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Quality of green beans (*Phaseolus vulgaris* L.) influenced by microwave and hot water pasteurization

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ABSTRACT

This research investigated the influence of 915 MHz microwave-assisted thermal pasteurization and conventional hot water pasteurization (F_{90}^{10}=10 min) on chlorophylls, greenness (a^*), ascorbic acid, and the growth of spoilage microorganisms in green beans in storage at 10, 7, and 2 °C for up to 100 days. Frozen cut green beans were processed in vacuum-sealed containers flushed with N_2. The beans suffered 28.3% and 33.9% losses of chlorophyll *a*, 9.2% and 15.3% losses of ascorbic acid during the microwave and hot water processing, respectively. During storage, slower spoilage (21 days at 10 °C, 42 days at 7 °C, and 100 days at 2 °C), superior preservation of chlorophyll (47–50%), ascorbic acid (47–62%), and a lower increase in a^* (2.8–5.2) were obtained in samples processed with microwave-assisted thermal pasteurization. *Paenibacillus* spp. were identified as the predominant bacteria in the spoiled green beans pasteurized with both methods. The results highlighted the potential of microwave pasteurization for producing safe, high-quality vegetable products.

1. Introduction

Vegetables are critical components of a healthy diet and account for an essential part of ready-to-eat (RTE) meals. RTE vegetables can be consumed either as recipe dishes intended for the retail market or as pre-cooked ingredients for caterers or other food services (Choma et al., 2000). Thermal processing is a classical method of food preservation that has been widely used in the production of RTE vegetables. In commercial preparation of RTE meals for cold chain distribution, a thermal process that exposes food products at 90 °C for 10 min, or an equivalent lethality, at the cold spot (the spot in the food which receives the least heat) in packages is often used to achieve a 6-log reduction of non-proteolytic *Clostridium botulinum*. This process would provide the products up to 6 weeks' shelf life at 5 °C (ECFF, 2006). The chill temperature storage serves as a prudent second barrier to maintain quality and extend the shelf life of the RTE meals.

The process mentioned above is sufficient to inactivate vegetative microbial flora; it may also activate dormant bacterial endospores (Krawczyk et al., 2017; Luu et al., 2015), leading to subsequent germination and outgrowth of nonpathogenic vegetative cells during cold storage. Sonar, Rasco, Tang, & Sablani (2019) reported that the aerobic mesophilic bacterial growth in pasteurized green pea puree (F_{90}^{10}=10 min) reached 6 log CFU/g after 80 days at 7 °C. Sous vide processed julienne carrots (85 °C) stored at 8 °C for four weeks had a total plate count of 6.48 log CFU/g (Nyati, 2000). Carlin et al. (2000) also observed that the count of aerobic mesophilic bacteria in pasteurized broccoli, carrot, zucchini, leek, potato, and split pea puree (80 °C, 30 min) increased up to 6–8 log CFU/g after 20-day storage at 10 °C. It has been reported that pasteurized zucchini puree showed the most rapid bacterial growth, while cook-chilled soybean sprouts (90 °C, 10 min) had no aerobic bacterial growth when stored at 10 °C for 24 days (Koo, Kim, Lee, Lyu, & Paik, 2008). It is evident from the literature that various vegetables after thermal pasteurization supported different bacterial growth, leading to a wide range of shelf life in cold storage at different temperatures. Thus, it is important to assess bacterial growth at different storage temperatures in studying quality losses and shelf life of RTE vegetables.

The thermal processing and storage of vegetables inevitably result in changes in physical characteristics and chemical composition, such as color, bioactive compounds, and antioxidants (Baardseth, Bjerke, Martinsen, & Skrede, 2010; Mazzeo et al., 2011; Peng et al., 2017). Consumers' demand for high-quality, fresh-like products has stimulated the

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development of new technologies aiming at reducing the adverse effects of processing and storage. Microwave heating has shown advantages in reduced heating time and improved heating uniformity when compared with conventional hot water heating (Khan, Tango, Miskeen, Lee, & Oh, 2017). The microwaves penetrate into the food and generate heat throughout the whole volume of food, making thermal conductivity and heat transfer coefficients no longer the limit of heat transfer (Burfoot, Griffin, & James, 1988). Another advantage of microwave heating is the direct conversion of electromagnetic radiation into heat within foods, which could improve energy efficiency (Regier & Schubert, 2001). These advantages over hot water heating often yield an increased production rate and a reduction in undesirable thermal degradation (Ahmed & Ramaswamy, 2007; Khan et al., 2017).

Several studies have compared the quality of different RTE foods preserved by microwave with hot water heating that delivered an equivalent level of control of foodborne pathogens. Pérez-Tejeda et al. (2016) reported less color changes in tomato puree after processing using microwaves (950W, $F_{93.3}^{8.9} = 1$ min). Marszałek, Mitek, and Skąpska (2015) concluded that microwave heating better preserved the color and nutrient of strawberry puree than conventional heat pasteurization. Published shelf life studies on pasteurized vegetables have been limited to puree or smoothie (Arjmandi et al., 2017; Benlloch-Tinoco, Igual, Rodrigo, & Martínez-Navarrete, 2015; Klug et al., 2018), which do not represent a more typical range of commonly consumed RTE meals. In addition, the studies on microwave pasteurization of vegetables are based on domestic microwave ovens, which do not represent the processing conditions used in industrial microwave systems for the commercial production of RTE meals. The objective of this study was to systematically evaluate the effects of 915 MHz microwave-assisted thermal pasteurization and conventional hot water pasteurization (F_{90}^{10}=10 min) on the quality of green beans stored at different temperatures (10, 7, and 2 °C). The green beans were characterized in terms of aerobic mesophilic bacteria count, pH, chlorophyll, color, and ascorbic acid.

2. Materials and methods

2.1. Sample preparation

Since our intention was to simulate industrial processes for year-around operation, we selected frozen green beans for this research. Frozen cut green beans (*Phaseolus vulgaris* L.), with a size of ~2.5 cm in length and 0.8 cm in diameter, and a moisture content of 90.5%, were purchased from the local market and stored in sealed bags at −18 °C. The green beans were blanched before flash freezing, as indicated by the manufacturer. Before testing, the green beans were defrosted in 4 °C water for 10 min and then drained off for 5 min. Approximately 226 g thawed green beans were filled into each rigid tray (Silgan Plastics, Chesterfield, MO) with a PP/regrind/tie/EVOH/tie/regrind/PP structure and an inner dimension of 140 × 95 × 30 mm. The trays were vacuum sealed with lidding film (metal oxide coated PET/biaxial oriented PA/PP) with a thickness of 101 ± 1.6 μm (Printpack., Atlanta, GA), using a vacuum sealer (Multivac T-200, Multivac Inc., Kansas City, MO). The sealing conditions were set at 200 °C for 8 s, with a vacuum (6.5 kPa absolute pressure) and nitrogen flushing at 45 kPa (absolute pressure).

2.2. Thermal processing and storage

Thermal processing was designed to achieve the lethality of $F_{90}^{10} = 10$ min at the cold spot in the trays. The lethality value was calculated according to Eq. (1):

$$F = \int_0^t 10^{\frac{T-T_{ref}}{z}} \quad (1)$$

where T (°C) is the temperature measured at the cold spot at time t (min), T_{ref} is the reference temperature (90 °C), and z represents the temperature sensitivity of the heat tolerance of the target microorganism. We selected a z-value of 10 °C for non-proteolytic *Clostridium botulinum* (ECFF, 2006). The cold spot in the trays during the hot water processing was located at the geometric center of the package. For microwave-assisted thermal pasteurization, a mashed potato model food containing chemical marker precursors was employed for cold spot determination (Bornhorst, Tang, Sablani, & Barbosa-Cánovas, 2017). The heating pattern inside the microwave processed model food, as indicated by the intensity of browning, was analyzed using a computer vision method developed by Pandit, Tang, Liu, and Mikhaylenko (2007). Based on the heating pattern, the cold spot was located off the geometric center and in the middle layer of the trays. Heat penetration studies were carried out by collecting time-temperature data at the cold spot in the food trays to determine the process schedules. The temperatures were recorded every 2 s by a mobile metallic temperature sensor and a TMI data logger workstation (TMI-USA Inc., Reston, VA) (Luan, Tang, Pedrow, Liu, & Tang, 2013, 2015). This measurement method has been validated with fiber optic temperature sensors for developing microwave-assisted pasteurization processes (Tang, 2015).

2.2.1. Microwave-assisted thermal processing

The microwave processing was performed in a pilot-scale 915 MHz semi-continuous Microwave-Assisted Pasteurization System (MAPS) developed at Washington State University. The system consisted of four sections (preheating, microwave heating, holding, and cooling), and each section had a separate water circulation system to control the water temperature and flow. The microwave section had four single-mode cavities connected to microwave generators. Approximately 5 kW of power was applied to each of the first two cavities, and the other two cavities equally split 8.7 kW. A metal carrier that could carry eight trays was immersed in the water and moved through the four sections mentioned above. The details of MAPS can be found in Tang, Hong, Inanoglu, and Liu (2018).

2.2.2. Conventional hot water processing

Conventional hot water (HW) processing was conducted by immersing the packages in a precision digital circulating water bath (Model 260, Thermo Scientific, Marietta, OH) set at 91 °C. Two trays were processed at a time.

2.2.3. Storage conditions

A total of 144 trays were processed and stored with protection from light at 10 ± 1 (a common refrigeration abuse temperature), 7 ± 1 (domestic refrigeration temperature), and 2 ± 0.5 °C (lower chill temperature) for 21, 42, and 100 days (Cronin & Wilkinson, 2009; James, Evans, & James, 2008). Three trays were randomly selected for each time point (Day 3, 7, 11, 14, 17, 21 at 10 °C; Day 5, 10, 15, 21, 28, 35, 42 at 7 °C; Day 7, 14, 28, 42, 60, 80, 100 at 2 °C), and each tray was measured in triplicate for quality quantification and microbiological analyses.

2.3. Microbiological analyses

Thawed or processed green beans were pureed using a hand blender (KHB100ER1, KitchenAid, Benton Harbor, MI). Before grinding, the blending arm and blade of the blender were sterilized using steam at 121 °C for 15 min, the motor body (holding part) was cleaned with 70% ethanol wipes. Every 30 s grinding followed by 30 s rest to allow ground green beans to cool down. The total grinding/resting time equaled 5 min. The total mesophilic bacteria were examined by diluting the green bean puree in 0.1% (w/v) of sterilized peptone water and plated on Plate Count Agar (PCA) and then incubated at 37 °C for 48 h, both aerobically and anaerobically. Yeast and mold were enumerated by dispensing

aliquots onto Potato Dextrose Agar (PDA) following incubation at 25 °C for 5 days. The PDA was acidified by adding 1 mL of tartaric acid per 10 mL of PDA. The aerobic mesophilic bacteria, yeast, and mold were analyzed before and after thermal pasteurization (Day 0), and at the selected sampling points as described in section 2.2.3. The anaerobic mesophilic count was only determined at the end of the storage (Day 21 at 10 °C, Day 42 at 7 °C, Day 100 at 2 °C). The selected dilutions from each green bean sample were plated in triplicate. All microbial counts were reported as $\log_{10}$ colony-forming units per gram of green bean (log CFU/g). The isolated colonies were identified by amplification of the 16S rRNA or the rpoB gene by PCR using universal eubacterial primers and comparing the results with sequences in the GenBank Database by the Washington Animal Disease Diagnostic Laboratory (Washington State University, Pullman, WA).

2.4. pH

The pH was determined by mixing 3 g of puree with 27 mL of deionized water and measuring at room temperature (23 °C) with a pH meter (Oakton Instruments, Vernon Hills, Il).

2.5. Chlorophyll

For chlorophyll determination, 3 g of green bean puree was homogenized with 25 mL of acetone-water (80:20, v/v) for 5 min at 7000 rpm (Polytron PT 10/35 GT, Kinematica AG, Luzern, Switzerland). The mixture was then put in a shaker rotating at 300 rpm at room temperature for 30 min and centrifuged at 8870 g for 6 min (Sorvall Biofuge Primo, Thermo Scientific, Waltham, MA). The supernatant was collected and brought up to a volume of 50 mL with acetone-water for spectrophotometric analysis (V-5000, Metash Instruments, Shanghai, China) at the wavelengths of 663 and 647 nm, measured against a blank. Eqs. (2)–(4) were used for calculation (Lichtenthaler, 1987), and the chlorophyll content was expressed as mg per 100 g.

$$\text{Chlorophyll } a = 12.25A_{663} - 2.79A_{647} \tag{2}$$

$$\text{Chlorophyll } b = 21.5A_{647} - 5.10A_{663} \tag{3}$$

$$\text{Total chlorophyll} = 7.15A_{663} + 18.71A_{647} \tag{4}$$

2.6. Color

The sample color was quantified in CIE $L^*a^*b^*$ color space using image analysis. L^* corresponds to lightness/darkness, a^* represents red (+) to green (−), and b^* represents yellow (+) to blue (−). The green beans from the topmost layer of the trays were taken out for image analysis, which aimed to simulate the first impression of the consumers as they opened the package. A computer vision system described by Zhang, Tang, Liu, Bohnet, and Tang (2014) was used to take photos (resolution: 5184 × 3456). The RGB images were calibrated using a color reference card (QPcard 203, QPcard AB, Helsingborg, Sweden) in Adobe Photoshop CC (Adobe system, Inc., San Jose, CA). Image analysis was performed in MATLAB R2019b (MathWorks, Inc., Natick, MA) by converting RGB to $L^*a^*b^*$. Thresholding was applied to select the region of interest through background removal. The color value obtained was the average color value of all pixels in the region of interest. The color difference was calculated based on Eq. (5):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \tag{5}$$

where L_0^*, a_0^*, and b_0^* correspond to the initial values of L^*, a^*, and b^*.

2.7. Ascorbic acid

Quantification of ascorbic acid (AA) was determined by HPLC following the method described by Scherer et al. (2012) with modifications. Five grams of green bean puree was homogenized with 10 mL 3% (w/v) meta-phosphoric acid for 1 min at 7000 rpm. The homogenate was incubated at room temperature (23 °C) for 2 h and centrifuged at 8870 g for 6 min. The supernatant was collected and filtered through a 0.45 μm nylon filter. Ten microliters of this filtered aliquot was injected into an Agilent 1100 HPLC system (Agilent Technology, Santa Clara, CA) to chromatographically separate the mixture into its component peaks through an RP18 5-μm 4.6 × 250 mm column (Waters Corporation, Milford, MA) equipped with a diode array detector. The mobile phase was a 0.01 mol/L monopotassium phosphate solution adjusted to a pH of 2.6 with o-phosphoric acid. The separation was accomplished using a 15-min isocratic elution procedure with a flow rate of 0.5 mL/min and a column temperature of 25 °C. The detecting wavelength was 250 nm. Pure L-Ascorbic acid was used to build a standard curve, and the AA content was expressed as mg per 100 g green beans.

2.8. Data analysis

Kinetic degradation of quality attributes was analyzed based on the analytical data collected during storage. The zero-order reaction model (Eq. (6)) and the first-order fractional conversion model (Eq. (7)) were found to best fit with the experimental data in this study:

$$C = C_0 - kt \tag{6}$$

$$\frac{C - C_\infty}{C_0 - C_\infty} = e^{-kt} \tag{7}$$

where C_0 is the initial value of the food quality attribute at $t = 0$ (day), C is the value at time t (day), C_∞ is the final equilibrium value, and k is the reaction rate constant (day^{-1}), which is temperature-dependent and follows the Arrhenius relationship (Peleg, Normand, & Corradini, 2012):

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \tag{8}$$

where E_a is the activation energy (kJ/mol), R is the universal gas constant (0.008314 kJ/mol K), T is the absolute temperature (K), and k_0 is the pre-exponential factor.

The analysis of variance and Tukey's honestly significant difference test at a 95% confidence level ($P < 0.05$) were performed to identify the differences among groups using SPSS v25 (IBM Corp., Armonk, NY). Two-tailed Pearson correlation analysis was carried out at the significance level of 0.01.

3. Results and discussion

3.1. Thermal processing

Typical temperature profiles at the cold spots in trays are shown in Fig. 1. MAPS processing included preheating for 35 min at 51 °C, microwave heating for 4.5 min in circulating water set at 91 °C, holding for 7.5 min in circulating water set at 91 °C, and cooling for 5 min at 23 °C. In HW processing, samples were heated for 47 min at 91 °C and cooled for 10 min in ice water. The lethality at the cold spots reached F_{90}^{10} = 14.1 ± 0.3 min and 13.3 ± 0.7 min for MAPS and HW processing, respectively.

In MAPS processing, the 35 min preheating step at 51 °C took up almost 60% of the whole processing time. This was to allow the samples to reach a uniform initial temperature before microwave heating. In an industrial process, this can be achieved by heating pre-packaged meals in a water flume at a constant temperature or through hot fill of the beans in packages before sealing. In the microwave heating section, the sample temperature increased from 51 to 89 °C at a heating rate of 8.4 °C/min. In HW processing, the temperature increase was relatively fast in the early part of the 47 min heating, as the temperature difference

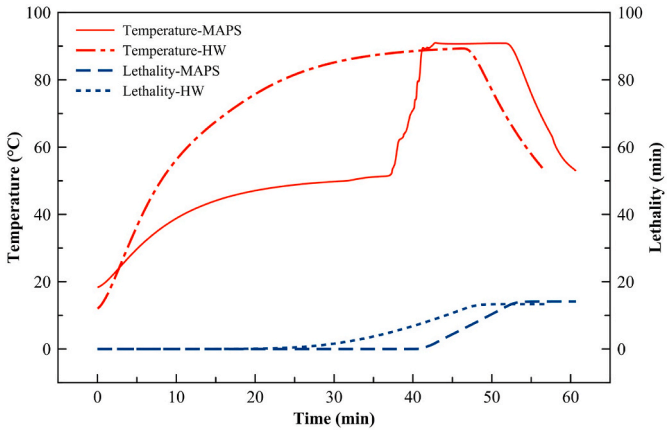

Fig. 1. Temperature profiles and thermal lethality at cold spots of microwave (MAPS) and hot water (HW) processing.

between the heating medium and green beans was large. The heating rate gradually decreased as the green bean temperature approached the medium temperature of 91 °C, due to the reducing temperature difference. The average heating rate of HW processing was 1.6 °C/min, about 1/5 of that in the microwave heating section of MAPS. The higher heating rate in MAPS meant less thermal exposure, which should have positive effects on the quality of food products.

3.2. Microbiological analyses

3.2.1. Shelf life determined by bacterial growth

The initial population of aerobic mesophilic bacteria in frozen-thawed green beans before thermal processing was 4.11 ± 0.15 log CFU/g. After processing, no plated colonies were detected in the samples processed by either method (limit of detection was 100 CFU/g), which was shown as 0 at day 0 in Fig. 2. The bacterial population increased dramatically to ~4 log CFU/g in the early part of the storage (day 3 at 10 °C, day 5 at 7 °C, and day 7 at 2 °C). The increase then slowed down to reach 7–8 log CFU/g at the end of the storage (Fig. 2). The initial rapid growth of the spoilage bacteria in the green beans may be explained by the availability of more nutrients for microbial growth from the damaged green bean plant cells and the lack of competing vegetative bacteria cells after the pasteurization. No yeast or mold counts were detected in any of the samples throughout the storage period, and no anaerobic growth was observed in any samples at the end of the storage.

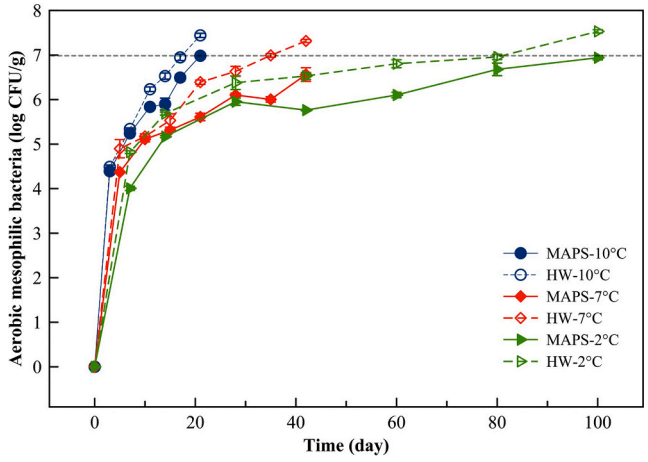

Fig. 2. Aerobic mesophilic bacteria count of microwave (MAPS) and hot water (HW) processed green beans during storage at 10, 7, and 2 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Pasteurized RTE meals could be considered as spoiled when the total aerobic microbial counts reach 7 log CFU/g or higher (FSANZ, 2001). In this study, package swelling was the first sign of spoilage, together with the presence of an off-odor that appeared when the package was opened. All swollen packages had aerobic mesophilic bacterial counts larger than 7 log CFU/g. During storage at 10 °C, the population reached 7 log CFU/g on day 17 and day 21 in HW and MAPS processed green beans, respectively (Fig. 2). Reducing the storage temperature from 10 °C to either 7 or 2 °C could delay spoilage by 2.5–4.5 times, as the lower temperatures reduced the growth of microorganisms. The MAPS processed samples took 42 and 100 days, while HW processed samples took 35 and 80 days, to reach the microbiological quality mark when stored at 7 and 2 °C, respectively. The results suggest that microwave heating had the potential to produce pasteurized green beans with longer shelf life.

3.2.2. Identification of spoilage bacteria in pasteurized green beans

Four morphologically different isolates from spoiled samples after 21 days at 10 °C were identified, of which three were classified as *Paenibacillus* spp. (*Paenibacillus* sp. F, *Paenibacillus terrae,* and *Paenibacillus* sp. FSL H7-0357) and one as *Bacillus* spp. (*Bacillus pumilus*). Only *Paenibacillus* spp. were identified from samples spoiled at 7 and 2 °C storage. All the bacteria identified are spore formers, which survived the pasteurization process and germinated into metabolically active cells during storage.

Similar to our study, Guinebretiere et al. (2001) reported high frequencies of the presence of *Paenibacillus* spp. in pasteurized zucchini puree stored at 10 °C. Helmond, Nierop Groot, and van Bokhorst-van de Veen (2017) isolated *P. terrae* from rice-vegetable RTE meals stored at 7 °C, and Carlin et al. (2000) isolated *B. pumilus* from pasteurized vegetable purees stored at 10 °C. Guinebretiere, Girardin, Dargaignaratz, Carlin, and Nguyen-The (2003) examined the contamination flows of spore-forming aerobic bacteria in a pasteurized zucchini puree processing line.*Paenibacillus* spp. and *Bacillus* spp. are known to be frequent contaminants in the soil, and the spore counts in the soil can be as high as 9 log CFU/g (Ammann, Kölle, & Brandl, 2011). The spore-forming bacteria may enter the plant through the root or attach to the surface of the vegetables by contact with soil or aerosols of soil. Another possible contamination pathway is the processing equipment, such as storage tanks and production lines (Soni, Oey, Silcock, & Bremer, 2016). Both *Paenibacillus* spp. and *Bacillus* spp. have been isolated from food processing plants as they can attach to stainless steel surfaces (Huck, Woodcock, Ralyea, & Boor, 2007; Salustiano et al., 2009). Consequently, they could enter the food chain easily.

3.3. pH

The initial pH of the green beans was 6.28. Thermal processing reduced the pH to 6.23 and 6.16 when MAPS and HW were employed, respectively. This may be due to the release of organic acids from the food matrix. The pH decreased in all samples during storage (Table 1). This was also demonstrated in other pasteurized vegetable puree during storage at 4–10 °C (Carlin et al., 2000; Sonar et al., 2019b). The pH drop during storage was caused by the growth of microbial flora, as observed in section 3.2. In the current study, the significant pH drop started somewhere around the middle of the shelf life. MAPS processed samples always had similar or higher pH as compared to HW processed ones. Referring to the microbial analysis in section 3.2, a pH value lower than 5.5 may suggest the end of the shelf life of pasteurized green beans due to spoilage.

3.4. Chlorophyll degradation

The total chlorophyll content of unheated green beans was 10.46 mg/100 g, out of which 68.0% was chlorophyll *a*. Both heating methods

Table 1
pH of microwave (MAPS) and hot water (HW) processed green beans during storage at 10, 7, and 2 °C.

10 °C			7 °C			2 °C		
Day	MAPS	HW	Day	MAPS	HW	Day	MAPS	HW
0	6.23 ± 0.01^{aA}	6.16 ± 0.01^{aB}	0	6.23 ± 0.01^{aA}	6.16 ± 0.01^{aB}	0	6.23 ± 0.01^{aA}	6.16 ± 0.01^{aB}
3	6.00 ± 0.01^{bcA}	5.99 ± 0.02^{bA}	5	6.19 ± 0.01^{aA}	6.13 ± 0.00^{aB}	7	6.16 ± 0.02^{bA}	6.06 ± 0.02^{bB}
7	6.03 ± 0.01^{bA}	6.04 ± 0.01^{bA}	10	6.21 ± 0.02^{aA}	6.14 ± 0.01^{aB}	14	6.14 ± 0.01^{bA}	6.08 ± 0.01^{bB}
11	5.99 ± 0.01^{cA}	5.72 ± 0.01^{cB}	15	6.18 ± 0.02^{aA}	6.13 ± 0.02^{aB}	28	6.12 ± 0.01^{bA}	6.11 ± 0.03^{abA}
14	5.95 ± 0.01^{dA}	5.52 ± 0.01^{dB}	21	6.17 ± 0.02^{abA}	6.01 ± 0.01^{bB}	42	6.06 ± 0.02^{cA}	5.93 ± 0.01^{cB}
17	5.81 ± 0.02^{eA}	5.27 ± 0.03^{eB}	28	6.08 ± 0.01^{bA}	5.57 ± 0.02^{cB}	60	6.02 ± 0.04^{cdA}	5.26 ± 0.03^{dB}
21	5.47 ± 0.02^{fA}	5.15 ± 0.02^{eB}	35	5.92 ± 0.03^{cA}	5.39 ± 0.03^{dB}	80	5.99 ± 0.02^{dA}	5.13 ± 0.03^{eB}
			42	5.60 ± 0.08^{dA}	5.17 ± 0.03^{eB}	100	5.69 ± 0.01^{eA}	

Values with different superscript letters have a significant difference ($P < 0.05$). Small superscript letters indicate the results in the same column, and capital superscript letters compare rows within the same temperature.

caused significant ($P < 0.05$) degradation of chlorophyll *a* and *b* (Table 2). Compared with HW, MAPS induced less loss in chlorophyll *a*. Chlorophylls continued to degrade during storage following the first-order fractional conversion model described in Eq. (7) (Fig. 3). The kinetic parameters of degradation are summarized in Table 3. As expected, the higher the storage temperature, the faster the degradation of chlorophylls over time, as indicated by higher degradation rates (*k*). Reducing the storage temperature from 10 to 2 °C could bring about a threefold reduction in *k* for both chlorophyll *a* and *b*. To better interpret the influence of storage temperature on chlorophyll degradation, chlorophyll content in samples after 17 days at 10 °C, 21 days at 7 °C, and 28 days at 2 °C were compared. MAPS processed green beans had total chlorophyll retentions of 49.6%, 53.2%, and 55.4%, while preservation of 45.4%, 49.3%, and 52.2% was obtained in the HW processed samples. Microwave technology provided green beans with greater chlorophyll retention over time, due to the lower *k* in MAPS processed samples than HW. The chlorophyll content of MAPS processed samples at the end of storage at 7 °C was significantly higher as compared with HW processed ones, and the values of those stored at 10 and 2 °C were still comparable (Fig. 3).

Schwartz and Von Elbe (1983) suggested that chlorophyll degradation in vegetables is the result of chlorophyll degrade to pheophytin and further to pyropheophytin. First-order degradation kinetics of chlorophyll were reported for fresh asparagus (Tenorio, Villanueva, & Sagardoy, 2004), pasteurized green pee puree (Sonar, Rasco, et al., 2019), and frozen green beans (Martins & Silva, 2002) during storage. But Benlloch-Tinoco et al. (2015b) reported second-order chlorophyll degradation kinetics in microwave and conventionally heated kiwifruit puree; the degradation rate decreased from 0.031 to 0.007 day^{-1} when the storage temperature changed from 22 to 4 °C. Besides storage temperature, the kinetic of chlorophyll degradation is also affected by pH and microbial growth. Gunawan and Barringer (2000) showed that microbial growth accelerates the color change in broccoli by producing acids. The decrease in pH favors converting chlorophyll to pheophytin by facilitating the replacement of Mg^{2+} by H^{+} (Gunawan & Barringer, 2000). Koca, Karadeniz, and Burdurlu (2007) reported that the degradation rate of chlorophyll *a* in green peas at pH 5.5 was twice that at pH 6.5. The higher pH in MAPS processed samples may be another reason that more chlorophyll was preserved.

Klug et al. (2018) reported that microwave (30s, 11 kW) caused less loss in chlorophyll in faba bean sauce compared to conventional pasteurization (85 °C, 5 min). Benlloch-Tinoco et al. (2015b) also confirmed the superiority of microwave heating in preserving chlorophylls and carotenoids in kiwifruit puree stored at 4–22 °C. The observed difference could be explained by the higher heating rate and less exposure time to high temperature in microwave heating.

Table 2
Quality attributes of green beans before and after thermal processing.

	Before processing	After processing	
		MAPS	HW
Typical image			
L^*	50.51 ± 1.41^{a}	50.55 ± 0.52^{a}	49.63 ± 1.48^{a}
a^*	-29.52 ± 0.08^{a}	-13.94 ± 0.71^{b}	-9.39 ± 0.29^{c}
b^*	44.06 ± 1.24^{a}	41.88 ± 0.12^{a}	43.45 ± 0.37^{a}
ΔE		15.82 ± 0.44^{a}	20.27 ± 0.52^{b}
pH	6.28 ± 0.02^{a}	6.23 ± 0.01^{b}	6.16 ± 0.01^{c}
Chlorophyll *a* (mg/100 g)	7.11 ± 0.10^{a}	5.10 ± 0.12^{b}	4.70 ± 0.13^{c}
Chlorophyll *b* (mg/100 g)	3.35 ± 0.18^{a}	1.86 ± 0.13^{b}	1.70 ± 0.16^{b}
Ascorbic acid (mg/100 g)	6.12 ± 0.10^{a}	5.56 ± 0.08^{b}	5.18 ± 0.03^{c}

Means in rows followed by different letters differed significantly ($P < 0.05$).

3.5. Color degradation

Both MAPS and HW processing induced considerable changes in color, as indicated by an increase in the a^* values (Table 2). No significant changes in L^* and b^* occurred during processing or storage, so a^* was used as the greenness indicator. A larger increase in a^* was observed when the green beans were conventionally heated. The more significant change in a^* of HW processed samples led to a significantly higher ΔE. A ΔE value of 3 is perceptible by most people (Paciulli, Palermo, Chiavaro, & Pellegrini, 2017), and the color changes were visually noticeable as shifting from bright green to olive green, as shown in typical images in Table 2. The increase of a^* during storage followed the first-order fractional conversion model (Eq. (7)). Storage temperature showed a considerable effect on the rate of reduction in greenness. A reduction in the storage temperature from 10 to 2 °C could reduce the color degradation rate from 0.149 and 0.173 to 0.0359 and 0.0363 day^{-1} in MAPS and HW processed green beans, respectively (Table 3). As shown in Fig. 4, microwave processed green beans had significantly ($P < 0.05$) lower a^* at each measured time point regardless of storage temperatures. The a^* of microwave heated green beans at the end of storage at all temperatures was between 2.8 and 5.2, while it was between 5.1 and 6.8 for conventionally processed samples. This suggests that MAPS better preserved the green color of green beans during storage.

An increase in a^* during thermal processing of green vegetables was observed previously (Aamir, Ovissipour, Rasco, Tang, & Sablani, 2014; Steet & Tong, 1996). Besides the increase of a^*, Kotani, Yamauchi, Ueda, Imahori, and Chachin (1999) reported that L^* and b^* were unchanged in boiled broccoli florets during storage at 10 °C. Several studies compared the effects of microwave and conventional heating on color. Faba bean sauce preserved by microwave had a smaller increase in a^* after processing (Klug et al., 2018). Microwave heated kiwifruit puree had a color change of 6.54 after 123 days storage at 4 °C, while the color change was

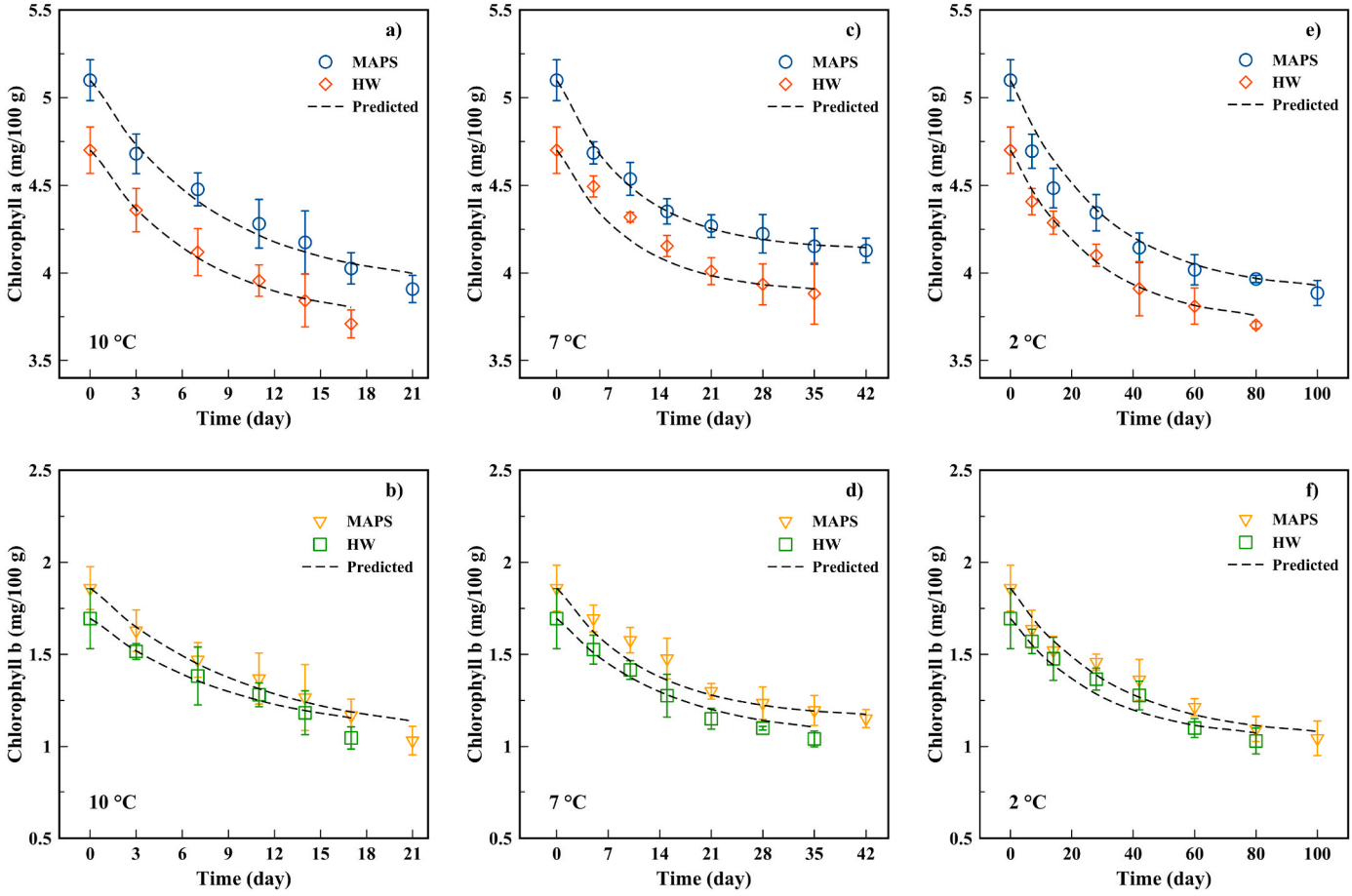

Fig. 3. Chlorophyll *a* and *b* content of microwave (MAPS) and hot water (HW) processed green beans during storage at 10 (a,b), 7 (c,d), and 2 °C (e,f). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Degradation reaction rate (k, day^{-1}) and activation energy (E_a, kJ/mol) of quality attributes of microwave (MAPS) and hot water (HW) processed green beans during storage at 10, 7, and 2 °C.

Temperature (°C)		Chlorophyll *a*		Chlorophyll *b*		*a**		Ascorbic acid	
		MAPS	HW	MAPS	HW	MAPS	HW	MAPS	HW
10	k	0.123	0.137	0.098	0.105	0.149	0.173	0.113	0.157
	R^2	0.957	0.990	0.972	0.979	0.964	0.955	0.958	0.980
7	k	0.0976	0.0988	0.0814	0.0890	0.0908	0.0959	0.0423	0.0685
	R^2	0.971	0.976	0.980	0.979	0.961	0.990	0.877	0.947
2	k	0.0334	0.0362	0.0308	0.0342	0.0359	0.0363	0.0140	0.0181
	R^2	0.991	0.994	0.936	0.922	0.991	0.974	0.826	0.956
	E_a	109.18	110.24	97.06	94.47	116.02	126.31	166.12	174.44
	R^2	0.959	0.982	0.951	0.946	0.999	0.999	0.986	0.999

7.80 in conventionally heated samples after 81 days storage at 4 °C (Benlloch-Tinoco et al., 2015a). These results all correlated with pigment changes, as the change in green color is attributed to the degradation of chlorophyll (Paciulli et al., 2017). In the current study, there was a strong negative correlation between total chlorophyll and *a** ($r = -0.958$, $P < 0.01$). This suggests that the objective color measurements could be employed for determining chlorophyll content in place of pigment analysis.

3.6. Ascorbic acid degradation

AA content in cut green beans was significantly ($P < 0.05$) reduced in thermal processing, from an initial value of 6.12 mg/100 g to 5.56 mg/100 g and 5.18 mg/100 g in MAPS and HW processing, respectively (Table 2). HW caused more loss than MAPS. The degradation of AA in the processed green beans during storage followed the trend described by the zero-order reaction model (Eq. (6)) (Fig. 5). The k increased with the increase of storage temperature (Table 3). Increasing the storage temperature from 2 °C to 7 and 10 °C resulted in a three- and eight-fold increase in k, respectively. Besides, AA degradation showed the highest activation energy compared to chlorophyll and color, which means that AA degradation has higher sensitivity to temperature changes. Retention of AA at the end of storage also varied with storage temperatures. MAPS processed green beans retained 47.2%, 54.9%, and 62.4% of AA after storage at 10, 7, and 2 °C for 21, 42, and 100 days, respectively, with significant differences among the temperatures. For HW processed

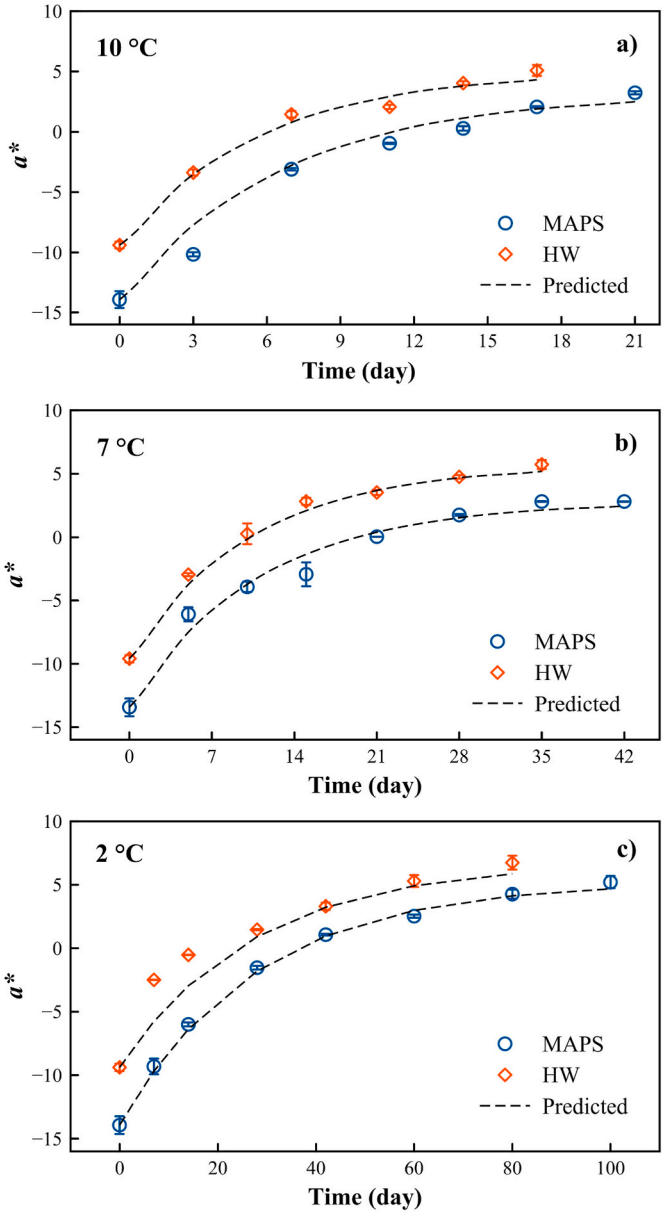

Fig. 4. Greenness (a^*) of microwave (MAPS) and hot water (HW) processed green beans during storage at 10, 7, and 2 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

samples, the retentions were 38.4% after 17 days at 10 °C, 40.7% after 35 days at 7 °C, and 59.0% after 80 days at 2 °C, with significantly lower loss at 2 °C compared to 7 and 10 °C. No significant difference was observed between 7 and 10 °C. MAPS processed samples had a lower AA degradation rate at all storage temperatures, resulting in higher AA retention than HW processed ones.

Other studies reported an 8–48% reduction of AA in different vegetables during thermal processing (Baardseth et al., 2010; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). Less degradation in AA after microwave heating compared with conventional heating was also reported in strawberry puree and capsicum (Kala & Prakash, 2004; Marszałek et al., 2015). Benlloch-Tinoco et al. (2015a) reported microwave pasteurized kiwifruit puree stored at 4 °C for 123 days has AA retention of 57.9%, while in conventionally heated samples, 40.8% of AA was preserved after 81 days at 4 °C. Koo et al. (2008) observed AA retentions of 45.3 and 44.4% in thermal pasteurized soybean sprouts

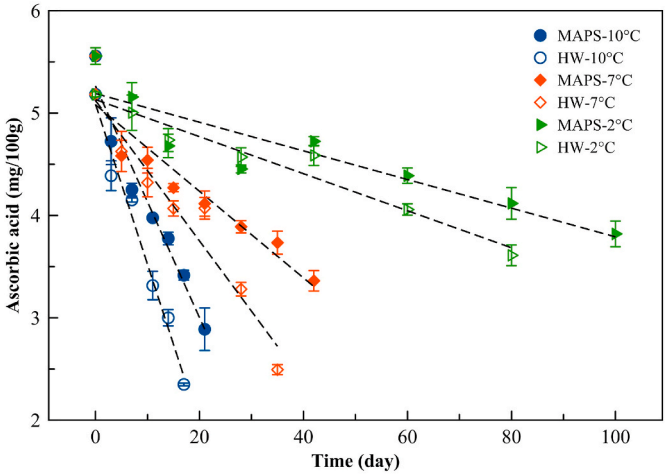

Fig. 5. Ascorbic acid content of microwave (MAPS) and hot water (HW) processed green beans during storage at 10, 7, and 2 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(90 °C, 10 min) after being stored for 24 days at 10 °C and 36 days at 3 °C. But in a recent study, Inanoglu et al. (2020) observed that MAPS processed green beans ($F_{70}^{7.5}$=2 min) lost 87.5% of AA after 8 days' storage at 2 °C and high pressure pasteurized green beans (600 MPa, 25 °C, 10 min) lost almost 100% of AA under the same storage conditions. The large loses of AA in green beans during storage in the study of Inanoglu et al. (2020) were likely caused by the presence of oxyen in the packages.

Aerobic degradation is considered the main degradation pathway of AA in which AA is oxidized to dehydroascorbic acid with the catalyst of metal ions, followed by hydrolysis and further oxidation (Wang, Law, Mujumdar, & Xiao, 2017). Therefore, the stability of AA during storage is highly affected by the oxygen present in the headspace and permeated from the package. In the current study, N_2 flush during sealing reduced the oxygen concentration in the package and lowered the oxidative degradation. The packages in Inanoglu et al. (2020) mentioned above were sealed at a similar vacuum level but not flushed with N_2. This highlights the importance of reducing oxygen concentration in AA preservation during storage. Van Bree et al. (2012) reported that the reaction rate of AA degradation in fruit juice during storage at 22 °C was 0.077 and 0.39 day^{-1} when the initial headspace oxygen concentration was 2.78 and 20.9%, respectively. Gómez Ruiz, Roux, Courtois, and Bonazzi (2018) also proved that lowering oxygen concentration in the headspace could reduce AA degradation rate during the thermal processing of a model solution. The use of lidding film with a low oxygen transmission rate (OTR) also helped AA preservation in our study. Sonar et al. (2019a) reported that the pasteurized fortified carrot puree (F_{90}^{10}=14 min) packed in pouches with OTRs of 1 and 30 cc m^{-2} day^{-1} retained 85–89% and 54–68% AA at the end of storage under 4–13 °C, while there was no AA retention in puree packed in pouches with OTR of 81 cc m^{-2} day^{-1}. Al-Ghamdi et al. (2020) used the same lidding film as the current study. The OTR of the film changed from 0.01 to 0.15 and 2.01 cc m^{-2} day^{-1} after MAPS and conventional hot water pasteurization (F_{90}^{10}=19.9 min). The significant lower OTR of the lidding film after the MAPS processing supported the results that higher AA retention was observed in MAPS processed green beans.

4. Conclusions

Both thermal processing and storage had adverse impacts on the quality of green beans. The 915 MHz microwave-assisted thermal processing led to less degradation in chlorophyll *a*, greenness, and smaller losses of ascorbic acid than conventional hot water processing. Lower

storage temperature reduced the quality degradation rate and extended the shelf life of pasteurized green beans. *Paenibacillus* spp. and *Bacillus* spp. were identified from spoiled pasteurized green beans. Microwave-assisted thermal processing showed superiority over conventional hot water processing when taking into account slower microbial growth and greater maintenance of chlorophylls, color, and ascorbic acid during storage. This study suggests that microwave-assisted pasteurization is a potential alternative to produce safe, high-quality vegetable products that preserve their quality during storage.

CRediT authorship contribution statement

Zhi Qu: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. **Zhongwei Tang:** Investigation, Data curation, Writing - review & editing. **Fang Liu:** Investigation, Data curation. **Shyam S. Sablani:** Writing - review & editing. **Carolyn F. Ross:** Writing - review & editing. **Sindhuja Sankaran:** Writing - review & editing. **Juming Tang:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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