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Impact of high-pressure and microwave-assisted thermal pasteurization on inactivation of *Listeria innocua* and quality attributes of green beans

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ABSTRACT

In response to the increasing consumer demand for high-quality, minimally processed ready-to-eat (RTE) meals, the food industry has shown strong interests in exploring new processing technologies for production of safe RTE meals with an adequate shelf life in cold distribution chains. The purpose of this research was to understand the effect of high-pressure processing (HPP) and microwave-assisted thermal pasteurization on the quality attributes of green beans.

The pasteurization conditions were selected as 600 MPa at 25 °C for 10 min for HPP and 70 °C for 2 min for processing with microwave-assisted pasteurization system (MAPS). Survival of *L. innocua* ATCC 51742 in green bean brine solution was analyzed right after the pasteurization processes. The quality attributes of the green beans, such as color, chlorophyll content, texture, vitamin C content, and pH, were further determined during storage at 2 °C for 36 days and 10 °C for 20 days.

High-pressure treatment resulted in a 3.7-log CFU/g reduction in *L. innocua* ATCC 51742, whereas MAPS processing showed a 9.0-log CFU/g reduction. Both pasteurization processes provided significantly better green color retention when the beans were stored at 2 °C than when they were stored at 10 °C. Regardless of the pasteurization methods, the change in the greenness (a^*) value of the beans followed similar trends during storage. Both methods caused an increase in the total color difference (ΔE) and a decrease in hue angle (yellowness) during storage ($p < 0.05$), but they had no significant effect on firmness, total chlorophyll content, and pH of the green beans. After the pasteurization processes, the vitamin C content of the HPP-treated green beans decreased significantly in comparison with the MAPS-treated beans ($p < 0.05$). This work reveals that both pasteurization treatments have a similar impact on quality attributes of green beans and that MAPS processing is more efficient in inactivating *L. innocua* ATCC 51742.

1. Introduction

The food industry uses thermal and nonthermal processes to pasteurize ready-to-eat (RTE) meals (Öztürk and Nilüfer-Erdil, 2015). Even though the RTE meal sector has seen significant growth in sales, most RTE meals are produced by conventional methods such as canning, which are considered prevalent methods for manufacturing microbiologically safe and stable products. However, these methods may introduce appearance, texture, flavor, and color changes that are undesirable for a premium quality product (Torres and Velazquez, 2005). Therefore, to meet consumer expectations, food companies seek new processing technologies for manufacturing microbiologically safe frozen and chilled RTE meals without compromising quality and with an adequate shelf life (Ramaswamy and Tang, 2008). Some frozen meals have not gone through a pasteurization process. They are often referred to as not-ready-to-eat (NRTE) meals. NRTE meals may present safety risks to consumers (Rounds et al., 2013). They must be thoroughly cooked following validated cooking instructions before consumption to assure the safety of the product. RTE meals, on the other hand, are safe over the intended shelf-life in proper storage, they are reheated at homes only for the purpose of better palatability (Tang et al., 2018).

Multiple studies have linked listeriosis outbreaks to the consumption

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of NRTE food products, including vegetables (Francis et al., 1999). The United States Department of Agriculture has zero-tolerance for *Listeria monocytogenes* (FSIS-USDA, 1989). *L. monocytogenes* can grow at refrigeration temperatures and, therefore, is the target microorganism in the production of chilled RTE foods. A pasteurization process should obtain a 6-log reduction of *L. monocytogenes* to produce safe, low-acid chilled RTE foods with a shelf life of $\leq$ 10 days at $<$ 5 °C (FDA, 2008). *Listeria innocua* has been used as a nonpathogenic surrogate for *L. monocytogenes* because of its close genetic relationship and metabolic similarity with *L. monocytogenes* and for studies conducted in pilot plants or in food processing facilities where other products might be manufactured for human consumption (Foegeding and Stanley, 1991; Milillo et al., 2012). *L. innocua* has been selected based on its higher resistance to heat and pressure than some of *L. monocytogenes* strains such as Scott A at 400 MPa and 30 °C for 1 min for HPP treatment and at 56, 60, 66 °C for thermal treatments (Foegeding and Stanley, 1991; Hu and Gurtler, 2017; Tay et al., 2003). Foegeding and Stanley (1991) found that two strains of *L. innocua* had $D_{56°C}$-values of 6.3 min, compared with a $D_{56°C}$-value of 3.0 min and 4.1 min for *L. monocytogenes* Scott A and strain F5069, respectively. According to Tay et al. (2003), 4 and 2 log reduction of *L. monocytogenes* Scott A and *L. innocua* were obtained at 400 MPa and 30 °C for 1 min, respectively. Nevertheless, specific strains, food matrices, and testing parameters may affect the suitability of *L. innocua* to be used as a potential surrogate (Mohan et al., 2019).

HPP provides several advantages including microbial inactivation at or near 25 °C and better results on quality characteristics due to low processing temperature compared to thermal treatments (Rastogi et al., 2007). Microwave heating offers a quick temperature rise of the food, which may result in better quality and renders safe foods with a shelf life comparable to that with conventional thermal processing (Tang, 2015). Even though there are insufficient data related to the effects of novel pasteurization treatments on the quality of RTE meals, Sorenson et al. (2011) reported that HPP has the potential to produce high quality chilled RTE meals. Studies have also shown that microwave-assisted pasteurization systems (MAPS) can be an efficient approach to attaining microbial safety without compromising quality (Joyner et al., 2016; Peng et al., 2017b; Sonar et al., 2020).

In the scientific literature, HPP and microwave-pasteurized products are reported to have superior quality in comparison with their conventionally treated (such as canned) counterparts (Knockaert et al., 2011; Pandrangi and Balasubramaniam, 2005). However, the processing conditions for conventional and novel processes are usually not selected on an equivalent basis, which is necessary for a fair comparison of the impact on quality. Although numerous authors have compared the effects of conventional and novel pasteurization methods, only a few studies have included this important consideration (Knockaert et al., 2011; Vervoort et al., 2012). The authors often claimed that HPP and thermal pasteurization conditions could attain the 6-log reduction in *L. monocytogenes* without presenting microbial data.

To the best of our knowledge, no studies have been published on the impact of both HPP and microwave heating as pasteurization methods on the quality of foods. The purpose of this study was to investigate the effect of selected HPP and MAPS treatments on inactivation of *L. innocua* and the quality of green beans during storage at 2 °C for 36 days and 10 °C for 20 days. The selected pasteurization conditions were 600 MPa, 10 min for HPP, and $P_{70\,°C}^{7.5\,°C} = 2$ min for MAPS to produce safe RTE green beans.

2. Materials and methods

2.1. Sample handling

Frozen green beans (*Phaseolus vulgaris* L.; Bird's Eye) were bought from a local market (Pullman, WA) and stored in a freezer until the experiments were performed. We selected green beans for this study because green beans are one of the most heat-sensitive vegetables that can be included in RTE meals. Growth and harvesting of green beans are seasonal and limited to specific regions with suited climate and soil conditions. Freezing is a common way to preserve green beans for year-round supply to markets elsewhere and could be used to manufacture RTE meals in all seasons. According to Koo et al., 2008, before pasteurization, blanching is necessary due to the selected processing conditions used in the present study. Therefore, we used blanched frozen cut beans. The frozen green bean samples were thawed at 5 °C for 20 h. Each pouch was filled with 136.5 ± 0.5 g green beans and 90.5 ± 0.5 g of brine solution containing 0.1% NaCl with 0.1% $CaCl_2$ in distilled water (w/w), and then was vacuum-sealed at 93.2 kPa using an UltraVac 250 (Ultrasource LLC., Kansas City, MO). Salt was added for taste and calcium chloride to reduce softening. The concentrations of salt and calcium chloride, as well as the ratio of green beans to brine, were adopted from Peng et al. (2017b). The selected pouches were made from laminate multilayer polymer (hyper-branched polyester (HBPET) 12 μm/biaxially oriented nylon (BON) 15 μm/cast polypropylene (CPP) 70 μm), had tear notch, rounded corners, 18.5 × 13.2 × 1.6 cm, and were supplied by Printpack Inc.(Atlanta, GA).

2.2. Pasteurization of green beans

The processing conditions for HPP and MAPS were selected based on recently published studies (Knockaert et al., 2011; Vervoort et al., 2012). A 6-log reduction in *L. monocytogenes* for the pasteurization of low-acid RTE meals was recommended by the European Chilled Food Federation (Peng et al., 2017a). Therefore, *L. monocytogenes* was considered as the pathogen of concern for the HPP and MAPS processes. According to Vervoort et al. (2012) and Knockaert et al. (2011), the food samples should receive a thermal treatment of $P_{70\,°C} = 2$ min (heating cold spot to 70 °C and holding for 2 min) and a HPP treatment of 600 MPa for 10 min to attain a 6-log reduction in *L. monocytogenes*. Accordingly, a treatment of 600 MPa for 10 min at 25 °C was selected for HPP, and 70 °C - 2 min (a condition that is equal to the pasteurization conditions for HPP to warrant microbial safety) was selected for microwave assisted thermal pasteurization.

2.2.1. High pressure processing

The packaged green beans were placed in the sample basket of an HPP isostatic pressing system (Engineered Pressure Systems, Inc., 165 Ferry Road Haverhill, MA 01835, USA) with a cylindrical pressure chamber (height = 0.25 m, diameter = 0.10 m). A 10% Hydrolubric 123-B aqueous solution was used as the pressurization medium where the green bean pouches were subjected to 600 MPa for 10 min at 25 °C followed by storage.

2.2.2. Microwave-assisted pasteurization

The packaged green beans were pasteurized with the pilot-scale MAPS developed at Washington State University, shown in Fig. 1 (Tang et al., 2018). The MAPS consists of four sections: preheating, microwave heating, holding, and cooling. In the microwave heating section, there are four single-mode 915 MHz microwave heating cavities, with a total microwave power of 18.7 kW (5 kW for each of the first two heating cavities and 8.7 kW for the last two cavities). Each section has a separate water circulation system to keep the water flow at a constant temperature. Food samples can be heated with microwave energy (for volumetric heating) and circulating hot water (for surface heating) during their residence on the food package carrier throughout the first three sections. The speed of the food package carrier in the microwave heating section and the holding time of the food package can be adjusted to achieve the desired P (pasteurization) values. In a MAPS process for this research, the green bean pouches were preloaded in stainless steel carriers (containing five pouches per carrier) and placed in the preheating section to heat up in circulating warm water (31 °C) in 25 min. After the preheating, the samples were transported through the

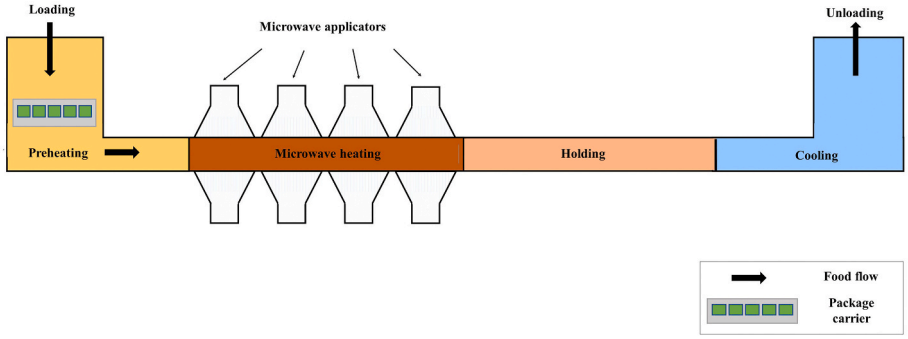

Fig. 1. Schematic diagram of a microwave-assisted pasteurization system (MAPS) developed at Washington State University (Tang et al., 2018).

microwave heating section at a speed of 116.8 cm/min and treated by the combination of microwave energy and circulating water at 72 °C. In the holding section, the samples were kept in circulating hot water at 72 °C for 3 min to reach the desired P value at the cold spot. The cooling was carried out by circulating cold water at 23 °C.

Initial MAPS runs were conducted to determine the locations of cold and hot spots in a mashed potato model food. The dielectric properties of mashed potato model food containing 0.1% salt and 0.1% $CaCl_2$ were similar to those of the mixture of green beans and brine (same ratio of green beans to brine in the pouch) based on our measurements in the range of 30 °C–100 °C. The loss factor of brine, green bean puree, and model food is 16.0 ± 1.4, 14.5 ± 1.6, and 17.3 ± 1.5 at 30 °C, respectively, and 22.4 ± 0.6, 17.6 ± 1.6, and 22.2 ± 2.0 at 70 °C (the treatment temperature for MAPS), respectively. The dielectric constant of brine, green bean puree, and model food is 73.0 ± 2.2, 69.3 ± 3.7, and 72.8 ± 4.8 at 30 °C, respectively, and 63.4 ± 0.9, 62.4 ± 2.9, and 67.0 ± 3.6 at 70 °C, respectively. Determining the locations of cold and hot spots within heterogenous food such as green beans in brine is difficult. Therefore, mashed potato model food having a homogenous structure was used for the heating pattern and cold/hot spot determination. The mashed potato model food with Maillard reaction products was developed and regarded as an optimal model food for pasteurization studies at pasteurization temperatures (70–100 °C) by Bornhorst et al. (2017). Because of the similarity of the dielectric properties of the model food and the green beans in brine, the identified cold and hot spots in the model food pouches were used as the cold and hot spots of the green beans pouches.

For the heating pattern and cold/hot spot determination, five pouches containing model food samples were processed by MAPS. The processed model food samples were sliced horizontally at the middle layer. Images of the middle layer of the samples were taken and analyzed to get the heating pattern by a computer vision system (Pandit et al., 2007). Finally, the cold and hot spots were located based on the color distribution in the heating pattern images.

During MAPS tests of the green beans and brine samples, the temperatures at the cold and hot spots were measured every 2 s using mobile metallic temperature sensors (TMI, Inc. Reston, VA, USA) as described by Luan et al. (2013). The temperature sensor with a sensor holder was supported by a thin Ultem frame to make the tip of the sensor located at the cold or hot spot inside the food pouch. The accumulative thermal lethality, $P_{70\,^{\circ}C}^{7.5\,^{\circ}C}$, was calculated based on the temperature measurements from Eq. (1) (Peng et al., 2017a):

$$P_{70}^{7.5} = \int_0^t 10^{\frac{T-70}{z}} dt \tag{1}$$

where $P_{70\,^{\circ}C}^{7.5\,^{\circ}C}$ is the equivalent thermal treatment time (in min) at 70 °C, T is the temperature (in °C) at time t (in min), and z is the thermal resistance constant with a value of 7.5 °C for *L. monocytogenes* (Peng et al., 2017a). The temperature measurement at the cold spot was to ensure that the products were safely pasteurized, while the temperature measurement at the hot spot was to determine the quality and the temperature history of the most-heated areas.

2.3. Storage

The three identical HPP- and MAPS-treated pouches for each data point were kept at 2 ± 0.5 °C and opened at storage days 0, 4, 8, 12, 16, 20, 24, 30, and 36 for quality analysis. For samples stored at 10 ± 0.5 °C, they were sampled at days 0, 2, 4, 6, 8, 10, 12, 16, and 20. The storage temperature of 10 °C was chosen as an abuse temperature, which has been used for shelf-life studies on RTE foods (Balamurugan et al., 2018; Koo et al., 2008).

2.4. Microbiological analyses

2.4.1. Preparation of L. innocua

L. innocua (ATCC 51742™) was obtained from the American Type Culture Collection and selected as a reference strain to compare the effectiveness of the two pasteurization methods in our pilot-scale tests. The same strain has been used in the studies on HPP and heat treatments (Al-Holy et al., 2004; Guerrero-Beltrán et al., 2011; Pokhrel et al., 2019; Rodrigues et al., 2016). 1 ml of *L. innocua* ATCC 51742 was transferred to 9 ml of tryptone soy broth (TSB, in screw cap tubes) and incubated at 37 °C for 24 h. The culture was centrifuged (4000×*g*, 15 min), and then the supernatant was discarded. The cells were washed twice in 0.1% peptone water to eliminate residual components of the media. The final cell pellets were kept in the freezer until the experiments.

2.4.2. Inoculation and processing of green bean samples

A piece of green bean (1.3 ± 0.1 g; negative for *L. monocytogenes* in 25 g by AOAC OMA, 2004.02 method) and 0.9 ml of brine solution (to mimic the food of 136.5 ± 0.5 g green beans and 90.5 ± 0.5 g of brine solution) were placed in a small pouch (8 × 6.5 cm), inoculated with *L. innocua* ATCC 51742 (stationary phase) to 9-log CFU/g. The small pouch was then double sealed by the vacuum sealer. The inoculated pouches were mixed by vortex to distribute the inoculum throughout the product and then kept at 4 °C overnight for physiological adaptation and attachment. The inoculated small pouch was placed at the cold spot of the pouches (containing 136.5 g of green beans and 90.5 g of brine solution), then the pouches were sealed by the vacuum sealer under a vacuum of 93.2 kPa for MAPS processing using the processing schedule as described in 2.2.2. The inoculated small pouches were treated by the HPP system located in the pilot plant as well. The inoculated small pouches were processed by HPP and MAPS in triplicate with three identical pouches (n = 9).

2.4.3. Enumeration

After the pasteurization treatments, the MAPS- (removed from the 227-g pouches) and HPP-treated inoculated small pouches were mixed by vortex. One gram of the sample from the inoculated small pouch was

decimally serially diluted in sterile 0.1% peptone water (9.0 ml) and enumerated as spread plates (0.1 ml) onto a TSAYE (trypticase soy agar with 0.6% yeast extract) plate. Typical colonies were counted after incubation for 48 h at 37 °C.

2.5. Color analysis

The CIE (Commission Internationale de l'éclairage) L^* (lightness/darkness), a^* (redness/greenness), b^* (yellowness/blueness), and hue angle for the untreated and pasteurized (by HPP and MAPS) samples were determined using a computer vision system following the procedures previously described in Bornhorst et al. (2017). The computer vision system consists of a digital camera (settings 1/100 s, ISO 640, F 4.5) connected to a computer with image acquisition and analysis software, a light pod, compact fluorescent light bulbs, and a sample stand with a black background. Green beans were placed on a black tray then put on the sample stand. The sample was illuminated with two compact fluorescent light bulbs (one from the right and the other from the left side). Images were captured by the digital camera (Canon EOS 60D, Canon Inc., Melville, NY) and saved in the computer. Image analysis, color correction, and conversion were then made with MATLAB R2019a. A color reference card (QPcard 203, QPcard AB, Sweden) was used for color correction and converting the image from RGB (R: red, G: green, and B: blue) to $L^*a^*b^*$ (León et al., 2006). The analysis was carried out in triplicate with three identical pouches for each time interval (n = 3). The L^*, a^*, and b^* values were used to calculate total color differences (ΔE) with Eq. (2) and hue angles (H, in °) with Eq. (3):

$$\Delta E = \sqrt{\left(L_t^* - L_0^*\right)^2 + \left(a_t^* - a_0^*\right)^2 + \left(b_t^* - b_0^*\right)^2} \quad (2)$$

$$H = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

where L_0^*, a_0^*, and b_0^* are the L^*, a^*, and b^* values of the untreated green beans, whereas L_t^*, a_t^*, and b_t^* represent the L^*, a^*, and b^* values of the green beans after the pasteurization treatments. The scales in Table 1 show how to interpret the difference between the colors of untreated and pasteurized green bean samples (Wang et al., 2014).

The hue angle is used as an indicator of color change and to present measurements of visual attributes. Angles of 90° ($+b^*$), 180° ($-a^*$), and 270° ($-b^*$) represent yellow, green, and blue hues, respectively, while 0° or 360° ($+a^*$) denotes a red hue. The hue angle of green-colored vegetables is between 90° and 180°; a change in hue angle values from 180° to 90° indicates a color shift from green to yellow in green foods (McLellan et al., 1995).

2.6. Measurements of chlorophyll content

The chlorophyll content of the green beans was measured according to the method of Sonar et al. (2019). The pasteurized green beans (15 g) and brine (10 ml) with 0.1 g of $CaCO_3$ were pureed in a grinder for 30 s. The puree of the processed green beans was homogenized with 25 ml of acetone (80% v/v) for 5 min at 7000 rpm by using a Polytron PT-3100 homogenizer (Kinematic, Luzerne, Switzerland). The extract was centrifuged (Sorvall Biofuge Primo model 75007588; Thermo Scientific, Osterode, Germany) at 8872×g for 6 min at 25 °C. The supernatant was diluted to 50 ml with acetone (80% v/v). The absorbance reading was conducted at 647 and 663 nm using a spectrophotometer (V-5000 Visible Spectrophotometer, M&A Instruments Inc., Shanghai, China). From the absorbance values, the chlorophyll contents (a, b, and total) were calculated using the following equations:

$$Chlorophyll\ a = 12.25\ A_{663} - 2.79\ A_{647}\left(\frac{\mu g}{ml\ of\ extract}\right) \quad (4)$$

$$Chlorophyll\ b = 21.50\ A_{647} - 5.10\ A_{663}\left(\frac{\mu g}{ml\ of\ extract}\right) \quad (5)$$

$$Total\ chlorophylls = 7.15\ A_{663} + 18.71\ A_{647}\left(\frac{\mu g}{ml\ of\ extract}\right) \quad (6)$$

where A_{647} and A_{663} are the absorbance values, which were read from the spectrophotometer at 647, 663 nm, respectively. The analysis was performed in triplicate with three identical pouches for each time interval.

Table 1
The degrees of difference for the ΔE (total color difference) scales (Wang et al., 2014).

Scale	Degrees of difference
ΔE<0.2	No perceptible difference
0.2<ΔE<0.5	Very small difference
0.2<ΔE<2	Small difference
2<ΔE<3	Fairly perceptible difference
3<ΔE<6	Perceptible difference
6<ΔE<12	Strong difference
ΔE>12	Different colors

2.7. Mechanical properties

The firmness of the green beans was determined by a TA. XT2 texture analyzer (Stable Micro Systems Ltd., Godalming, UK) in a cut test. A 3 mm thick stainless-steel Warner-Bratzler blade was used for the cut test. A piece of green bean (length: 17 ± 1 mm, diameter: 8 ± 1 mm) was positioned on a slot surface and cut with the blade at a speed of 1 mm/s. The shearing forces (F_{max}, N) to quantify firmness were obtained from the force-time curves. For a single data point, eight green beans were measured from three identical pouches for each time interval (n = 24).

2.8. Quantification of vitamin C

Vitamin C was quantified using the method published in Zhang et al. (2019) by an Agilent 1100 high-performance liquid chromatography system (HPLC; Agilent Technology, Santa Clara, CA) with a 250 mm 5 μm XTerra C18 column (Waters Corporation, Milford, MA) and a diode array detector at 250 nm wavelength. The analysis was performed in triplicate with three identical pouches for each time interval.

2.9. pH

The pH of the pasteurized green bean puree was measured by a handheld meter (LAQUA twin-Ph-22, Horiba, Montpellier, France). The analysis was performed in triplicate with three identical pouches for each time interval.

2.10. Data analysis

All statistical results were acquired from IBM SPSS Statistics 22. One-way ANOVA, followed by Tukey's post hoc means comparison ($p < 0.05$) test, was performed to evaluate the effect of the pasteurization treatments and storage time on quality attributes. An independent t-test was performed to compare the storage temperatures of 2 and 10 °C for each pasteurization treatment.

3. Results and discussion

3.1. Pasteurization of green beans

Typical pressure-temperature profiles of the samples treated by high pressure are presented in Fig. 2A. The HPP process included 50 s of come-up time, 10 min of holding at a pressure of 600 MPa, and 15 s of decompression time.

Typical temperature profiles of the samples processed by MAPS are presented in Fig. 2B. The MAPS process started with a preheating at 31 °C for 25 min by circulating water. The 4.3 min of heating by microwaves and 72 °C circulating water led to a temperature increase inside the food pouch. The 3 min holding section in a 72 °C circulating water helped to obtain the required lethality value. The MAPS process was completed by 5 min of cooling in 25 °C circulating water. This process provided a lethality of $P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min at the cold spot, which was the geometric center of the pouch, and $P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 23.6$ min at the hot spot, which was 53 mm away from the center and close to an end of the pouch.

The microbiological inactivation study showed that the MAPS treatment resulted in 9.0-log CFU/g reduction of *L. innocua* ATCC 51742 in green bean broth (pH 5.8 ± 0.2), whereas the HPP treatment (600 MPa for 10 min at 25 °C) resulted in a 3.7-log CFU/g reduction (Fig. 3).

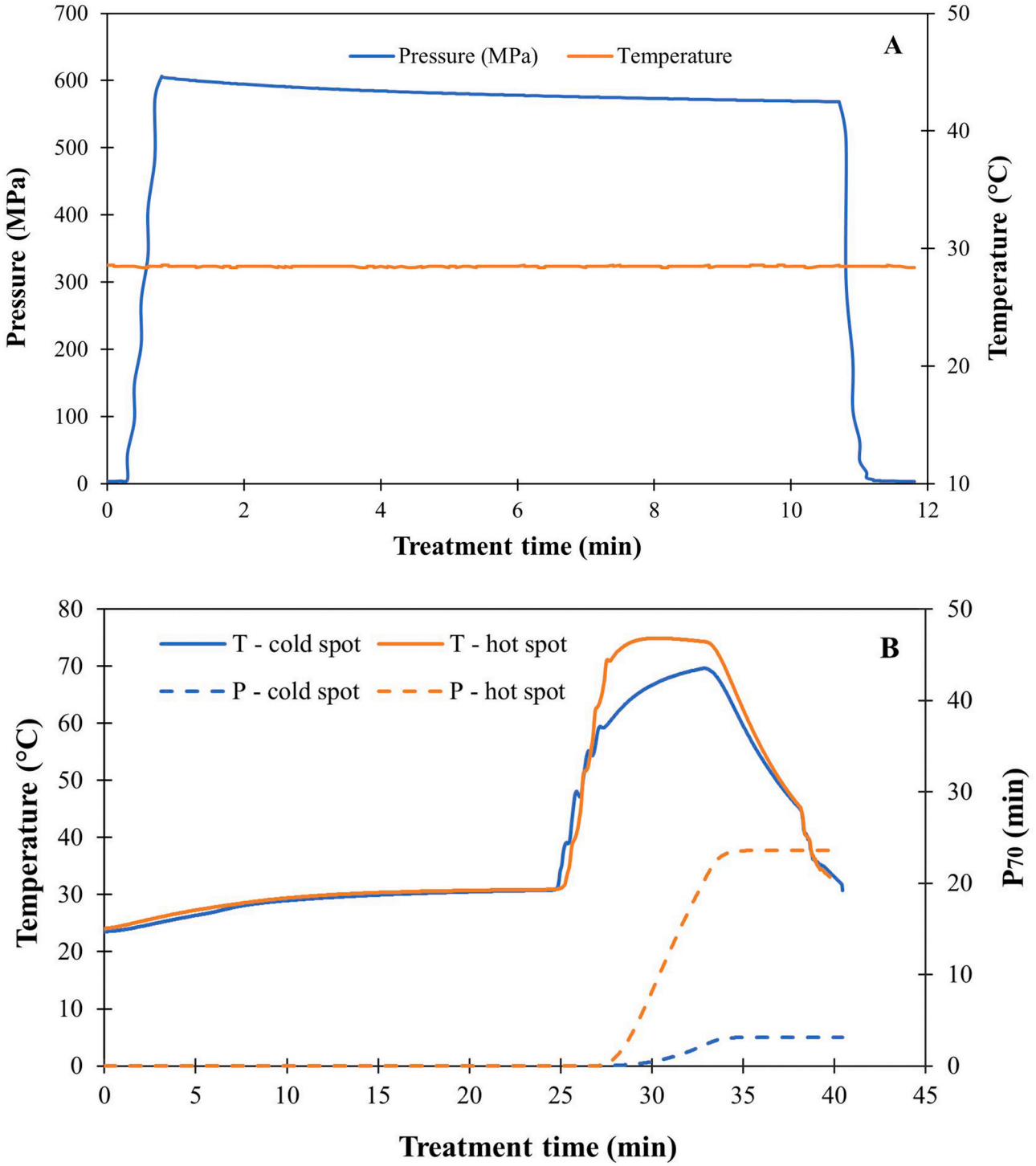

Fig. 2. (**A**) The typical pressure (blue line) and temperature (orange line) profiles in an HPP process (600 MPa for 10 min at 25 °C). (**B**) The typical temperature-time profiles of the cold (blue line) and hot (orange line) spots in the green bean pouch during a MAPS process designed for $P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 2$ min. The corresponding pasteurization value at the cold spot was calculated to be $P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min at the end of the MAPS process (blue dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Heating at 70 °C for 2 min resulted in a more than 6.0-log reduction in *L. monocytogenes* inoculated on chicken surface (Coote et al., 1991) and beefsteak and carrot (Gaze et al., 1989). The same HPP pasteurization conditions resulted in a 6.0-log reduction in *L. monocytogenes* in low pH (6.65) brain heart infusion broth (Dogan and Erkmen, 2004). According to Ates et al. (2016), the HPP conditions of 600 MPa and 25 °C for 5 min resulted in a more than 6.0-log inactivation in *L. monocytogenes* in soup (pH 6.1). However, our HPP test results showed a less than 4.0-log reduction of *L. innocua* ATCC 51742 in green beans. This lower inactivation level might be attributed to the higher resistance to pressure of *L. innocua* ATCC 51742 compared with that of *L. monocytogenes*. It might also be caused by the food constituents and the pH of green beans, which might influence the heat and pressure resistance of *L. innocua* (Simpson and Gilmour, 1997).

3.2. Quality evaluation

3.2.1. Color

Fig. 4A and 4B show the a^* values (greenness) of the untreated, pasteurized (by HPP and MAPS) green beans stored at 2 and 10 °C, respectively. The MAPS processing resulted in an initial bright green color (a^* value) but no significant change in green color in comparison with the untreated green beans. Lau et al. (2000) observed a similar increase in green color in green asparagus in a heated treatment of 70 °C (water bath). Woolfe (1979) attributed the enhancement in green color to the expulsion of air between the cells, resulting in air removal from the surface. The HPP pasteurization (600 MPa for 10 min at 25 °C) also resulted in no significant change in green color compared with the untreated green beans. This retention of green color can be explained by the permeabilization of plant cells, resulting in chlorophyll leakage into the intercellular space under pressures of 100–800 MPa (Préstamo and Arroyo, 1999). After pasteurization, the green beans treated by MAPS were significantly greener than those treated by HPP ($p < 0.05$). However, the loss of greenness was observed throughout all storage periods, irrespective of the pasteurization method (Table 2). Koo et al. (2008) observed a color decrease in soybean sprouts processed by the cook-chilled method at 70 °C for 2 min during storage at 3 and 10 °C. The shelf-life based on sensory acceptance in terms of color was eight days in a study of sous-vide cooked green beans stored at 3 °C (Knøchel et al., 1997). The greenness of the beans stored at 10 °C decreased more than that of the beans stored at 2 °C. A decrease in greenness with increasing storage temperature (from 5 to 25 °C) was observed in guacamole processed under an HPP treatment (two cycles at 689 MPa with holding times of 5 min each) by Palou et al. (2000). A decrease in greenness during 60 days of storage at 30 °C was observed in microwave-treated broccoli by Koskiniemi et al. (2013).

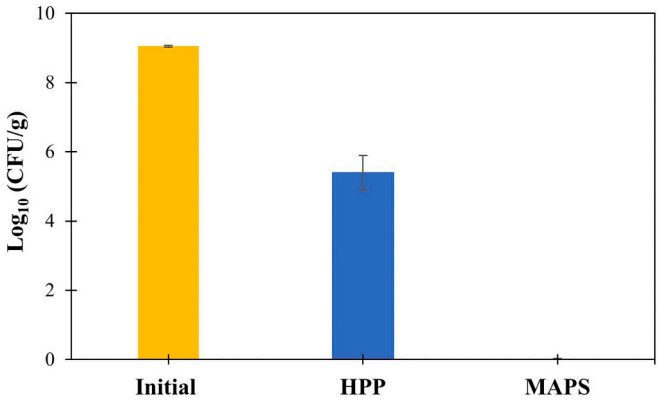

Fig. 3. The effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS processing ($P_{70\,^\circ C}^{7.5\,^\circ C} = 3.2$ min) on the survival of *L. innocua* in small pouches of green beans and brine.

The L^* value (lightness) of the green beans pasteurized by MAPS was not significantly different from that of HPP-treated samples right after the pasteurization processes, and during storage at 2 °C for 36 days and 10 °C for 20 days (data not shown). For the samples processed by both pasteurization methods, similar to the a^* values (greenness), an increase in yellowness was observed with an increase in storage time (data not shown). Since both pasteurization treatments had a similar effect on the L^*, a^*, and b^* values of the green beans, we calculated the total color difference (ΔE) for the green beans with Eq. (2) to characterize the changes in color caused by processing and storage. Fig. 5A and 5B show the ΔE values of the pasteurized (by HPP and MAPS) green beans stored at 2 and 10 °C, respectively. The ΔE value of MAPS processed samples was initially 7.77 ($6 < \Delta E < 12$), which indicates that a strong difference in the color of the green beans was caused by the MAPS treatments (Table 1). The ΔE value of HPP processed samples was initially 4.61 ($3 < \Delta E < 6$), which indicates a noticeable difference in color before and after the HPP treatments. Green bean samples have changed to a different color after day 8 and 16 at 2 °C and day 6 and 8 at 10 °C for HPP and MAPS, respectively. Zhang et al. (2016) used $\Delta E = 12$ as the end point of shelf life, which denotes that the color of food has changed to a different color. Both pasteurization processes gave rise to similar ΔE values, denoting similar color changes in the green beans at the end of storage at 2 and 10 °C. An increase in storage temperature from 2 to 10 °C induced a higher ΔE value at the same storage time, i.e., the increase in storage temperature reduced the color retention of the green beans. Although the MAPS treatments resulted in a greater log reduction in *L. innocua* ATCC 51742 compared with the HPP treatments, both treatments caused a similar degree of color change in the green beans during the storage except for day 8, 12, 16 at 2 °C and day 6 at 10 °C. On those days, HPP resulted in larger color change as compared to MAPS.

Fig. 6A and 6B show the hue angle value (another indicator of greenness) of the HPP and MAPS pasteurized green beans stored at 2 and 10 °C, respectively. The initial hue angle values of the processed samples (124.8° for HPP and 123.5° for MAPS) were not significantly different from that of the untreated samples (128°), which also represents that both the HPP and the MAPS treatments did not cause much color changes in the samples. However, regardless of the pasteurization methods, a significant decrease in the hue angle of processed green beans was observed when the storage time increased either at 2 °C or 10 °C. The hue angle decreased faster at 10 °C storage than at 2 °C storage. An et al. (2008) reported a decrease in hue angle in terms of storage period and storage temperature on green asparagus (stored at 2 °C and 10 °C).

3.2.2. Chlorophyll content

The effects of the pasteurization methods and storage conditions on the total chlorophyll content of the green beans are shown in Fig. 7A and 7B. A similar chlorophyll degradation was observed in the HPP- and MAPS-treated green beans. Ahmed and Ramaswamy (2006) reviewed the effect of HPP and thermal treatments on chlorophyll pigment and reported that some chlorophyll-containing green products were affected similarly by both methods. Regardless of pasteurization methods, the degradation of chlorophyll in green beans was faster at 10 °C as compared with at 2 °C storage. The considerable effect of storage temperature on the degradation of chlorophyll was confirmed by Manolopoulou and Varzakas (2016), who reported that the reduction in chlorophyll at temperatures of 10 and 20 °C is higher than that at 0 and 5 °C in broccoli and lettuce.

Chlorophyll degradation occurs as a result of thermal treatments due to chlorophyll's inherent sensitivity to heat (Petzold et al., 2014). According to Erge et al. (2008), the lowest degradation in chlorophyll occurs at 70 °C among temperatures ranging from 70 to 100 °C in a water bath. Chlorophyllase, the leading enzyme in chlorophyll degradation, losses its activity when heated to 100 °C (Erge et al., 2008), and can still act at temperatures between 65 and 75 °C and give rise to pheophorbides, which decrease green color (Aamir et al., 2014). Hence,

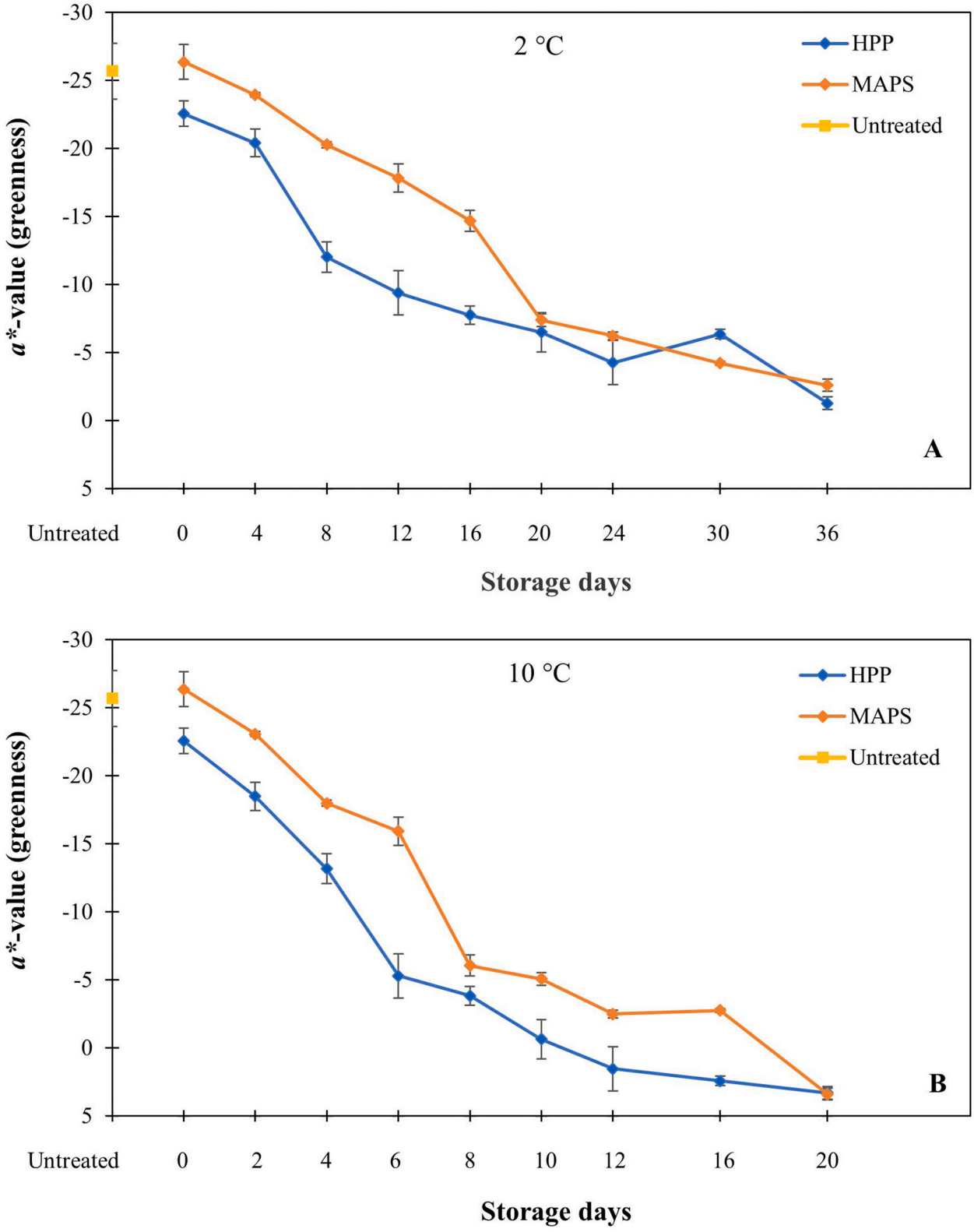

Fig. 4. Effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS processing ($P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min) on the a^* (greenness) values for green beans during storage at 2 °C over 36 days (**A**) and at 10 °C over 20 days (**B**). Data are the means ± SD (n=3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the degradation of chlorophyll might be attributed to the chlorophyllase activity because of low treatment temperatures in HPP and MAPS processes.

Van Loey et al. (1998) reported that pressures up to 800 MPa did not have a noticeable effect on chlorophyll degradation, and the reduction of chlorophyll became noticeable in combined pressure-temperature (>50 °C) treatments. The authors observed green color retention in broccoli juice treated by HPP (pressures combined with temperatures up

Table 2
Effect of storage on the color change of MAPS ($P^{7.5\,^{\circ}C}_{70\,^{\circ}C} = 3.2$ min) and high pressure (600 MPa for 10 min at 25 °C) processed green beans.

Treatment	Storage period at 2 °C									
	Frozen	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 30	Day 36
MAPS										
HPP										
Treatment	**Storage period at 10 °C**									
	Frozen	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 16	Day 20
MAPS										
HPP										

to 50 °C). The extreme stability of chlorophyll during pressure treatments at low temperatures can be attributed to the negligible compressibility of covalent bonds. Similar chlorophyll degradation in green beans pasteurized by HPP at 25 °C and MAPS at 70 °C might be due to the longer treatment time of HPP (10 min) as compared with that of MAPS (4.3 min).

3.2.3. Mechanical properties

The firmness values of the untreated and the pasteurized green beans during storage are shown in Table 3. No significant differences in firmness were obtained between the untreated and the pasteurized green beans by both treatments. Vervoort et al. (2012) and Knockaert et al. (2011) observed no significant differences in the firmness of carrot pieces between HPP (600 MPa for 10 min at 25 °C) and thermal pasteurization ($P_{70\,^{\circ}C} = 2$ min) after treatments. No significant difference was also observed between the green beans treated by MAPS and HPP right after the treatments. The retention in firmness might be due to the low-temperature exposure, which causes less β-elimination of pectin. Contrary to this, Vervoort et al. (2012) highlighted that a processing temperature lower than 80 °C is not adequate to initiate the β-eliminative depolymerization of pectin. Van Buggenhout et al. (2009) reported that no or limited β-elimination, consequently, prevent softening, which could occur under HPP conditions.

The firmness values of green beans after both pasteurization treatments did not change significantly over 30 days of storage at 2 °C. This retention in firmness during storage might be attributed to the addition of calcium chloride in the brine and the storage temperature. Calcium pectate occurs in the reaction of calcium with pectic acid in the cell wall, helps molecular bonding responsible for tissue firming (Sila et al., 2004). This observation is consistent with a study by Peng et al. (2017b). The retention of firmness in green beans processed by HPP (500 MPa for 1 min at 25 °C) during storage of 31 days at 6 °C was reported by Krebbers et al. (2002). No significant changes in firmness were detected for sous-vide cooked ($P_{70\,^{\circ}C} = 14$ min) green beans during storage of 25 days at 3 °C by Knøchel et al. (1997). The firmness of the HPP- and MAPS-treated green beans stored at 10 °C decreased after eight days. This texture loss might be caused by breaking down pectins occurring due to microbial growth (Giménez et al., 2003). The firmness of potatoes (cooked in boiling water for 10 min) decreased with an increase in storage temperature from 4 to 20 °C (Nourian et al., 2003).

3.2.4. Quantification of vitamin C

A significant amount of vitamin C was lost after the HPP and MAPS treatments (Fig. 8). The vitamin C content in the untreated green beans was 8.6 ± 0.6 mg/100 g wb (wet basis), but in the green beans after the HPP and MAPS treatments were 5.6 ± 0.3 mg/100 g wb and 3.3 ± 0.1 mg/100 g wb, respectively. The higher vitamin C loss in the HPP-treated samples could be due to the longer treatment time in HPP (10 min) than in MAPS (4.3 min). Similar results were found in tomato puree when processed by high-pressure or thermal treatments under the following conditions, thermal treatments at 70 °C for 30 s and 90 °C for 1 min retained vitamin C in tomato puree as similar as HPP under 400 MPa for 15 min at 25 °C (Sánchez-Moreno et al., 2006).

Further loses of vitamin C occurred during storage regardless of pasteurization methods. The HPP-treated green beans lost all vitamin C on the 8th day of storage at 2 °C, whereas at the same temperature, MAPS-treated samples retained 12.5% of the initial vitamin C. Almost all the vitamin C was lost in the green beans pasteurized by both pasteurization methods after 2 days of storage at 10 °C (data not shown). The degradation of vitamin C could be due to oxygen present in the headspace of the polymer pouch or permeation of oxygen through the polymer during storage (Patel et al., 2020; Zhang et al., 2019). The pouches used in the present study have PET and nylon as a barrier layer with an oxygen transmission rate < 1 $cm^3\ m^{-2}\ day^{-1}$. According to Sonar et al. (2020), 12–76% of vitamin C was lost in pasteurized mashed potatoes stored in different polymer pouches (oxygen transmission rate range = 1.7–111 $cm^3\ m^{-2}\ day^{-1}$) at 5 °C for 90 days. Therefore, vitamin C degradation highly depends on the total treatment time, oxygen level in the package, packaging material, food matrix, and storage temperature.

3.2.5. pH

The pH value of the green beans before the pasteurization treatments was 5.8 ± 0.2. The pH value of the green beans pasteurized by HPP was the same as that of the untreated samples, while the pH value of the MAPS-treated green beans was slightly lower (5.6 ± 0.1) without a

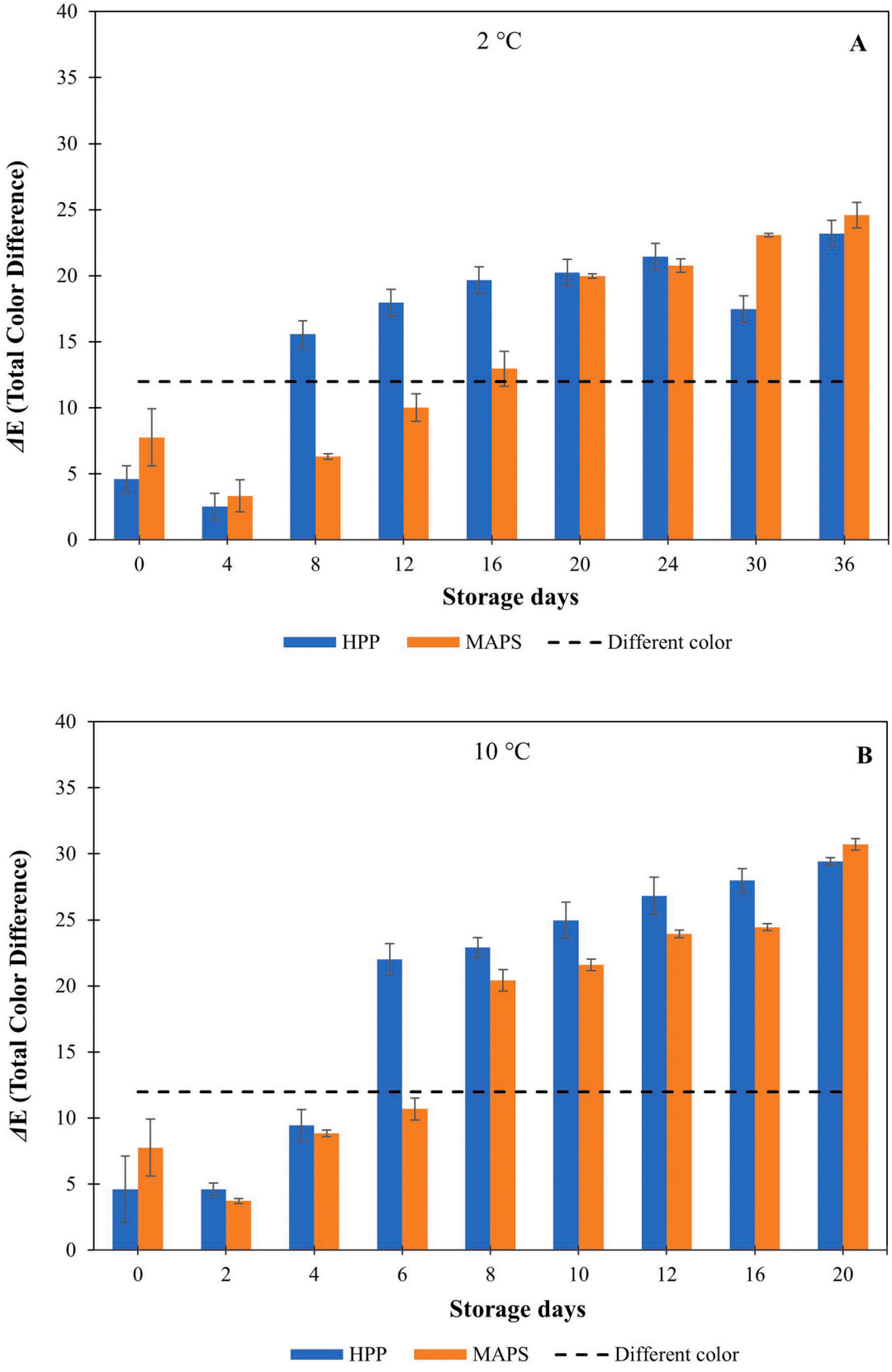

Fig. 5. Effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS processing ($P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min) on the ΔE (Total color difference) values in green beans during storage at 2 °C for 36 days (**A**) and at 10 °C for 20 days (**B**). Data are the means ± SD (n=3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

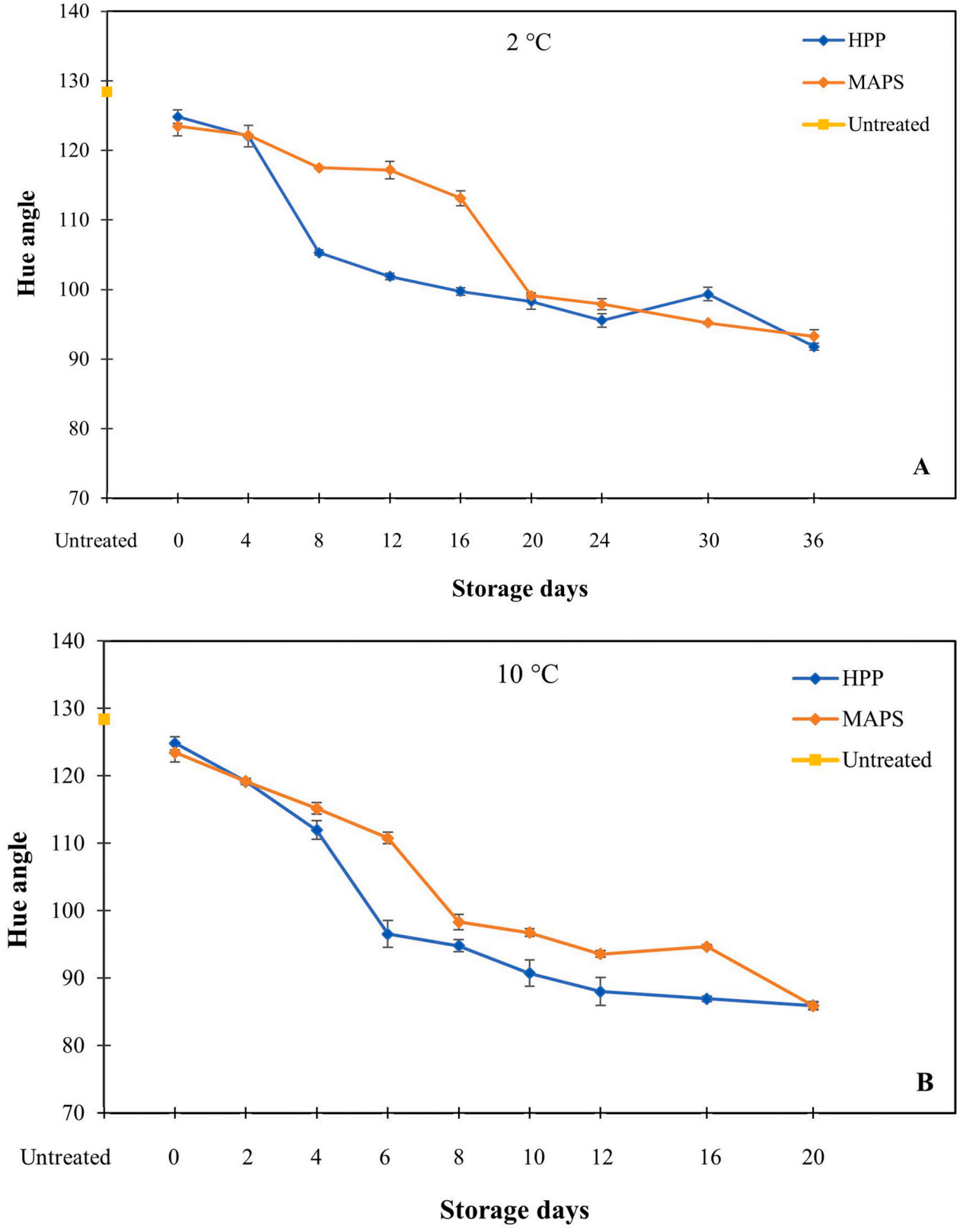

Fig. 6. Effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS processing ($P^{7.5\,^{\circ}C}_{70\,^{\circ}C} = 3.2$ min) on the hue angle of green beans during storage at 2 °C for 36 days (**A**) and at 10 °C for 20 days (**B**). Data are the means ± SD (n=3).

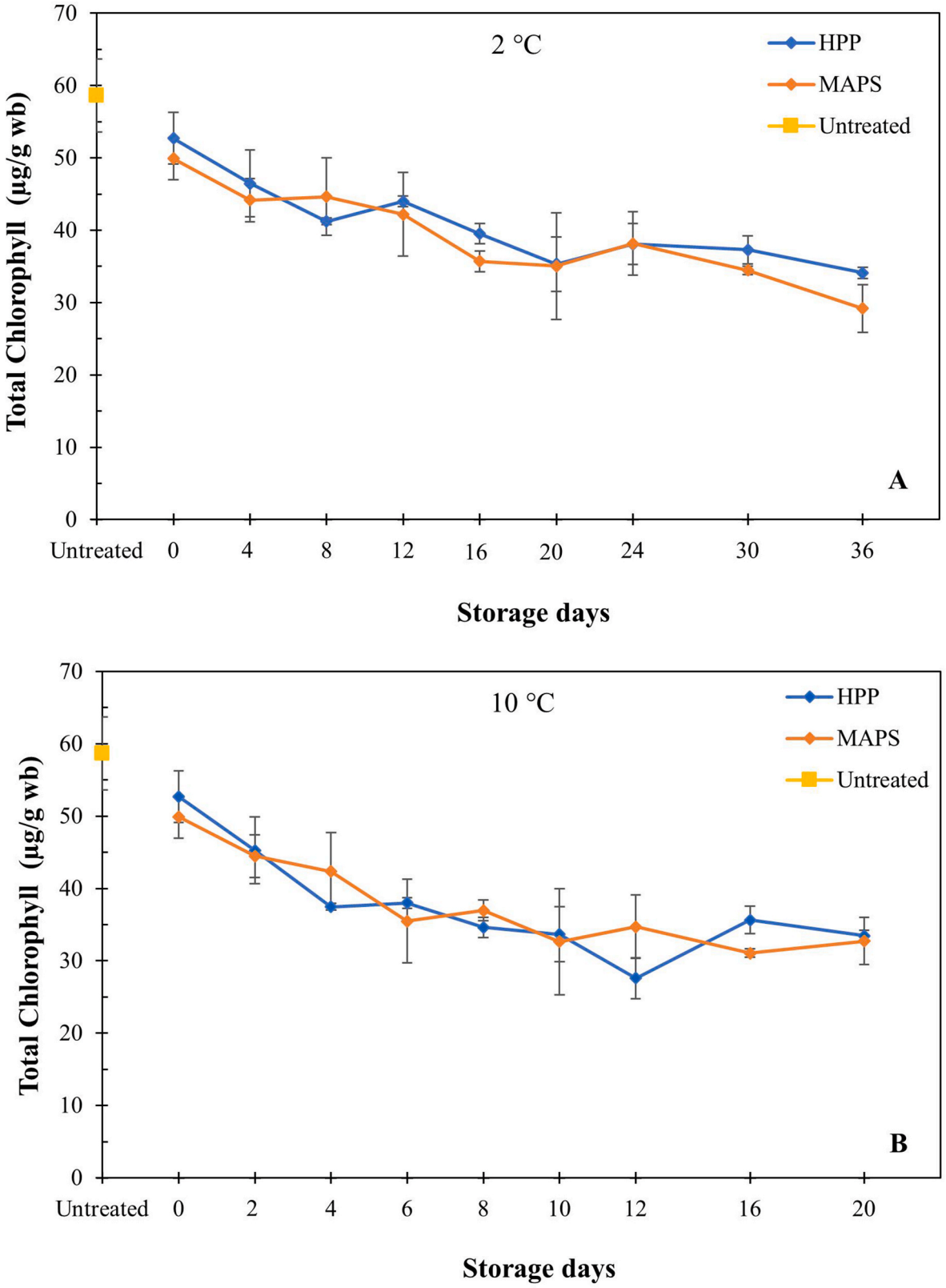

Fig. 7. Effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS processing ($P^{7.5\ °C}_{70\ °C} = 3.2$ min) on the chlorophyll content of green beans during storage at 2 °C for 36 days (**A**) and at 10 °C for 20 days (**B**). Data are the means ± SD (n=3).

Table 3
Effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS ($P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min) on firmness and pH of green beans during storage.

Storage period	Stored at 2 °C				Storage period	Stored at 10 °C			
	Treatments					Treatments			
	HPP		MAPS			HPP		MAPS	
	Texture	PH	Texture	pH		Texture	pH	Texture	pH
Frozen	24.2 ± 3.5^{A}	5.8 ± 0.2^{a}	24.2 ± 3.5^{A}	5.8 ± 0.2^{a}	Frozen	24.2 ± 3.5^{A}	5.8 ± 0.2^{a}	24.2 ± 3.5^{A}	5.8 ± 0.2^{a}
0	22.1 ± 2.5^{A}	5.8 ± 0.0^{a}	22.2 ± 2.4^{A}	5.6 ± 0.1^{a}	0	22.1 ± 2.5^{A}	5.8 ± 0.0^{a}	22.2 ± 2.4^{A}	5.6 ± 0.1^{a}
4	20.9 ± 2.4^{A}	6.0 ± 0.1^{a}	24.4 ± 3.0^{A}	6.0 ± 0.1^{a}	2	22.9 ± 2.6^{A}	5.8 ± 0.1^{a}	23.8 ± 2.8^{A}	5.7 ± 0.1^{a}
8	22.5 ± 3.3^{A}	6.1 ± 0.2^{a}	22.4 ± 3.1^{A}	5.9 ± 0.1^{a}	4	21.8 ± 3.0^{AB}	6.1 ± 0.1^{a}	24.9 ± 2.6^{A}	6.0 ± 0.0^{a}
12	21.6 ± 2.7^{A}	5.9 ± 0.0^{a}	21.4 ± 3.6^{A}	5.7 ± 0.1^{a}	6	21.3 ± 2.1^{AB}	6.0 ± 0.3^{a}	22.2 ± 3.4^{A}	6.0 ± 0.1^{a}
16	20.7 ± 4.2^{A}	5.8 ± 0.1^{a}	19.4 ± 5.0^{A}	5.9 ± 0.1^{a}	8	22.7 ± 3.7^{A}	5.5 ± 0.2^{a}	21.5 ± 4.0^{A}	5.5 ± 0.1^{a}
20	22.0 ± 3.7^{A}	5.9 ± 0.1^{a}	21.5 ± 3.4^{A}	5.8 ± 0.3^{a}	10	21.8 ± 2.6^{AB}	5.2 ± 0.2^{a}	19.9 ± 3.9^{AB}	5.0 ± 0.1^{a}
24	22.9 ± 1.7^{A}	5.9 ± 0.1^{a}	20.5 ± 4.8^{A}	5.9 ± 0.1^{a}	12	17.4 ± 5.1^{BC}	5.3 ± 0.5^{a}	17.1 ± 4.0^{BC}	5.8 ± 0.5^{a}
30	21.8 ± 2.9^{A}	5.8 ± 0.2^{a}	20.9 ± 4.6^{A}	5.4 ± 0.1^{a}	16	16.4 ± 3.6^{BC}	5.4 ± 0.8^{a}	16.7 ± 4.7^{BC}	5.8 ± 0.6^{a}
36	20.7 ± 3.0^{A}	5.4 ± 0.3^{a}	18.3 ± 3.9^{B}	5.7 ± 0.2^{a}	20	20.2 ± 4.4^{AB}	4.5 ± 0.5^{b}	14.1 ± 4.0^{C}	4.4 ± 0.1^{b}

Values with different superscript letters are significantly different ($p < 0.05$), between the means of each column within the same temperature.

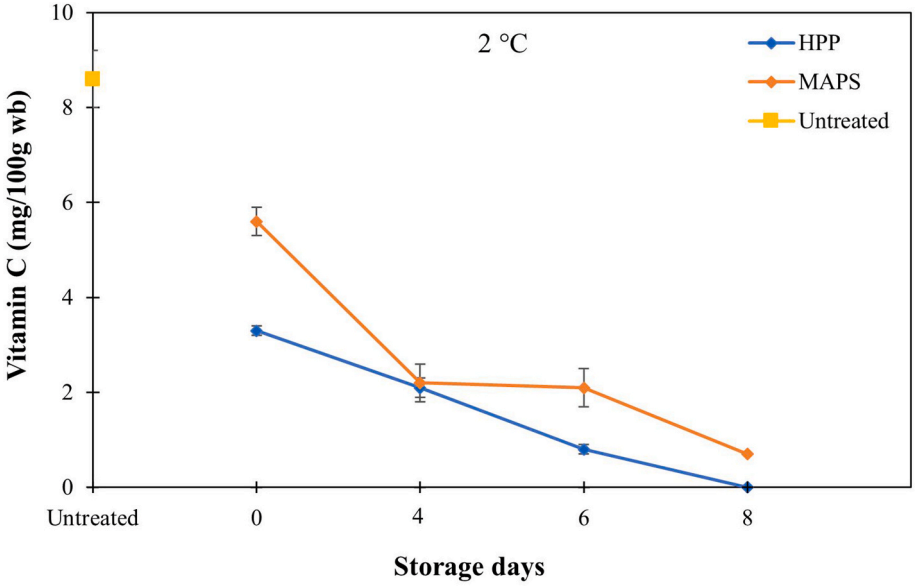

Fig. 8. Effect of high pressure (600 MPa for 10 min at 25 °C) and MAPS processing ($P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min) on vitamin C content of green beans during storage at 2 °C for 8 days. Data are the means ± SD (n=3).

significant difference (Table 3). Jacobo-Velázquez and Hernández--Brenes (2010) and Barba et al. (2010) have reported similar results for HPP-treated avocado puree and vegetable juices, respectively. However, storage temperature significantly influenced the pH of the pasteurized green beans during the storage. The pH of the pasteurized green beans stored at 2 °C was in the range of 5.4–6.0 and 5.4 to 6.1 for MAPS and HPP, respectively. However, the pH of the green beans significantly decreased to 4.4 (MAPS) and 4.5 (HPP) on storage day 20 at 10 °C ($p < 0.05$) meanwhile, the sample pouches were bulging, and the sample had putrid smell and discoloration. Microbial growth, mainly of acid-producing microorganisms, might be responsible for the decrease in pH and the undesirable appearance and smell. Zhao et al. (2013) observed a pH drop in cucumber juice treated by HPP and thermal pasteurization during 50 days of storage at 4 °C.

4. Conclusion

For the green beans tested in this study, the MAPS process ($P_{70\,^{\circ}C}^{7.5\,^{\circ}C} = 3.2$ min) is more effective than HPP (600 MPa for 10 min at 25 °C) in inactivation of *L. innocua* ATCC 51742. All the green beans pasteurized by HPP and MAPS had similar a^*, ΔE, and hue angle values at the end of the storage period for both storage temperatures (2 and 10 °C). A color change in the green beans from bright green to olive green/yellow was observed during storage regardless of the pasteurization methods. The chlorophyll content, texture, and pH of the pasteurized green beans were similar for both pasteurization treatments. After the pasteurization process, the vitamin C content of the green beans pasteurized by HPP decreased more than that of the green beans pasteurized by MAPS. The processing temperature of 70 °C for MAPS did not negatively affect the color and vitamin C content of the green beans after pasteurization.

This work shows that HPP and MAPS treatments at the processing condition used in this study had different efficacies against *L. innocua* ATCC 51742 in green beans. The impacts of HPP and MAPS on the quality of the green beans were similar. Both HPP and MAPS offer advantages to food companies as a pasteurization method for preserving green beans.

Declaration of competing interest

None.

CRediT authorship contribution statement

Sumeyye Inanoglu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Gustavo V. Barbosa-Cánovas:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision. **Juhi Patel:** Methodology, Data curation, Writing - original draft. **Mei-Jun Zhu:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Shyam S. Sablani:** Conceptualization, Writing - review & editing, Supervision. **Frank Liu:** Conceptualization, Methodology, Data curation. **Zhongwei Tang:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Juming Tang:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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