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# Stability of color, β-carotene, and ascorbic acid in thermally pasteurized carrot puree to the storage temperature and gas barrier properties of selected packaging films

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## Abstract

Quality of pasteurized products can be affected by the combination of temperature abuse and gas barrier properties, especially oxygen transmission rate (OTR), of packaging films. Carrot puree was thermally pasteurized (90 °C, 14 min) and stored in three different types of pouches (OTR—0.99 ± 0.05 [F-1], 29.8 ± 1.38 [F-30], and 80.9 ± 2.15 [F-81] $cm^3\ m^{-2}\ day^{-1}$) at different temperatures (4, 8, and 13 °C). Weight loss, pH, instrumental color, β-carotene, and ascorbic acid were evaluated over a 100-day storage period. Results show that film OTR and temperature had a significant ($p < 0.05$) effect on food quality and nutritional attributes. High-barrier film (F-1) retained quality, while low-barrier film (F-81) increased losses during processing and storage for all parameters tested. High-barrier film demonstrated stable color values ($a^*$ and $b^*$) and significantly ($p < 0.05$) higher retention of β-carotene during storage period. Ascorbic acid retention varied from 0 to 89%, depending upon packaging type. Based on ascorbic acid degradation kinetics, activation energy and $Q_{10}$ values ranged from 20.3–72.3 kJ/mol and 1.36–2.96, respectively.

## Practical applications

Pasteurized food products have limited shelf life under refrigerated conditions. The shelf life of these products can be reduced by the combined effect of temperature abuse and gas barrier properties of packaging films. There is scarce information in the literature focusing on the shelf life studies of in-pack thermally pasteurized products as a function of gas barrier properties. The selection criteria of films for pasteurized products are complex. Generally, higher the barrier properties needed higher is the cost of packaging. This study demonstrated the suitability of medium-barrier films for in-pack pasteurization; and combined effect of temperature abuse and gas barrier properties on the nutrient retention.

## 1 | INTRODUCTION

Carrots are an excellent source of carotenoids, an important micronutrient for human health (Chen, Peng, & Chen, 1996). β-Carotene is the major carotenoid in carrots with antioxidant and provitamin A activity (Knockaert, Lemmens, Van Buggenhout, Hendrickx, & Van Loey, 2012). However, since β-carotene is unsaturated, it is highly prone to oxidation and isomerization due to heat, oxygen, light, acid, metal ions, and free radicals. This results in a loss of color and nutritive benefits (Boon, McClements, Weiss, & Decker, 2010). β-Carotene stability

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depends on the physical form of carotenoids in the food matrix (Provesi, Dias, & Amante, 2011). Since carrots are not high in vitamin C, processed carrot products such as juice and puree are often fortified with vitamin C.

Vitamin C, also known as L-ascorbic acid, cannot be endogenously synthesized in human body, and is an essential dietary component that is necessary for many biological and physiological functions (Capuano, Oliviero, & van Boekel, 2018). However, it is the most labile vitamin, and is highly susceptible to oxidation. The deterioration of vitamin C depends upon temperature, oxygen concentration, pH, metal cations, light, and water activity (Torregrosa, Esteve, Frígola, & Cortés, 2006). Studies show that the gas barrier properties of packaging material, especially the oxygen transmission rate (OTR), can negatively affect ascorbic acid stability in processed food products (Robertson, 2012).

Carrots are often consumed either raw or processed in the form of juice, puree, canned, frozen and as an ingredient in many food items. Processing increases shelf life but can affect quality depending upon the type of process selected. Thermal pasteurization enhances shelf life of foods to few days or few weeks under refrigerated conditions depending on the degree of heat treatment given to the product. According to the European Chilled Food Federation (2006), pasteurization processes equivalent to $F_{70\ ^\circ C}$ = 2 min for 6-D inactivation of *Listeria monocytogenes* can provide ≤10 days of shelf life and pasteurization processes equivalent to $F_{90\ ^\circ C}$ = 10 min for 6-D inactivation of nonproteolytic *Clostridium botulinum* can provide shelf life of up to 6 weeks at 5 °C. Any temperature abuse during this distribution channel can negatively affect the quality and safety of a product, and thus its shelf life. Therefore, an effective cold chain throughout the distribution system of pasteurized products followed by proper refrigerated storage at home plays a major role in keeping pasteurized products safe. The mean refrigerator temperature in consumers' homes is one of the most vulnerable part of the distribution chain. This often varies between 6–10 °C, and many domestic refrigerators are operated above 10 °C (James, Evans, & James, 2008).

The stability of pasteurized products in terms of chemical, nutritive and microbiological qualities can be influenced by the combinations of storage temperatures and gas barrier properties of polymeric packaging. Gas barrier properties, that is, the OTR and water vapor transmission rate (WVTR), play a crucial role in maintaining the shelf life of products. Selection of optimum packaging material for pasteurized products is complex. The selection process is determined by several factors including product composition, package properties (mechanical, optical, and gas barrier), and cost. High-barrier films typically used for sterilized products are not ideal for pasteurized products due to over-packaging and increased cost. Because pasteurized products are stored at refrigerated conditions with few days to few weeks of shelf life, low to medium-barrier films can generally be used. Storage studies using a range of films with different barrier properties can inform the selection of appropriate films for products with a short shelf life. However, few storage studies have examined the effects of gas barrier properties of polymeric packaging material on thermally pasteurized products (Bhunia, Ovissipour, Rasco, Tang, & Sablani, 2017; Kim, Paik, & Lee, 2003).

The temperature dependency of a chemical reaction on a small temperature range can be expressed with a temperature quotient, $Q_{10}$ value. This value indicates how rapidly a reaction proceeds when the temperature is increased by 10 °C (Robertson, 2012). Petrus, Loiola, and Oliveira (2010) reported the $Q_{10}$ value of pasteurized milk packed in high-density polyethylene bottle and low-density polyethylene pouch, but there is little data on other pasteurized and refrigerated food products.

The objective of this study was to investigate the quality of thermally pasteurized carrot puree in polymeric packaging materials with varied OTRs and at three different refrigerated storage temperature conditions. Water loss, color change, pH, β-carotene content, and ascorbic acid content were used as quality parameters. Quality analysis data was then used to conduct a kinetic study and determine the $Q_{10}$ value. Findings demonstrate the cumulative effects of oxygen permeation through packaging film and storage temperature on the quality of carotenoid-rich food products. This study improves our understanding of degradation kinetics to inform the selection of suitable packaging material for thermally pasteurized products.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials and reagents

Fresh carrots (Bolthouse Farms, Bakersfield, CA) were purchased from a local supermarket. Vitamin C (ascorbic acid, pure powder) used for fortification was purchased from Now Foods Co., Bloomingdale, IL. Vitamin C (L-Ascorbic Acid, BioXtra, >99%) and meta-phosphoric acid for high performance liquid chromatography (HPLC) measurement were purchased from Sigma-Aldrich Co. (St. Louis, MO). Potassium phosphate monobasic and HPLC grade water were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Butylated hydroxytoluene (BHT) was purchased from ACROS Organics (Geel, Belgium). Acetone (ACS grade), hexane (ACS grade) and o-phosphoric acid (ACS grade) were purchased from Avantor Performance Materials (Center Valley, PA). Ethanol (USP grade) was purchased from Decon Labs, Inc. (King of Prussia, PA).

### 2.2 | Polymeric film pouches

Three types of polymeric films with varied gas barrier properties were selected from three different manufacturers and designated as F-1, F-30, and F-81 based on their OTR. Films were used to make uniform pouches of 110 × 150 mm ($W \times H$) dimension using Koch Easy Pack vacuum sealer (Ultrasource LLC., Kansas City, MO). The OTR of the films ($n$ = 3) was measured following ASTM standard method D3985 using an Ox-Tran 2/21 instrument (Mocon, Inc., Minneapolis, MN) at 23 °C, 55 ± 1% RH and 1 atm (ASTM 1995). The WVTR ($n$ = 3) was measured following the ASTM standard method F372-99 using a Permatran 3/33 instrument (Mocon, Inc., Minneapolis, MN) at 100% RH and 38 °C (ASTM 1990). Film structure, thickness, OTR, and WVTR of three films were given in Table 1.

**TABLE 1** Details of packaging films used for study

| Film | Structure | Thickness (μm)[a] | OTR ($cm^3\ m^{-2}\ day^{-1}$)[b] | WVTR ($g\ m^{-2}\ day^{-1}$)[b] |
|---|---|---|---|---|
| F-1 | PET/LLDPE/LDPE/Tie/Nylon66/Tie/LLDPE/LDPE | 102 ± 2.73 | 0.99 ± 0.05 | 3.94 ± 0.03 |
| F-30 | LDPE/Tie/Nylon/Tie/LDPE | 86.4 ± 1.36 | 29.8 ± 1.38 | 4.18 ± 0.06 |
| F-81 | PET-PE based | 70.0 ± 1.26 | 80.9 ± 2.15 | 6.59 ± 0.03 |

Abbreviations: LDPE, low density polyethylene; LLDPE, linear low density polyethylene; OTR, oxygen transmission rate; PET, polyethylene terephthalate; WVTR, water vapor transmission rate.
[a]Based on five replicates ($n$ = 5).
[b]Based on three replicates ($n$ = 3).

## 2.3 | Puree preparation

Carrots were washed with water, peeled and cut into 2 or 4 pieces depending on the size (longitudinally in half and then each half into halves) before blanching. Blanching was done with food grade steam at 98 °C for 5 min. The blanched carrots were cooled down immediately by rinsing in room temperature water. Blanched carrots were mixed with equal amount of water and fortified with 600 mg/kg of ascorbic acid. They were then blended for 1 min using kitchen blender to obtain a uniform puree. The puree was prepared in small batches of 700 g each and mixed together for another 5 min with a mixer after every 15 batches. Two hundred-gram portions of puree were filled into each pouch and vacuum sealed at a 0.8 bar pressure using Ultravac vacuum sealer (Ultrasource LLC., Kansas City, MO). Headspace in the vacuum-packed pouches ($n$ = 6) was measured following Dunno, Whiteside, Thomas, and Cooksey (2015) and found to be 0.6 ± 0.3 $cm^3$.

## 2.4 | Pasteurization and storage

In-pack thermal pasteurization was carried out by immersing pouches in hot water in a steam-jacketed kettle maintained at water temperature of 92 ± 1 °C. Total processing time was determined by preliminary experiments, in which the core temperature of the product in the pouch was monitored with a T-type thermocouple (Omega Engineering, Stamford, CT). Time–temperature data ($n$ = 2) was recorded by a data logger system. Processing time was designed to achieve 6-log reduction of psychrotrophic, nonproteolytic *C. botulinum* type E that is, $P^{10^\circ C}_{90^\circ C}$ = 10 min. The following equation was used to calculate the $P$ value:

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

where $T$ ( °C) is the temperature measured at the cold spot at time $t$ during processing, $T_{ref}$ is the reference temperature (90 °C), and $z$ is the $z$-value of the target bacteria in the products (10 °C). The pouches were cooled with room temperature water at 23 °C and kept in a chilled room at 4 °C for approximately 3 hr before transfer into the storage area. Total processing time was 50 min including heating for 35 min and cooling for 15 min with a cumulative lethality of 14.0 ± 0.07 min.

A total of 144 pouches was randomly divided and stored at three different temperatures: 4, 8, and 13 °C for 100, 80, and 45 days, respectively, in dark conditions. The higher storage temperature was selected to understand the temperature abuse scenario during rupture of cold chain. Domestic refrigerators (365 L; GE Company, Louiseville, KY) were used to store pouches at 4 and 8 °C, and a lab incubator (238 L, MIR-254, Panasonic Healthcare Corporation of North America, Wood Dale, IL) was used for pouches at 13 °C. The relative humidity of storage environment was about 20%. Before storage, the initial weight of the pouches was measured. Duplicate pouches were taken out at each time interval (Day 16, 31, 45, 60, 80, 100 at 4 °C; Day 10, 21, 32, 45, 60, 80 at 8 °C; and Day 8, 14, 20, 27, 34, 45 at 13 °C), with two replicates drawn from each pouch for quality analysis. The 0th day sample was drawn before transferring pouches into storage area and used to see the pasteurization effect. Before drawing sample for quality analysis, the pouch contents were uniformly mixed.

## 2.5 | Weight loss and moisture content

Weight loss was measured to determine migration of water from the inside of the pouch to the storage environment. Duplicate pouches were used to calculate weight loss. Moisture content of the puree ($n$ = 4) was measured gravimetrically using a hot air oven at 105 °C for 24 hr (AOAC, 950.46).

## 2.6 | pH

For pH measurement, 5 g puree was uniformly mixed with 45 mL of deionized water. The pH of the solution ($n$ = 4) was measured by immersing the electrode into the sample. The pH value was obtained until the value stabilized, as indicated by the pH meter (Seven Go SG2, Mettler Toledo, Schwerzenbach, Switzerland).

## 2.7 | Color measurement

Instrumental color was measured using reflectance measurement with a CM-5 spectrophotometer (Konica Minolta, Ramsey, NJ) with the specular component excluded, an observer angle of 10°, an 8 mm measurement area, and illuminant D65 (Daylight, color temperature 6,504 K). Two samples were drawn from each pouch. Approximately the same amount of puree was place into a plastic petri dish, and color was measured at three locations for each sample. Color ($n$ = 12) was recorded using the CIE $L^*$ $a^*$ $b^*$ color space, where $L^*$ corresponds to lightness, $a^*$ to red/green chromacity, and $b^*$ to yellow/blue chromacity. Total color difference was calculated using the following equation:

$$\Delta E = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2}, \quad (2)$$

where $L_0^*$, $a_0^*$, and $b_0^*$ stand for the initial values of $L^*$, $a^*$, and $b^*$ immediately after processing. An SLR camera system (EOS 60D, Canon, Inc., NY) was used to observe visual changes during the storage period.

## 2.8 | β-Carotene

β-Carotene was extracted from the carrot puree and quantified by a spectrometer, following Leong and Oey (2012) with some modifications. Five grams of puree was homogenized with 30 mL extraction solvent of 50% hexane, 25% acetone, and 25% ethanol with 0.1% (w/v) BHT, using a Polytron PT 2500E (Kinematica, Bohemia, NY) homogenizer in a 50 mL tube for 5 min at 7,000 rpm. The homogenate was vacuum filtered through a filter paper (Whatman No. 1, GE Healthcare Bio-Sciences, Pittsburgh, PA), and the remaining solids were homogenized again in 10 mL of solvent for 1 min. The homogenate was again filtered, combined into a separatory funnel, and manually shaken for few seconds, then held until the organic and aqueous phase separated. The lower aqueous phase was discarded, while the upper organic phase was filtered using filter paper and collected in a 50 mL volumetric flask. The final solution was diluted to 50 mL with extraction solvent and measured for β-carotene content using a spectrometer (Ultraspec 4000, Pharmacia Biotech, Inc., Piscataway, NJ). The absorbance of the solution was measured at 450 nm against a blank of extraction solvent. The β-carotene content ($n$ = 4) was quantified using the equation:

$$\text{Carotenoid content}\,(\mu\text{g/g}) = \frac{A \times \text{volume}\,(\text{mL}) \times 10^4}{E_{1\text{cm}}^{1\%} \times \text{sample weight}\,(\text{g})}, \quad (3)$$

where $A$ is absorbance at 450 nm, $E_{1\text{cm}}^{1\%}$ is extinction coefficient (2,560 for β-carotene in hexane).

## 2.9 | Ascorbic acid

The ascorbic acid content was analyzed by HPLC following the method of Scherer et al. (2012) with some modifications. Three grams of puree was homogenized with 10 mL 3% (w/v) meta-phosphoric acid using a Polytron PT 2500E (Kinematica, Bohemia, NY) homogenizer in a 50 mL tube for 1 min at 7,000 rpm. The homogenate was left for extraction at room temperature (23 °C) for 2 hr. Next, tubes of the homogenates were centrifuged at 8,000 rpm (accuSpin 400, Fisher Scientific, Pittsburgh, PA) for 6 min at room temperature. The supernatant was filtered through 0.45 μm nylon filter. Ten microliters of aliquot was injected into Agilent 1100 HPLC system (Agilent Technology, Santa Clara, CA) using a RP18 5 μm 4.6 × 250 mm column (Waters Corporation, Milford, MA) that was equipped with a diode array detector. The mobile phase was 0.01 mol/L monopotassium phosphate solution adjusted to pH = 2.6 with o-phosphoric acid. The separation was a 15-min isocratic elution procedure with a flow rate of 0.5 mL/min and column temperature of 25 °C. The detection wavelength was 250 nm. Quantification was performed with a calibration line that was built using 2, 5, 10, 15, 20, 30, 40, 50, 60, and 100 mg ascorbic acid/100 g. Results were expressed as mg of ascorbic acid per 100 g of carrot puree ($n$ = 4).

## 2.10 | Data analysis

Quality changes at a given temperature, in general, can be expressed using the following equation:

$$\frac{dC}{dt} = -kC^n, \quad (4)$$

where $k$ is the reaction rate constant, $C$ is the quality attribute at a given time, and $n$ is the order of the reaction. Integrated forms of the Equation (4), zero-order and first-order kinetic models, were used to find the best empirical relationship:

$$\text{Zero}-\text{order}: C_t = C_0 - kt, \quad (5)$$

$$\text{First}-\text{order}: \ln(C_t) = \ln(C_0) - kt. \quad (6)$$

The Arrhenius relationship was used to determine the effects of temperature on degradation rate:

$$k = k_o \exp\left(-\frac{E_a}{RT}\right), \quad (7)$$

where $E_a$ is the activation energy of the reaction (kJ/mol), $R$ is the universal gas constant (8.314 J/K mol), and $T$ is the absolute temperature (K). The activation energy was obtained from the slope of the graph on plotting rate constant against the reciprocal of the absolute temperature ($1/T$) on a semi-logarithmic scale.

The rate of quality change with temperature was also described using $Q_{10}$ value. When the temperature difference is not 10 °C, $Q_{10}$ can be expressed using equation (Robertson, 2012):

$$Q^{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2 - T_1}}, \quad (8)$$

where $k_1$ and $k_2$ are reaction rate constant at temperature $T_1$ and $T_2$, respectively.

Data was analyzed with OriginPro 8 (OriginLab, Inc., MA) and JMP 13 (SAS Institute, Inc., Cary, NC), using Tukey's honestly significant difference (HSD) at $\alpha$ = 0.05. The General Linear Model (GLM) was employed using the packaging film OTR, storage temperature and time as independent variables. The effect of thermal treatment on quality parameters was also analyzed using one-way ANOVA with Tukey's test at a 95% confidence level.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Weight loss

The WVTRs of three selected films were similar, between 3.94 and 6.59 g $m^{-2}$ $day^{-1}$ (Table 1). The initial moisture content of carrot

puree was 94.5 ± 0.1% (wet basis) and the moisture content remained stable throughout the storage period for all three types of pouches. For the F-1, F-30 and F-81 pouches, the total weight loss was less than 0.5% during storage irrespective of temperature. Maximum weight loss was observed in F-81 pouches which correlated with the highest WVTR among the selected films. Weight loss for F-81 pouches at the end of the storage period was 0.35 ± 0.01%, 0.42 ± 0.01%, and 0.26 ± 0.01% at 4, 8, and 13 °C, respectively. Weight loss, which is mainly due to moisture migration from inside of the pouch to the environment, can be very high for low barrier films if they are stored at elevated temperatures for long periods. Zhang, Tang, Rasco, Tang, and Sablani (2016) reported weight losses of 14.2, 9.2, and 1.5% in microwave assisted thermally sterilized mashed potato packed in polymeric pouches with a WVTR of 2.64, 1.78, and 0.29 g $m^{-2}$ $day^{-1}$, respectively, after 12-weeks of storage at 50 °C. Lesser weight loss in our study could be due to the low storage temperature and relatively short storage period.

## 3.2 | pH

The pH of the fortified unprocessed carrot puree was 5.85 ± 0.04. Results show that the packaging film OTR, storage temperature and time significantly ($p < .05$) affected pH values during storage period (Table 2). After pasteurization, the pH of the puree decreased slightly, and remained stable in F-1 and F-30 pouches during the storage period except for F-30 pouch on the 45th day of storage at 13 °C. However, the pH of carrot puree packed in F-81 pouches started decreasing from the 31st day of storage period at all three temperatures. The pH drop in the F-81 pouch was slowest at 4 °C followed by 8 and 13 °C.

Microbiological analysis was not performed since this study focused on changes in physiochemical properties during storage. The pH drop in the F-81 pouch (OTR—80.9 $cm^3$ $m^{-2}$ $day^{-1}$) may be due to the ingress of oxygen into the pouch during storage, supporting growth of aerobes, and facultative anaerobes. However, visible spoilage indicators such as bulging, putrid smell, or discoloration were not observed in any of the pouches at any time. The pasteurization process was designed for a 6D inactivation of nonproteolytic type *C. botulinum* which is not sufficient for inactivation of other thermoduric spores of *Bacillus* and *Clostridium* species. These species are widely distributed in soil and can grow at refrigeration temperatures (Postollec et al., 2012). Carlin, Guinebretiere, Choma, Pasqualini, and Braconnier (2000) identified *Bacillus* species as a dominant spoilage bacteria in commercially pasteurized vegetable purees including carrots at 4 °C and abused temperatures. They also observed a pH drop to 5.5 in carrot puree after storage at 10 °C from an initial average pH between 6.0 and 6.5 for all vegetables.

## 3.3 | Instrumental color

Results show a significant ($p < 0.05$) decrease in $a^*$ (redness) value in carrot puree in all three types of pouches following thermal processing (Table 3). However, only $L^*$ (lightness) and $b^*$ (yellowness) changed

**TABLE 2** pH of carrot puree during storage period at three different storage temperatures

| 4 °C | | | | 8 °C | | | | 13 °C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | F-1 | F-30 | F-81 | Day | F-1 | F-30 | F-81 | Day | F-1 | F-30 | F-81 |
| 0 | 5.78 ± 0.06$^{aA}$ | 5.74 ± 0.00$^{aA}$ | 5.79 ± 0.00$^{aA}$ | 0 | 5.78 ± 0.06$^{aA}$ | 5.74 ± 0.00$^{aA}$ | 5.79 ± 0.00$^{aA}$ | 0 | 5.78 ± 0.06$^{aA}$ | 5.74 ± 0.00$^{aA}$ | 5.79 ± 0.00$^{aA}$ |
| 16 | 5.79 ± 0.03$^{abA}$ | 5.84 ± 0.01$^{bB}$ | 5.82 ± 0.01$^{aAB}$ | 10 | 5.81 ± 0.01$^{abA}$ | 5.82 ± 0.03$^{abA}$ | 5.80 ± 0.02$^{aA}$ | 8 | 5.83 ± 0.09$^{aA}$ | 5.85 ± 0.03$^{abA}$ | 5.76 ± 0.02$^{abA}$ |
| 31 | 5.74 ± 0.03$^{aA}$ | 5.73 ± 0.03$^{bB}$ | 5.73 ± 0.01$^{bA}$ | 21 | 5.88 ± 0.02$^{abA}$ | 5.81 ± 0.03$^{abB}$ | 5.78 ± 0.03$^{aB}$ | 14 | 5.84 ± 0.05$^{aA}$ | 5.88 ± 0.03$^{abA}$ | 5.73 ± 0.01$^{abB}$ |
| 45 | 5.81 ± 0.05$^{abA}$ | 5.82 ± 0.00$^{bA}$ | 5.70 ± 0.01$^{bB}$ | 32 | 5.80 ± 0.02$^{abA}$ | 5.84 ± 0.09$^{abA}$ | 5.69 ± 0.00$^{bB}$ | 20 | 5.77 ± 0.06$^{aA}$ | 6.08 ± 0.21$^{bB}$ | 5.73 ± 0.04$^{abA}$ |
| 60 | 5.90 ± 0.06$^{bA}$ | 5.86 ± 0.01$^{bA}$ | 5.64 ± 0.04$^{cB}$ | 45 | 5.82 ± 0.07$^{abA}$ | 5.83 ± 0.08$^{abA}$ | 5.64 ± 0.01$^{cB}$ | 27 | 5.85 ± 0.03$^{aA}$ | 5.91 ± 0.03$^{abA}$ | 6.14 ± 0.40$^{aA}$ |
| 80 | 5.85 ± 0.06$^{abA}$ | 5.76 ± 0.02$^{aB}$ | 5.54 ± 0.02$^{dC}$ | 60 | 5.91 ± 0.04$^{bA}$ | 5.79 ± 0.02$^{abB}$ | 5.61 ± 0.01$^{cC}$ | 34 | 5.76 ± 0.04$^{aA}$ | 5.85 ± 0.04$^{aA}$ | 5.56 ± 0.03$^{bB}$ |
| 100 | 5.86 ± 0.02$^{abA}$ | 5.76 ± 0.01$^{aB}$ | 5.51 ± 0.02$^{dC}$ | 80 | 5.88 ± 0.06$^{abA}$ | 5.89 ± 0.05$^{bA}$ | 5.56 ± 0.01$^{dB}$ | 45 | 5.88 ± 0.02$^{aA}$ | 5.49 ± 0.12$^{cB}$ | 5.48 ± 0.15$^{bB}$ |

*Note.* Values with different superscript letters are significantly different ($p < 0.05$). Small letters compare numbers in the same column, and capital letters compare rows within the same temperature.

**TABLE 3** Effect of thermal processing on select quality attributes of carrot puree

| Attribute | Before pasteurization | After pasteurization | | |
|---|---|---|---|---|
| | | F-1 | F-30 | F-81 |
| Ascorbic acid (mg/100 g) | 50.6 ± 0.60$^{a}$ | 43.2 ± 1.29$^{b}$ | 43.0 ± 1.54$^{b}$ | 42.0 ± 0.94$^{b}$ |
| β-Carotene (μg/g) | 50.3 ± 1.85$^{a}$ | 51.2 ± 4.66$^{a}$ | 46.9 ± 2.91$^{a}$ | 48.4 ± 2.88$^{a}$ |
| Lightness ($L^*$) | 37.8 ± 0.11$^{a}$ | 37.5 ± 0.43$^{a}$ | 37.6 ± 0.12$^{a}$ | 37.1 ± 0.09$^{b}$ |
| $a^*$ | 22.5 ± 0.03$^{a}$ | 21.7 ± 0.62$^{b}$ | 21.7 ± 0.20$^{b}$ | 20.8 ± 0.12$^{b}$ |
| $b^*$ | 34.7 ± 0.13$^{a}$ | 34.8 ± 0.40$^{a}$ | 35.2 ± 0.62$^{a}$ | 33.1 ± 0.30$^{b}$ |
| $\Delta E$ | | 1.02 ± 0.64$^{A}$ | 1.09 ± 0.20$^{A}$ | 2.47 ± 0.31$^{B}$ |

*Note.* Values with different superscript letters are significantly different ($p < 0.05$).

significantly ($p < 0.05$) in the F-81 pouch. Studies have identified a variable effect of thermal processing of carrots and its products on $L^*$ and $b^*$ values. Some have reported a decrease in the $L^*$ value (Araya et al., 2009; Picouet, Sárraga, Cofán, Belletti, & Guàrdia, 2015; Vervoort et al., 2012), while others found no significant difference (Ayvaz et al., 2012; Patras, Brunton, Da Pieve, Butler, & Downey, 2009). Araya et al. (2009), Ayvaz et al. (2012) and Vervoort et al. (2012) reported a decrease in $b^*$. Picouet et al. (2015) observed no significant difference. However, all of them have reported significant changes in the $a^*$ (redness) value. Patras et al. (2009) reported a significant reduction in color intensity after thermal treatment. In our study, the maximum reduction in color parameters was observed for puree in the F-81 pouch leading to a significant ($p < 0.05$) color difference between puree in the F-1 and F-30 pouches after pasteurization. This suggests that a higher film OTR may affect some quality parameters during processing.

Our results indicate a significant ($p < 0.05$) effect of packaging OTR, storage temperature and time on the color parameters of carrot puree during storage. Lightness ($L^*$) for the puree in the F-1 pouch was significantly higher than F-81 but not for the puree in the F-30 pouch. Redness ($a^*$) differed significantly for the three types of pouches irrespective of temperature with higher values in the F-1 pouch. Yellowness ($b^*$) was significantly higher in puree from the F-1 pouch than the others. However, puree in the F-30 and F-81 did not show any significant difference ($p > 0.05$) irrespective of storage temperature.

The initial difference in color parameters was likely due to pasteurization, which continued during the storage period. The $L^*$ values for puree in all three pouches were stable throughout the storage period at all temperatures, except for a small drop in the F-81 pouch at the end of storage period at 4 °C (Figure 1). The $a^*$ and $b^*$ values remained constant for puree packed in the F-1 films irrespective of temperature; and were significantly higher ($p < 0.05$) than puree in the F-30 and F-81 pouches. In the F-30 and F-81 pouches, $a^*$ and $b^*$ values were stable at 4 °C for the first 8 weeks and then decreased slightly as shown in Figure 1. A similar trend was observed at other temperatures (data not shown). Araya et al. (2009) reported a retention of $L^*$ and decrease in $a^*$ and $b^*$ values in sous-vide cooked carrot strips in high oxygen barrier films stored at 4 °C for 14 days. Ayvaz et al. (2012) also reported similar trends of $L^*$ retention and a decrease in $a^*$ and $b^*$ values in high pressure sterilized baby carrots packed in low-barrier (47.8 $cm^3\ m^{-2}\ day^{-1}$) and high-barrier (0.91 and 1.33 $cm^3\ m^{-2}\ day^{-1}$) films, as well as a retention of redness in 0.91 cc film during 12-weeks of storage at 25 and 37 °C.

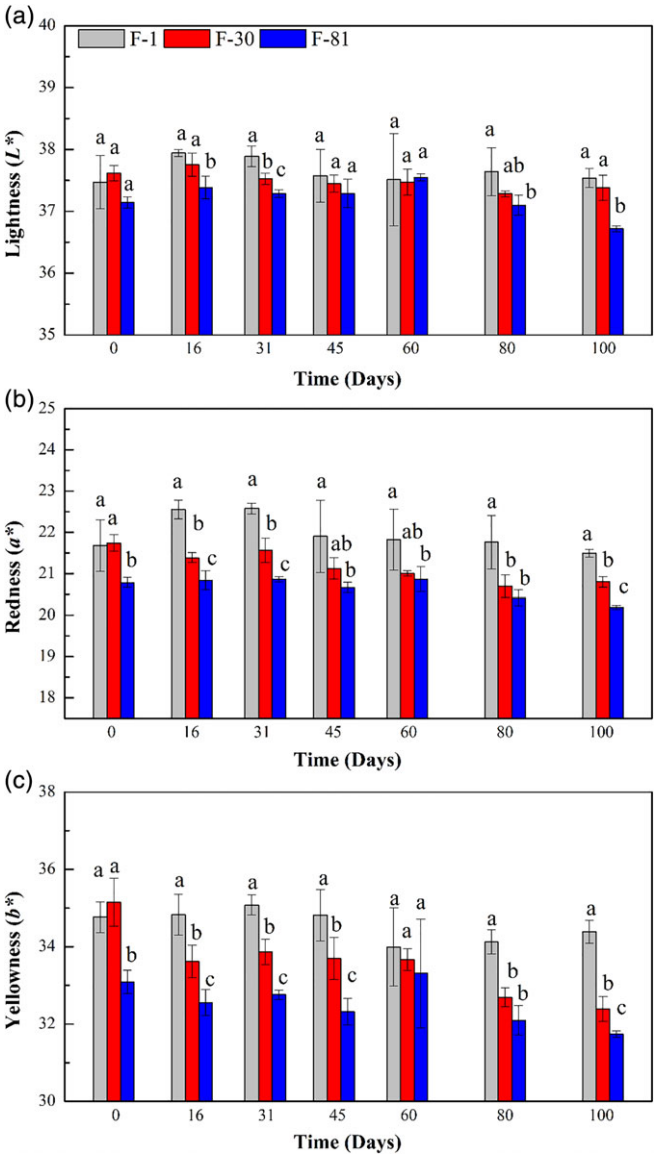


**FIGURE 1** Color values for carrot puree packed in different packaging films at 4 °C: (a) lightness, (b) redness, and (c) yellowness

The puree in the F-1 pouch exhibited significantly ($p < 0.05$) lower color difference than that in the F-30 and F-81 pouches during storage. Although these values were significantly different, the overall color differences for puree in all three types of pouches were less than 3. The color differences in the puree packed in F-1, F-30, and F-81 pouches were between 0.49–1.66, 1.11–2.93, and 0.37–2.63, respectively. Zhang et al. (2016) reported a color difference of 3 and above as visually perceptible. Figure 2 represents the overall visual color of the puree during the storage and correlates with the instrumental color analysis and color difference analysis for F-1, F-30, and F-81 pouches. A yellow tint was observed on the inside surface of all three types of pouches during storage, regardless of temperature. Retention of yellow compounds on the surface was variable; and was more pronounced in F-81 pouches. Polymeric film properties may play a role in either color or carotenoid retention, suggesting an avenue for further research.

### 3.4 | β-Carotene content

β-Carotene is responsible for the orange color in carrots, as well as providing nutritional benefits. High temperature processing may result in oxidation and/or isomerization of β-carotene affecting its concentration and bioavailability (Knockaert et al., 2012). Our results show that thermal pasteurization did not significantly affect ($p > 0.05$) β-carotene content for carrot puree in F-1, F-30, and F-81 pouches (Table 3). Others have observed similar effects of thermal pasteurization on the amount of β-carotene in carrots and carrot products. These studies include—Patras et al., 2009 (puree); Knockaert et al., 2011 (discs); Knockaert et al., 2012 (puree); Vervoort et al., 2012 (dices); Picouet et al., 2015 (juice). Patras et al. (2009) observed a 26% increase in β-carotene content in carrot puree after thermal pasteurization, due to the enhanced extraction efficiency from heat-treated sample (Chandler & Schwartz, 1988). Picouet et al. (2015) reported no significant effect of mild heat treatment on β-carotene content in acidified juice (pH–5.5) compared to a loss in nonacidified juice (pH–6.48).

In our study, puree in F-1 pouches showed a significantly ($p < 0.05$) higher retention of β-carotene during storage. There was no significant difference in retention of β-carotene in the F-30 and F-81 pouches. However, the differences between F-1, F-30, and F-81 pouches were small (Figure 3). Provesi et al. (2011) reported no significant changes in sterilized pumpkin puree stored at room temperature for 180 days in glass bottles; and in mild heat treated (80 °C, 27 min) acidified and nonacidified carrot juice stored at 5 ± 2 °C for 29 days (Picouet et al., 2015). However, Chen et al. (1996) found a decrease in the concentration of β-carotene storage temperature increased for acidified, pasteurized (105 °C, 30 s) carrot juice stored at 4, 25, and 35 °C for 3 months. They observed a decrease in β-carotene content from 54.7 to 50.9 μg/mL at 4 °C with a formation of 13-*cis* isomer in dark storage conditions.

To our knowledge, there are no published studies on the effect of packaging films on thermally pasteurized carrots/carrot products during storage at refrigeration temperatures. However, Ayvaz et al. (2012) studied the effect of pressure assisted thermal sterilization on baby carrots in packaging films with OTRs 0.91 (Nylon/EVOH/EVA), 1.33 (Nylon/EVA), and 47.79 $cm^3\ m^{-2}\ day^{-1}$ (MetPET/PE), respectively; and stored at 25 and 37 °C for 12 weeks. Storage in high-barrier pouches (0.91 $cm^3\ m^{-2}\ day^{-1}$ OTR) helped retain color as well as β-carotene during storage at both temperatures. However, continuous degradation of β-carotene was observed for product packed in 1.33 and 47.79 $cm^3\ m^{-2}\ day^{-1}$ OTR pouches during the storage period. These losses were attributed to a significant increase in the OTR of the films due to structural changes on the film surface after pressure-heat

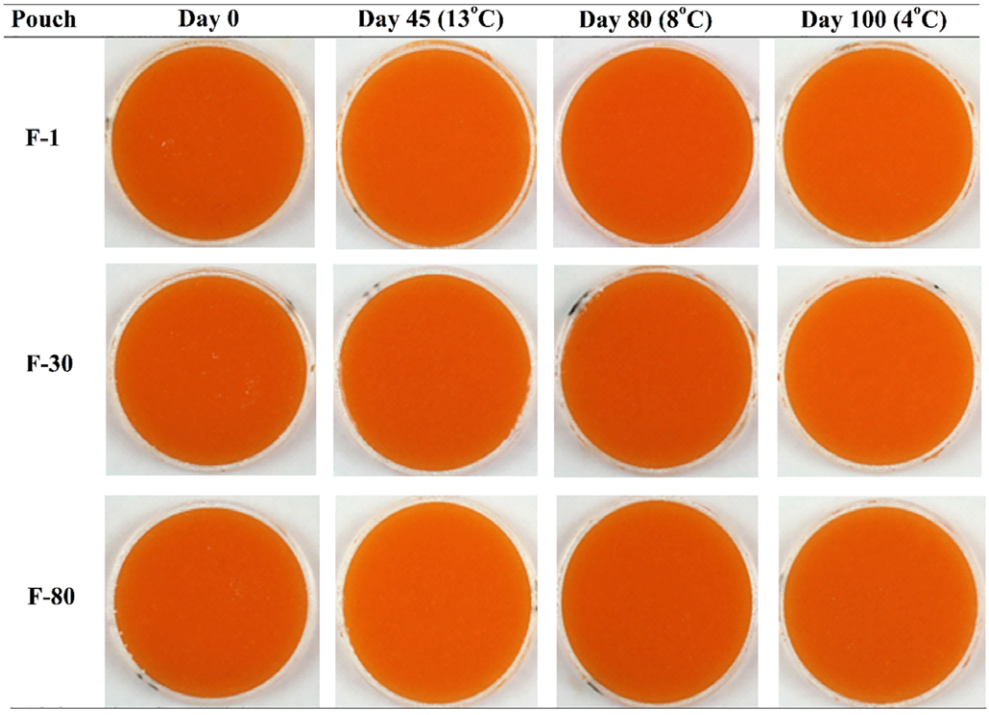


**FIGURE 2** Visual color change in carrot puree at the end of storage period at respective temperature

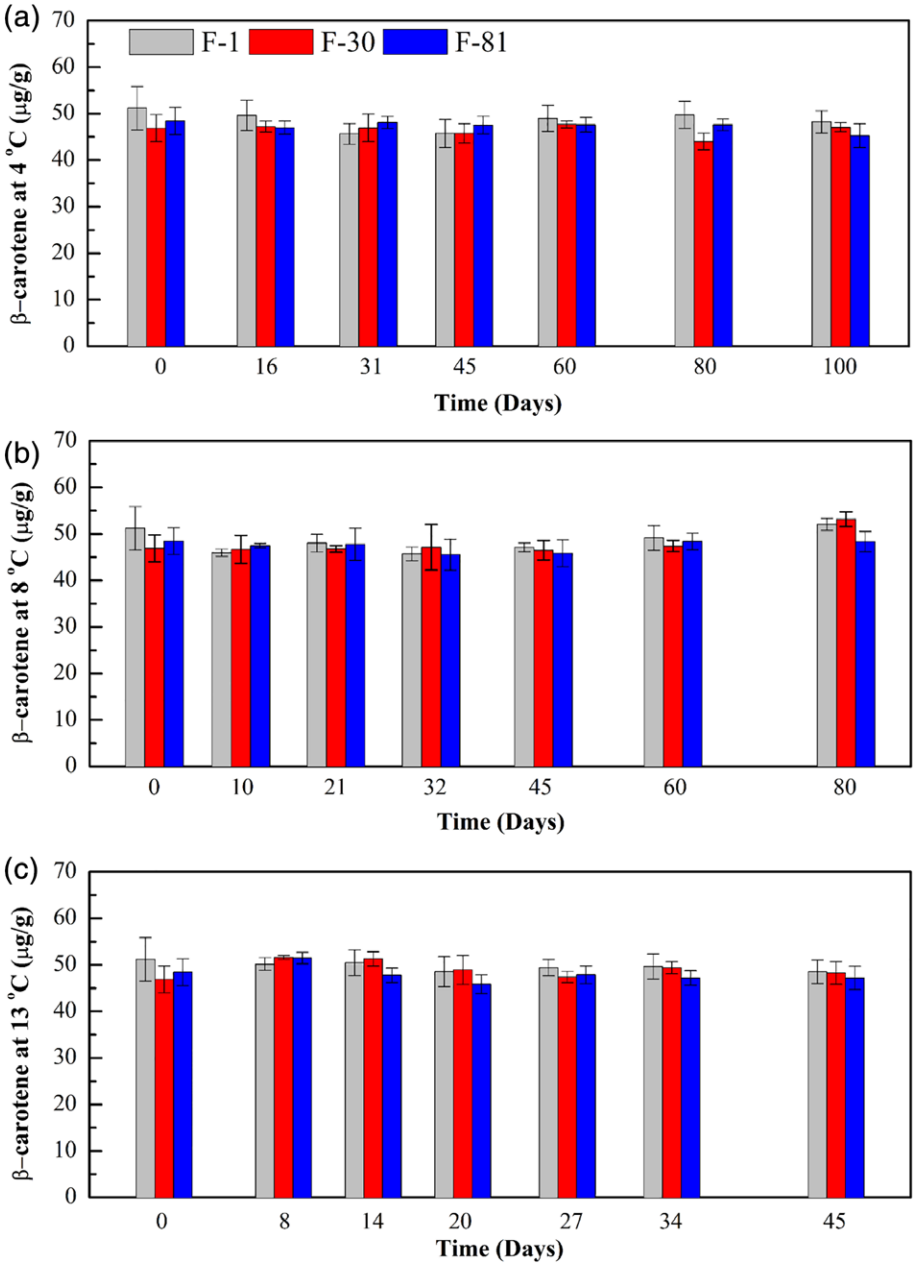


**FIGURE 3** β-Carotene content of carrot puree packed in different packaging films at three storage conditions: (a) 4 °C, (b) 8 °C, and (c) 13 °C

treatment. In another study, after an initial decline β-carotene content remained stable following microwave assisted thermally sterilized sweet potato puree packed in four types of pouches with OTRs in the range of 0.016–1.404 $cm^3$ $m^{-2}$ $day^{-1}$, stored at 23 and 35 °C for 9 and 18 months, respectively (Zhang et al., 2019, submitted). They suggested initial decline to the residual oxygen content; and storage stability to the recovery of gas barrier properties to initial level during storage. The apparent increase was due to the higher extractability after thermal treatment.

*a** value can provide direct correlation of β-carotene content (Patras et al., 2009). In our study, β-carotene content during storage correlated with color changes in carrot puree. Figures 1 and 3a display similar trend between the retention of β-carotene and color values for carrot puree packed in F-1 pouches and small losses in F-81 pouches. Ayvaz et al. (2012) reported a similar trend in high pressure sterilized baby carrots packed in high and low barrier films. In our study for a given type of pouch, the temperature effect was significantly ($p < 0.05$) higher at 13 °C than for the other two temperatures. This may be attributed to more efficient extraction of carotene after storage due to cell wall softening and membrane destabilization (Imsic, Winkler, Tomkins, & Jones, 2010). Imsic et al. (2010) reported a 25 and 34% increase in all-trans-β-carotene in raw carrots stored at 4 and 20 °C, respectively, followed by a gradual reduction to level similar to the initial value.

Although in our study the F-81 pouch had very high OTR compared to the Nylon/EVA and MetPET/PE pouches in Ayvaz et al. (2012), the lower storage temperature and the presence of ascorbic acid may have contributed to a higher relative stability of β-carotene. Morais et al. (2002) found positive correlation between ascorbic acid concentration and the stability of β-carotene in paprika powder stored at 22 °C for 8 weeks. Sánchez-Moreno, Plaza, de Ancos, and Cano (2003) attributed losses of carotenoids

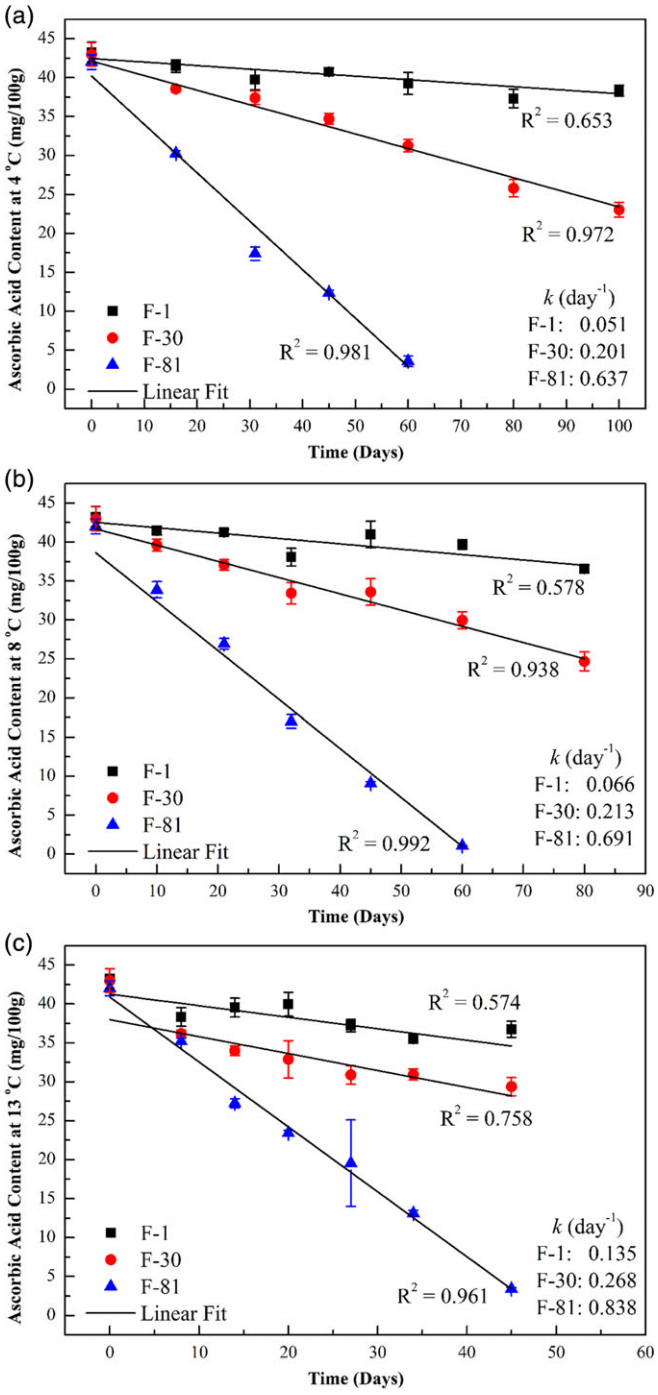


**FIGURE 4** Ascorbic acid content of carrot puree packed in different packaging films at three storage conditions: (a) 4 °C, (b) 8 °C, and (c) 13 °C

to the depleted protection of vitamin C in high-pressure processed orange juice at the end of storage at 4 °C.

## 3.5 | Ascorbic acid content

Pasteurization process significantly ($p < 0.05$) reduced ascorbic acid content in carrot puree by 14.6–17.0% for all pouch types (Table 3). Higher processing losses for puree in the F-81 pouch may be due to the higher oxygen permeability of the film. Other researchers found a 17–84% reduction of ascorbic acid in fruits and vegetables during thermal pasteurization (Koo, Kim, Lee, Lyu & Paik, 2008; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008; Torregrosa et al., 2006; Zhou et al., 2014). Variability in losses during thermal processing may be due to the differences in food composition, the presence of metal cations, pH, residual headspace in the package or dissolved oxygen content in the food. Capuano et al. (2018) reported that factors such as different tissue structure, mechanical damage during harvesting, intrinsic enzyme (ascorbate oxidase) and sulfhydryl group content, and the presence of metal ions, such as $Fe^{3+}$ and $Cu^{2+}$ can affect the thermal stability of vitamin C in various foods. Herbig and Renard (2017) observed a slightly higher stability of vitamin C and a lower degradation rate in carrot puree at pH 5.5 than for apple puree serum at pH 3.5 when heated at 80 °C for several hours. These results contradict findings on a citrate–phosphate buffer at pH 3.5, 5.5 and 7.5, in which ascorbic acid exhibited the highest stability at pH 3.5 (Herbig & Renard, 2017). This variation was attributed to the composition of food matrix.

In our study, degradation of ascorbic acid occurred fastest at 13 °C followed by 8 and 4 °C during storage (Figure 4). This depended on the OTR of the packaging and storage temperature. The effect of packaging film OTR, temperature and time on ascorbic acid retention during storage was significant ($p < 0.05$). For puree packed in F-1 pouches there was a 5.8, 5.2, and 15.0% loss in ascorbic acid during storage for 45 days at 4, 8 and 13 °C, respectively. For puree packed in F-30 pouches, the loss was 19.3, 21.9, and 31.7%, respectively under the same storage conditions. Similarly, there was 70.5, 78.4, and 91.9% reduction in ascorbic acid for puree packed in the F-81 pouches. Ascorbic acid was not detected in carrot puree packed in F-81 pouches after the 80th day of storage at 4 or 8 °C. Carrot puree packed in F-1 pouches retained 85–89% ascorbic acid whereas puree in F-30 pouches retained 54–68% ascorbic acid at the end of storage.

The stability of ascorbic acid during storage is affected by the temperature and oxygen present in the headspace, dissolved in the matrix, or permeating through the packaging material. In this study, the maximum degradation of carrot puree occurred in F-81 pouches, which had the highest OTR (OTR—80.9 $cm^3\ m^{-2}\ day^{-1}$). Degradation was significantly ($p < 0.05$) higher than the F-1 and F-30 pouches, regardless of storage conditions. These results accord with those in the literature (Ayhan, Yeom, Zhang, & Min, 2001; Polydera, Stoforos, & Taoukis, 2003). However, few studies have examined the influence of OTR of packaging material on nutrient retention in pasteurized food products. Polydera et al., 2003 reported a higher degradation rate in pasteurized reconstituted orange juice packed in polypropylene bottles (medium-barrier) than in polyethylene/aluminum/cellophane laminated pouches (high-barrier) at refrigeration temperatures (0–15 °C). Ayhan et al. (2001) reported a significantly higher retention of ascorbic acid in pulsed electric field treated orange juice packed in glass and polyethylene terephthalate bottles than in polyethylene-based bottles. Our literature search revealed no studies on how nutrient retention in low-acid pasteurized products may be affected by the gas barrier properties of flexible polymeric pouches.

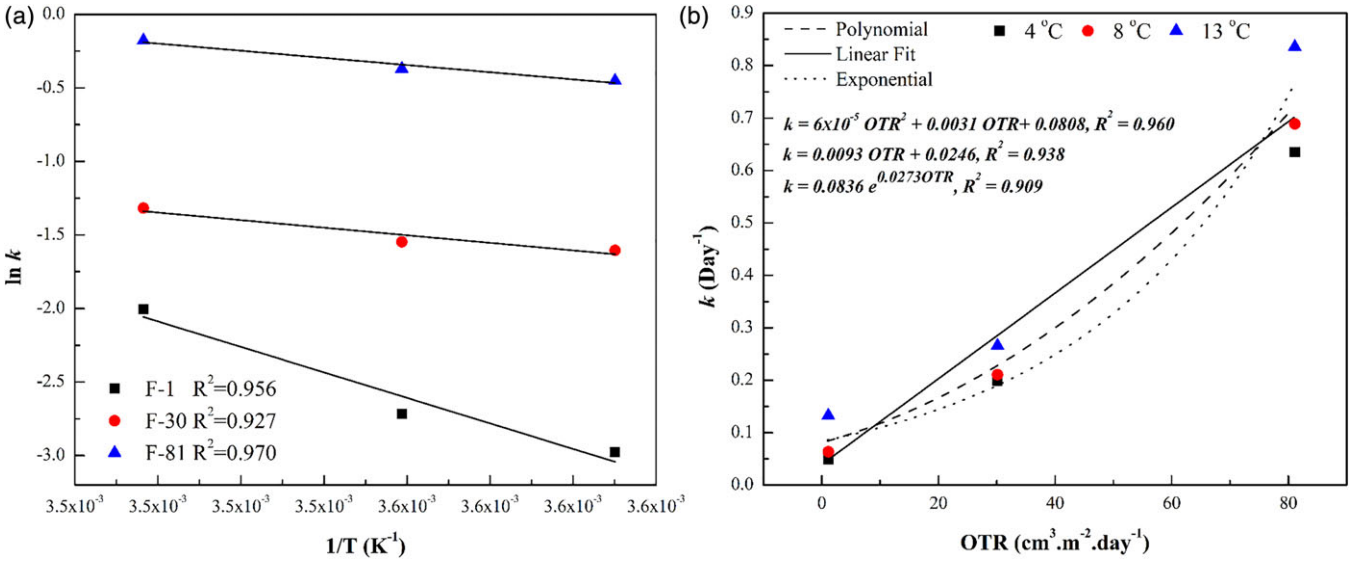


**FIGURE 5** (a) Arrhenius plot of ascorbic acid degradation and (b) relationship between OTR of films and rate constant at three temperatures. Abbreviation: OTR, oxygen transmission rate

In our study, the effect of storage temperature was not significant ($p > 0.05$) for a given type of packaging films, except for F-1 pouches, in which significant differences were observed between 4 and 13 °C; and between 8 and 13 °C. The ascorbic acid content in carrot puree packaged in F-1 pouches stored at 4 and 8 °C differed significantly from that stored at 13 °C. No significant difference ($p > .05$) was found between 4 and 8 °C. However, temperature had no significant effect on ascorbic acid in carrot puree packaged in F-30 and F-81 pouches. Therefore, it can be stated that for low oxygen barrier films (F-30 and F-81), higher oxygen permeation through the film mainly controls the stability of ascorbic acid, regardless of storage temperature. However, in the case of high-barrier films (F-1), ascorbic acid is more sensitive to temperature since it degrades faster at abused conditions.

There was a marginal decrease in redness and yellowness as well as β-carotene toward the end of storage period at 4 and 8 °C in F-81 pouch, when no ascorbic acid was found. This suggests the protective effect of ascorbic acid on the β-carotene and color.

### 3.5.1 | Degradation kinetics

Ascorbic acid degradation during storage followed zero-order kinetics. A good zero-order fit was observed for degradation in the F-30 and F-81 pouches. However, for puree in the F-1 pouch both zero-order and first-order kinetic models fit; and had similar correlation factor ($R^2$) values. Hence, further data analysis was conducted using zero-order for puree packed in the F-1 pouch. Authors have reported first-order kinetics of ascorbic acid degradation during refrigerated storage (Bosch et al., 2013; Polydera et al., 2003; Rodrigo et al., 2003). In all of these studies the product was packaged in a high-barrier packaging such as polypropylene/ethylene–vinyl alcohol pouches, aluminum laminates and cartons, and information about the gas barrier properties was not reported. Miyawaki, Sugiyama, and Inoue (2016) reported that zero-order kinetics fits well for ascorbic acid oxidation in open systems where oxygen supply is not a limiting factor. However, it follows first-order in closed reaction systems. In our study, we may consider that enough oxygen permeated through low-barrier pouches (F-30 and F-81) and was not a limiting factor for ascorbic acid degradation. An initial drop in ascorbic acid content was observed for puree in the F-1 pouches at all three storage temperatures, and ascorbic acid content gradually decreased thereafter. A similar trend was observed for product in the F-30 pouch at 13 °C. The dissolved oxygen in the matrix rapidly reduced ascorbic acid at the start (Zerdin, Rooney, & Vermuë, 2003), followed by anaerobic degradation during storage. In most cases, the rate constants for anaerobic degradation were 2–3 times less than those of oxidative degradation (Gregory III, 2008). The higher permeability of the F-30 and F-81 pouches may be a contributing factor for continuing aerobic degradation of ascorbic acid.

Puree packed in the F-81 pouches showed the highest rate constant for ascorbic acid degradation, while puree in the F-1 pouch showed the lowest rate constant, regardless of storage temperature (Figure 4). Polydera et al., 2003 observed that rate constant nearly doubled for orange juice packed in polypropylene bottles (medium-barrier) compared to high-barrier laminated pouches at 15 °C. The rate constants of the F-1, F-30, and F-81 pouches at three temperatures were used to calculate activation energy, $E_a$, following the Arrhenius plot (Figure 5a). The activation energy for ascorbic acid degradation was found to be 72.3, 21.5, and 20.3 kJ/mol for carrot puree packed in the F-1, F-30, and F-81 pouches, respectively. Polydera et al., 2003 reported $E_a$ of 18.3 kJ/mol (initial storage period) and 13.1 kJ/mol (extended storage period) for ascorbic acid degradation in thermally pasteurized reconstituted orange juice stored at 0–15 °C. The higher $E_a$ of F-1 pouch suggests greater temperature dependence of the ascorbic acid degradation rate, which is also reflected in the higher $Q_{10}$ value. The $Q_{10}$ value for F-1, F-30, and F-81 pouches was calculated as 2.96, 1.38, and 1.36, respectively. Thus, higher $E_a$ and $Q_{10}$ value suggest the temperature dependence of ascorbic acid degradation in high-barrier

films. The lower $E_a$ and $Q_{10}$ values in F-30 and F-81 pouches suggests higher dependence of ascorbic acid degradation on oxygen permeation through the film rather than temperature in low barrier films. Therefore, for low oxygen barrier films (F-30 and F-81), higher oxygen permeation mainly controls the stability of ascorbic acid, regardless of storage temperature. For high-barrier films (F-1), ascorbic acid is more sensitive to temperature since it degrades faster at higher temperature.

In this study, there was a correlation between the rate constant and the OTR at all storage temperatures (Figure 5b). Since the storage temperature effect was not significant except in case of the F-1 pouch, a single relationship was obtained to determine the effect of OTR on the ascorbic acid degradation rate constant. There is no such relationship in the literature for ascorbic acid degradation rate with gas barrier properties. However, Bhunia et al. (2017) reported a relationship between the lipid oxidation rate constant and the OTR for pasteurized mussels in tomato sauce; and Zhang et al. (2016) reported a relationship between moisture permeation and WVTR for sterilized mashed potato. OTR range selected for the study was wide and representative of the commercially used films for pasteurized products. This relationship can be used to predict the ascorbic acid degradation rate constant and thus the shelf life, based on ascorbic acid content at refrigerated storage conditions for the films within the studied range of OTR.

## 4 | CONCLUSIONS

Findings of this study reveal that thermal pasteurization affects the quality and nutritional attributes of carrot puree depending upon the pouch type. Maximum losses occurred in low-barrier film (F-81), suggesting the influence of the high OTR during processing. Packaging film OTR and storage temperature significantly ($p < 0.05$) affected pH, color, β-carotene, and ascorbic acid content of the carrot puree. The main mechanism determining quality was ascorbic acid degradation. The degradation of ascorbic acid in low-barrier pouches during later stages of storage was correlated with losses in color and β-carotene. This suggests the protective effect of ascorbic acid.

Performance of low-barrier film (F-81) was very low in terms of nutrient retention with no detection of ascorbic acid, whereas medium and high-barrier films retained more than 50% of ascorbic acid at the end of storage. Our findings demonstrate that medium-barrier film (F-30) is sufficient to retain nutritive value and can be used for in-pack pasteurized food products with shelf life of 12–14 weeks at refrigerated conditions. Further research may explore the upper OTR range to reduce costs and maintain shelf life.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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