



Applications of different oxygen scavenging systems as an active packaging to improve freshness and shelf life of sliced bread

Elif Kütahneçi¹ · Zehra Ayhan²

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Abstract

The objective of this study was to extend the shelf life of sliced sourdough bread by active packaging using oxygen absorbers of different capacity in combination with a modified atmosphere. The sourdough bread slices were packaged using biaxially oriented polypropylene/polyvinylidene chloride (BOPP/PVDC) bags with two oxygen absorbers of different capacity (100 and 300 cc) under different atmospheres (air, 50% carbon dioxide [CO₂]:50% nitrogen [N₂], 100% N₂) and stored at 22 °C for 18 days. The high capacity absorbers kept the O₂% concentration < 1% in 100% N₂ atmosphere during the entire storage. In the headspace containing the high capacity absorber, the initial oxygen (21%) decreased below 1% in six days, and in nine days in the packages with the low capacity absorber. Whiteness index decreased and browning index increased during storage, but the highest whiteness index, lowest browning index and hardness were obtained for 100% N₂ combined with the high capacity absorber. The chemical properties were affected by the storage time but not by the packaging treatments. The microbial growth was kept at a lower level in bags with 100% N₂ and high capacity absorbers. The study indicated that modified atmosphere packaging (50% CO₂ and 50% N₂) with no initial oxygen was not sufficient to preserve microbial quality, possibly due to transfer of trapped oxygen from the pores of the sliced bread to the headspace during the storage period. The product packaged with 100% N₂ and the high capacity absorber received the highest sensory scores and had the longest shelf life (12 days) among all treatments.

Keywords Active packaging · Modified atmosphere packaging · Oxygen absorbers · Shelf life · Sliced sourdough bread

1 Introduction

Bread is one of the main and widely consumed foods in Turkey and worldwide due to its nutritional and sensory characteristics (Galic et al. 2009). Bread is mostly consumed fresh, and preservation of freshness is still a major challenge for the bakery industry due to fast staling which reduces consumer acceptability (Fik et al. 2012). Thus, bread is one of the most wasted foods (FAO 2011). Melini and Melini (2018) reported that spoilage of bread results in 5–10% losses in world bread production and economic losses for the bakery industry and the consumer besides health risks from mycotoxin contamination. The average shelf life of bread

is about 3–4 days at room temperature for genuine bread made of flour, yeast, water and salt without any preservatives and enzymes (Nielsen and Rios 2000; Fik 2004; Galic et al. 2009; Khoshakhlagh et al. 2014). Enzymes such as amylases and lipases, emulsifiers and lipids are often included in bread formulations to retard staling (Pasqualone 2019). Sourdough lowers the pH and reduces mold growth. However, the pH drop and the acidification can extend bread shelf life only to a limited extent and/or do not extensively inhibit mold growth (Melini and Melini 2018). Apparently, the formulation has an influence on bread shelf life.

The main limiting factors on shelf life are staling and microbial spoilage mostly due to fungal activity (Nielsen and Rios 2000; Rodriguez et al. 2000; Kotsianis et al. 2002; Galic et al. 2009; Khoshakhlagh et al. 2014). There has been a growing interest in packaging and preservation technologies to retard staling and microbial spoilage and thus, increase the shelf life for whole and sliced bread (Nielsen and Rios 2000; Gutierrez et al. 2009); e.g. active packaging with absorbing and/or releasing compounds effective against

✉ Zehra Ayhan
zehraayhan@sakarya.edu.tr

¹ Culinary Arts Department, Cappadocia Vocational College, Cappadocia University, Nevşehir, Turkey

² Department of Food Engineering, Faculty of Engineering, Sakarya University, Sakarya, Turkey

bread staling and/or antimicrobials preventing the growth of undesirable microorganisms (Melini and Melini 2018). Pasqualone (2019) reviewed various packaging solutions to extend the shelf life of bread including all types of potential packaging materials and innovative packaging solutions from active and intelligent packaging to edible coating and nanomaterials coupled with modified atmosphere packaging (MAP). The author reported that active packaging in the form of active sachets could be less expensive than MAP as it does not need high-barrier films and special packaging machines for displacing atmospheric air from the headspace. The most widely used preservation methods are MAP technologies and the use of antimicrobials, basically chemical additives based on propionates and sorbates, especially for sliced products (Pasqualone 2019). However, consumers prefer additive free foods, especially when they are frequently and daily consumed (Melini and Melini 2018). MAP is reported to prevent the microbial spoilage and bread staling during storage (Rasmussen and Hansen 2001; Degirmencioglu et al. 2011; Khoshakhlagh et al. 2014). MAP aims to remove the atmospheric air by a gas mixture commonly composed by CO₂ and N₂ (Pasqualone 2019). CO₂ is mostly used with MAP packaging of bakery products due to its bacteriostatic and fungistatic effects (Sivertsvik et al. 2002). It is also challenging to reduce the oxygen (O₂) content in the package headspace to a very low concentration due to a large number of pores in the bread crumb which tend to trap O₂ (Galic et al. 2009; Fik et al. 2012). Molds could grow in the headspace with an O₂ concentration as low as 1–2% (Tabak and Cook 1978). However, a high CO₂-MAP combined with nitrogen may not be sufficient to remove the trapped O₂ in the pores.

Oxygen absorbers are proposed as an alternative to overcome this drawback of MAP packaging (Berenzon and Saguy 1998; Del Nobile et al. 2003; Melini and Melini 2018).

There are some researches concentrated on combination of the MAP and active packaging techniques to extend the shelf life of different types of bread (Fernandez et al. 2006; Degirmencioglu et al. 2011; Suhr and Nielsen 2013).

It was reported that no microbial growth was observed in sliced rye bread packaged with oxygen absorbers during 42 days of storage (Salminen et al. 1996). Del Nobile et al. (2003) prolonged the shelf life of the bread from three days to approximately 18 days in 80% CO₂ + 20% N₂ atmosphere using oxygen absorbers.

MAP technologies in combination with oxygen absorbers can be used more effectively to remove the O₂ remaining in the pores of the sliced product and thus, retard the microbial spoilage and extend the shelf life. There is limited literature on a combination of MAP and oxygen absorbers as called active modified atmosphere for packaging of bakery products (Nielsen and Rios 2000; Guynot et al. 2003). Exploring

active packaging applications as an alternative to chemical additives in frequently consumed products such as sliced bread could be an alternative for both industry and consumers who prefer a clean label.

Upasen and Wattanachai (2018) demonstrated that the combination of active packaging using oxygen scavengers and MAP has a high potential for bakery product industries. However, they reported that if the packaging film possesses a high barrier for O₂ transmission, the use of oxygen scavenger and MAP is almost unnecessary. However, this is not valid for the sliced bread with pores trapping O₂ which will eventually transfer into the package headspace even if the O₂ transmission is prevented by the high barrier material. This study aimed to improve the quality indices and extend the shelf life of sliced sourdough bread using different capacity oxygen absorbers combined with MAP. The novelty of this study is to explore the combination of different capacity oxygen absorbers under 100% N₂ for low cost active MAP packaging, and also different capacity oxygen absorbers under air atmosphere to investigate whether active packaging without using special MAP gases and packaging machines is possible.

2 Material and methods

2.1 Material

Sourdough bread slices consisting of wheat flour, water, sourdough starter, yeast and salt were freshly supplied from a local bakery. The packaging material was supplied by Polinas Packaging Company (Manisa, Turkey). The packaging material is of corona treated printable layer/transparent BOPP film/PVDC coating with a thickness of 31 µm. The O₂ transmission rate (OTR) and water vapor transmission rate (WVTR) are 30.0 cm³/m²/24 h (at 23 °C and 0% relative humidity [RH]) and 3.5 g/m²/24 h (at 38 °C and 90% RH), respectively. Two different concentrations of oxygen absorbers (Fresh Life[®]) were provided by CML Food Company (Istanbul, Turkey): a low capacity absorber of 100 cc and a high capacity absorber of 300 cc.

The oxygen absorbers used in this study are iron based oxygen absorbers. In iron based O₂, the reduced iron is oxidized to the non-toxic ferric oxide trihydrate complex under appropriate moisture conditions [4Fe(OH)₂ + O₂ + 2H₂O → 4Fe(OH)₃]. Thus, it consumes the O₂ in the package headspace (Galic et al. 2009; Prasad and Kochhar 2014; Yildirim et al. 2018).

2.2 Packaging operation and shelf life study

The packaging film provided by the company was cut into sizes of 30 cm × 37 cm, and sealed with a constant heat

sealer (ME-400 CFN, Mercier Corporation, Taiwan) at 125 °C to produce a pouch. The heat seal was tested for leaks according to dye penetration test method of Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM) (Arndt 2001).

Ten slices of bread (approx. 350 ± 30 g and each slice in 12×11 cm size) were placed in BOPP/PVDC bags hygienically and packaged with a single chamber packaging machine (Reepack, RV 300, Germany) combined with a triple gas mixer (KM60-3, Witt, Germany). Six different packaging applications were prepared (Table 1). Triplicate packages were prepared for each application and were stored at 22 °C for 18 days and analyzed on 0, 3, 6, 9, 12, 15 and 18 days for headspace gas, physical, chemical, microbiological and sensory attributes. 21 packages for each packaging application and a total of 126 packages for six packaging applications were prepared. Three packages on each analysis day were tested for each application.

2.3 Analyses

2.3.1 Headspace analysis

The headspace O_2 (%v/v) and CO_2 (%v/v) concentrations were determined using a gas analyzer (Witt, Oxybaby, Hamburg, Germany) before opening the packages. Gas analysis was performed by inserting the needle attached to the gas analyzer through an impermeable rubber seal attached to the outer surface of the packaging film. The results are expressed as $O_2\%$ and $CO_2\%$. The measurements were taken twice from each bag and averages of six measurements were calculated for each application (Nalçabasmaz et al. 2017).

2.3.2 Physical analyses

The color of sliced bread samples was analyzed by a color analyzer (PCE-CSM 7, PCE Instruments, Meschede, Germany), using the CIE L^*a^* and b^* scale. Five slices were taken from each bag, and the color measurements were performed on both sides of each slice. Averages of 30 measurements were taken for each application (Khosshakhlagh et al. 2014). Whiteness index (WI) and browning index (BI) were calculated based on the color values (L^* , a^* and b^*) of sourdough bread with Eqs. 1–3.

$$WI = L^* - 3b^* + 3a^* \quad (1)$$

$$BI = \frac{100(x - 0.31)}{0.17} \quad (2)$$

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (3)$$

Texture analysis was performed by TA-TX Plus (Stable Micro System, Surrey, UK) with the texture analyzer using a 50 mm diameter cylinder probe with 1 mm/s test speed and 30 kg load cell under compression mode. The samples were cut into 8×8 cm dimensions. Five slices were taken from each bag, and 15 measurements were taken for each application (Rasmussen and Hansen 2001). The results were expressed as compressive force (N) and bread firmness was measured according to the American Association of Cereals Chemists (AACC) method 74–09 (AACC 1999).

Table 1 Packaging applications

Application abbreviation	Application details
MAP1-OA1	100% N_2 modified atmosphere with a low capacity (100 cc) oxygen scavenger
MAP1-OA2	100% N_2 modified atmosphere with a high capacity (300 cc) oxygen scavenger
Air atmosphere-OA1	Air atmosphere with a low capacity (100 cc) oxygen scavenger
Air atmosphere-OA2	Air atmosphere with a high capacity (300 cc) oxygen scavenger
MAP2	50% CO_2 + 50% N_2 modified atmosphere
Air atmosphere	Air atmosphere (control)

Table 2 Sensory attributes and scale used for sliced bread

Attribute	1	5
Crust color	Matt, pale color	Unique bright color of bread
Crumb color	Non-homogenous dark color	Homogenous white-beige bread color
Smell	Weak bread smell, musty smell	Strong and distinctive smell of bread
General acceptability	Very bad	Very good

2.3.3 Chemical analyses

A total of 10 g was taken from five different slices of each bag and homogenized with 100 ml of distilled water for 1 min and the pH was measured with a calibrated pH meter (WTW-315, Germany). Two measurements were performed for each bag and averages of six measurements were taken for each application (American Association of Cereal Chemists International [AACCI], 2010). Analysis of water activity was performed on crust and crumb of the bread samples with a water activity meter (AquaLab®, Model Series 3, Decagon Devices, Inc., Pullman, WA., USA).

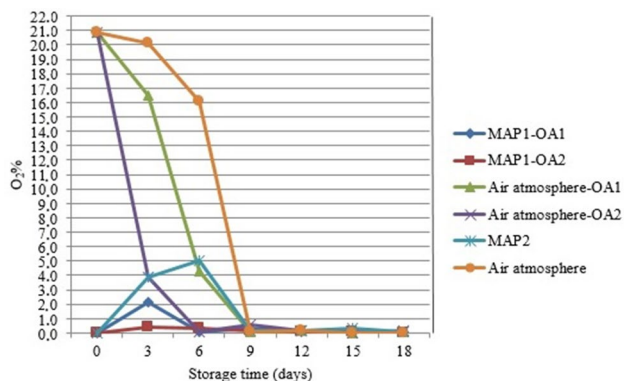
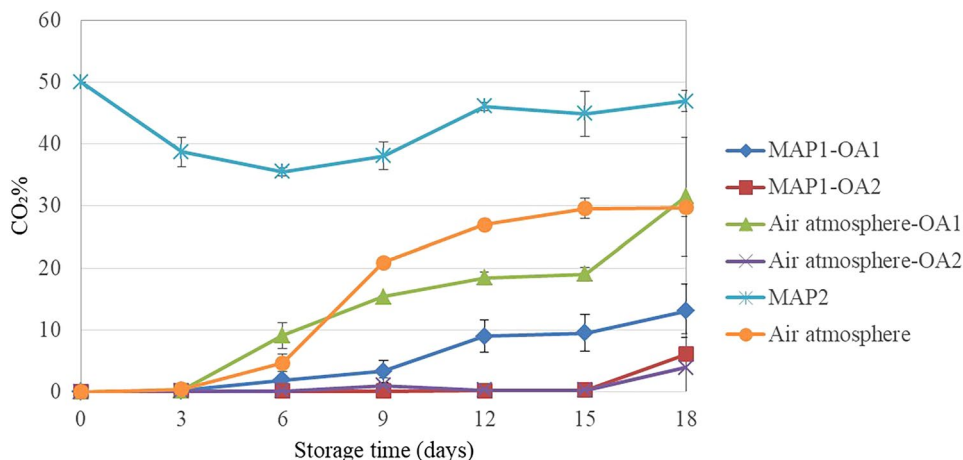


Fig. 1 The effect of oxygen absorbers and MAP on headspace oxygen content (%) of sliced bread during the storage period (MAP1-OA1: 100% N₂ + low capacity oxygen absorber, MAP1-OA2: 100% N₂ + high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂)

Fig. 2 The effect of oxygen absorbers and MAP on headspace carbon dioxide content (%) of sliced bread during the storage period (MAP1-OA1: 100% N₂ + low capacity oxygen absorber, MAP1-OA2: 100% N₂ + high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂)



Two measurements were made per crumb and crust for each bag and averages of six measurements were taken for each application.

2.3.4 Microbiological analyses

Total mesophilic aerobic bacterial count were measured. Total mesophilic aerobic bacteria were determined using plate count agar (PCA, Merck) with incubation at 37 °C for 48 h. Microbial counts were expressed as log cfu/g. The microbiological quality (visible mold growth) of the bread slices was also observed visually and was calculated by dividing the number of slices having visible mold growth to the total number of slices per package on each analysis day.

2.3.5 Sensory evaluation

Sensory evaluation was performed with a panelist group of six experienced panelists. Crust and crumb color, smell and general acceptability properties were evaluated on a five points hedonic scale with the score of three and above indicating acceptability (Certel et al. 2009). Table 2 shows the evaluation criteria for each attribute.

2.3.6 Statistical analysis

Effects of experimental factors (packaging application and storage time) were evaluated by using a two-way analysis of variance (ANOVA) and Duncan multiple comparison range tests at 95% confidence level. SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) for Windows was used as the statistical software. All results are presented by mean values \pm standard deviation.

Table 3 Effect of active packaging and MAP on WI and BI values during storage

Application	Storage time							
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	
	WI							
MAPI-OA1	51.70 ± 2.28 ^{Aa}	50.87 ± 2.15 ^{ABa}	46.92 ± 3.45 ^{Cb}	48.34 ± 2.25 ^{Ab}	43.95 ± 7.23 ^{ABc}	43.95 ± 2.98 ^{Ac}	42.35 ± 4.90 ^{Bc}	
MAPI-OA2	51.70 ± 2.28 ^{Aa}	49.57 ± 2.30 ^{Bb}	47.52 ± 2.94 ^{Cc}	48.20 ± 2.27 ^{Abc}	42.79 ± 3.23 ^{Bd}	41.74 ± 3.56 ^{ABd}	46.99 ± 6.85 ^{Ac}	
Air atmosphere-OA1	51.70 ± 2.28 ^{Aa}	50.88 ± 2.50 ^{ABab}	46.92 ± 2.69 ^{Cb}	40.55 ± 12.75 ^{Cc}	41.17 ± 9.24 ^{BCc}	38.92 ± 11.78 ^{Bc}	39.29 ± 8.53 ^{Bc}	
Air atmosphere-OA2	51.70 ± 2.28 ^{Aa}	50.22 ± 2.49 ^{ABa}	47.95 ± 2.88 ^{ABb}	46.30 ± 3.76 ^{ABc}	41.46 ± 3.19 ^{BCe}	43.39 ± 2.69 ^{Ad}	41.15 ± 2.90 ^{Bc}	
MAP2	51.70 ± 2.28 ^{Aa}	48.23 ± 2.03 ^{Cb}	49.29 ± 1.91 ^{Ab}	46.02 ± 2.58 ^{ABcd}	46.55 ± 2.78 ^{Ac}	44.65 ± 4.68 ^{Ad}	49.18 ± 3.91 ^{Ab}	
Air atmosphere	51.70 ± 2.28 ^{Aa}	51.35 ± 3.26 ^{Aa}	46.35 ± 3.25 ^{Cb}	42.42 ± 12.65 ^{BCbc}	38.55 ± 10.39 ^{Ccd}	38.83 ± 13.21 ^{Bcd}	34.20 ± 12.38 ^{Cd}	
	BI							
MAPI-OA1	14.84 ± 0.96 ^{Ac}	14.97 ± 1.26 ^{Bc}	16.16 ± 1.99 ^{Bb}	16.81 ± 1.31 ^{ABb}	18.45 ± 3.17 ^{BCa}	18.98 ± 1.68 ^{ABa}	18.10 ± 1.57 ^{BCa}	
MAPI-OA2	14.84 ± 0.96 ^{Ac}	15.15 ± 1.32 ^{Bc}	15.21 ± 1.35 ^{CDc}	15.77 ± 1.37 ^{Bc}	18.27 ± 1.72 ^{Ca}	18.97 ± 2.17 ^{ABa}	16.90 ± 3.00 ^{Cb}	
Air atmosphere-OA1	14.84 ± 0.96 ^{Ad}	16.23 ± 1.81 ^{Ad}	17.06 ± 1.72 ^{Ad}	18.37 ± 6.91 ^{Ac}	20.28 ± 5.40 ^{Aa}	20.13 ± 7.43 ^{ABb}	19.78 ± 2.21 ^{ABa}	
Air atmosphere-OA2	14.84 ± 0.96 ^{Ad}	15.61 ± 1.58 ^{ABd}	15.59 ± 1.65 ^{BCd}	17.28 ± 2.09 ^{ABc}	19.92 ± 2.13 ^{ABa}	18.63 ± 1.65 ^{ABb}	19.94 ± 2.29 ^{ABa}	
MAP2	14.84 ± 0.96 ^{Ac}	15.65 ± 1.61 ^{ABb}	14.57 ± 1.40 ^{Dc}	17.41 ± 1.77 ^{ABa}	17.55 ± 1.72 ^{Ca}	18.06 ± 2.07 ^{Ba}	16.37 ± 1.16 ^{Cb}	
Air atmosphere	14.84 ± 0.96 ^{Ac}	15.41 ± 1.84 ^{ABde}	17.71 ± 2.28 ^{Acde}	17.26 ± 2.88 ^{ABcd}	20.01 ± 2.77 ^{ABabc}	21.89 ± 12.11 ^{Aa}	21.64 ± 7.25 ^{Aab}	

For each column, mean values of similar capital letters are not statistically significant among applications ($p > 0.05$). For each line, mean values of similar small letters are not statistically significant during storage ($p > 0.05$)

MAPI-OA1: 100% N₂ + low capacity oxygen absorber, MAPI-OA2: 100% N₂ + high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂

Table 4 Effect of active packaging and MAP on bread texture during storage

Application	Compressive Force (N)						
	Storage time						
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
MAP1-OA1	4.90 ± 0.97 ^{Af}	13.86 ± 2.25 ^{ABCe}	19.53 ± 4.71 ^{BCd}	26.66 ± 3.32 ^{Ac}	29.52 ± 6.68 ^{ABbc}	37.49 ± 3.86 ^{Aa}	30.26 ± 3.92 ^{Ab}
MAP1-OA2	4.90 ± 0.97 ^{Ae}	14.55 ± 3.50 ^{ABd}	18.54 ± 3.38 ^{CDc}	22.07 ± 2.79 ^{Bb}	23.82 ± 3.28 ^{Cb}	30.82 ± 5.31 ^{BCa}	31.11 ± 6.08 ^{Aa}
Air atmosphere-OA1	4.90 ± 0.97 ^{Ad}	14.92 ± 1.97 ^{Ac}	27.11 ± 3.52 ^{Ab}	26.58 ± 5.20 ^{Ab}	33.15 ± 6.81 ^{Aa}	32.85 ± 3.79 ^{Ba}	27.08 ± 6.35 ^{Ab}
Air atmosphere-OA2	4.90 ± 0.97 ^{Ad}	12.23 ± 1.19 ^{Cc}	17.88 ± 3.19 ^{CDb}	20.82 ± 3.34 ^{Bb}	30.83 ± 6.35 ^{ABa}	28.73 ± 6.02 ^{Ca}	28.42 ± 6.59 ^{Aa}
MAP2	4.90 ± 0.97 ^{Af}	13.18 ± 2.22 ^{ABCe}	16.77 ± 2.14 ^{DD}	26.37 ± 5.37 ^{Ac}	32.48 ± 6.20 ^{Aa}	29.25 ± 5.00 ^{BCb}	29.88 ± 2.36 ^{Aab}
Air atmosphere	4.90 ± 0.97 ^{Ad}	12.79 ± 2.18 ^{BCc}	21.13 ± 2.68 ^{Bb}	22.16 ± 5.91 ^{Bb}	27.27 ± 4.76 ^{BCa}	28.00 ± 6.35 ^{Ca}	28.31 ± 5.97 ^{Aa}

For each column, mean values of similar capital letters are not statistically significant among applications ($p > 0.05$). For each line, mean values of similar small letters are not statistically significant during storage ($p > 0.05$)

MAP1-OA1: 100% N₂ + low capacity oxygen absorber, MAP1-OA2: 100% N₂ + high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂

Table 5 Effect of active packaging and MAP on pH during storage

Application	pH						
	Storage time						
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
MAP1-OA1	4.94 ± 0.08 ^{Ab}	5.05 ± 0.17 ^{BCab}	5.06 ± 0.1 ^{BCab}	4.97 ± 0.09 ^{Bb}	5.22 ± 0.16 ^{BCa}	5.11 ± 0.11 ^{Bab}	5.22 ± 0.15 ^{ABa}
MAP1-OA2	4.94 ± 0.08 ^{Abc}	5.05 ± 0.13 ^{BCbc}	4.96 ± 0.21 ^{Cbc}	4.94 ± 0.18 ^{Bbc}	5.31 ± 0.04 ^{ABa}	4.86 ± 0.03 ^{Cc}	5.08 ± 0.24 ^{Bb}
Air atm-OA1	4.94 ± 0.08 ^{Ade}	4.91 ± 0.04 ^{De}	4.98 ± 0.03 ^{Ccd}	5.02 ± 0.04 ^{Bbc}	5.09 ± 0.05 ^{Da}	5.10 ± 0.04 ^{Ba}	5.04 ± 0.04 ^{Bab}
Air atm-OA2	4.94 ± 0.08 ^{Ac}	5.19 ± 0.03 ^{Ab}	5.16 ± 0.04 ^{ABb}	4.92 ± 0.23 ^{Bc}	5.31 ± 0.02 ^{ABa}	5.32 ± 0.03 ^{Aa}	5.30 ± 0.05 ^{Aa}
MAP2	4.94 ± 0.08 ^{Abc}	5.00 ± 0.12 ^{CDabc}	5.10 ± 0.10 ^{BCab}	4.92 ± 0.19 ^{Bc}	5.15 ± 0.08 ^{CDa}	5.15 ± 0.18 ^{Ba}	5.07 ± 0.14 ^{Babc}
Air atmosphere	4.94 ± 0.08 ^{Ae}	5.14 ± 0.04 ^{ABd}	5.25 ± 0.04 ^{Aabc}	5.30 ± 0.04 ^{Aab}	5.35 ± 0.04 ^{Aa}	5.19 ± 0.13 ^{Bbcd}	5.18 ± 0.15 ^{ABcd}

For each column, mean values of similar capital letters are not statistically significant among applications ($p > 0.05$). For each line, mean values of similar small letters are not statistically significant during storage ($p > 0.05$)

MAP1-OA1: 100% N₂ + low capacity oxygen absorber, MAP1-OA2: 100% N₂ + high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂

3 Results and discussion

3.1 Headspace gas concentration (O₂% and CO₂%)

The headspace evolution of O₂ and CO₂ is shown in Fig. 1 and 2, respectively. The high capacity oxygen absorbers (300 cc) were able to keep the O₂% concentration below 1% in 100% N₂-MAP for the entire storage. However, with the low capacity absorbers (100 cc) the O₂ level reached up to 2.1% on the 3rd day of storage possibly to the transfer of O₂ from the pores of the bread. After the 3rd day the low capacity absorbers kept the O₂% concentration below 1%. The initial O₂ concentration (21%) of air atmosphere significantly decreased below 1% in six days in the headspace containing high capacity oxygen absorbers, while it took nine days for

the packages with low capacity absorbers. This indicates that air atmosphere combined with the oxygen absorbers did not effectively lower the O₂ content in a short time causing a higher microbial count during storage. Janjarasskul et al. (2016) reported that using an oxygen absorber (400 cc) combined with a high barrier pouch is a useful solution for eliminating the O₂ remaining in the pores of sponge cake.

Although there is no initial O₂ in the application of 50% CO₂ + 50% N₂, the O₂ increased up to 5% possibly due to transfer of trapped O₂ from the sliced bread pores to the headspace on 6th day. This concentration of O₂ decreased below 1% after 9 days of storage possibly due to aerobic microbial growth. Although 50–60% CO₂ combined with N₂ is widely suggested for bread packaging, it was not effective to remove the trapped O₂ from the pores of the sliced

Table 6 Effect of active packaging and MAP on water activity (a_w) of crumb and crust during storage

Application	Storage time								
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18		
MAPI-OA1	0.941 ± 0.003 ^{Aabc}	0.949 ± 0.006 ^{Ba}	0.946 ± 0.004 ^{Ab}	0.943 ± 0.003 ^{BCabc}	0.937 ± 0.012 ^{Bc}	0.948 ± 0.002 ^{Ab}	0.940 ± 0.010 ^{Abc}		
MAPI-OA2	0.941 ± 0.003 ^{Ab}	0.938 ± 0.005 ^{Cb}	0.950 ± 0.003 ^{Aa}	0.949 ± 0.004 ^{Aa}	0.949 ± 0.004 ^{Aa}	0.939 ± 0.007 ^{Bb}	0.943 ± 0.003 ^{Ab}		
Air atm-OA1	0.941 ± 0.003 ^{Abc}	0.939 ± 0.006 ^{Cc}	0.952 ± 0.002 ^{Aa}	0.938 ± 0.006 ^{Cc}	0.944 ± 0.007 ^{ABbc}	0.947 ± 0.006 ^{Ab}	0.945 ± 0.003 ^{Ab}		
Air atm-OA2	0.941 ± 0.003 ^{Ab}	0.940 ± 0.010 ^{Cab}	0.936 ± 0.006 ^{BCb}	0.944 ± 0.004 ^{ABa}	0.947 ± 0.005 ^{Aa}	0.948 ± 0.006 ^{Aa}	0.943 ± 0.009 ^{Ab}		
MAP2	0.941 ± 0.003 ^{Aa}	0.941 ± 0.009 ^{Ca}	0.943 ± 0.008 ^{ABa}	0.942 ± 0.005 ^{BCa}	0.941 ± 0.005 ^{ABa}	0.941 ± 0.009 ^{ABa}	0.944 ± 0.002 ^{Aa}		
Air atmosphere	0.941 ± 0.003 ^{Ab}	0.959 ± 0.003 ^{Aa}	0.933 ± 0.013 ^{Cbc}	0.939 ± 0.007 ^{BCb}	0.941 ± 0.003 ^{ABb}	0.941 ± 0.007 ^{ABb}	0.929 ± 0.008 ^{Bc}		
MAPI-OA1	0.766 ± 0.023 ^{Ac}	0.908 ± 0.013 ^{ABCb}	0.913 ± 0.006 ^{BCDb}	0.941 ± 0.002 ^{Aa}	0.933 ± 0.007 ^{Aa}	0.937 ± 0.007 ^{ABa}	0.931 ± 0.006 ^{Ba}		
MAPI-OA2	0.766 ± 0.023 ^{Ad}	0.892 ± 0.006 ^{Cc}	0.911 ± 0.011 ^{Bb}	0.905 ± 0.014 ^{Bbc}	0.926 ± 0.008 ^{Aa}	0.927 ± 0.005 ^{CDa}	0.936 ± 0.005 ^{ABa}		
Air atm-OA1	0.766 ± 0.023 ^{Ad}	0.918 ± 0.021 ^{ABc}	0.929 ± 0.005 ^{Abc}	0.933 ± 0.004 ^{Abc}	0.928 ± 0.004 ^{Abc}	0.945 ± 0.002 ^{Aa}	0.939 ± 0.003 ^{Ab}		
Air atm-OA2	0.766 ± 0.023 ^{Ac}	0.912 ± 0.018 ^{ABCb}	0.922 ± 0.003 ^{ABab}	0.909 ± 0.005 ^{Bb}	0.930 ± 0.003 ^{Aa}	0.934 ± 0.005 ^{BCa}	0.936 ± 0.007 ^{ABa}		
MAP2	0.766 ± 0.023 ^{Ad}	0.899 ± 0.032 ^{BCc}	0.912 ± 0.012 ^{CDbc}	0.933 ± 0.006 ^{Ab}	0.932 ± 0.013 ^{Ab}	0.931 ± 0.010 ^{BCDab}	0.938 ± 0.003 ^{Aa}		
Air atm	0.766 ± 0.023 ^{Ac}	0.928 ± 0.004 ^{Aa}	0.921 ± 0.004 ^{ABCab}	0.910 ± 0.004 ^{Bb}	0.931 ± 0.004 ^{Aa}	0.924 ± 0.010 ^{Da}	0.923 ± 0.010 ^{Ca}		

For each column, mean values of similar capital letters are not statistically significant among applications ($p > 0.05$). For each line, mean values of similar small letters are not statistically significant during storage ($p > 0.05$)

MAPI-OA1: 100% N₂ + low capacity oxygen absorber, MAPI-OA2: 100% N₂ + high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂

Table 7 Effect of active packaging and MAP on microbial growth during storage

Application	Total aerobic mesophilic bacterial count (log cfu/g)						
	Storage time						
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
MAP1-OA1	<2 ^a	<2	5.65 ± 0.14 ^{Bd}	7.56 ± 0.25 ^{Aa}	7.49 ± 0.24 ^{Aa}	7.07 ± 0.37 ^{Ab}	6.82 ± 0.46 ^{Ac}
MAP1-OA2	<2	<2	4.24 ± 0.16 ^{Dd}	4.90 ± 0.44 ^{Dc}	4.47 ± 0.25 ^{Ed}	5.32 ± 0.81 ^{Cb}	5.90 ± 0.13 ^{Ca}
Air atmosphere-OA1	<2	<2	5.64 ± 0.42 ^{Bd}	5.97 ± 0.43 ^{Cbc}	5.87 ± 0.40 ^{Ccd}	6.25 ± 0.42 ^{Bab}	6.36 ± 0.42 ^{Ba}
Air atmosphere-OA2	<2	<2	5.03 ± 0.42 ^{Ca}	4.84 ± 0.51 ^{Da}	5.07 ± 0.56 ^{Da}	5.16 ± 0.45 ^{Ca}	5.16 ± 0.53 ^{Da}
MAP2	<2	<2	6.57 ± 0.05 ^{Ab}	6.55 ± 0.30 ^{Bb}	6.96 ± 0.12 ^{Ba}	7.20 ± 0.19 ^{Aa}	6.94 ± 0.73 ^{Aa}
Air atmosphere	<2	4.83 ± 0.26 ^{Ad}	5.77 ± 0.41 ^{Bc}	6.47 ± 0.23 ^{Bb}	6.95 ± 0.34 ^{Ba}	7.14 ± 0.64 ^{Aa}	7.03 ± 0.16 ^{Aa}

For each column, mean values of similar capital letters are not statistically significant among applications ($p > 0.05$). For each line, mean values of similar small letters are not statistically significant during storage ($p > 0.05$)

^a<2, No development even in the lowest dilution (10^{-2})

MAP1-OA1: 100% N₂+low capacity oxygen absorber, MAP1-OA2: 100% N₂+high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂+79% N₂+low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂+79% N₂+high capacity oxygen absorber, MAP2: 50% CO₂+50% N₂, Air atmosphere: (Control) 21% O₂+79% N₂

Table 8 Effect of active packaging and MAP on %visual mold growth during storage

Application	Visible mould growth (%)						
	Storage time						
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
MAP1-OA1	0.00	0.00	0.00	53.33	63.33	100.00	100.00
MAP1-OA2	0.00	0.00	0.00	0.00	0.00	20.00	100.00
Air atmosphere-OA1	0.00	0.00	30.00	100.00	100.00	100.00	100.00
Air atmosphere-OA2	0.00	0.00	0.00	0.00	30.00	36.67	100.00
MAP2	0.00	0.00	10.00	100.00	100.00	100.00	100.00
Air atmosphere	0.00	0.00	40.00	100.00	100.00	100.00	100.00

MAP1-OA1: 100% N₂+low capacity oxygen absorber, MAP1-OA2: 100% N₂+high capacity oxygen absorber, Air atmosphere-OA1: 21% O₂+79% N₂+low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂+79% N₂+high capacity oxygen absorber, MAP2: 50% CO₂+50% N₂, Air atmosphere: (Control) 21% O₂+79% N₂

bread. Similar results obtained by Öz et al. (2006) indicating that the headspace of packaged bread samples with MAP (30% CO₂+70% N₂) had no O₂ initially, but O₂ was detected between 2–3% due to the transfer of trapped O₂ from the pores of bread slices during storage.

CO₂ concentrations remained below 1% in the applications with high capacity oxygen absorbers for 15 days. CO₂% levels increased up to 31.5% at the end of the storage with air atmosphere with low capacity oxygen absorbers, whereas the application of 100% N₂ with the low capacity oxygen absorbers reached 13.1%. The released O₂ from the pores possibly provided suitable growth conditions for aerobic microorganisms which produce CO₂, resulting in CO₂ increase for applications without oxygen absorbers.

Modified gas compositions without O₂ are generally reported to be successful in slowing down the microbial growth. However, the negative effect of the trapped O₂ in

bread pores could only be eliminated by the use of oxygen absorbers (Piergiovanni and Fava 1997; Janjarasskul et al. 2016).

3.2 Physical properties

The WI decreased and BI increased in all treatments during increased storage (Table 3). Considering the recommended longest shelf life of sliced bread as 12 days, 100% N₂ atmosphere with both oxygen absorbers had a higher WI than the air atmosphere. However, there was no significant difference in WI between products packaged under air atmosphere (control) and air atmosphere with both oxygen absorbers.

The bread slices packaged under 100% N₂ atmosphere with both high and low capacity oxygen absorbers had lower browning index than the air application with both high and low capacity oxygen absorbers on 12th day of storage.

The decrease in WI and increase in BI in bread could be explained by starch retrogradation and staling. Majzoobi et al. (2011) reported a time-dependent decrease in the L^* value of the bread samples which was attributed to retrogradation of starch. In a study conducted on Sangak bread, color changes of bread were attributed to the bread staling rather than a moisture loss since there were negligible changes in moisture content during storage (Khoshakhlagh et al. 2014).

The effects of oxygen absorbers and MAP on the textural property of sliced bread are given in Table 4. The results are expressed in Newton (N) as the compressive force, and the increasing values refer to the hardening in texture. The hardness of bread slices increased for all applications during storage ($p \leq 0.05$). Although there was no significant difference among packaging applications on the 18th day in terms of product hardness, the bread slices packaged under 100% N_2 with high capacity oxygen absorbers had the highest softness on the 12th day of storage. Some studies indicated that packaging under CO_2 atmosphere reduced the hardness of bread during storage (Knorr and Tomlins 1985; Cencic et al. 1996). On the other hand, it is also reported that hardness of bread is not affected by the atmosphere (Black et al. 1993; Rasmussen and Hansen 2001; Khoshakhlagh et al. 2014).

Increase in hardness is mostly attributed to the transfer of moisture through the bread crumb to the crust (Baik and Chinachoti 2000), moisture loss or retrogradation of starch in bread (Certel et al. 2009). Water acts like a plasticizer in the structure of bread. When the moisture content decreases, hydrogen bonds are formed between starch polymers or between starch and proteins, increasing the hardness (Schiraldi and Fessas 2001; Majzoobi et al. 2011).

3.3 Chemical properties

The pH increased slightly with increasing storage period at all treatments, but there was no apparent effect of any packaging treatment on pH (Table 5). Gerçekaslan (2006) and Kotancılar et al. (2009) reported that the pH values of sliced bread increased during storage. There was a positive correlation reported between pH and softness of the bread (Kotancılar et al. 2009).

There was gradual increase in water activity of the crust and very slight changes in water activity of the crumb for all applications during storage ($p \leq 0.05$) (Table 6). On the 12th day of storage, there were no significant differences among packaging treatments in terms of water activity of the crust. As a result, chemical properties are mostly affected by the storage time but slightly by the packaging treatments with different capacity oxygen absorbers and modified atmosphere. Licciardello et al. (2014) and Ertugay (2006) reported similar results.

3.4 Microbiological quality

Bread slices packaged under 100% N_2 and air atmosphere with the high capacity oxygen absorbers had the lowest bacterial growth during storage (Table 7). The total aerobic mesophilic bacterial growth in air atmosphere (control) reached up to 7.03 log cfu/g on the 18th day. Similar results were reported for sponge cake packaged with oxygen absorbers, suggesting that active packaging systems show great potential for delaying microbial growth (Janjarasskul et al. 2016).

The effects of oxygen absorbers and MAP on visual microbial (mold) growth during storage time are shown in Table 8, and the photograph of the bread slices taken on 12th day of storage can be seen in Fig. 3. There was no visual mold growth until 6 days for all applications. However, bread slices packaged with 50% CO_2 + 50% N_2 atmosphere had 10%, a low capacity oxygen absorber combined with air atmosphere had 30%, and the air atmosphere group (control) had 40% of visible microbial growth on the 6th day. There was no visual mold growth on the slices packaged with high capacity oxygen absorbers under 100% N_2 for 12 days and under air atmosphere for 9 days. Nevertheless, the visual mold growth was observed in all applications on 15th and 18th days. High CO_2 (50%) with no oxygen absorbers was not enough to control the mold growth.

The use of low and high capacity oxygen absorbers with 100% N_2 and high capacity O_2 oxygen absorbers with air

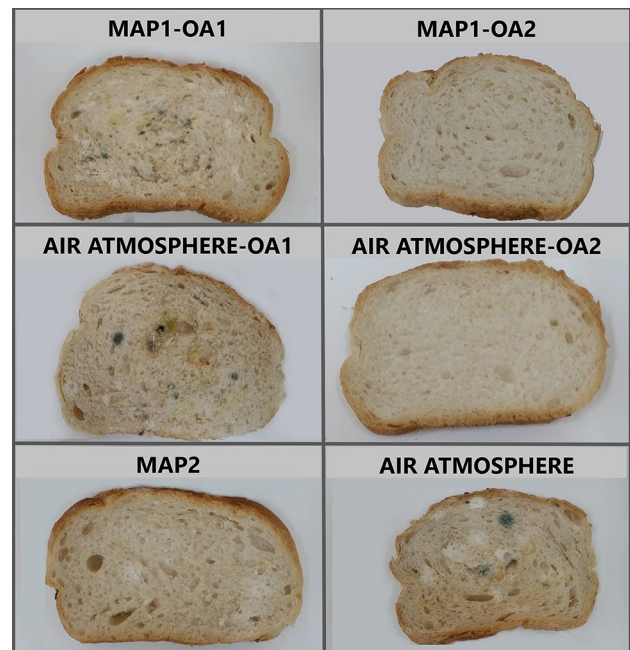


Fig. 3 The pictures of bread slices treated with different applications on the 12th day of storage

Table 9 Effect of active packaging and MAP on sensory attributes during storage

Application	Storage time							
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	
MAPI-OA1	5.00 ± 0.00 ^{Aa}	4.33 ± 0.52 ^{Aab}	3.67 ± 0.82 ^{Abc}	3.50 ± 1.22 ^{ABbc}	3.00 ± 0.63 ^{Ac}	2.00 ± 0.63 ^{ABd}	1.00 ± 0.00 ^{Ae}	
MAPI-OA2	5.00 ± 0.00 ^{Aa}	4.17 ± 0.75 ^{Ab}	3.67 ± 1.03 ^{Abc}	3.67 ± 0.52 ^{Abc}	3.33 ± 1.03 ^{Abc}	2.83 ± 0.75 ^{Ac}	2.00 ± 0.00 ^{Ad}	
Air atmosphere-OA1	5.00 ± 0.00 ^{Aa}	4.33 ± 0.52 ^{Aa}	2.83 ± 0.75 ^{Ab}	2.33 ± 1.03 ^{Bbc}	2.00 ± 0.89 ^{BCcd}	1.33 ± 0.52 ^{Bde}	1.00 ± 0.00 ^{Ae}	
Air atmosphere-OA2	5.00 ± 0.00 ^{Aa}	4.50 ± 0.55 ^{Aa}	3.67 ± 0.82 ^{Ab}	3.33 ± 1.03 ^{ABbc}	2.83 ± 0.75 ^{ABc}	2.83 ± 0.41 ^{Ac}	2.00 ± 0.00 ^{Ad}	
MAP2	5.00 ± 0.00 ^{Aa}	4.33 ± 0.52 ^{Aa}	3.50 ± 0.55 ^{Ab}	2.67 ± 1.21 ^{ABc}	2.00 ± 0.63 ^{BCc}	1.83 ± 0.98 ^{Bc}	1.00 ± 0.00 ^{Ad}	
Air atmosphere	5.00 ± 0.00 ^{Aa}	4.33 ± 0.52 ^{Ab}	3.33 ± 0.82 ^{Ac}	2.50 ± 0.55 ^{ABd}	1.67 ± 0.52 ^{Cc}	1.50 ± 0.55 ^{Bef}	1.00 ± 0.00 ^{Af}	
MAPI-OA1	5.00 ± 0.00 ^{Aa}	4.67 ± 0.52 ^{Aab}	4.00 ± 0.63 ^{Abc}	3.33 ± 0.82 ^{Ac}	2.33 ± 1.03 ^{BCd}	1.83 ± 0.75 ^{Bd}	1.00 ± 0.00 ^{Ae}	
MAPI-OA2	5.00 ± 0.00 ^{Aa}	4.67 ± 0.52 ^{Aab}	4.00 ± 0.89 ^{Abc}	3.17 ± 0.41 ^{Ad}	3.33 ± 1.03 ^{Ac}	2.83 ± 0.75 ^{Ad}	1.00 ± 0.00 ^{Ae}	
Air atmosphere-OA1	5.00 ± 0.00 ^{Aa}	4.67 ± 0.52 ^{Aa}	1.67 ± 0.82 ^{Bb}	1.50 ± 0.84 ^{Bb}	1.17 ± 0.41 ^{Bb}	1.50 ± 0.55 ^{BCb}	1.00 ± 0.00 ^{Ab}	
Air atmosphere-OA2	5.00 ± 0.00 ^{Aa}	4.50 ± 0.55 ^{Aab}	4.00 ± 0.63 ^{Ab}	3.00 ± 1.10 ^{Ac}	2.67 ± 0.52 ^{ABc}	2.67 ± 0.52 ^{Ac}	1.00 ± 0.00 ^{Ad}	
MAP2	5.00 ± 0.00 ^{Aa}	4.33 ± 0.52 ^{Aab}	3.83 ± 0.75 ^{Ab}	2.00 ± 0.89 ^{Bc}	1.50 ± 0.84 ^{CDcd}	1.67 ± 0.52 ^{BCcd}	1.00 ± 0.00 ^{Ad}	
Air atmosphere	5.00 ± 0.00 ^{Aa}	4.67 ± 0.52 ^{Aa}	3.33 ± 0.82 ^{Ab}	1.67 ± 0.52 ^{Bc}	2.00 ± 0.63 ^{BCDc}	1.00 ± 0.00 ^{Cd}	1.00 ± 0.00 ^{Ad}	
MAPI-OA1	5.00 ± 0.00 ^{Aa}	4.67 ± 0.52 ^{Aa}	3.83 ± 0.98 ^{Ab}	3.17 ± 0.75 ^{ABb}	2.17 ± 0.75 ^{BCc}	1.83 ± 0.98 ^{BCc}	1.00 ± 0.00 ^{Ad}	
MAPI-OA2	5.00 ± 0.00 ^{Aa}	4.67 ± 0.52 ^{Aab}	4.00 ± 0.89 ^{Abc}	3.33 ± 0.52 ^{Ac}	3.00 ± 0.63 ^{Ade}	2.50 ± 0.84 ^{Abe}	3.00 ± 0.00 ^{Ade}	
Air atmosphere-OA1	5.00 ± 0.00 ^{Aa}	4.50 ± 0.55 ^{Aa}	2.17 ± 0.75 ^{Bb}	1.50 ± 0.84 ^{Cc}	1.00 ± 0.00 ^{Dc}	1.00 ± 0.00 ^{Ac}	1.00 ± 0.00 ^{Ac}	
Air atmosphere-OA2	5.00 ± 0.00 ^{Aa}	4.33 ± 0.52 ^{Aab}	3.83 ± 0.75 ^{Ab}	3.00 ± 1.10 ^{ABc}	2.83 ± 0.75 ^{ABc}	2.67 ± 0.52 ^{Ac}	2.00 ± 0.00 ^{Ad}	
MAP2	5.00 ± 0.00 ^{Aa}	4.50 ± 0.55 ^{Aa}	3.50 ± 1.05 ^{Ab}	2.17 ± 0.75 ^{BCc}	1.67 ± 0.82 ^{CDcd}	1.50 ± 0.55 ^{CDcd}	1.00 ± 0.00 ^{Ad}	
Air atmosphere	5.00 ± 0.00 ^{Aa}	4.50 ± 0.55 ^{Aa}	3.17 ± 0.75 ^{ABb}	1.50 ± 0.84 ^{Cc}	1.33 ± 0.52 ^{Bc}	1.17 ± 0.41 ^{CDc}	1.00 ± 0.00 ^{Ac}	
MAPI-OA1	5.00 ± 0.00 ^{Aa}	4.00 ± 0.00 ^{Ab}	3.33 ± 0.2 ^{Abc}	3.00 ± 0.89 ^{Ac}	1.83 ± 0.75 ^{Bd}	1.33 ± 0.82 ^{Bde}	1.00 ± 0.00 ^{Ae}	
MAPI-OA2	5.00 ± 0.00 ^{Aa}	4.17 ± 0.41 ^{Ab}	3.33 ± 1.03 ^{Ac}	3.17 ± 0.41 ^{Ac}	3.17 ± 1.33 ^{Ac}	2.17 ± 0.41 ^{Ad}	1.00 ± 0.00 ^{Ae}	
Air atmosphere-OA1	5.00 ± 0.00 ^{Aa}	4.17 ± 0.41 ^{Ab}	1.00 ± 0.00 ^{Cc}	1.33 ± 0.82 ^{Bc}	1.00 ± 0.00 ^{Bc}	1.00 ± 0.00 ^{Bc}	1.00 ± 0.00 ^{Ac}	
Air atmosphere-OA2	5.00 ± 0.00 ^{Aa}	4.00 ± 0.00 ^{Ab}	3.50 ± 0.84 ^{Abc}	2.83 ± 0.98 ^{Ac}	3.00 ± 0.63 ^{Ac}	2.33 ± 0.52 ^{Ad}	1.00 ± 0.00 ^{Ae}	
MAP2	5.00 ± 0.00 ^{Aa}	4.00 ± 0.00 ^{Ab}	3.13 ± 1.03 ^{Ac}	1.83 ± 0.75 ^{Bd}	1.17 ± 0.41 ^{Be}	1.17 ± 0.41 ^{Be}	1.00 ± 0.00 ^{Ae}	
Air atmosphere	5.00 ± 0.00 ^{Aa}	4.00 ± 0.00 ^{Ab}	2.7 ± 0.75 ^{Bc}	1.33 ± 0.52 ^{Bd}	1.17 ± 0.41 ^{Bd}	1.00 ± 0.00 ^{Bd}	1.00 ± 0.00 ^{Ad}	

For each column, mean values of similar capital letters are not statistically significant among applications ($p > 0.05$). For each line, mean values of similar small letters are not statistically significant during storage ($p > 0.05$)

MAPI-OA1: 100% N₂ + low capacity oxygen absorber, MAPI-OA2: 100% N₂ + high capacity oxygen absorber, MAP1-OA1: 21% O₂ + 79% N₂ + low capacity oxygen absorber, Air atmosphere-OA2: 21% O₂ + 79% N₂ + high capacity oxygen absorber, MAP2: 50% CO₂ + 50% N₂, Air atmosphere: (Control) 21% O₂ + 79% N₂

atmosphere applications resulted in lowest mold growth throughout the entire storage. Black et al. (1993) showed that MAP (100% CO₂) with oxygen absorbent sachets was the best technique for slowing down the yeast and mold growth for pita bread compared to MAP (15% CO₂) without the sachets. Brasava et al. (2012) indicated that packages with oxygen absorbers were effective in slowing down mold growth until the 14th day.

Upasen and Wattanachai (2018) tested linear low-density polyethylene (LDPE) based materials with an oxygen scavenger and also sachets on bread and reported that although the scavenging capability of the oxygen absorber sachet lasted for only four days, the fungi and mold development thereafter was still lower compared to the package without the sachet. Combined applications of oxygen sensors as smart packaging technology and ethanol emitters as active packaging with MA (10% CO₂ and 90% N₂) were applied to Ciabatta bread (consisting of wheat flour, yeast, milk, water, sugar, salt and butter). oxygen sensors demonstrated that O₂ was utilized by yeasts/moulds within the packs over time while the use of ethanol emitters prevented mycological growth, thereby extending the product shelf life (Hempel et al. 2013). However, the ethanol may have an adverse effect on taste and may not be acceptable by the consumer.

Results indicated that the most effective packaging technique was the use of high capacity oxygen absorbers to control the microbial growth for sliced bread where the low capacity oxygen absorbers were insufficient. N₂ replaces the O₂ in the headspace initially and then the O₂ trapped in the pores (which could not be removed initially) and also possibly transmitted through the packaging material was absorbed by the high capacity oxygen absorbers providing low level of O₂ (< 1%) in the headspace through the entire storage and preventing the mold growth for 12 days of storage.

3.5 Sensory evaluation

The panelist's scores for crust and crumb color decreased during storage (Table 9). Products packaged with 100% N₂ with high and low capacity oxygen absorbers were acceptable on the 12th day in terms of crust color. However, the products packaged only with 100% N₂ and high capacity oxygen absorbers were acceptable on the 12th day of storage in terms of crumb color and odor. The acceptability for crust and crumb colors of the bread slices packaged with high CO₂ application and air atmosphere (control) was limited to six days.

In terms of overall product acceptability, the bread slices packaged with 100% N₂ and high capacity oxygen absorbers, and air atmosphere with high capacity oxygen absorbers were acceptable on the 12th day of storage. However, this period was nine days for air atmosphere with high capacity oxygen absorber due to visible mold growth on the 12th day.

It has been demonstrated that oxygen absorbers had no negative effects on the sensory quality of the packaged bread (Salminen et al. 1996; Latou et al. 2010). Freshly prepared bread slices packaged with oxygen absorbers were acceptable for longer time compared to bread slices packaged without oxygen absorbers (Matche et al. 2011).

4 Conclusions

In conclusion, the most effective application was the high capacity oxygen absorber combined with 100% N₂. The O₂ concentration (%) was below the critical concentration in 100% N₂ with high capacity oxygen absorbers for the entire storage. However, in air atmosphere the high capacity oxygen absorbers reduced the O₂ concentration in the headspace below the critical concentration within six days. Although there was no initial O₂ in 50% CO₂ + 50% N₂ MAP application, the O₂ concentration increased above 2% due to the trapped O₂ in the pores of bread which caused aerobic microbial growth accordingly, and the shelf life was limited to three days. The product packaged with 100% N₂ in combination with high capacity oxygen absorber received highest sensory scores among all applications and was acceptable for 12 days, and the shelf life was suggested as 12 days at 22 °C. MAP technologies in combination with the use of sufficient capacity oxygen absorbers can be used more effectively to remove the O₂ trapped in the product and thus, retard microbial spoilage and extend the shelf life. Exploring oxygen absorbers as an active packaging application instead of using chemical additives in frequently consumed products like bread will be a good commercial alternative for the industry and consumers.

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Declarations

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

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