Food Control 114 (2020) 107233

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

High pressure-assisted thermal sterilization of low-acid fruit and vegetable purees: Microbial safety, nutrient, quality, and packaging evaluation

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ARTICLE INFO

Keywords:
High pressure-assisted thermal sterilization
Bacillus amyloliquefaciens
Total plate counts
Ascorbic acid
β-carotene
Chlorophyll
Betalains
And anthocyanin

ABSTRACT

The objective of this study was to assess the influence of pressure-assisted thermal sterilization (PATS) on nutrients and quality of pumpkin, butternut squash, pea, beetroot, and purple potato purees. PATS processing parameters included preheating of purees at 98 °C for 5 min and pressurizing at 600 MPa in a vessel set at 90 °C for 5 min. Colorimetric temperature sensors, two types of *Bacillus amyloliquefaciens* spores, total aerobic and anaerobic plate count, HPLC, as well as spectrometer and spectrophotometer were utilized. The colorimetric temperature sensor based on its color value when extrapolated suggested the purees' temperature reached 122 °C. Thus, at least 9-$\log_{10}$ reduction of *B. amyloliquefaciens* spores was achieved in a purple potato puree, and no detectable aerobic and anaerobic microorganisms were found after PATS in all purees. Vitamin C showed 3–14% reduction after PATS. Total chlorophyll and β-carotene did not vary significantly ($p > 0.05$) after PATS, while betalains and anthocyanin were sensitive and decreased significantly. Interestingly, total color change (ΔE) of pumpkin, butternut squash, and beetroot purees ranged between 2.5 and 3.4 below the threshold ($\Delta E = 6$) value that can be observed by the naked eyes. EVOH-based packages did not show any visual deformation, but their oxygen and water vapor transmission rates increased significantly ($p < 0.05$) after PATS. The present research suggests that PATS can be used to produce safe and high-quality shelf-stable fruit and vegetable products.

1. Introduction

High hydrostatic pressure (HPP) processing is a growing technology with a projection of about $55 billion in global food market sales by 2025; more than 300 industrial machine sales took place in 2015 alone (Huang, Wu, Lu, Shyu, & Wang, 2017). The HPP success and popularity can be attributed to the food quality retention, short processing time, and easy access to the machine (Balasubramaniam, Barbosa-Cánovas, & Lelieveld, 2016). The HPP food market statistics showed that 25% of sales were for meat products, 20% for vegetables, 20% for juices and beverages, 5% for seafood, and 30% for toll processing and other food categories in 2014–2015, including ready-to-eat meals (Huang et al., 2017).

High hydrostatic pressure-assisted thermal sterilization (PATS) is still a developing technology for sterilization of shelf-stable prepackaged low-acid foods (pH > 4.6) (U.S. Food and Drug Administration, 2018). PATS offers the advantage of short processing time because of the adiabatic compression heat, which is generated upon applying pressure. Additionally, prepacked food has minimal possibilities of recontamination. This technology has the potential to produce shelf-stable foods with minimal deterioration in food nutrients and quality, unlike conventional thermal processes (Holdsworth & Simpson, 2016). The synergistic effect of high pressure and high temperature helps to minimize the processing time, the effect on food quality, and inactivate microorganisms of concern. In February 2009, the U.S. Food and Drug Administration (FDA) accepted sterilization of shelf-stable low-acid food using PATS (Stewart, Dunne, & Keener, 2016) at set conditions including an initial temperature of 90 °C, pressure 690 MPa and holding time of 3 min. The packages were inoculated with *Clostridium botulinum* spores, and the inactivation attained was 6 $\log_{10}$ (Stewart et al., 2016). Later, the Institute for Food Safety and Health (IFSH) at Chicago, IL, obtained a second FDA acceptance for pressure enhanced sterilization

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https://doi.org/10.1016/j.foodcont.2020.107233
Received 2 December 2019; Received in revised form 9 March 2020; Accepted 11 March 2020
Available online 14 March 2020
0956-7135/

(PES) in 2015 (IFSH, 2015).

PATS mainly relies on high temperature, specifically 120 °C, to inactivate spores. Furthermore, the continued effort showed that sterilization can be achieved with a low starting temperature (approximately 90 °C) by counting the pressure > 600 MPa effect (Margosch, Ehrmann, Gänzle, & Vogel, 2004; Mills, Earnshaw, & Patterson, 1998). Inactivation of 6 $\log_{10}$ of *C. botulinum* (TMW 2.357) proteolytic type B was achieved for different types of foods with some tailing effect (Maier, Lenz, & Vogel, 2017). The adiabatic heat of high pressure can increase the temperature by 3, 3.7, 4.8, or 5 °C/100 MPa depending upon the initial water temperatures such as 25, 50, 60, and 90 °C, respectively (Otero & Sanz, 2003). Food products with 70% moisture content have a comparable elevated temperature profile to water (Buzrul, Alpas, Largeteau, Bozoglu, & Demazeau, 2008), and food containing fat content can increase the temperature up to 10 °C per 100 MPa. The combination of high pressure > 600 MPa and starting temperature of 120 °C may be overprocessing, disregarding the adiabatic heat and pressure effect. Thus, low initial temperature 90 °C can achieve the same lethality with the aid of pressure and taking into account the adiabatic heat of food products (Wilson, Dabrowski, Stringer, Moezelaar, & Brocklehurst, 2008). In general, high-pressure pasteurization shows superior quality for various foods (Mussa & Ramaswamy, 1997). It already has existing marketed products, but high pressure-assisted thermal sterilization is not well studied in terms of retaining quality, pH, nutrients, sensorial attributes, and package barrier properties.

Fruits and vegetables are the heart of a meal; however, they come in different forms and are produced by various processes. Pureed forms of fruits and vegetables can be utilized as main food ingredients for immediate consumption, such as baby or elderly foods that have higher market value for the convenience and comfort compared to fresh produce of the same. Overall, processed prepackaged food is meant to deliver safe, nutritious, and high-quality products (Holdsworth & Simpson, 2016). Carrot, pea, pumpkin, and butternut squash purees are among the popular purees marketed by large quantities from global companies. These purees are often thermally sterilized using traditional thermal sterilization processes. During thermal sterilization, several heat-sensitive nutrients, such as vitamin C, are significantly decreased due to the excessive and long applied heat (Zhang et al., 2019). Also, quality decay is possible, including color and texture changes (Patel et al., 2019). Natural color pigments also could be affected by heat because they have complex chemical structures that give each product its unique color. For instance, β-carotene comes from a carotenoid family and gives pumpkin and butternut squash their yellow-orange coloration. Chlorophylls are responsible for the green color of plants and specifically green products. Betalains come in red and yellow colors that are water-soluble and known for antioxidant properties. Anthocyanin is one of the unique pigments of many fruits and vegetables, giving them a purple color (Rodriguez-Amaya, 2019a; 2019b). PATS may help in preserving the most wanted food qualities like color and important nutrients. Nevertheless, the research concerning the influence of PATS on food quality and nutrients is limited (Lau & Turek, 2007).

To address this knowledge gap, the objective of this study was to examine the influence of PATS on selected fruit and vegetable purees fortified with vitamin C. The combined effect of high pressure and high temperature was investigated on five homogeneous purees in flexible packaging: pumpkins, butternut squash, peas, beetroots, and purple potatoes. Each puree carries a different predominant color pigment for checking the suitability of various foods for the PATS process. Pumpkin and butternut squash were selected because of the β-carotene domination as a pigment in their composition; peas were chosen because they are rich in chlorophylls, beetroots are rich in betalains, and finally, purple potatoes contain a substantial amount of anthocyanin. These fruits and vegetables are rich in natural color pigments that may be sensitive to high temperatures. A quantitative understanding of the effect of PATS on natural color pigments will help develop PATS fruits and vegetable products. Paper-based colorimetric temperature measurement, microbial validation, aerobic and anaerobic plate counts, vitamin C, color pigments quantification, pH, and instrumental color analyses were carried out to reveal PATS potential. To the best of the authors' knowledge, a limited number of studies have reported the influence of PATS on the physicochemical properties of selected food purees.

2. Materials and methods

2.1. Puree preparation and processing

Fresh pumpkin, butternut squash, beetroots, purple potatoes, and frozen peas were purchased from local markets Wal-mart and Safeway (Pullman, WA). Fruit and vegetable choices were based on their diverse composition and sensitivity to the process. Five-hundred grams of pumpkin, butternut squash, peas, beetroots, and purple potatoes were cut into small cubes 0.02 m (L × W × H) and blanched in saturated steam at 100 °C for 5 min for inactivation of enzymes and partial cooking. The cut fruits and vegetables (500 g) were added to the blender with 500 mL of purified water and blended for 2 min (set as a puree) using a blender (Kitchenaid®, Benton Harbor, MI) with an approximate speed of 3000 round per minute (rpm). Crystallite vitamin C powder (ascorbic acid) 0.5 g/L (w/v) was added into the purees and additionally stirred for 30 s. This amount was carefully chosen not to increase the puree acidity and give the daily recommended dose for adults 75–120 mg/day and children 15–50 mg/day, according to the U.S. National Institutes of Health (U.S. Department of Health & Human Services, 2018). Aluminum foil- and two newly designed multilayer EVOH-based packages for high-pressure processing applications with high barrier properties were prepared and tailored into 0.08 m × 0.10 m (L × W) pouches and filled with 40 g of each puree. The filled packages were vacuumed and hermetically sealed using an Easy-Pack vacuum sealer (UltraSource. LLC. Kansas, MO). EVOH-based multilayer film rolls were provided by Novolex, WA, and Kuraray America, Inc. Houston, TX. Packages of fruit and vegetable purees were preheated at 98 °C for 5 min fully immersed in a water kettle and monitored with thermocouples inserted in the cold spot inside the package. The come-up time for puree temperature to reach 98 °C was approximately 3 min. Immediately after preheating, the packages were then transferred to a high-pressure machine with 2-L vessel capacity (Engineered Pressure Systems, Inc. Haverhill, MA). Three packages of each puree were prepared ($n = 3$).

The high-pressure vessel was preheated and set at its maximum temperature and pressure 90 °C and 600 MPa for 5 min processing time. The pressure medium was 10% Hydrolubric 123-B aqueous solution. The temperature inside the processing vessel was recorded using three fixed (type K) thermocouples (Omega Engineering Inc., Stamford, CT) at different locations in the top part of the vessel. A cylindrical polyoxymethylene-based insulator (liner) was used to reduce the heat transfer to the minimum as described elsewhere (Al-Ghamdi, Rasco, Tang, Barbosa-Cánovas, & Sablani, 2019).

The following equation can calculate the adiabatic heat of purees $\frac{\Delta T}{\Delta P} = \frac{T \times \alpha}{\rho \times C_p}$, where α is volumetric thermal expansion coefficient (K^{-1}), ρ is the density, T is the initial temperature (K), P is pressure (Pa), and C_p is the specific heat at the initial temperature (J/kg. K). By knowing that the puree has about 94% water content, the adiabatic heat can be calculated for water. The adiabatic heat was about 5 °C/100 MPa when the starting temperature is 90 °C. Preheating of the purees before processing was to ensure full utilization of the adiabatic heat induced by high pressure and precaution of heat loss during package transfer and processing. Thus, 98 °C (i.e., boiling temperature without countered pressure) was selected for the purees as initial product temperature. This was sufficient preheating to elevate the puree temperature more

than 120 °C or 5 °C × 6 (600 MPa), which would be 128 °C without the heat loss (Grauwet, der Plancken, Vervoort, Hendrickx, & Loey, 2016). The samples after PATS were cooled using water at room temperature (23 °C) and kept overnight at room temperature for additional analyses.

An independent colorimetric paper-based temperature sensor was introduced into the high-pressure vessel and puree packages to record their temperatures since the vessel thermocouples were in a fixed place and cannot measure the puree's temperature. A Thermex temperature-indicating sensor was cut into small stripes 0.01 m × 0.02 m and protected by multilayer transparent film. This sensor has sensing ability from 90 to 150 °C and a response time of 0.1 s (Sensor Products, Inc., NJ). The sensors change color from white to gray/black as a response to the temperature intensity, with a higher temperature leading to a darker sensor color. To build a calibration curve of temperature vs. lightness of the sensor, the pressure and process time were fixed at 600 MPa and 5 min holding time. Next, the initial vessel temperature was set at 70, 75, 80, 85, and 90 °C to check the sensor response. Two stripes were introduced into the vessel in each run, and the maximum vessel temperature was recorded as read by three fixed thermocouples in the vessel. Finally, sensors' lightness at each temperature was correlated with temperature. After establishing the calibration curve of the vessel temperature, the sensors were introduced into the food in the geometric center of the package. Lightness (L^*) of the sensor was measured using a spectrophotometer, as detailed in the following section.

2.2. Microbial validation and total plate counts

The sterilization validation of the process was carried out using two types of strains *Bacillus amyloliquefaciens* (Fad 82 and 11/2) following the exact methods of (Ahn & Balasubramaniam, 2007; Margosch et al., 2004; Rajan, Ahn, Balasubramaniam, & Yousef, 2006). *B. amyloliquefaciens* strains were obtained from Dr. Michael Gänzle, University of Alberta. Vegetative cells were received at 100 μL/L concentration, and 20 mg were diluted in 5 mL of tryptic soy broth with 0.1% yeast extract overnight (12–18 h). The incubation was in a shaker with its speed set at 200 rpm and temperature at 37 °C. The cells were streaked on 25 mL tryptic soy agar (TSA, Becton Dickinson Corp., Sparks, MD) plate and 10 mg/L (ppm) $MnSO_4$ (Sigma-Aldrich Co., MO). The plates were incubated at 32 °C for 10–15 days. The plates were then flooded with 10 mL of sterile distilled water and the agar surface was scraped. The collected suspension and water were centrifuged at 3000 × *g* using AccuSpin 400 (Fisher Scientific, Pittsburgh, PA) and washed 3 times with 10 mL of sterile distilled water. The pellet of centrifugation of the bacteria was diluted in 5 mL of sterile distilled water. The suspension of bacteria was pasteurized at 80 °C for 10 min to eliminate vegetative cells in 50 mL polypropylene tubes. The initial spores count was 9.9 $\log_{10}$ CFU/mL for both strains Fad 82 and Fad 11/2. The suspension (1 mL) was inoculated in 1 g of sterilized purple mashed potatoes (highest pH = 6.24) in a small plastic bag 0.02 × 0.04 m that was thoroughly mixed, sealed, and placed in 40 g of sterilized purple potatoes puree and packaged in a larger package as specified above 0.08 m × 0.10 m. The inoculated 1 g of purple potatoes puree was placed in the center in the larger package containing 40 g sterilized purple potatoes puree. Inactivation was done for the preheating step and combined preheating and PATS together. At least three independent runs were performed. An inoculated sample containing a mixture of 1 g of purple potatoes puree and 1 mL of bacteria suspension that went through PATS was serially diluted 1 mL in peptone water 9 mL up to 8 times. The liquid portion (0.1 ml) was extracted and placed to a Petri dish, to which melted TSA (25 mL) was poured. The plates were incubated aerobically at 37 °C (n = 3). The lower detection limit of the enumeration method was 1 $\log_{10}$ CFU/g.

For further assurance, aerobic and anaerobic plate count was conducted from unprocessed and processed purees. First, the media was prepared using tryptic soy agar (Becton, Spark, MD). Forty grams of the agar were added to 1 L purified water and diluted thoroughly for 10–15 min. The media was autoclaved at 120 °C for 20 min. A dilute of 15 g of peptone powder (Sigma-Aldrich Co., MO) in 1 L of purified water was also autoclaved at 120 °C for 20 min. The purees as unprocessed and processed were extracted and diluted in peptone water. The dilution was in a plastic stomacher bag, taking 9 g of puree to 90 mL of peptone water. A pour plate method was followed where 0.1 ml of the above mixture was taken into a Petri plate and then 25 mL of the media, set at 48 °C in a water bath, was added. All plates were allowed to cool in a sterile environment and placed in a closed chamber with anaerobic sachets GasPak. Plates with anaerobic sachets were labeled as anaerobic plates, whereas plates set in the open incubator were labeled as aerobic. All aerobic and anaerobic plates were placed in a 37 °C incubator for 48 h (Sonar, Rasco, Tang, & Sablani, 2019). Unopened processed packages of purees were kept at 37 °C for a year to ensure proper inactivation and no inflation would occur, whereas unprocessed packages of butternut squash purees bulged in 1 day as demonstrated in the pictures in the supplementary material (see Fig. S1). Two samples were drawn from 3 packages with a total sampling of 6 before and 6 after PATS (n = 6).

2.3. Ascorbic acid quantification

High-performance liquid chromatography (HPLC) was used to quantify the ascorbic acid content in all purees. Five g of puree was extracted in 50 mL tube and homogenized with 15 mL of meta-phosphoric acid for 1 min at 7000 rpm using a Polytron homogenizer model PT 2500E (Kinematica, Bohemia, NY). The solution was then left for 2–3 h at 23 °C to extract. The solution was centrifuged at 5000 × *g* for 6 min using an AccuSpin 400 (Fisher Scientific, Pittsburgh, PA) then filtered through a 0.45 μm filter. Ten μL of the filtered extract was injected to Agilent 1100 HPLC (Agilent Technology, Santa Clara, CA) equipped with a diode array detector RP18, 5 μm, and 4.6 × 250 mm column. The flow rate was set at 0.5 mL/min, column temperature was set at 25 °C, and the vitamin C retention time was about 7 min. A calibration curve was previously constructed of the ascorbic acid quantity versus peak area (Zhang et al., 2019). Three samples were drawn from different packages (n = 3).

2.4. Color pigments quantification and pH

The quantification of natural color pigments was performed following the methods of Zhang et al. (2019) and Sonar, Paccola, et al. (2019) and Sonar, Rasco, et al. (2019). A simple approach using Ultraspec 4000 spectrometer (Pharmacia Biotech Inc., Piscataway, NJ) was utilized. Since it is a comparative study between unprocessed and processed purees, the spectrometer method was considered sufficient, as have been demonstrated in previous studies (Sonar, Paccola, et al., 2019; Zhang et al., 2019). Each color pigment extraction and measurement explained in detail next (n = 3). All readings were compared against the same extraction solvent without the food product as a blank reading.

2.4.1. Beta-carotene

The total beta-carotene was extracted using 5 g of the pumpkin and butternut squash samples in 20 mL of an organic solvent consisting of 50% of hexane, 25% acetone, 25% of ethanol and 0.1% of butylated hydroxytoluene w/v. The mixture was homogenized at 7000 rpm using Polytron PT 2500E (Bohemia, NY), filtered using Whatman filters No.1 and the remaining residuals were collected again. Another 10 mL of the organic solvent was added remaining solids and homogenized once again for 1 min and filtered. The collected solvent was placed in a separation funnel. The separated yellow part was collected and diluted (1:1 v/v) with the same extraction solvent that also served as blank for the spectrometer measurement at 450 nm wavelength. Carotenoid content was calculated using equation (1) where A is the absorbent

area, 2560 is the extinction coefficient of β-carotene:

$$Carotenoid\ content\left(\frac{\mu g}{g}\right) = \frac{A \times extraction\ volume\ (mL) \times 10^4}{2560 \times Sample\ weight\ (g)} \quad (1)$$

2.4.2. Chlorophylls

Chlorophylls extraction was done using 5 g of peas puree before and after processing. The extraction solvent consisted of distilled water with 80% acetone (v/v), and 25 mL was added to the 5 g puree in 50 mL PP tubes. The mixture was homogenized using at 7000 rpm for 5 min, shook at 300 rpm for 30 min, and centrifuged at 3000 × *g* for another 5 min all at the room temperature (23 °C). The remaining supernatant was collected and diluted up to 25 mL in a 25 mL flask. Absorbent wavelength area (A) in the spectrometer was measured at 663 and 647 nm. The following equations (2)–(4) were used to calculated chlorophylls.

$$Chlorophyll\ a\left(\frac{\mu g}{g}\right) = 12.25 \times A_{663} - 2.79 \times A_{647}\left(\frac{mL\ ofextract}{weight\ of\ sample}\right) \quad (2)$$

$$Chlorophyll\ b\left(\frac{\mu g}{g}\right) = 21.50 \times A_{647} - 5.10 \times A_{663}\left(\frac{mL\ ofextract}{weight\ of\ sample}\right) \quad (3)$$

$$Total\ chlorophyll\left(\frac{\mu g}{g}\right) = 7.15 \times A_{663} - 18.71 \times A_{647}\left(\frac{mL\ ofextract}{weight\ of\ sample}\right) \quad (4)$$

2.4.3. Betalains

Five g of beets puree was taken for extraction and homogenized using 15 mL of distilled water at 7000 rpm for 3 min. The mixture was filtered using Whatman No. 1 and the remaining solids were filtered again at 7000 rpm for 1 min. The filtered homogenate was then diluted into 50 mL of distilled water. The betalains consisted of two betacyanin and betaxanthin that were calculated using the absorbent area under the curve $A = A_{536} - A_{650}$ for betacyanin or $A_{485} - A_{650}$ for betacyanins.

$$Betacyanin\ or\ betaxanthin\left(\frac{mg}{L}\right) = \left(\frac{A \times M_w \times DF \times 1000}{\varepsilon \times l}\right) \quad (5)$$

where M_w is the molecular weight of betacyanins and betaxanthins in H_2O are 550 and 339 g/mol, respectively, *DF* is the dilution factor, l is path length (1.25 cm), and ε is the molar extinction coefficients of betacyanins and betaxanthins in H_2O are 60000 and 48000 L/mol.cm, respectively.

2.4.4. Anthocyanins

Similarly, the anthocyanins extraction involved 5 g of the purple mashed potatoes puree in 10 mL of methanol and 0.1% hydrogen chloride (HCL). The acidified solvent was homogenized at 7000 rpm from 5 min with 5 g puree and filtered. Then the solids were collected and homogenized two times again at the same speed and time. The filtered mixture was then diluted by (1:1 v/v ratio) in 0.025 mol/L potassium chloride (KCl) buffer (adjusted to pH 1.0) and 0.4 mol/L sodium acetate ($C_2H_3NaO_2$) buffer (adjusted to pH 4.5).

$$Anthocyanins\left(\frac{mg}{L}\right) = \left(\frac{A \times M_w \times DF \times 1000}{\varepsilon \times l}\right) \quad (6)$$

where $A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$, ($M_w$) is the molecular weight = 449.2 g/mol for cyanidin-3-glucoside, *DF* is the dilution factor; l is path length (1.25 cm) and ε is the molar extinction coefficient = 26900 L/cm.mol.

2.4.5. pH

The pH value was taken by dipping the electrode into 30 mL sample of puree using a pH meter (Seven Go SG2, Mettler Toledo, Schwerzenbach, Switzerland). The pH meter was calibrated at 4, 7, and 10 pH before each use. The sampling was in triplicates from different packages ($n = 3$).

2.5. Color analysis

The instrumental color of pumpkin, butternut squash, pea, beetroot, and purple potato purees was measured using a CM-5 spectrophotometer (Minolta CR 200, Konica Sensing America Inc., Ramsey, NJ). The result was reported based on the International Commission on Illumination (CIE) color's system (*L* Darkness to Lightness, a* Red to Green, b* Yellow to Blue*) and the color difference calculated using ($\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$). Three samples from different packages were taken comparing before and after PATS purees ($n = 3$). For apparent visual observation, images were taken using a DSLR camera stationary (EOS 60D, Canon Inc., Tokyo, Japan) equipped with an EF-S, 100 mm, f/4.5 lens (Canon Inc., Tokyo, Japan). The following camera settings were used: aperture = 4.5 mm, shutter speed = 1/50 s, view angle = 100 mm, no flash and autofocus. To eliminate natural variation, no natural lights were permitted.

2.6. Oxygen and water vapor transmission rates of EVOH-based multilayer films

Combined high pressure and high temperature effect on the gas barrier properties of packaging films is important to the shelf life of processed products. For two 7-layer EVOH-based films, we measured the oxygen transmission rate (OTR) according to ASTM D3985 standard using Ox-Tran 2/21 MH (Minneapolis, MN) and water vapor transmission rate (WVTR) according to ASTM F 372-99 using Permatran 3/33 (Minneapolis, MN). The films' thickness was measured using an electronic disc micrometer (model 15769, Flexbar Machine Co. Islandia, NY) ($n = 5$).

2.7. Statistical analysis

The data were analyzed using JMP version 14 (SAS Institute Inc., Cary, NC). Tukey's Honestly Significant Difference (HSD) test was conducted to determine the significant difference between the observations' means at a 95% confidence interval, $\alpha = 0.05$.

3. Results & discussion

3.1. Pressure and temperature profiles

Fig. 1 shows the pressure and temperature profiles of PATS used in this study. The adiabatic heat helped to raise the vessel temperature from 90 °C to 115 °C as recorded by type (K) thermocouples inside the vessel. Similar trends were observed by others (Al-Ghamdi et al., 2019; Dhawan et al., 2014; Rasanayagam et al., 2003). Due to the heat loss, the vessel temperature did not reach 120 °C even though there was an insulator installed in the vessel. Gradually, after applying high pressure (600 MPa) the vessel reached 115 °C. However, during the holding time, there was loss of heat until the vessel temperature reached 100 °C after 5 min of holding time. Fig. 2 shows the colorimetric sensor calibration curve with sensors' images. The sensor introduced to the food package showed a darker intensity (far right image). By taking the lightness of the sensor and extending the linear relationship, the food temperature has likely reached higher temperatures than the vessel, and it was extrapolated to be specifically 122 °C. The sensor gave a higher intensity of black/gray color inside the package compared to the one in the vessel, which may indicate a higher temperature in the food. This was generated from the adiabatic heating, as observed previously (Balasubramanian & Balasubramaniam, 2003).

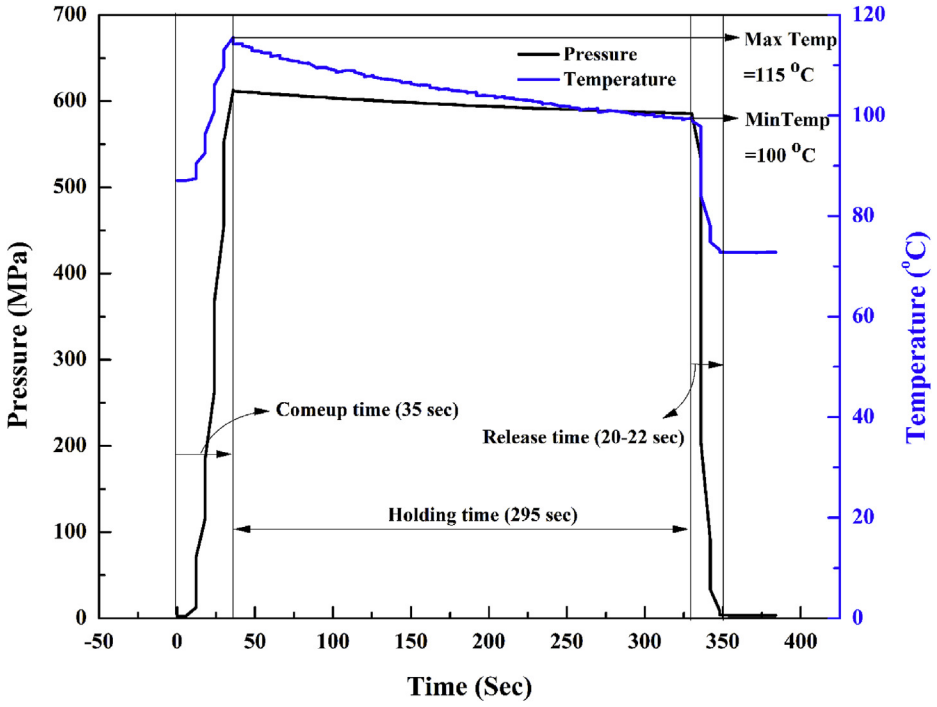

Fig. 1. PATS profile illustrating the pressure and temperature as measured inside the high hydrostatic pressure vessel.

3.2. Microbial validation and total plate counts

The initial inoculum population was 9.6 $\log_{10}$ CFU/g of sample for Fad 82 and 9.4 $\log_{10}$ CFU/g of sample for Fad 11/2. PATS completely inactivated the initial microbial load of *B. amyloliquefaciens* Fad 82 and 11/2, and no colonies were detected. This indicated at least 9.6 and 9.3 $\log_{10}$ CFU/g reduction was achieved after PATS process for Fad 82 and 11/2, respectively. Since *B. amyloliquefaciens* Fad 82 has decimal reduction time D = 0.25 min at 121 °C at 1 atm (Rajan, Ahn, Balasubramaniam, & Yousef, 2006) and *Clostridium botulinum* has D = 0.20 min at 121 °C (Margosch et al., 2006), the PATS process used in this study was sufficient to reduce the initial microbial population

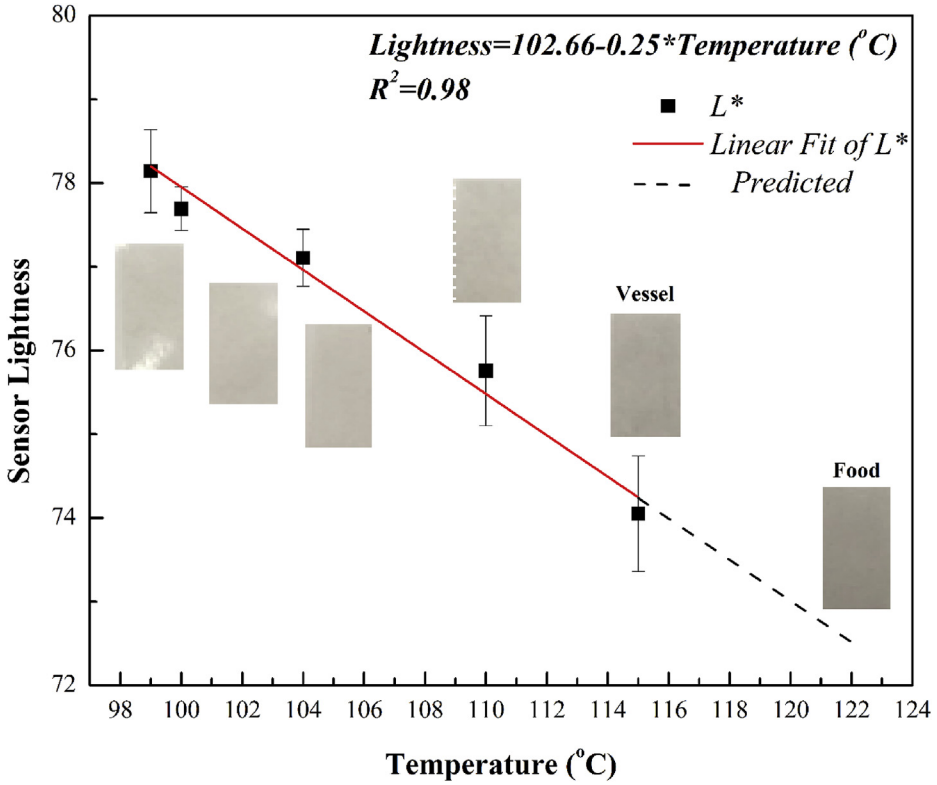

Fig. 2. Sensor color intensity vs. temperature as recorded by thermocouples inside the vessel and sensor color intensity inside the food (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

count by > 9 $\log_{10}$ CFU/g of purple mashed potatoes puree. *B. amyloliquefaciens* is more thermally resistant compared to *C. botulinum* (Margosch et al., 2006; Rajan, Ahn, Balasubramaniam, & Yousef, 2006). Interestingly, the preheating step alone at 98 °C for 5 min was able to reduce the initial population by 3 and 2 $\log_{10}$ CFU/g sample for Fad 82 and 11/2, respectively. This heat inactivation during the preheating was similar to that of *C. botulinum* TMW 2.357 but different than the *B. amyloliquefaciens* TMW 2.479, as reported by Margosch et al. (2006). The differences in food matrices and pH may influence the surrogates' survival. The FDA requirement is at least a 6-$\log_{10}$ reduction of *C. botulinum* during PATS (Acidified & Low-Acid Canned Foods Guidance Documents & Regulatory Information, 2018; Stewart et al., 2016). The first FDA filing for pressure-assisted thermal sterilization achieved 6 $\log_{10}$ reduction of *C. botulinum* that was considered sterilization (Stewart et al., 2016). Also, Sevenich et al. (2014) stated that commercial sterility, i.e. 12-$\log_{10}$ reduction of *B. amyloliquefaciens* TMW 2.479, is theoretically feasible with temperature ranging from 107 to 115 °C using 600 MPa pressure for 9.80 min and 0.45 min, respectively. Due to the heat loss from the high-pressure vessel during processing, microbial death kinetics cannot be obtained based on non-isothermal processing. Other studies have also shown full inactivation of *B. amyloliquefaciens,* as summarized in Table 1. The higher the initial temperature, the higher is the inactivation, but once the temperature reaches 120 °C the surrogate tends to be affected more by thermal stress. An attempt to start with lower initial temperatures (70 and 80 °C) has been made, but this showed some tailing effect. An additional measure was taken, and total plate count was done to estimate the microbial safety of the processed purees. Table 2 shows that the aerobic and anaerobic colonies count before and after PATS. All processed fruit and vegetable purees tested below the detection limits (1 $\log_{10}$ CFU/g) for aerobic and anaerobic microorganisms. For blanched purees that were not PATS processed, the initial aerobic plate counts were higher than anaerobic counts, and this could be because of the initial natural load and varieties of microorganisms considering different sources of these fruits and vegetables.

Researchers have demonstrated that the highest inactivation of proteolytic type-B *Clostridium botulinum* TMW 2.357 min surrogate was approximately 6 $\log_{10}$ cycle reduction, which was observed in braised veal at 600 MPa and 110 °C for 5 min without preheating (Maier et al., 2017). Four types of foods, including green peas with ham, steamed sole, braised veal, and vegetable soup, were examined in high pressure-assisted thermal sterilization. The products were not preheated but achieved 5 to 6 log cycle reduction of proteolytic type-B*Clostridium botulinum* at different pressures (e.g., 300, 450, and 600 MPa) and temperature combination 100–110 °C (Maier et al., 2017). Margosch et al. (2006) described that the high pressure-assisted thermal processing > 600 MPa showed a tailing effect on inactivation of the spores counts of *Clostridium botulinum* type B TMW 2.357. A preheating step is important. Thus, preheating at 98 °C was selected before PATS processing in this study to ensure safe and reliable inactivation of spores by elevating temperature through the induced adiabatic heating. In the FDA filing and acceptance process of high pressure-assisted thermal sterilization (Stewart et al., 2016), the mashed potato packages were preheated at 98 °C for 16 min.

Table 2
Total microbial aerobic and anaerobic plate count of fruit and vegetable purees (n = 6).

Fruits & Vegetables	Aerobic microbial count $\log_{10}$ (Log_{10} CFU/g)		Anaerobic microbial count $\log_{10}$ (Log_{10} CFU/g)	
	Unprocessed	PATS	Unprocessed	PATS
Pumpkin	3.4 ± 0.1	N.D.	3.0 ± 0.3	N.D.
Butternut Squash	3.9 ± 0.1	N.D.	2.7 ± 0.8	N.D.
Peas	3.7 ± 0.1	N.D.	2.5 ± 0.1	N.D.
Beetroots	3.6 ± 0.7	N.D.	2.4 ± 0.2	N.D.
Purple Potato	3.4 ± 0.5	N.D.	2.0 ± 0.2	N.D.

(N.D.) means Not Detectable.

3.3. Vitamin C content

Fig. 3 demonstrates Initial and final vitamin C content after fortification and PATS process in the five selected purees. The initial fortified vitamin C contents were 49.8 ± 1.2, 56.9 ± 1.1, 60.2 ± 0.8, 47.1 ± 1.0, and 43.7 ± 0.5 mg/100g in pumpkin, butternut, peas, beetroots, and purple potato, respectively. Butternut squash and peas purees showed higher initial vitamin C content compared to other fruits and vegetables; this may be attributed to the natural presence of vitamin C in both products. Butternut squash contains about 21 mg/100 g of vitamin C, and peas contain about 40 mg/100 g according to the USDA data. Even though the fortified amount was 50 mg of vitamin C to 100 g of puree, the initial detected amount was less in some purees, and this could be the result of a possible reaction that occurs during the addition of ascorbic acid with present food components. Vitamin C content reduced as influenced by PATS process by 3–14% as illustrated in Fig. 3, where the final content after PATS was 42.8 ± 1.5, 51.5 ± 2.2, 58.4 ± 0.5, 42.2 ± 0.7 and 41.8 ± 0.9 mg/100g in pumpkin, butternut, peas, beetroots, and purple potato, respectively. High temperature may have played a role in this loss of vitamin C after PATS. The total reduction was highest in pumpkin by 14% while it was only 3% in peas. Different chemical composition, acidity, and various pigments of the studied products may have played a protective role for vitamin C loss variation, such as the presence of other vitamins, namely vitamin A and vitamin E (Patel et al., 2019). Vitamin C losses in PATS in this study were less than the one reported by Raj, Chakraborty, and Rao (2019) for amla juice, where up to 35% loss was observed in pasteurization temperature 60 °C for 20 min. Vitamin C loss was less, approximately > 10% when the heat was not involved only high pressure for 20 min for the same product (Raj et al., 2019). High pressure has led

Table 1
Inactivation studies of *B. amyloliquefaciens* strain TMW 2.479 (Fad 82) after high pressure and temperature processing.

Process conditions	Product	Inactivation Log_{10} CFU/g	Reference
800 MPa, 80 °C, and 16 min	Mashed carrots	2	Margosch et al. (2004)
700 MPa, 121 °C, and 1 min	Egg patty mince	> 7	Rajan, Ahn, Balasubramaniam, & Yousef, 2006
1200 MPa, 120 °C, and 2 min	Tris-His buffer (pH 5.15)	> 4	Margosch et al. (2006)
700 MPa, 105 °C, and 10.5 min	Deionized water	~7.7	Ahn and Balasubramaniam (2007)
600 MPa, 120 °C, and 1 s	Baby food puree	No detectable colonies	Sevenich et al. (2014)
600 MPa, 115 °C, and 0.25 min	Baby food puree and ACES-buffer (pH 7)	5	(Sevenich et al., 2014)
600 MPa, 115 °C, and 0.45/1.5 min[a]	Baby food puree and ACES-buffer (pH 7)	12 (extrapolated)	(Sevenich et al., 2014)
600 MPa, 115 °C, and 5 min[b]	Purple potatoes puree	9.6 & 9.4[c]	This study

[a] Extrapolated baby food processing time was 0.45 min and ACES-buffer was 1.5 min.
[b] Included 5 min preheating at 98 °C.
[c] Based on two strains Fad 82 and 11/2, respectively (Li, Schottroff, Simpson, & Gänzle, 2019).

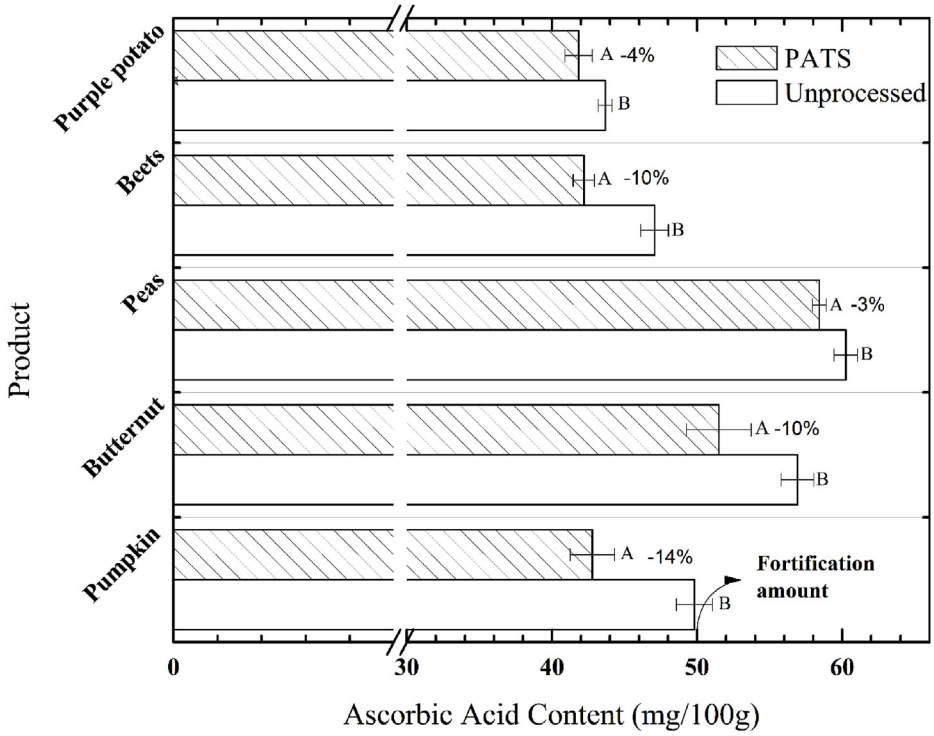

Fig. 3. Vitamin C content in pumpkin, butternut squash, peas, beets, and purple potato purees before and after PATS processing ($n = 3$). Different capital letters (A and B) indicate significant differences between means of processed and unprocessed samples ($p < 0.05$).

to a loss of 13% of ascorbic acid in asparagus juice at 600 MPa for 20 min from 108 mg/L, which was significantly lower compared to the processing at 120 °C for 3 min, which lost about 23% in their study (Chen et al., 2015). High-pressure processing alone does not affect the vitamin C content as much as high temperature, as can be seen by the surface response of rate constant reported by (Raj et al., 2019). The greater retention of vitamin C was because of the mild PATS processing in terms of reduced overall processing time. Involvement of heat in the preheating stage and actual processing stage (98 and > 120 °C, respectively) may cause this small vitamin C reduction. In contrast, a short exposure time to heat may have played a significant role in reducing the degraded amount of vitamin C that is still sufficient for the daily recommended dose.

Xanthakis, Gogou, Taoukis, and Ahrné (2018) found that conventional blanching of mango by low temperature and longtime (LTLT) caused 24% vitamin C loss, while high temperature and short time cased about 15% loss. Processing time and temperature make an impact independently on the degradation of vitamin C. Commercially available purees containing small amounts of vitamin C, therefore, could be fortified and processed by PATS to help maintain a vitamin C rich product that will satisfy the daily recommended dose needed.

3.4. Natural color pigments and pH

Overall, PATS did not significantly ($p < 0.05$) influence β-carotene in pumpkin (2.4×10^{-2} to 1.9×10^{-2} mg/g) and butternut squash (4.4×10^{-2} to 4.1×10^{-2} mg/g) (see Fig. 4). This original quantity found in pumpkin and butternut squash before PATS was similar to the one previously reported (García-Parra, González-Cebrino, Delgado, Cava, & Ramírez, 2016; Zaccari & Galietta, 2015). It should be noted that the β-carotene was neither heat nor pressure-sensitive, as demonstrated in previous research (Butz et al., 2002; Lee & Coates, 2003; Sonar, Paccola, et al., 2019; Zhang et al., 2019). High pressure alone (400 and 600 MPa) for 2 min did not affect the quantity of alpha-and-beta-carotenes in carrots and broccoli, respectively, at low processing temperature (McInerney, Seccafien, Stewart, & Bird, 2007) or in soy-smoothies (Andrés, Mateo-Vivaracho, Guillamón, Villanueva, & Tenorio, 2016). Total chlorophyll (3.0×10^{-2} to 2.0×10^{-2} mg/g) and chlorophyll *b* (1.2×10^{-2} to 0.5×10^{-2} mg/g) decreased but they were not significantly ($p < 0.05$) affected by PATS, but chlorophyll *a* (1.9×10^{-2} to 1.4×10^{-2} mg/g) significantly decreased after PATS. Butz et al. (2002) showed that chlorophyll *a* and *b* were not affected by the pressure, but a slight decrease occurred when the heat was applied (75 °C) for minced broccoli. Betalains in red beet, including betacyanin and betaxanthins, have shown sharp and significant ($p > 0.05$) decrease (42–57%) after PATS processing. Betalains are heat sensitive and decreased after boiling, roasting, and sterilization by 7–45% (Jiménez-Aguilar et al., 2015; Ravichandran et al., 2013) in red beet and prickly pears, but increased by 4–8% in HPP treatment in prickly pears, as discussed by (Jiménez-Aguilar et al., 2015). This increase may be due to better extraction of betalains under high pressure only. Anthocyanin in purple potatoes also significantly decreased by 57% from 146.5 ± 0.7 to 63.2 ± 3.8 mg/g as affected by PATS processing. Researchers showed that high pressure (200–800 MPa) altered the content of anthocyanin in strawberry approximately 0–20% after one day of storage at refrigeration temperature (4 °C) (Zabetakis, Leclerc, & Kajda, 2000). On the other hand, anthocyanin is heat-sensitive, as demonstrated by Jimenez et al. (2010), and that is probably why PATS influenced the initial content of anthocyanin in purple potato puree. In addition, anthocyanin content in wild berry was not effected by HPP from 200 to 600 MPa for 2–15 min treatment time but reduced significantly in the equivalent pasteurization process at 70 °C for 30 min in the same study (Liu et al., 2016).

The pH of pumpkin, butternut squash, peas, beetroots, and purple potato was above 4.6 in low-acid foods (see Table 3). For all fruits and vegetables, pH decreased after processing and significantly different from each other except for purple potato. The acidity of the product changed after processing due to the release of the acidic compounds from fiber and binding sites. The pH reduction for different types of foods such as fruit juices, salad dressing, yogurt, and guacamole was attributed to the hydrogen bonding breakdown and forming of hydrophobic moieties (Torres, Serment-Moreno, Escobedo-Avellaneda,

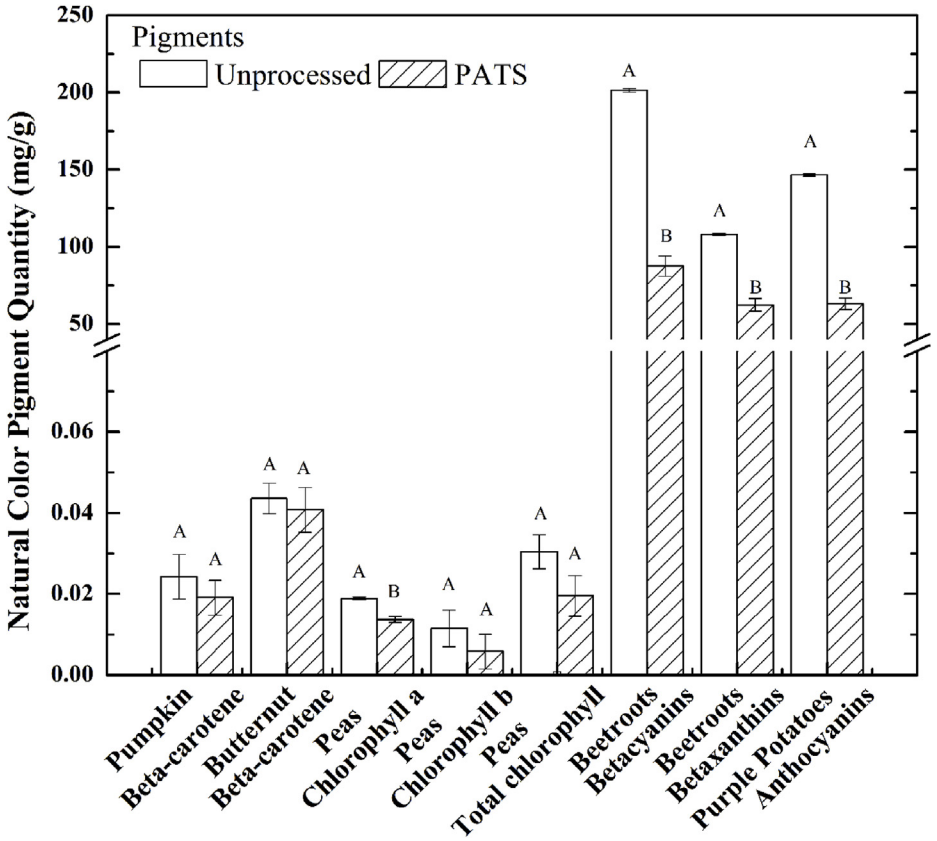

Fig. 4. Natural color pigments content in fruit and vegetable purees before and after PATS ($n = 3$). Different capital letters (A and B) indicate significant differences between means of processed and unprocessed samples ($p < 0.05$).

Table 3
pH of fruit and vegetable purees before and after PATS processing ($n = 3$).

Fruits &Vegetables	pH	
	Unprocessed	**PATS**
Pumpkin	5.01 ± 0.03[a]	4.89 ± 0.08[b]
Butternut Squash	4.79 ± 0.00[a]	4.79 ± 0.03[b]
Peas	5.26 ± 0.01[a]	5.04 ± 0.01[b]
Beetroots	5.41 ± 0.02[a]	5.28 ± 0.03[b]
Purple Potato	6.24 ± 0.08[a]	6.14 ± 0.17[a]

Different subscript letters indicate a significant difference between the means ($p < 0.05$).

Velazquez, & Welti-Chanes, 2016).

3.5. Color and visual appearance

Color is the most attractive feature of fruits and vegetables. Fig. 5 displays instrumental color parameters as well as the apparent visual image of each puree. Overall, the color difference in pumpkin, butternut, and beetroots was not distinct when observed with the naked eye where ΔE was approximately 3 or less. Two products that were sensitive to the heat applied in PATS are peas and purple potatoes. Purple potato showed lighter color that could be associated with loss of anthocyanin discussed earlier, whereas peas showed browning coloration instead of their green original fresh color because of the pathway reactions that chlorophyll can take with heat. The color difference in peas was indicating a different color ($\Delta E > 12$) as described by (Zhang et al., 2016), which reveals the sensitivity of green color to heat. The instrumental color indicators L^*, b^*, and a^* varied depending on the product. In general, lightness (L^*) value slightly decreased only in butternut and peas but interestingly increased in the other three examined products (e.g., pumpkin, beets, and purple potatoes). Yellowness (b^*) also decreased in butternut and beets, but the rest either remained the same or increased in the other three products, including pumpkin. This may be attributed to the high-temperature extractability of β-carotene in pumpkin (García-Parra et al., 2016). Green color ($-a^*$) of peas has changed significantly after processing similar to that avocado puree during storage observed by López-Malo, Palou, Barbosa-Canovas, Welti-Chanes, and Swanson (1998). Redness ($+a^*$) either increased or remained the same except in purple potato, which showed a sharp decrease due to the anthocyanin content reduction explained above. Minimum discoloration of the product may be the advantage of this PATS technology. Images in Fig. 5 show the actual product appearance before and after PATS processing. Pumpkin and butternut squash were not affected by the PATS process. Beetroots and purple potato purees looked lighter, which is also in line with L^*, b^*, and a^* values. The only one distinct difference was for the peas puree, as it appeared to be brownish green rather than light green. The link between natural pigments and the instrumental color was not clear in this study and this probably because of the complex nature of pigment in each product. PATS may have the advantage of preserving the natural attributes of heat-sensitive foods.

3.6. Oxygen and water vapor transmission rates of EVOH-based multilayer films

The EVOH-based pouches did not show any visual defects. However, OTR and WVTR of films increased significantly ($p < 0.05$) (Table 4). The OTR of film #2 after PATS process was < 0.5 cm^3/m^2. day. This film can be considered suitable for shelf-stable products, as described by Zhang et al. (2019). However, the OTR of film #1 increased from 0.16 to 3.32 cm^3/m^2. day probably due to the differences in the grade, thickness, or high pressure and high-temperature tolerance of the EVOH

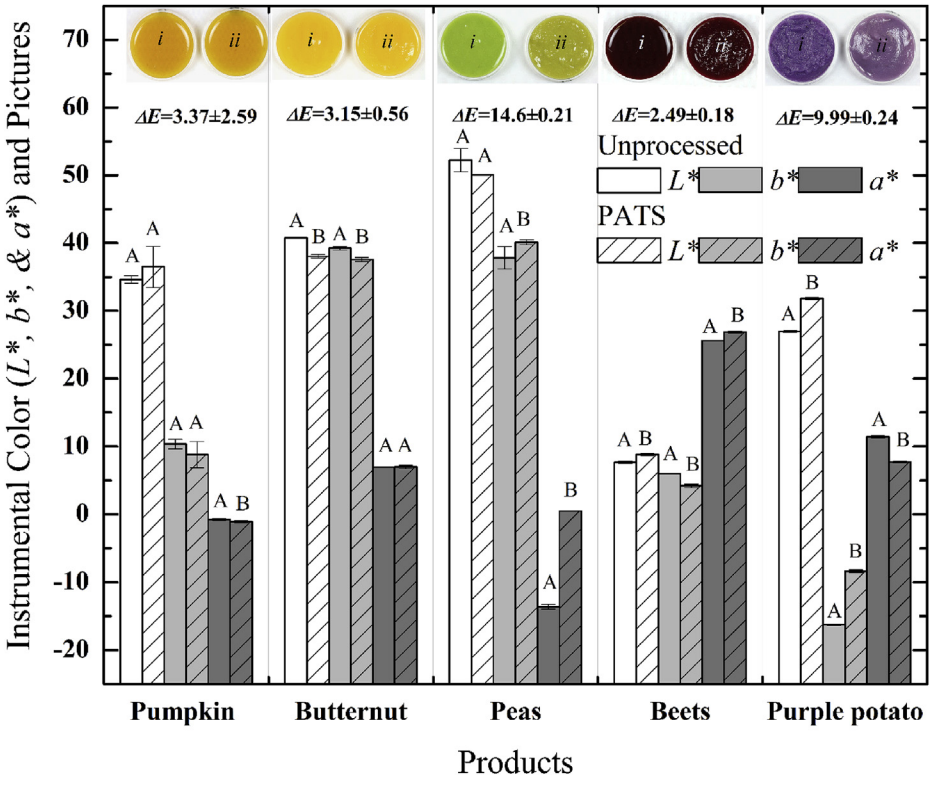

Fig. 5. Color measurements of pumpkin, butternut squash, peas, beetroots, and purple potato puree before and after processing, and their representative pictures before (***i***) and after PATS (***ii***) (n = 3). Different capital letters (A and B) indicate significant differences between means of processed and unprocessed samples (p < 0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

layer.

4. Conclusions

High pressure-assisted thermal sterilization (PATS) was utilized to examine microbial safety, nutrient, and quality retention of homogeneous fruit and vegetable purees. Overall, PATS showed a potential in its sterility of the product, nutrient preservation, and excellent overall food quality. Temperature sensor readings suggested that the purees have reached 122 °C. The *B. amyloliquefaciens* Fad 82 and 11/2 were fully inactivated by > 9 $\log_{10}$ cycle after PATS in purple potatoes puree. The aerobic and anaerobic microbial count did not show any detectable colonies after PATS and 6 months of storage confirming the safety of the purees. The vitamin C retention after PATS was 85–97%, depending upon the puree. Natural color pigments such as β-carotene and chlorophyll were not sensitive to PATS, but betalains and anthocyanin were sensitive to PATS. The instrumental color change was classified as below the distinct change for pumpkin, butternut squash, and beetroot. The visual observation showed that the pea and purple potato purees were the most influenced by PATS. One of the EVOH-based films (film #2) was suitable for shelf-stable products despite some increase in OTR and WVTR. The overall changes in microbial load, food quality, and packaging by PATS process depend upon pressure and temperature sensitivity of spores, types of pigment, and polymer film structure. The results indicated that PATS is a suitable process and can potentially be used to produce shelf-stable prepackaged purees with superior physical and chemical quality. PATS could be beneficial to the food industry since it offers short processing time and good quality retention. A shelf-life study of PATS processed food may reveal an interesting nutrient retention with high consumer acceptance, and likely opening a new venue in the growing market of HPP.

CRediT authorship contribution statement

Saleh Al-Ghamdi: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft, Writing - review & editing. **Chandrashekhar R. Sonar:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing. **Juhi Patel:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - review & editing. **Zeyad Albahr:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Shyam S. Sablani:** Conceptualization, Funding acquisition, Supervision, Visualization, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Table 4
Structures, thicknesses, and oxygen and water vapor transmissions rates before and after PATS (n = 3–5).

#	Structure	Thickness (μm)	OTR (cm3/m2.day)		WVTR (g/m2.day)	
			Control	PATS	Control	PATS
1	PE/PA6//EVOH//PA6/PE	109 ± 4	0.16 ± 0.06 ^{aA}	3.32 ± 0.71^{bA}	3.72 ± 0.13 ^{aA}	5.07 ± 0.74 ^{aA}
2	PP/PA//EVOH*//PA/PP	107 ± 4	0.23 ± 0.04 ^{aA}	0.47 ± 0.02^{bB}	2.61 ± 0.11 ^{aB}	3.88 ± 0.08 ^{bA}

(//) Two dashes indicate tie layer in between layers. * EVOH grade is EVAL 171 with density of 1.2 g/cm^3 density. Small subscript letters indicate significant differences between processed and unprocessed film and capital letters indicate significant differences the two examined films.

Acknowledgment

This research was supported by the (USDA) National Institute of Food and Agriculture Research grants numbers 2016-67017-24597 and 2016-68003-24840, and Hatch project #1016366. The authors would like to thank Dr. Gänzle, University of Alberta, for providing the *B. amyloliquefaciens*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107233.

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