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RESEARCH

Pressure-Assisted Thermal Sterilization and Storage Stability of Avocado Puree in High Barrier Polymeric Packaging

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Abstract

The main objective of this study was to evaluate the feasibility of developing shelf stable avocado puree processed with pressure-assisted thermal sterilization (PATS). A pressure of 600 MPa and initial vessel temperature of 90 °C was used over 5 min processing time. Two high barrier polymeric packages were used: Film A-PE/PA6//EVOH and Film B-AlO_x-coated PET//AlO_x-coated PET//AlO_x-coated PET//ONy//CPP. Ascorbic acid with concentration of 0.4 g/kg of avocado was added to puree to enhance its color stability (pH 6.6). Results showed that the weight loss was lower in package B (0.5%) than in package A (4–5%) over 104 days of storage at 23 °C. Instrumental color (greenness) of the puree in both packaging changed significantly ($p > 0.05$) during storage despite the addition of ascorbic acid; however, total color changes of puree in film B were lower than film A. There was no significant ($p > 0.05$) degradation of chlorophyll concentrations of PATS-processed avocado during storage. The dissolve oxygen concentration in avocado puree during storage was stable and the maximum normalized dissolve oxygen was less than 1%. Lipid oxidation of the fortified and plain avocado puree packaged in films A and B was stable with no significant ($p > 0.05$) changes during storage. Total plate count of avocado puree after PATS and during the 3 months of storage was below the detection limit. The results suggest that shelf stable avocado puree with limited shelf life could be developed using the PATS process.

Keywords Ascorbic acid · Chlorophyll · Oxygen content · Polymer packaging · Storage · Total plate counts

Highlights

- Total plate counts of PATS avocado puree were below the detection limit after 3-month storage.
- TBARS values of avocado puree were stable during storage at 23 °C.
- Total color change of puree in film B was lower than film A after 3-month storage.
- Chlorophyll concentrations were stable during the three months of storage.
- Ascorbic acid was used to enhance avocado qualities and as a nutrient supplement.

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Introduction

Avocado is an exceptionally nutritious fruit that is consumed raw or added to our meals and salads. The origin of this delightful fruit is Central and South America, and a moderate climate is necessary for it to grow and mature. The market for avocados is largely North America, Europe, and Asia, but avocado is consumed in all seven continents (Salazar-López et al., 2020). Avocado contains large amounts of carbohydrates (8.53 g per 100 g of avocado), fats (14.7 g per 100 g of avocado), and vitamins according to USDA database. Apart from salads, it is also used in sandwiches and side dishes. The most popular form of avocado is guacamole, which is mass marketed all over the world. Guacamole may be available as a dipping sauce, condiment, or as a topping on sandwiches. Avocado is highly susceptible to enzymatic browning catalyzed by polyphenol oxide (PPO) activates during after-harvest handling, processing, and storage (Weemaes et al., 1999). The release of PPO which caused by cell distribution can accelerate the oxidation of phenolic compounds in the presence of oxygen to o-quinones, which eventually non-enzymatically polymerize to form brown pigments (Weemaes et al., 1998). In addition to that, lipid oxidation is a factor that can shorten the shelf life of avocado (Elez-Martinez et al., 2005). Presently, the most common process for preserving avocado is a high-pressure process (HPP) due to the gentle effect on the avocado's green color and the inactivation of enzyme activities as well as microorganisms (Jacobo-Velázquez & Hernández-Brenes, 2010; López-Malo et al., 1998; Palou et al., 2000). On the contrary, treated avocado with conventional thermal processing can induce off-flavor (Bates, 1970), alternating the original taste of avocado.

Commercially available HPP guacamole has superior sensory and nutritional qualities compared to many thermally processed fruits and vegetables. Guacamole is prepared with avocado pulp, diced onion, salt, and citric acid, lime juice, or vinegar to attain a pH lower than 4.6 to avoid spore germination. The shelf life of HPP guacamole is around 8–10 weeks under refrigeration conditions, requiring additional energy for safe distribution through cold chain supply. The avocado is a temperature and oxygen-sensitive fruit; hence, novel methods for processing and packaging are required to produce shelf stable avocado-based products.

Pressure-assisted thermal sterilization (PATS) can potentially be utilized to produce higher quality shelf stable avocado puree due to shorter heat exposure. The application of pressure facilitates uniform temperature rise in the entire product (Otero & Sanz, 2003). In a PATS process, pressure ranging from 600 to 800 MPa and a short time holding at product temperature of 121 ℃ would result in a shelf-stable products (Al-Ghamdi et al., 2020, 2022). PATS has shown to inactivate bacterial spores in fruit purees (Al-Ghamdi et al., 2020; Sevenich et al., 2014; Skinner et al., 2014, Margosch et al., 2004). PATS also has minimal impact on food quality parameters and nutrient content (Al-Ghamdi et al., 2020; Rastogi et al., 2007). An earlier PATS study showed potential to produce shelf-stable avocado puree; however, shelf-life studies are needed to further develop commercial shelf-stable guacamole (Al-Ghamdi et al., 2022). In addition, robust high barrier packaging that can withstand thermal and mechanical stresses of PATS process is required.

Multilayer polymeric packages are the most suitable choice among other packaging materials due to their processability and flexibility (Parhi et al., 2020). Selecting the right type of packaging is an important factor to commercially produce safe, high-quality foods that can reach consumers. Ethylene–vinyl alcohol (EVOH)-based multilayered films and metal oxide (AlO_x)-coated polyethylene terephthalate (PET)-based multilayered films can potentially be used for PATS-processed products due to their high barrier performance and robustness with food processing technologies.

The objective of this study was to evaluate the stability of PATS-processed avocado puree by assessing the microbial, physical, and chemical quality of puree packaged in EVOH and metal oxide-coated PET-based packaging during storage at room temperature for 104 days. In addition, the influence of PATS on physical properties of multilayer packaging was also evaluated.

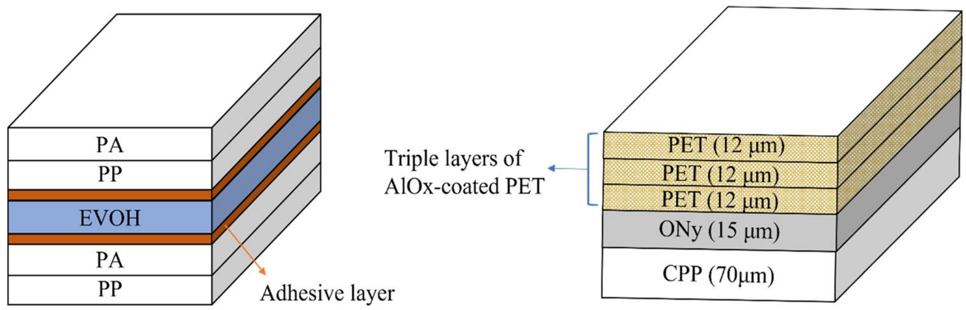
Materials and Methods

Avocado Puree Preparation and Packaging Films' Barrier Properties

Ripe avocados (*Persea americana*) were purchased from a local store (Walmart, Pullman, WA). The ripe avocados ($n=45$) were washed, cut into halves, peeled by hand, and placed together in a dish and mashed manually to create a homogenous avocado puree. The prepared avocado puree was divided into two batches. One batch was left plain (i.e., only avocado puree) and the second batch was fortified with vitamin C, pure powder, and ascorbic acid (New Foods, Bloomingdale, IL) with a concentration of 0.4 g/kg of avocado. The two batches of avocado were degassed twice using an Easy-Pack vacuum sealer (UltraSource. LLC. Kansas, MO) under 99% vacuum setting for 5 s and kept at room temperature (~24 °C) until the packing process.

Al-Ghamdi et al. (2022) showed that both EVOH- and PET-AlO_x-based packages are suitable for PATS processes as they maintained high barrier properties after PATS treatment. Therefore, two types of multilayer packaging films were used in this study to package the avocado puree. The first film A was a coextruded EVOH-based film with a seven-layer structure. This film includes a EVOH core layer as a barrier layer, laminated with polyamide (PA) from both sides for protection. The structure of the EVOH-film was PP/PA//EVOH//PA/PP. The second Film B was a newly designed PET-metal oxide-based film. This film included three metal oxide-coated PET films and its structure was AlO_x-coated PET (12 µm)//AlO_x-coated PET (12 µm)//AlO_x-coated PET (12 µm)//ONy(15 µm)//CPP(70 µm) as shown in Fig. 1. The oxygen transmission rate (OTR) and the water vapor transmission rate (WVTR) of the two films after PATS in the present study were measured using an Ox-Tran 2/21 MH (Mocon Inc., Minneapolis, MN, USA) and a Mocon Permatran 3/33 instrument (Modern Control, Minneapolis, MN) following the method described by Al-Ghamdi et al. (2019) and Parhi et al. (2020). The film sample preparation involved cutting the films to have an appropriate size of 50 cm^2 surface area and then it was placed in an aluminum mask. The aluminum mask that contained the film sample was mounted inside the instrument and subjected to OTR and WVTR tests according to ASTM D3985 standard (at 23 °C, 55% RH, and 1 atm) and ASTM F372-99 standard (33 °C, 100% RH) respectively. The OTR values of film A before and after

Fig. 1 EVOH-based and PET-coated based films' structure representation; PP, polypropylene; PA, polyamide; EVOH, ethylene vinyl alcohol copolymer; PET, polyethylene terephthalate; ONy, biaxially oriented nylon 6; CPP, cast polypropylene; and AlOx, aluminum oxide



PATS were 0.23 ± 0.04 (cm^3/m^2.day) and 0.47 ± 0.02 (cm^3/m^2.day), respectively, as shown in Table 1. The OTR values of film A before and after PATS were under the detective limit of the instrument (< 0.05). The WVTR values of film A before and after PATS were 3.72 ± 0.06 (g/m^2.day) and 4.97 ± 0.14 (g/m^2.day), respectively, while the WVTR values for film B before and after PATS were 0.10 ± 0.00 (g/m^2.day) and 0.41 ± 0.01 (g/m^2.day) respectively.

Multiple pouches (0.10 m length, and 0.05 m width) were prepared using these two multilayer films. The tailored pouches were filled with approximately 25–30 g avocado puree and then vacuum-sealed twice using an Easy-Pack vacuum sealer (UltraSource. LLC. Kansas, MO) under 99% vacuum setting for 5 s to minimize the oxygen content inside the package. The vacuum-sealed avocado pouches were wrapped with aluminum foil to minimize the photo oxidation and stored at 4 °C for 12 h before PATS. The packages used in this study were labeled as follows: package A, package B, package A + AA, and package B + AA as shown in Table 2.

Pressure-Assisted Thermal Sterilization (PATS) Treatment

PATS treatment of avocado puree in flexible pouches was carried out in a 2-L high-pressure machine (Engineered Pressure Systems, Inc., Haverhill, MA) following the protocol described by Al-Ghamdi et al. (2020). Firstly, the vessel of the machine was filled with the pressure medium, 10% Houghton Hydrolubic 123B soluble oil/water solution (Houghton & Co., Valley Forge, PA), and set to its maximum preheating temperature (90 °C) allowing the vessel and the pressure medium to be equilibrated at 90 °C. The pouches were preheated to nearly (~ 98 °C) (selected as the initial temperature of the pouches) in a kettle with boiling water. To record the temperature of the packaged avocado during the preheating step, a type K thermocouple (Omega Engineering Inc., Stamford, CT) was installed in the geometric center of the package (cold spot). The come-up time of the avocado puree ($n = 3$) was 5 min, as determined in a separate heat penetration study. After the pouches ($n = 3$) reached 98 °C after heating for 5 min in the water kettle, they were immediately placed in the pressure vessel and pressurized at 600 MPa for 5 min using an electrohydraulic pump (Hochdruck-Systeme GmbH, AP 10–0670-1116, Sigless, Austria). To minimize the heat loss from the pouches to the vessel walls during PATS processing, a cylindrical insulator (liner) made of polyoxymethylene (McMaster-Carr, Atlanta, GA) was used. The liner had a thickness, an internal diameter, and a height of 0.1 m, 0.08 m, and 0.22 m, respectively. Prior to pressurization, the temperature of the liner was equilibrated to the vessel and pressure medium preheated to 90 °C. The temperature inside the vessel was recorded using three thermocouple probes. After PATS processing, the pouches were removed from high pressure unit and cooled in a water bath at room temperature. In multiple runs performed, a total of 85 pouches were treated with the PATS process. All the processed pouches were stored at room temperature in the dark for 104 days.

Table 1 Film structure, and thickness, and barrier properties before and after PATS (adapted from Al-Ghamdi et al., 2020 and Parhi et al., 2020)

Polymeric film	Film structure	Thickness (μm)	OTR (cm^3/m^2.day) Control	OTR PATS	WVTR (g/m^2.day) Control	WVTR PATS
Film A	PP/PA//EVOH//PA/PP	107 ± 04	0.23 ± 0.04^a	0.47 ± 0.02^b	3.72 ± 0.06^a	4.97 ± 0.14^b
Film B	AlOx-coated PET(12 μm)//AlO_x-coated PET(12 μm)//AlO_x-coated PET(12 μm)//ONy(15 μm)//CPP(70 μm)	121 ± 0.8	ND	ND	0.10 ± 0.00^a	0.41 ± 0.01^b

ND not available/detectable

Values with the same superscript letters are not significantly ($p < 0.05$) different

Table 2 Illustration of labeled packages and the contained avocado product

Package	Packaged product
Package A	EVOH-based film and contained plain avocado puree
Package B	PET-AlO_x-based film and contained plain avocado puree
Package A + AA	EVOH-based film and contained ascorbic acid-fortified avocado puree
Package B + AA	PET-AlO_x-based film and contained ascorbic acid-fortified avocado puree

Weight Loss

Weight loss of avocado pouches during storage was measured by periodically weighing the pouches ($n = 3$) using a balance (model GK703, Sartorius, Goettingen, Germany). The results were expressed as a percentage (%) of weight loss.

Instrumental Color

The color of the avocado after PATS and during storage period was measured using a CM-5 spectrophotometer (Konica Minolta, Ramsey, NJ) under reflectance mode with an 8-mm measurement, an observer angle of 10°, and the illuminant D65. Five grams of avocado was filled and spread evenly on the bottom of a petri dish (60 mm × 15 mm) and its color parameters L^*, a^*, and b^* (CIELAB) were quantified by the spectrophotometer. The color measurements for each set of packages were performed in triplicate, and the total color difference was calculated as follows:

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \tag{1}$$

According to Zhang et al. (2016), a value of ΔE that is > 6 indicates a strong change between two colors, and a ΔE value that is > 12 indicates a different color of the food samples.

Chlorophyll Measurements

The amount of chlorophyll in the avocado puree was measured using the same method described in a previous study (Sonar et al., 2019) with minor modifications. Five grams of avocado puree was added to 25 mL of acetone (80% v/v) in a 50-mL centrifuge tube (polypropylene). The mixture was then homogenized for 3 min at 6500 rpm using a Polytron PT 2500E homogenizer (Kinematica, Bohemia, NY, USA) and incubated in the dark at room temperature for 30 min. After incubation, the homogenate was centrifuged at 4000 × g for 10 min at room temperature using (AccuSpin 400; Fisher Scientific, Pittsburgh, PA, USA). The supernatant was then collected and filtered through filter paper (Whatman No. 1; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), and its absorbance (A) was measured using a Spectrophotometer (Ultraspec 4000, Pharmacia Biotech Inc., Piscataway, NJ) at wavelengths of 663 nm and 647 nm. The chlorophyll in the avocado puree was quantified using the following equations (Sonar et al., 2019):

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

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

Oxygen Content in Packaged Purees

The oxygen content in the avocado puree inside the package was established by measuring the dissolved oxygen using an OxySense 325i noninvasive oxygen analyzer (Oxysense Inc., Dallas, TX, USA). The measurement system consisted of two parts: the first part is a hand-held pen, and the second is an adhesive oxygen sensor (Oxydot). The oxydot was located inside the transparent package at the center of the inner side after which the package was filled with avocado puree and sealed. The dissolved oxygen concentration in the packaged avocado was measured after PATS and during storage period using hand-held pen. The oxygen content measurements were performed in triplicate, with the results expressed in fraction (between 0 and 1) of normalized oxygen concentration values assuming the maximum oxygen concentration outside the packaged food is 21%, as follows:

$$C = \frac{C_t - C_0}{C_{eq} - C_0} \tag{4}$$

where C_t is oxygen concentration at any time, C_0 is the initial oxygen concentration, and C_{eq} is the maximum oxygen concentration outside the packaged food, which is 21%.

Lipid Oxidation

Lipid oxidation in puree was measured using a modified 2-thobarbituric acid reactive substance (TBARS) method as described by Bhunia et al. (2017). The absorbance of the avocado samples ($n = 6$) was quantified using a Spectrophotometer (Ultraspec 4000, Pharmacia Biotech Inc., Piscataway, NJ) at wavelength of 532 nm, and the results are expressed in mg of malondialdehyde kg^{-1} of the sample.

Total Plate Counts

To ensure the safety of the sterilized avocado, total anerobic and aerobic plate counts were measured. Approximately 10 g of avocado (e.g., whether fresh or processed) was homogenized and mixed with 90 mL of peptone water (0.1%) in stomacher bag. Then an amount of 1 mL of the avocado-peptone water mixture was used to prepare the predetermined serial dilutions in 10-mL test tube containing 9 mL of peptone water (0.1%) under sterilized conditions. Following the preparation of the serial dilutions, aliquots of 1 mL of each serial dilution were poured on tryptic soy agar (BD and Company, Sparks, MD, USA) ($n = 2$). Prior to the incubation, plates for anerobic analysis were placed in airtight jars that were conditioned with anaerobic sachets. The plates for both total anerobic and aerobic plate counts were incubated at 37 °C for 48 h. The plate count results were expressed in log colony-forming units (CFU) $\log_{10}$ ($n = 6$).

pH Measurements

A pH meter (Seven Go SG2, Mettler Toledo, Schwerzenbach, Switzerland) was used to measure the pH value of avocado puree. Three grams of the avocado was mixed with 10 mL of distilled water, and then the electrode of the pH meter was immersed in the mixture. The pH value of the samples ($n = 3$) was recorded until the device gave a steady reading.

Data Analysis

An analysis of variance (ANOVA) test was used to determine the differences among means of the collected data. This was followed by post-hoc comparison using Tukey's test to determine the significant difference among means. Both tests were performed at significant level of 0.05 with SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results and Discussion

PATS Treatment Profile

The total processing time was ~ 350 s: 36 s for pressurization, 285 s for holding, 28 s for decompression. Figure 2 illustrates PATS pressure and temperature profiles in the pressure vessel of the packaged avocado puree. The temperature of the medium in the pressurized vessel during the come-up time increased from the initial temperature of 90 °C to a maximum temperature of 115 °C. This temperature increase was the result of the compression heating under quasi-adiabatic conditions inside the vessel (Al-Ghamdi et al., 2019, 2020; Dhawan et al., 2014). The maximum temperature of the pressure medium inside the vessel reached 115 °C and it decreased to 100 °C during the holding time. The decrease in temperature is attributed to the heat loss from the pressure vessel to the surroundings as explained by Al-Ghamdi et al. (2019, 2020).

Visual Defects of PATS on the Packages

In this study, both films A and film B used for packaging of avocado puree showed minor visual defects after PATS. These visual physical defects were in the forms of white spots and tiny bubbles. For film A, only white spots were observed around the headspace area of the pouches.

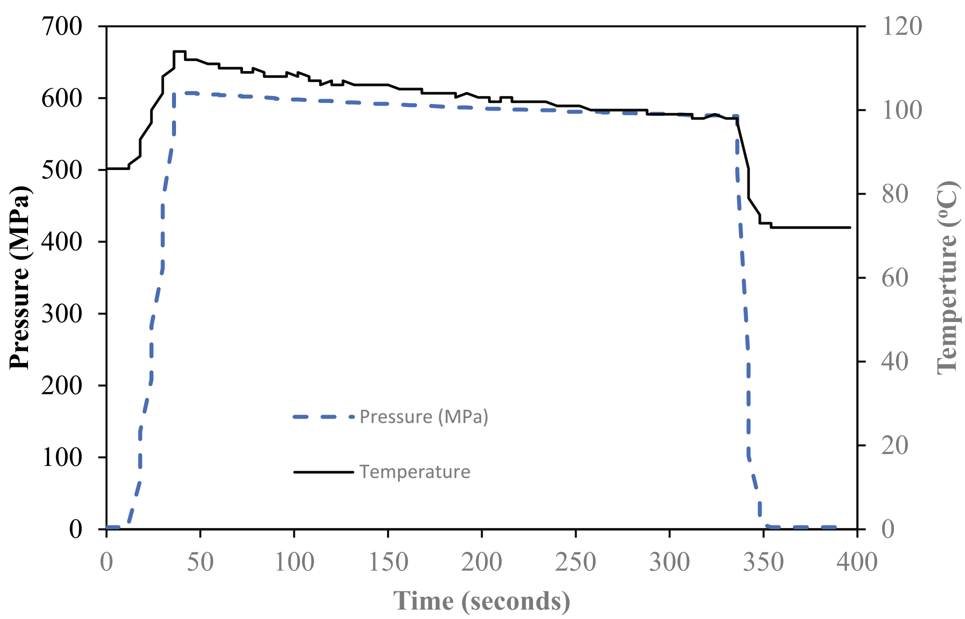


Fig. 2 Pressure and average temperature profiles during PATS at 600 MPa and an initial product temperature of 90 °C

However, for film B, both white spots and small bubbles were observed on different locations on the pouches. The visual defeats on packaging in the present study were similar to the defects reported earlier by our previous work (Al-Ghamdi et al., 2019). The presence of residual air in the package headspace might have resulted in damaging the integrity of multilayer polymeric packages (Al-Ghamdi et al., 2019; Sterr et al., 2015). Al-Ghamdi et al. (2019) reported that headspace levels of 0.05 cc air to 0.30 cc air induced damages on the package when temperature above 30 °C and working pressure of 600 MPa are combined for high pressure processing. The release of entrapped air in avocado puree to the headspace of the package during the pre-heating step along with stresses from the vacuum sealing may have led to the observed defects on films A and film B. Hence, degassing of puree and a very high level of vacuum before packaging may be employed to minimize the damage to barrier packaging.

Weight Loss

Weight loss of thermally processed food products is caused mainly by moisture loss from the food through the package and is directly related to the water vapor transmission rate (WVTR) of the film, as documented in previous studies (Patel et al., 2020; Sonar et al., 2019; Zhang et al., 2016). The measured data for weight loss of the plain and ascorbic acid-enriched avocado puree packaged in package A and package B stored at 23 °C for 104 days are shown in Fig. 3. There were no significant ($p > 0.05$) changes in puree weight observed in all packages immediately after PATS. However,

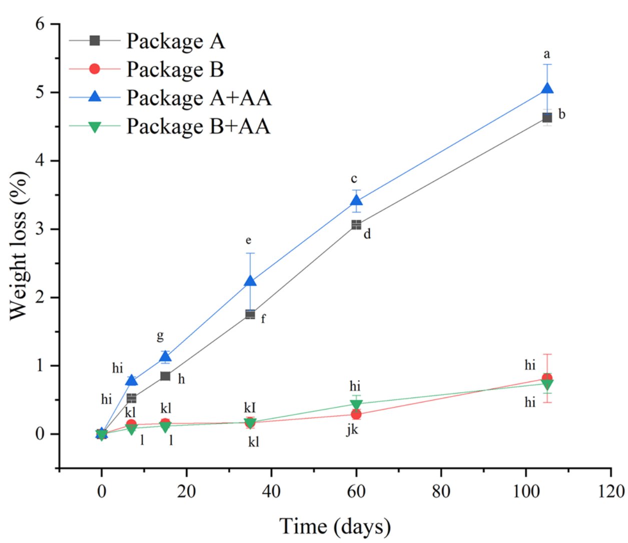


Fig. 3 Weight loss in avocado puree pouches stored at room temperature 23 °C for 104 days. Values with the same superscript letters are not significantly ($p < 0.05$) different

after 104 days storage at room temperature, 4–5% of weight loss was observed for avocado puree package A and package A + AA. The weight loss in package B and package B + AA was around 0.5%. This difference can be attributed to higher water vapor barrier property of metal oxide-coated PET. The WVTR of film B 0.41 g/m^2.day was an order of magnitude lower than film A, which had WVTR of 4.97 g/m^2.day. Patel et al. (2020) reported that weight loss in product packaged in metal oxide–coated PET film–based pouches with WVTRs of 0.83 g/m^2.day and 0.81 g/m^2.day was around 2.7% and 1.9%, respectively. In this study, the results showed that three layers of metal oxide–coated PET-based multilayer film had a better performance in maintaining the moisture of the packaged avocado in comparison with the EVOH-based film. The benefits of lower WVTR to the packaged foods lie in preserving the food color and texture as well as minimizing its weight loss (Patel et al., 2021; Zhang et al., 2019).

Instrumental Color

Color is an attractive feature of any vegetables or fruits and is an important quality parameter to monitor throughout the product shelf life. The most predominant color in avocado is green. The greenness ($-a^*$) of the avocado puree that was packaged in package A and package B, whether with the addition of ascorbic acid or without, was significantly affected ($p > 0.05$) by PATS process, as a^* increased significantly ($p < 0.05$) from an initial value -8.6 ± 0.04 to -4.3 ± 0.21 (average value of all the packages). Similarly, PATS significantly ($p > 0.05$) reduced L^{**} value, from 56.8 ± 0.21 to 52.8 ± 0.36. However, a significant ($p > 0.05$) increase was observed of b^* values after PATS. The total color difference (ΔE) of the avocado puree in different packages just after PATS was similar, ranging between 6.23 ± 0.59 and 6.55 ± 0.32. These ΔE values indicated a noticeable change in the color of the avocado puree by PATS compared to the fresh puree color before processing. The change in puree color may be attributed to a combined effect of the pre-heating and PATS process conditions (Fig. 4). Al-Ghamdi et al. (2022) also observed that the light green color of avocado puree was sensitive to PATS processing conditions, as the light green color of puree turned to dark green color after PATS.

During storage, a^* values of avocado puree in different packages increased significantly ($p < 0.05$). The largest increase of a^* value of all the packaged avocado was during the first month and later, the value did not change significantly, as shown in Fig. 5a. The addition of ascorbic acid did not help in retaining the color of avocado puree during storage. Due to better barrier properties, package B retained the maximum a^* (-0.48 ± 0.07) value, which also falls within the consumer acceptance limit of avocado puree, as reported by López-Malo et al. (1998). At the end

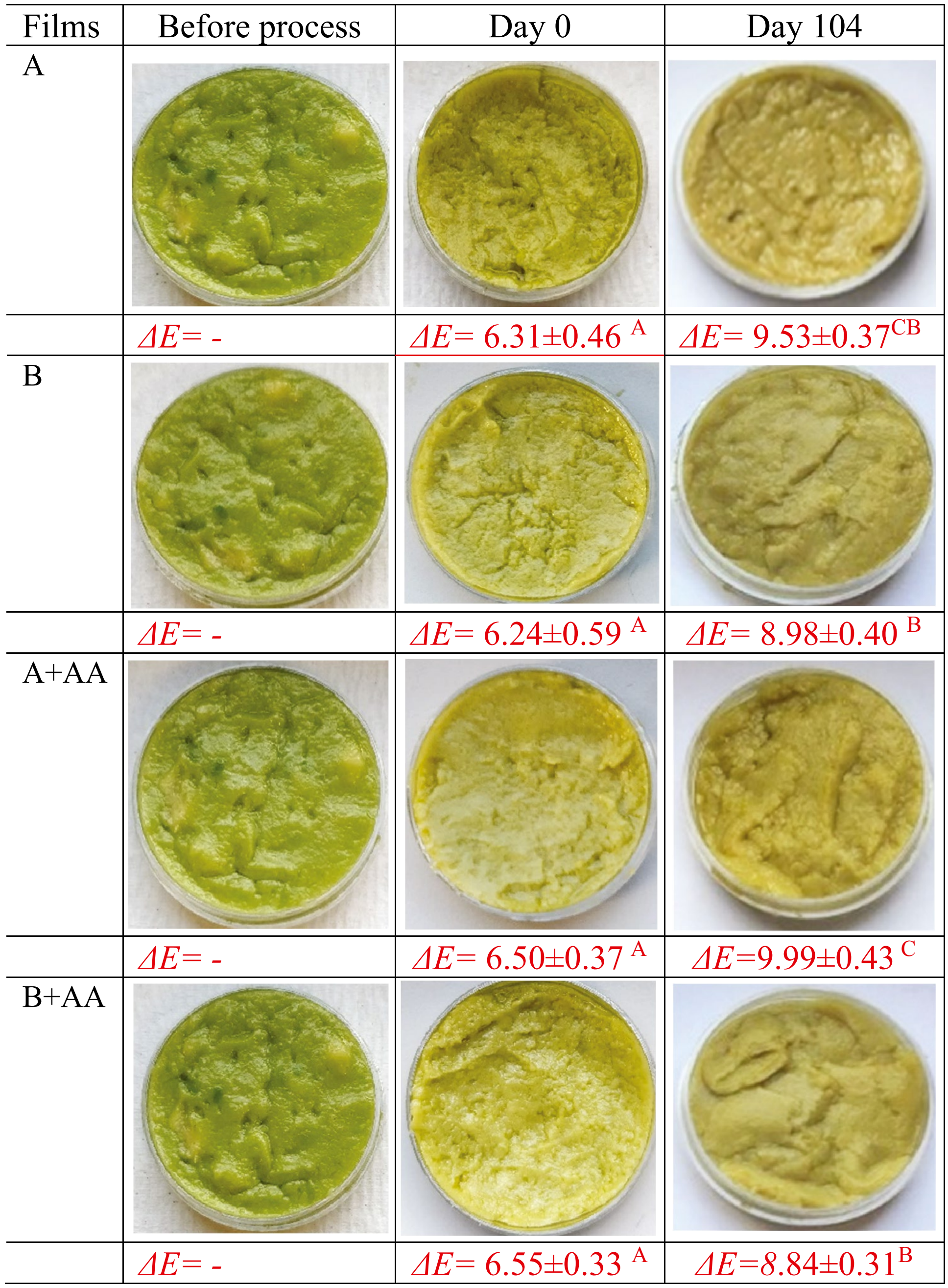


Fig. 4 Representative pictures of the plain and the ascorbic acid avocado purees before, after PATS and at the end of 104-day storage. ΔE, the total color difference calculated by Eq. 1. Values with the same superscript letters are not significantly ($p < 0.05$) different

of storage, the L^* values of puree were as follows: package A, 52.6 ± 0.07; package A + AA, 52.7 ± 0.41; package B, 55.5 ± 0.41; and package B + AA, 55.7 ± 0.13 (Fig. 5b). The changes in instrumental color parameters of packaged avocado puree could be because of PPO activities during storage in the avocado puree. The reactivation of PPO activities in avocado puree during storage after subjected to high pressure treatment was observed by Jacobo-Velázquez and Hernández-Brenes (2010). The higher the PPO activities, the more instrumental color changes will be observed such as decreases in lightness (L^*) and the loss of greenness (a^*) as described by Soliva et al. (2000) in minimally processed avocado. In the present study, the lower L^* values of puree in package A and package A + AA may be attributed to the higher weight loss compared to the puree in package B and package B + AA. Patel et al. (2021) also attributed the decrease in L^* values in chicken pasta to the weight loss in EVOH-based packaging. However, no significant ($p < 0.05$) changes were observed for b^* values in all packages at the end of storage except for package A. At the end of storage, the total color difference (ΔE) values differed significantly ($p < 0.05$) from their encounters after PATS (day 0), ranging between 9.99 ± 0.043

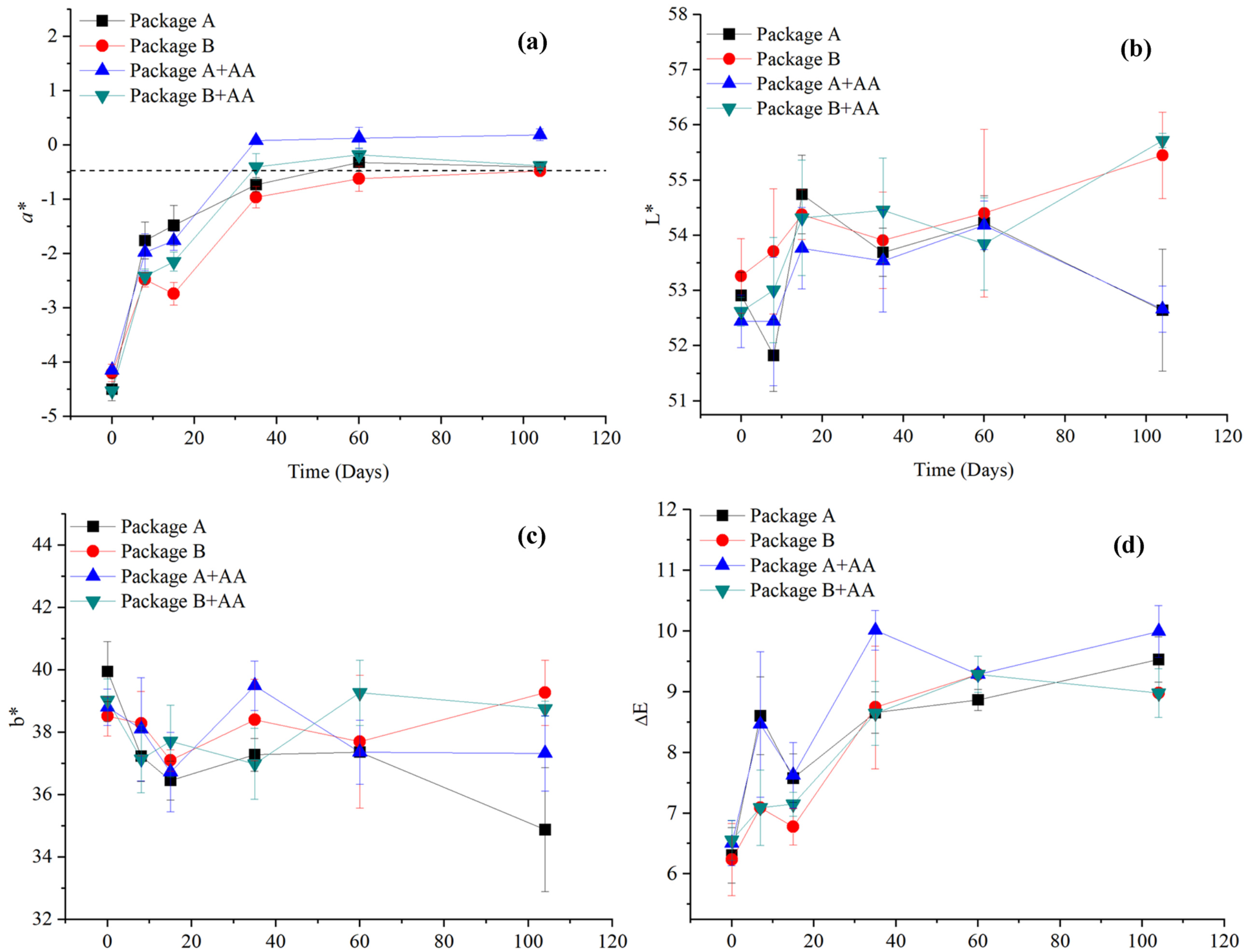


Fig. 5 Changes in instrumental color of the avocado puree packaged in films A and B during the storage period: **a** greenness (dashed line represents the consumer acceptance limit of avocado puree based on (López-Malo et al., 1998), **b** lightness, **c** redness, and **d** total color difference

and 9.53 ± 0.037 additionally. The ΔE values of the avocado puree in film B were lower than film A. However, all packaged avocados maintained ΔE values that are less than 12 at the end of storage period, which indicates that they did not change to a different color. Pasteurized guacamole with high pressure was reported as maintaining a negative a^* value of -6.15 for a few days, and avocado paste with a negative a^* value of -0.47 to approximately 2 months at storage refrigeration temperatures (López-Malo et al., 1998; Palou et al., 2000).

Chlorophyll Measurements

The quality of green vegetables and fruits is often coupled with chlorophyll concentrations. It is thus important to monitor chlorophyll degradations in the processed food during storage. The initial concentration of chlorophyll types a and b in unprocessed avocado was 12.8 ± 1.14 and 9.79 ± 0.68, respectively. PATS processing induced a significant ($p < 0.05$) reduction in the chlorophyll concentration of both the plain and fortified avocado purees. The values of chlorophyll types a and b of PATS processed avocado were 6.93 ± 0.36 (average of all packages) and 4.99 ± 1.23 (average of all packages), respectively. This reduction in chlorophyll concentration can be attributed to thermal and pressure degradation in accordance with the data reported by Al-Ghamdi et al., (2020, 2022). Al-Ghamdi et al. (2022) observed a significant ($p < 0.05$) reduction in chlorophyll types a and b in avocado puree subjected to PATS treatment; similarly, Al-Ghamdi et al. (2020) reported a significant decreased in chlorophyll type a in green peans puree after PATS treatments. The alteration of the green color of thermally processed vegetables is resulted from the formation of pheophytin as well as to pyropheophytin (Schwartz et al., 2008). The degradation of both chlorophyll types a and b in green vegetables was also observed when a high pressure and high temperature treatment at 117 °C was applied (Sánchez et al., 2014). The degradation in green color was accompanied with the production of pheophytins.

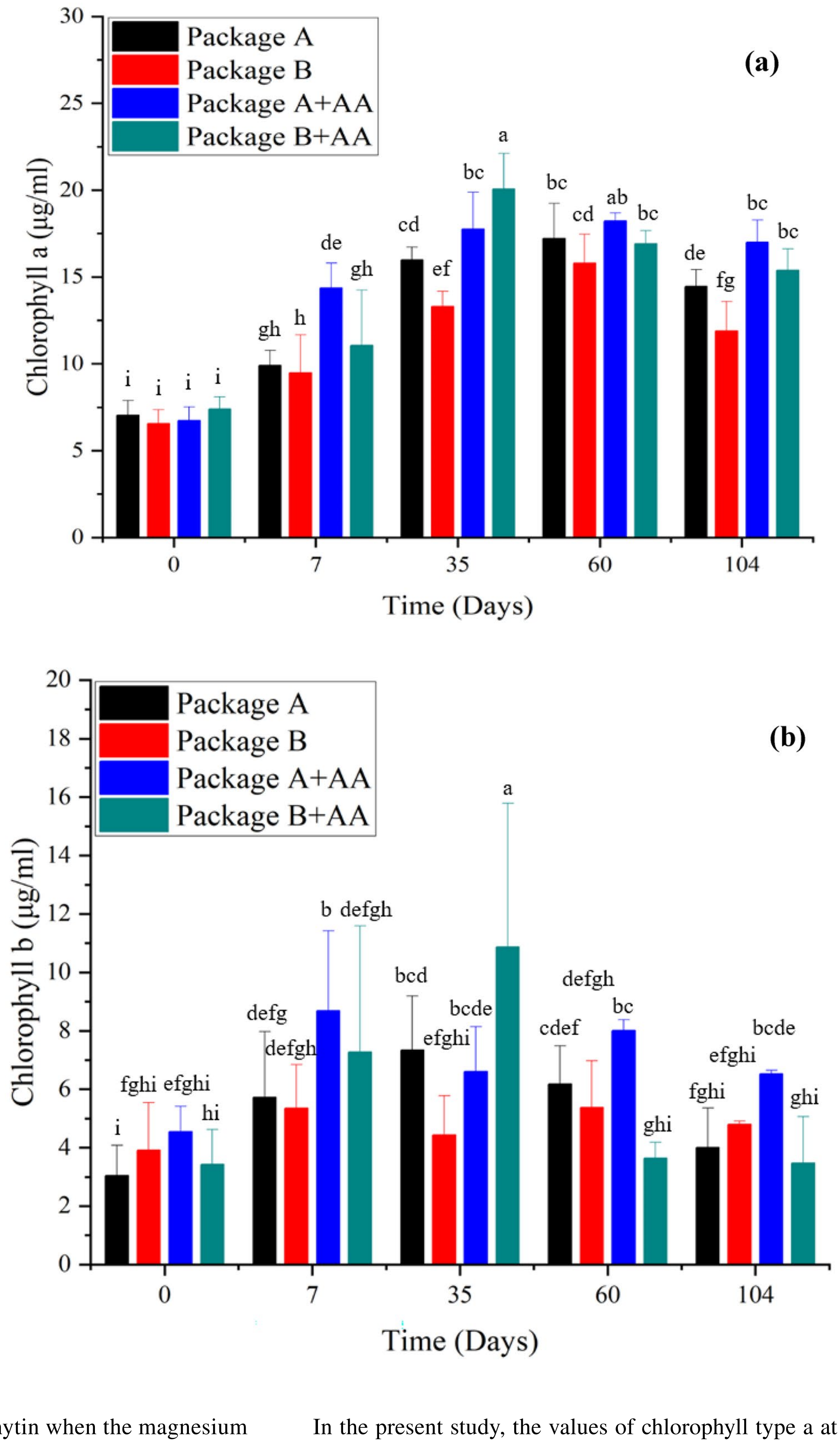


Fig. 6 Chlorophyll trend of PATS processed avocado during storage for 104 days at 23 °C: **a** chlorophyll type a concentration, **b** chlorophyll type b concentration. Values with the same superscript letters are not significantly ($p < 0.05$) different

Chlorophyll degrades to pheophytin when the magnesium atom in chlorophyll is replaced with two hydrogen ions during thermal treatment (Schwartz et al., 2008) leading to an olive-brown color.

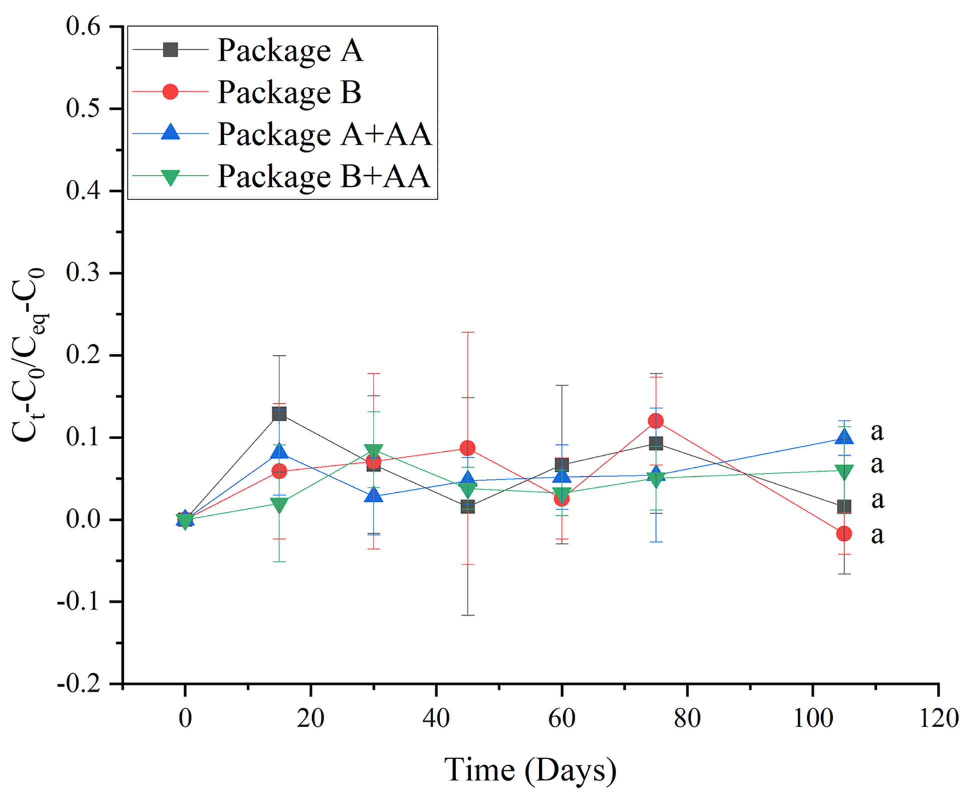
In the present study, the values of chlorophyll type a at the end of the storage period ranged between 17 ± 1.26 and 15.4 ± 1.22, while chlorophyll type b ranged between 4 ± 1.36 and 6.5 ± 0.13. At the end of storage, the concentration of chlorophylls a and b increased significantly, as shown in Fig. 6. The PATS process could have caused a rupture of the plant cell wall and thus more chlorophylls released during storage and the extraction process. However, product stored in medium and low barrier films showed degradation of chlorophylls. For example, Inanoglu et al. (2022) reported a degradation of chlorophyll types a and b by 23% and 67%, respectively, for green beans stored at 2 °C after subjected to a high-pressure treatment combined with a temperature of 48 °C. Moreover, Sonar et al. (2019) observed degradation in chlorophyll types a and b by approximately 30% and 50%, respectively, in thermally-processed green peas stored at 7 °C for almost 3 months. In the present study, although the packaged avocado puree was stored at room temperature for 3 and a half months, there was no significant differences in both chlorophyll types a and b of the plain and ascorbic acid-fortified avocado puree. The relative stability of pH values during storage could have led to chlorophyll pigments stability as pH is a factor that affect chlorophyll stability during storage (Buckle & Edwards, 1970). Overall, the addition of ascorbic acid did not influence the total concentration of chlorophylls in packaged avocado puree during storage.

Oxygen Content in Packaged Purees

Figure 7 shows oxygen concentration in packaged purees measured in this study, and it is very important due to the oxygen sensitivity of avocado puree. The initial dissolved oxygen concentration measured in avocado puree was 6.3% and 6.5% in package A and package B, respectively. The initial oxygen concentration in avocado puree in package A + AA and package B + AA was 3.1% and 3.4%, which was lower than package A and package B, likely due to the presence of ascorbic acid. After the PATS process, the oxygen concentration in avocado purees did not change significantly ($p > 0.05$) in all packages. Sonar et al. (2020) reported that the oxygen concentration in thermally processed beet mixed mashed potato fortified with ascorbic acid was less than salmon in sauce. They attributed this reduction in oxygen content to the consumption of oxygen due to the presence of both ascorbic acid and betalains.

No significant ($p < 0.05$) changes occurred to the dissolve oxygen concentration in the avocado puree for all packages during the storage of nearly 3 and half months. The oxygen concentration in product during storage depends on oxygen ingress through the multilayer films, which is affected by oxygen transmission rates of the films used, and oxygen being consumed by food matrix, which is affected by its chemical composition. Avocado is rich in proteins and unsaturated fats, making it prone to oxidation reactions, and the addition of ascorbic acid can also react with oxygen and lower its concentration in the product. Zhang et al. (2016) observed different trends for the oxygen content in thermally processed mashed potato packaged in polymeric films having

Fig. 7 Dissolve oxygen concentration ($n = 3$) in avocado puree during storage at 23 °C for 104 days. Values with the same superscript letters are not significantly ($p < 0.05$) different



different OTRs, ranging from 0.07 to 2.11 cc/m^2·day, stored at 50 °C for 12 weeks. There was a reduction in the dissolve oxygen concentration in packaged mashed potato that was packaged in packaging materials with low OTR during the first week of the storage at room temperature. On the other hand, there was a significant ($p < 0.05$) increase in the dissolved oxygen concentration that was packaged in packaging materials with high OTR during the first week of the storage period as well. These changes in oxygen content were linked to the barrier properties of the packaging materials. In a complex food matrix such as avocado puree enriched with ascorbic packaged in finite oxygen barrier packaging, complex chemical and biological reactions could take place where oxygen is consumed and released, which makes it challenging to predict the oxygen content in the food system without quantifying the rate of oxygen consumption in various reactions and knowledge of oxygen ingress through packaging films and leaks (Chaix et al., 2014; Zhang et al., 2016).

Lipid Oxidation

The lipid oxidation trend (TBARS, mg of MDA kg^{-1} of the sample) in avocado puree after PATS and during storage period is illustrated in Fig. 8. After the PATS process, the TBARS values in avocado puree were 1.50 ± 0.22, 1.57 ± 010, 1.51 ± 0.24, and 1.53 ± 0.17 mg of MDA kg^{-1} for package A, package B, package A + AA, and package B + AA, respectively. At the end of storage, these values became 1.14 ± 0.10, and 1.11 ± 0.29, 1.10 ± 0.18, and 1.11 ± 0.26 mg of MDA kg^{-1} for package A, package B, package A + AA, and package B + AA, respectively. Overall, the TBARS values were relatively stable with downward trends in all packages during storage. In addition, there were no significant ($p < 0.05$) changes observed in the TBARS values despite the addition of ascorbic acid or the package types. Additionally, at the end of the storage, there were no significant ($p < 0.05$) differences in the TBARS values between the films. Elez-Martínez et al. (2006) reported that K_{270}, which is an indicator of secondary oxidation, of minimally processed avocado purees, ranged between 0.517 and 1.587 stored at refrigeration conditions for almost 6 months.

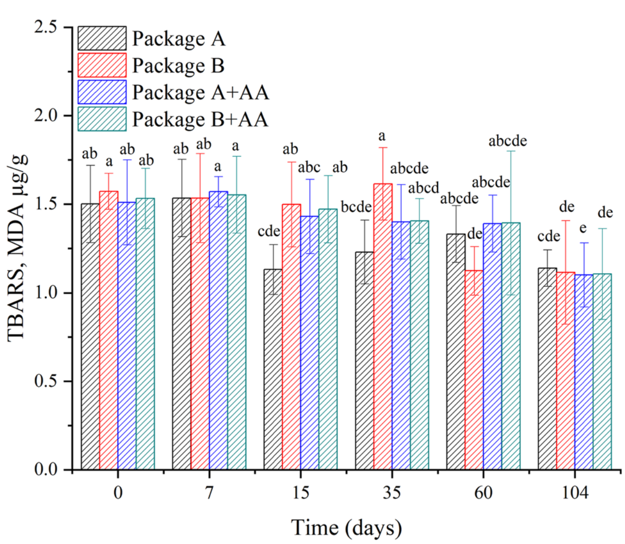


Fig. 8 2-thobarbituric acid reactive substance (TBARS) ($n = 6$) of the plane and fortified avocado puree packaged in films A and B during storage at room temperature for 104 days. Values with the same superscript letters are not significantly ($p < 0.05$) different

The rate of lipid oxidation reaction during storage in packaged foods is dependent on the availability of oxygen inside the package, which is related to the oxygen transmission rate (OTR) of the packaging film (Bhunia et al., 2017; Patel et al., 2021). In this study, the maximum normalized dissolve oxygen concentration in avocado puree during storage was 0.11–0.13 Thus, the low oxygen content inside the packages and higher oxygen barrier property of multilayer films might have led avocado puree to undergo less of an oxidation reaction during storage. Sonar et al. (2019) reported a correlation between the headspace oxygen level inside the package and the trend of TBARS values in several food products that were stored at 4 °C for 10 days. On the other hand, the addition of ascorbic acid did not show any significant ($p < 0.05$) effect on the TBARS values in the avocado during the 3 months at room temperature. This could be because the storage period was short and not enough to show the effect of ascorbic acid on the TBARS values. We did not conduct formal sensory analysis to correlate with the TBARS value; however, the TBARS value of < 1.51 mg kg^{-1} is considered not or slightly rancid and acceptable to consumer for fish products (Secci & Parisi, 2016). Sensory studies need to be conducted for development of commercially acceptable shelf stable PATS avocado puree.

pH and Total Plate Counts

The pH value of the unprocessed plain avocado puree was 6.66 ± 0.06, while the value of ascorbic acid enriched unprocessed avocado puree was 6.76 ± 0.01. After PATS, the pH value for all packaged purees decreased significantly ($p < 0.05$) to values of 5.35 ± 0.04 for package A and 5.38 ± 0.03 for package B, and 5.35 ± 0.03 for package A + AA, and 5.40 ± 0.02 for package B + AA (Table 3). The decrease in pH values could be attributed to the release of the acidic compounds from the food matrix (Al-Ghamdi et al., 2020). However, during the storage, the pH values were stable, and no significant ($p < 0.05$) changes were observed for all packages, except for package B. The total plate counts for both anaerobic and aerobic microorganisms were below the detection limit after PATS as well as at the end of storage, as no plated samples were detected for growing colonies in

Table 3 pH values of the processed avocado after PATS and during storage

Storage time (days)	pH values			
	Package A	Package B	Package A + AA	Package B + AA
0	5.35 ± 0.04[efg]	5.38 ± 0.03[defg]	5.35 ± 0.03[efg]	5.40 ± 0.02[bcde]
7	5.29 ± 0.15 [fg]	5.39 ± 0.02[cdefg]	5.35 ± 0.01[efg]	5.36 ± 0.04[defg]
15	5.38 ± 0.02[defg]	5.42 ± 0.03[bcde]	5.34 ± 0.04[efg]	5.47 ± 0.04[bcd]
35	5.49 ± 0.06[abc]	5.58 ± 0.07[a]	5.36 ± 0.16[defg]	5.43 ± 0.03[bcde]
60	5.29 ± 0.06 [g]	5.40 ± 0.01[bcdef]	5.36 ± 0.05[defg]	5.33 ± 0.08[efg]
104	5.35 ± 0.05[defg]	5.51 ± 0.031[ab]	5.35 ± 0.04[defg]	5.45 ± 0.03[bcde]

Values with the same superscript letters are not significantly ($p < 0.05$) different

the avocado puree samples. These observations are consistent with the data reported by Al-Ghamdi et al. (2022) after similar processing conditions.

Conclusion

The results of this study show the feasibility of developing PATS-processed shelf-stable avocado puree. Both EVOH and metal oxide–coated PET-based packages were able to withstand selected high pressure and high temperature treatment; however, there was minor deterioration in the barrier properties of EVOH-based packaging and a very good vacuum is required to minimize the damage to the packaging. Three-layer AlO_x-coated PET-based packaging was superior in terms of reducing the weight loss and total color change at the end of 3-month storage at 23 °C. The high barrier packaging also controlled the oxidation of lipids. The addition of ascorbic acid did not help maintain the color of avocado puree; hence, other natural ingredients must be investigated in the future studies. Chlorophyll types a and b retention was not different for the puree fortified with ascorbic acid during storage. The PATS process produced shelf-stable avocado puree, as confirmed by undetectable anaerobic and aerobic total plate counts. However, based on the outcome of this study, optimization of process and puree formulation as well as more than three months storage and sensory studies are needed to develop consumer acceptable PATS-processed avocado puree with extended shelf life at room temperature.

Author Contribution Zeyad Albahr: conceptualization, methodology, investigation, data curation, writing original draft, formal analysis, resources. Saleh Al-Ghamdi: methodology, data curation, investigation, reviewing and editing. Juming Tang: reviewing and editing, resources, supervision. Shyam Sablani: conceptualization, reviewing and editing, supervision, project administration, funding.

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Availability of Data The data that support the findings of this study are available from the corresponding author, Shyam Sablani, upon reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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