

CHEMICAL AND MICROBIOLOGICAL QUALITY DURING STORAGE: HALF-DRIED SALTED ROUND SCAD (*DECAPTERUS MARUADSI*)

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<https://doi.org/10.34302/crpjfst/2020.12.5.15>

Article history:

Received:

29 August 2020

Accepted:

25 December 2020

Keywords:

Half-dried salted round scad;

Packaging;

Quality change;

Shelf life

ABSTRACT

Half-dried salted round scad can be stored for only a short time due to high-moisture content. The objective of this study was to evaluate the quality changes during storage under 3 packaging; air (T1), vacuum (T2), and air with oxygen absorber (T3). Moisture content, pH, TVB value, TMA value, TBA value, total viable count as well as yeast and mold of half-dried salted round scad significantly increased along with the longer storage time ($p < 0.05$). The vacuum packaging had the lowest effect on the quality changes of half-dried salted round scad. When stored at room temperature (30 ± 2 °C) half-dried salted round scad gained higher amounts of yeast and mold than the standard of TISI. On day 15 of storage, yeast and mold of the sample were 6.4×10^2 CFU/g. When stored under chilling condition (4 ± 1 °C) for 30 days, half-dried salted round scad gained higher TMA value as well as yeast and mold values than the standard of TISI at 11.48 mg/100 g and 5.2×10^2 CFU/g, respectively. Therefore, the shelf life of half-dried salted round scad packed under vacuum condition were 10 and 25 days for samples stored at room temperature and cold storage, respectively.

1. Introduction

Half-dried salted fish is one of the most popular traditional products. It has high nutrition, soft texture, unique taste and flavor. It is also a great source of high protein which contains all essential amino acid. Half-dried salted fish is different from normal salted fish in term of the moisture and salt content in their final products. For salted fish, the a_w is usually under 0.85 and the salt content is over 10% (Thai industrial standards institute; TISI. 312/2006). For half-dried salted fish, it is a high-moisture content product and the salt content is less than 10% (light cure). Moreover, the main characteristics of salted fish affecting consumer buying decision are light salty taste, special flavor without stinky smell and absence of fish bone (Sukjuntra, 2014). Due to the lower salt

content in half-dried salted fish, food spoilage bacteria and food-borne pathogenic bacteria can easily grow in this product which leads to the product's shorter shelf life. Nowadays, snake-head fish and sepat siam, freshwater fish, are commonly used as half-dried salted fish products.

Among the sea water fish species, the round scad (*Decapterus maruadsi*), a fatty fish, has been chosen as the raw material for half-dried salted fish production because of its appearance and taste which are better than those of lean fish. Half-dried salted round scad also has soft texture during to the low salt added which leads to no denaturation of fish protein (Ana and Rui, 2010; Magnus and Turid, 2012). Moreover, the half-dried salted round scad is healthy food for consumer, due to its low salt content (Nuwanthi

et al., 2016). For the above reasons, half-dried salted round scad is widely consumed and available in local markets especially in the lower southern part of Thailand. However, the supply of half-dried salted round scad are still low because of its traditional sun drying process with the lack of proper technology to improve the production. Furthermore, during the rainy season, the half-dried salted round scad is not available in the market because of the sun drying process. The factors determining the half-dried salted round scad product's quality are raw material quality, salt quantity and quality, good and hygienic processing, packaging and suitable storage condition (Koral, 2013; Lorentzen *et al.*, 2016).

In order to improve the quality and extend the shelf life of half-dried salted round scad, appropriate packaging is crucial. Nowadays, there are many studies on various packaging for shelf life extension of fish and fishery products including vacuum packaging, oxygen absorber, moisture absorber and modified atmosphere packaging (Mohan *et al.*, 2009). The vacuum packaging is a widely used method in the market as its efficiency and low cost. This packaging can prevent oxidative rancidity, and inhibition of the growth of aerobic bacteria and spoilage bacteria, especially *Micrococcus* and *Aeromonas* (Masniyom, 2011; Renato, 2012; Pinar, 2013; Kumar and Ganguly, 2014).

Appropriate packaging is able to increase the shelf life of half-dried salted round scad, increase the marketing competition capacity and also enhance reputation of this product. Thus, the objectives of this study were to select the practical packaging methods conforming to entrepreneur in the lower southern part of Thailand for increasing the shelf life of half-dried salted round scad. Samples were stored at room temperature (30 ± 2 °C) and at cold storage (4 ± 1 °C) for 15 and 30 days, respectively. The changes of physical, chemical and microbiological properties of sample were determined at 5 days interval.

2. Materials and methods

2.1. Round Scad Preparation

The A grade to low B grade of round scad (*Decapterus maruadsi*) (8-12 fish/kg) according to EU grade was beheaded, gutted, butterflied and deboned. After that, the butterflied fish was soaked in 6% (w/v) brine solution for 20 min at a ratio of 1:1 (fish: brine solution). Then the soaked fish was dried in a solar dryer (Electricity Generating Authority of Thailand; SD-050 model) until a_w of fish fresh ranged from 0.85 to 0.90. Then, the fish was stored in low density polyethylene bags (LDPE) under 3 types of packaging; air (T1), vacuum (T2) (VAC-STAR S220, Switzerland), and air with an iron-based oxygen absorbent (T3) which utilized in sachet form and meant to be used in products with maximum water activity (a_w) of 0.85 and minimum oxygen absorption of 300 ml. Afterwards, half-dried salted round scad was stored at room temperature (30 ± 2 °C) for 15 days, chilled in condition (4 ± 1 °C) for 30 days, and sampled every 5 days for evaluation of the shelf life.

2.2. Quality Analysis

Chemical Analysis

The pH measurement was determined by homogenizing samples with distilled water at ratio of 1:5 (w/v), as described by Manthey *et al.* (1988). First, pH values of homogenate were measured by using a pH meter (Schott, model G 0840). Also, moisture content was determined using an oven method (Association of official Analytical Chemists, 2000). Then, salt content was analyzed using AOAC method. Moreover, the Total volatile base nitrogen (TVB) and Trimethylamine (TMA) value were analyzed using conway unit (Siang and Kim, 1992) by grinding 2 g of grounded sample with 8 ml of 4% (w/v) Trichloroacetic acid (TCA) in a mortar and then filtrating the texture through filter paper (Whatman No. 41). The volume of filtrate was adjusted to 10 ml using 4% (w/v) TCA. After that, 1 ml of sample extract was pipetted into outer ring of conway unit. Then 1 ml of inner ring solution (boric acid in ethanol) was

pipetted into inner ring of conway unit. Subsequently, 1 ml of saturated K_2CO_3 was pipetted into another side outer ring of conway unit. Moreover, The Trimethylamine value was carried out according to the methodology of TVB with a slightly different. After the sample extraction, the sample extract, inner ring solution and saturated K_2CO_3 were added into conway unit. Then, 1 ml of 10% (v/v) Formaldehyde solution was pipetted into the sample extract portion. After conway unit was closed, both of the TVB and TMA portion were gently mixed and incubated at $37^\circ C$ for 45 - 60 min, respectively. After incubation, the inner ring solution was titrated with 0.02 N Hydrochloric acid (HCl) using micro-burette until its green color turned pink color. The blank test was done using 1 ml of 4% (w/v) TCA instead of sample extract. TVB and TMA value was calculated as follows.

$$\begin{aligned} & \text{TVB (mg/100mg)} \\ &= \frac{N \times 14 \times (A - C) \times 10 \times 100}{W} \quad (1) \end{aligned}$$

$$\begin{aligned} & \text{TMA (mg/100mg)} \\ &= \frac{N \times 14 \times (B - C) \times 10 \times 100}{W} \quad (2) \end{aligned}$$

Where; N = Normality of HCl

A = Titration volume (ml) of 0.02N HCl for sample extract (TVB)

B = Titration volume (ml) of 0.02N HCl for blank

C = Titration volume (ml) of 0.02N HCl for sample extract (TMA)

W = Weight of sample (g)

Besides, the Thiobarbituric acid (TBA) value was determined by a distillation method (Egan *et al.*, 1981). First, 10 g of sample was homogenized with 50 ml of distilled water, then adding 4 N HCl. After that, the mixture was heated with steam distillation until 50 ml of distillate was collected. A 5 ml of distillate was mixed with 5 ml of TBA reagent and incubated in boiling water for 35 min. After cooling, the absorbance of the solution was read at 538 nm.

The blank test was done using distilled water instead of sample. TBA value was calculated as follows.

$$\text{TBA(mg MAD/kg)} = 7.8 \times A \quad (3)$$

Where A; Absorbance of the solution was read at 538 nm

Microbiological analysis

A microbiological analysis was carried out by examining total plate count (TPC) as well as yeast and mold according to the technique recommended by Bacteriological Analytical Manual (2001). *Staphylococcus aureus* and *Escherichia coli* MPN (3 tubes method) were analyzed using the technique recommended by Bacteriological Analytical Manual (2002).

2.3. Statistical analysis

The experiment was carried out with a completely randomized design (CRD). Data obtained from chemical parameters were analyzed by analysis of variance (ANOVA) using computer software. Duncan's New Multiple Range Test (DMRT) was used to compare the means with a significance level of 95%.

3. Results and discussions

3.1. Chemical quality changes

pH value

The result of this study indicated that the storage at room temperature ($30 \pm 2^\circ C$) had affected the pH value of half-dried salted round scad. pH values of the samples significantly increased with the increasing storage time. All 3 samples packed in air (T1), vacuum (T2) and air with oxygen absorber (T3) had the pH values of 5.98 on day 0 and their pH values reached to 6.73, 6.63 and 6.74 at the end of storage (day 15), respectively ($p > 0.05$) (data is not shown). Corresponding to the result of Farid *et al.* (2014) who found that the pH values of 2 dried salted snake-head fish products stored at room temperature ($27-30^\circ C$) increased from 6.3 and 6.5 (day 0) to 8.1 and 7.9 (day 150). For half-dried salted round scad stored under chilling

condition) 4 ± 1 °C (in the present study, the pH value used for indicating fish quality of all 3 samples slightly increased with the longer storage time ($p > 0.05$) (data is not shown). During the post-mortem period, the decomposition of nitrogenous compounds occurred by microorganisms and formed volatile amines, ammonia, amine and trimethylamineoxide, which are basic alkalinity compounds affecting on the increasing pH value of fish (Farid *et al.*, 2014; Frangos *et al.*, 2010; Lorentzen *et al.*, 2015). Thus, the storage of dried salted round scad in chilling temperature is able to retard microorganism activities leading to the minor changes in pH.

Moisture content

Moisture content of half-dried salted round scad stored at room temperature significantly increased with the longer storage time ($p < 0.05$). The moisture content of samples packed in air (T1), vacuum (T2) and air with oxygen absorber (T3) were 54.93% on day 0 and increased to 61.27, 57.43 and 58.39% on day 15 of storage, respectively (data not shown). From these results, the moisture content of the sample stored in non-vacuum packaging (T1) was higher than other packaging. ($p < 0.05$). The increase in moisture content of half-dried salted round scad was due to the moisture absorption of products an environment with from higher humidity (Farid *et al.*, 2014). This is in agreement with the study of Adenike (2014), Ikutegbe & Sikoki (2014) and Reza *et al.* (2015) who found that moisture content of smoked catfish, dried chela (*Laubuka dadiburjori*) and smoked long-neck croaker (*Pseudotolithus typus*) increased with increasing the storage time ($p < 0.05$). The moisture content of smoked catfish increased from 62.52% to 69.13% (on week 6), the moisture content of dried chela increased from 5.88% to 10.83% (on day 30) and the moisture content of smoked long-neck croaker increased from 10.90% to 13.10% (on week 4).

The result of half-dried salted round scad stored in chilling condition indicates that the changes of moisture content in the final products were lower than those stored at room temperature. The moisture content of sample stored in air (T1), vacuum (T2) and air with oxygen absorber (T3) packaging was 54.93% on day 0 and increased to 59.87, 58.68 and 58.77% on day 30 of storage, respectively (data not shown). This was because dried fish slowly re-absorbed moisture from the relatively humidity condition like a chilling storage. However, moisture content of half-dried salted round scad is a factor of shelf life because it affects microbial growth and chemical changes including oxidation reaction and browning reaction. Normally, high moisture food is highly perishable.

Salt content

The salt content analysis of half-dried salted round scad stored at room temperature showed that the salt contents of samples packed in air (T1), vacuum (T2) packaging significantly decreased on day 5 of storage with a constant value on day 5-15 of storage. Whereas, the salt content of samples packed in air with oxygen absorber (T3) was stable during 15 days of storage ($p > 0.05$). On day 15, salt content of 3 half-dried salted round scad were 3.38, 3.51 and 3.83% respectively (Figure 1(a)). In refrigerator storage (4 ± 1 °C) condition, salt contents of all 3 samples varied with increasing storage time and showed the difference of salt content on day 15 of storage (Figure 1(b)). It can be seen that a decrease in salt content of products corresponded to the increased moisture content similar to Oyarekua (2014) who found that the salt content of smoked catfish slice decreased with an increase in storage time ($p < 0.05$). The salt content decrease from 16.66% to 12.05% on week 6 of storage. In order to increase the shelf life of salted fish with 55-58% moisture content, Loannis (2014) demonstrated that salt content of salted fish should be in the range of 18-21%. From this study, high moisture content with 0.85 -0.90 of a_w and low salt content (less than 5%)

in half-dried salted round scad promoted the growth of microorganism and has effects the on short shelf life of this product.

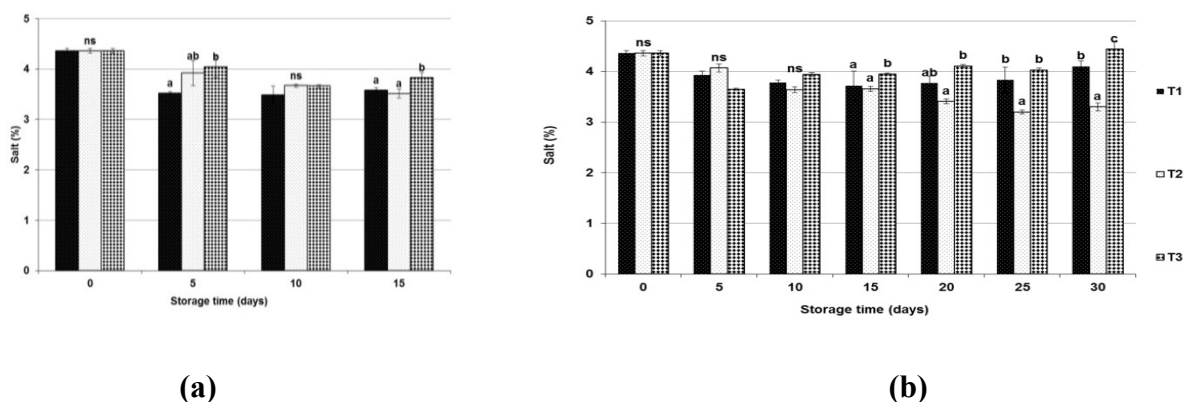


Figure 1. Salt content of half-dried salted round scad stored at (a) room temperature and (b) chilling condition under air (T1), vacuum (T2) and air with oxygen absorber (T3) packaging

Total volatile base nitrogen (TVB)

The TVB value in half-dried salted round scad stored at room temperature significantly increased along with the longer storage time ($p < 0.05$). After 15 days of storage, TVB value in sample packed by vacuum packaging (T2) was lowest at 32.47 mg/100 g as shown in Figure 2(a). Moreover, the TVB value in half-dried salted round scad stored in refrigerator significantly increased with the longer storage time ($p < 0.05$). The results showed that TVB value increased from an initial value of 10.99 mg/100 g on the day 0 to 24.24, 23.33 and 25.92 mg/100 g on the day 30 of storage in T1, T2 and T3, respectively (Figure 2(b)). Similar results were reported by Latifa et al. (2014) about refrigerated storage (4 °C) of smoke-dried

chapila (*Gudusia chapra*) that TVB value increased from 8.84 to 20.04 mg/100 g after 9 months of storage. In addition, Farid et al. (2014) reported that TVB value in sun-dried salted shoal fish at room temperature (27-30 °C) storage increased from 4.89 to 30.86 mg/100 g after 5 months of storage. TVB is a group of biogenic amine produced by bacteria including ammonia, dimethylamine and trimethylamine. This increasing values correspond to the increasing fish spoilage; therefore, TVB is used for indicating the spoilage of fish and the consumer acceptance. The acceptability level of TVB in salted fish is 35 - 40 mg/100g. Above that level, fish products are considered unsuitable for human consumption (Bilgin and Degirmenci, 2019).

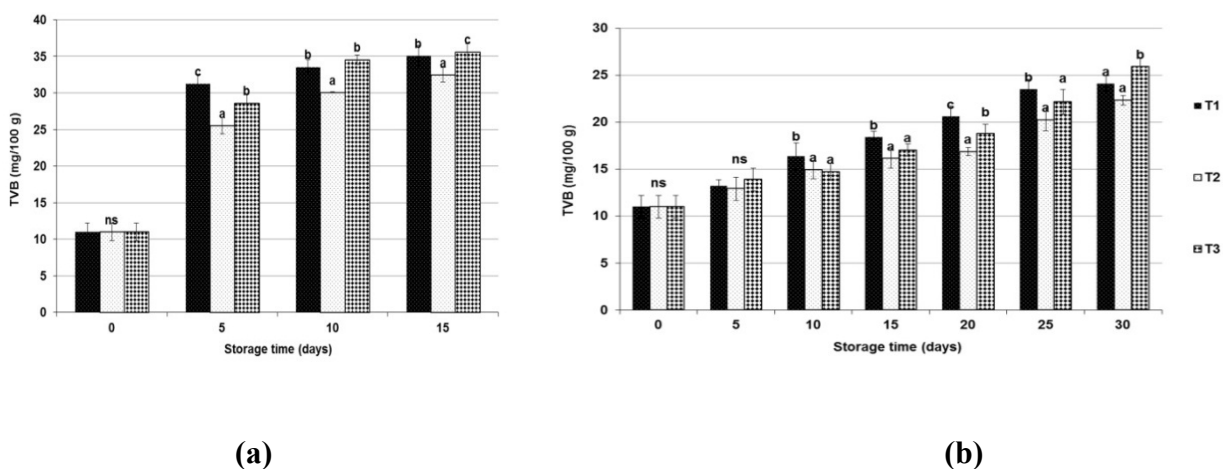


Figure 2. Total volatile base (TVB) values of half-dried salted round scad stored at (a) room temperature and (b) chilling temperature under air (T1), vacuum (T2) and air with oxygen absorber (T3) packaging

The results of this study also showed that the different packaging effects on the growth of spoilage microorganism in fish products. Sample stored in air packaging had the highest spoilage. Whereas, sample stored in vacuum condition was not able to inhibit the growth of aerobic bacteria (Fuentes *et al.*, 2011). In addition, the storage under chilling condition was able to prevent the growth of microorganism and enhance the shelf life of product. This study suggested that samples packed in air (T1), and air with oxygen absorber (T3) conditions and stored at room temperature had TVB value over the limitation of 35 mg/100 g. on day 15 of storage at 35.09 and 35.61 mg/100 g, respectively. On the contrary, TVB values of all 3 samples stored at chilling condition were within the standard of 35 mg/100 g TVB during the storage time. These results indicated that the quality of half-dried salted round scad stored at chilled condition was acceptable.

Trimethylamine (TMA)

The TMA value of half-dried salted round scad stored at room temperature significantly increased along with the longer

storage time ($p < 0.05$). After 15 days of storage, TMA value of sample packed by vacuum packaging (T2) was lowest at 7.42 mg/100 g (Figure 3(a)). Likewise, the values of half-dried salted round scad stored in refrigerator significantly increased along with the longer storage time ($p < 0.05$). The results showed that TMA value increased from an initial value of 2.81 mg/100 g on the day 0 to 12.23, 11.48 and 12.57 mg/100 g on the day 30 of storage in T1, T2 and T3, respectively (Figure 3(b)). Similar results have been report by Bilgin and Degirmenci (2019) during refrigerated storage (4 ± 1 °C) of hot-smoked meager (*Argyrosomus regius*). TMA value increased from 1.35 to 5.57 mg/100 g after 56 days of storage. TMA was derived from non-protein nitrogen compounds (trimethylamine oxide; TMAO) in fish by trimethylamine oxide reductase and degraded to TMA, dimethylamine (DMA), formaldehyde (FA) and ammonia (Benjakul *et al.*, 1997). Thus, the increasing TMA value stimulates the increase of TVB value. The fresh fish normally has TMA value of 10-15 mg/100 g (Venugopal, 2006). TMA value of sample packed in air (T1) condition and stored at room temperature was >10 mg/100

g on day 15 of storage. Whereas, TMA value of samples stored under chilling condition was >10 mg/100 g on day 25, 30 and 20 of storage for samples packed in air (T1), vacuum (T2) and air with oxygen absorber

(T3) conditions, respectively. Therefore, the vacuum packaging of half-dried salted round scad is the best packaging in slowing down the growth of aerobic bacteria.

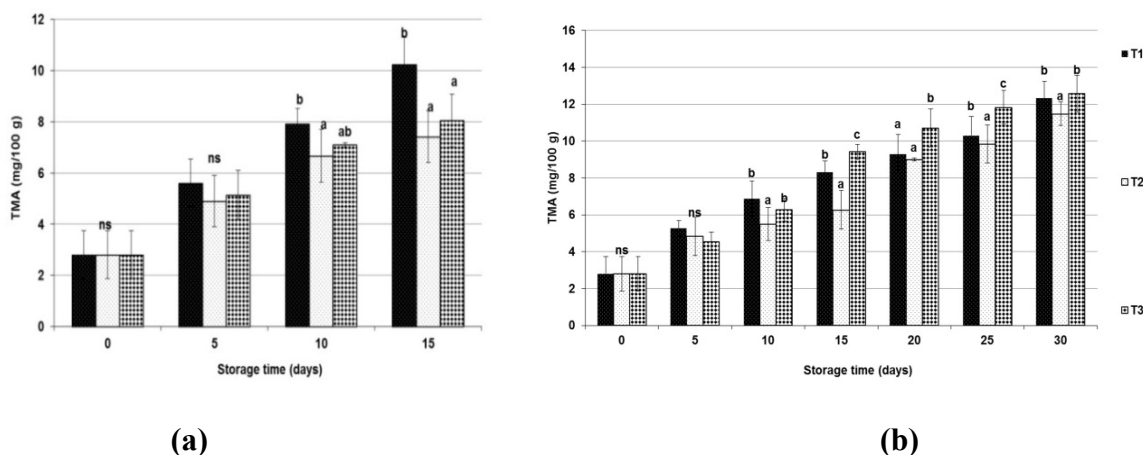


Figure 3. Trimethylamine values (TMA) of half-dried salted round scad stored at (a) room temperature and (b) chilling condition under air (T1), vacuum (T2) and air with oxygen absorber (T3) packaging

Thiobarbituric acid (TBA)

The TBA value of half-dried salted round scad stored at room temperature significantly increased along with the longer storage time ($p < 0.05$). The sample packed under vacuum condition (T2) had the lowest increasing TBA value with an initial of 0.39 mg MAD/kg and increased to 1.89 mg MAD /kg on day 15 of storage. The TBA value of samples stored under chilling condition significantly increased with an increased storage time ($p < 0.05$) with 2.45 mg MAD /kg on day 30 of storage. The increase in TBA led to the oxidation reaction of products and degraded to malonaldehyde. The increased TBA of half-dried salted round scad also has effects on food spoilage, nutrition and consumer health safety. Comparing to striped snaked-head fish, catfish and snapper queen fish, fatty fish with highly unsaturated fat, such as round scad, tend to have more

oxidation reaction (Oyarekua, 2014; Lohalaksanadech and Sujarit, 2016). Moreover, Oxygen was a factor leading to oxidation reaction. So half-dried salted round scad packed in vacuum condition had the lowest TBA value according to the result of Antonios and Michael (2007) who found that the packaging of mackerel (*Scomber japonicas*) under vacuum condition resulted in the lowest increase of TBA when comparing to modified atmosphere packaging and air packaging. However, the limitation of TBA in fish was lower than 8 mg MAD /kg and fresh fish has <3 mg MAD /kg. Among fishery products, the TBA of 3-4 mg MAD /kg indicates the low quality product. When considering the TBA in dried salted round scad, the results indicated that half-dried salted fish still had acceptable levels of TBA even at the end of storage time.

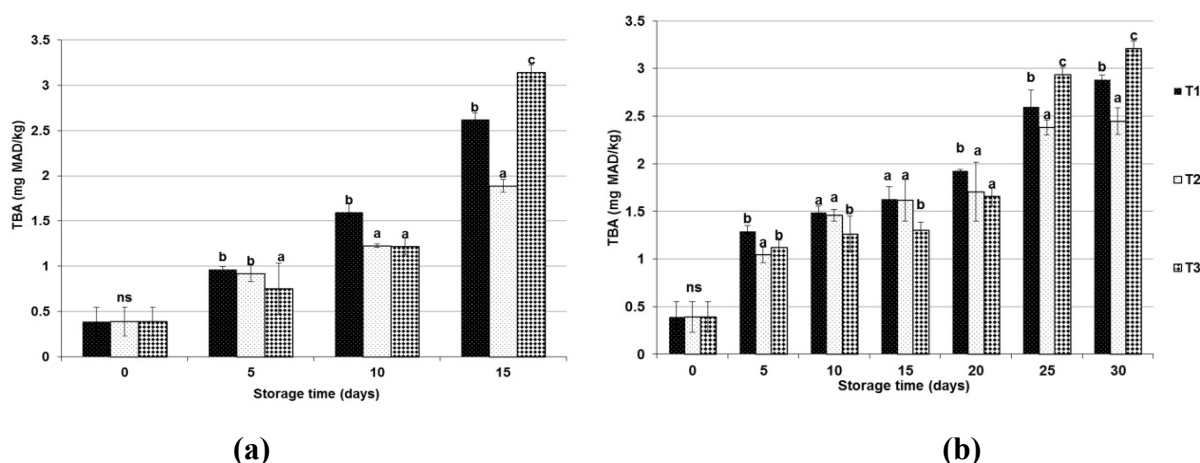


Figure 4. Thiobarbituric acid values (TBA) of half-dried salted round scad stored at (a) room temperature and (b) chilling condition of air (T1), vacuum (T2) and air with oxygen absorber (T3) packaging

3.2. Microbiological quality changes

Total plate count (TPC)

An initial number of TPC in half-dried salted round scad was 1.6×10^2 CFU/g in room temperature storage, and reached 2.9×10^6 , 2.2×10^5 and 3.0×10^5 CFU/g on day 15 of storage for 3 samples packaging, respectively. Whereas, The TPC in samples stored in refrigerator reached 3.3×10^6 , 2.0×10^5 and 3.5×10^5 CFU/g on day 30 of storage for samples packed in air (T1), vacuum (T2) and air with oxygen absorber (T3) packaging respectively (Table 1-2). The highest numbers of TPC were observed in dried salted round scad packed in air packaging and stored at room temperature and refrigerator. Meanwhile, the lowest number of TPC was observed in samples packed in vacuum packaging and stored at room temperature and refrigerator. Even though, the Thai Community Products Standards does not define the TPC in sun-dried fish (Thai industrial standards institute; TISI. 298/2006), the TPC is still necessary

for indicating the spoilage of products, especially the high moisture and low salt content products. If salt content in salted products is above 10% and their moisture content is below 40%, toxigenic-halophilic amine which forms bacteria will be inhibited (Koral *et al.*, 2013).

Escherichia coli and *Staphylococcus aureus*

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) of all 3 half-dried salted round scad packaging stored at room temperature and refrigerator were less than Thai Community Products Standards for sun-dried fish. The standard limitation of *E. coli* is less than 50 MPN/g and *S. aureus* is less than 200 CFU/g (data not shown). *E. coli* is used as a hygienic indicator for food processing plants, indicating the fecal contamination from animals and humans. *S. aureus* is a toxigenic microorganism which is harmful to consumers

Table 1. The microbial count of half-dried salted round scad stored at room temperature ($30\pm 2^\circ\text{C}$)

| Storage time (days) | Total plate count (CFU/g) | | | Yeast and mold (CFU/g) | | |
|---------------------|---------------------------|------------------|------------------|------------------------|------------------|------------------|
| | Air (T1) | Vacuum (T2) | Absorber (T3) | Air (T1) | vacuum (T2) | absorber (T3) |
| 0 | 1.6×10^2 | 1.6×10^2 | 1.6×10^2 | 2.0×10^2 | 2.0×10^2 | 2.0×10^2 |
| 5 | 1.9×10^3 | 1.8×10^3 | 1.9×10^3 | 1.6×10^2 | 1.7×10^2 | 2.6×10^2 |
| 10 | 2.0×10^6 | 1.3×10^4 | 2.2×10^4 | 2.6×10^2 | 1.5×10^2 | 1.6×10^2 |
| 15 | 2.9×10^5 | 2.2×10^5 | 3.0×10^5 | 2.7×10^3 | 6.4×10^2 | 5.4×10^2 |

Table 2. The microbial count of half-dried salted round scad stored in chill condition ($4\pm 1^\circ\text{C}$)

| Storage time (days) | Total plate count (CFU/g) | | | Yeast and mold (CFU/g) | | |
|---------------------|---------------------------|------------------|------------------|------------------------|------------------|------------------|
| | Air (T1) | Vacuum (T2) | absorber (T3) | Air (T1) | vacuum (T2) | absorber (T3) |
| 0 | 1.6×10^2 | 1.6×10^2 | 1.6×10^2 | 2.0×10^2 | 2.0×10^2 | 2.0×10^2 |
| 5 | 1.3×10^3 | 1.4×10^3 | 1.8×10^3 | 1.9×10^2 | 1.8×10^2 | 2.5×10^2 |
| 10 | 1.1×10^4 | 1.6×10^4 | 2.3×10^4 | 1.7×10^2 | 2.0×10^2 | 3.0×10^2 |
| 15 | 2.4×10^4 | 1.9×10^4 | 2.8×10^4 | 2.9×10^2 | 3.4×10^2 | 2.1×10^2 |
| 20 | 2.9×10^5 | 2.2×10^4 | 3.0×10^4 | 2.7×10^2 | 3.7×10^2 | 2.3×10^2 |
| 25 | 3.1×10^6 | 2.7×10^4 | 3.2×10^4 | 6.9×10^2 | 3.5×10^2 | 5.5×10^2 |
| 30 | 3.3×10^6 | 2.0×10^5 | 3.5×10^5 | 7.2×10^2 | 5.2×10^2 | 5.1×10^2 |

4. Conclusions

Half-dried salted round scad stored in vacuum packaging showed the lowest quality changes comparing to samples stored in air and air with oxygen absorbers packaging. The chilling storage was able to increase the shelf life of half-dried salted round scad. It can be concluded that, the shelf life of half-dried salted round scad packed in air condition was 5 and 10 days length when stored at room temperature and chilled condition, respectively. On the other hand, the shelf life of half-dried salted round scad

stored in vacuum package was 10 and 25 days under room temperature and chilled condition, respectively. For the greater amount of half-dried salted round scad as an industrial product, food additives are recommended to extend the product's shelf life.

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Acknowledgment

This research was funded by Yala Rajabhat University