



Determining Shelf Life of Ready-to-Eat Macaroni and Cheese in High Barrier and Oxygen Scavenger Packaging Sterilized via Microwave-Assisted Thermal Sterilization

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Abstract

Ready-to-eat macaroni and cheese filled in novel oxygen scavenger and metal oxide-coated high-barrier polymer packages were processed in pilot scale 915-MHz microwave-assisted thermal sterilization system (MATS). Also, aluminum foil packages were processed in Allpax retort system to compare packaging performance. Physicochemical and sensory attributes of macaroni and cheese packaged in different oxygen and water vapor transmission rates were evaluated and stored for 6 months at 37.8 °C. Findings showed oxygen transmission rate (OTR) increase by 2–7 times and water vapor transmission rate (WVTR) increase by 2.5–24 times after MATS processing. OTR of polymeric packaging had no significant effect on vitamin A and vitamin E, shear force, and food color. Comparable results between polymeric and aluminum foil packaging were observed throughout the shelf life. This indicates that oxygen scavenger and high-barrier packaging with OTRs ~ 0.03–0.34 cc/m² day and WVTRs ~ 0.62–7.19 g/m² day can be used for ready-to-eat meals with extended shelf life for soldiers and astronauts.

Keywords Oxygen scavenger packaging · Oxygen transmission rate · Water vapor transmission rate · Vitamin A · Vitamin E · Shelf life

Introduction

Shelf-stable packaged food requires 3–5 years of shelf life for long missions such as military combat or NASA journeys (Catauro and Perchonok 2012; Perchonok et al. 2012). Therefore, long shelf life requires sterilization and high-barrier packaging. Conventional thermal sterilization processes are reliable means of controlling food pathogens and producing safe, shelf-stable food (Holdsworth et al. 2008). However, the excessive heat in thermal processing may affect

food quality (Van Boekel 2008; Ling et al. 2015), as well as the gas barrier properties of polymeric packaging (Pascall and Han 2018). Microwave-assisted thermal sterilization (MATS) offers a shorter processing time than retort processing, with similar lethality (Tang 2015). This minimizes quality degradation and is less detrimental to package barrier properties (Dhawan et al. 2014).

Packaging with a high oxygen and water vapor barrier is required to achieve a longer shelf life of sterilized foods. Aluminum foil-based flexible packaging offers very high gas barrier properties but is not compatible with microwave-based thermal processes. Ethylene vinyl alcohol (EVOH) and metal oxide-coated polyethylene terephthalate-based packaging are suitable alternatives, with low density, transparency, and high gas barrier properties (Zhang et al. 2017). This multilayered packaging is coextruded or laminated to provide an excellent barrier to oxygen and water vapor (Al-Ghamdi et al. 2019). Oxygen and water vapor barrier properties are known to compromise the color and vitamin C content of pasteurized carrot puree (Sonar et al. 2019). However, high gas barrier packaging can maintain the color and sensory quality of sterilized foods (Zhang et al. 2019).

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Recently, oxygen scavenger (OS)–based high-barrier packaging technology has been developed to improve the shelf life of food. Oxygen scavenger packaging is comprised of a protective layer, a metal oxide–coated barrier layer, an oxygen absorbent layer, and a heat-seal layer. This packaging prevents external oxygen from permeating inside until the oxygen scavenging elements are saturated. However, studies on the shelf life of thermally sterilized, ready-to-eat meals packaged in high-barrier and OS packaging are limited. Johnson et al. (2018) reported that oxygen scavenger packaging significantly reduces the oxidation of ascorbic acid and alpha-tocopherol in liquid food systems. It can also inhibit lipid oxidation; however, there is an initial 1–2-day delay in oxygen scavenging. This is due to the time that water from food systems takes to penetrate the innermost layer of the pouch and activate the iron-based oxygen scavenging system. Zhang et al. (2019) observed that oxygen barrier polymeric packaging with an oxygen transmission rate below 0.3 cc/m² day showed similar vitamin C retention to aluminum foil-based packaging over 9 months of storage at 35 °C. Understanding the oxidative stability of vitamins in ready-to-eat meals packaged in high-barrier and OS pouches will inform the development and selection of appropriate packaging for thermally sterilized foods.

This study investigated oxygen scavenger packaging and other high-barrier polymer packaging used to extend the shelf life of ready-to-eat thermally sterilized (MATS vs. retort) macaroni and cheese. Specifically, physicochemical quality parameters were determined including vitamin A and vitamin E, color, instrumental texture, and sensory attributes of macaroni and cheese. State-of-the-art OS and metal oxide–coated multilayer packaging with aluminum foil-based packaging were also compared. The effect of thermal sterilization on vitamin retention, physical, and sensory quality parameters was also evaluated.

Materials and Methods

Food Ingredients, Reagents, and Chemicals

In this study, shredded mild cheddar cheese made by the Kraft and Heinz companies containing annatto colorant was purchased from a local market (Walmart, Pullman, WA) to make cheese sauce in-house. Other ingredients in the sauce included xanthan gum, salt, canola oil, resistant starch, disodium phosphate, sodium caseinate, and water. Xanthan gum was purchased from Bob's Red Mill (Milwaukie, OR). Macaroni, canola oil, resistant starch, and sodium caseinate were provided by the US Army Natick Soldier Systems Center. Vitamin A (vitamin A palmitate) and vitamin E 700 IU powder (d-alpha-tocopherol acetate) were purchased from Bulk Supplements (Hard Eight Nutrition LLC, Henderson, NV). Other ingredients in the vitamin A palmitate powder were Arabic gum,

starch, and DL-alpha-tocopherol, while the vitamin E powder contained cornstarch. Other chemicals such as potassium hydroxide and disodium phosphate were purchased from Sigma-Aldrich Co. (St. Louis, MO). Butylated hydroxytoluene was obtained from Acros Organics (Belgium). Chemical reagents including hexane, ethanol, acetone, and phosphoric acid were purchased from Avantor Performance Materials (Center Valley, PA).

Preparation of Macaroni and Cheese

First, cheese sauce was prepared with water, starch, canola oil, disodium phosphate, cheddar cheese, sodium caseinate, and xanthan gum at 70 °C, as per the recipe developed by the US Army Natick Soldier Systems Center. The elbow-shaped macaroni was comprised of durum wheat semolina and egg protein. Pieces were 1.94 ± 0.18 cm in length and approximately 0.46 ± 0.04 g in weight. The macaroni was boiled in water at (approx. 98 °C) for 8 min in a 5-L stainless steel pot. The cooked macaroni was then drained and allowed to cool for 30 min at 25 ± 2 °C. The macaroni and the cheese sauce were mixed together at a 70% cheese sauce:30% macaroni (w/w) ratio. To fortify the formulation, vitamin A and vitamin E were added, with a total concentration of 0.02% and 0.06% (w/w), respectively. Vitamins were mixed thoroughly in the cheese sauce for 30 min at a constant speed of 107 rpm using A200 Hobart mixer with 20-L stainless steel bowl and flat beater (Hobart GmbH, Offenburg, Germany). Packages were filled with 227 ± 2 g of macaroni and cheese.

Packaging of Macaroni and Cheese

Four different types of pouches (labeled as A to D, 8 oz capacity) based on multilayer films were used for MATS processing. All pouches had a metal oxide–coated polyethylene terephthalate as an oxygen barrier layer. Pouch D also incorporated an OS layer. An aluminum (Al) foil-based, multilayer pouch (labeled as R) was used as a reference. Only conventional retort can be used to process products in these pouches, as aluminum foil packages are not transparent to microwaves and thus are not suitable for in-package MATS processing.

Pouches were provided by packaging polymer companies (Kuraray America Inc., Houston, TX, USA; Toppan Printing Co, LTD., Japan and Mitsubishi Chemical Corporation Japan). Vacuum sealing was conducted using Easy-Pack (UltraSource, LLC, Kansas, MO), with a sealing time of 2.5 to 5 s and temperatures of 180 to 200 °C, depending on the type of package. The total thickness of each package was measured in at least at three different locations using an electronic disc micrometer model 15769 manufactured by Flexbar Machine Co. (Islandia, NY). See Table 1.

The oxygen-absorbing package D illustrated in Fig. 1 had four layers: the protective layer, gas barrier layer, oxygen

Table 1 The following list provides details (package label, structure, dimensions length \times width, total thickness) of multilayer pouches used in this study

Package	Structure	Dimensions (L \times W) (mm)	Total thickness (μm)
A	AlOx PET 12 μm //OPA 15 μm //CPP 70 μm	185 \times 140	112
B	AlOx 13 μm PET//OPA 15 μm //CPP 50 μm	180 \times 130	92
C	PET/coating 12 μm //PA 15 μm //PP 50 μm	185 \times 130	92
D	PET//PA//AlOx//MXD6//EVOH//iron-based oxygen absorbent agents//PP	180 \times 130	114
R	PET 12 μm //aluminum 9 μm //nylon 15 μm //PP 80 μm	210 \times 120	120

absorption layer, and heat-seal layer that enable oxygen consumption in the package and preventing external O_2 to permeate inside. These pouches provided a range of oxygen and water vapor transmission rates (OTR = 0.01–0.04 cc/m^2 day and WVTR = 0.13–0.35 g/m^2 day).

Thermal Processing

A single-mode microwave-assisted thermal sterilization (MATS) pilot system (25 kW, 915 MHz) at the Washington State University was used to process 190 pouches of macaroni and cheese (Tang 2015; Zhang et al. 2016b). Processing conditions were set as follows: preheating at 60 $^\circ\text{C}$ for 25 min; microwave heating for 3.7 min at 124 $^\circ\text{C}$; holding time for 3.8 min at 121 $^\circ\text{C}$; cooling in tap water for 5 min at 20 $^\circ\text{C}$; and system pressure at 240 kPa (35 psi). The average obtained lethality was $F_0 = 11.1$ min.

A pilot-scale (207 kPa) retort (Allpax 2402 Products, LLC, LA) was used to sterilize 20 aluminum foil-based pouches (R) of macaroni and cheese in one run, with four pouches aligned in a single rack. Five racks were stacked upon each other in one run to achieve uniform processing. Three runs were made to process the 60 Al foil pouches. The processing conditions were come-up time of 11 min, cooking time of 19 min at 121 $^\circ\text{C}$, and cooling time of 38 min at 25 $^\circ\text{C}$. The average calculated lethality at the cold spot was $F_0 = 6$ min. The water spray processing type was selected to obtain higher heat transfer

rates. The MATS-processed products showed a better quality than retort-processed products for a similar lethality (Tang 2015). Hence, these retort conditions (smaller F_0 value) were selected to match the initial quality of the MATS-processed product.

Storage Conditions

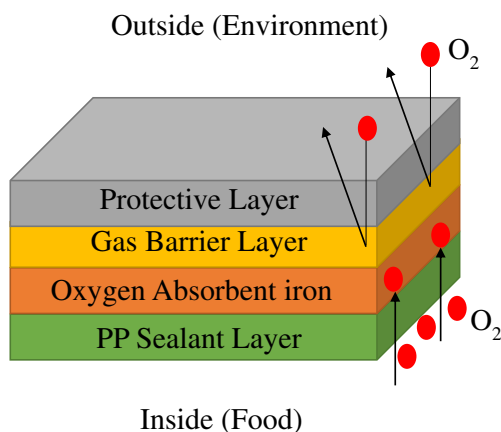
The processed pouches were stored in a closed incubator, KB055 (Darwin Chambers, Saint Louis, MO), set at higher temperature of 37.8 ± 0.2 $^\circ\text{C}$ for the accelerated shelf life study (Robertson 2005). According to Zhang et al. (2016b), model kinetics based on the color of mashed potatoes gave a Q_{10} value ranged from 2.85–3.15 for high-barrier polymer packages. This shelf life prediction model using color difference $\Delta E = 12$ as the endpoint predicted that 5.5 months of shelf life of mashed potatoes at 37 $^\circ\text{C}$ was equivalent to 2.5 years at 23 $^\circ\text{C}$. If $Q_{10} = 3$, 6 months at 40 $^\circ\text{C}$ is equivalent to 4.5 years at 20 $^\circ\text{C}$, noting that thermally processed food Q_{10} value ranges from 1–4 (Robertson 2005).

Oxygen and Water Vapor Transmission Rates

The oxygen transmission rate (OTR) was measured using a Mocon Ox-Tran 2/21 MH permeability instrument (Modern Control, Minneapolis, MN) at 23 $^\circ\text{C}$, 55% RH, and 1 atm. The test was performed according to ASTM D3985 standards. The water vapor transmission rate (WVTR) was measured utilizing a Mocon Permatran 3/33 (Modern Control, Minneapolis, MN) at 100% RH and 38 $^\circ\text{C}$. Samples were prepared by cutting the films into an exact size of 50 m^2 and mounting them inside Mocon chambers. The oxygen transmission rates and water vapor transmission rates were measured before and after processing (Zhang et al. 2017; Bhunia et al. 2016).

Weight Loss

The weight of stored macaroni and cheese pouches was recorded monthly to determine the influence of WVTR on weight loss in different packaging stored at 37.8 $^\circ\text{C}$ for 6 months using a simple balance scale (model GK703, Sartorius, Goettingen, Germany).

**Fig. 1** Oxygen scavenging package mechanism and structure

Quantification of Vitamin A and Vitamin E

Quantification of vitamin A and vitamin E was performed using an Agilent 1100 high-performance liquid chromatography system (HPLC) (Agilent Technology, Santa Clara, CA) with a 250-mm 5- μ m XTerra C18 column (Waters Corporation, Milford, MA) and a diode-array detector. Vitamin content was determined following (Lopez-Cervantes et al. 2006) method, with minor modifications. In this study, 100-g macaroni and cheese was blended in a Kitchen Aid blender for 2 min. Five-gram blended sample was used for extraction, and 15 mL of 85% KOH and 500 μ L of 0.5% BHT were then added. Saponification was carried out at 80 °C for 15 min. Next, 3 mL of water and 15 mL of hexane were added to the saponified mixture, which was then centrifuged at 500g force for 2 min. Five milliliters of the supernatant was collected and dried using 98% pure N₂ gas. Three milliliters of the buffer methanol:acetonitrile:water (68:28:4) (w/w) ratio was added to reconstitute the evaporated residue. Then, the mixture was passed through 0.45- μ m syringe and collected in amber-colored vials and injected into HPLC system at a 1.4 mL/min flow rate and a column temperature of 25 °C. Calculations of vitamin content were based on calibration curves constructed earlier ($R^2 > 0.99$) as diluted solid in liquid (μ g/mL) following (Lopez-Cervantes et al. 2006). Vitamin A and vitamin E were measured at 325-nm and 208-nm wavelengths, respectively.

Instrumental Texture of Macaroni

Maximum peak force (N) required to shear through the macaroni was recorded as shear force. This force was determined using a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 25-kg load cell. First, the macaroni was separated from the cheese sauce and wiped clean using soft tissue paper. Two pieces of macaroni were placed parallel to each other spaced 1 cm apart on the cutting plate and then cut using a 3-mm-thick cutting blade (Sissons et al. 2008). The blade moving speed was set at 1 mm/s, and peak force (N) was recorded (Wang et al. 2018). The initial distance between the blade and macaroni was set to 50 mm. Then, the blade was allowed to cut the entire macaroni pieces. The test was performed at room temperature (approx. 25 ± 2 °C).

Color Measurement

The color of the macaroni and cheese was measured using a CM-5 spectrophotometer (Konica Minolta, Ramsey, NJ). Results were reported based on the CIE color system (L^* (lightness), a^* (red to green), b^* (yellow to blue)) and color difference ($\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$). Differences in perceivable color can be analytically classified as very distinct

($\Delta E > 3$), distinct ($1.5 < \Delta E < 3$), and a small difference ($1.5 < \Delta E$) (Zhang et al. 2016a). Approximately 25–35g sample was taken in a Petri dish, and the spectrophotometer was set to reflectance mode (Bornhorst et al. 2017).

Sensory Evaluation

A sensory evaluation was performed by 12 trained panelists at the US Army Natick Center. Packages were stored at three temperatures as follows: 4.4 °C for 6 months, which served as a control; 48.8 °C for 1 month; and 37.8 °C for 3 and 6 months. This storage time and temperature were designed to be equivalent to 2–3 years of shelf life at ambient temperature (25 °C). Evaluation was carried out based on appearance, odor, flavor, texture, and overall acceptance on a labeled effective magnitude (LAM) scale (Cardello et al. 2007). The scale ranged from 1 to 9 (worst to best quality) for all attributes in each package at different storage times and temperatures. Samples were warmed for serving and numbered to prevent bias in panelist judgments. The serving size was 15–25 g.

Data Analysis

OTR and WVTR, vitamin quantification, instrumental texture, and color data were analyzed by JMP 13 (SAS Institute Inc., Cary, NC) using pairwise Tukey's honestly significant difference (HSD) method between the means at $\alpha = 0.05$. Sensory data were collected by Compusense-at-hand software (Compusense Inc., Guelph, ON) and analyzed using ANOVA.

Results and Discussion

Thermal Processing Effect

Oxygen and Water Vapor Transmission Rates

Table 2 displays the OTR and WVTR of different polymeric packages (A, B, C, and D) before and after MATS processing. The OTR of the packages ranged from 0.01–0.04 cc/m² day before MATS processing. However, after MATS processing, the OTR increased significantly ($p < 0.05$) to 0.03–0.34 cc/m² day, except for package A. The increase in OTR was due to changes in polymer morphology and minor cracks in the metal oxide coating after thermal processing (Zhang et al. 2017; Dhawan et al. 2014; Mokwena et al. 2009). Package A sustained thermal and mechanical stresses during sterilization and the OTR change was not significant. The OTR of D (OS package) was not measured before processing due to the presence of active elements that absorb oxygen upon permeation. The WVTR of the pouches increased significantly ($p < 0.05$) from 0.13–0.35 g/m² day before processing to 0.76–7.3 g/m² day.

Table 2 OTR and WVTR of polymer pouches before and after MATS processing ($n = 3$)

Package	OTR (cc/m ² day)		WVTR (g/m ² day)	
	Before	After	Before	After
A	0.01 ± 0.00 ^a	0.03 ± 0.01 ^a	0.26 ± 0.04 ^a	0.62 ± 0.26 ^a
B	0.02 ± 0.01 ^a	0.18 ± 0.00 ^b	0.35 ± 0.03 ^a	2.67 ± 0.41 ^b
C	0.04 ± 0.02 ^a	0.31 ± 0.09 ^b	0.13 ± 0.02 ^a	0.76 ± 0.27 ^b
D	NA	0.34 ± 0.15 [*]	0.31 ± 0.05 ^a	7.19 ± 0.32 ^b

Values with the same small subscription letter are not significantly different from each other at ($p > 0.05$) comparing process influence between columns. NA, not applicable because of the oxygen scavenging elements present in the package

^{*}This value was measured after oxygen scavenging elements were saturated

The changes in the metal oxide coating and polymer morphology were likely due to an increase in WVTR after MATS processing (Zhang et al. 2016a). Nevertheless, these selected packages provided a range of OTR and WVTR values to reveal the influence of film barrier properties on physical and chemical quality of macaroni and cheese during long-term storage. Although the OTR and WVTR of packaging may slightly change during storage, the values obtained just after thermal processing were considered to explain physicochemical changes in macaroni and cheese during shelf life (Zhang et al. 2017). The influence of package barrier properties on the physicochemical quality of pasteurized foods has been previously reported (Zhang et al. 2019; Bhunia et al. 2017). However, the degree of influence depends upon process severity and the chemical composition of food.

Nutrients and Physicochemical Quality

Table 3 shows the nutrient content and quality of macaroni and cheese before and after thermal processing. Both sterilization methods MATS and retort significantly ($p < 0.05$) increased vitamin A and vitamin E content. Vitamin E is known for its stability and an increase in content with high-temperature processing. In this study, vitamin E increased by 39%. Other researchers found that vitamin E increased during the heating of tomato soup and baked tomato slices above 180 °C (Seybold et al. 2004). As cooking or baking time increased, vitamin E significantly increased. A short heating time helped release the vitamin E, which is naturally present, from its binding sites in tomato (Anderson and Sunderland 2002; Seybold et al. 2004).

Vitamin A concentration increased by 28% after processing in this study. This may be attributed to the presence of Arabic gum and starch as ingredients in vitamin A palmitate powder. Arabic gum is a known capsule material with very good emulsifying properties and provides protection to the heat-labile

material (Wilson and Shah 2007). Sterilization temperatures may have enabled the release of vitamin A from microcapsules. This, in turn, facilitated efficient solvent extraction during measurements and thus increased the concentration.

The shear force of macaroni increased significantly ($p < 0.05$) after retort processing, while MATS-processed macaroni showed a softer texture compared with retort-processed macaroni. This was probably because the volumetric heating by MATS in a short time at a high temperature softened the texture of macaroni compared with conduction and convection heating of retort for a longer time. Joyner et al. (2016) found that a longer parboiling time resulted in higher firmness of the pasteurized fettuccine pasta. Interestingly, color measurements did not differ significantly ($p > 0.05$) after processing.

Sensory Analysis

The macaroni and cheese packages were not analyzed for sensory tests immediately after processing. Instead, the packages were stored at 4.4 °C for 6 months. It was expected that the physicochemical changes in macaroni and cheese stored at refrigeration temperature would be minimal and that it could serve as a control for the sensory analysis. This is because there are minimal changes in sensory attributes during refrigerated storage (Zhang et al. 2019). Overall, for all sensory attributes, there was no significant ($p > 0.05$) differences observed among retort-processed (package R) and MATS-processed (packages A, B, C, and D) macaroni and cheese.

Storage Effects

Weight Loss

The weight loss in macaroni and cheese during 6-month storage at 37.8 °C ranged from 0–16% for different packaging (Fig. 2a). The aluminum foil pouch (R) showed no significant weight loss, while the highest weight loss (16%) was observed in package D with OS layer. Weight loss ranged from 2.5 to 5% in pouches A–C. At the end of the sixth month of storage, the weight loss percentage in the different packages differed significantly ($p < 0.05$), except for pouches A and C. This weight loss correlated well ($R^2 = 0.97$) with the WVTR of pouch films (Fig. 2b). Zhang et al. (2016a) also reported a weight loss of around 14% in pouches (WVTR of 8.7 g/m² day) stored at 50 °C for 3 months (Zhang et al. 2016a). A high-water vapor barrier property (lower WVTR) may be essential to minimize weight loss in packaged food.

Vitamins A and E

The vitamin A content in the processed macaroni and cheese packages R, A, B, C, and D was 10.8 µg/mL, 11.2 µg/mL, 10.5 µg/mL, 10.1 µg/mL, and 12.0 µg/mL, respectively

Table 3 Vitamin content and food quality attributes of macaroni and cheese before and after processing ($n = 3$)

Attributes	Before process	Retort* (R) $F_o = 6$ min	Microwave-assisted thermal sterilization (MATS), $F_o = 11.1$ min			
			A	B	C	D
Vitamin E ($\mu\text{g/mL}$)	108.0 ± 3.6^b	142.7 ± 5.2^a	139.4 ± 9.1^a	138.6 ± 5.9^a	127.8 ± 17.8^{ab}	146.6 ± 10.4^a
Vitamin A ($\mu\text{g/mL}$)	7.52 ± 0.26^b	10.83 ± 0.85^a	11.16 ± 0.96^a	10.45 ± 0.26^a	10.10 ± 1.61^a	11.96 ± 0.92^a
Shear force (N)	3.97 ± 0.30^b	4.55 ± 0.27^a	3.73 ± 0.11^{bc}	3.73 ± 0.21^{bc}	3.67 ± 0.19^{bc}	3.49 ± 0.16^c
Lightness (L^*)	68.4 ± 0.39^a	70.5 ± 0.57^a	70.0 ± 2.46^a	70.0 ± 2.20^a	68.1 ± 1.34^a	71.7 ± 1.89^a
Redness (a^*)	0.47 ± 0.25^a	1.29 ± 0.51^a	0.97 ± 0.27^a	0.86 ± 0.50^a	0.70 ± 0.39^a	1.16 ± 0.09^a
Yellowness (b^*)	29.0 ± 0.18^a	26.6 ± 1.16^a	26.6 ± 1.22^a	26.8 ± 0.92^a	27.4 ± 0.85^a	27.0 ± 0.76^a

*Processed in aluminum foil-based pouches (R). Values with small letters subscription in the same row are not significantly different from each other at ($p > 0.05$) and values after plus-minus (\pm) sign indicate standard deviation

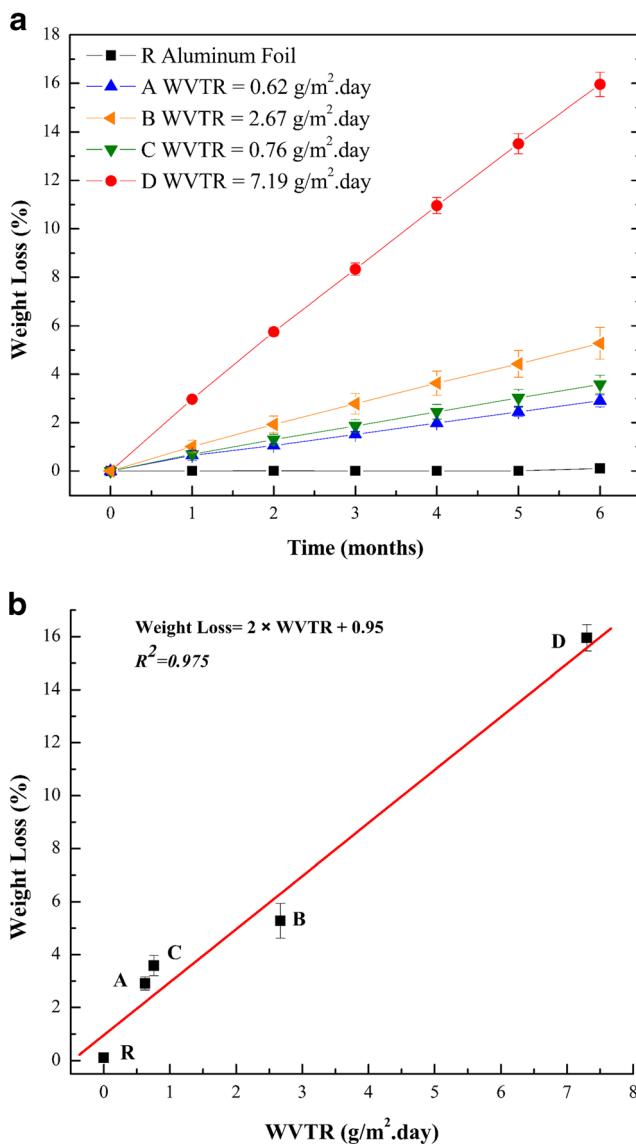


Fig. 2 Weight loss during the storage time at 37.8 °C (a) and water vapor transmission rates after processing and weight loss after 6-month correlation (b) ($n = 5$)

(Table 4). During the first month of storage at 37.8 °C, the vitamin A content decreased significantly ($p < 0.05$) but did not show any further significant reduction after the first month (Table 4). Interestingly, the effect of package OTR on vitamin A retention was not significant ($p > 0.05$) except during the first month of storage. In the first month, packages A, B, and C showed a similar vitamin A content that was significantly lower than in package D and R. The reduction in the first month was likely because the initial oxygen concentration entrapped in the headspace or within the food was decreased by initial oxidation. During the first month, packages R and D provided a superior barrier compared with the other packages. Package R contained aluminum foil, while package D contained an oxygen scavenger layer, which provided a superior barrier against oxygen ingress. All packages provided a high oxygen barrier and limited oxygen ingress. Therefore, no significant changes occurred until the sixth month of storage, except for package D. Vitamin A was reported to retain 99% of its original content in soybean oil after 9 months of storage at 23 °C, according to Favaro et al. (1991). Favaro et al. (1991) found that the retention of vitamin A was about 88–90% for different cooking methods within the same oil. NASA conducted a study on nutritional quality in space food stored at 21 °C and found that vitamin A content decreased by 15% after 3 years (Cooper et al. 2017).

Similarly, the vitamin E in macaroni and cheese showed a significant initial reduction to that of vitamin A but was stable during the remaining storage period (Table 4). The starting concentrations varied among the packages, but a slight increase was observed in 3rd to 4th month of storage time at 37.8 °C and the sixth month for some packages. A large standard deviation was noted for vitamin E in the sixth month of storage. This may have occurred because the samples were drawn from the packages and stored frozen for an extra month, as HPLC was under maintenance and unavailable to measure vitamin E. Data are limited on the stability of vitamin A and vitamin E in sterilized, ready-to-eat meals. Vitamin E content did not differ significantly ($p > 0.05$) in different OTR

Table 4 Stability of vitamins A and E in macaroni and cheese in all pouches during 6-month storage at 37.8 °C ($n = 3$)

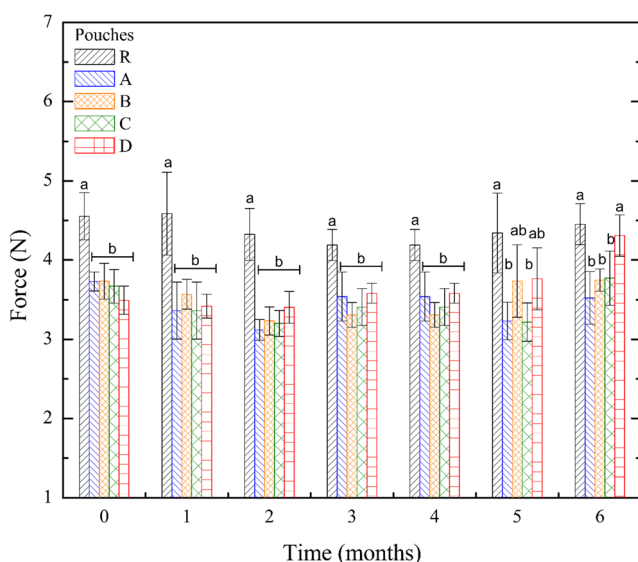
Time (months)	Retort	MATS			
	R	A	B	C	D
Vitamin A content ($\mu\text{g/mL}$)					
0	10.83 \pm 0.85 ^{aA}	11.16 \pm 0.92 ^{aA}	10.45 \pm 0.26 ^{aA}	10.10 \pm 1.61 ^{aA}	11.96 \pm 0.92 ^{aA}
1	4.85 \pm 0.25 ^{bAB}	3.24 \pm 0.27 ^{bC}	3.49 \pm 0.54 ^{bBC}	3.17 \pm 0.27 ^{bC}	5.27 \pm 0.82 ^{bcdA}
2	4.38 \pm 0.84 ^{bA}	3.71 \pm 0.39 ^{bA}	3.73 \pm 0.20 ^{bA}	3.41 \pm 0.19 ^{bA}	4.01 \pm 0.09 ^{cdA}
3	5.08 \pm 0.91 ^{bA}	4.06 \pm 0.02 ^{bA}	4.26 \pm 0.19 ^{bA}	4.54 \pm 0.67 ^{bA}	5.07 \pm 0.04 ^{bcdA}
4	4.93 \pm 0.31 ^{bA}	4.85 \pm 0.69 ^{bA}	4.72 \pm 0.88 ^{bA}	4.83 \pm 0.41 ^{bA}	5.54 \pm 0.04 ^{bA}
5	3.54 \pm 0.33 ^{bA}	3.86 \pm 0.47 ^{bA}	3.66 \pm 0.35 ^{bA}	4.15 \pm 0.35 ^{bA}	4.55 \pm 0.44 ^{bcdA}
6	4.21 \pm 0.42 ^{bA}	3.75 \pm 1.01 ^{bA}	3.61 \pm 1.50 ^{bA}	3.76 \pm 1.41 ^{bA}	3.73 \pm 0.46 ^{dA}
Vitamin E content ($\mu\text{g/mL}$)					
0	142.7 \pm 5.2 ^{aA}	139.4 \pm 9.1 ^{aA}	138.6 \pm 5.9 ^{aA}	127.8 \pm 17.8 ^{aA}	146.6 \pm 10.4 ^{aA}
1	77.5 \pm 9.7 ^{bAB}	58.9 \pm 6.2 ^{cC}	63.1 \pm 3.0 ^{cBC}	64.1 \pm 8.2 ^{bABC}	81.4 \pm 4.3 ^{bcA}
2	78.7 \pm 9.08 ^{bA}	74.3 \pm 5.4 ^{ca}	74.3 \pm 4.8 ^{bcA}	69.6 \pm 2.5 ^{bA}	73.3 \pm 2.6 ^{ca}
3	91.5 \pm 16.4 ^{bA}	83.1 \pm 3.1 ^{bcA}	80.5 \pm 7.3 ^{bcA}	89.5 \pm 7.3 ^{abA}	88.3 \pm 5.0 ^{bcA}
4	83.1 \pm 7.4 ^{bA}	85.3 \pm 7.4 ^{bcA}	86.8 \pm 16.8 ^{abcA}	85.5 \pm 5.4 ^{abA}	93.8 \pm 5.5 ^{bA}
5	70.7 \pm 4.0 ^{bA}	64.2 \pm 9.1 ^{ca}	70.0 \pm 3.4 ^{bcA}	74.0 \pm 9.3 ^{bA}	79.1 \pm 2.8 ^{bcA}
6	85.3 \pm 6.8 ^{bA}	100.7 \pm 25.0 ^{bA}	120.5 \pm 46.7 ^{abA}	107.0 \pm 42.1 ^{abA}	77.2 \pm 7.3 ^{bcA}

Values with the same small subscription letter are not significantly different from each other at ($p > 0.05$) comparing time influence between rows, capital letters are comparing between packages in each column at ($p > 0.05$), and a plus-minus (\pm) sign indicates standard deviation

packages during the sixth month of storage at 37.8 °C. Results show that the OS and high-barrier packages aided in the preservation of vitamin A and vitamin E in ready-to-eat macaroni and cheese, similar to aluminum foil-based packaging.

Shear Force

Storage time did not significantly ($p > 0.05$) affect the shear force of macaroni in the retort pouch (R) over 6 months (Fig. 3).

**Fig. 3** Changes in shear force of macaroni in different packages (R, A, B, C, and D) during 6-month storage at 37.8 °C ($n = 3$)

Polymeric packages A, B, and C also did not show any significant change during storage. However, package D showed a significant difference in shear force at the end of 6-month storage at 37.8 °C compared with previous months. The increment in the shear force was most likely due to loss of weight (approx. 16%). There was mainly moisture loss from package D, as it had highest WVTR of the packages. This loss of moisture may have led to a harder texture of macaroni, increasing the shear force at the end of storage. Diantom et al. (2016) found that the moisture helped maintain the softer texture of macaroni in ready-to-eat pasta (Diantom et al. 2016). Carini et al. (2013) found that the hardness of ready-to-eat pasta in sauce packaged in a PP tray significantly decreased in 20 days of storage at 23 °C, but recovered after a month over a storage period of 34 days (Carini et al. 2013).

Color Analysis

Figure 4 shows the color change of macaroni and cheese during storage based on redness a^* (a), yellowness b^* (b), lightness L^* (c), and total color difference ΔE (d). Macaroni and cheese showed an initial red color range ($a^* = 0.7$ – 1.3), then increased significantly over time to reach an average of 7.5 redness (Fig. 4a). Figure 4 b shows the yellowness of macaroni and cheese during storage, which did not change significantly ($p > 0.05$) from the month 0 samples. The yellowness in the recipe mainly came from the cheese sauce. During the first month, a minor reduction in yellowness may indicate

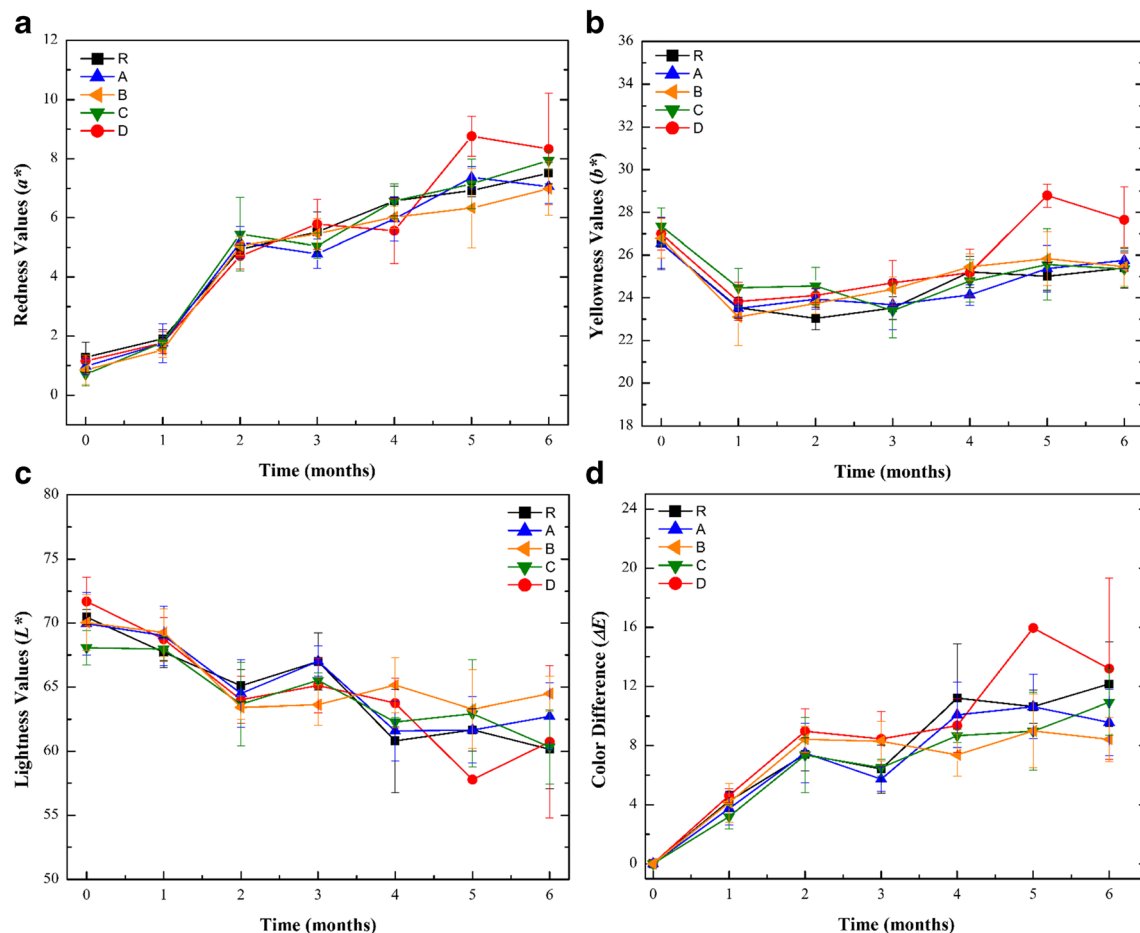


Fig. 4 Color changes during 6 months of shelf life of ready-to-eat meal (macaroni and cheese) at 37.8 °C; redness (a), yellowness (b), lightness (c), and total color difference (d) ($n = 3$)

lipid oxidation, since the formulation was rich in lipids (Fig. 4b). Residual air may have contributed to the initial low level of lipid oxidation (Frankel 2012).

On the other hand, lightness (Fig. 4c) did show a small reduction in the first 2 months of storage, although the change was not significant ($p > 0.05$) and remained mostly constant during the remaining 4 months of storage. In terms of overall color difference (ΔE), there was a trend of redness and lightness during 6-month storage of macaroni and cheese as shown in Fig. 4d. Overall, ΔE showed a significant difference ($p < 0.05$) in its value at the end of the accelerated shelf life storage compared with the values at the start of storage. The package OTR had no influence on color difference by the end of the shelf life study. Interestingly, the performance of polymeric-based packaging A, B, C, and D was similar to that of aluminum foil-based packaging. Figure 5 shows the visual color of macaroni and cheese after processing and at the end of 6 months of storage at 37.8 °C. Visual observation shows a minor pink hue in the sample after 6 months of storage in packages due to the annatto colorant present in the cheddar cheese (Shumaker and Wendorff 1998).

The main driving force of rapid color change especially during the first month of storage at 37.8 °C may be due to non-enzymatic Maillard browning and minor lipid oxidation (Baisier and Labuza 1992). The latter was limited due to the low level of oxygen available, since the packages have excellent barrier properties, with an OTR after processing of < 0.3 cc/m² day (Zhang et al. 2016a). A similar trend in color difference, L^* and a^* was observed previously for sterilized cheese stored in glass jars for 1 year, in mashed potatoes packaged in high-barrier films stored at 37 °C for 6 months, and in sweet potato puree stored at 35 °C for 9 months (Zhang et al. 2019; Kristensen et al. 2001). This color difference is attributed to non-enzymatic Maillard browning. In this study, despite differences in formula, ingredients and moisture content, browning, or general color difference in macaroni and cheese reached $\Delta E = 12$ in pouches D and R, which is classified as a different color by (Zhang et al. 2016a) and could be considered as the end of shelf life by (Zhang et al. 2016b). However, conclusions on instrumental color measurements announcing the end of shelf life should not be drawn. Other pouches had $\Delta E < 12$, indicating that the macaroni and cheese had not yet reached the end of shelf life.

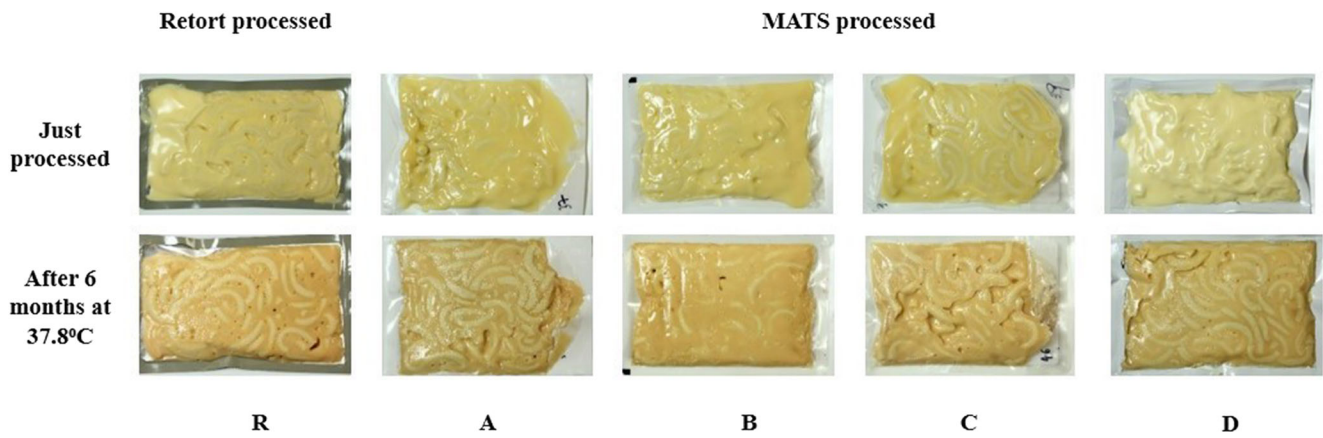


Fig. 5 Color of macaroni and cheese after the process and after 6-month storage at 37.8 °C

This accelerated shelf life study for 6-month storage at 37.8 °C was designed to be equivalent to a shelf life of approximately 3 years at ambient temperature (approx. 23–25 °C). The findings that the OTR has no influence are

Table 5 Sensory attributes score of macaroni and cheese in aluminum foil (R) and polymer packages (A, B, C, and D) stored at different conditions ($n = 12$)

Package	Control at 4.4 °C	4 weeks at 48.8 °C	3 months at 37.8 °C	6 months at 37.8 °C	<i>p</i> value
Appearance					
R	6.60 ± 0.39 ^a	5.60 ± 0.52 ^b	6.45 ± 0.37 ^a	5.80 ± 0.35 ^b	0.0001
A	6.75 ± 0.26 ^a	5.75 ± 0.45 ^b	6.46 ± 0.50 ^a	5.92 ± 0.73 ^b	0.0001
B	6.58 ± 0.36 ^a	5.50 ± 0.77 ^c	6.08 ± 0.51 ^b	5.75 ± 0.62 ^{bc}	0.0001
C	6.75 ± 0.34 ^a	5.5 ± 0.48 ^c	6.17 ± 0.25 ^b	5.75 ± 0.62 ^c	0.0001
D	6.75 ± 0.40 ^a	5.75 ± 0.75 ^c	6.29 ± 0.45 ^{ab}	5.96 ± 0.62 ^{bc}	0.0001
Odor					
R	6.55 ± 0.28 ^a	6.00 ± 0.53 ^b	6.30 ± 0.48 ^{ab}	6.10 ± 0.46 ^b	0.0013
A	6.38 ± 0.53 ^a	5.79 ± 0.66 ^b	6.29 ± 0.45 ^a	5.96 ± 0.62 ^b	0.0001
B	6.38 ± 0.48 ^a	5.58 ± 0.67 ^b	6.00 ± 0.48 ^{ab}	5.92 ± 0.60 ^b	0.0006
C	6.42 ± 0.36 ^a	5.92 ± 0.56 ^b	6.25 ± 0.34 ^{ab}	5.92 ± 0.42 ^b	0.0013
D	6.50 ± 0.30 ^a	6.04 ± 0.62 ^b	6.13 ± 0.61 ^b	5.96 ± 0.62 ^b	0.0022
Flavor					
R	6.40 ± 0.46 ^a	5.90 ± 0.66 ^a	6.00 ± 0.62 ^a	5.90 ± 0.46 ^a	0.0364
A	6.33 ± 0.54 ^a	5.58 ± 0.76 ^c	6.08 ± 0.67 ^{ab}	5.75 ± 0.92 ^{bc}	0.0001
B	6.17 ± 0.58 ^a	5.42 ± 0.70 ^b	5.71 ± 0.54 ^{ab}	5.54 ± 0.62 ^b	0.0027
C	6.21 ± 0.50 ^a	5.67 ± 0.44 ^b	6.00 ± 0.48 ^{ab}	5.79 ± 0.50 ^b	0.0007
D	6.33 ± 0.39 ^a	5.83 ± 0.78 ^b	5.92 ± 0.63 ^{ab}	5.88 ± 0.71 ^b	0.0097
Texture					
R	6.60 ± 0.39 ^a	6.10 ± 0.39 ^b	6.35 ± 0.48 ^{ab}	5.95 ± 0.37 ^b	0.0073
A	6.63 ± 0.38 ^a	6.13 ± 0.61 ^{bc}	6.42 ± 0.51 ^{ab}	6.00 ± 0.67 ^c	0.0001
B	6.50 ± 0.43 ^a	5.67 ± 0.86 ^b	5.96 ± 0.54 ^b	5.88 ± 0.61 ^b	0.0001
C	6.63 ± 0.38 ^a	5.88 ± 0.43 ^c	6.25 ± 0.45 ^b	5.92 ± 0.36 ^c	0.0001
D	6.50 ± 0.52 ^a	5.96 ± 0.84 ^b	6.17 ± 0.54 ^{ab}	6.08 ± 0.73 ^b	0.0736
Overall acceptance					
R	6.35 ± 0.47 ^a	5.65 ± 0.47 ^b	6.05 ± 0.60 ^{ab}	5.75 ± 0.42 ^b	0.0043
A	6.38 ± 0.48 ^a	5.58 ± 0.60 ^c	6.17 ± 0.69 ^{ab}	5.83 ± 0.86 ^{bc}	0.0001
B	6.25 ± 0.45 ^a	5.38 ± 0.74 ^b	5.71 ± 0.50 ^b	5.63 ± 0.61 ^b	0.0001
C	6.29 ± 0.45 ^a	5.58 ± 0.42 ^c	6.04 ± 0.50 ^{ab}	5.71 ± 0.45 ^{bc}	0.0001
D	6.42 ± 0.47 ^a	5.71 ± 0.89 ^b	5.96 ± 0.66 ^{ab}	5.83 ± 0.62 ^b	0.0028

Values with the same small subscription letter are not significantly different from each other at ($p > 0.05$) comparing time influence between columns

confirmed by Zhang et al. (2016b), who found that high-barrier polymeric packaging with $\text{OTR} < 0.30 \text{ cc/m}^2 \text{ day}$ could replace conventional aluminum foil-based packaging that is commonly used for retort-processed products.

Sensory Analysis

Table 5 presents results of the sensory evaluation by trained panelists. Samples stored at low temperature, 4.4°C , were considered as control samples. For accelerated shelf life, storage periods of 6 months at 37.8°C and 1 month at 48.8°C are considered to be equivalent to 3-year storage at 23°C (Robertson 2005). The overall acceptance for macaroni and cheese stored at 37.8°C for 3 months (equivalent to 18-month storage at 23°C) did not differ significantly from the control, except package B. This suggests that minimal sensory changes occurred during this period. At end of 6 months, macaroni and cheese in all packages showed a significant reduction in acceptance by panelists. However, the panel preferred the texture of macaroni and cheese in package D. The product in package D was well liked for its texture by panelists, probably because the loss in moisture content during storage made the cheese sauce viscous and the macaroni firmer. In general, macaroni and cheese in package B ($\text{OTR} = 0.18 \text{ cc/m}^2 \text{ day}$) and C ($\text{OTR} = 0.31 \text{ cc/m}^2 \text{ day}$) scored lower in all attributes compared with package R (aluminum foil) and D (oxygen scavenger). All packages had a sensory score of close to 6 after 6 months of storage at 37.8°C , indicating that the macaroni and cheese was still liked by the panelists and had the potential to last three years at 23°C .

Conclusions

Results of this study demonstrate that thermal sterilization selectively affects the physicochemical quality and vitamin content of ready-to-eat macaroni and cheese. The color parameters of macaroni and cheese were not affected, while vitamin A and vitamin E increased after MATS and retort processing. The retort process resulted in a higher shear force of cutting macaroni compared with MATS processing. On average, the concentration of vitamin A and vitamin E decreased by 49% and 9%, respectively, during 6-month storage at 37.8°C . The color change was significant, while the shear force of macaroni and cheese did not change during storage, except in package D.

Findings show that the OTR of MATS-processed packages in the range of 0.03 to $0.34 \text{ cc/m}^2 \text{ day}$ did not impact physicochemical and vitamin stability in macaroni and cheese though out the accelerated shelf life. However, WVTR influenced the shear force of macaroni at the end of storage in package D. Sensory analysis also confirmed that the shelf life of macaroni and cheese is at least 3 years at 23°C . Overall,

this study implies that the high-barrier packaging used in this study has a similar shelf life as aluminum pouches. Since polymeric packages have a lower density than aluminum foil packages and are also compatible with MATS, they could offer a suitable alternative in the packaging and transportation of food for the US Army and NASA space programs.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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