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Packaging to prolong shelf life of preservative-free white bread

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Abstract

This research studied various types of packaging to prolong the shelf life of non-preservative white bread. Three types of blown film packages were used, i.e. a single LDPE layer incorporated with an oxygen scavenger, a single LDPE layer containing an oxygen absorber sachet, and three layers of LDPE laminated with O-nylon. The effects of modified packaging atmosphere, i.e. 5, 10, and 21 vol. % of oxygen in nitrogen balance, on the shelf life was also included. Characterization of the packaging films was carried out using several techniques, such as Oxygen Transmission Rate (OTR) and an optical microscopy. Headspace gases, microbial count, as well as physical appearance were used to evaluate the shelf life. The optical microscopic images showed that incorporating the oxygen scavenger into the plastic film produced small pores, contributing to a passive function of the films as their OTRs were significantly enhanced. However, the microbial growth on bread stored in those packages was suppressed, implying that the intermediate generated from scavenging reaction might act as a fungistatic. Even though the scavenging capability of the oxygen absorber sachet lasted only for 4 days, the fungi and mould development thereafter was still lower compared to the package without the sachet. The prolonging white bread shelf life was found to be primarily dependent on two factors. The package with a high oxygen barrier such three-layer films defeated microorganisms. With a

low initial oxygen level of around 5% by volume, the bread shelf life could be prolonged up to 5–7 days.

Keywords: Food science, Chemical engineering

1. Introduction

Bakery products, bread, in particular, are widely consumed in Western societies and becoming more common for Asians with a modern and busy lifestyle. Bread can be consumed directly or used as a part of other food such as pizza and pudding. It is easy to prepare and hence, a popular choice for breakfast to suit a city lifestyle. Lately, there are a large variety of types of bread to fulfil consumer need for beneficial nutrients such as dietary fibre, minerals and vitamins. However, bakery products usually have a short shelf life of only a few days at room temperature due to their high water activity. Refrigerating storage of these types of products is not applicable to their texture and taste change with temperature and therefore, they are best consumed at room temperature. Even though some viable fungi are destroyed during baking, some spores can survive or fungi contamination from other processes such as cooling and packaging can occur and are the most spoilage reason in bakery products. The use of proper packaging has been the main choice for satisfying the “green” consumerism market demands to extend the shelf life of bakery products without using any preservatives [1, 2].

Advanced packaging technologies have been playing an important role in food industries for more than a decade and becoming more and more common in recent years especially in a health-concerned society. As these types of packaging successfully extend the shelf life for several foodstuffs including bakery products, uses of chemical preservatives which cause health hazard problems can be reduced significantly. According to Smith [3], food packages are required to have multi-functions in terms of chemical, physical, and biological alterations of the food to prolong its shelf life. Chemically, the packages should be able to control and/or prevent oxidation and some other chemical reactions, such as hydrolytic rancidity and Maillard reaction, in food products. The packages should maintain the moisture level in the products which is a critical parameter controlling bread staling and changes in texture and physical appearance. Most importantly, microbial growth in food during storage is a serious problem and a major cause to shorten food shelf life. Packages that can inhibit microbial proliferation are currently in demand.

Modified atmosphere packaging (MAP) is one of the methods used to extend product shelf life. This can be done by the use of gases to replace air around non-respiring foods regardless of whether or not the atmosphere changes over time of storage or packing. Most MAP for bakery products is mainly high CO₂ contents with the

elimination of O₂ due to the fact that CO₂ is considered as an antimicrobial agent. In high water content food like bakery products, it is found that CO₂ can dissolve in water to form carbonic acid, lowering the pH. This acidification of the cell contents causes the death of bacteria. Smith et al [4] concluded that mould growth could not be prevented, but could be delayed by N₂ and/or CO₂ up to 5–10 days. The only possibility to prevent mould growth was to maintain the level of O₂ to be below 0.4%. Results of the dependency of O₂ content on fungi growth on other types of products under MAP were confirmed [5, 6, 7, 8]. However, the high concentration of CO₂ may lead to increased perceived acidity in the taste [5, 9, 10]. Furthermore, the conclusive effect of CO₂ in the MAP on the bread quality currently cannot be made due to conflicting results from various research. While Hasan [11] found no trend of effects of CO₂ on pita bread texture, Sourki et al [12] reported that Iranian flatbread hardness increased significantly as CO₂ concentration increased. On the other hand, Rasmussen and Hansen [13] and Khoshakhlagh et al [14] concluded that CO₂ altered neither a staling rate nor texture of white and part-baked Sangak bread, respectively. The ability of CO₂ on delaying the firming of bread and biscuits was also testified [15].

Another packaging technique that has been focused is active packaging (AP), the packages that not only act as a protective barrier but interact with the product in order to protect food from adulteration by, for example, moisture, oxygen, ethylene, and microorganisms. Two types of AP, i.e. sachet-based and plastic film-based packaging, have been developed. Sachets, containing active ingredients such as oxygen absorber, ethylene scavenger, and moisture absorber, were developed in the late 1970s in Japan. Although this technique is considerably practical for packaging industries, some disadvantages are inevitable. They cannot be used in liquid products or in the tight fitting film as their functionalities would be stifled. Furthermore, risks of accidental ingestion of these sachets are a serious concern. These drawbacks can be alleviated if those functionality components are incorporated to the plastic film itself. In addition to that, recent studies have revealed that combinations of various packaging techniques to prolong shelf life are more effective. The study of Berenzon and Saguy [16] suggested that even though using oxygen absorber sachets was very effective in terms of controlling the headspace oxygen, they could not slow down lipid oxidation of crackers when stored at high temperatures, attaining comparable sensory evaluation of crackers kept in absence of oxygen absorber sachets. An oxygen absorber sachet was found to extend the shelf life of pita bread and bakery products by impeding mould and yeast growth even in CO₂/N₂ MAP due to the fact that it absorbed oxygen trapped in food and in the air that permeated through the package [4]. As ethanol was well known as a disinfectant in the medical field, the ability to delay the microbial growth on rye bread depended on the size of ethanol emitter sachets [17]. While Latou et al [18] could not see any advantages of using a combination of an oxygen absorber sachet and an ethanol emitter sachet over to the

use of an ethanol emitter sachet alone, Janjarasskul et al [19] reported a synergistic action of the two. Several research disclosed more effectiveness of sachet based AP or the combination of AP with a MAP over MAP alone [4, 10, 20]. Saladino et al [21] added paper filters and a small plastic bag containing paper filters soaked with allyl, benzyl and phenyl isothiocyanates oil into bread plastic trays and found that these essential oils could reduce aflatoxins (AFs) and prevent microflora growth on the bread. Similarly, Balaguer et al [22] adopted the use of essential oil, namely cinnamaldehyde, as an antibacterial and antifungal substance to bioplastic wheat gliadin films and found the dependency of fungicidal and fungistatic activities against *Penicillium expansum* and *Aspergillus niger* on the cinnamaldehyde loading. Drastic reduction in fungi and hence the extension of shelf life of bread and cheese were clearly observed. Busolo and Lagaron [23] prepared active polyolefin composites by melt extrusion of iron particles, kaolinite clay and high and linear low-density polyethylene (HDPE and LLDPE) and the nanocomposites films were subsequently prepared by compression moulding. The agglomeration of nanoclay was more pronounced in HDPE, resulting in less oxygen scavenger activity. Besides an active role, by incorporating the nanoclay into the films could reduce the oxygen and water permeability in passive action by 22–28 and 33%, respectively [23, 24]. Particle sizes of iron powder were one of the factors controlling the oxygen scavenging activity [25]. As one expected, the nanoscale iron powder had a higher oxygen absorption rate and capacity compared to a commercial iron-based oxygen scavenger masterbatch (Shelfplus® O₂). While these advanced packaging technologies using nanocomposites continue to remain competitive in a global market, an emerging issue of food safety due to direct contact with those nanocomposites should be widely explored and concerned [26].

Considering that a high number of studies have been carried out on advanced packaging to prolong the shelf life of bread, there is a limitation of the MAP that has been focused on which was the usage of CO₂ to replace O₂. However, in terms of economic point of view, air and N₂ are cheap and easy to use. This study investigated and compared the effects of O₂/N₂ MAP incorporating with plastic-based AP on the shelf life of white bread by considering headspace oxygen contents, microbial counts and appearance. In addition, the effects of film thickness were also examined using multilayer film packages. Besides the scavenging activity, barrier properties of the films were also determined.

2. Experimental

2.1. Materials and bread packaging and storage

Chiba® Shelfplus O₂-2400 (currently owned by Albis Plastic GmbH, Germany) masterbatch was purchased from a local supplier. Low-density polyethylene

(LDPE) resins were obtained from Thai Polyethylene Co., Ltd. The packaging film was manufactured using a film blowing technique at Seesan 56 Co., Ltd., Thailand, for a single layer film and at Prepack Co., Ltd., Thailand, for a three layer film laminated with O-nylon. The blown film packages were classified as three types, i.e. (1) single layer of LDPE incorporated with different oxygen scavenger concentrations, (2) single layer of LDPE containing an oxygen absorber sachet, and (3) three layers of LDPE incorporated with different oxygen scavenger concentrations in the middle layer and laminated with O-nylon. Details of the three types of packages are illustrated in Table 1. The packages of the size of $200 \times 150 \text{ mm}^2$ were heat sealed at the bottom. The 2.5 g KEEPIT oxygen absorber sachets purchased from a local supplier were 100% iron oxide powder. White bread prepared without preservatives was purchased from Big C supermarket in Chonburi, Thailand, on the day it was baked. According to the bakery advice, the bread shelf life was only 2 days. The bread was packed in the packages which were subsequently vacuum sealed and then filled with the $300 \text{ cm}^3 \text{ O}_2/\text{N}_2$ gas mixture. For the O_2/N_2 gas mixture, O_2 was obtained from the air in the atmosphere with approximately 65% relative humidity (RH) and N_2 used as a balanced gas was from a Linde gas cylinder with a purity of 99%. Each package containing one slice of the bread under three different packaging atmosphere of 5, 10, and 21% by volume of O_2 was stored in a room temperature of around 30°C during the period of the study of 7 days. As one package of the samples was used to perform the tests each day, hence 7 packages of each sample were prepared to complete the test.

2.2. Oxygen scavenger characterization

Scanning Electron Microscopy equipped with Energy Dispersive X-Ray Analysis (SEM-EDX) technique was used to provide detailed high-resolution images of the Ciba® Shelfplus O_2 -2400 oxygen scavenger masterbatch along with the elemental identification. The analysis using an LEO 1450 VP operated at 20 kV. A Polaron Range Model SC 7620 Ion Sputter Coater was used for gold sputtering. In addition,

Table 1. Details and packaging codes of packages used in this research.

Packaging code	Details	Oxygen scavenger content (% w/w)	Thickness (μm)
1L0	1 layer LDPE film	0	37 ± 5
1L5	1 layer LDPE film	5	46 ± 6
1L10	1 layer LDPE film	10	47 ± 7
1LOS	1 layer LDPE film with a 2.5 g oxygen absorber sachet	0	37 ± 5
3L0	3 layer LDPE films with O-nylon lamination	0	87 ± 2
3L10	3 layer LDPE films (the middle layer contain oxygen scavenger) with O-nylon lamination	10	87 ± 5

the quantitative compositional information of the oxygen scavenger masterbatch was carried out using a Thermogravimetric Analysis (TGA). Mettler TGA850 was heated from 30–1000 °C at a heating rate of 10 °C/min under a nitrogen blanket.

2.3. Packaging film characterization

The film thickness was measured using an ID-C112B Digimatic Indicator, Mitutoyo, Japan, having a resolution of 1 µm. Five measurements were done for each film and four films for each packaging sample were carried out to obtain an average and standard deviation values as shown in [Table 1](#). The opacity of the film was verified using percent haze measured by an UltraScan XE, HunterLab, Virginia, USA. It is a dual beam xenon flash spectrophotometer with a wavelength range from 360 to 750 nm. The test was performed in a transmission mode with an area viewport size of 1 inch. An investigation on the microstructure of the films was carried out using a Carton Micro System CM 400 series optical microscope (Japan) equipped with a digital DinoLite eyepiece camera (Anmo Electronics Corporation, Taiwan). Image processing and analysis were performed using the DinoCapture 2.0 software. Oxygen transmission rate (OTR) of the films was tested using N500 Gas Permeability Analyzer (GBPI Packaging Instrument Co., Ltd) according to standard method ASTM D1434. The films of the size of 110 cm in diameter were tested at 23 °C, 0% RH, and 0.1 MPa partial pressure difference. The flow oxygen was an ultrahigh purity grade. Film compositions were analyzed using the TGA technique in a similar way to the analysis of the oxygen scavenger masterbatch.

2.4. Oxygen permeability of the films and headspace gases analysis

The oxygen permeability of the films and oxygen and carbon dioxide contents in the headspace of the packages were investigated using a CP 3800 Variant Gas Chromatography Analyzer (GC). The analysis was performed using two columns, i.e. HP Plot Q and MolSieve, connecting in series in an oven which was set at 60 °C. A Thermal Conductivity Detector (TCD) was equipped with the GC. For oxygen permeability, the packages only contained gas mixture without a slice of bread and the gas measurements were performed every 2 hours for 24 hours and then every 12 hours after that until reached 72 hours. The headspace gas analysis of the packages containing a slice of bread was, on the other hand, carried out daily for a storage period of 7 days.

2.5. Physical appearance

Inspection for visible microbial growth on white bread was performed prior to the microbial count analysis. Photos of the fungi and moulds, if detectable, were taken

and the sizes of the visible colonies were measured. The period from packaging to when visible microorganism was detected was considered as “bread mould-free shelf life”.

2.6. Microbial analysis

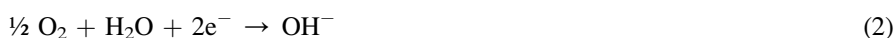
Spread plate technique was used to analyze yeast and mould counts. 39 g of potato dextrose agar (PDA) was dissolved in 1 L of distilled water and the solution was autoclaved at 121 °C under 15 psi for 15 min. 15–20 ml of the solution was poured into each petri dish and kept in a laminar flow clean bench. 22 g of crumb bread were added to 198 ml of 0.85% sodium chloride (NaCl) solution previously autoclaved and stirred until becoming homogeneous. Serial decimal dilutions were made using the 0.85% NaCl solution. Once the desired concentration was reached, 0.1 ml of the solution was spread on the surface of the prepared culture medium and incubated at 35 ± 2 °C for 48 hours prior to colony counts. According to food safety guidance of the Department of Medical Sciences, Ministry of Public Health, Thailand, microbial counts for bread products must not exceed 10^4 CFU/g while yeast and mould counts must not exceed 10^2 CFU/g.

3. Results and discussion

3.1. Characterization of oxygen scavenger masterbatch and packaging films

3.1.1. SEM-EDX

Fig. 1 illustrates SEM images of the Ciba® Shelfplus O₂-2400 oxygen scavenger masterbatch. It can be seen that there are a number of particles of the sizes of few microns with few agglomerates of the size up to 30–50 microns dispersed on the surface of the material. Once the EDX technique was utilized, Fig. 2(a) and (b) showed that those particles were pure iron whereas the main component of the material (base polymer in the masterbatch) was carbon, respectively. It can be concluded that the oxygen scavenger masterbatch composes of iron powder in a low-density polyethylene matrix. Similarly to the oxygen absorber sachets, the mechanism of this type of oxygen scavenger follows the following reactions.



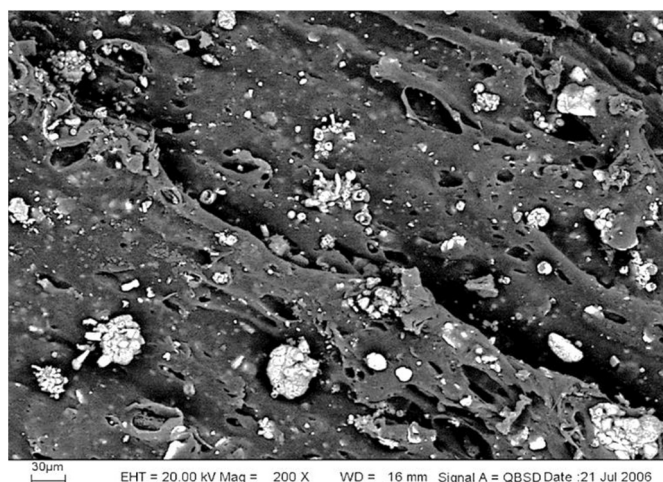


Fig. 1. SEM image of Shelf-plus O₂-2400 oxygen scavenger master-batch.

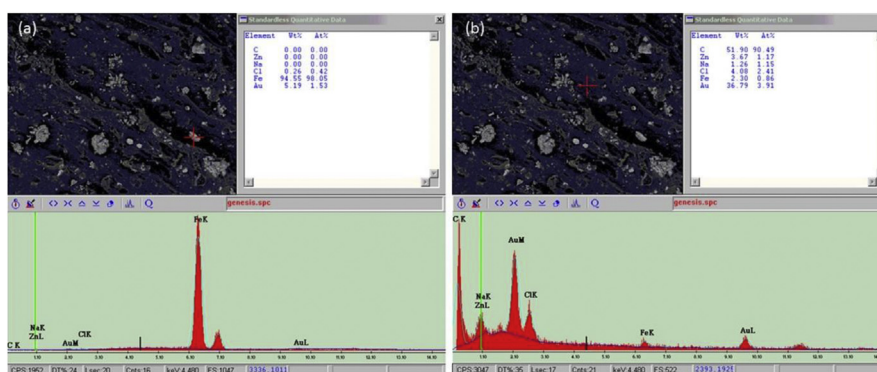
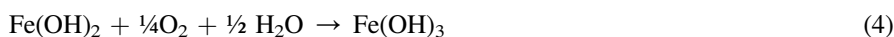


Fig. 2. SEM-EDX of dispersion phase (a), and matrix phase (b) of Shelf-plus O₂-2400 oxygen scavenger master-batch.



3.1.2. TGA

The TGA analysis in Fig. 3 shows that the oxygen scavenger masterbatch composed of around 50% of a base polymer which was LDPE and 50% of iron powder which still remained at the temperature of 1000 °C. The decomposition temperature of the base polymer in the masterbatch at 475 °C (see DTG curve) was similar to that of LDPE, confirming that it was LDPE. The unlevelled baseline of the TGA could be seen from the results especially with the tests of three layer films and hence, % remained residues in the curves could not be read directly. Percent residues were calculated from % weight loss at the decomposition temperature of 475 °C and found that for the single layer films containing oxygen scavenger at 0, 5, and 10% w/w were 1,

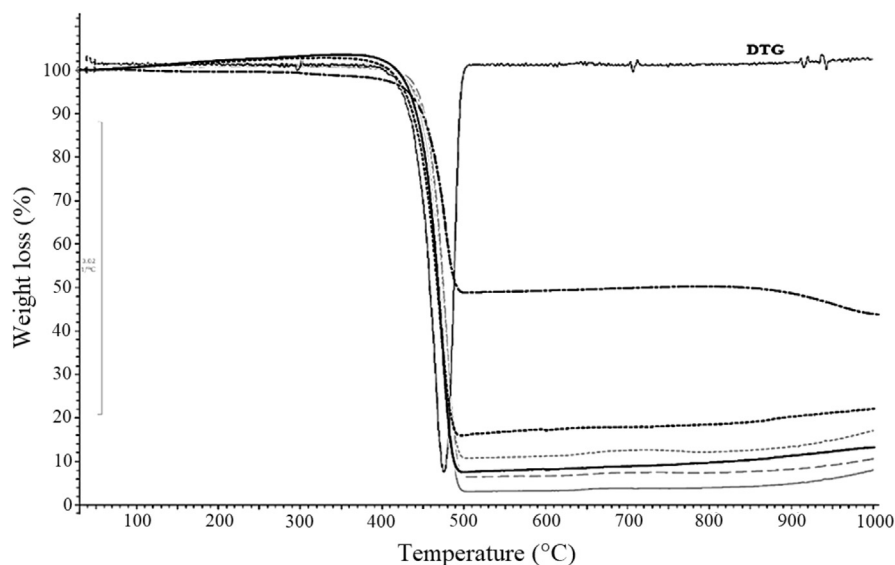


Fig. 3. TGA and DTG chromatograms of Shelfplus O₂-2400 oxygen scavenger masterbatch and packaging films.

6, and 10.5%, respectively. The slightly higher amount of residues found from TGA might be due to some other impurities in the LDPE such as metal catalysts and/or reaction terminators. For the three layer films, the remainings were also in the same range of % oxygen scavenger contents in the packages.

3.1.3. Film appearance and microstructure

Film thickness and % haze are illustrated in Fig. 4. When incorporating the oxygen scavenger into the LDPE film, the thickness of the films increased from 37 μm to

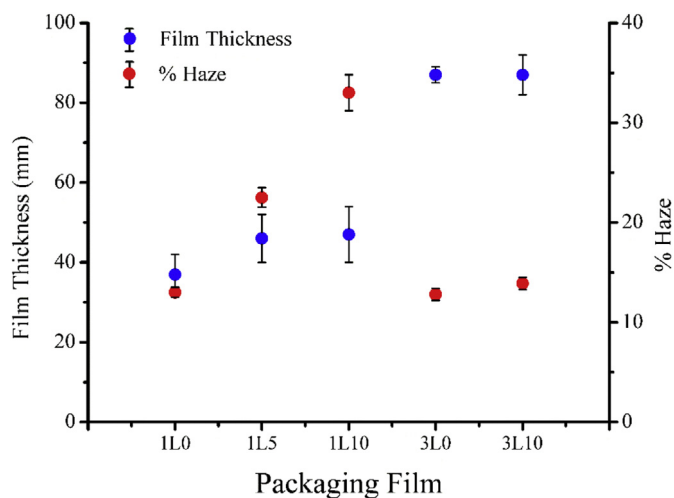


Fig. 4. Thickness and opacity of the packaging films.

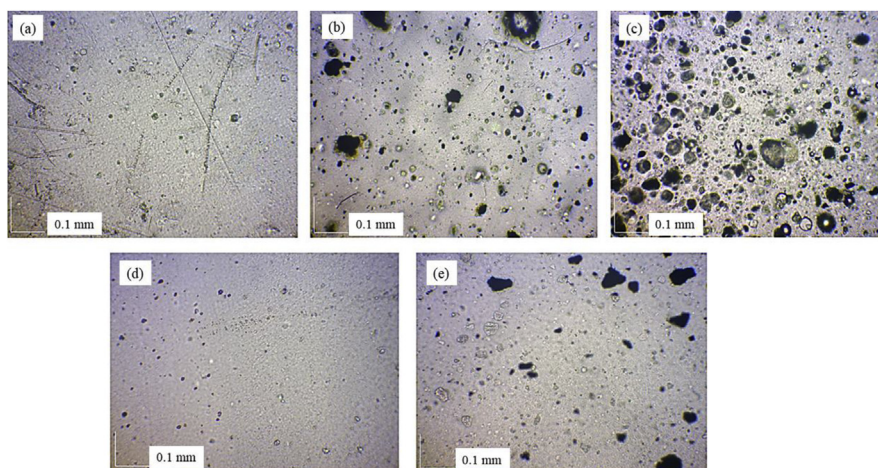


Fig. 5. Optical microscopy images of packing films: (a) 1L0, (b) 1L5, (c) 1L10, (d) 3L0, and (e) 3L10.

around $46\ \mu\text{m}$, equivalent to 24% increase. However, there was no difference between the thickness of the films with 5 and 10% w/w oxygen scavenger. In opposite, %haze increased significantly and monotonically with the oxygen scavenger concentration. With 5 and 10% w/w oxygen scavenger, the % haze increased 73 and 154%, respectively. On the other hand, with three layer films, there was no dissimilarity between film thickness as well as % haze of the films with (3L10) and without (3L0) incorporating oxygen scavenger. In addition, % haze of the films was similar to that of the single layer neat film (1L0). This might be because the iron powder was incorporated only with the middle layer. As one would expect, the three-layer films had thickness almost 3 times of the thickness of the single layer neat film.

Fig. 5(a–e) show optical microscopy images of different packaging films. It can be seen that the single layer neat film (1L0) had few small pores of the size of few microns. These small holes might occur during the film processing by blown film extrusion. However, once the oxygen scavenger was incorporated into the film, those pores became larger in size. The pore size and amount of the pores increased as the oxygen scavenger concentration increased. In addition, the images show iron powder heterogeneously distributed in the film with some agglomeration. Busolo and Lagaron [23] suggested that the non-homogeneity of these particles may be due to insufficient shear during the film processing and/or affinity with the matrix. This is reasonable since blown film technique does not achieve high shear. The affinity of iron particles and the base polymer was a crucial issue in terms of aggregation of the particles in polymer matrix since it was found that with a higher viscosity matrix, a less homogeneity the film. This iron powder agglomeration was the cause of an increase in film thickness as well as opacity as previously presented in Fig. 4. In addition, a large number of small holes in the film incorporating with oxygen scavenger was due to the fact that those particles could not be bonded with the polymer matrix. Those small pores had an effect on the gas transmission rate of the films as

will be discussed in the next section. For the three layer films, those small pores could barely be seen. Even though holes were generated during the film processing for the three layer films, the O-nylon lamination could obscure the gas permeability as observed by the OTR presented in Table 2. Similar to the single layer films, iron particle agglomerations were also detected in the three layer films (3L10).

3.1.4. OTR for passive function

Table 2 summarizes the OTR of the packaging films used in this research as well as in other works. The OTR was measured under 0% HR in which the scavenging activity was expected to be inefficient according to the results of Foltynowicz [25] and Busolo and Lagaron [23]. For single layer films, even though the neat film (1L0) was thinnest, the OTR was the lowest. The OTR of the LDPE film of 37 μm in this study ($154.44 \text{ cc/m}^2 \cdot 24 \text{ h} \cdot 0.1 \text{ MPa}$) were found to be closed to that of the OPP of the similar thickness of 33 μm studied by Sourki et al [12] ($186.19 \text{ cc/m}^2 \cdot 24 \text{ h} \cdot 0.1 \text{ MPa}$). Incorporating the films with the oxygen scavenger (1L5 and 1L10) generated small holes as seen in Fig. 5, consequently the OTRs were increased. As the amount of incorporating oxygen scavenger increased, the number of small pores increased

Table 2. Oxygen transmission rate of the packaging films.

Packaging films	Thickness (μm)	OTR ($\text{cc/m}^2 \cdot 24 \text{ h} \cdot 0.1 \text{ MPa}$)	Testing conditions	Storage product	Reference
1L0	37	154.44	23 °C, 0% RH, 0.1 MPa	White bread	
1L5	46	219.65	23 °C, 0% RH, 0.1 MPa	White bread	
1L10	47	287.89	23 °C, 0% RH, 0.1 MPa	White bread	
3L0	87	7.94	23 °C, 0% RH, 0.1 MPa	White bread	
3L10	87	8.09	23 °C, 0% RH, 0.1 MPa	White bread	
PVDC/nylon/ CPP	2/14/44	6.73	23 °C, 0% RH, 0.1 MPa	Sponge cake	Janjarasskul et al [19]
nylon/LLDPE	15/65	66.03	23 °C, 0% RH, 0.1 MPa	Sponge cake	Janjarasskul et al [19]
PET-SiOx/LDPE	62	4	25 °C, 75% RH, 0.1 MPa	Wheat bread	Latou et al [18]
OPP	33	186.19	N/A	Iranian flatbread	Sourki et al [12]
PET-Al-LLDPE	12/7/65	0.04	N/A	Iranian flatbread	Sourki et al [12]
PET/PET/LLDPE	12/12/65	0	N/A	Iranian flatbread	Sourki et al [12]
Laminated EVA	95	<2	25 °C, 75% RH	Wheat bread	Rasmussen and Hansen [13]
Laminated PA	90	90	20 °C, 50% RH	Mackerel	Stamatis and Arkoudelos [5]
PE/EVOH/PE	N/A	7	23 °C, 0% RH, 0.1 MPa	Beef steak	Limbo et al [20]
PE/TIE/PVDC/TIE/E	100	6.89	23 °C, 75% RH	Buckwheat noodles	Bai et al [27]
PET	30	56	23 °C	Pasta	Sanguinetti et al [7]
EVOH/OPET/PE	54	4	23 °C	Pasta	Sanguinetti et al [7]

and so did the OTR. However, the result showed here conflicted with the findings from previous studies where the OTR reduction was achieved with the iron-based oxygen scavenger active films [23, 24]. This is because those research works incorporated iron powder into nanoclay and the nanoclay acted as a media to bond with polyolefin matrix. As a result, there were no holes appearing in the films.

For the three layer films (3L0 and 3L10), OTRs of the film were much lower than those of the single layer films and comparable to those of multi-layer and/or laminated films in other studies. Even though small pores appeared in the oxygen scavenger incorporating film in the middle layer, the OTRs of the three layer films were identical.

3.1.5. Oxygen permeability in active mode

The O₂ contents in the packages under the initial O₂ atmosphere of 5 and 10% by volume during the storage period are shown in Fig. 6(a) and (b), respectively. The experiments of the packages containing air (21% O₂ atmosphere) were not performed as there was no driving force for mass transfer between inside and outside the packages. It was obvious that three-layer films were a very good barrier for oxygen transfer, and probably for other gases as well. This can be confirmed from the OTR shown in Table 2. As the oxygen scavenger was incorporated in the middle layer of the film, its scavenging activity was impeded and hence there was no difference in O₂ content for the films without (3L0) and with (3L10) incorporating oxygen scavenger. For the single layer film, the initial oxygen permeable rate was higher in the packages with 5% O₂ atmosphere compared to that with 10% O₂ atmosphere due to the fact that the pressure gradients between inside and outside the packages which were a driving force for oxygen transfer were higher. However, once the pressure differences were smaller as O₂ permeated into the packages the permeable rates of the two packages decreased and were closed to each other. As the initial O₂ concentration in the packages with 10% O₂ atmosphere was higher, the environment inside the packages reached the normal air atmosphere (21% O₂) quicker. It took a couple days for oxygen in the air to permeate into both packages completely. It seemed that

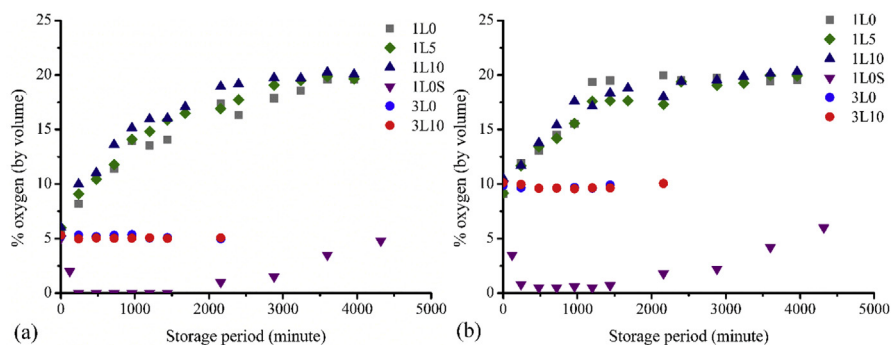


Fig. 6. Oxygen permeability test of packaging films under an initial 5% O₂ atmosphere (a) and an initial 10% O₂ atmosphere (b).

the passive function of the films as a barrier medium was more pronounced than the active function as an oxygen scavenger. In accordance with the film morphology, the films with higher amounts of incorporating oxygen scavenger resulted in a slightly higher O_2 content and a faster permeable rate due to a larger number of holes in the films. This was again agreed with OTR results shown in Table 2.

Oppositely, the single layer neat film containing an oxygen absorber sachet (1LOS) acted differently. The O_2 rapidly diminished within a couple hours. For the packages with an initial 5% O_2 atmosphere, O_2 was completely disappeared; however, there was a very low level of O_2 (<1% v/v) left in the packages with an initial 10% O_2 atmosphere and a low level of around 2.2% v/v in the packages with air (21% v/v O_2) as illustrated in Fig. 7. This was sensible since the initial amount of O_2 was the highest in the package filled with air and lowest in the package with the initial 5% O_2 atmosphere. The very high O_2 absorbing performance of sachet based AP in comparison to the scavenging ability of the film based AP was due to the fact that the iron powder exposed to the O_2 easily without any barrier. In addition, the amount of the iron powder in the sachet was considerably higher than that incorporating into the LDPE films. In all three packages, the low O_2 levels were remained constant for about a day before they started to gradually emerge. The increased level of O_2 was because the ambient air continued to permeate through the films while the sachets were already filled up with O_2 completely and became inactive.

3.2. Characterization of white bread after storage

3.2.1. Headspace gases

One slice of white bread was packed in each package under different packaging atmosphere. The headspace oxygen was monitored daily and shown in Fig. 8(a–c). In

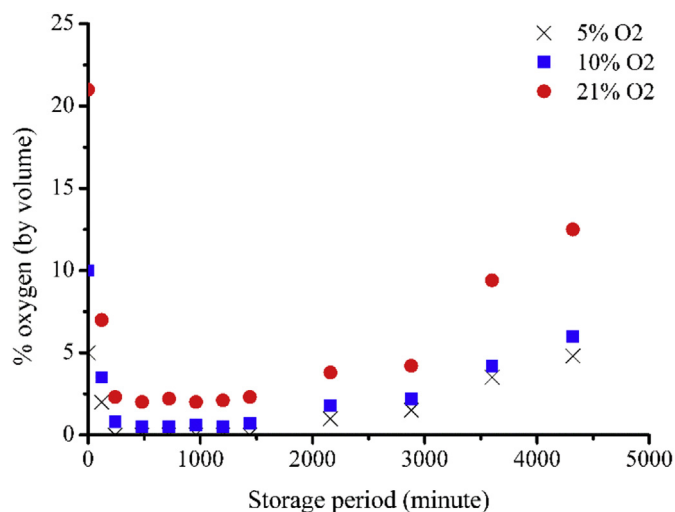


Fig. 7. Oxygen permeability test of sachet based packages under different oxygen content atmosphere.

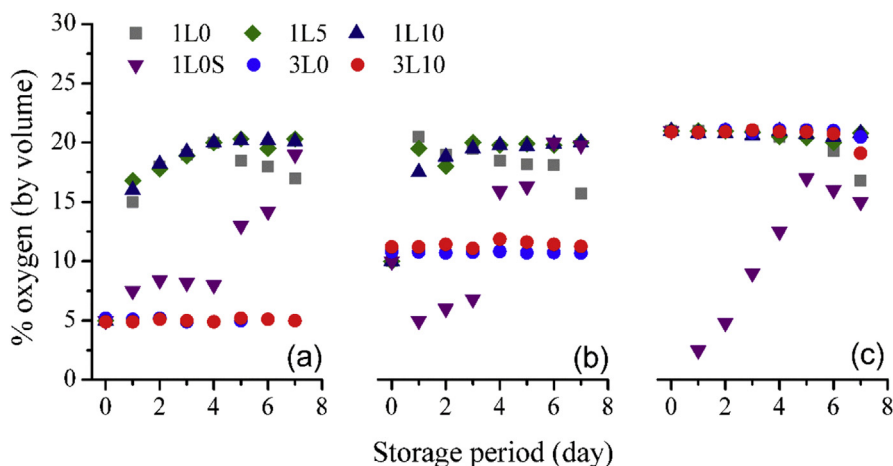


Fig. 8. Headspace oxygen during the storage period of different packages under initial 5% O₂ atmosphere (a), 10% O₂ atmosphere (b), and 21% O₂ atmosphere (c).

the gas permeability experiments, O₂ content in the packages monotonically increased due to air outside the packages permeated into the packages; however, for these experiments, as microbial contaminated in the bread used O₂ to cultivate, O₂ consumption occurred at the same time as the permeation of the outside air. Consequently, the headspace O₂ was a net result of these two phenomena. For three layer packages, it was obvious that there was no O₂ permeated from outside air so the reduction of O₂ in the packages due to fungi and mould cultivation was expected. However, the O₂ levels were barely changed except for the last couple days of the packages filled with air, implying there were particularly low levels of microorganism proliferation. Oppositely, the headspace O₂ content of the single layer film packages under 5 and 10% O₂ atmosphere increased sharply and within the first couple days, the levels were considered similar to those empty packages. This was because, for the first couple days, the number of microbial counts was still low and hence minimal amounts of O₂ were consumed. But on the 4th and 5th day, the headspace O₂ levels started to decline for the packages with the initial 10 and 5% O₂ atmosphere, respectively, because of the microbial growth. The results were in accordance with the results of headspace CO₂ and microbial counts which would be discussed later on. Among the packages with different initial O₂ levels, it was revealed that the packages with higher O₂ concentrations, the higher O₂ consumption due to the microbial growth was more accelerated. Interestingly, the reduction in the O₂ level of the air packages was not clear and indistinguishable with the packages with 10% O₂ atmosphere. The microbial count results confirmed this finding. This suggested that to minimize the spoilage of the bread from microorganisms, the O₂ level must be lower than 10% v/v. For the single layer films incorporating without and with different oxygen scavenger contents, the slightly lower O₂ levels were found in the packages without the oxygen scavenger and no difference in O₂ levels was found in the packages with 5 and 10% w/w oxygen scavenger,

conflicting with the results of OTR and oxygen permeability in active mode. The hypothesis for this phenomenon was that the oxygen scavenger inhibited the microbial growth not only physically by scavenging headspace O_2 as mentioned in previous publications [4, 10, 20, 28], but also biochemically as confirmed by the results from the microbial count. The intermediate agent from the scavenging reaction might act as a fungistatic.

For the sachet based packaging, the headspace O_2 was absorbed immediately but soon started to gradually increase after 1 day of storage due to the air permeation. While the sachets in the 5% O_2 atmosphere packages absorbed less O_2 , the headspace O_2 levels were lowest. Whereas the sachets in the air packages absorbed more O_2 , the headspace O_2 levels were highest. The decrease of headspace O_2 at the final days of storage (6th and 7th days) was observed only in the packages filled with air. This was due to the fact that a large number of fungi and mould had visibly grown on the bread as would be mentioned in appearance and mould-free shelf life section.

The results of headspace CO_2 presented in Fig. 9(a–c) were corresponding to the results of headspace O_2 previously discussed. Even though the headspace O_2 levels of the three layer film packages was unchanged, very low levels of around 0.2% v/v of headspace CO_2 were detected except for the air packages on the last day of storage that approximately 1–2% v/v CO_2 were identified. For the single layer film packages, the headspace CO_2 levels started to progressively increase from 4th day of storage for the packages of neat LDPE with and without an oxygen absorber sachet whereas the headspace CO_2 was noticeable only in the last couple days of storage for the 5 and 10% w/w oxygen scavenger incorporating film packages. This could be due to two reasons. Firstly, as the oxygen scavenger incorporating films contained relatively more pores than the neat films, CO_2 could easily diffuse through the films to outside environment. Secondly, the low amounts of CO_2 were simply due to the low numbers of

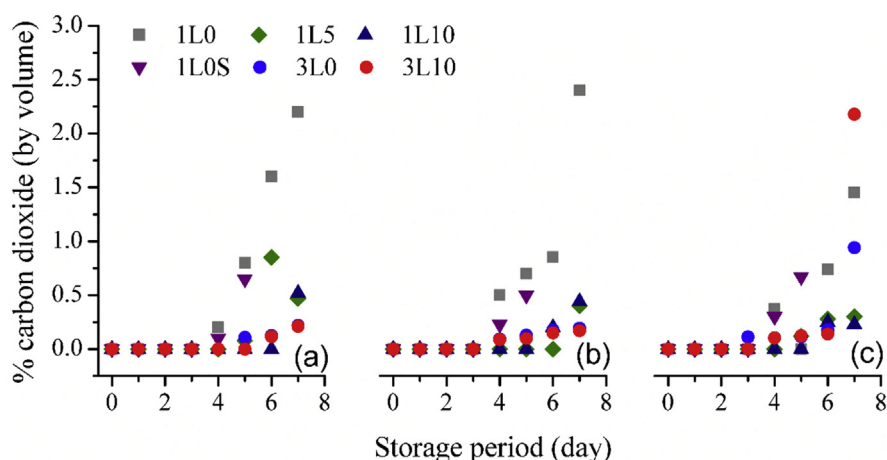


Fig. 9. Headspace carbon dioxide during the storage period of different packages under initial 5% O_2 atmosphere (a), 10% O_2 atmosphere (b), and 21% O_2 atmosphere (c).

microorganisms and this hypothesis was proved by the results from the microbial investigation, revealing lower amounts of fungi and mould in the oxygen scavenger incorporating film packages compared to those in the neat film packages. Since the low levels of less than 3% v/v of CO₂ were detected, the dependency of initial O₂ concentration in packaging atmosphere on the headspace CO₂ was not cleared.

3.2.2. Microbial counts

As mentioned earlier that low levels of headspace CO₂ were identified in the three-layer film packages, the microbial investigation disclosed that the found headspace CO₂ was a result of microorganisms as seen in Fig. 10(a–c). Even though the results of headspace gases could not differentiate the differences between the three layer films incorporating with (3L10) and without (3L0) oxygen scavenger, higher microbial counts in the neat films were noticeable. A similar trend was observed for the single layer film packages especially in those packages with the low initial O₂ headspace (5% O₂ atmosphere). By incorporating an oxygen scavenger into the LDPE films, microbial growth could be delayed. It was likely that the higher oxygen scavenger concentration, the lower microbial counts although the headspace gases of the 5 and 10% w/w oxygen scavenger film packages were not distinguishable. The scavenging capacity of the oxygen absorber sachet started to deplete after 4 days of storage; however, during those periods, the results showed that the microbial growth rate was suppressed. As a result, one might conclude that there was no significant advantage of using an oxygen absorber sachet in the single layer neat film. Nevertheless, the number of fungi and mould appeared after 4 days of storage period were slightly lower in the packages contained the oxygen absorber sachet, proving its beneficial role in active packaging. Another factor influencing a delay in the microbial development was the amount of O₂ content in the package.

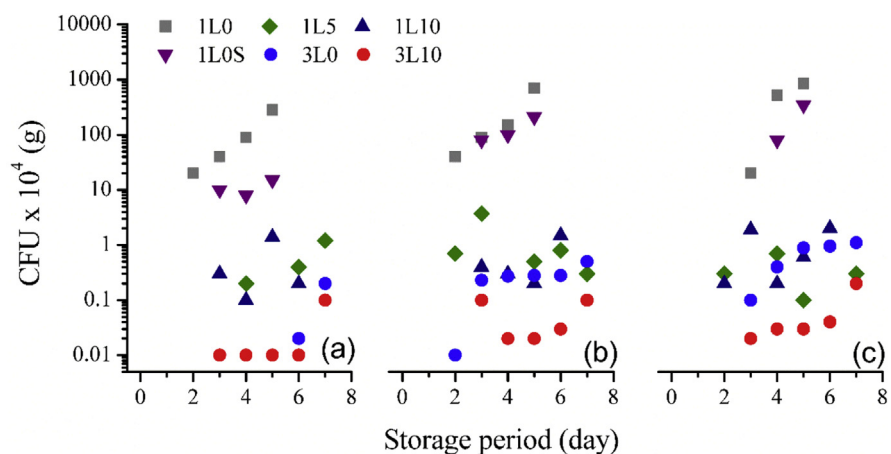


Fig. 10. Microbial counts during the storage period of different packages under initial 5% O₂ atmosphere (a), 10% O₂ atmosphere (b), and 21% O₂ atmosphere (c).

With a low O₂ atmosphere of 5% v/v, the number of microorganisms was about a magnitude lower than those in the 10 and 21% O₂ atmosphere. The findings were agreed with the work of Knorr and Tomlins [15] who reported that 100% N₂ in packaging atmosphere was as effective as 100% CO₂ in terms of extending the white bread shelf life from 5 days in air packaging to 13 days. However, this was contradicted to the conclusion made by Bai et al [27] who did not discover any significant difference in the number of total plate counts in buckwheat noodles packed in normal air and in 100% N₂. Comparable microbial counts in the packages with 10 and 21% O₂ atmosphere implied that 10% v/v O₂ content in the head-space of the packages were sufficient for the microbial growth. Interestingly, the food safety guidance of the Department of Medical Sciences, Ministry of Public Health, Thailand, sets the limitation of yeast and mould counts as 10² CFU/g where those bread with invisible fungi and mould colony possessed microbial counts over the limit and might be mistakenly consumed by customers.

3.2.3. Appearance and mold-free shelf life

During the storage period of 7 days, fungi and mould colonies on slides of bread in the three-layer film packages both with (3L10) and without (3L0) incorporating with oxygen scavenger under all three O₂ concentration atmosphere (5, 10, and 21% v/v) were not visible. The appearance of fungi and mould on the bread in the single layer films packages depended mainly on the initial O₂ contents in the packaging head-space as well as the oxygen scavenger concentrations. With the packages containing air inside, the microbial development appeared earlier than those with lower O₂ concentrations (5 and 10% O₂ atmosphere). Similar findings were reported by Smith et al [4] that 100% N₂ atmosphere could prolong the crusty rolls' shelf life compared to a normal air package. By incorporating an oxygen scavenger into film packages,

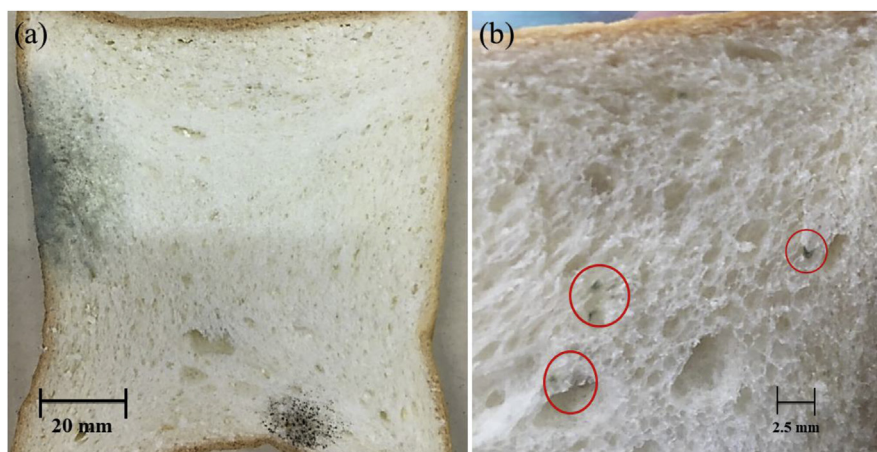


Fig. 11. Bread appearance after storage in most packages (a) and in 1L0 package under 21% O₂ atmosphere (b).

Table 3. Bread mould-free shelf life.

Packaging code	Headspace oxygen content (with N ₂ balance)		
	5%	10%	21%
1L0	5 days	5 days	4 ^a days
1L5	-	-	6 days
1L10	-	-	6 days
1L0S	-	5 days	4 days
3L0	-	-	-
3L10	-	-	-

- Means no visible microbial on bread and hence the shelf life >7 days.

^aIndicates large colonies as shown in Fig. 11(b).

the visible microorganisms growth could be delayed for air headspace packages and prevented for low O₂ headspace packages (5 and 10% O₂ atmosphere). Unlike the report on the substantial extension of shelf life of bakery products using an oxygen absorber sachet by Salminen et al [17] and Smith et al [4] the use of the oxygen absorber sachet in this research did not impede the appearance of fungi and mould; however, the colony sizes were much smaller as seen in Fig. 11(a) and (b). Except for an extreme condition of a single layer neat film (1L0) packed under air headspace (21% O₂), the detectable microbial colony sizes were considerably small as shown in Fig. 11(a). Only with the bread kept under the extreme condition showed extensive fungi and mould colony sizes illustrated in Fig. 11(b). Table 3 summarized the storage day that visible microbial on bread was spotted, or in other words, the mould-free shelf life of the bread.

4. Conclusions

Prolonging of white bread shelf life was accomplished with the use of novel advanced packaging technologies. The combination of AP and MAP demonstrated a high potential for bakery product industries. It was illustrated that CO₂ was not a necessity in the MAP as long as the amount of O₂ was minimized by using N₂ as a balance gas. By incorporating an iron-based oxygen scavenger into the petroleum plastic films, the intermediate from the scavenging reaction acted as an antimicrobial agent in a similar way to CO₂. If the packaging film possesses a high barrier for O₂ transmission property, the usage of incorporating oxygen scavenger as well as the MAP is almost unnecessary. These technologies could be extended to other bakery products.

Declarations

Author contribution statement

Settakorn Upasen: Performed the experiments.

Piyachat Wattanachai: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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