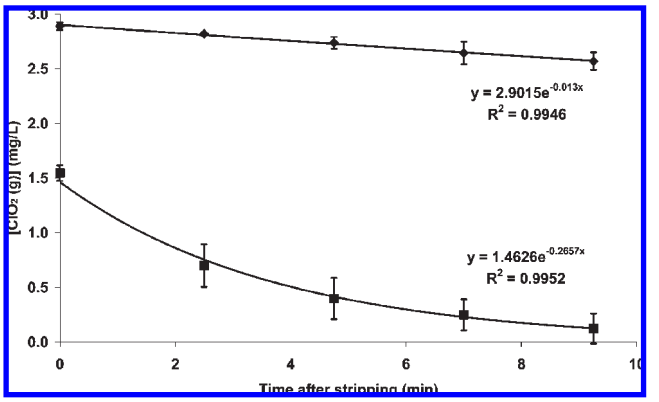

Figure 2. Evolution of the gaseous chlorine dioxide concentration [ClO_2 (g)] (mg L^{-1}) in the empty chamber (◆, n = 3) and during the treatment of 2 kg of fresh-cut iceberg lettuce (■, n = 3). Data points represent means; errors bars are standard deviations. Lines represent exponential regression of which the equations are shown.

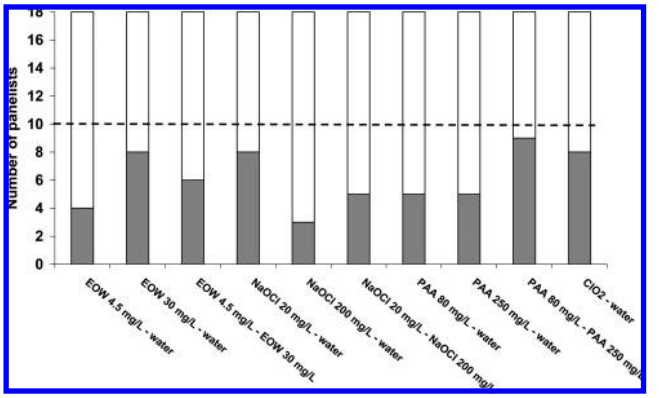

Figure 3. Evaluation of the difference in overall sensory quality by means of triangle tests (n = 18) between water-washed iceberg lettuce and iceberg lettuce treated with a sanitizer and between iceberg lettuce washed with a low concentration of a specific sanitizer and lettuce washed with a high concentration of the same sanitizer: gray shading, number of panelists choosing the correct sample as the odd sample; no shading, number of panelists choosing the wrong sample as the odd sample; - - -, limit (n = 10) indicating significant difference in sensory quality between both series (α = 0.05).

Table 1. Effect of Peroxyacetic Acid (PAA) Treatment on Different Nutritional Parameters[a] and on the Color[a] of Fresh-Cut Iceberg Lettuce

parameter	control	water	PAA (80 mg L^{-1})	PAA (250 mg L^{-1})
vitamin C (AA + DHA) (mg/100 g of fw)[b]	4.18 ± 0.19 a	3.56 ± 0.25 ab	3.22 ± 0.21 b	3.18 ± 0.18 b
all-trans-β-carotene (μg/100 g of fw)[c]	89.91 ± 10.10 a	89.64 ± 7.50 a	97.87 ± 8.79 a	61.70 ± 8.62 b
9-*cis*-β-carotene (μg/100 g of fw)[c]	14.93 ± 1.59 a	14.83 ± 1.27 a	17.78 ± 1.29 a	10.75 ± 1.83 b
neoxanthin (μg/100 g of fw)[c]	12.75 ± 4.12 a	12.45 ± 2.36 a	9.33 ± 2.62 a	9.87 ± 1.45 a
lactucaxanthin (μg/100 g of fw)[c]	49.94 ± 12.89 a	53.49 ± 3.80 a	34.51 ± 2.52 a	44.00 ± 7.35 a
lutein (μg/100 g of fw)[c]	79.51 ± 16.47 a	85.41 ± 3.79 a	62.62 ± 2.42 a	70.64 ± 8.40 a
total phenols (mg of GAE/100 g of fw)[b]	24.50 ± 2.12 a	22.47 ± 0.16 a	25.69 ± 0.46 a	25.80 ± 1.13 a
α-tocopherol (μg/100 g of fw)[c]	121.86 ± 7.86 a	109.43 ± 7.91 a	120.20 ± 13.19 a	101.05 ± 10.01 a
FRAP antioxidant capacity (μmol of Fe^{2+}/100 g of fw)[c]	19.37 ± 1.16 a	25.35 ± 0.64 b	22.59 ± 0.48 c	20.79 ± 1.30 ac
L^*[d]	64.90 ± 6.74 a	64.00 ± 5.04 a	63.01 ± 4.77 a	62.34 ± 6.46 a
a^*[d]	−4.67 ± 1.92 a	−3.03 ± 1.22 b	−4.47 ± 1.74 a	−3.34 ± 1.44 b
b^*[d]	18.94 ± 5.62 a	13.16 ± 5.47 b	17.26 ± 4.52 a	13.85 ± 4.50 b

[a] Mean ± standard deviation. Values with different letters in a row show statistical significance (α = 0.05). [b] n = 2. [c] n = 3. [d] n = 30.

Table 2. Effect of Sodium Hypochlorite (NaOCl) Treatment on Different Nutritional Parameters[a] and on the Color[a] of Fresh-Cut Iceberg Lettuce

parameter	control	water	NaOCl (20 mg L^{-1})	NaOCl (200 mg L^{-1})
vitamin C (AA + DHA) (mg/100 g of fw)[b]	6.72 ± 0.53 a	5.71 ± 0.02 a	5.42 ± 0.16 a	4.47 ± 0.70 a
all-trans-β-carotene (μg/100 g of fw)[c]	66.42 ± 19.32 a	81.37 ± 16.12 a	88.36 ± 18.59 a	75.47 ± 4.55 a
9-*cis*-β-carotene (μg/100 g of fw)[c]	8.23 ± 2.74 a	8.65 ± 1.04 a	9.15 ± 2.65 a	6.83 ± 0.77 a
neoxanthin (μg/100 g of fw)[c]	3.34 ± 2.20 a	7.37 ± 3.31 a	3.77 ± 0.62 a	1.87 ± 1.24 a
lactucaxanthin (μg/100 g of fw)[c]	16.93 ± 3.24 ab	26.86 ± 8.47 a	24.35 ± 2.19 ab	12.18 ± 5.88 b
lutein (μg/100 g of fw)[c]	40.04 ± 10.91 ab	47.15 ± 13.87 a	24.98 ± 6.77 ab	16.93 ± 11.84 b
total phenols (mg of GAE/100 g of fw)[b]	40.71 ± 1.10 a	36.15 ± 2.54 a	37.48 ± 3.04 a	35.36 ± 3.31 a
α-tocopherol (μg/100 g of fw)[c]	98.27 ± 18.83 a	101.13 ± 6.93 a	98.24 ± 3.60 a	99.61 ± 6.75 a
FRAP antioxidant capacity (μmol of Fe^{2+}/100 g of fw)[c]	23.23 ± 1.57 ab	24.12 ± 1.36 ab	24.73 ± 1.06 b	21.03 ± 0.57 a
L^*[d]	65.98 ± 4.88 a	65.21 ± 5.76 a	63.57 ± 5.43 a	64.76 ± 5.40 a
a^*[d]	−3.76 ± 1.63 a	−2.84 ± 1.37 b	−2.74 ± 0.96 b	−1.85 ± 0.89 c
b^*[d]	16.65 ± 5.33 a	13.97 ± 4.71 ab	13.99 ± 3.85 ab	12.73 ± 5.29 b

[a] Mean ± standard deviation. Values with different letters in a row show statistical significance (α = 0.05). [b] $n = 2$. [c] $n = 3$. [d] $n = 30$.

Table 3. Effect of Neutral Electrolyzed Oxidizing Water (EOW) Treatment on Different Nutritional Parameters[a] and on the Color[a] of Fresh-Cut Iceberg Lettuce

parameter	control	water	EOW (4.5 ± 0.5 mg L^{-1})[e]	EOW (30 ± 1 mg L^{-1})[e]
vitamin C (AA + DHA) (mg/100 g of fw)[b]	7.08 ± 0.24 a	6.36 ± 0.62 a	6.74 ± 0.19 a	6.22 ± 0.08 a
all-trans-β-carotene (μg/100 g of fw)[c]	72.99 ± 8.36 a	67.95 ± 5.14 a	63.16 ± 9.78 a	74.21 ± 7.12 a
9-*cis*-β-carotene (μg/100 g of fw)[c]	13.37 ± 1.73 a	12.42 ± 1.20 a	12.21 ± 2.36 a	13.72 ± 1.44 a
neoxanthin (μg/100 g of fw)[c]	7.67 ± 1.11 a	11.97 ± 1.34 a	9.64 ± 2.30 a	10.63 ± 3.30 a
lactucaxanthin (μg/100 g of fw)[c]	28.64 ± 0.98 a	37.01 ± 4.04 a	32.35 ± 7.30 a	37.48 ± 7.59 a
lutein (μg/100 g of fw)[c]	56.48 ± 2.44 a	65.13 ± 6.07 a	58.17 ± 8.77 a	63.11 ± 10.28 a
total phenols (mg of GAE/100 g of fw)[b]	31.56 ± 0.51 a	25.27 ± 5.43 a	27.89 ± 1.66 a	22.70 ± 3.84 a
α-tocopherol (μg/100 g of fw)[c]	136.75 ± 2.95 a	139.90 ± 1.60 a	112.34 ± 4.06 b	118.36 ± 2.78 b
FRAP antioxidant capacity (μmol of Fe^{2+}/100 g of fw)[c]	32.44 ± 2.49 a	33.44 ± 1.41 a	32.30 ± 1.83 a	29.74 ± 1.73 a
L^*[d]	64.12 ± 4.09 a	65.47 ± 6.00 b	64.28 ± 5.39 b	64.96 ± 6.90 c
a^*[d]	−4.99 ± 2.06 a	−3.53 ± 1.25 b	−3.38 ± 1.54 b	−2.30 ± 1.13 c
b^*[d]	17.12 ± 4.61 a	13.91 ± 4.40 bc	14.17 ± 4.79 ac	12.51 ± 5.00 c

[a] Mean ± standard deviation. Values with different letters in a row show statistical significance (α = 0.05). [b] $n = 2$. [c] $n = 3$. [d] $n = 30$. [e] Free chlorine.

Table 4. Effect of Gaseous Chlorine Dioxide Treatment on Different Nutritional Parameters[a] and on the Color[a] of Fresh-Cut Iceberg Lettuce

parameter	control	water	chlorine dioxide (1.54 ± 0.07 mg L^{-1})
vitamin C (AA + DHA) (mg/100 g of fw)[b]	2.04 ± 0.18 a	1.33 ± 0.15 b	1.14 ± 0.37 b
all-trans-β-carotene (μg/100 g of fw)[b]	160.20 ± 8.55 a	139.54 ± 3.56 a	138.20 ± 6.35 a
9-*cis*-β-carotene (μg/100 g of fw)[b]	28.39 ± 1.60 a	28.85 ± 7.91 a	24.98 ± 1.17 a
neoxanthin (μg/100 g of fw)[b]	13.74 ± 2.96 a	10.88 ± 3.03 a	10.16 ± 1.81 a
lactucaxanthin (μg/100 g of fw)[b]	45.18 ± 4.11 a	39.13 ± 11.17 a	35.43 ± 3.19 a
lutein (μg/100 g of fw)[b]	84.31 ± 5.21 a	83.54 ± 3.70 a	68.16 ± 1.16 b
total phenols (mg of GAE/100 g of fw)[b]	32.88 ± 2.74 a	27.38 ± 0.86 b	26.26 ± 1.51 b
α-tocopherol (μg/100 g of fw)[b]	151.90 ± 17.42 a	118.43 ± 16.92 ab	97.09 ± 13.56 b
FRAP antioxidant capacity (μmol of Fe^{2+}/100 g of fw)[b]	22.69 ± 1.56 a	21.66 ± 1.30 a	20.23 ± 2.14 a
L^*[c]	64.95 ± 5.96 a	63.98 ± 6.46 a	59.63 ± 3.72 b
a^*[c]	−4.65 ± 2.36 a	−3.23 ± 1.33 b	−1.17 ± 0.55 c
b^*[c]	18.27 ± 7.00 a	14.39 ± 4.46 b	11.66 ± 4.31 b

[a] Mean ± standard deviation. Values with different letters in a row show statistical significance (α = 0.05). [b] $n = 3$. [c] $n = 30$.

18% when chlorine dioxide gas was used (**Tables 3** and **4**). Contrary to neutral EOW, the effect of washing with both peroxyacetic acid and sodium hypochlorite was dependent on the concentration used as well as on the particular carotenoid. A rinsing step with 80 mg L^{-1} peroxyacetic acid or with 20 mg L^{-1} sodium hypochlorite did not affect the content of the individual carotenoids present in minimally processed iceberg lettuce, whereas washing with 250 mg L^{-1} peroxyacetic acid additionally decreased the *all-trans*-β-carotene and the 9-*cis*-β-carotene contents by, respectively, 27.5 and 31.2% when compared with the contents in the washed lettuce (**Table 1**). The content of the other carotenoids (neoxanthin, lutein, and lactucaxanthin) was not affected after a decontamination step with 250 mg L^{-1} peroxyacetic acid. Opposite to washing with 250 mg L^{-1} peroxyacetic, a rinsing step with 200 mg L^{-1} sodium hypochlorite decreased the lactucaxanthin and lutein contents, whereas the content of the other carotenoids remained stable (**Table 2**).

Total Phenols. Apart from the leaching of phenols to the wash water, none of the studied decontamination agents (peroxyacetic acid, sodium hypochlorite, neutral electrolyzed oxidizing water, chlorine dioxide) decreased the total phenol content of fresh-cut iceberg lettuce assessed by the Folin−Ciocalteu method (**Tables 1**−**4**).

α-*Tocopherol*. Washing lettuce with water did not significantly decrease the α-tocopherol content. Similarly, adding peroxyacetic acid, in either in a low or high concentration, or adding sodium hypochlorite (20 or 200 mg L^{-1}) to the wash water did not influence the α-tocopherol content of fresh-cut iceberg lettuce (**Tables 1** and **2**). Conversely, applying a decontamination step with neutral EOW decreased the α-tocopherol content significantly regardless of the used free chlorine concentration

(**Table 3**). Although not statistically significant, contact with 1.54 mg L^{-1} chlorine dioxide gas reduced the α-tocopherol content of minimally processed iceberg lettuce by an additional 18% to the recorded loss after washing with water (**Table 4**).

Total Antioxidant Capacity. Up until now only the effect of a decontamination step on specific antioxidants present in iceberg lettuce was studied within the framework of the current research. To include also the antioxidant activity of other antioxidants as well as to take into account the synergistic action of the antioxidants present in iceberg lettuce, the antioxidant capacity was determined. On the basis of the previous paragraphs it can be noted that the nutrient losses caused by using a decontamination agent were limited. This was confirmed by comparing the results of the FRAP assay (**Tables 1−4**). Washing fresh-cut iceberg lettuce did not significantly reduce the total antioxidant capacity of fresh-cut iceberg lettuce (**Tables 1−4**). Besides, the use of neutral EOW (4.5 and 30 mg L^{-1} free chlorine) or contact with 1.54 mg L^{-1} gaseous chlorine dioxide did not change the total antioxidant capacity of the lettuce (**Tables 2−4**) when compared with the water-washed lettuce. Although quite limited, the application of 200 mg L^{-1} sodium hypochlorite tended to decrease the total antioxidant capacity when compared with the water series (**Table 2**). The FRAP value of lettuce after washing with peroxyacetic acid was reduced when compared with the FRAP value of water-washed lettuce, but tended to increase when compared with the unwashed lettuce (**Table 1**).

DISCUSSION

The objective of the current study was to evaluate different decontamination agents for their suitability to reduce the microbial load of fresh-cut iceberg lettuce without unacceptable effect on both the sensory quality and the nutritional value. Because of practical considerations the experiments were conducted on different vegetable batches bought at the same wholesale business. The advantage of this approach is that in this way the situation of the consumer arbitrarily buying lettuce in the supermarket is imitated. A disadvantage of this approach is the difference in nutrient content over the batches. This was circumvented by including a control series (cut, but unwashed) and a water series (cut and washed with water) in each experiment. Each experiment was completed with two series washed with a liquid decontamination agent, used in respectively low and high concentrations, or one series when the lettuce was treated with gaseous chlorine dioxide.

In a first step the effect of the decontamination agents on the initial plate count of fresh-cut iceberg lettuce was examined. The initial microbial load of lettuce is usually situated in the range of ∼4−6 log cfu g^{-1}, and the counts from the exterior leaves exceeded those from interior layers by 1−2 log cfu g^{-1}, which corresponds with the initial microbial load of the lettuce used in the current study (4.2−5.6 log cfu g^{-1}) (*26*). Many papers have dealt with the effect of the traditionally used sodium hypochlorite on the microbial quality of minimally processed iceberg lettuce. A decontamination step with 100−200 mg L^{-1} NaOCl generally reduced the microbial load of fresh-cut iceberg lettuce by 0.9−2.1 log cfu g^{-1} (*26*−*28*). Abadias et al. (*9*) reported that a treatment with neutral EOW (50 mg L^{-1} free chlorine, pH 8.6) or with 120 mg L^{-1} sodium hypochlorite reduced the total aerobic mesophilic count by 0.8 and 0.9 log cfu g^{-1}, respectively, which was significantly different from the inactivation achieved after washing with distilled water (0.4 log cfu g^{-1}). Washing with neutral EOW reduced the total plate count of fresh-cut iceberg lettuce by 3.3 log cfu g^{-1} when compared with the unwashed lettuce and by 2.4 log cfu g^{-1} when compared with the water-washed lettuce (*29*). The slightly better results in that study can be due to the higher water-to-produce ratio (50 g L^{-1}) than the one used in our study (100 g L^{-1}). In a recent Spanish study microbial reductions achieved in fresh-cut iceberg lettuce after a washing treatment with 80 mg L^{-1} peroxyacetic acid ran up to about 1.5 log cfu g^{-1} (*28*). With regard to the use of 1.82 mg L^{-1} gaseous chlorine dioxide, Gómez-López et al. (*12*) obtained a microbial reduction of 1.1 log cfu g^{-1} when compared with the initial plate count. Generally, the inactivation of the established natural microflora obtained as a part of this study were analogous with those reported elsewhere. Nevertheless, it should be stated that the effect of a decontamination step on the natural microflora is smaller than the effect on artificially inoculated bacteria because of the formation of biofilms, their better attachment to or trapping in the vegetable leading to a decreased accessibility of the bacteria (*9*). Furthermore, when literature data on the inactivation achieved after a decontamination step in lettuce are compared, factors such as temperature, pH, water-to-produce ratio, and contact time as well as the concentration used should be considered (*30*). Finally, only three of all the tested treatments resulted in a significantly higher reduction than the ones achieved with water washing. These laboratory-scale experiments were conducted with fresh tap water. In the fresh-cut industry, water is frequently reused, allowing micro-organisms to accumulate in the water and resulting in a recontamination of the produce. Adding a decontamination agent to the wash water may control the microbial load of the wash water and can in some cases improve the microbial quality of the produce itself.

In a second phase the effect of the tested sanitizing treatments on the overall sensory quality was evaluated by means of triangle tests. None of the treatments changed the sensory quality significantly. Moreover, the panelists did not observe browning after the use of any of the sanitizers. Because differentiation between individual fruits and vegetables by consumers is based primarily upon appearance, objective color measurements were undertaken. A major defect of minimally processed lettuce is the browning of cut edges, which consequently limits the shelf life and marketability of fresh-cut lettuce. A decrease in luminosity L^* and an increase of the a^* value are associated with browning appearance and quality loss (*31*). Within this study no decreased luminosity was observed after washing with peroxyacetic acid and sodium hypochlorite. However, neutral EOW as well as chlorine dioxide resulted in a significant decrease of the lightness L^* and an increase of the a^* value, which can be an indication of browning. Nevertheless, assessors could not observe a significant difference in sensory quality between water-washed lettuce and lettuce washed with a decontamination agent. Because it takes several days for the section of cut lettuce to turn brown due to the de novo biosynthesis of polyphenols, differences in browning were not observed immediately after the decontamination step, which was the moment panelists evaluated the sensory quality (*32*). Contrary to our results, another study found that the luminosity in lettuce treated with electrolyzed oxidizing water (pH 6.5; 12.5, 60, and 120 mg L^{-1} free chlorine) was higher than in the chlorinated samples (pH 8.0; 120 mg L^{-1}) (*30*). Martín-Diana et al. (*33*) reported an increased lightness in fresh-cut iceberg lettuce after a decontamination step with 120 mg L^{-1} sodium hypochlorite (pH 8). In both papers it was stated that the increased luminosity could be due to the blanching effect on the tissue due to the oxidizing capacity of neutral EOW and sodium hypochlorite, which could cause air removal around the fine hairs on the surface of the plant and the expulsion of air between the cells (*31*, *33*). Higher concentrations of electrolyzed water might have produced more damage to the tissues, increasing the chances of nonenzymatic browning appearance (*31*). An increased a^* value after washing with sodium hypochlorite or neutral EOW or

contact with chlorine dioxide gas observed in this study can indicate the degradation of chlorophyll pigments as well as the appearance of browning (*33*). Sy et al. (*7*) also reported that immediately after a treatment with 1.4 mg L^{-1} chlorine dioxide fresh-cut lettuce underwent a slight browning, which became more severe with increasing chlorine dioxide concentrations.

In a last phase the impact of a sanitizing procedure on beneficial nutrients present in minimally processed iceberg lettuce was determined. Considerable differences in nutrient content were observed between different vegetable batches. The profile and/or the content of the phenolic micronutrients, vitamin C, carotenoids, and tocopherols of lettuce could vary according to the genotype but could also be influenced by various environmental factors such as agronomic practices (fertilization, irrigation), soil composition, temperature, sun irradiation, and light intensity (*13*). The vitamin C content, which is the sum of ascorbic acid and dehydroascorbic acid, of iceberg lettuce encountered within the framework of the current study fluctuated between 2.0 and 7.1 mg/100 g of fw, which corresponded with values reported in the literature before (*34*). Apart from the mechanical losses induced by water washing of the lettuce, no additional losses of vitamin C caused by adding a decontamination agent were observed in this study. This corresponds with the results reported elsewhere. Washing fresh-cut iceberg lettuce with distilled water, ozonated water (5 mg L^{-1}), NaOCl (200 mg L^{-1}), or hot water (50 °C) followed with ozonated water (5 mg L^{-1}) did not change the vitamin C content significantly (*27*). Similarly, the vitamin C contents of chlorine- and calcium lactate-treated lettuce did not differ (*33*). This also agrees with Akbas and Ölmez (*35*), who reported a decrease of the vitamin C content during storage but could not find differences between washing treatments.

Analogous to vitamin C none of the tested decontamination procedures decreased the total phenol content when compared with water-washed lettuce. With regard to phenols, storage conditions more significantly affected the phenol content than the use of a washing solution (*36*). Similarly, changes in individual phenolic compounds such as chlorogenic and isochlorogenic acid and monocaffeoyltartaric acid and dicaffeoyltartaric acid present in iceberg lettuce were independent of the washing treatments (water, ozonated water, chlorine) (*17*).

The main carotenoids retrieved in lettuce in a study by Nicolle et al. (*13*) were lutein [150−337 μg g^{-1} of dry weight (dw)], β-carotene (119−201 μg g^{-1} of dw), neoxanthin, violaxanthin, and lactucaxanthin, which were in good agreement with the results within the current study. Data on the effect of decontamination on the carotenoid content of lettuce are scarce. Dipping fresh-cut iceberg lettuce in chlorine (100 mg L^{-1} free chlorine, pH 8.6) did not significantly affect the β-carotene content (*35*). Also, in another study chlorine-treated iceberg lettuce maintained its carotenoid content (*16*). In the current study a washing step with sodium hypochlorite (20 and 200 mg L^{-1}) did not affect the β-carotene content, whereas 250 mg L^{-1} peroxyacetic acid significantly reduced its content. These opposite effects may be explained by a higher oxidative stress during a peroxyacetic acid treatment than during a sodium hypochlorite treatment.

However, the variability in lettuce constituents due to washing and/or disinfecting on day 0 is minimal compared with the sample variability between different parts of the tissue.

Besides the effect of decontamination on individual carotenoids in iceberg lettuce, their impact on the total antioxidant capacity of lettuce was determined, too. The total antioxidant capacity measured by means of the FRAP assay is a measurement for the presence of especially the water-soluble antioxidants described earlier and the presence of other antioxidants as well as for the synergistic action of the different antioxidants. Because a rather hydrophilic extraction solvent was used to extract the antioxidants, carotenoids as well as α-tocopherol will only be partly extracted. Consequently, vitamin C as well as phenols would be responsible for an important part of the antioxidant capacity. Given that the effect of a sanitation procedure on the content of the individual antioxidants is limited when compared with the nutrient losses induced by water rinsing, additional effects of a disinfection process on the total antioxidant capacity were not observed for electrolyzed oxidizing water and gaseous chlorine dioxide. In the case of sodium hypochlorite, a significant but incipient reduction of the total antioxidant capacity was indeed observed. Conversely, the FRAP antioxidant capacity after washing with peroxyacetic acid showed a decrease when compared with the antioxidant capacity of water-washed lettuce, but the FRAP value remained higher than the one of the unwashed lettuce. Opposite effects on FRAP antioxidant capacity were observed after using different sanitizers. This may be due to differences in the quality of the starting material and the different oxidation capacities of the treatments.

On the basis of the presented results it can be deduced that alternative decontamination agents can be applied during lettuce processing to reduce the use of sodium hypochlorite, which is associated with different drawbacks such as the potential formation of harmful disinfection byproducts. From a microbial point of view a treatment with neutral electrolyzed oxidizing water (30 mg L^{-1}) or with 250 mg L^{-1} peroxyacetic acid or contact with gaseous chlorine dioxide was the most effective in fresh-cut iceberg lettuce without influencing its sensory quality. Given that vegetable oils are the main source of tocopherols in the diet, neutral electrolyzed oxidizing water retained the nutrient content of fresh-cut iceberg lettuce the best. When these data are evaluated, the following remarks should be considered: (1) the used sanitation procedure within this study does not completely reflect the recommended procedure used by the fresh-cut industry, (2) the higher tested doses of sodium hypochlorite and peroxyacetic acid cannot be applied for fresh-cut washing, and (3) shelf-life studies should be conducted to evaluate the effect of these sanitation steps on the microbial and sensory quality and on the nutrient content throughout storage.

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LITERATURE CITED

(1) Nicola, S.; Fontana, E.; Torassa, C.; Hoeberechts, J. Fresh-cut produce: postharvest critical issues. *Act. Hortic.* **2006**, *712*, 223–230.
(2) Nguyen-the, C.; Carlin, F. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 371–401.
(3) Abadias, M.; Usall, J.; Anguera, M.; Solsona, C.; Viñas, I. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.* **2008**, *123*, 121–129.
(4) Carrasco, E.; Pérez-Rodríguez, F.; Valero, A.; García-Gimeno, R. M.; Zurera, G. Growth of *Listeria monocytogenes* on shredded, ready-to-eat iceberg lettuce. *Food Control* **2008**, *19*, 487–494.
(5) Park, C.-M.; Hung, Y.-C.; Doyle, M. P.; Ezeike, G. O. I.; Kim, C. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *J. Food Sci.* **2001**, *66*, 1368–1372.
(6) Koseki, S.; Yoshida, K.; Kamitani, Y.; Isobe, S.; Itoh, K. Effect of mild heat pre-treatment with alkaline electrolyzed water on the efficacy of acidic electrolyzed water against *Escherichia coli* O157:H7 and *Salmonella* on lettuce. *Food Microbiol.* **2004**, *21*, 559–566.

(7) Sy, K. V.; Murray, M. B.; Harrison, M. D.; Beuchat, L. R. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *J. Food Prot*. **2005**, *68*, 1176–1187.
(8) Huang, T.-S.; Xu, C.; Walker, K.; West, P.; Zhang, S.; Weese, J. Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. *J. Food Sci*. **2006**, *71*, 134–139.
(9) Abadias, M.; Usall, J.; Oliveira, M.; Alegre, I.; Viñas, I. Efficacy of neutral electrolyzed oxidising water (NEW) for reducing microbial contamination on minimally-processed vegetables. *Int. J. Food Microbiol*. **2008**, *123*, 151–158.
(10) Piagentini, A. M.; Mendez, J. C.; Guemes, D. R.; Pirovani, M. E. Modeling changes of sensory attributes for individual and mixed fresh-cut leafy vegetables. *Postharvest Biol. Technol*. **2005**, *38*, 202–212.
(11) Roura, S. I.; Pereyra, L.; del Valle, C. E. Phenylalanine ammonia lyase activity in fresh cut lettuce subjected to the combined action of heat mild shocks and chemical additives. *LWT* **2008**, *41*, 919–924.
(12) Gómez-López, V. M.; Ragaert, P.; Jeyachchandran, V.; Debevere, J.; Devlieghere, F. Shelf-life of minimally processed lettuce and cabbage treated with gaseous chlorine dioxide and cysteine. *Int. J. Food Microbiol*. **2008**, *121*, 74–83.
(13) Nicolle, C.; Carnat, A.; Fraisse, D.; Lamaison, J.-L.; Rock, E.; Michel, H.; Amouroux, P.; Remesy, C. Characterisation and variation of antioxidant nutrients in lettuce (*Lactuca sativa folium*). *J. Sci. Food Agric*. **2004**, *84*, 2061–2069.
(14) Kalt, W. Effects of production and processing factors on major fruit and vegetable antioxidants. *J. Food Sci*. **2005**, *70*, 11–19.
(15) Kaur, C.; Kapoor, H. C. Antioxidants in fruits and vegetables—the millennium's health. *Int. J. Food Sci. Technol*. **2001**, 703–725.
(16) Martín-Diana, A. B.; Rico, D.; Barry-Ryan, C.; Frías, J. M.; Henehan, G. T. M.; Barat, J. M. Efficacy of steamer jet-injection as alternative to chlorine in fresh-cut lettuce. *Postharvest Biol. Technol*. **2007**, *45*, 97–107.
(17) Beltran, D.; Selma, M. V.; Marin, A.; Gil, M. I. Ozonated water extends the shelf life of fresh-cut lettuce. *J. Agric. Food Chem*. **2005**, *53*, 5654–5663.
(18) Vandekinderen, I.; Van Camp, J.; Devlieghere, F.; Veramme, K.; Denon, Q.; Ragaert, P.; De Meulenaer, B. Effect of decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (*Daucus carota* L.). *J. Agric. Food Chem*. **2008**, *56*, 5723–5731.
(19) Dodson, K. Y.; Young, E. R.; Soliman, A. G. M. Determination of total vitamin C in various food matrices by liquid-chromatography and fluorescence detection. *J. AOAC Int*. **1992**, *75*, 887–891.
(20) Taungbodhitham, A. K.; Jones, G. P.; Wahlqvist, M. L.; Briggs, D. R. Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. *Food Chem*. **1998**, *63*, 577–584.
(21) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem*. **1998**, *46*, 3630–3634.
(22) Waterman, P. G.; Mole, S. *Analysis of Phenolic Plant Metabolites*; Blackwell Scientific Publications: Oxford, U.K., 1994.
(23) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr*. **2003**, *133*, 2812–2819.
(24) Kaur, C.; Kapoor, C. H. Antioxidant activity and phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol*. **2002**, *37*, 153–161.
(25) Meilgaard, M. C.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*, 4th ed.; CRC Press: Boca, Raton, FL, 2006.
(26) Baur, S.; Klaiber, R.; Wei, H.; Hammes, W. P.; Carle, R. Effect of temperature and chlorination of pre-washing water on shelf-life and physiological properties of ready-to-use iceberg lettuce. *Innovative Food Sci. Emerg. Technol*. **2005**, *6*, 171–182.
(27) Koseki, S.; Isobe, S. Effect of ozonated water treatment on microbial control and on browning of iceberg lettuce (*Lactuca sativa* L.). *J. Food Prot*. **2006**, *69*, 154–160.
(28) Allende, A.; Selma, M. V.; López-Gálvez, F.; Villaescusa, R.; Gil, M. I. Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biol. Technol*. **2008**, *49*, 155–163.
(29) Ongeng, D.; Devlieghere, F.; Debevere, J.; Coosemans, J.; Ryckeboer, J. The efficacy of electrolyzed oxidizing water for inactivating spoilage microorganisms in process water and on minimally processed vegetables. *Int. J. Food Microbiol*. **2006**, *109*, 187–197.
(30) Pirovani, M.; Piagentini, A.; Güemes, D.; Arkwright, S. Reduction of chlorine concentration and microbial load during washing-disinfection of shredded lettuce. *Int. J. Food Sci. Technol*. **2004**, *39*, 341–347.
(31) Rico, D.; Martín-Diana, A. B.; Barry-Ryan, C.; Frías, J. M.; Henehan, G. T. M.; Barat, J. M. Use of neutral electrolysed water (EW) for quality maintenance and shelf-life extension of minimally processed lettuce. *Innovative Food Sci. Emerg. Technol*. **2008**, *9*, 37–48.
(32) Murata, M.; Tanaka, E.; Minoura, E.; Homma, S. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement on sensory evaluation during storage. *Biosci., Biotechnol., Biochem*. **2004**, *68*, 501–507.
(33) Martín-Diana, A. B.; Rico, D.; Barry-Ryan, C.; Frías, J. M.; Mulcahy, J.; Henehan, G. T. M. Comparison of calcium lactate with chlorine as a washing treatment for fresh-cut lettuce and carrots: quality and nutritional parameters. *J. Food Sci. Agric*. **2005**, *85*, 2260–2268.
(34) Llorach, R.; Martínez-Sánchez, A.; Tomás-Barberán, F. A.; Gil, M. I.; Ferreres, F. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem*. **2008**, *108*, 1028–1038.
(35) Akbas, M. Y.; Ölmez, H. Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. *J. Food Sci. Agric*. **2007**, *87*, 2609–2616.
(36) Martínez-Sánchez, A.; Allende, A.; Bennett, R. N.; Ferreres, F.; Gil, M. I. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biol. Technol*. **2006**, *42*, 86–97.

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