

## Effect of different storage conditions on quality of White-Scar oyster (*Crassostrea belcheri*)

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**Abstract:** Changes in some chemical, microbiological and sensory qualities of white-scar oyster (*Crassostrea belcheri*) during different storage conditions were studied. Lower changes in chemical and microbiological qualities were found in chilled shell-on oysters when compared with those stored at ambient temperature. Shell-on oyster packed in 2.5% brine showed a slower decrease in pH but increased in total volatile basic nitrogen (TVB-N), total viable count (TVC) and psychrotrophic bacteria at a faster rate than those stored in air. *Escherichia coli* and *Vibrio parahaemolyticus* were less than 1.8 and 3 MPN/g, respectively. Irrespective of packing medium and storage temperature, sensory scores for every parameter decreased while microorganisms increased with the storage duration. Therefore, with respect to microbiological and sensory qualities, shell-on oyster could be kept in air at chilled temperature for 9 days. Shucked oyster packed in 4% brine showed a decrease in pH and increases in TVB-N, TVC and psychrotroph at a slower rate than those packed in 2.5% brine and water. Whereas *E. coli* and *V. parahaemolyticus* were less than 1.8 and 3 MPN/g, respectively in every packing medium and stored at chilled temperature for 12 days. The decreased in sensory score of all attributes with the increase in TVC was found in shucked oyster packed in different salt concentrations. Therefore, shucked oysters could be kept for 10 days in 4% brine at chilled temperature.

**Keywords:** Oyster, *Crassostrea belcheri*, quality changes, chilled storage, ambient storage

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### Introduction

White-scar oyster (*Crassostrea belcheri*) is mainly cultured in Thailand and generally sold with shell-on and consumed fresh. However, some constraints of limiting domestic and export markets may occur because of their short shelf-life (Hu *et al.*, 2008). There is no report on the shelf-life of fresh white-scar oyster; nevertheless Thai oyster farmers recommended that the fresh oysters could maintain the quality not more than 2 days at ambient temperature (30±2°C) storage in normal air. Pacific oysters (*Crassostrea gigas*) stored in air with water sprinkling at 7°C had 52-80% of survival after 20 weeks storage (Seaman, 1991). In addition, flat oysters (*Ostrea edulis*) stored in running seawater at 9°C, freshwater ice at 1°C and cold wood wool without ice at 5°C were mostly alive after 3 weeks with only 1-4% of dead oysters (Aaraas *et al.*, 2004).

Like other seafood, quality deterioration and spoilage of fresh oysters occur very rapidly due to enzymatic autolysis, microbial growth and physical alterations. The post-harvest changes of oyster tissue depend very significantly upon factors affecting the concentration of substrates and metabolite in the tissue, the activity of endogenous enzymes, the microbial contamination and the conditions after harvesting (Sikorski *et al.*, 1990).

The loss of quality and spoilage of seafood due to bacterial growth and endogenous enzymatic changes are faster at ambient than at chilled temperature conditions (Huss, 1995). During storage, the bacteriological quality of the flat oyster (*O. edulis*) were changed i.e. black bacterial colonies appeared on all plates from day 12 and 19 onward in iced and cold stored samples, respectively, with approximately 10<sup>4</sup> colonies/ml mantle fluid. Whereas black colonies

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were absent in the running seawater (control) samples, except one single colony in 1 sample from day 19. It was also reported that tissue pH in all live oysters ranged from 5.6-6.3 and tissue pH of dead oysters ranged from 5.2 to 5.4, and decreased during iced (1°C) and chilled (5°C) storage and had a tissue pH of 5.5 at day 16 (Aaraas *et al.*, 2004). Storage conditions may also influence the sensory profile especially the smell of seaweed, fish and mud, and the appearance of the mantle and plum color. Iced flat oysters and chilled flat oysters had significantly more pronounced smell of seaweed, fish, and mud, and more shrunken and contracted mantle appearance than those kept in running seawater (Aaraas *et al.*, 2004). Sodium chloride (NaCl) is added to food products for various purposes, including a decrease in water activity, less availability to microbial attack and enhancement of functional properties, leading to an increase of the shelf-life time (Takiguchi, 1989).

Information about the postmortem changes and preservation method of oysters is scarce which limits its commercialization as a fresh product. Therefore, the present study was undertaken to investigate the effect of different storage conditions on the quality of white-scar oyster (*C. belcheri*) both in shell-on and shucked forms.

## Materials and Methods

### Storage conditions and sampling

White-scar oysters (*C. belcheri*) of market size, 300-350 g in weight and 13-15 cm in length, were obtained from Bandon bay, Suratthani province, south of Thailand in June 2005. After harvesting, the live oysters were placed in nylon sacks and transported to the laboratory within 4-5 h at ambient temperature (30±2°C) which was a normal procedure used in the region. Fresh oysters were washed with tap water (total viable count; TVC was approximately 2.97 log CFU/g, *Escherichia coli* was not found and coliforms were < 2 Most Probable Number; MPN/100 ml) for 10 min and drained for 5 min. Two trials with three replications for each trial were carried out at different conditions as follows: (1) shell-on oysters packed in air (one hundred oysters were packed in a hemp sack, size 70 x 100 cm), and stored at ambient (30±2°C) (SSA) and chilled (4±2°C) (SSC) temperatures; (2) shell-on oysters packed in plastic bucket containing 2.5% brine at a ratio of oyster:brine of 7:3 w/w and stored at ambient (SBA) and chilled (SBC) temperatures, with the brine changed every day during storage and (3) shucked oyster packed in different media, i.e. water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) in polypropylene cup at the ratio of

shucked oyster:media of 7:3 (w/w) with a net weight of 180 g, covered with polypropylene film and heat sealed, then stored at chilled (4±2°C) temperature.

The shell-on oysters stored only at ambient temperature for 4 days were removed every 12 h for chemical and microbiological analyses (15 oysters each time), and every 24 h for sensory evaluation (25 oysters each time), whereas both shell-on oysters and shucked oysters stored at chilled temperature for 10 and 12 days, respectively, were removed every 24 h (40 oysters each time) for all analyses. The oysters for day 0 were analyzed immediately after arrival in the laboratory.

### Chemical analysis

**pH.** Ten g of oyster tissue was homogenized with 20 ml of distilled water at 12,000 rpm for 1 min. The pH of the homogenate was measured using a pH meter (Woyewoda *et al.*, 1986).

**Total Volatile Basic-Nitrogen (TVB-N).** Two g of oyster tissue was homogenized with 8 ml of 4% trichloroacetic acid (TCA) at 12,000 rpm for 2 min and kept at ambient temperature for 30 min. The homogenate was centrifuged at 3,000 rpm for 10 min. The supernatant was made up to 10 ml using 4% TCA and TVB-N was determined according to the method of Hasegawa (1987).

### Microbiological analysis

For microbiological counts, twenty five g of oyster tissue was stomached with 225 ml of sterile Butterfield's phosphate-buffered water. From the 10<sup>-1</sup> dilution, other decimal dilutions were prepared. Total viable count (TVC) and psychrotrophic count were determined by pour plate method using plate count agar and incubated at 35°C for 48 h and at 7°C for 10 days, respectively (BAM, 2001). For *Escherichia coli* (*E. coli*) detection, lauryl sulfate tryptose (LST) broth and EC broth were used for the preliminary screening followed by selective streaking on eosin methylene blue (EMB) agar. Typical *E. coli*-like colonies were confirmed by IMViC test (BAM, 2002). For *Vibrio parahaemolyticus* (*V. parahaemolyticus*) detection, glucose salt teepol broth (GSTB) was used for the preliminary screening. Growth of *V. parahaemolyticus* in GSTB tubes was confirmed by streaking onto thiosulfate citrate bile salts sucrose (TCBS) agar, as described according to the method of BAM (2004).

### Sensory evaluation

The sensory quality of oyster tissue was assessed by a ten trained panelists using the modified guidelines from Jeong *et al.* (1990) and Aaraas *et al.* (2004) with the scale from 1 to 9: 1, reject; 2,

extremely poor; 3, very poor; 4, poor; 5, acceptable (border line); 6, good; 7, very good; 8, extremely good and 9, excellent. Panelists were asked to score without consuming for appearance (loss of shape integrity and uniform texture), color of plum (Brownish-yellow and white/creamy), texture (soft and firm/elastic) and odor (smell/spoilage-like and melon-like/seaweed) of oysters. Individual samples from each treatment were placed on dishes (diameter 7.0 cm), covered with aluminium foil and stored at 4°C for at least 15 min prior to serving.

#### Statistical analysis

The data from three replications of two trials were combined and subjected to the analysis of variance (ANOVA) and comparison of means by Duncan's multiple range test for significant differences at  $P < 0.05$  (Steel and Torrie, 1980).

### Results and Discussion

#### Chemical quality

**pH:** Changes in pH can be used as indicator for the postmortem changes of glycogen to lactic acid and the degradation of muscle components e.g. proteins and nucleotides during long term of storage (Jay, 2000). The initial pH of white-scar oyster (*C. belcheri*) was 6.1 and gradually decreased ( $P < 0.05$ ) in all treatments during storage (Figure 1). Similar results on pH of live flat oyster (*O. edulis*) were reported ranging from 5.6 to 6.3 and after death from 5.2 to 5.3 (Aaraas *et al.*, 2004). The shell-on oysters in different media showed slower change in pH when stored at chilled temperature than those stored at ambient temperature. This was probably due to the lower temperature storage could retard the decomposition of glycogen (Huss, 1995). Using 2.5% brine resulted in a slower decrease in pH of shell-on oyster than those packed in air under both ambient and chilled storage. Because ionic strength of salt solution could affect the solubility of myofibrillar proteins and hence produced volatile bases which could delay the decreasing rate of pH (Huss, 1995). Shucked oyster packed in 4% brine showed a slower decrease in pH than those packed in 2.5% brine and in water during storage at chilled temperature.

**TVB-N:** Total volatile base-nitrogen (TVB-N), a decomposed both protein and non-protein nitrogenous compounds (trimethylamine (TMA), dimethylamine (DMA) and ammonia), of all samples increased with storage time, probably caused by bacterial and endogenous proteolytic enzymatic actions (Hernandez-Herrero *et al.*, 1999). The initial TVB-N value of white-scar oyster (*C. belcheri*) samples was

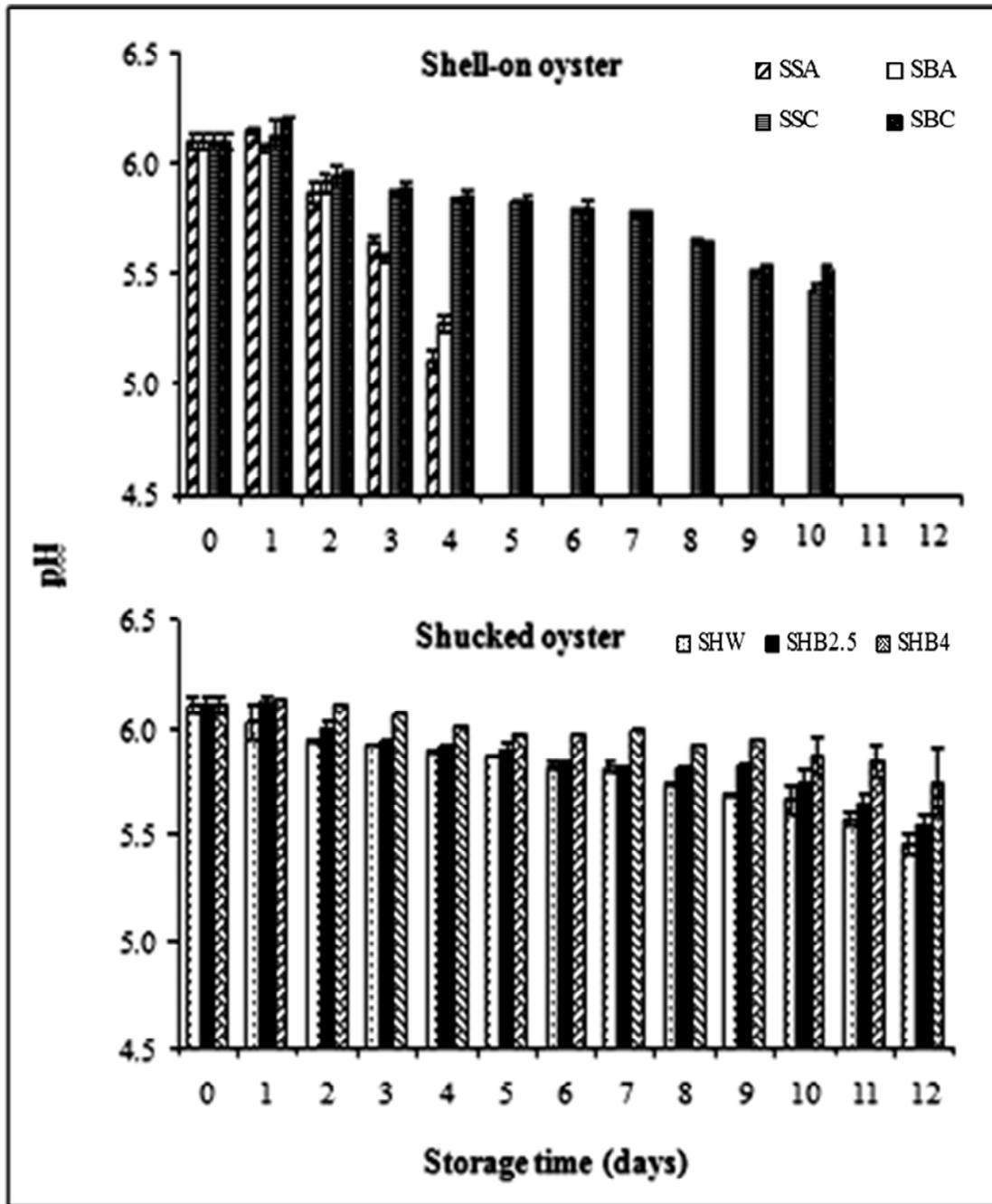
1.8 mg N/100 g (Figure 2), whereas of purified and nonpurified with high pressure flat oysters (*O. edulis*) was 11.2 and 13.3 mg N/100 g, respectively (Lopez-Caballero *et al.*, 2000). The TVB-N values of the shell-on oyster in different media stored at chilled temperature increased at a slower rate than those stored at ambient temperature (Figure 2), probably due to the low temperature most likely delay the growth of protease-producing bacteria and inhibit the protease activity (Huss, 1995). Shell-on oyster packed in 2.5% brine showed an increase in TVB-N at a faster rate than those packed in air, probably because the higher level of NaCl in the samples supported higher growth of halophilic bacteria and thus contributed to the higher contents of TVB-N (Tsai *et al.*, 2005). The TVB-N values of shucked oysters packed in different media were not significantly changed ( $P \geq 0.05$ ) during chilled storage. After storage for 12 days, they were 7.4, 5.3 and 4.4 mg N/100 g for shucked oyster packed in water, 2.5% brine and 4% brine, respectively (Figure 2).

TVB-N values of all white-scar oysters (*C. belcheri*) after storage under different conditions in this study were 4.4-14.8 mg N/100 g. Although these values were considerably low levels, the oyster showed spoilage due to the microbiological quality (Table 1). Whereas raw flat oyster (*O. edulis*) showed spoilage containing TVB-N of 25-30 mg N/100 g after 10 days of chilled (5°C) storage (Lopez-Caballero *et al.*, 2000).

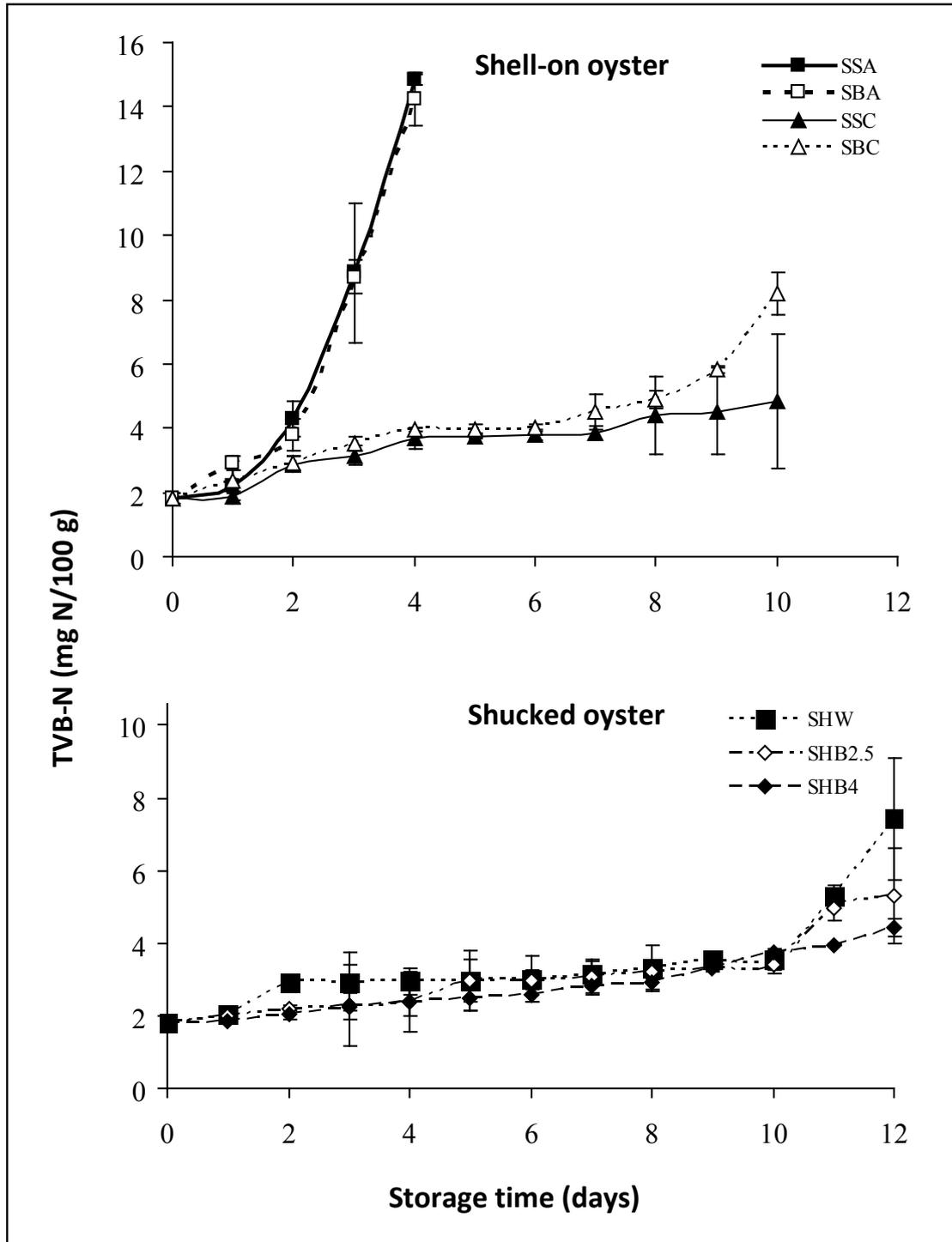
#### Microbiological quality

Changes in TVC and psychrotroph of shell-on and shucked white-scar oysters (*C. belcheri*) during storage under chilled and ambient temperature are shown in Table 1. The initial TVC of fresh oysters in this study was 3.7 log CFU/g and gradually increased throughout the duration of storage under the studied media. It was reported that the initial TVC in mussels (*Mytilus galloprovincialis*) was 4.5 log CFU/g and dramatically increased during storage at 4°C under modified atmosphere packaging (Goulas *et al.*, 2005). Refer to the acceptable limit of TVC for chilled bivalve mollusks (not more than 5.7 log CFU/g, Department of Fisheries, 2004), shell-on oysters packed in both air and 2.5% brine, stored at ambient temperature (SSA, SBA) and chilled temperature (SSC, SBC) could be kept for less than 3, 9 and less than 7 days, respectively. With the same reference, shucked oyster packed in water, 2.5% brine and 4% brine (SHW, SHB2.5 and SHB4) stored at chilled temperature could be kept for 9 and 11 days, respectively.

After storage, salt content of oyster increased from initially 1.5% w/w to different value under



**Figure 1.** Changes in pH of shell-on oyster stored in air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC), and shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature storage.



**Figure 2.** Changes in TVB-N of shell-on oyster stored in air at ambient (SSA) and chilled temperatures (SSC), and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC), and shucked oyster stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature storage.

**Table 1.** Changes in microbiological quality of shell-on and shucked oysters during storage

Storage time (days)	Total Viable Count (log CFU/g)						
	SSA	SBA	SSC	SBC	SHW	SHB2.5	SHB4
0	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>
1	3.8±0.3 <sup>ab</sup>	4.1±0.0 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.2 <sup>a</sup>	3.7±0.4 <sup>a</sup>	3.8±0.3 <sup>a</sup>
3	5.8±0.1 <sup>c</sup>	6.3±0.3 <sup>c</sup>	4.4±0.0 <sup>b</sup>	4.8±0.1 <sup>b</sup>	4.5±0.5 <sup>ab</sup>	4.4±0.1 <sup>ab</sup>	4.3±0.3 <sup>ab</sup>
5	NT	NT	5.3±0.2 <sup>d</sup>	5.3±0.0 <sup>cd</sup>	5.3±0.2 <sup>bcd</sup>	5.0±0.2 <sup>bcd</sup>	4.9±0.4 <sup>cd</sup>
7	NT	NT	5.4±0.1 <sup>de</sup>	5.8±0.2 <sup>e</sup>	5.6±0.8 <sup>cd</sup>	5.4±0.1 <sup>bcd</sup>	5.2±0.2 <sup>cdef</sup>
9	NT	NT	5.6±0.0 <sup>ef</sup>	6.0±0.1 <sup>e</sup>	5.7±0.4 <sup>cd</sup>	5.4±0.5 <sup>cd</sup>	5.5±0.1 <sup>ef</sup>
11	NT	NT	NT	NT	5.8±0.6 <sup>cd</sup>	5.6±0.6 <sup>d</sup>	5.6±0.2 <sup>ef</sup>
Acceptable limit	5.7 log CFU/g						
Storage time (days)	Psychrotrophic bacteria (log CFU/g)						
0	NT	NT	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>
1	NT	NT	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>a</sup>	3.7±0.0 <sup>ab</sup>	3.7±0.1 <sup>a</sup>	3.7±0.1 <sup>ab</sup>
3	NT	NT	4.4±0.1 <sup>bc</sup>	4.5±0.1 <sup>b</sup>	4.0±0.6 <sup>abcd</sup>	4.2±0.1 <sup>bc</sup>	4.0±0.1 <sup>abc</sup>
5	NT	NT	4.7±0.7 <sup>c</sup>	4.9±0.2 <sup>bc</sup>	4.3±0.2 <sup>abcd</sup>	4.2±0.2 <sup>bcd</sup>	4.1±0.1 <sup>abc</sup>
7	NT	NT	4.8±0.2 <sup>c</sup>	5.0±0.1 <sup>c</sup>	4.4±0.2 <sup>bcd</sup>	4.3±0.2 <sup>bcd</sup>	4.2±0.2 <sup>abc</sup>
9	NT	NT	4.9±0.2 <sup>c</sup>	5.1±0.1 <sup>c</sup>	4.5±0.3 <sup>cd</sup>	4.4±0.0 <sup>cde</sup>	4.3±0.0 <sup>bc</sup>
11	NT	NT	NT	NT	4.6±0.4 <sup>cd</sup>	4.6±0.1 <sup>de</sup>	4.4±0.3 <sup>c</sup>
Storage time (days)	<i>Escherichia coli</i> (MPN/g)						
0-11	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8
Storage time (days)	<i>Vibrio parahaemolyticus</i> (MPN/g)						
0-11	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0

Data are expressed as means ± standard deviation of two trials. NT, not test.

a-f in the same column indicating significantly different ( $P < 0.05$ ).

SSA and SBA, SSC and SBC = shell-on oyster stored in air and in 2.5% brine at ambient and chilled temperatures, respectively. SHW, SHB2.5 and SHB4 = shucked oyster stored in water, 2.5% and 4% brine at chilled temperature, respectively.

different packing media and storage temperature as follows: 1.5, 2.3, 1.5 and 1.8% w/w for shell-on oyster (SSA, SBA, SSC and SBC) and 1.1, 2.1 and 2.6% w/w for shucked oyster (SHW, SHB2.5 and SHB4), respectively. The increase in salt content may affect the growth of microorganisms showing from some microorganisms could survive in the presence of 2.5% salt (Huss, 1995). Furthermore, the inhibition of microbial growth in shucked oysters stored in both 2.5% brine and 4% brine was not significantly different throughout the duration of storage. The higher salt contents (> 5.0%) in salted mackerel apparently had some inhibitory effect on bacterial growth (Tsai *et al.*, 2005). Wheaton and Lawson (1985) showed that fish with salt content above 1%, the bacteria associated with fish spoilage were increasingly stressed. Most of these bacteria would die or at least stop growing as the salt content of the fish was increased from 6% to 8%.

The psychrotroph of shell-on and shucked oysters in different storage conditions gradually increased during chilled storage (Table 1). The psychrotroph of shell-on oyster packed in 2.5% brine increased faster than those packed in air during chilled storage, probably due to some of the spoilage bacteria can grow well in the sodium-containing condition (Huss, 1995). Whereas those microorganisms of shucked oysters packed in 4% brine increased with a slower rate than those packed in 2.5% brine and in water. The result suggested that the growth of spoilage bacteria might be inhibited by a high salt concentration.

*E. coli* and *V. parahaemolyticus* of shell-on and shucked oysters under various media, storage temperatures were less than 1.8 and 3 MPN/g, respectively, throughout the storage time (Table 1). Although the TVC of oysters after storage exceed the standard value, the pathogenic bacteria was still lower than 2.3 MPN/g (for *E. coli*) and 10<sup>4</sup> MPN/g (for *V. parahaemolyticus*) (Department of Fisheries, 2004), probably due to the storage conditions were not optimum for their growth.

#### Sensory quality

The sensory qualities of oyster tissue after storage were observed from shrunken and contracted mantle, dissolved gill, soft in texture, dark in color and strong odor. Decrease in sensory scores of shell-on oysters both packed in air and in 2.5% brine was not significantly different ( $P \geq 0.05$ ) but under ambient temperature were greater than those stored at chilled temperature during storage (Figure 3). The appearance, color, texture and odor scores of the shucked oysters in water slightly decreased at a greater rate than those packed in 2.5% and 4% brine

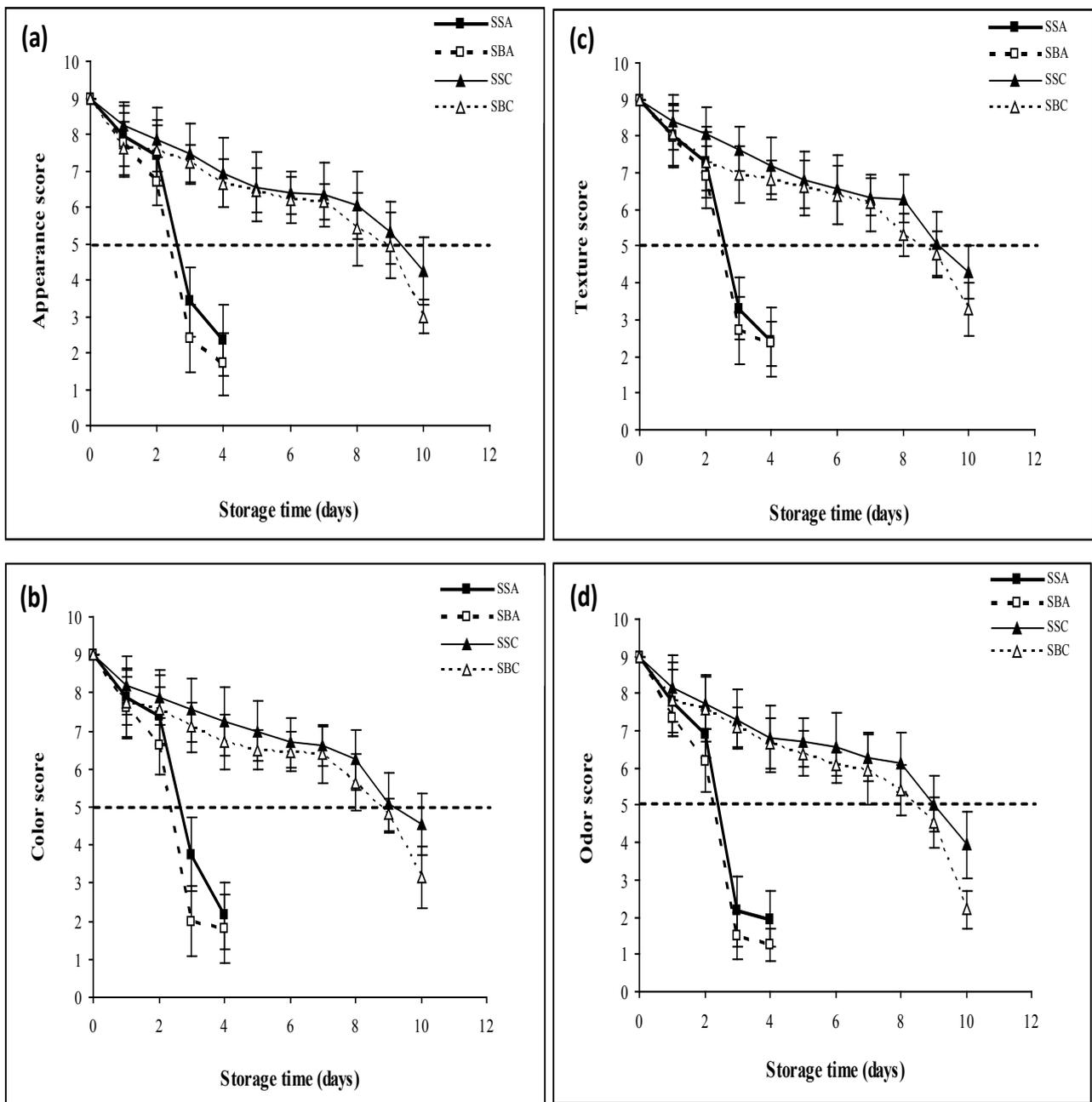
during chilled storage (Figure 4). The acceptable shelf-life will be considered from the sensory score of more than 5.0. The results showed that shell-on oysters packed in air and in 2.5% brine could be accepted at less than 3 days at ambient temperature and 8-9 days under chilled storage. Whereas shucked oysters packed in water was up to 9 days and those in 2.5% and 4% brine were up to 10 days. Aaraas *et al.* (2004) recommended that the immersed flat oysters (*O. edulis*) could be consumed within a week but should not be used after 12 days of storage.

#### Conclusion

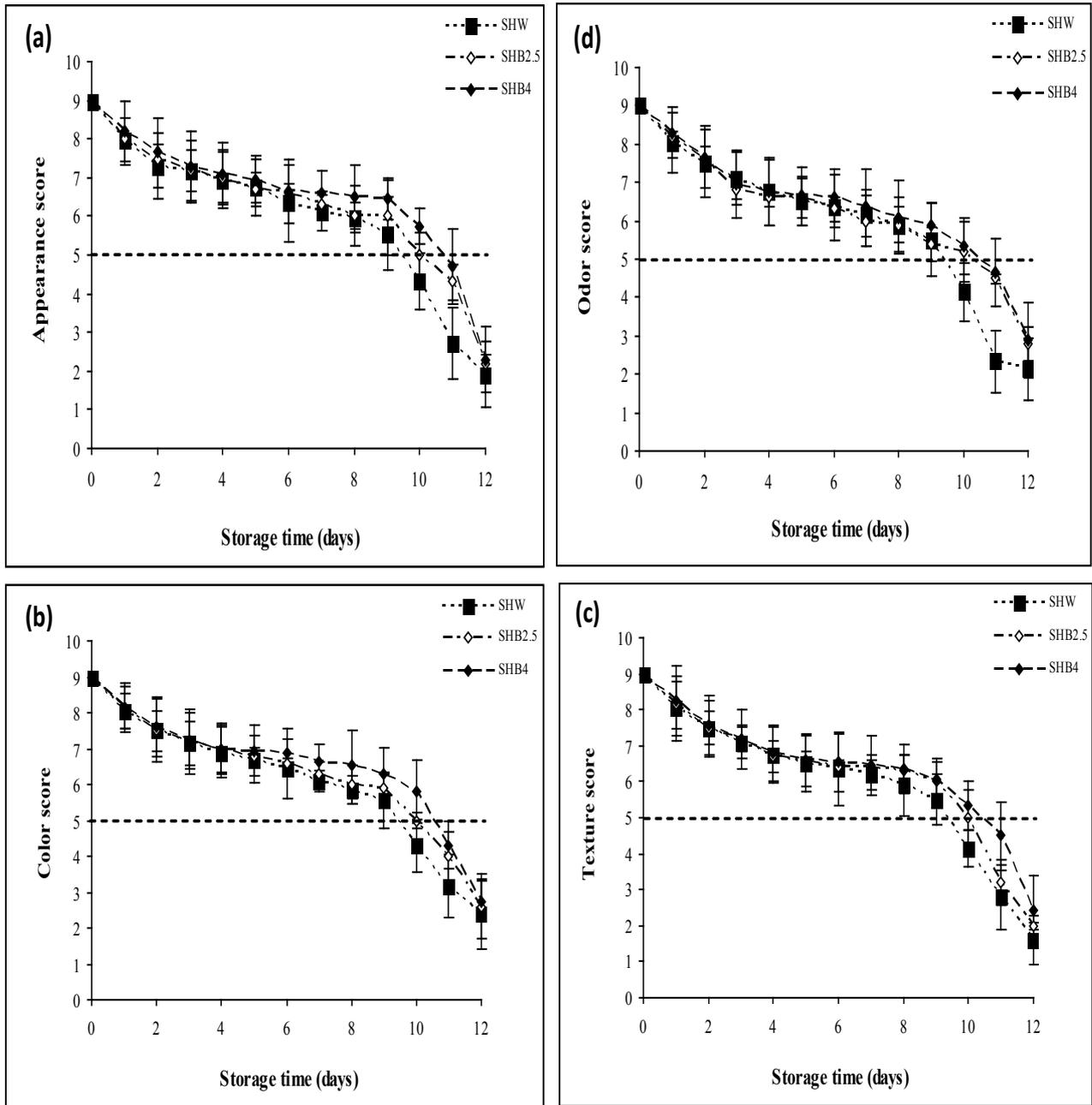
Shell-on oysters, under chilled storage both in air and 2.5% brine showed slower changes in chemical and microbiological qualities than those under ambient temperature. Considering from microbiological and sensory quality, it was found that shell-on oysters packed in air and in 2.5% brine could be accepted at less than 3 days at ambient temperature and 7-9 days under chilled storage. Whereas shucked oysters packed in water, 2.5% brine and 4% brine were accepted at 9, 10 and 10 days, respectively of chilled storage. Though keeping shucked oyster in 4% brine showed the greatest effect on the shelf-life extension, it might affect to the acceptable taste of oyster.

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**Figure 3.** Sensory quality of shell-on oyster (a: appearance score; b: color score; c: texture score; d: odor score) stored in hemp sack at ambient (SSA) and chilled temperatures (SSC) and in 2.5% brine at ambient (SBA) and chilled temperatures (SBC).



**Figure 4.** Sensory quality of shucked oyster (a: appearance score; b: color score; c: texture score; d: odor score) stored in water (SHW), 2.5% brine (SHB2.5) and 4% brine (SHB4) at chilled temperature.

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