Thesis for the Degree of Master of Fisheries Science

Effects of Table Salt and Thymol Oil on Shelf Life of Common Carp (*Cyprinus carpio*) Fillets during Iced Storage

by

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KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

February 2013

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Advisor: Professor Ji-Young Yang

by

Abida Saeed

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Fisheries Science

in KOICA-PKNU International Graduate Program of Fisheries Science,

The Graduate School,

Pukyong National University

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A Dissertation

by

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February 22, 2013

아비다 사이드의 수산학석사 학위논문을

인준함

2013 년 2 월 22 일

주 심 농학박사 이양봉 인

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Effects of Table Salt and Thymol Oil on Shelf Life of Common Carp (*Cyprinus carpio*) Fillets during Iced Storage

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Abstract

The study evaluated the effects of table salt and thymol oil used as natural preservatives on the quality and shelf life of the common carp (*Cyprinus carpio*) fillets exposed to different treatments during iced storage. The effects were investigated by measuring of microbiological, chemical and sensory parameters for 21 days at 3 day intervals. Treatments included the following: S1 (control sample) and dip treatments for S2 (12% salt solution, w/v), S3 (0.7% thymol oil solution, v/v) and S4 (salt 12%, w/v + thymol oil 0.7%, v/v). The S3 and S4 produced significantly lower ($p<0.05$) total viable count of bacteria (TVC), pH, total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) values as compared to S1 and S2 samples after day 6 up to the end of the storage period. TVC of fillets dipped in S1 and S2 was significantly higher than those of fillets dipped in S3 and S4. TVB-N of fillets in S1 was significantly higher than that of fillets in S3 and S4. However, no significant difference was observed among TVB-N of fillets in S2 and S3; and among S3 and S4. TMA-N of fillets in S2, S3 and S4 was significantly lower

than that of fillets in S1. However, no significant difference was found among the fillets in S2, S3 and S4. Positive significant correlations were established within and between microbial and chemical parameters. According to the results of TVC, pH, TVB-N, TMA-N and sensory parameters, the shelf life of the common carp (*C. carpio*) fillets has extended upto 9, 18 and more than 21 days in S1, S2, S3 and S4 respectively. However, no significant difference ($p>0.05$) was observed among S3 (0.7% thymol oil solution, v/v) and S4 (salt 12%, w/v + thymol oil 0.7%, v/v). Therefore, for extending the shelf life of the fillets thymol oil could be the effective treatment.

Keywords: *Cyprinus carpio,* thymol oil, table salt, shelf life, iced storage

Introduction

Fish is a perishable product that requires effective preserving methods to keep quality and avoid food poisoning. Therefore, numerous techniques have been used to increase its shelf life and prevent seafood poisoning (Ababouch *et al*., 1996; Ashie *et al*., 1996; Attouchi and Sadok, 2010; Stammen *et al*., 1990). Iced storage is, therefore, an efficient way of reducing the rate of deterioration and also of extending the shelf life of fish. However, the shelf life of fish stored in ice is limited by enzymatic and microbiological spoilage. Many fish species have been examined during storage in ice (Fan *et al*., 2008; Karungi *et al*., 2004; Namulema *et al*., 1999; Ryder *et al*., 1993). However, the quality of fish muscle will also deteriorate during iced storage as the fish muscle is abundant in proteins and unsaturated fatty acid. Endogenous proteases, which are able to hydrolyze different proteins in the fish muscle, are important in the early deterioration process (Cepeda *et al*., 1990), so taking some measures to delay the decline of fish quality and extending the preservation life of fish during ice storage is worthwhile. Icing reduces temperature to about 0°C, which lowers the growth rate of spoilage and pathogenic microorganisms (Huss, 1995).

Spoilage reactions can be inhibited by traditional processing and preservation procedures (Gould, 1996; Huss *et al*., 1997). Salt-preservation is one of the oldest preservation methods, which is still commonly practiced in the world. Microbial activity is prevented by the bactericidal effect of salt so that fish meat is protected from microbial spoilage. The level of protection depends upon the concentration of salt and as the salt level increase in the product, shelf life extends further (Del-Valle and Gonzales-Inigo, 1968; Horner, 1997). In modern days, in addition to salt, some other antimicrobial additives are used to inhibit antimicrobial activity which is the major spoilage factor in foods (Patir *et al*., 2009).

Due to the growing interest in using natural preservatives, essential oils (EOs) are obtaining success in food research activity as natural compounds with appreciable antimicrobial properties (Dorman and Deans, 2000; Manohar *et al*., 2001). EOs are claimed to possess a broad spectrum of active properties because of their high content in phenolic derivatives such as thymol, carvacrol and eugenol (Aureli *et al*., 1992; Hammer *et al*., 1999). Essential oil compounds, such as carvacrol and thymol, will both prevent the microbial and chemical deterioration when added to food (Burt, 2004;

Mahmoud *et al*., 2004; Ultee *et al*., 1999). The antimicrobial effect of these phenolic compounds can be enhanced by combining with other natural preservatives (Ramanathan and Das, 1992; Yamazaki *et al*., 2004). Thymol is a monocyclic phenolic compound, the common natural source being the essential oil of *Thymus vulgaris* (Lamiaceae). Its main therapeutic application is in dental preparations to kill odour-producing bacteria. It is also employed as a preservative on the strength of its antimicrobial (Cosentino *et al*., 1999; Venturini *et al*., 2002) and antioxidant properties (Aeschbach *et al*., 1994). There is, in general, limited information in the literature on the effect of thymol oil, used in combination with other preservative technologies.

The common carp (*Cyprinus carpio*), as one of the most widely cultured species due to its fast growth rate, easy cultivation, high feed efficiency ratio (Tokur *et al*., 2006). The spoilage of fish is a complex process in which physical, chemical and microbiological mechanisms are implicated. We aim to study the effect of salt and thymol oil on fresh fillets of *C. carpio*: (a) to determine the shelf life of the carp fillets treated with different levels of salt and thymol oil; (b) to evaluate the degree of spoilage of treated

samples by microbial, chemical and sensory tests; (c) to determine whether there is a synergism in the combined effect of salt and thymol oil as antimicrobial and antioxidant agents on carp fillets during iced storage.

Materials and Methods

Raw materials

The common carp (*Cyprinus carpio*), varying from 500-600 g in weight, were obtained from Young Nam Fresh Water Fish Market (Bujeon-Dong, Jin-Gu, Busan, Korea). The fish were deheaded and filleted manually by a sterile scalp and delivered to the laboratory in polystyrene boxes while stored in ice. The fillets were washed with tap water, drained well and stored at –70°C in a deep freezer (NF-400SF, Nihon Freezer, Japan).

Essential oil and table salt (Sodium chloride)

Thymol oil (100%) natural essential oil of *Thymus vulgaris* was purchased from Neumond Co. (Düfte der Natur, Germany). Saline solution (12%, w/v) was made with common table salt (NaCl) bought from Hanjoo Co. (Busan, Korea).

Treatment and storage of carp fillets

Fillets were divided into four batches with each batch receiving one of the treatments shown in Table 1.

Table 1. Treatments given to the samples

Sample No.	Sample Name	Sample Treatment
1	S1	Control
2	S2	Salting by immersing 12% (w/v) in NaCl brine
3	S3	Thymol oil 0.7% (v/v)
4	S4	Salt 12% (w/v) + Thymol oil 0.7% (v/v)

The samples were given dip treatment for 30 min at room temperature and drained well. After that the fillets were individually packed in plastic bags, the packs were iced with ice flakes in the ratio of 1:1 (fish: ice) in insulated polystyrene boxes, and the boxes were kept at room temperature for 21 days. The melted ice was replaced daily, to maintain the ratio. Fillet samples were taken randomly and analyzed microbiologically, chemically and sensorially on day 0, 3, 6, 9, 12, 15, 18, and 21.

Bacteriological analyses

Sample preparation

Portions, each of weight 25 g, from each of the carp fillet (ca. 50 cm^2, 50 g), were weighed by electronic scale, transferred aseptically into a stomacher bag containing 225 ml of sterilized 0.9% saline and homogenized

for 1 min by a stomacher 400 (Easy Mix, AES Laboratories, France) at room temperature in laboratory.

Preparation of test suspension

Saline solution was used to dilute the sample for counting the number of bacteria. Concentration of saline solution 0.9% was made by dissolving 9 g of laboratory grade sodium chloride (NaCl) into 1 liter of distilled water and was mixed by Embo Mixer (Korea) for 15 min. Each of 9 ml of the prepared saline solution was kept in a glass test tube of 20 ml volume which was autoclaved at 121°C for 15 min and then cool down to room temperature. Fresh sterilized 0.9% saline solution was daily prepared.

Counting number of bacteria

The samples were further diluted by 10 dilution factor series for each sample. The homogenized fillet sample was further serially diluted using 9 ml sterile saline by 10 dilution factor series for each sample for bacteriological analysis. The 3M Petrifilms^{TM} were used to determine the total viable counts for bacteria (TVC), *Escherichia coli* and coliforms and *Staphylococcus aureus*. Serial dilutions were done in duplicate up to 10^5

times for TVC, 10^2 times for *Escherichia coli* and coliforms and 10^0 for *Staphylococcus aureus*.

The 3M Petrifilm ^{TM}, a dry-film for aerobic plate count, was placed on a flat surface. The top film was lifted and 1 ml test suspension was inoculated onto the center of the film base. The top film was carefully placed down on inoculum. Test suspension was distributed over prescribed growth area with downward pressure in the center of a plastic spreader device (recessed side down). The plate was left undisturbed for 1 min to permit gel to solidify. Incubation of the plates was done at 48 ± 3 hrs. at 35 ± 1°C for TVC (Advantec Model C1-310, Toyo- Seisakusho Co., Japan). In the incubator, the plates were placed in horizontal position, clear side up, in stacks not exceeding 20 units. The plates were counted promptly after incubation period. A magnifier-illuminator was used to facilitate counting. Colonies stained in various shades of red were found. All colonies in countable range (30-300 colonies) were counted.

To compute bacterial count, the total numbers of colonies per plate (or average number of colonies per plate for duplicate plates of the same dilution) were multiplied by the reciprocal of the dilution factor used.

During counting, for colonies on duplicate plates of consecutive dilutions, the mean number of colonies was computed for each dilution before determining average bacterial count. Estimated counts were made on plates with > 300 colonies.

The same procedure was used for *Escherichia coli* and coliforms and results were expressed by logarithms of colony forming units (log CFU) per gram of sample (AOAC, 2002). In this case, the plates were incubated for 24 ± 1 hrs. at 35°C followed by the enumeration of the bacterial counts. In the case of *Staphylococcus aureus* the plates were incubated for 24 ± 2 hrs. at 35°C then incubated by Petrifilm™ reactive disk at 35 ± 1°C for 1 hr followed by the enumeration of the bacterial colonies.

Chemical analyses

Determination of pH

Fish fillet (ca. 10g) was homogenized in 100 ml of distilled water, and the mixture was filtered. The pH of the filtrate was measured by a digital pH meter (Orion 3 Star, Thermo Electron Co., USA). Duplicate measurements were taken, and average results were obtained for each fish sample.

Conway's microdiffusion method

TVB-N and TMA-N were determined according to the procedure of Siang and Kim, 1992 by Conway's Microdiffusion Unit.

Sample Extraction: The extract was prepared by mixing 2 g of the ground fish fillet with 8 ml of 4% TCA in a 50 ml beaker and homogenized. It was left for 30 min at ambient temperature with occasional grinding. It was then filtered through a filter paper (Whatman No 41). The filtrate was kept in a Mackertny bottle that was subsequently labeled.

Determination of TVB-N and TMA-N: Two Conway's units which had been thoroughly cleaned with a neutral detergent to remove any containment. To the edge of the outer ring of each unit was applied sealing agent (Vaseline). Using a micropipette, 1 ml of the solution was pipetted into the inner ring of each unit. Into the outer ring of each unit, 1 ml of the sample extract was pipetted. One ml of saturated K_2CO_3 solution was carefully pipetted into the outer ring of each unit carefully, to prevent any from entering the inner ring, and immediately the units were covered and closed with a clip. The solutions in the units were then mixed gently, to prevent any solution in one ring from mixing with the other. The units were placed in an incubator

(Advantec Model C1-310, Toyo- Seisakusho Co., Japan) at 37°C for 60 min. After that, the unit's covers were removed and the inner ring solution, now a green colour, was titrated with 0.02N HCl by a burette (50ml) until the green coloured solution turned pink. An average titrate volume of HCl was determined from the results of two titrations for each fish fillet sample. For each titration the TVB-N values were calculated. A blank test was also carried out using 1 ml of 1% TCA instead of the sample extract.

TMA-N in fish fillet was determined by the Conway's Microdiffusion Technique, which is the same as TVB-N determination but prior to addition of (K_2CO_3), 1ml of 10% neutralized formalin was pipetted to the extract to react with ammonia and thus allow only the TMA-N to diffuse over the unit.

Sensory assessment

The sensory quality of fish fillet was evaluated by six member panelists from the laboratory staff. Panelists scored for colour, odour, texture and general acceptability, using a nine–point hedonic scale (Table 2) as described by Amerine *et al*. (1965). A sensory score of four was taken as the borderline of acceptability.

Table 2. Sensory scores for panel studies

Observation (raw sample)	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Statistical analyses

Two-way analysis of variance (ANOVA) was used to evaluate significant difference within and between factors by the Statistical Analysis System (SAS) software (version 9.1.3, SAS Institute Inc., Cary, North Carolina, USA) and Student's *t*-test, and for sensory assessment having ordinal data the Wilcoxon rank sum test was applied. The Fisher's Least Significant Difference (LSD) procedure was used for multiple comparisons of means after ANOVA with the level of significance fixed at 5%. Pearson's correlation coefficient (r) was used to determine correlations.

Results and Discussion

Bacteriological analyses of the carp fillets during iced storage

In this study, bacteriological analyses were carried out for total viable counts (TVC), *Escherichia coli*, coliforms and *Staphylococcus aureus*. Only TVC were detected while the rest of three remain not detected. Since *Escherichia coli* and coliforms are agents of faecal contamination, they remained undetected probably because the samples were not contaminated by faeces. *Staphylococcus aureus* is an agent of cross contamination and hence it may not have been detected because the samples were not contaminated.

Microbial growth is important factor to shelf life and consequently to consumer acceptance of fresh fish. TVC value of S1 at zero time of storage was significantly higher than those values in S2, S3 and S4 in sequence (Table 3 and Fig. 1). The storage life of fish is affected by the initial microbial load of the fish and the storage temperature (Erkan *et al*., 2006). The low initial TVC counts indicate very good fish quality. TVC is an important criterion for quality evaluation; the maximum recommended bacterial count for good quality products is 5.7 $\log_{10}$ cfu/g, and the

Table 3. Total viable counts (log_{10} cfu/ g) of *Cyprinus carpio* fillet samples during iced storage

		Storage period (days)							
Microorganisms (log_{10} cfu/g)	**Sample Treatment[1]**	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**21**
Total Viable Count (TVC)	**S1**	**3.22±0.11^{aE}**	**3.32±0.00^{aE}**	**4.65±0.02^{aDE}**	**5.67±0.03^{aDE}**	**5.88±0.01^{aD}**	**6.30±0.01^{aC}**	**6.70±0.00^{aB}**	**7.01±0.04^{aA}**
	S2	**2.97±0.10^{abD}**	**2.62±0.04^{bE}**	**2.92±0.32^{bD}**	**4.06±0.00^{bD}**	**5.01±0.03^{aCD}**	**5.14±0.02^{bC}**	**5.62±0.03^{bB}**	**6.04±0.06^{bA}**
	S3	**2.89±0.16^{bD}**	**2.61±0.00^{bE}**	**2.65±0.00^{bDE}**	**2.80±0.01^{bDE}**	**2.85±0.05^{aDE}**	**3.24±0.02^{cC}**	**3.60±0.00^{cB}**	**3.44±0.06^{cA}**
	S4	**2.63±0.12^{bDE}**	**2.29±0.05^{cE}**	**2.65±0.01^{bDE}**	**2.72±0.03^{bD}**	**2.86±0.02^{aD}**	**3.02±0.09^{cC}**	**3.15±0.04 ^{dB}**	**3.24±0.05^{cA}**
***E.coli*/coliforms**	S1	ND	ND	ND	ND	ND	ND	ND	ND
	S2	ND	ND	ND	ND	ND	ND	ND	ND
	S3	ND	ND	ND	ND	ND	ND	ND	ND
	S4	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	S1	ND	ND	ND	ND	ND	ND	ND	ND
	S2	ND	ND	ND	ND	ND	ND	ND	ND
	S3	ND	ND	ND	ND	ND	ND	ND	ND
	S4	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not Detected

[1]See Table 1

Values indicate means ± SD of two replicates.

Different small character superscripts denote significant differences ($p<0.05$) between different treatments in the same storage periods.

Different superscript capital characters means significant differences ($p<0.05$) between different storage periods in the same treatment.

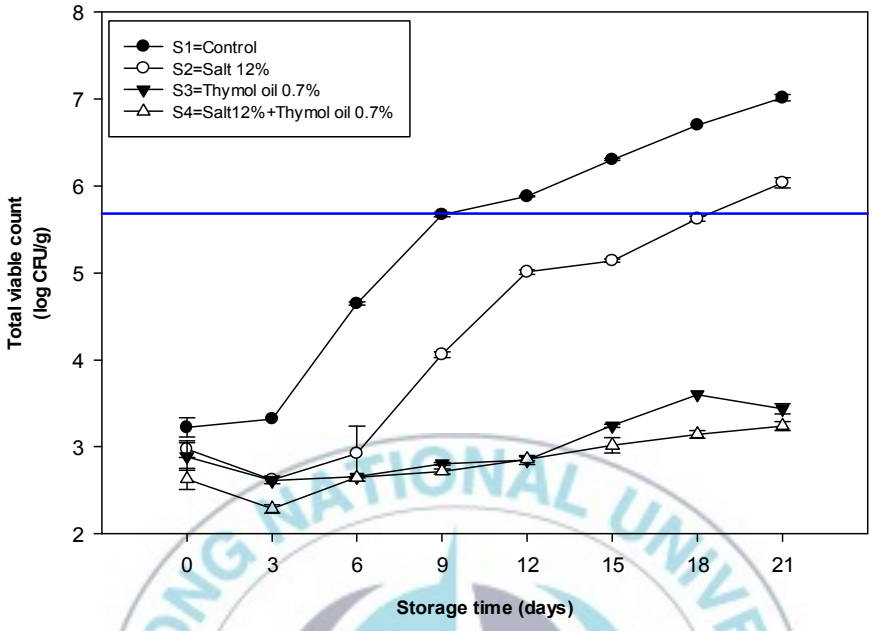

Fig. 1. Changes in total viable counts (TVC) of *Cyprinus carpio* fillet samples during iced storage. The horizontal line shows the good quality acceptability limit of 5.7 $\log_{10}$ cfu/ g.

maximum recommended bacterial count for marginally acceptable quality products is 7 $\log_{10}$ cfu/g (ICMSF, 1986). TVC in all samples, gradually increased during storage period (21 days) (Fig. 1) and the values of TVC at the end were significantly higher than those at the zero day in S1, S2, S3 and S4 consecutively (Table 3). The incremental pattern of TVC in the fillet samples showed the following ascending order; S4< S3< S2< S1.

The increase of TVC in fillets samples during iced storage was demonstrated by Leung *et al*. (1992) and Lyon and Reddmann (2000); it is also in agreement with the results reported by Fan *et al*. (2008) for fresh fillets of silver carp dipped in 0.2% tea polyphenol. In this study, TVC rose continuously and reached the good quality limit on day 9 in control (S1) on day 18 in S2 meanwhile in S3 and S4 on day 21. The lower values in TVC observed in S3 and S4 can be attributed to the inhibitory effect of the salt and thymol oil on growth of spoilage bacteria. The result of treatment with the salt 12% + thymol oil 0.7% (S4) can be considered as most effective inhibitory factor for the growth of spoilage bacteria by extending the shelf life of fillets samples during iced storage. Significant positive correlations were established between TVC and storage periods for different samples

during iced storage; S1 (r= +0.97), S2 (r= +0.96), S3 (r= +0.84) and S4 (r= +0.92). It is well known that the use of salt and essential oil does not allow or delay bacterial occurrence and especially the phenolic components, carvacrol and thymol, are known to exert antimicrobial activity. On the other hand, storage time and temperature have the maximum effect on bacterial occurrence (Burt, 2004). Mejlholm and Dalgaard (2002) showed that the essential oils can cause cells to die by means of increasing the permeability of cytoplasmic membrane of microorganisms. They found that oregano oil reduced the growth of *Photobacterium phosphoreum* and extended the shelf life of cod fillets kept in modified atmosphere packages.

Chytiri *et al*. (2004) and Özlem (2012) stated that on zero day TVC can be in between 3.0 and 4.0 $\log_{10}$ cfu/ g in carp fillets and similar results also were shown by this study. The present results are in agreement with those of Mahmoud *et al*. (2004) who reported that dipping carp fillets in carvacrol/thymol solution (1%) both reduced the initial TVC load and extended the shelf life from 4 days to at least 12 days at 5°C. Furthermore, Harpaz *et al*. (2003) observed that total viable counts of Asian sea bass treated with 0.05% (v/w) oregano and/or thymol oil decreased after 7 days

of storage and rose at the end of the storage period (33 days). Erkan *et al*. (2011) reported that the result of the treatment with 1 % thymol and laurel essential oil was equally effective in inhibiting the spoilage bacterial growth and extending the shelf life of fish samples during iced storage from 9 days to 13 days.

Moreover, Giatrakou *et al*. (2008) reported that the combined use of Modified Atmosphere Packaging (MAP) and oregano oil 0.1% v/w extended the shelf life of swordfish fillets by 8 days, as determined by sensory and microbiological analyses. Frangosa *et al*. (2010) found that the addition of salt extended the product's shelf life by 9 days, whereas the combination of salt, oregano EO (0.2% v/w) under Vacuum Packaging (VP) conditions resulted in a significant shelf life extension in trout fillets, i.e. by approximately 11-12 days, according to sensory data, as compared to the control, kept under aerobic refrigerated conditions.

Chemical analyses of the carp fillets during iced storage

Changes in pH

Changes in pH values of all samples showed a similar trend and decreased over the first two days and increased thereafter (Table 4; Fig. 2).

Table 4. The pH levels of *Cyprinus carpio* fillet samples during iced storage

Storage period (days)	Treatments[1]			
	S1	**S2**	**S3**	**S4**
0	5.96 ± 0.01^{aFG}	5.96 ± 0.01^{aF}	5.96 ± 0.01^{aE}	5.96 ± 0.01^{aD}
3	5.90 ± 0.02^{aG}	5.84 ± 0.01^{aF}	5.54 ± 0.01^{bG}	5.59 ± 0.03^{bF}
6	6.02 ± 0.04^{aF}	5.87 ± 0.01^{bE}	5.55 ± 0.02^{cG}	5.55 ± 0.02^{cG}
9	6.25 ± 0.04^{aE}	6.01 ± 0.02^{bE}	5.86 ± 0.02^{cF}	5.87 ± 0.02^{cE}
12	6.38 ± 0.04^{aD}	6.24 ± 0.02^{bD}	6.17 ± 0.01^{bcD}	6.14 ± 0.01^{cC}
15	6.60 ± 0.03^{aC}	6.40 ± 0.03^{bC}	6.28 ± 0.02^{cC}	6.25 ± 0.01^{cB}
18	6.81 ± 0.02^{aB}	6.47 ± 0.01^{bB}	6.36 ± 0.01^{cB}	6.33 ± 0.01^{cA}
21	7.00 ± 0.07^{aA}	6.69 ± 0.03^{bA}	6.40 ± 0.01^{cA}	6.36 ± 0.01^{cA}

[1]See Table 1

Values indicate means ± SD, of two replicates.

Different small character superscripts denote significant differences ($p<0.05$) between different treatments in the same storage periods.

Different superscript capital characters means significant differences ($p<0.05$) between different storage periods in the same treatment.

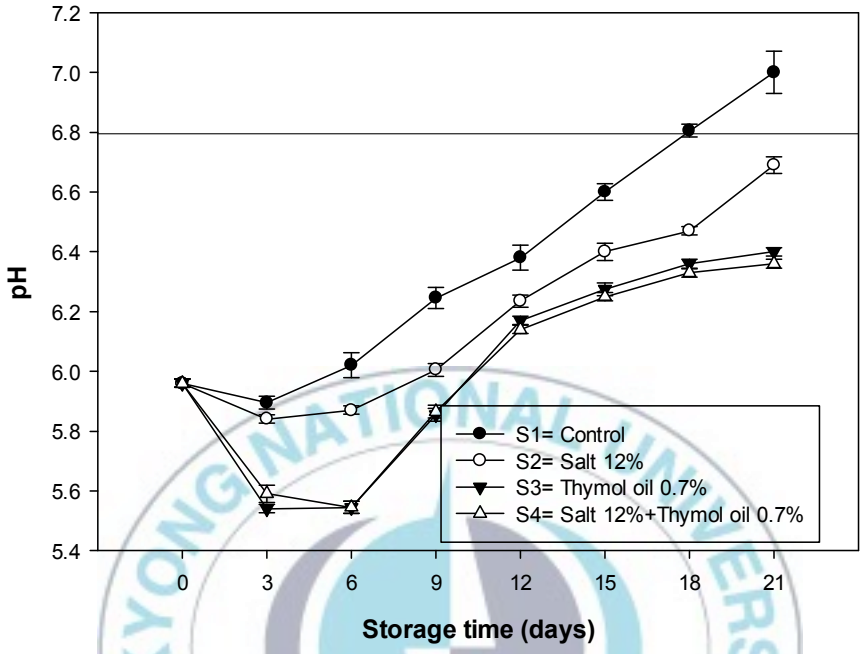

Fig. 2. Changes in pH values of *Cyprinus carpio* fillet samples during iced storage. The horizontal line shows the good quality acceptable limit of 6.8.

Similar trend was observed by Fan *et al.* (2008) in *Hypopthalmicthys molitix* (Silver carp) fillets dipped in 0.2% tea polyphenol; Lannelongue *et al*. (1982); Meekin *et al*. (1982) have reported a decrease in the pH of fillet samples with increase in the concentration of CO_2. The decline in initial pH may be attributed to the dissolution of CO_2 in the fillet samples due to respiratory activities by microbial enzymes.

It is well documented that increasing pH values during storage may be attributed to the production of volatile basic components, such as ammonia, dimethylamine, trimethylamine as well as other biogenic amines as a result of fish spoilage bacterial action by either endogenous or microbial enzymes (Goulas and Kontominas, 2007; Huss, 1995; Hyytia *et al*., 1999; Ludorff and Meyer, 1973; Ruiz-Capillas and Moral, 2001). The pH limit of acceptability is usually 6.8–7.0 for fillet samples (Ludorff and Meyer, 1973).

The data showed that pH values increased in all samples. At the end, pH value of control was significantly higher than that in S1, S2 and S3 in sequences. Meanwhile, pH of S2 is significantly higher than in S3 and S4 in sequence. However, no significant difference was observed in pH of S3 and S4. Furthermore, it can be expected that the lower pH in S3 and S4 can

enhance the microbial inhibition and contributes to the extending of the preservation of fillet samples by inhibiting the activity of the endogenous proteases (Fan *et al*., 2009). Significant positive correlations were established between the pH levels and storage periods: S1 (r= +0.98), S2 (r= +0.94), S3 (r= +0.82) and S4 (r= +0.81). The results of the present study showed that there was a constant increase in pH of all samples throughout the period of iced storage, which agreed with the findings of Indira *et al*. (2010) for *Cyprinus carpio* samples during refrigerated storage.

Changes in total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) increased gradually in all samples during the iced storage (Table 5 and Fig. 3). The results of the experiment showed that there was a constant increase in TVB-N values during iced storage, these results agreed with the TVB-N study by Indira *et al*. (2010)

Table 5. Total volatile basic nitrogen levels of *Cyprinus carpio* fillet samples during iced storage (mg N/100 g wet samples)

Storage period (days)	Treatments[1]			
	S1	**S2**	**S3**	**S4**
0	11.31 ± 0.16^{aF}	11.03 ± 0.08^{abG}	10.64 ± 0.16^{bcH}	10.30 ± 0.32^{cG}
3	18.09 ± 0.24^{aE}	15.90 ± 0.32^{bF}	15.34 ± 0.16^{bcG}	15.12 ± 0.16^{cF}
6	21.17 ± 0.16^{aD}	18.59 ± 0.32^{bE}	16.46 ± 0.16^{cF}	16.91 ± 0.16^{cE}
9	34.94 ± 0.32^{aC}	19.26 ± 0.32^{bE}	17.81 ± 0.16^{cE}	18.26 ± 0.16^{cD}
12	42.78 ± 0.32^{aB}	20.22 ± 0.24^{bD}	18.48 ± 0.16^{cD}	18.70 ± 0.16^{cC}
15	48.47 ± 1.06^{aA}	24.14 ± 0.08^{bC}	19.26 ± 0.32^{cC}	18.98 ± 0.08^{cC}
18	*	27.22 ± 0.48^{aB}	21.39 ± 0.16^{bB}	20.05 ± 0.16^{cB}
21	*	30.38 ± 0.43^{aA}	22.06 ± 0.16^{bA}	21.22 ± 0.08^{bA}

* Not analyzed

[1]See Table 1

Values indicate means ± SD, of two replicates.

Different small character superscripts denote significant differences ($p<0.05$) between different treatments in the same storage periods.

Different superscript capital characters means significant differences ($p<0.05$) between different storage periods in the same treatment.

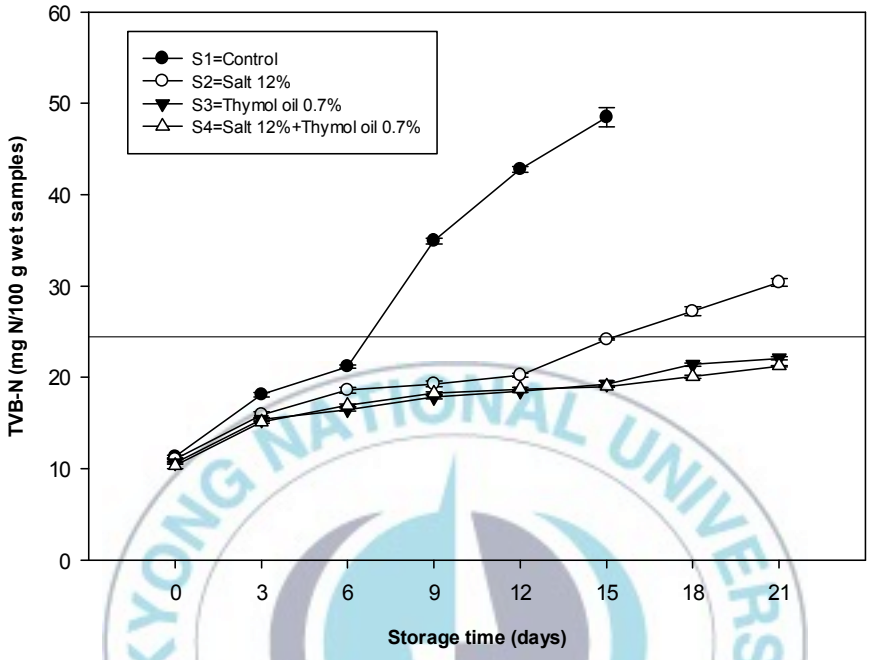

Fig. 3. Changes in total volatile basic nitrogen (TVB-N) of *Cyprinus carpio* fillets during iced storage. The horizontal line shows the limit of acceptability; 25 mg-N/100 g.

for *Cyprinus carpio* during refrigerated storage. Similar results were found by Fan *et al*. (2008) in *Hypopthalmicthys molitix* (Silver carp) fillets dipped in 0.2% tea polyphenol in iced storage, and also by Harpaz *et al*. (2003) in whole sea bass on addition of oregano or thymol oil (0.05% v/v) during storage at 0-2°C. Erkan *et al*. (2011) also reported that TVB-N values in fresh bluefish increased in control after 9 days and in 1% thymol oil and 1% laurel oil after 13 days of iced storage.

TVB-N which is mainly composed of ammonia, and primary, secondary and tertiary amines resulting from degradation of proteins and non-protein nitrogenous compounds, is chiefly caused by microbial activity and endogenous enzymes (Ruiz-Capillas and Moral, 2005). TVB-N is widely used as an indicator for meat deterioration (Olafsdottir *et al*., 1997). Erkan and Ozden (2008) reported that the limit of acceptability for fish fillets was 25 mg TVB-N/100 g. The TVB-N in S1, S2 reached this acceptable limit on day 9, 18 respectively; meanwhile S3 and S4 reached on day 21. Mahmoud *et al*. (2004) reported a TVB-N value of 30 mg N/100g after 12 days of storage at 5°C, after dipping carp fillets in a solution of 0.5% carvacrol and

thymol oil (v/v), whereas the control reached this value after only 4 days. Furthermore, Goulas and Kontominas (2007) and Frangosa *et al*. (2010) reported that sea bream fillets stored under MAP with the addition of salt and oregano oil (0.4%-0.8%, v/w) exceeded the upper acceptability limit for TVB-N proposed by the European Commission of 35 mg N/100g (EEC, 1995) after 29-30 days of storage, thereby extending the shelf life of fresh sea bream by ca. 11-18 days. Giatrakou *et al*. (2008) reported that addition of oregano oil (0.1%, v/w) in filleted swordfish resulted in exceeded TVB-N proposed acceptability limit (20-21 mg N/100g) on day 10 of ice storage. During this study, at the day 15, TVB-N value of control was significantly higher than that in S2, S3, and S4 in sequences. Meanwhile, TVB-N of S2 is significantly higher than in S3 and S4 in sequence. However, no significant difference was observed in TVB-N of thymol oil 0.7% alone (S3) and the combination of salt 12% + thymol oil 0.7% (S4).

This can be attributed to either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds, or both (Banks *et al*., 1980). Significant positive correlations were also established between TVB-N and storage periods for

different samples during iced storage; S1 (r= + 0.99), S2 (r= +0.98), S3 (r= +0.95) and S4 (r= +0.91).

Changes in trimethylamine nitrogen (TMA-N)

Trimethylamine nitrogen (TMA-N) values increased constantly in all samples during the iced storage (Table 6 and Fig. 4). These results are well agreed with work done by Indira *et al*. (2010) on *Cyprinus carpio* samples during refrigerated storage. During this study, on day zero, the TMA-N values of control (S1) was significantly higher than those of S2, S3, S4; meanwhile no significant difference was observed among S2, S3 and S4 respectively. Similar results were found by Fan *et al*. (2008) in *Hypopthalmicthys molitix* (Silver carp) fillets dipped in 0.2% tea polyphenol in iced storage and also Erkan *et al*. (2011) reported that TMA-N value of fresh bluefish increased for control groups after 9 days and in 1% thymol oil and in 1% laurel oil samples after 13 days of iced storage.

Concentration of TMA-N in fish muscle has been used as a more specific index of bacterial spoilage. The volatile amines TMA-N and total volatile basic nitrogen (TVB-N) have been widely proposed as quality indicators in fish since they show a close relationship with the sensory score

Table 6. Trimethylamine-nitrogen levels of *Cyprinus carpio* fillet samples during iced storage (mg N/100 g wet samples)

Storage period (days)	Treatments[1] S1	S2	S3	S4
0	1.68 ± 0.16^{aG}	1.18 ± 0.08^{bG}	1.01 ± 0.16^{bFG}	0.95 ± 0.08^{bD}
3	3.02 ± 0.16^{aFG}	1.06 ± 0.24^{bG}	0.78 ± 0.16^{bcG}	0.50 ± 0.08^{cD}
6	4.48 ± 0.32^{aF}	2.35 ± 0.48^{bF}	1.29 ± 0.08^{cF}	0.73 ± 0.24^{cD}
9	10.33 ± 0.55^{aE}	3.45 ± 0.61^{bE}	1.90 ± 0.16^{cE}	1.12 ± 0.16^{cCD}
12	15.18 ± 1.57^{aD}	6.83 ± 0.61^{bD}	3.30 ± 0.24^{cD}	2.35 ± 0.48^{cBC}
15	20.93 ± 0.89^{aC}	8.18 ± 0.16^{bC}	4.82 ± 0.16^{cC}	3.24 ± 0.63^{dB}
18	24.36 ± 0.55^{aB}	10.08 ± 0.32^{bB}	5.66 ± 0.24^{cB}	4.83 ± 0.13^{cA}
21	31.54 ± 0.79^{aA}	12.04 ± 0.08^{bA}	6.16 ± 0.16^{cA}	5.99 ± 1.41^{cA}

[1]See Table 1

Values indicate means ± SD, of two replicates.

Different small character superscripts denote significant differences ($p<0.05$) between different treatments in the same storage periods.

Different superscript capital characters means significant differences ($p<0.05$) between different storage periods in the same treatment.

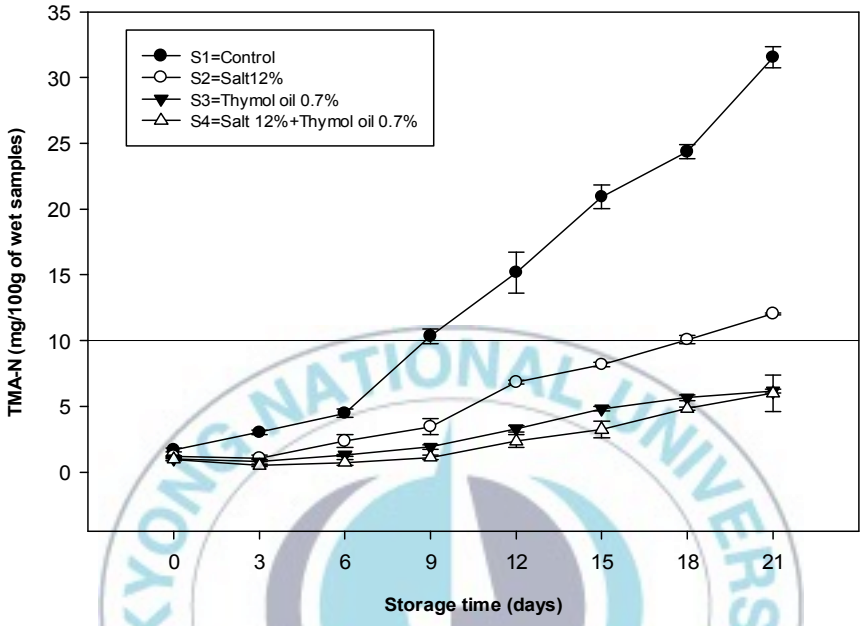

Fig. 4. Changes in the trimethylamine nitrogen (TMA-N) of *Cyprinus carpio* fillet samples during iced storage. The horizontal line shows the limit of acceptability of 10 mg-N/100 g.

(Erkan, 2003). TMA-N is considered a valuable tool in the quality evaluation of fish stored in ice, thanks to its quick accumulation in muscle under chilling conditions. TMA-N is one of the main compounds responsible for the characteristic fishy odour and is produced by the reduction of TMA-O by the trimethylamine reductase enzyme activity of certain bacteria (Huss, 1995). During this study, the control (S1) reached the acceptability limit for human consumption (10-15 mg N/100 g) on day 9 as recommended by Connell (1975), S2 on day 18 while in S3 and S4 by day 21 the values were still acceptable. Significant positive correlations were also established between TMA-N and storage periods for different samples during iced storage; S1 (r= + 0.98), S2 (r= +0.98), S3 (r= +0.97) and S4 (r= +0.93).

Correlations between TVC, pH, TVB-N and TMA-N in carp fillets during iced storage were observed in the samples (Table 7).

Table 7. Correlations between the parameters in *Cyprinus carpio* fillet samples during iced storage

Treatment	**TVC** and **pH**	**TVB-N** and **pH**	**TMA-N** and **pH**	**TVC** and **TVB-N**	**TVC** and **TMA-N**	**TVB-N** and **TMA-N**
S1	+0.93	+0.97	+0.99	+0.96	+0.93	+0.98
S2	+0.97	+0.90	+0.98	+0.90	+0.98	+0.95
S3	+0.90	+0.63	+0.91	+0.69	+0.93	+0.86
S4	+0.91	+0.54	+0.88	+0.72	+0.93	+0.72

Sensory assessment

The acceptability of fish and fishery products during cold storage depends on the changes in their sensory attributes. The sensory qualities of fish samples were evaluated in terms of colour, odour, texture and overall acceptability using a nine-point hedonic scale (Table 2). The fish samples were considered to be acceptable for human consumption until the sensory score reached to score 4 (Amerine *et al*., 1965). The results of sensory assessment of the common carp fillet samples are shown in Table 8 and Figs. 5 and 6. The results indicate that sensory scores showed a decline in all

samples with increasing time of storage period. Results of sensory scores of S3 (thymol oil 0.7%) and S4 (salt 12% + thymol oil 0.7%) were significantly higher than those in S1 (control) and S2 (salt 12%).

It is well known that when fish spoiled, it gives rise to the subsequent development of strongly fishy, rancid and putrid odour, and then the fish are clearly rejected by any scoring panel (Fan *et al*., 2009). Thus, from the data, it is clear that S3 and S4 samples were in an acceptable condition and had good quality characteristics in terms of sensory assessment during the storage period. This decline trend of sensory properties during iced storage is in agreement with the results reported by Fan *et al*. (2008) for fresh fillets of silver carp dipped in 0.2% tea polyphenol.

The shelf life of Asian sea bass (*Lates calcarifer*) treated with 0.05% oregano and/or thymol oil was found to be 33 days at 0–2°C (Harpaz *et al*., 2003). Furthermore, Mahmoud *et al*. (2004) reported that the dipping treatment of carp (*Cyprinus carpio*) fillets in 1% (carvacrol and thymol) mixture extended the shelf-life of the product from 4 to 12 days at 5°C. The

Table 8. Sensory properties of *Cyprinus carpio* fillet samples during iced storage

Storage period (days)	Treatments[1] S1	S2	S3	S4
Colour				
0	9.00^{aA}	9.00^{aA}	9.00^{aA}	9.00^{aA}
3	8.00^{aB}	8.00^{aB}	8.50^{aA}	9.00^{aA}
6	7.50^{aC}	8.00^{aBC}	8.00^{aAB}	8.00^{aAB}
9	7.00^{abCD}	7.50^{aCD}	7.50^{aBC}	8.00^{aBC}
12	4.50^{cE}	6.00^{bE}	6.50^{abCD}	7.00^{aBD}
15	3.00^{cF}	5.00^{bF}	6.00^{aCDE}	7.00^{aBDE}
18	3.00^{dFG}	4.50^{cG}	6.00^{bDEF}	7.00^{aBDF}
21	2.00^{dGH}	3.50^{cGH}	6.00^{bEFG}	7.00^{aDEFG}
Odour				
0	9.00^{aA}	9.00^{aA}	9.00^{aA}	9.00^{aA}
3	8.00^{aA}	8.00^{aA}	9.00^{aA}	9.00^{aA}
6	7.50^{aAB}	7.00^{aAB}	8.00^{aAB}	8.00^{aB}
9	5.50^{bC}	7.00^{aBC}	8.00^{aABC}	8.00^{aABC}
12	3.00^{cD}	7.00^{bBCD}	7.50^{aBCD}	7.50^{aABCD}
15	3.00^{cDE}	5.50^{bDE}	7.50^{aBCDE}	7.50^{aBCDE}
18	2.00^{cDEF}	5.00^{bDEF}	7.00^{aEF}	7.00^{aEF}
21	2.00^{cEFG}	3.00^{bG}	6.00^{aFG}	6.50^{aEFG}
Texture				
0	9.00^{aA}	9.00^{aA}	9.00^{aA}	9.00^{aA}
3	8.00^{aB}	8.50^{aA}	9.00^{aA}	9.00^{aA}
6	7.00^{aB}	7.50^{aBC}	8.00^{aAB}	8.00^{aAB}
9	5.50^{abCD}	7.50^{aABC}	8.00^{aABC}	8.00^{aBC}
12	3.00^{cE}	7.00^{bACD}	7.50^{aABCD}	7.50^{aABCD}
15	3.00^{cEF}	5.50^{bDE}	7.00^{aBCDE}	7.50^{aCDE}
18	2.00^{cEFG}	5.00^{bEF}	7.00^{aEF}	7.00^{aEF}
21	2.00^{dFGH}	3.00^{cG}	6.00^{bFG}	6.50^{aEFG}
Overall acceptability				
0	9.00^{aA}	9.00^{aA}	9.00^{aA}	9.00^{aA}
3	8.00^{aB}	8.50^{aA}	9.00^{aA}	9.00^{aA}
6	7.00^{aC}	7.50^{aB}	7.50^{aB}	7.50^{aA}
9	5.50^{bCD}	7.50^{aABC}	8.00^{aABC}	8.00^{aAB}
12	3.00^{bE}	7.00^{aBCD}	7.50^{aBCD}	7.50^{aABC}
15	3.00^{cEF}	5.50^{bDE}	7.50^{aBCDE}	8.00^{aABCD}
18	2.00^{cEFG}	5.00^{bCEF}	7.00^{aEF}	7.00^{aDE}
21	2.00^{cFGH}	3.00^{bG}	6.00^{aFG}	6.50^{aEF}

[1]See Table 1

Different small character superscripts denote significant differences ($p<0.05$) between different treatments in the same storage periods.

Different superscript capital characters means significant differences ($p<0.05$) between different storage periods in the same treatment.

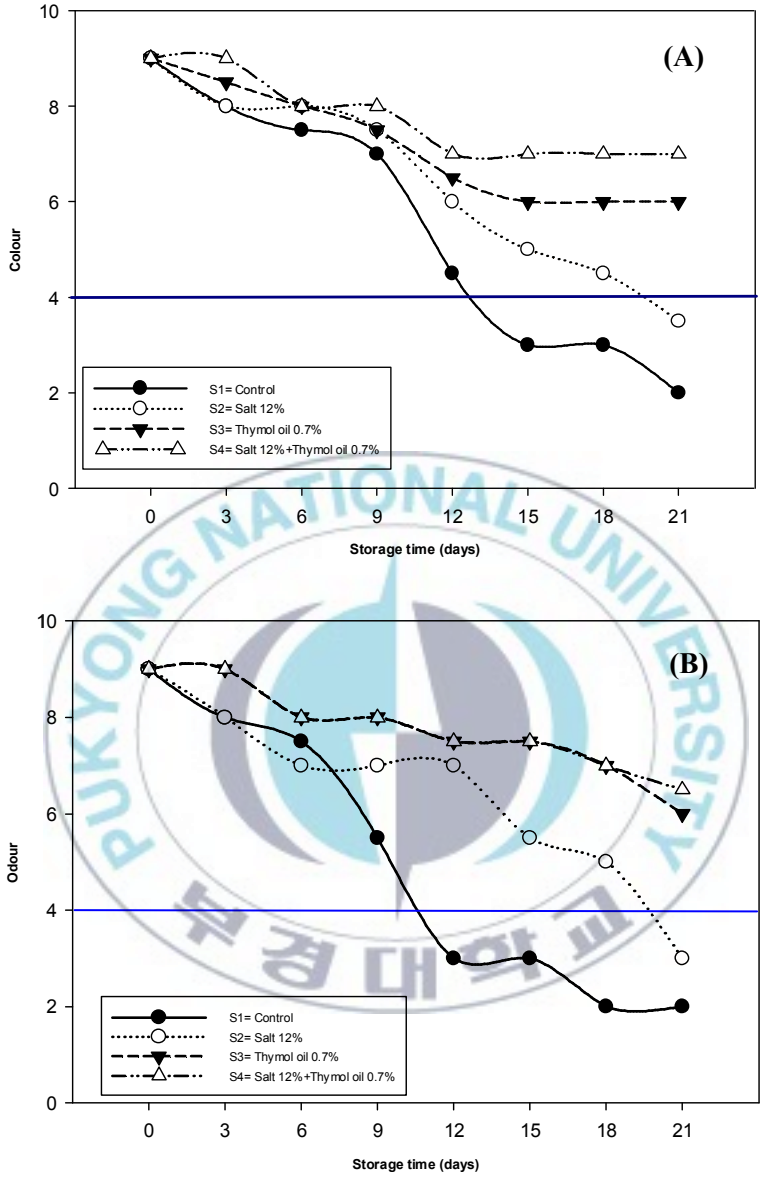

Fig. 5. **(A)** Changes in colour, **(B)** Changes in odour, during sensory analysis of *Cyprinus carpio* fillet samples during iced storage. The horizontal line at score 4 shows the good quality acceptable limit.

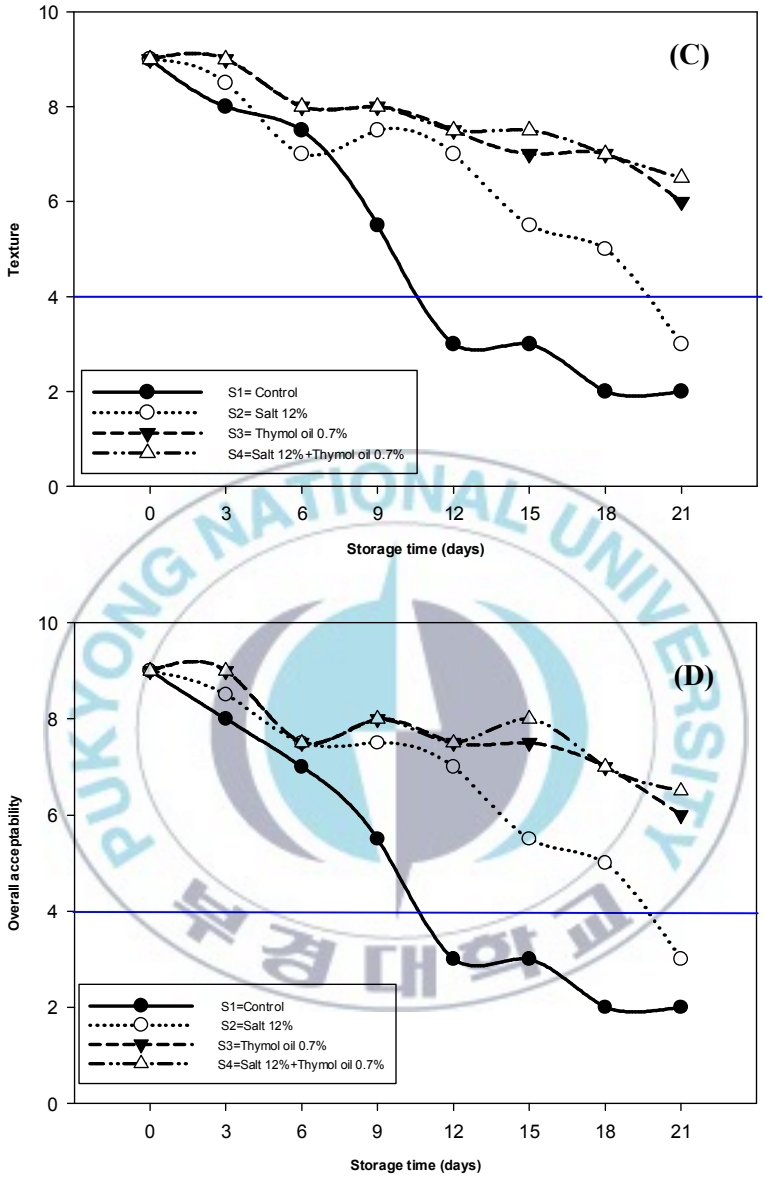

Fig. 6. (C) Changes in texture, **(D)** Changes in overall acceptability during sensory analysis of *Cyprinus carpio* fillet samples during iced storage. The horizontal line at score 4 shows the good quality acceptable limit.

application of MAP-essential oil and electrolyzed NaCl solutions-essential oil has been previously reported to extend the shelf life of sea bream (Goulas and Kontominas, 2007) and carp fillets (Mahmoud *et al*., 2006). Likewise, Erkan *et al*. (2011) reported that the shelf life of fresh bluefish during ice storage was 9 days, and that by adding 1 % thymol and laurel oil extended the products' shelf life up to 3–4 days. The results of microbiological analyses (TVC) and chemical analyses of this study also followed the similar trend as results of sensory evaluation. Frangosa *et al*. (2010) found that the addition of salt extended the products' shelf life by 9 days, whereas the combination of salt, oregano EO (0.2%, v/w) under VP conditions resulted in a significant shelf life extension of trout fillets, i.e. by ca. 11-12 days, according to sensory data, as compared to the control, kept under aerobic refrigerated conditions.

Conclusion

Effects of table salt and thymol oil on the quality and shelf life of the common carp (*Cyprinus carpio*) fillets were investigated by measuring of microbiological, chemical and sensory parameters for 21 days at 3 day intervals. Treatments of S3 (0.7% thymol oil) and S4 (salt 12% + thymol oil 0.7%) on the common carp (*Cyprinus carpio*) fillets led to retention of the good quality characteristics, and the both recipes (S3 and S4) showed similar trends in all parameters (TVC, pH, TMA-N, TVB-N and sensory). Both had an extended shelf life during iced storage of more than 21 days. No significant difference ($p>0.05$) was found among the effectiveness of treatments given to S3 and S4. The synergistic effect of salt and thymol oil in extending the shelf life of the fillets was not significant. Hence, thymol oil can be one of the most important factor for extending the shelf life of *Cyprinus carpio* during iced storage.

Acknowledgements

All praises for Almighty ALLAH, the most beneficent and the most merciful and with His blessings; I am able to complete this thesis.

I would like to thanks and highly appreciate the financial and academic support of Korean International Cooperation Agency (KOICA) and Pukyong National University (PKNU) for enabling my dream to study abroad come true.

My deepest gratitude goes to Professor Ji-Young Yang for his supervision and help to drive this study. My special thanks and appreciation for Professor Yang-Bong Lee for being helpful and available all the time and Professor Chul-Woong Oh, the members of my thesis evaluation committee for guidance and assistance.

My sincere thanks for untiring support and encouragement of Professor Young-Ki Hong, Dr. Kyoung-Mi Kang and all professors and coordinators of KOICA-PKNU International Graduate Programme of Fisheries Science.

I am highly obliged to Professor Sung-Yun Hong for correcting and offering suggestions for improvement to write my thesis.

It would not have been possible to write my dissertation successfully without the help, support and guidelines of my sincere friends Benrick Ogutu, M. Waheed Amiry, Rochana Weerasingha, my labmates and all KOICA-PKNU colleagues.

My hearty gratitude to my beloved parents, Saeed Ahmad and Shahida Saeed, and my sweet sisters who have given me their endless love, unequivocal support and countless prayers which enabled me to reach this success.

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