MICROBIOLOGICAL DESIGN AND VALIDATION OF THERMAL AND HIGH PRESSURE PROCESSING OF ACIDIFIED CARROTS AND ASSESSMENT OF PRODUCT QUALITY

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Received for Publication April 12, 2015
Accepted for Publication July 14, 2015

doi:10.1111/jfpp.12552

ABSTRACT

Modification of pH and combined use of novel processing methods may be a good strategy to improve the quality of canned vegetables. In this study, selected thermal (TP) and high pressure-assisted thermal (HP-T) processing methods were validated for citric acid-infused carrots (pH ≤ 4.5) using *Bacillus licheniformis* spores. Previously established thermal inactivation kinetics data were used to setup the target process times (to achieve 7-log kill of *B. licheniformis*). The microbial spores were inoculated at the center of simulated carrot alginate beads and subjected to different processing methods. Delivered process lethalities, evaluated by the microbial count/re-count method and measured time–temperature data, were equal to or higher than the targeted values. No survivors were found after the treatments, demonstrating the adequacy of the processes. Texture, color and β-carotene retention in processed carrots, evaluated and compared with those processed under conventional canning, showed higher texture retention ($P < 0.05$) in the modified processing methods. Residual hardness values of carrots were 86% with HP-T, 70% with ohmic heating and 8% with conventionally canned product. The same trend was observed with chewiness value. However, processing methods showed no differences ($P > 0.05$) with respect to color change. In terms of β-carotene, carrots subjected to a relatively more severe heat treatment (water immersion mode in static retort) showed better β-carotene extractability than samples from HP-T.

PRACTICAL APPLICATIONS

Conventional thermal processing of "low acid" foods (pH > 4.6) experiences significant quality loss due to the long thermal processing times required to inactivate spores of the key pathogen *Clostridium botulinum*. Alternative thermal processing techniques have evolved to shorten the processing times by enhancing heating rate to the product through process modifications or using thin profile packages. Even though quality retention can be enhanced through these modifications, thermal damage to quality is still indispensable since the sensitively microbial destruction to heat remain unaltered. Product acidification moves the "low acid" foods to "acid" category, thereby shifting the process regulation from the high temperature sterilization to the lower temperature pasteurization conditions. Savings in energy and reduction in process time immediately become obvious. Novel acidification methods are necessary for making the process efficient. *Bacillus licheniformis* is important and a suitable microorganism for validating thermal processing of acidified foods. The combined use of acid infusion methods and alternative thermal processing technologies could play a significant role to improve quality of acidified foods as compared to current practices.

INTRODUCTION

In the food industry, the recent focus has been to produce better quality products without compromising safety. Depending on their intensity, thermal and high pressure (HP) processes are classified into two categories: pasteurization and commercial sterilization. Pasteurization is a relatively mild temperature process (70–100C) or a moderate HP process (~650 MPa) that destroys the vegetative cells of microorganisms but has almost no effect on spores. However, commercial sterilization is a thermal process at high temperatures (above 100C) or elevated temperature–pressure (HP-T) combination processes (700–900 MPa, with product initial temperature of 70–90C) with the objective of destroying the pathogenic and spoilage-causing microorganisms. Commercial sterilization is defined as the condition achieved by the application of heat (sufficient alone, or in combination with other appropriate treatments) to render the food free of microorganisms capable of growing in the food at normal nonrefrigerated conditions at which the food is likely to be held during distribution and storage. Obviously, it means the processed product could have some dormant microbial spores that are incapable of showing their activity during normal storage and distribution conditions.

Different processing scenarios can be used to meet the objectives of enhanced quality retention. The efficacy of a given process for a specific food product is evaluated on the basis of protocols and/or scientific data to demonstrate that the process and associated control procedures can be reproduced to deliver safe and better quality products. Food and Drug Administration (FDA) of US defines validation as a "documented program which provides a high degree of assurance that a specific process will consistently and repeatedly produce a product meeting its predetermined specification and quality attributes" (WHO 1999). Validation is a critical step for the assurance of product safety (Leaper and Richardson 1999) not only for those which receive a thermal process but also for other alternative processing methods. In validation studies, microbial destruction data are used to determine whether thermal or nonthermal technologies individually or in combination with pH, water activity, preservatives and other adjuncts will provide sufficient lethality to render a food product is safe (National Advisory Committee on Microbiological Criteria for Foods [NACMCF] 2010) or inhibit the germination and growth of other heat-resistant spores.

In thermal processing, pathogen survival depends on treatment time and temperature employed to achieve the target lethality. In addition to thermal destruction, there are other complementary strategies that could be adopted to control the growth of survivor spores. For instance, it is indicated that the probability of growth and toxin-producing activity of the pathogen, types A and B of *Clostridium botulinum*, is low in products with pH < 4.6 (Stumbo 1973), and therefore, this microorganism is considered to have no public health concern in acidic foods. However, this might not always be the case. For example, if *Bacillus licheniformis* spores are present in the product and if the given pasteurization treatment is insufficient to kill them, their growth and activity could elevate the pH to levels beyond 4.6 and thus could create conditions conducive for germination and growth of *C. botulinum* spores (Rodriguez *et al*. 1993). Therefore, the pasteurization process schedule for high acid or acidified foods should be sufficient enough to inactivate spores of *B. licheniformis*. A carefully validated pasteurization process would result in shelf-stability in acidic foods (pH < 4.5) at room temperature without compromising public health (Ramaswamy and Abbatemarco 1996). Evaluation of the impact of various processes on the quality attribute should be important in addition to assuring product safety. This allows for a processing method to comply with both safety and quality issues. Therefore, the focus of this study was to evaluate the combined use of acid infusion and thermal/pressure-assisted pasteurization methods for process validation using *B. licheniformis* spores and their influence on the quality of the processed carrots.

MATERIALS AND METHODS

Preparation of Carrot Sample and Acid Infusion

Carrots were purchased from a grocery store and stored under refrigerated conditions (4C) until use. The top and bottom portions of each carrot were first cut and then they were peeled, cut to ~1 cm^3 and blanched in water at 90C for 3 min to inactivate enzymes. Blanched carrot cubes were heat-sealed in two polyethylene bags: one with 1.15% (w/v) citric acid (AI) the other with distilled water (control), in the proportion of 3:1 (v/v) liquid : solid. These bags were then subjected to a short HP treatment (255 MPa, 5 min) to rapidly infuse the acid solution to achieve a pH of 4.4 ± 0.1 at the core of the AI cubes. The HP treatment was carried out with water as the pressurization medium, which was operated at a pressurization rate of 4 MPa/s and a depressurization time of less than 10 s. The samples were introduced at room temperature (24 ± 1C) and reached a maximum temperature of 30 ± 1C during pressure treatment due to adiabatic heating (Tola and Ramaswamy 2013). No significant pH variation occurred in control samples after high pressure treatment, and it remained at 6.0 ± 0.2. HP-treated carrots were then removed from the bags and briefly rinsed with distilled water and used for subsequent tests.

Carrot Alginate Bead Preparation, Acid Infusion and Sterilization

Leaching of inoculated spores from real food particles during processing is a major limitation in biological validation of thermal processing and often results in overestimation of delivered lethality. In order to overcome this limitation, simulated food particles like those fabricated using alginates, egg albumin, etc., are commonly used in validation studies. Following this trend, alginate particles were prepared using carrot puree according to method indicated by Brown *et al.* (1984) with under the optimum conditions established in Hassan and Ramaswamy (2011). Briefly, 300 g of peeled carrots was boiled for 20 min and then blended with 100 mL of distilled water to make fine puree. The puree was thoroughly mixed with 0.225 g of trisodium citrate and 14.1 g of sodium alginate. A quantity of 0.9 g of calcium sulfate suspended in 30 mL of distilled water was added to the puree and mixed thoroughly. The pure was then rolled into balls of approximately 2 cm diameter and soaked for 32 h in 2.6% calcium chloride solution. Hard enough beads can save their integrity until the end of a given process and will have similar thermophysical properties to a real carrot particle (Marcotte *et al.* 2000; Hassan and Hosahalli 2012). Soaked balls were withdrawn and rinsed with distilled water and then cut into 1-cm cube sizes. The cubes then were infused with citric acid solution (1.15% w/v) as indicated previously. The cubes were then autoclaved at 121C for 15 min for inoculated pack study. Additional details of the methodology are detailed elsewhere (Tola and Ramaswamy 2014a,b).

Chemical Sterilization of Real Carrot Cubes Used as Filler

For validation study and to simulate actual processing condition, the following procedures were used: pre-sterilized cans (for conventional thermal processing, TP), ohmic heating cell (for ohmic heating treatment, OH) and polyethylene bags (for HP-T treatment) were filled with sterile carrot cubes and sterile liquid (water acidified with citric acid to pH 4.4 ± 0.1). Carrot cubes were first washed with soap solution and thoroughly rinsed in running tap water. Washed cubes were transferred to a sterile beaker and soaked in sufficient volume of sodium hypochlorite solution (2.5%) for 5 min. Soaked samples were removed and again washed three times with sterile distilled water and further soaked for 20 min (two times) in excess sterile water to remove residues of the hypochlorite solution. The carrot cubes were then acid infused using sterile 1.15% citric acid solution according to the previous protocol to adjust the pH to 4.4 ± 0.1 and then were used with sterile alginate beads from the previous step for validation study.

Target Microorganism and Inactivation Kinetic Parameters

B. licheniformis spore is recommended as a surrogate for acid-tolerant, pH-elevating and heat-resistant *Bacillus* species to validate processing methods of acidified low-acid foods (FDA 2010). *B. licheniformis* can grow in pasteurized acidified foods because of its capability to survive and grow at low pH. It has higher heat (Janštová and Lukášová 2001) and pressure (Nakayama *et al.*, 1996) resistance as compared to other *Bacillus* species. Lack of complete inactivation of these spores can contribute to an indirect food safety risk. Survivor spores can germinate and grow in acid or acidified product and neutralize lower pH to higher level (pH > 4.6) at which spores of *C. botulinum* are able to grow. Due to this reason, complete inactivation of this bacterium in acid or acidified foods is a critical step to ensure food safety. Therefore, for this study, the bacterium was selected as a target surrogate for validation of acidified low-acid foods. Inactivation kinetic parameters of *B. licheniformis* spores determined in our previous work were used as an input to validate the processes. Thermal inactivation data of the spores for conventional ($D_{97C} = 1.2$ min at pH = 4.5 $z = 12.6$C) and ohmic ($D_{97C} = 1.1$ min at pH = 4.5 $z = 16.3$C) heating methods are detailed in Tola and Ramaswamy (2013) and parameters for HP-T ($D_{600\ MPa,97C} = 0.6$ min at pH = 4.5 $z = 23.3$C) are detailed in Tola and Ramaswamy (2014a,b).

Processing Methods

In this study, six alternative pasteurization methods and one conventional sterilization schedule were included, as indicated in Table 1, to conduct microbial validation and product quality evaluation. Among six pasteurization methods, four of them were based on conventional thermal methods and the other two were novel food processing methods (ohmic heating and HP-T processing). All processing methods were validated mimicking actual commercial processing conditions. Selected processing methods were as follows: (i) cans (307×306) in water immersion mode (WIM) in static retort at 97C; (ii) cans in WIM in a shaking water bath to simulate agitation heating (100 strokes/min at 97C), orienting of cans either in horizontal (SK-H) or vertical (SK-V) position; (iii) cans in static retort using steam (97C) as a heating medium (ST-P); (iv) ohmic heat using a custom-made ohmic heating unit at 97C (Fig. 1); (v) high pressure-thermal treatment (600 MPa at 60C); and (vi) commercial canning in static retort (121.1C) using steam as a heating medium (ST-S) for non-acid-infused carrot to represent low-acid food processing. All test samples, except for the conventional method, were infused with 1.15% citric acid and their pH were reduced to

TABLE 1. TYPES OF PASTEURIZATION METHODS VALIDATED FOR INACTIVATION OF SPORES OF *BACILLUS LICHENIFORMIS* AND EVALUATION OF QUALITY PARAMETERS FOR CARROT

No.	Treatments	Cans/heating cell/bag orientation during processing	*P* values based on kinetic data (Eq. 2)
1	Water immersion mode at 97C in static retort (WIM)	Vertical	$P_{97C}^{12.6} = 8.4$ min
2	Steam heating mode at 97C in static retort (ST-P)	Vertical	$P_{97C}^{12.6} = 8.4$ min
3	Shaking of cans in water immersion mode at 97C in water bath (SK-V)	Vertical	$P_{97C}^{12.6} = 8.4$ min
4	Shaking cans in water immersion mode at 97C in water bath (SK-H)	Horizontal	$P_{97C}^{12.6} = 8.4$ min
5	Ohmic heating in heating cell at 97C (OH) (static heating)	Vertical	$P_{97C}^{16.3} = 7.7$ min
6	High pressure-assisted thermal treatment (HP-T) (600 MPa-60C)	Polyethylene bag	$P_{60C\text{ at }600\text{ MPa}}^{23.3} = 4.2$ min *
7	Commercial sterilization in steam static retort at 121C (ST-S)	Vertical	$F_{121C}^{10} = 3$ min †

* Based on determined kinetic data, combined pressure and heat treatment at pH 4.5 of medium a 7-log reduction can be achieved for 4.2-min holding time.† Literature values to deliver minimum process lethality to achieve 12D reduction for *Clostridium botulinum* spores.

4.4 ± 0.1 for pasteurization treatments However, test samples under commercial canning (processing at 121C) were not acidified. In all cases, cooling was done by immediately removing and immersing the containers in cold water. All pasteurization conditions were selected based on microbial inactivation kinetic results as indicated earlier. However, for sterilization treatment, $D_{121C} = 0.21$ and $z = 10$C for *C. botulinum* were used (Stumbo 1973).

Thermal Treatments

For thermal treatment, a pilot-scale retort, a water bath and a custom-made OH unit were used. Cans (307×306, Home Canning Co., Montreal, QC, Canada), ohmic heating cell (12 cm length × 9 cm internal diameter) for OH and polyethylene bag (12 cm length × 12 cm width) for HP were filled with equal weight (200 g) of carrot and liquid (160 g). The liquid was water acidified with citric acid to pH 4.4 for pasteurization treatments or 2% brine for commercial canning. For thermal processing methods, tips of two thermocouples (type-T, Omega Engineering Corp., Stamford, CT) were located at the center of alginate beads and carrot cubes (two for each) and placed close to each electrode (preliminary study showed that samples close to electrodes heated slower than cubes in other parts) and geometric center of cans, respectively. Thermocouple's attachment was made for those non-inoculated samples to gather time/temperature data. Output was recorded using data acquisition system (HP34970A, Hewlett Packard, Loveland, CO) at 10-s intervals. Thermal treatments (97C or RT 121C) were carried out using the retort in water immersion or steam heating mode or using water bath (Julabo Labortechnik GMBH, model SW22, Seelbach, Germany) for the shaking mode.

OH treatment was given using a custom-made static ohmic heating unit. A schematic of setup is shown in Fig. 1. Briefly, the unit consisted of a treatment chamber (a cylindrical glass beaker, 12 cm long, internal diameter of 9.0 cm and wall thickness of 4 mm) and two stainless steel electrodes (15 cm long and 20 mm thick and the distance between them was 7.5 cm) bent to the same contour of the cylindrical glass beaker. The system included an 1,10V 60 Hz AC input power supply, an AC/DC transformer and a variable frequency generator (Scientifix Ltd., Guelph, ON, Canada) to generate alternative current at specific frequency for ohmic heating. The unit was operational up to 170 V and the adjustable frequency range was 1–30 kHz. A frequency of 4 kHz square-wave electrical power was maintained with the help of oscilloscope (Tektronix TPS2012, CA) and with maximum applied current of 9 A.

High Pressure-Thermal (HP-T) Treatment

The HP-T treatment was given in the HP equipment indicated in Tola and Ramaswamy (2014a). A high-capacity

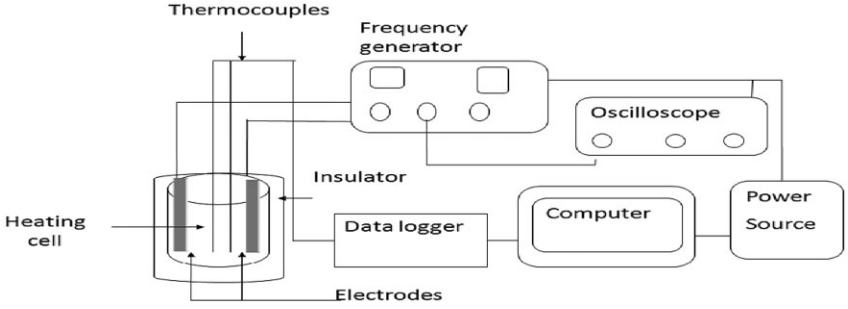

FIG. 1. EXPERIMENTAL SETUP OF STATIC OHMIC HEATING (OH) SYSTEM

water bath (FP45, Julabo Labortechnik GMBH) was used to circulate temperature-controlled hot water around the pressure shell in order to regulate the shell temperature to +2C above the target temperature to compensate heat dissipation to maintain test temperature. To maintain required temperature stability at a given pressure, combined HP-T treatments were conducted using a thick-walled polyoxymethylene insulating chamber as sample holder during treatments. Full details of this chamber and its performance are presented in Shao *et al.* (2010). Prior to treatment, the insulator and sample were preheated in a temperature-controlled water bath to stabilize their initial heating temperature (T_i) and achieve the target heating temperature (Nguyen *et al.* 2007):

$$T_i = T_t - \left(H_C \times \frac{\Delta P}{100} + \Delta T_H \right) \quad (1)$$

where T_i is the initial temperature of the sample required to reach the target temperature, T_t is the target temperature, H_C is the heat of compression of the sample (positive $\Delta T°$ per 100 MPa pressure build-up), ΔP is the processing pressure during the holding time and ΔT_H is the temperature gain by the sample from surrounding medium.

Given the carrot's high moisture content and the fact that they were soaked in citric acid solution, H_C can be assumed to follow that of water. Moreover, since samples were kept in a well-insulated chamber (Shao *et al.* 2010), one can assume that they gained no heat ($\Delta T_H = 0$) from the surrounding medium before pressurization. Samples were subjected to pressure/temperature combination of 600 MPa/60C for 4.2 min. After each treatment, samples were immediately withdrawn and cooled in cold water.

Determination of Process Lethality

In order to compare different processes regarding their lethality, the concept of the "*P* value" was used. The *P* value is defined as the duration (min) required to achieve a given reduction ratio in the number of microorganism at a given constant temperature (Holdsworth 1985). Designed *P* values were calculated from the initial and final spore counts and available inactivation kinetic data of target microorganism using Eq. (2), assuming the traditional thermal death time model. This is a first-order reaction model that is defined by the decimal reduction time (D_T) and a temperature sensitivity kinetic parameter (z value). This equation indicates the severity of a given thermal pasteurization method with respect to microbial destruction and it is a key element to establish processing time at constant temperature:

$$P_T^z = P \text{ value} = D_T \log\left(\frac{N_0}{N} \right) \quad (2)$$

where P_T^z is the *P* value at 97C and certain z value, N is the final count of recovered spores after a specific time–temperature history, N_0 is the initial spore count at time zero and D_T is the decimal reduction time (min) at a fixed temperature to reduce the spore concentration by a factor of 10.

The improved general method based on the numerical integration of lethality based on gathered time–temperature data allows comparisons to be made between different processes at a given reference temperature. Due to temperature gradient or residence time distributions, every spatial element of the food product will experience an different time–temperature history which needs to be integrated to quantify the cumulative loss in a given food quality parameter or microbial destruction. These will yield ultimately a singular integrated impact of the process. By integrating these point values (from collected time–temperature profile data at the slowest heating and cooling point), an actual average process value can be obtained using Eq. (3):

$$P_T^z = \int_0^t 10^{(T-T_r)/z} \times dt \quad (3)$$

where P_T^z is the *P* value at 97C and certain z value, T is the temperature of the product at time t (C), T_r is the reference temperature (97C) and z is the temperature change required to effect a 10-fold change in the D_T value (C).

The *P* value calculated using Eq. (2) will be equivalent to that calculated from time–temperature integration (Eq. 3) provided that first-order kinetics have been followed for the spores destruction throughout the heat process. By combining the above two equations, cumulative log reduction from a given integrated pasteurization treatment can be estimated using Eqs. (4) and (5):

$$P_T^z = \int_0^t 10^{(T-T_r)/z} \times dt = D_T \times \log\left(\frac{N_0}{N} \right) \quad (4)$$

$$\text{Cumulative log reduction} = \log\left(\frac{N_0}{N} \right) = \frac{P_T^Z}{D_T} \quad (5)$$

Before processing, all samples were test-run to ensure that consistent processing conditions were maintained and to establish required *P* values (i.e., time [min] required to achieve 7-log reduction on *B. licheniformis* spores). The above equations are widely used for canned food thermal process evaluation and calculation, and equally applicable for samples subjected to OH. The efficacy of HP-T treatment to inactivate the spores evaluated using Eq. (2) considering specific D_T value as indicated earlier.

Microbial Validation Study

Bacterial Spore Preparation, Inoculation and Recovery. For the inoculated pack study, the initial spore

concentration was first adjusted to approximately 10^9 cfu/mL. Then, this suspension was inoculated to the center of five sterile carrot alginate beads (20 μL spore suspension per bead, and five beads per package to give a cumulative volume concentration of 100 μL per package) using sterile syringe targeted to give approximate inoculation level of 10^7 spores/can (or per heating cell for OH or polyethylene bag for HP-T). The center of the cube was considered to be the slowest heating part and spore inactivation at this spot to ensure total spore inactivation in the container. In this study, carrot cubes, alginate beads, liquid, cans, the heating cell and electrodes of OH, thermocouples, polyethylene bags (for HP-T) and other equipment were kept under sterile conditions and all can seaming and thermal sealing of polyethylene bags were performed under aseptic conditions. Ohmic heating was carried out in a laminar flow hood to avoid any contamination. Sterile condition was maintained to exclude contamination of other heat-resistant spores since the validation studies in terms of inactivation of *B. licheniformis* spores were conducted below 100C.

The survival fraction of *B. licheniformis* spores in processed samples was analyzed by count–recount method by determining the spore concentration before and after processing. All inoculated alginate beads from each can (all five beads) were collected aseptically and crushed with 20 mL of sterile 0.1% peptone water in stomacher (Interscience, Model W, Markham, Ontario, Canada) for 6 min to release the spores from alginate bead matrix. Four 1 mL portion of homogenate from each container were spread plated (from 10^{-3} to 10^0 serial dilution) on nutrient agar as the recovery medium and incubated at 37C for 48 h. Survival colony counts from containers were expressed as average viable cfu/can/heating cell/bag. If no viable spores were detected in the processed samples, the survival number of spores in a sample was recorded as below the detection limit. Three samples subjected to each processing condition were evaluated in the study.

In this study, processing methods were custom designed through preliminary tests to yield equivalent effect of inactivation of spores of *B. licheniformis* (7 log CFU/container) in acid-infused carrot cubes (Table 1). For each run of conventional thermal treatment, two containers per treatment were run simultaneously. One was filled with sterile carrot cubes but with inoculated alginate beads for microbial validation study. In this case, inoculated beads (five per container) were placed at the center of sterile can filled with sterile carrot and liquid and then hermetically sealed under aseptic condition. However, the other container was filled with acid-infused nonsterile carrot, liquid and non-inoculated alginate beads (two beads per container) for the purpose of quality assessment study. Time–temperature profiles from the second can were collected from two carrot and two alginate beads attached with thin flexible thermocouples (type-T, Omega Engineering Corp., Stamford, CT) at the center of the can and assumed that both inoculated and non-inoculated cans experienced the same time/temperature profile since they are exposed to the same heating conditions. Therefore, the validation was evaluated from inoculated alginate bead test cans, while quality retention studies were completed with the control samples.

Quality Evaluation

Texture. A texture profile analysis (TPA) of samples was measured using a Texture Analyzer (TA/XT/PLUS Stable Micro System, Ltd., Godalming, U.K.) using cylindrical compression flat head probe of 25 mm diameter. Compression was performed twice (up to 50% sample thickness) at a compression rate of 1 mm/s. Each measurement was repeated 10 times and 8 data points (with CV less than 10%) were selected to estimate mean values. TPA involves analyzing the force–time curve for a product to evaluate various textural parameters. However, in this study, hardness and chewiness values of samples were considered. Hardness was measured as the residual amount of hardness left at the end of the first compression. Chewiness was defined as the product of hardness, cohesiveness and springiness results of TPA.

β-Carotene Extraction and Measurement. For extraction of the total β-carotene content, the method of Sadler *et al.* (1990) was followed with minor modifications. Carrot puree was made to fine paste with a pestle and mortar. A sample of fine paste (1 g) was mixed with of 1 g $CaCl_2{\cdot}2H_2O$ and 50 mL of extraction solvent (50% hexane, 25% acetone and 25% ethanol, containing 0.1% butayelated hydoxyl tolune [BHT]) and shaken 30 min at 4 ± 1C. After adding 15 mL of distilled water, the solution was frequently shaken for a further 15 min at 4 ± 1C. The organic phase, containing the carotenoids, was separated from the water phase, using a separation funnel, and was filtered using Whatman filter paper No. 1 (Sigma-Aldrich, Saint Louis, MO). The extraction procedure was carried out under subdued light to avoid degradation of carotenoids. β-Carotene was estimated from a standard curve ($R^2 = 0.997$) of β-carotene (Sigma-Aldrich, Saint Louis, MO) dissolved in the same solvent combination at a wave length of 450 nm using spectrophotometer (Novaspec II Visible, England).

Color Measurement. Visual total color of samples was determined using CIE (Commission Internationale de L'Eclairage) $L^*a^*b^*$ color space to evaluate the effect of processing methods on color change of processed samples using tri-stimulus colorimeter (Minolta camera CM-500d, Osaka, Japan), which was calibrated using white tiles. The measurements were taken using D-65 illuminant and 10°

observer. Total color change was expressed in terms of L^* value (lightness, ranging from zero (black) to 100 (white), a^* (redness) value and b^* (yellowness) value. Color measurement was made after making carrot puree and filling it in to in small Petri dish. Raw carrot was considered a target sample and color changes were evaluated as compared the raw color. At least three measurements were taken at different locations on the surface of each puree and the procedure was repeated three times. Eventfully total color change (ΔE) between raw and final color of processed carrot was calculated using the Hunter–Scotfield equation (Eq. 6):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (6)$$

where ΔE is the total color change as compared to raw, L^* and L are the initial and final lightness values, respectively, a^* and a are the initial and final redness values, respectively, and b^* and b are the initial and final yellowness values, respectively.

Statistical Analysis

Data are presented as means ± standard deviation of three observations. Analysis of variance (ANOVA) was performed with general linear model using Minitab 16.1.0.0 (Minitab, Inc., MN) computer software program. For significant results, mean separation was conducted using Tukey's pairwise mean comparison at 5% significance level.

RESULTS AND DISCUSSION

Heating Profiles of Carrot and Carrot Alginate Particles

Biological validation of thermal processing can be categorized based on whether or not the bacterial spores are in contact with a food (Dignan *et al.* 1989). In the contact method, a given thermal process is evaluated through inoculating bacterial spore suspension in food particles (Berry *et al.* 1985). Direct inoculation of spore suspension into food particle has some limitations in terms of possible spore leaching, but it offers many advantages including uniform inoculum and controlled particle size. In earlier studies, Perspex (poly-methylacrylate) beads containing spores were used to monitor processing of particles in continuous flow systems (Hunter 1972). Later attempts involved spores immobilized in calcium alginate gel beads (Dallyn *et al.* 1977) and large food alginate cubes containing spores (Brown *et al.* 1984). Sodium alginate-based food particles are more commonly used to validate current thermal processing methods. In the presence of calcium ions, alginate-based particle has the ability of forming thermally stable hard gels (Phillips and Williams 2000). Texture integrity of these particles during thermal processing minimizes the leaching of inoculated spores to the surrounding liquid. Therefore, inoculated carrot alginate fabricated particles can be used as a carrier of spores to determine the process lethality considering initial and final counts (Abdelrahim 1994; Walsh *et al.* 1996; Marcotte *et al.* 2000). Based on this, carrot-based alginate particles were prepared and their heating profile was compared with carrot cubes both in ohmic and in conventional heating (water immersion) methods (Hassan and Hosahalli 2012). The heating rate of alginate beads was found to be almost the same with real carrot particles (Fig. 2). Practically identical heating behavior was observed during OH and conventional heating. This might be because of the very low percentage of sodium alginate added (4.7%) in carrot pure during particle preparation which did not impose a significant change on the thermal property of alginate beads. In addition to this, Hassan and Hosahalli (2012) reported that both calcium chloride concentration and immersion time had insignificant effect on the thermophysical properties of alginate-based particles. This confirmed that alginate-based beads and carrot cubes had the same thermal diffusivity.

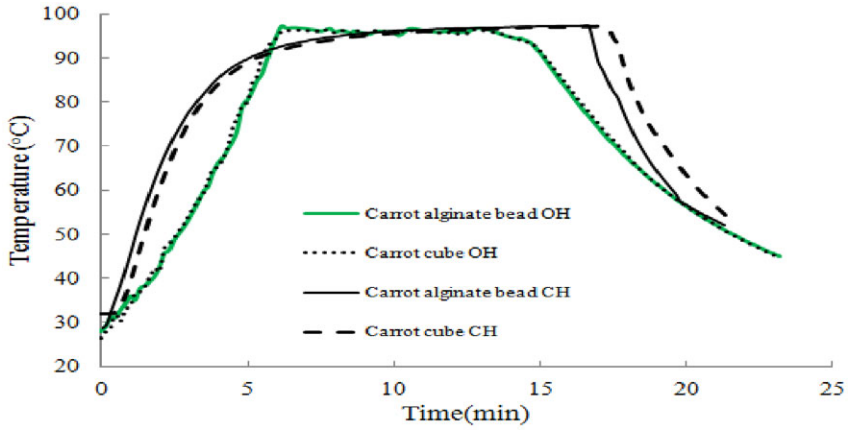

FIG. 2. HEATING PROFILES OF ACID-INFUSED CARROT CUBES (BROKEN) AND CARROT ALGINATE BEADS (SOLID) FOR OHMIC AND CONVENTIONAL HEATING IN WATER IMMERSION MODE

Furthermore, structural integrity of carrot alginate beads during heating and cooling phases was retained intact, which is an important property to avoid leaching of inoculated spores during lethality study. Therefore, carrot-based alginate particles were used as a carrier of spores to represent carrot during validation study of various processing methods.

Heat Penetration Data and Process Lethality Verification

In thermal processing, heat penetration parameters together with microbial inactivation kinetic data are commonly used to establish, validate and optimize thermal processes. Time/temperature profiles to validate different processing methods are indicated in Fig. 3. Sterilization temperature (steam, at 121C) was included to compare the quality advantages of other pasteurization methods as compared to conventional commercial canning. Preliminary tests were made to calculate the duration in minutes (the *P* value) of an equivalent process time (min) at a given constant temperature (retort temperature = T_r = 97C) that would result in the same reduction ratio in microbial count. Because lethality is additive, the integration lethal effect of different time–temperature profiles was used to get the effective time as is commonly performed in thermal process calculations. Based on data collected from the heating curves, the integrated *P* values (Eq. 3) of each processing method calculated according to improved general method are indicated in Table 2. Designed *P* values (Eq. 2) were established based on *B. licheniformis* spores inactivation kinetic parameters as indicated in Tola and Ramaswamy (2013, 2014a,b). The thermal processing methods yielded the same *P* value of 8.4 min (Eq. 2), while the OH yields a slightly lower value (7.7 min) due to some electrical effects of OH on inactivation kinetics and HP-T process has a relatively much different value of 4.2 min due to HP effects on microbial destruction. The conventional processing value is based on commercial sterility (F_0 10.6 min at 121C) (Eq. 3). Approximate come-up times for the particle to reach target temperature of 97 or 121C are also indicated in Table 2. The fastest was for ohmic heating (6.2 min) and followed by steam heating in static retort at 97C (ST-P) (7.8 min). In case of OH, heating of carrot cubes and carrot alginate beads takes place via volumetric resistance heating (Fryer 1995) of both particles and liquid and hence shorten the come-up time. Steam retorting had a lower come-up-time due to high heat transfer rates associated with condensation of steam on cans. Other methods, except WIM (under static condition) (19.5 min), exhibited similar come-up times (11.2–12.3 min). This steam showed that OH and heating resulted in shorter come-up time, which could contribute to quality improvement through reducing processing time.

P value (Eq. 2) obtained from integration of kinetic effects of time–temperature profiles from different thermal processing method are also indicated in Table 2. On the average, a *P* value of 10.1 ± 0.4 min was observed for all thermal pasteurization treatments, except for OH, due to differences in the spore inactivation kinetic data. Equivalent *P* values implied the same degree of pasteurization even if

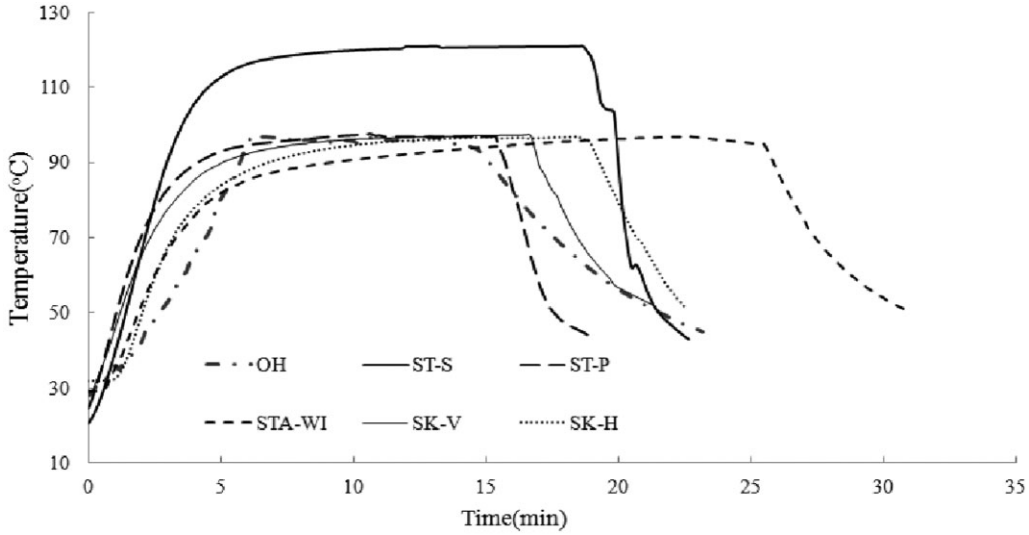

FIG. 3. HEATING PROFILE OF CARROT PARTICLES IN DIFFERENT THERMAL TREATMENT METHODS
ST-S = steam sterilization (121C) in static retort; ST-P = steam pasteurization (97C) in static retort; WIM = water immersion mode (97C) in static retort; SK-V = shaking mode of heating (97C) in water bath cans in vertical orientation; SK-H = shaking mode of heating (97C) in water bath cans in horizontal orientation; OH = ohmic heating (97C).

TABLE 2. DIFFERENT THERMAL PROCESSING METHODS AND THEIR CORRESPONDING PASTEURIZATION VALUE (*P* VALUE) DETERMINED FROM *BACILLUS LICHENIFORMIS* SPORES INACTIVATION KINETIC AND HEAT PENETRATION DATA

Treatments	Inoculated level (spores/can/heating cell/bag)	Come up time (min) to 97C/60C	*P* value (min) required to achieve 7 log reduction (Eq. 1)	*P* value (min) delivered (Eq. 2)	Cumulative log reduction (cfu/container) achieved (Eq. 3)	log cfu/container observed from actual experiment†
WIM	1×10^8	19.5	8.4	9.7 ± 0.4*	8.1 ± 0.7	>7§
SK-V	1×10^8	11.2	8.4	10.5 ± 0.5	8.8 ± 0.5	>7
SK-H	1×10^8	12.3	8.4	10.3 ± 0.4	8.6 ± 0.5	>7
ST-P	1×10^8	7.8	8.4	9.8 ± 0.5	8.2 ± 0.6	>7
ST-S	1×10^8	12	8.4	10.6 ± 0.6	8.8 ± 0.6	>7
OH	1×10^8	6.2	7.7	8.3 ± 0.1	7.5 ± 0.1	>7
HP-T	1×10^8	2‡	4.2	nd	nd	>7

* Standard deviation.† No colony was observed after 48 h culturing on nutrient agar medium.‡ Samples preheated to 42C to reach target temperature of 60C during pressure build-up step.§ No colony growth observed from cultures samples after each processing treatment.nd, not determined.WIM = water immersion mode (97C) in static retort; SK-V = shaking mode of heating (97C) in water bath cans in vertical orientation; SK-H = shaking mode of heating (97C) in water bath cans in horizontal orientation; ST-P = steam pasteurization (97C) in static retort; ST-S = steam sterilization (121C) in static retort; OH = ohmic heating (97C); HP-T = high pressure-assisted thermal treatment.

different processes produce different heat penetration curves (Fig. 3). In general, the integrated *P* values (Eq. 3) were higher than those required for achieving the appropriate processes (due to accommodation of additional effects during come-up and come-down periods). Further, these could not be compared for commercial sterilization and HP-T processing due to conditions outside the normal range of temperature conditions employed for acidified thermal processing.

Ultimately, the effectiveness of a given thermal/pressure-assisted thermal treatment is evaluated in terms of log reduction of spores which gives the equivalent biological lethality. Selected processing method should be capable enough to produce safe product to avoid issues of public health safety. To achieve such objectives, thermal or HP-T processes are commonly planned on a more conservative procedure. In this study, microbiological validation study was conducted to achieve 7 or more log reduction of *B. licheniformis* spores under worst pasteurization conditions (high level of inoculum, considering the slowest heating spots both in the product and container). Designed (Eq. 2) and delivered (Eq. 3) process schedules as well as cumulative log reduction (Eq. 4) achieved from actual processes are presented in Table 2. Delivered *P* value ranged from 8.3 to 10.6 min with a corresponding cumulative minimum log reduction of spores ranging from 7.5 to 8.8 cfu/can/heating cell/bag, which was slightly more than planned 7-log reduction of spores (Table 2). The number of surviving spores in processed samples after incubation were monitored and no colonies (>7 log) were observed for any of the treated sample (Table 2). The same efficacy was observed in HP processing with no spore survival after delivered process. This showed that designed and delivered process schedules were sufficient enough to inactivate spores of *B. licheniformis*. From safety point of view, processing severity greater than these could enable to produce *B. licheniformis* free acidified foods. Therefore, the concern of pH elevation due to the action of *B. licheniformis* and creation of optimum germination and growth environment for spores of *C. botulinum* could be controlled, since growth and toxin production of *C. botulinum* under reduced pH (<4.6) environment is very unlikely.

Quality Retention Evaluation

The purpose of a scheduled process is to deliver a predetermined amount of lethal agent (vary depending on the type of heat, pressure), in a given time under the right conditions, to ensure eradication of target microorganisms of concern with minimal damage to quality properties. Therefore, the effect of the different types of pasteurization methods for acidified thermal processing were also compared with relative to conventional canning for quality retention.

Texture Retention

Perception of canned vegetable palatability is derived from a complex interaction of sensory and physical properties during chewing. The objective mechanism used to evaluate the effect of a given food preservation method on the texture properties of a product varies. The development of TPA is an important milestone in food science to understand how and to what extent processing methods influence texture of foods (Pons and Fiszman 2007). In most cases, a linear correlation has been observed between TPA results and subjective texture measurements (Meulleneti *et al.* 1998).

In this work, the processing effects of thermal and HP-T processing methods on the textural properties of carrot are compared. Hardness and chewiness were the two textural properties evaluated. The hardness value was measured by the peak force of the first compression of the sample simulating the first bite during chewing. Chewiness is defined as the energy required to chew a solid food to a state where it is ready for swallowing, but mathematically expressed as a product of hardness, cohesiveness and springiness. Figure 4 shows that there is a significant difference ($P < 0.05$) in retention of hardness and chewiness among processing methods. Better hardness and chewiness were obtained from samples subjected to HP-T and OH treatments. When the residual hardness was compared with the raw, 86 and 70% of the hardness was preserved in HP-T and OH treatments, respectively. The chewiness values were also superior in these methods as compared to others. These results are also in agreement with Knockaert *et al.*'s (2011) study on carrots. This quality advantage mainly originated from inherent processing nature of the two methods. In OH, the heating is rapid and volumetric, which reduces total processing time and its determinant impact. The food responds for passing current by generating volumetric heat internally due to its inherent resistance (Sastry and Barach 2000) which facilitate fast heating. Such a heating behavior results in a uniform and fast heating throughout the food and reduce the overall processing time with reduced come-up time as indicated in Table 2 and hence contributes for better quality retention (Palaniappan and Sastry 1991). In addition to this, the energy conversion efficiency and heating behavior of OH is high since no packaging barrier is there for the food since it was heated directly in a beaker. HP-T treatment, considered as the nonthermal alternative to conventional thermal processing, allows uniform and rapid pressure treatment (at relatively low or moderate temperatures) which ultimately minimizes the sever effects of conventional heating (Nguyen *et al.* 2007). The advantage of pressure processing is also confirmed by other studies (McClements *et al.* 2001; Nguyen *et al.* 2007). These studies reported that the process can be considered equivalent to a high-temperature-short-time treatment (Leadley 2005) without significantly affecting the quality of the product. The benefit of combining use of moderate pressure and temperature in terms of production cost effectiveness and safety is also indicated by de Heij *et al.* (2003). Because of these behaviors of the two processing methods, a better texture retention could be achieved.

When other pasteurization methods were compared, no significant ($P > 0.05$) difference was observed with respect to the texture of carrots processed in ST-P, WIM, SK-V and SK-H. Since equal volume of solid and liquid were used during all treatment conditions, samples in the shaking water bath might not have had enough headspace and moving liquid during shaking to enhance better mixing of samples for rapid heat penetration. Even so, texture retention from these processing methods was considerably higher than samples processed in conventional canning for non-acidified carrot. Residual hardness and chewiness observed from conventional canning (ST-S) using steam as a heating medium were at the bottom with low retention values of 8 and 2.6%, respectively (Fig. 4). This shows that an extensive thermal treatment in conventional canning causes pronounced damage to quality attributes of low-acid canned vegetables. Therefore, the application of moderate processing conditions with strategy of using different complementary preservation methods could be immensely attractive to improve quality of canned vegetables. The combined use of moderate processing methods (thermal/pressure-assisted thermal) with novel pH reduction method (HP acid infusion) of low-acid vegetables could significantly contribute to minimize the impact of intensive thermal or high-pressure-high temperature sterilization process on

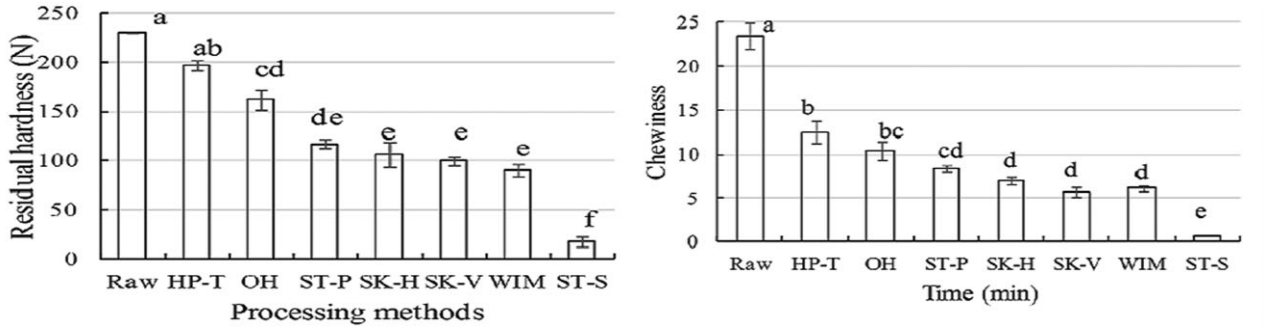

FIG. 4. EFFECT OF DIFFERENT PROCESSING METHODS ON RESIDUAL HARDNESS AND CHEWINESS OF CARROT
ST-S = steam sterilization (121C) in static retort; ST-P = steam pasteurization (97C) in static retort; WIM = water immersion mode (97C) in static retort; SK-V = shaking mode of heating (97C) in water bath cans in vertical orientation; SK-H = shaking mode of heating (97C) in water bath cans in horizontal orientation; OH = ohmic heating (97C). Bars with the same letter are not significantly different.

quality attributes. Barbosa-Canovas and Juliano (2008) also indicated that synergetic approaches through pH reduction or use of antimicrobial preservatives could help to reduce limitations of intensive processing methods but ensures safety and better quality product with reduced processing cost.

Color Retention

Color change of many foods after processing is an important quality attributes in marketing since it tells the extent of quality damage on the product. Because of this, color measurement is one of objective parameters that can be used as quality index in quality control of processed foods (Giese 2000). From market point of view, although it does not reflect nutrition value, but it is important as it relate consumer preference based on appearance. The color of the food material could change during processing due to certain enzymatic and/or nonenzymatic reactions. The effect of various processing methods on carrot color was evaluated in terms of total color change observed according to Eq. (6). The effect of processing methods was insignificant ($P > 0.05$) in terms of total color change of carrot, except sample treated in conventional canning method (ST-S) which showed more color change as compared to others (Fig. 5). As indicated also in Liesbeth *et al.* (2012), no noticeable difference in total color was observed from mild (600 MPa, 25C) and severe (600 MPa, 61.3C) pasteurization treatments, even though better retention of color was observed for samples in high pressure treatments than thermal. The insignificant difference in color change among pasteurization methods might be due to reduced impact of treatments on color responsible microstructure of carrot tissue. Even though statically no difference was observed among pasteurization methods, slightly better retention of color is observed for samples from HP-T and OH (Fig. 5). Color change observed in case of conventional canning (ST-S) could be due to Maillard reaction because of intensive heat effect or due to loss of certain color components.

β-Carotene Content

Carrots are the richest source of α- and β-carotene of common fruits and vegetables consumed all over the world. β-Carotene is an important nutrient that is responsible for the orange color of carrots. It can act as a pro-vitamin A and as an antioxidant, by which it can help protect humans from several diseases (Britton 1995). The impact of thermal and HP processing on the carotenoid content of carrots was largely dependent on the processing intensity applied. The result in Fig. 6 shows that the samples in WIM resulted in better β-carotene yield. This is because more β-carotene could be released from carotene responsible matrices of carrot tissue during relatively extended heat (come-up time of 19.5 min; Table 2) treatment. The more sever treatment enhances the extractability of β-carotene as compared to less sever treatment in case of HP-T. Different researchers (Vásquez-Caicedo *et al.* 2006; Lopez *et al.* 2008) reported that β-carotene exists in crystalline form in carrot chromoplast. This intracellular localization while bounded with other macromolecules implies that the compound should be released from such a matrix for easy extractability through relatively more severe treatments. Aguilera (2005) and Waldron *et al.* (2003) also indicated that the cell walls could influence the release of β-carotene from the tissue matrix, which was evident in case of HP-T, since better texture retention was observed as compared to low β-carotene content (Fig. 6). As processing can affect the food matrix and cell wall, it can also influence the accessibility of carotenoids. However, with more intensive thermal treatment (ST-S), an increase in β-carotene extractability was not observed. This might be associated with enhanced degradation of the compound due to oxidation and other mechanisms than mechanisms enhancing extractability

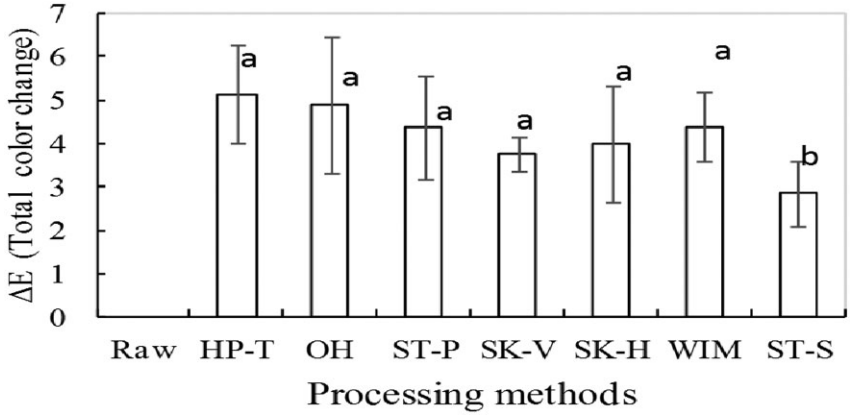

FIG. 5. EFFECT OF DIFFERENT PROCESSING METHODS ON TOTAL COLOR CHANGE OF CARROT
ST-S = steam sterilization (121C) in static retort; ST-P = steam pasteurization (97C) in static retort; WIM = water immersion mode (97C) in static retort; SK-V = shaking mode of heating (97C) in water bath cans in vertical orientation, SK-H = shaking mode of heating (97C) in water bath cans in horizontal orientation; OH = ohmic heating (97C). Bars with the same letter are not significantly different.

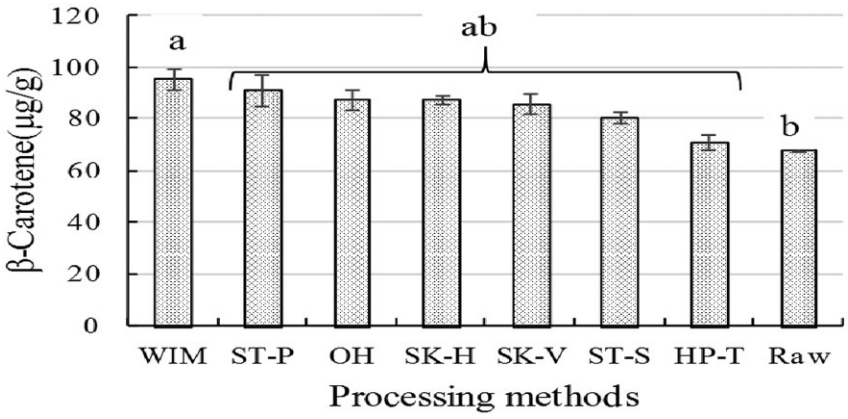

FIG. 6. EFFECT OF PROCESSING METHODS ON B-CAROTENE CONTENT OF PROCESSED CARROT
ST-S = steam sterilization (121C) in static retort; ST-P = steam pasteurization (97C) in static retort; WIM = water immersion mode (97C) in static retort; SK-V = shaking mode of heating (97C) in water bath cans in vertical orientation; SK-H = shaking mode of heating (97C) in water bath cans in horizontal orientation; OH = ohmic heating (97C). Bars with the same letter are not significantly different.

(Lemmens *et al*. 2009) and also the result was in line with color change of the sample. But in general, no considerable difference was observed in terms of β-carotene extractability among other thermal pasteurization methods, which is in agreement with Liesbeth *et al*.'s (2012) result.

CONCLUSIONS

It is understood that in conventional processing of acidic foods (pH < 4.6), the presence of *C. botulinum* (type A and B) spores might not be a health concern and moderate processing conditions are commonly practiced. However, the coexistence of spores of *B. licheniformis* in such type of foods, or acidified low-acid foods, along with spores of *C. botulinum* could endanger public health because the former has the capacity to grow in acid or acidified foods and hence elevate the pH above 4.6 in which the latter situation can grow and produce toxin. Therefore, for acidified low-acid foods, complete inactivation of spores of *B. licheniformis* should be used as a critical step to ensure food safety. Designed processing schedules based on predetermined inactivation kinetic parameters were found sufficient to achieve the required degree of spore inactivation of *B. licheniformis* in production of acid or acidified low-acid foods.

ACKNOWLEDGMENTS

The authors express their gratitude to Health Canada, Ottawa, for donating *B. licheniformis* culture, and one of the authors (Yetenayet B. Tola) also expresses his gratitude to the Canadian International Development Agency for the financial support to conduct this research (CIDA, Project No. S-064360).

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