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MICROBIAL CONTAMINATION IN PEELED CHESTNUTS AND THE EFFICACY OF POSTPROCESSING TREATMENTS FOR MICROBIAL SPOILAGE MANAGEMENT

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ABSTRACT

High undesirable spoilage of peeled chestnuts after thawing prompted a 2006–2007 survey in which chestnuts were quantitatively examined for microbial contaminants after harvest and peeling. Chestnuts (*C. sativa* × *C. crenata* cv. "Colossal") were collected after harvest from seven Michigan farms, and peeled using a commercial-brulage-peeler. Average mesophilic aerobic bacteria (MAB), yeast and molds populations in peeled chestnuts were 2.70, 2.74 and 2.51 after harvest; 3.46, 3.27 and 2.40 during peeling; and 5.39, 3.09 and <1.70 log CFU/g after peeling, respectively. Two bacteria *Rahnella* sp., and *Curtobacterium* sp. and the yeast *Candida* sp. were the primary causes of spoilage. With the objective of reducing microbial contamination, six postprocessing sanitizer treatments, as well as X-ray irradiation and warm water (65C) were evaluated (18 days of storage at 4C). X-ray irradiation, 3 min immersion in 92 ppm hydrogen dioxide and 65C water were most effective in reducing MAB and yeast.

PRACTICAL APPLICATIONS

High undesirable spoilage of peeled chestnuts after thawing prompted a 2006–2007 survey in which chestnuts were quantitatively examined for microbial contaminants after harvest and peeling. Two bacteria *Rahnella* sp., and *Curtobacterium* sp. and the yeast *Candida* sp. were the primary causes of spoilage. With the objective of reducing microbial contamination, six postprocessing sanitizer treatments, as well as X-ray irradiation and warm water (65C) were evaluated (18 days of storage at 4C). X-ray irradiation, 3 min immersion in 92 ppm hydrogen dioxide and 65C water were most effective in reducing MAB and yeast. This treatments can be practically used to reduce microbial decay and enhance peeled chestnut quality.

INTRODUCTION

As an alternative to fresh chestnut sales, peeled chestnuts are processed and sold to diversify the Michigan chestnut industry. Demand for products such as peeled chestnuts, considered to be a fresh processed (cut) product, is increasing as a result of consumer demand for healthy, fresh, easy to use and appetizing foods (Kader 2002; IFPA 2006). In Michigan, fresh chestnuts are commercially peeled with a commercial peeling system made in Italy and purchased and imported in 2001 (Boema; Neive, Italy; Fig. 1). After peeling, the chestnuts are typically vacuum-packed and stored frozen (Guyer *et al.* 2003). Other mechanical methods of peeling, for example, air-impingement de-shelling technologies, are also available but are not used in Michigan (Gao *et al.* 2008). Removing the shell and pellicle, which are considered to be natural physical barrier that protects the kernel, causes water loss and contamination of the kernel with pathogens and opportunistic organisms (Cantwell 1995; Mencarelli 2001), potentially affecting their final quality and safety (Field *et al.* 2006).

Within 10 to 15 days (~12 days) during storage of vacuum-sealed peeled chestnuts at 4C, sticky, yellow ooze on the surface of thawed peeled chestnuts can be developed.

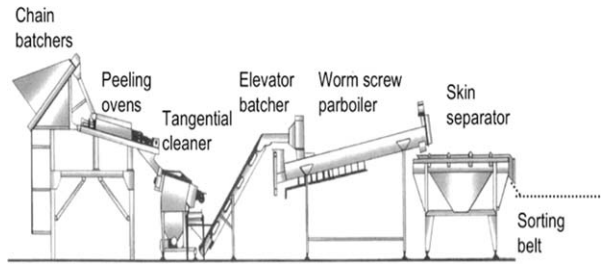

FIG. 1. SCHEMATIC DIAGRAM OF A BRULAGE CHESTNUT PEELING LINE (BOEMA; NEIVE, ITALY)

This ooze affects peeled chestnuts final quality and acceptability. Similar problems have never been reported in peeled chestnuts, but presence of slime or ooze is also common in processed fruits, vegetables, juices, ready-to-use salads and meats (Guerzoni *et al.* 1996; James *et al.* 2005; Tournas 2005; Ragaert *et al.* 2006; Brightwell *et al.* 2007; Ng 2007).

Growth of spoilage organisms is usually accompanied by the accumulation of metabolites, such as ethanol, lactic acid and ethyl acetate, among others. The organoleptic changes due to such metabolic activity are associated with the enzymatic oxidation of various compounds leaking from the injured tissues. Spoilage is detectable by sensory or microbiological methods when the microbial population reaches a certain level, which is dependent on the microbial species in question and the compositions of food material. In the worst case, shelf life of commercial products may not exceed five days even under conditions of refrigeration temperature (4C) (Guerzoni *et al.* 1996; James *et al.* 2005; Tournas 2005; Ragaert *et al.* 2006; Brightwell *et al.* 2007; Ng 2007). Zagory (1998) and Tournas (2005) indicated that after harvest, a wide range of economically important vegetables and specifically fresh-cut products, are often spoiled by a wide variety of microorganisms including various bacteria (*Curtobacterium, Rahnella, Erwinia, Pseudomonas* spp., among others) and fungal species. Spoilage significantly increases when the product is injured or the skin has been damaged or removed. Packaged sliced onions, shredded mixed lettuce and other commodities stored under air or modified atmosphere suffer from extreme colonization of diverse spoilage microorganisms, including bacteria, molds and several yeasts (*Pichia fermentans, Cryptococcus laurentii* and *Candida* spp., among others) (Liu and Li 2006; Ragaert *et al.* 2006). All these usually lead to undesirable quality and economic loss, which confirm the significance of microbial growth during production, harvest, processing and during storage (Zagory 1998; Tournas 2005).

Increasing attention has been focused on the microbial safety of processed fruits and vegetables, mainly on intervention methods to kill or remove human pathogens and spoilage microorganisms in fresh produce (Perish *et al.* 2003; Sapers 2003). In the majority of cases, sanitizing agents are added to processing water to reduce microbial populations and prevent cross-contamination of the product. Of these, chlorine has been used for several decades and is still the most widely used sanitizer in the food industry (IAFP 2002; Baur *et al.* 2004). However, chlorine does not always reduce microbial populations, including foodborne pathogens, and may be harmful due to the formation of toxic chlorine byproducts (Richardson *et al.* 2000; Foley *et al.* 2004; Vitro *et al.* 2005). New technologies, including food irradiation has been reported to achieve greater than a 5-log reduction of pathogens and other microorganisms (pasteurization treatments), having the potential to greatly improve the microbiological quality and safety of produce (Niemira 2003).

Therefore, the objectives of this study were to determine which spoilage organisms negatively impact peeled chestnuts before, during and after processing and how they were surviving and growing on the processed product. In addition, the efficiency of various postprocessing treatments to reduce microbiological populations and prolong the shelf life of peeled chestnuts was also assessed.

MATERIALS AND METHODS

Chestnut Samples

During the 2006 and 2007 growing seasons, chestnut samples (cv. "Colossal") from seven Michigan farms were assessed for microbial populations at seven points: Two points before processing (peeling), and five points during commercial brulage processing (Boema; Neive, Italy), at the Michigan State University Rogers Reserve, Jackson. MI. Triplicate chestnut samples (250 g) were collected in Whirl-Pak bags (NASCO, International Inc., 120 mm × 60 mm) (1) after transport to the facility (recollection buckets), (2) pre-peeling at the facility (receiver in chain batcher), (3) on the elevator batcher after passing through the burner, (4) passing through the steamer (worm screw parboiler), (5) passing through the counter-rotating rollers and through the brushes (skin separator), (6) transport on the sorting belt and (7) before packaging at the end of the peeling line. Samples were immediately transported to the laboratory on ice and analyzed within 24 h of collection.

Determination of Microbial Populations in Fresh Unpeeled Chestnut, and Peeled Chestnut Samples

Fresh unpeeled chestnuts were surface sterilized by dipping each chestnut in 99% ethanol and flaming after which the shell was aseptically removed. Each kernel sample was weighed (∼ 25 g) into a sterile Whirl-Pak stomacher bag diluted 1:5 in phosphate buffer solution (PBS) (pH 7.4) and

homogenized for 1 min (normal speed) in a stomacher Model 400 (Seward Lab. System, England). The same procedure, with exception of shell removal was repeated for all of the collected peeled chestnuts. The suspension was serially diluted and 100 µL aliquots were inoculated onto trypticase soy agar (Becton and Dikinson Sparks, MD) plus 0.6% yeast extract containing 100 ppm of cycloheximide (Sigma-Aldrich, St. Louis, MO) (TSA-YEC) for quantification of mesophilic aerobic bacteria (MAB) and onto potato dextrose agar containing 20 ppm streptomycin and 50 ppm ampicillin (PDA-SA) for enumeration of yeasts and molds.

Identification of Microorganisms

Microorganisms isolated from 25 samples (25 g) of symptomatically thawed peeled chestnuts, containing sticky, yellow ooze on the surface were picked and subsequently purified after isolation. Spoilage symptoms were described, photographed and classified.

MAB were identified using sequencing rDNA subunits (White *et al.* 1990). Bacterial genomic DNA was extracted using the protocol proposed by Jacobs *et al.* (2008). The region including the two spacers (UFLP and URPL) was amplified using the method of LiPuma *et al.* (1999). A total volume of 25 µL for each reaction contained 60 ng of DNA, 23 µL of AFLP amplification core mix (Applied Biosystems, Foster City, CA), 2.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems) and 10 pM of each primer. The primers UFPL (5′-AGTTTGATCCTGGCTCAG-3′) and URPL (5′-GGTTACCTTGTTACGACTT-3′) were used for targeting the 16S rDNA region from the kingdom Procaryotae (bacteria) (LiPuma *et al.* 1999).

The extracted DNA region was amplified in a 2,720 thermal cycler (Applied Biosystems). The program included a cycle at 95C for 2 min, 30 cycles of 95C for 30 s, 55C for 30 s and 71C for 1 min and a final elongation at 71C for 10 min. The PCR product was placed in wells on an agarose gel (1.25%), and electrophoresed for 3 h at 70 volts. After electrophoresis, the amplified region was purified using a QIAquick PCR Purification kit (Qiagen, MD). The PCR product was sequenced in both directions using moderate throughput sequencing (Research Technology and Support Facility, MSU, East Lansing, MI). Sequences were deposited in the Lasergene software (DNA Star Inc., Madison, WI) and used as queries for similarity searches in the NCBI nucleotide database (NCBI 2008). Species reported had a 98 to 100% match in both directions.

Yeasts were inoculated on potato dextrose agar, incubated for 72 h at 25C (± 3C) and then sent to the Michigan State University Diagnostic Center for Population and Animal Health (DCPHA) (MSU, East Lansing, MI) for further identification. Yeasts were identified using the API 20C yeast identification system together with microscopic morphology determinations. This computer-assisted system for rapid identification of yeast provides results comparable to those obtained of conventional morphological methodologies (Land *et al.* 1979).

Preparation of Inoculate

Based on visual observations, the identified cultures determined to be associated with spoilage in peeled chestnuts were used in further inoculation studies. Bacteria were subcultured on TSA-YEC and yeasts were subcultured onto PDA-SA and incubated for 48 h at 25C. A single colony of each strain was transferred to 5 mL of Lauria–Bertani (LB) soft media (Becton and Dikinson, Sparks, MD), incubated for 24 h at 25C, and constantly aerated on a rotator shaker at 150 rpm (model Classic C10, New Brunswick Scientific Co. Inc., Edison, NJ). Broth cultures were then sterilely transferred by pouring the subculture into sterile 100 mL LB media. This process was exponentially repeated, each 24 h until desired amount of inoculate from each organism was acquired.

Sterilization and Inoculation of Peeled Chestnuts

Whole chestnuts (cv. "Colossal") were mechanically peeled, vacuum-sealed in low-density polyethylene bags (Kent Buthcers Supply, Grand Rapids, MI) stored at – 5C for five weeks until used and then were completely thawed for four hours at 25C before use.

The thawed chestnuts were sterilized by two successive 10 min immersions in 20% sodium hypochlorite (1 L per 0.5 kg of chestnuts) with constant agitation on a rotator shaker (190 rpm) (model Classic C10, New Brunswick Scientific Co. Inc., Edison, NJ) and then subjected to three 15 min washings with sterile-deionized water (1 L per 0.5 kg of chestnuts) with agitation (190 rpm) to eliminate residual chlorine. Chestnuts were then placed on sterile blotting paper and air-dried in a bio-safety hood with for 25 min.

To confirm chestnut spoilage associated with isolated microorganisms, individual organisms and equal organism cocktail, containing between 10^8 and 10^9 CFU/mL of each organism were used as inoculate in the test. Sterilized chestnuts were placed in flasks contained mixed inoculate (1 L per 1 kg of chestnuts) and agitated on a rotator shaker (200 rpm) for 15 min. Inoculated chestnuts were placed on sterile blotting paper and air-dried in a bio-safety hood for 30 min.

Treatments of artificially inoculated peeled chestnuts, storage and microbial analysis. Eight treatments were implemented in an attempt to mimic industrial processes (Table 1). All liquid-solution treatments, except heat

TABLE 1. POSTPROCESSING TREATMENTS USED FOR INOCULATED PEELED CHESTNUTS

Commercial name	Active agent	Composition
X-ray irradiation (Rayfresh Foods Inc., Ann Arbor, MI)	low-energy X-ray irradiation, 70 kV / 57 mA	0.5, 1, 1.5, 2 kGy
Storox (BioSafe Systems, Glastonbury, CT)	27% Hydrogen dioxide + 2% peracetic acid	2,700-ppm hydrogen dioxide + 200-ppm peracetic acid
Agri-Cide (Life Science Group, Inc. Monticello, IN)	18.25 – 21.75% Copper sulfate pentahydrate	1 ppm
Chlorine dioxide solution (ICA TriNova, LLC Forest Park, GA)	$ClO_2 + H_2O$	10 ppm
Ozone solution (Aqua Air Technologies, Inc., Bloomfield, NI)	$O_3 + H_2O$	0.70 ppm
Peracetic Acid (Lenntech, Rotterdamseweg, The Netherlands)	Peracetic acid	80 ppm
Chlorine solution (Champion packaging, Inc. Woodridge, IL)	6% Sodium hypochlorite	100 ppm
Sodium chloride (Sigma-Aldrich, St. Louis, MO)	Sodium chloride	0.2 M
Warm water	Heat	65C

treatment (warm water), X-ray irradiation and ozone solutions, were thoroughly mixed, to achieve the desired concentration in 30 L of water. Inoculated ∼500 g of chestnuts were immersed in each solution for 2 min. After treatment, three ∼150g (∼12 chestnuts) samples per treatment were collected.

For warm water treatments, peeled chestnuts were treated with heated water generated by a laboratory water bath (model Durabath, Baxter Scientific, Melrose Park, IL). After the desired temperature was reached, inoculated chestnuts were immediately submerged in the water for 2 min. Temperature was constantly monitored using a 7 mm immersion thermometer (Fisherbrand Scientific by Ertco, UK). Five hundred grams of inoculated chestnuts were dipped at once in warm water for 2 min. After treatment, three ∼150 g (∼12 chestnuts) samples per treatment were collected.

Ozone was generated using a commercial ozone generator (Aqua Air Technologies, Inc., Bloomfield, MI) equipped with an oxygen concentrator model CD 10/AD Corona Discharge ozone generator system (Clear Water Tech., Inc., San Luis Obispo, CA). Gas was delivered through an inlet line directly into the water, which was constantly circulated through a 30 L water container. Water containing up to 0.7-ppm ozone was obtained within 25 min. After the desired concentration was achieved, ∼500 g of inoculated chestnuts were immersed in the ozone solution for 2 min. The ozone concentration was constantly monitored using a portable dissolved ozone meter (model OZ-21P, DKK-TOA Corporation, Takadanobaba, Shinjuku-ku, Tokyo, Japan).

Inoculated peeled chestnuts were irradiated at target doses of 0.5, 1.0, 1.5 and 2.0 kGy using a low-energy X-ray food irradiator (Rayfresh Foods Inc., Ann Arbor, MI) at 70 kV and 57 mA. The sample consists of a single layer of chestnuts (∼12 chestnuts, ∼150 g) in a Whirl-Pak plastic bag. Each sample was irradiated on both sides by flipping the sample halfway through treatment (double treatment), to maximize dose uniformity. Incident dose was measured at six locations on the surface using radiochromic film dosimeters (GAF3001DS, GEX Corp., CO). The dosimeters were read 24 h after irradiation using a standard spectrophotometric method (Spectronic Genesys 20, Thermo Fisher Scientific, Inc., Waltham, MA) based on calibration curves at 500/550 nm.

All populations of MAB and Y were determined on the day of treatment (day 0), and after 10 and 18 days of storage at 4C.

Statistical Analysis

Microbial Population in Peeled Chestnuts. One-factor analysis of variance (ANOVA) was done on all microbial populations obtained from peeled chestnuts. Significance among the means was determined by the Tukey post hoc multiple comparisons of means test at the 95% family-wise confidence level ($P = 0.05$). Calculations were performed by using the "R: A language and environment for statistical computing" statistical package (Ott and Longnecker 2001; R Development Core Team 2007).

Microbial Population in Treated Peeled Chestnuts. Repeated measurement design with analysis of variance (ANOVA) was done to average microbial count data (MAB and yeast) obtained from treated peeled chestnuts. Since the assignment of samples to the different treatment conditions as well as the choice of subsamples at each point of measurement were completely at random, sphericity can be assumed making multivariate as well as degrees of freedom corrections unnecessary (Keselman *et al.* 2001; Ott and Longnecker 2001). This procedure will indicate if a treatment significantly reduces the microbial population during 18 days of storage. Significance among the means was determined using the Tukey post hoc multiple comparisons of means test at the 95% family-wise confidence level ($P < 0.05$). These calculations were performed using the same statistical package listed above.

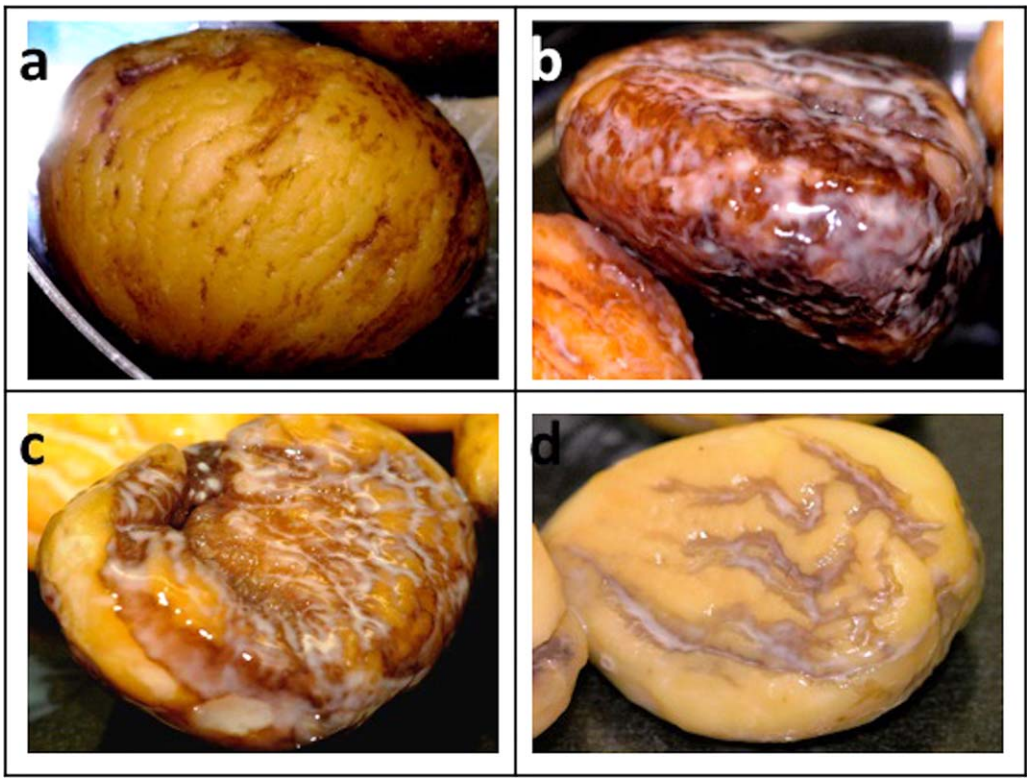

FIG. 2. PEELED CHESTNUTS, 10 DAYS AFTER INOCULATION AND STORED AT 4C
a) Noninoculated sterilized control, b) inoculated with *Curtobacterium* sp., c) inoculated with *Rahnella* sp., d) inoculated with *Candida* sp.

RESULTS

Microorganisms Associated with Spoiled Peeled Chestnuts

Two bacteria (*Rahnella* sp. and *Curtobacterium* sp.), and one yeast (*Candida* sp.) were consistently isolated from the samples (~ 25 g) of vacuum-packaged, peeled chestnuts thawed and stored for 10 to 15 days at 4C. These three microbes were considered the primary cause of spoilage as they each produced yellow, sticky ooze when artificially inoculated on sterile chestnuts. *Rahnella* sp. and *Candida* sp. were also found in fresh unpeeled chestnut kernels, and therefore the source of contamination of peeled chestnuts may be fresh chestnuts that are naturally contaminated when brought from the field. Although some browning was also associated with the spoilage, this can occur without the development of ooze as the chestnuts oxidize in time.

In most cases, all three spoilage organisms were present in spoiled chestnut; however, each alone, was also capable of causing spoilage. No obvious visual difference in terms of symptoms produced if either one or more organisms causing spoilage were observed (Fig. 2). The chestnuts were visually spoiled when the MAB and yeast populations exceeded 8-log CFU/g.

Chestnut Samples Microbial Population. Chestnut samples were collected at different locations before, during and after peeling, to determine how spoilage organisms were becoming established on the processed peeled product. Populations of MAB, molds and yeast before processing (buckets and receiver) varied from 2.50 to 2.90 logs CFU/g. After peeling, MAB ($P < 0.01$) and yeast ($P < 0.01$) increased significantly up to 5.39 and 3.09 logs CFU/g, respectively. In contrast, mold populations at the end of processing decreased to <1.7 logs CFU/g (undetectable level; Fig. 3). A significant difference was seen within all microbial populations determined from samples collected before, during and after processing ($P < 0.01$). In general, the brushes played an important role in significantly increasing MAB and yeast populations in peeled chestnuts.

Postprocessing Treatments

Sanitizers and Warm Water. To determine if the shelf life of chestnuts could be extended after thawing, several microbial reduction strategies were assessed using chestnuts inoculated with the three identified spoilage organisms. The mean, plus or minus the standard deviation, for populations

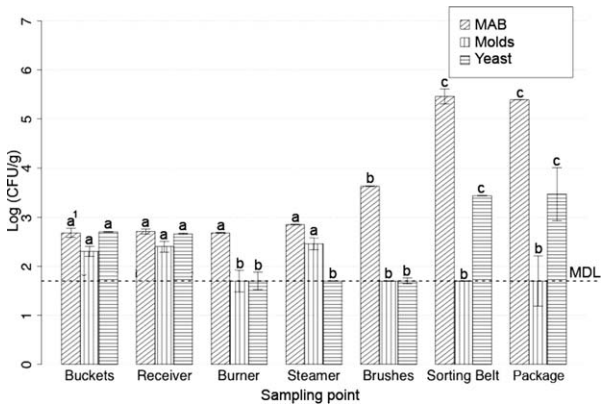

FIG. 3. TOTAL MAB, MOLDS AND YEAST COUNTS (LOG CFU G^{-1}) ON PEELED CHESTNUT SAMPLES (BRULAGE PEELER – BOEMA; NEIVE, ITALY)
[1]Values followed by the same letter within organisms are not significantly different at $P < 0.05$ (ANOVA) (Tukey multiple comparison of means). Minimum detectable level (MDL), represented by a dotted horizontal line = Minimum possible count (0.5) x minimum dilution factor x inoculated aliquot (100 µL). Error bars indicate standard deviation.

of MAB and yeast were evaluated immediately after each treatment, and after 10 and 18 days of storage at 4C (Fig. 4).

Two minutes exposure time to 10 ppm chlorine dioxide, 1 ppm copper sulfate pentahydrate and 80 ppm peracetic acid significantly reduced MAB and yeast population during 18 days of storage in comparison with the nontreated control (F [8, 2] = 43.89, $P < 0.01$). However, on day 18 the microbial populations had already surpassed the desired levels and spoilage could be observed. Only 2 min exposure time to 2,700 ppm hydrogen dioxide + 200 ppm peracetic acid and warm water treatment significantly reduced the mean MAB and yeast population during 18 days of storage in comparison with the nontreated control (F [8, 2] = 43.89, $P < 0.01$). In both cases, the mean microbial population on day 18 was significantly lower by 1.24 and 1.55 logs CFU/g respectively, in comparison with the nontreated control and no spoilage could be observed.

X-Ray Irradiation. To determine if spoilage could be managed and shelf life extended after thawing, various X-ray irradiation doses were tested on chestnuts inoculated with the three microbes associated with spoilage. The mean of MAB and yeast populations from the various doses of X-ray irradiation treatment were determined immediately after treatment, and 10 and 18 days of storage at 4C (Fig. 5).

All X-ray irradiation doses significantly reduced the MAB and yeast populations during 18 days of storage in comparison with the nontreated control ($P < 0.01$). The mean microbial reductions when compared with the nontreated control on day 18 for 0.5, 1.0, 1.5 and 2.0 kGy were 1.47, 2.33, 3.69 and 4.02 logs CFU/g, respectively. After 18 days no spoilage could be observed in any of the treated chestnuts.

DISCUSSION

Within 12 days after thawing, commercially brulage peeled chestnuts (Fig. 1) developed sticky, yellow ooze over the surface (Fig. 2) that affected the final quality, acceptability and shelf life. The three primary spoilage microorganisms identified included two bacteria *Rahnella* sp., *Curtobacterium* sp. and one yeast, *Candida* sp. There is no evidence indicating that filamentous fungi play an important role during spoilage.

Contamination of peeled chestnuts was strongly influenced by the peeling process. The traditional method of peeling chestnuts (e.g., manual elimination of the shell and pellicle from fresh chestnuts) is laborious and requires heat and knives. Other mechanical methods, such as air-impingement de-shelling, are also available but are not used in Michigan (Gao *et al.* 2008). Michigan chestnut samples indicated that average MAB and yeast populations significantly increased during peeling with the skin separator (brushes) and sorting belt identified as key points of contamination.

Similar results have been reported for other commodities and processing plants. Monitoring of several organisms, like *Listeria monocytogenes* during produce processing has indicated that cross-contamination from contaminated zones in the processing line, floor surface and gloves is critical and extremely difficult to control (Pappelbaum *et al.* 2008). Others have reported that utensils, processing tables and lines may also increase the levels of MAB, coliforms and pathogenic organisms like *Escherichia coli* 0157:H7 (Christison *et al.* 2008). All of these studies have strongly indicated

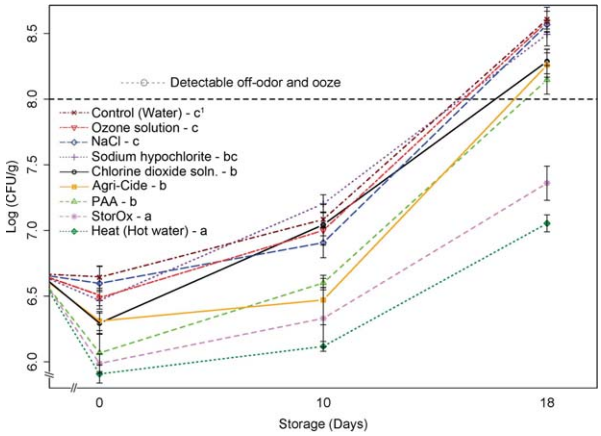

FIG. 4. MEAN MAB AND YEAST COUNTS (LOG CFU G^{-1}) FROM PEELED CHESTNUTS TREATED WITH DIFFERENT SANITIZERS DURING 18 DAYS OF STORAGE AT 4C
[1]Overall data point followed by the same letter within treatments are not significantly different at $P = 0.05$ (Repeated measurement design – ANOVA with post hoc Tukey multiple comparison of means). Error bars indicate standard deviation.

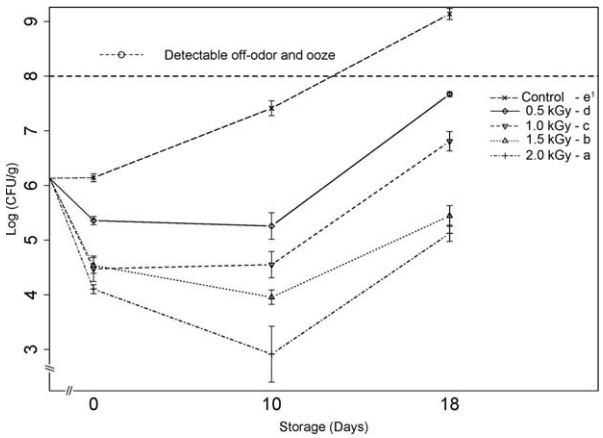

FIG. 5. MEAN MAB AND YEAST COUNTS (LOG CFU G^{-1}) FROM PEELED CHESTNUTS TREATED WITH DIFFERENT X-RAY IRRADIATION DOSES DURING 18 DAYS OF STORAGE AT 4C

[1]Overall data point followed by the same letter within dose levels are not significantly different at $P = 0.05$ (Repeated measurement design – ANOVA with post hoc Tukey multiple comparison of means). Error bars indicate standard deviation.

that the processing environment may play an important role in maintaining and enhancing pathogen populations. Because of these possibilities, and since MAB and yeast populations significantly increased by more than 2 logs in chestnuts between harvest and after peeling, improved and more effective microbial reduction strategies after and during processing are needed to ensure the quality and prolong the shelf life of peeled chestnuts.

A method to reduce microorganisms in the final product is to eliminate microbial presence by constantly disinfecting sections from the processing line, using different detergents, sterilizing agents and enzymes. These methods have been evaluated in dairy and meat processing lines (Anon. 1970; Manvi and Anand 2001) and have also proven to be effective in removing microbial biofilms from dispensing equipment (Walker *et al.* 2007). Nevertheless, further studies must be done to apply these methods to the chestnut peeler.

Another alternative is to try to inactivate these spoilage microorganisms after processing and prior to storage. In most cases, sanitizing agents are added to processing water and consequently to the product, to reduce microbial populations and prevent cross-contamination of the processed commodity (Sapers 2003). Hydrogen dioxide concentration of 1 to 10% have been used to reduce the microbial population and extend the shelf life of whole fruits, like blueberries, fresh-cut fruits and vegetables (Sapers 2003; Crowe *et al.* 2005). Hydrogen dioxide is Generally Recognized As Safe (GRAS), because it is reduced to water and oxygen after treatment (Sapers 2003). Although treatments containing high concentrations of hydrogen dioxide may be useful in reducing microbial populations and extending shelf life, the use of these treatments in commodities like lettuce, blueberries and others, might be limited because of possible plant surface oxidation resulting in product damage (Block 1991; Aharoni *et al.* 1994; Simmons 1997; Ukuku and Sapers 2001; Ukuku *et al.* 2001). The present study indicated that hydrogen dioxide (1:100 dilution of Storox, BioSafe Systems, Glastonbury, CT) significantly reduced the mean populations of MAB and yeast in peeled chestnuts, during 18 days of storage in comparison with the nontreated control.

Warm water (65C) was found to be among the best methods in significantly reducing spoilage as well as MAB and yeasts in peeled chestnuts, during 18 days of storage, in comparison with the nontreated control. Heat treatments are often applied for a relatively short time (Fallik 2004), as hot water dips or rinsing, vapor heat, hot dry air (Fallik 2004) and other relatively new developed technologies like far infrared radiation (Tanaka *et al.* 2007). Historically, water has been the preferred medium for most applications since it is a more efficient heat transfer medium than air (Fallik 2004). Immersion of avocado fruit (cv. "Hass") for 20–30 min at 40–42C controls decay and enhances the storage quality (Fallik 2004). Immersion of sweet chestnuts in 45C water for 45 min enhanced storage quality of fresh product (Jerimini *et al.* 2006). A five log reduction of *Escherichia coli* O157:H7 was also observed after immersing apples in 80 and 90C water for 15 s (Fallik 2004).

Studies have demonstrated that irradiation may also be capable of reducing pathogens and other microorganisms more than five logs in many foods. Food irradiation is approved for specific foods by the Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA)/World Health Organization (WHO), but irradiated products must be labeled properly using the Radura symbol. Given the lack of evidence for toxic residues, irradiation may become an attractive alternative to chemical sanitizers. However, irradiation remains expensive, requires special facilities and still lacks consumer acceptability (Diehl 1995; Howard *et al.* 1995; Kilcast 1995; Gamage *et al.* 1997; Luh 1997; Niemira 2003; Smith and Pillai 2004; Saroj *et al.* 2006). All evaluated X-Ray irradiation doses (0.5, 1, 1.5 and 2 kGy) significantly reduced the mean population of MAB and yeast during 18 days of storage and decreased spoilage in comparison with the nontreated control. Using the highest dose of radiation, population of MAB and yeast were 4 log CFU/g lower after 18 days of storage. Similar results have been reported for other commodities (Niemira 2003). Nevertheless, it is important to consider that, both warm water treatments and irradiation may damage produce tissue and leak nutrients by breaking down cell wall material, producing off-flavors in the treated produce (Lydakis and Aked 2003; Niemira 2003; Soto-Zamora and Yahia 2005; Saroj *et al.* 2006). Further sensory evaluation is recommended to discard possible effects.

CONCLUSION

The present study showed that two bacteria *Rahnella* sp., and *Curtobacterium* sp. and the yeast *Candida* sp. were the primary causes of spoilage of mechanically peeled chestnuts. Overall, MAB and yeast in chestnuts significantly increased during peeling, with the skin separator (brushes) and sorting belt being key points of contamination. Nevertheless, several postprocessing treatments were able to inhibit the growth of these microorganisms. Among these, 2,700 ppm hydrogen dioxide + 200 ppm peracetic acid, warm water immersion and X-ray irradiation were most effective. The prospect of adopting these methods as commercial postprocessing treatments needs to be examined and may have several advantages for the peeled chestnut industry. Cost, efficient application, monitoring and implementation of each method must also be examined to maximize economic gains. All of the evaluated treatments are presently used in food processing and are generally regarded as safe at the appropriate concentration. These may be implemented as part of a sustainable integrated pest management strategy for peeled chestnut spoilage.

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