

The Impact of Carbon-Monoxide Treatment on Biochemical and Sensorial Quality of Tilapia Fillet during Low Temperature Storage

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Abstract Carbon monoxide (CO) has been applied to fish muscle for colour stability and consumer preference during frozen storage and transportation. This study compared the changes in aerobic plate counts (APC), pH, total volatile basic nitrogen (TVB-N), K-value, colour, and sensory analyses between CO-treated and untreated tilapia fillets stored in ice and refrigerated at 5°C up to 14 days. Except for Hunter a* value, the two test fillet groups resembled each other in the freshness quality profiles during storage although the initial levels of APC, pH, and K-value varied slightly. The CO-treated fillets had significantly higher a* values and higher freshness grading scores than that of the untreated fillets. Good relationships among K-value, overall, colour and odour qualities were demonstrated. The shelf life was 8-9 days and 4-5 days stored in ice and refrigerated temperatures, respectively on the basis of sensory characteristics and K-values. The consumers surveyed in this study had a preference for tilapia fillets with enhanced red muscle color; however, it did not bias acceptability.

Keywords: *tilapia fillet, carbon monoxide, muscle colour, freshness, sensory evaluation*

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1. Introduction

Meat purchasing decisions are influenced by colour more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness [1]. The colour and appearance of aquatic food products can also have a major influence on perceived quality [2]. Fish are very susceptible to both microbiological and chemical deterioration. Modified atmosphere packaging (MAP) can effectively inhibit bacterial growth and oxidative reactions, and then extends the shelf-life of fish products [3,4]. In particular, the containing of <0.4% carbon monoxide (CO) as a part of modified atmospheres provides a highly acceptable colour with improved colour life [5,6]. CO reacts with oxy-myoglobin/hemoglobin (oxy-Mb/Hb) to form carboxy-Mb/Hb, being more stable against autoxidation and less prooxidant and peroxidase activities than oxy-Mb/Hb [7].

In recent years the treatment of CO gas or filtered smoke (FS) on fishery products is widely used in order to stabilize or enhance muscle colour [8]. Several studies have reported the effects of post-mortem treatment with CO or FS on fish products such as tuna [9,10,11,12]; mahi mahi [13] and tilapia [14]. Overall, the main advantages of CO or FS treatment compared to untreated controls are: giving significantly enhanced colour (Hunter a* value) on treatment and colour stability after freezing, thawing and subsequent cold storage, lowering aerobic microbial

counts, and extending microbial shelf life. Besides, a more effective technique enabling to provide much higher a* values than CO-gassed fillets by euthanizing live tilapia with CO-saturated water has also been introduced [14,15,16]. However, CO/FS treated products are not approved for commerce in countries other than the United States, where the fish treated with CO or FS must display labeling indicative of that process. As concerned, CO-treated fish muscle is characterized by an abnormal cherry colour contrast to normal muscle color [17]. CO treatment may mask spoilage because the stable cherry-red colour can last beyond the microbiological shelf life of the fillets. High concentrations of CO can restore the red colour of grade C brown tuna to grade B or better and make the week-old mahi mahi to look better than that of fresh one [18].

Many investigations on the use of CO and FS with fish have been reported [8]. But information on the CO enhanced muscle red colour affecting the freshness assessment of fish during refrigerated storage is scanty. Thus the objective of this investigation was to compare the changes in microbial growth, pH, total volatile basic nitrogen (TVB-N), K-value, colour stability, and sensory assessments of CO-treated and untreated tilapia fillets stored in ice and at 5°C for up to 14 days. The relationships between colour measurement, K-value, and sensory results were also examined to evaluate how the CO enhanced muscle colour influenced consumers' acceptance and judgment on freshness of fish fillets.

2. Materials and Methods

2.1. Sample Preparation and Storage

CO-treated and untreated frozen tilapia skinned fillets (average weight: 192 ± 25 g) were collected from a seafood manufacturer in Pintung prefecture in the southern area of Taiwan. All fillets of sashimi grade were produced using cultured tilapia, *Omnireochromis* hybrids as the raw materials. Contrast to untreated fillets, CO-treated fillets had previously been treated with an exposure to about 100% CO for 40-60 min prior to liquid nitrogen freezing. The vacuum-packed specimens were transported in dry ice to the laboratory in the Department of Food Science, National Taiwan Ocean University and stored at -65°C until use. Before use, frozen fillets were thawed in a cold room (4°C). Each of the fillets was placed in a polyethylene bag without seal and then stored in ice for 10-14 days. Four fillets were taken randomly from each treatment at different times, cut with sterilized knife and chopping board into small pieces, and mixed to prepare homogenates as representative samples for microbiological, pH, and chemical analyses.

2.2. Aerobic Plate Count (APC)

Ten grams of fish muscle was blended with 90 ml of 0.85% saline solution, followed by a serial dilution in the same solution. Each diluted sample (1 ml) was dispensed and poured into Plate Count Agar (Difco Co., Detroit, MI) supplemented with 0.5% NaCl. The APC plates were incubated at 35°C for 2 days.

2.3. Measurement of pH and Chemical Analyses

Muscle pH was determined on the homogenates 94 of fish muscle mixed with distill water (1:5, w/v) at room temperature using an automatic pH-meter. For total volatile basic nitrogen (TVB-N) analysis, 5 g of fish muscle was homogenized with 30 ml of 7% cold trichloroacetic acid and centrifuged at 4000 g for 20 min. The supernatant was then used for analysis according to the micro-diffusion method of Conway [19].

Determinations of adenosine triphosphate and its breakdown products including adenosine diphosphate, adenosine monophosphate, inosine monophosphate, inosine, and hypoxanthine were according to the extraction and high performance liquid chromatography (HPLC) procedures described previously [20]. The results were expressed as K-value using the formula [21].

2.4. Colour Measurement

The surface color of fillets was determined by measuring the Hunter tristimulus parameters L^* (lightness), a^* (redness), and b^* (yellowness) values using a colour difference meter (TC-10, Nippon Denshoku Co., Tokyo, Japan). The white standard plate ($X = 90.84$; $Y = 92.60$; $Z = 109.11$) was used for calibration.

2.5. Sensory Evaluation

CO-treated and untreated tilapia fillets with the same storage time were evaluated together using two forms of

sensory analysis: a freshness quality grading test and an acceptance test. The panel consisted of 50-52 graduate students (half females and half males of 23-27 years) and 2 faculty members (males, 50-52 years) from the Department of Food Science at the National Taiwan Ocean University. To conduct freshness quality grading, panelists had previously been trained by introducing fresh and faintly putrid fillets which were prepared from untreated tilapia, to familiarize the differences between acceptable and unacceptable samples. Panelists were asked to examine characteristics such as surface colour, texture, odour, and overall quality. Accordingly, the degree of freshness of each characteristic was graded using a scale of 1-9 (score 9 = very fresh; 5 = borderline; 1 = very stale). Prior to conduct acceptance test, panelists had been prescreened for not dislike sashimi and being normal in sight, smell, and taste sensations. CO-treated and untreated fillets (15-20 g) at the same storage time were offered simultaneously. The panelists were asked to taste samples and to score the appearance in colour, texture, odour, taste and overall acceptance using a 7-point hedonic scale (score 7 = like very much; 5 = neither like nor dislike; 1 = dislike very much).

2.6. Statistical Analysis

Each experiment was repeated two to four times. One-way analysis of variance and *t*-test were performed on data to determine the effect of storage time and significant differences between CO-treated and untreated fillet samples by using the General Linear Models procedure of SAS software and Microsoft Excel. The Linear Fit procedure of ORIGIN software (version 5.0, Microcal Software, Inc., Northampton, MA, USA) was used for correlation analyses between the mean values of chemical and sensory parameters.

3. Results and Discussion

3.1. Changes of APC, pH, TVB-N and K-Value

Changes of APC in the 2 groups of tilapia fillets during storage in ice or refrigerated at 5°C are shown in Figure 1. The initial APC in CO-treated fillets (2.92 ± 0.08 log CFU/g) was lower ($p < 0.05$) than that in the untreated fillets (3.44 ± 0.25 log CFU/g). After 14 days of iced storage, APC reached 5.17 ± 0.55 and 5.97 ± 0.68 log CFU/g in CO-treated and untreated fillets, respectively. The CO-treated tilapia fillets had lower ($p < 0.05$) microbial proliferation with 7-10 storage days. Other researchers [22] demonstrated similar microbial growth trend of CO-treated and untreated tilapia fillets in ice storage at the period of day 6 to 15. The APC increased rapidly during storage at 5°C and on day 5 reached 6.38 ± 0.07 and 6.83 ± 0.19 log CFU/g in CO-treated and untreated fillets, respectively. The results agreed with research on FS-treated yellowfin tuna [23] and CO-treated tilapia [22,24] during storage at refrigerated 5°C . When the APC reaches 10^6 CFU/g in a food product, it is assumed to be at, or near, spoilage. According to the microbial safety criterion, the shelf life for CO-treated and untreated fillets was about 14 days in ice and 4 days at 5°C .

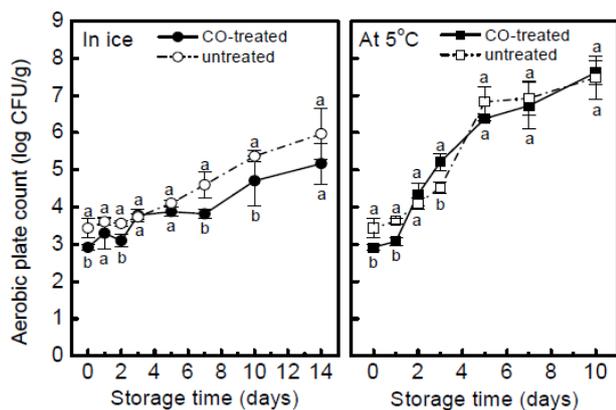


Figure 1. Changes of aerobic plate count in CO-treated and untreated tilapia fillets during storage in ice and at 5°C. Data are mean \pm SD ($n = 3$). Means within the same day not sharing the same letter differ significantly ($p < 0.05$).

The initial pH of CO-treated and untreated fillets was 6.36 ± 0.04 and 6.58 ± 0.07 , respectively (Figure 2). The pH values changed little during iced storage and within 5 days of storage at 5°C. The initial TVB-N levels (11.4–11.7 mg/100 g) were almost the same between the 2 groups of fillets (Figure 3). The TVB-N increased slowly to a range of 12–18 mg/100 g during storage at both conditions except that CO-treated and the untreated fillets sharply increased to 64.2 and 88.2 mg/100 g after 10 days of storage at 5°C, respectively. On the contrary, CO-treated tilapia fillets contained higher TVB-N levels throughout refrigerated and iced storage [22]. The freshness of tilapia fillets 165 stored at $2 \pm 2^\circ\text{C}$ was rejected on the 12th day, with a TVB-N level of 19.5 mg/100 g [25]. Different temperatures and atmospheric conditions (under air and modified atmosphere packaging, MAP) affected both the shelf-life and spoilage potential of bacteria as well as composition profile of the volatile organic compound. *Pseudomonas* spp. was the most abundant spoilage microorganism; however, growth of *Brochothrix thermosphacta* and Lactic Acid Bacteria (LAB) were favoured under MAP compared to air during storage of sea bream fillets at 0, 5 and 15°C [26]. The potential chemical spoilage index of sea bream fillets was suggested to be 3-methylbutanal, acetic acid, ethanol and the ethyl esters, which as microbial origin. TVB-N was not recommended to as an indicator because its value never reached concentration of legislation limit during storage [26].

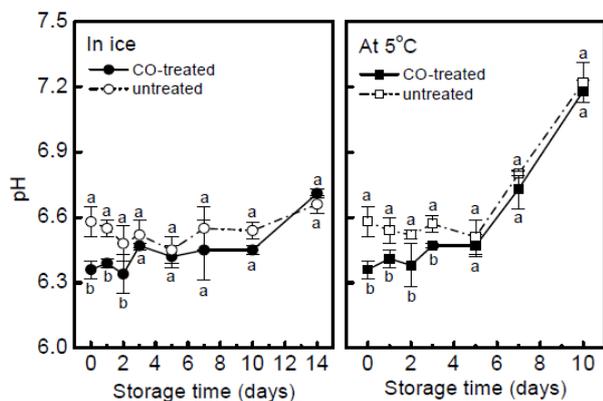


Figure 2. Changes of pH in CO-treated and untreated tilapia fillets during storage in ice and at 5°C. Data are mean \pm SD ($n = 3$). Means within the same day not sharing the same letter differ significantly ($p < 0.05$).

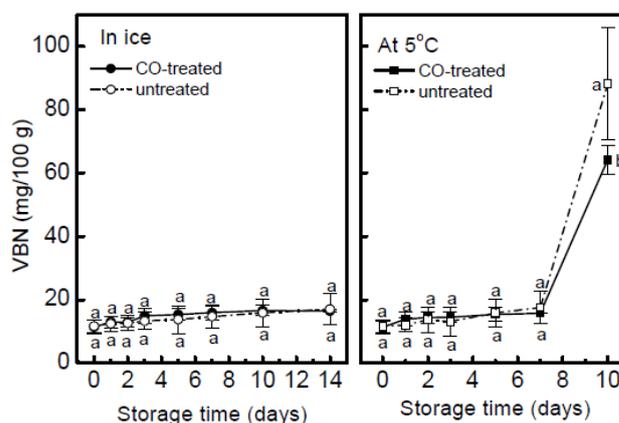


Figure 3. Changes of total volatile basic nitrogen (TVB-N) in CO-treated and untreated tilapia fillets during storage in ice and at 5°C. Data are mean \pm SD ($n = 2$). Means within the same day not sharing the same letter differ significantly ($p < 0.05$).

The bacteria predominately involved in shrimp spoilage were *B. thermosphacta*, *Serratia liquefaciens*-like and *Carnobacterium maltaromaticum* whose main characteristic odours were cheese-sour, cabbage-amine and cheese-sour-butter, respectively [27]. The volatile compounds were as 3-methyl-1-butanol, 2,3-butanedione, 2-methyl-1-butanol, 2,3-heptanedione and trimethylamine. *C. divergens* inoculation into cooked and peeled shrimps exhibited a maximum production rate of TVB-N ranging from 80 to 90 mg-N 100 g⁻¹. All the six spoilage microorganisms inoculation exhibited a very low TMA production, less than 10 mg-N 100 g⁻¹ [27]. The dominant bacteria were *C. maltaromaticum*, *Hafnia alvei* and *Photobacterium phosphoreum* from spoiled raw salmon fillets under MAP storage at 8°C. The dominant odour created by *P. phosphoreum* inoculation were TMA-amine and acetic acid-sour which was suggested as a raw salmon spoilage marker. TVB-N and TMA-N were observed during storage with a maximal level of 38 and 21 mg-N 100 g⁻¹, respectively [28].

LAB and *B. thermosphacta* were co-dominant with predominant *Pseudomonas* spp. and H₂S producing bacteria of gutted European sea bass under MAP storage at 2°C. TVB-N and TMA-N were increased largely only at the late stages of storage or after rejection of the products, making them poor indicators for freshness/spoilage. The level of microbial origin volatile organic compounds such as ethanol, 2-ethyl-1-hexanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 3-methylbutanal, 2-methylbutanal and some ethyl esters increased during storage [29].

The initial K-value in CO-treated fillets (12.3%) was somewhat higher than that in untreated fillet (7.0%). K-values increased linearly with storage times to 62–64% and 82–86% at the end of storage in ice and refrigerated at 5°C, respectively (Figure 4). High correlations ($R^2 \geq 0.98$) between K-value and storage time were observed independent of samples (Supplementary information), suggesting that K-value is a good freshness indicator for tilapia fillets stored at refrigerated temperatures. On the other hand, the shelf life of tilapia fillets packed in high-barrier film under modified atmospheres, indicated that K-values were independent of spoilage because the packed fillets that had a high K-value (93–95%) were still judged by sensory characteristics to be acceptable [30,31].

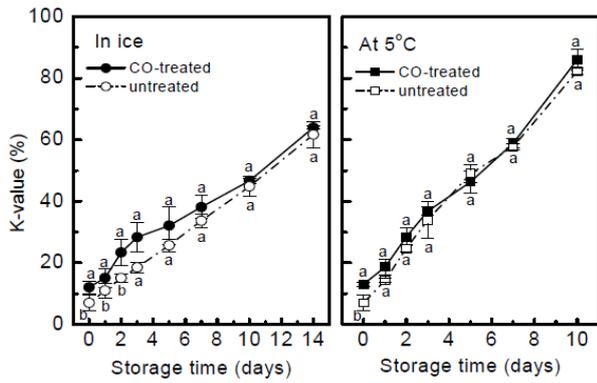


Figure 4. Changes of K-value in CO-treated and untreated tilapia fillets during storage in ice and at 5°C. Data are mean ± SD (n = 2). Means within the same day not sharing the same letter differ significantly (p < 0.05)

Table 1. Hunter tristimulus colour values of CO-treated and untreated tilapia fillets during storage in ice and at 5°C^a

Days		L*	a*	b*
<i>In ice</i>				
0	CO	29.0 ± 0.5a	16.2 ± 0.6a	7.8 ± 0.5a
	UN	24.8 ± 2.5b	8.9 ± 2.0b	7.1 ± 0.3b
1	CO	29.6 ± 1.1a	14.0 ± 1.3a	8.1 ± 0.5a
	UN	25.7 ± 1.8b	7.3 ± 2.3b	8.3 ± 1.5a
2	CO	30.5 ± 1.7a	12.0 ± 1.5a	8.1 ± 0.4a
	UN	26.5 ± 1.4b	5.8 ± 0.7b	7.8 ± 1.4a
3	CO	32.9 ± 1.5a	9.7 ± 0.7a	8.1 ± 0.4a
	UN	30.4 ± 3.3a	5.4 ± 0.6b	9.0 ± 1.3a
5	CO	35.5 ± 2.1a	7.1 ± 0.5a	8.0 ± 0.6a
	UN	32.6 ± 2.8b	3.9 ± 0.2b	8.2 ± 1.1a
7	CO	36.2 ± 2.2a	5.1 ± 0.4a	8.1 ± 0.7a
	UN	32.3 ± 1.5b	3.6 ± 0.5b	8.3 ± 0.9a
10	CO	37.4 ± 1.7a	3.7 ± 2.1a	8.3 ± 0.8a
	UN	33.0 ± 0.8b	3.0 ± 0.8a	8.3 ± 0.7a
14	CO	38.5 ± 1.1a	1.1 ± 0.8a	8.8 ± 0.8a
	UN	37.6 ± 2.4a	1.4 ± 0.5a	9.3 ± 1.0a
<i>At 5°C</i>				
0	CO	29.0 ± 0.5a	16.2 ± 0.6a	7.8 ± 0.5a
	UN	24.8 ± 2.5b	8.9 ± 2.0b	7.1 ± 0.3b
1	CO	29.7 ± 0.9a	13.0 ± 0.7a	7.9 ± 0.2a
	UN	28.0 ± 2.6a	6.2 ± 1.9b	9.1 ± 1.3a
2	CO	31.7 ± 0.5a	11.5 ± 0.4a	8.1 ± 0.5a
	UN	28.6 ± 2.0b	3.5 ± 0.6b	8.8 ± 1.3a
3	CO	35.7 ± 0.9a	8.5 ± 0.8a	7.3 ± 1.0a
	UN	30.1 ± 2.0b	2.8 ± 0.4b	8.6 ± 0.8a
5	CO	37.5 ± 1.0a	5.0 ± 1.3a	8.2 ± 1.5a
	UN	31.3 ± 1.8b	2.0 ± 0.3b	9.1 ± 0.8a
7	CO	37.4 ± 0.5a	3.2 ± 1.5a	7.9 ± 1.1a
	UN	37.4 ± 2.6b	1.5 ± 1.1b	8.6 ± 0.7a
10	CO	39.8 ± 4.5a	3.7 ± 2.0a	7.9 ± 1.2a
	UN	39.2 ± 0.3a	1.8 ± 1.3b	8.5 ± 0.9a

3.2. Changes of Colour

The initial a* value in untreated and CO-treated fillets was 8.9 and 16.2, respectively (Table 1). Fresh CO-treated fillets also had a higher L* value. All a* values declined immediately after storage. In comparison to the untreated 211 fillets, CO-treated fillets within 7 days of storage in both conditions still had higher (p < 0.05) a* values. The L* values tended to increase slowly with storage time, whereas b* values staged unchanged. The increase in L* value might be due to loss in wetness on muscle surface because the fillets stored for long times were lack of sheen.

The L* values of CO-treated tilapia fillets increased during all storage temperatures were also observed [22]. The b* values increased by previous CO-treated tilapia fillets in 0°C storage possibly due to oxidation of heme protein [22], which was not seen in this study. The increase of a* values in this study was in agreement with tuna [9,10,12,20]; mahi mahi [13]; Spanish mackerel [25] and tilapia [14,16,22]. Unexpectedly, a more rapid decline in a* value during storage in 0°C for CO-treated tilapia than untreated fillets was demonstrated [22]. This might be caused by the speedy oxidation of the heme protein upon thawing, especially for previously frozen fish under 100% CO-treatment [32]. The difference of a* and b* value variances under CO-treatment needs to be further elucidated.

As shown in Table 2, surface colour, odour, texture, and overall quality scores in CO-treated and untreated fillets decreased gradually with storage time. Under the same storage time, the colour, texture, and overall quality of CO-treated fillets were scored much higher (p < 0.05) than that of untreated fillets with the exception of no significant difference (p > 0.05) in odour scores. Thus it might imply that muscle colour was the determinant attribute for assessment of freshness of tilapia fillets, because CO-treated fillets were characterized with higher a* values as compared to untreated fillets. Table 2 also shows the changes in attribute scores by using an acceptance test. Colour, odour, texture, taste, and overall acceptability scores decreased with storage time in ice and at 5°C. Inconsistent with previous findings, only the colour and 234 overall acceptability scores of CO-treated fillets stored within 5 days in ice or refrigerated for 2-3 days at 5°C were significantly higher (p < 0.05) than those of the untreated fillets. In contrast, there were no significant differences (p > 0.05) in scores of odour, texture, and taste between samples before and after storage. The data evidenced again that CO-treated fillets were preferable to untreated fillets was due to enhanced muscle colour. However, the CO-treated tilapia failed sensory assessment than untreated products when stored refrigerated and in ice [22], which was opposite this study. This difference might be principally due to the colour (a* value) preference. A study investigated whether consumer preference for beef colours influenced taste scores of beef steaks and patties, indicated that despite the effects of colour on appearance and likelihood to purchase, colour did not affect taste scores [33].

3.3. Relationships among K-value, Colour, and Sensory Scores

In the present study most data from APC, pH, TVB-N and K-value suggested that under the same storage time, CO-treated and untreated fillets resembled each other in quality profile with the exception of the big differences in a* value (muscle colour). Changes of adenosine triphosphate and its breakdown products during storage were shown in supplementary information. K-values increased linearly over time and its increases were not affected by muscle colour. Figure 5 shows the high correlation of a* value with log₁₀ K-value, but slopes varied depending on the kind of samples. The initial a* value in untreated fillets averaged 8.9. Due to initial high a* value (16.2), the decrease of a* value to 8.9 in CO-

treated fillets corresponded to the samples having been stored for about 4 days in ice or 2.5 days at 5°C. As concerned by Schubring (2008), the main drawback of the use of CO treatment in fishery products is leading to mask

deterioration after storage and thus to mislead consumers into thinking that it is fresher or higher value than it actually is [8].

Table 2. Sensory scores for freshness grading test and acceptance test of CO-treated (CO) and untreated (UN) tilapia fillets during storage in ice and at 5°C^a

Days		Freshness grading test				Acceptance test				
		color	texture	odor	overall	color	texture	odor	taste	overall
<i>In ice</i>										
0	CO	7.2a	6.7a	6.2a	6.7a	5.8a	5.4a	5.2a	5.4a	5.6a
	UN	6.0b	5.9b	5.9a	5.8b	4.9b	5.2a	5.2a	5.2a	5.1a
1	CO	7.3a	6.7a	6.1a	6.8a	5.7a	5.3a	5.1a	5.2a	5.4a
	UN	5.6b	5.6b	5.7a	5.6b	4.7b	5.0a	5.0a	5.0a	5.0a
2	CO	7.3a	6.6a	5.9a	6.7a	5.4a	4.9a	4.9a	4.9a	5.2a
	UN	5.4b	5.5b	5.7a	5.4b	4.2b	4.5a	4.5a	4.7a	4.6a
3	CO	7.0a	6.5a	5.9a	6.5a	5.2a	4.6a	4.5a	4.5a	4.9a
	UN	5.1b	5.2b	5.4a	5.2b	4.1b	4.5a	4.5a	4.4a	4.4a
5	CO	6.2a	6.0a	5.6a	6.1a	4.7a	4.4a	4.4a	4.4a	4.6a
	UN	4.6b	5.1b	5.3a	4.8b	4.1b	4.2a	4.2a	4.3a	4.1a
7	CO	6.1a	5.8a	5.6a	5.8a	4.1a	4.2a	4.3a	4.1a	4.2a
	UN	4.0b	5.0b	5.3a	4.7b	3.9a	4.1a	4.1a	4.1a	4.0a
10	CO	5.9a	5.5a	5.4a	5.6a	3.9a	3.9a	3.9a	3.8a	3.9a
	UN	3.9b	4.8b	5.1a	4.5b	3.6a	4.0a	4.0a	3.7a	3.7a
14	CO	5.2a	5.2a	4.9a	5.0a	–b	–	–	–	–
	UN	3.6b	4.4b	4.6a	4.2b	–	–	–	–	–
<i>At 5°C</i>										
0	CO	7.2a	6.7a	6.4a	6.7a	5.8a	5.4a	5.2a	5.4a	5.6a
	UN	6.0b	5.9b	5.9a	5.8b	4.9b	5.2a	5.2a	5.2a	5.1a
1	CO	6.9a	6.2a	5.7a	6.2a	5.1a	4.9a	4.8a	4.8a	4.9a
	UN	5.1b	5.5b	5.7a	5.3b	4.2b	4.7a	4.7a	4.6a	4.5a
2	CO	6.4a	5.9a	5.6a	6.1a	4.8a	4.6a	4.6a	4.4a	4.6a
	UN	4.3b	4.8b	5.3a	4.8b	4.1b	4.5a	4.4a	4.4a	4.2a
3	CO	6.1a	5.8a	5.2a	5.8a	4.3a	4.6a	4.2a	4.3a	4.3a
	UN	3.7b	4.7b	5.1a	4.2b	3.8b	4.4a	4.1a	4.1a	4.2a
5	CO	5.8a	5.3a	5.0a	5.3a	3.8a	4.0a	3.9a	4.1a	3.9a
	UN	3.5b	4.5b	4.7a	4.0b	3.5b	4.0a	3.8a	3.8a	3.7a
7	CO	5.1a	5.1a	3.6a	4.2a	–	–	–	–	–
	UN	2.8b	3.1b	3.4a	3.2b	–	–	–	–	–

^aData are mean values of 52-54 panelists. For freshness grading test, a 9-point scale was used: score 9 = extremely good; 5 = limit of reject to purchase; 1 = extremely bad. For acceptance test, a 7-point hedonic scale was used: score 7 = like very much; 4 = neither like nor dislike; 1 = dislike very much. Means in the same storage time sharing different letters (a,b) are significantly different ($p < 0.05$).

^bNot determined.

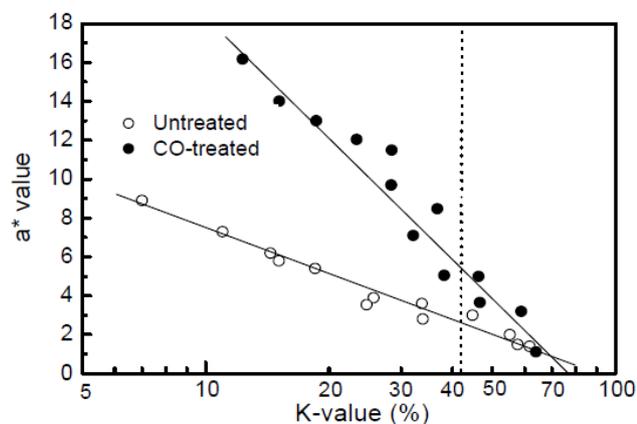


Figure 5. Log10 K-value correlation with Hunter a* value. Regression coefficients are $-0.97 \sim -0.99$

Good correlations of K-value with sensory results (e.g., $r = -0.93 \sim -0.97$ for overall and color quality/acceptability scores) were observed. Under the same K-value, however, the corresponding overall and colour quality/acceptability scores of CO-treated fillets

were much higher than that of untreated fillets as a consequence of initially enhanced a* value. Thus it can be said that after storage the CO-treated fillets looked top-quality by virtue of intensively red muscle colour, but it actually was not. Since K-value correlated well with odour quality and acceptability scores ($r = -0.85 \sim -0.99$) and the scoring on odour quality and acceptability was independent of sample treatments, the data on K-value (X) from both test fillets were thus combined to predict shelf life (Y, days) by linear equation: for odour quality score (X), $Y = 6.1178 - 0.02226X$; for odour acceptability score (X), $Y = 5.3058 - 0.03045X$. When odour quality and acceptability scores reached the borderlines of rejection, the time corresponded to the samples having a K-value of 50.2% and 42.9%, respectively (Supplementary information). Lower K-value limit obtained from odour acceptability score indicated the fact that tasting sample was more sensitive in odour detection. When K-value of 42.9% was the acceptable limit for freshness quality of tilapia fillets, the shelf life was 8-9 days of storage in ice and 4-5 days refrigerated at 5°C. The shelf life of tilapia fillets packaged in 100% air and stored at 4°C was 9-13

days on the basis of sensory characteristics, whereas the K-value reached 62.2% on day 9 [30,31].

4. Conclusion

This study showed that except for muscle colour (a* value) frozen-thawed tilapia fillets, which had previously been treated or untreated with CO, had similar quality profiles during storage in ice or at 5°C. The increased a* values led CO-treated tilapia fillets being graded a much better freshness quality by colour than untreated fillets. CO-treated tilapia fillets demonstrated a masking effect on the consumers' preference due to redness muscle colour. The consumers surveyed in this study had a preference for the tilapia fillets with enhanced muscle colour. However, preference for muscle colour did not bias taste, odour, and texture acceptability.

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Supplementary

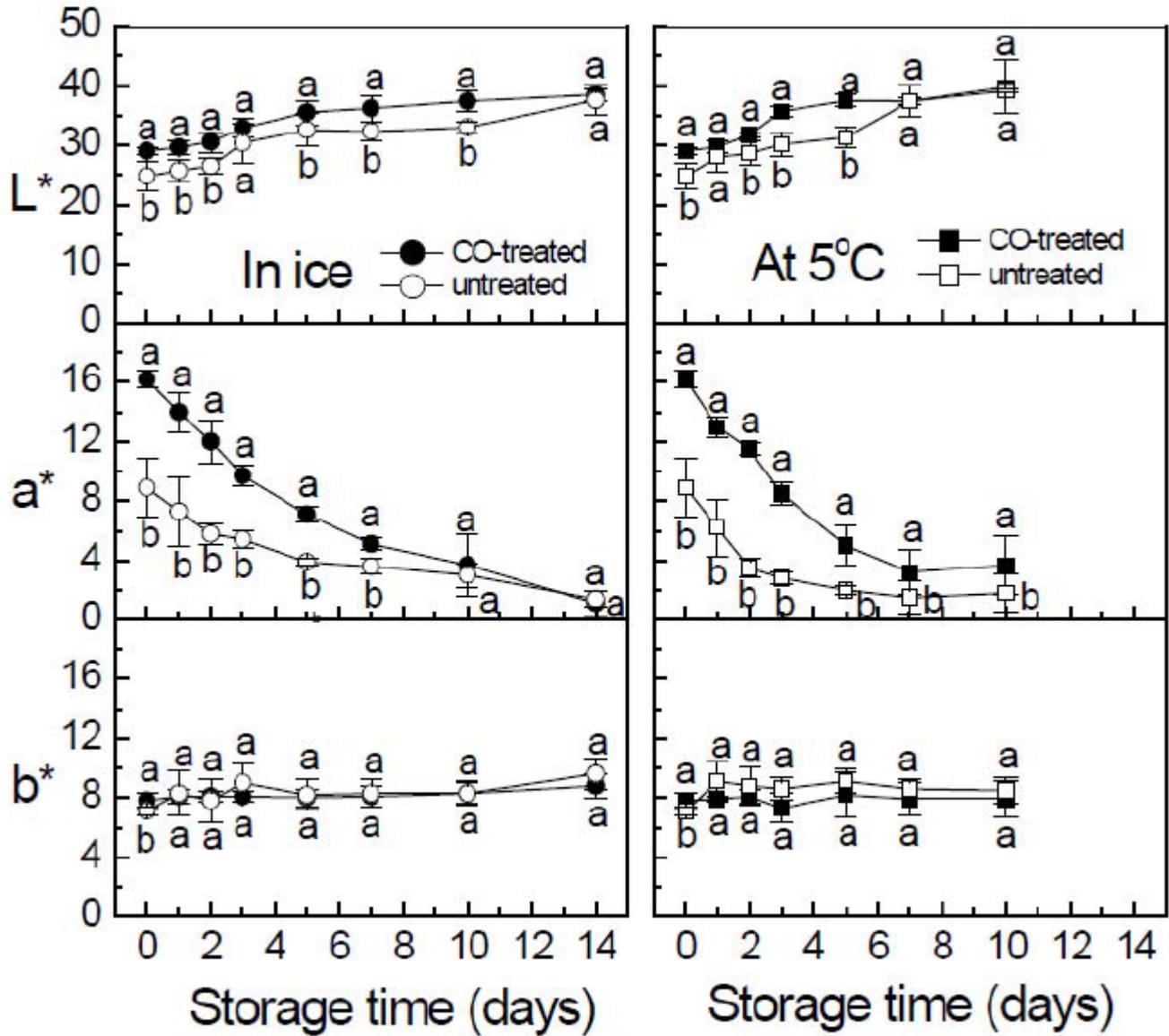


Figure 1. Hunter tristimulus color values of CO-treated and untreated frozen-thawed tilapia fillets during storage in ice and at 5°C. Data are mean ± SD (n= 4). Means in the same storage time sharing different letters (a,b) are significantly different (P < 0.05)

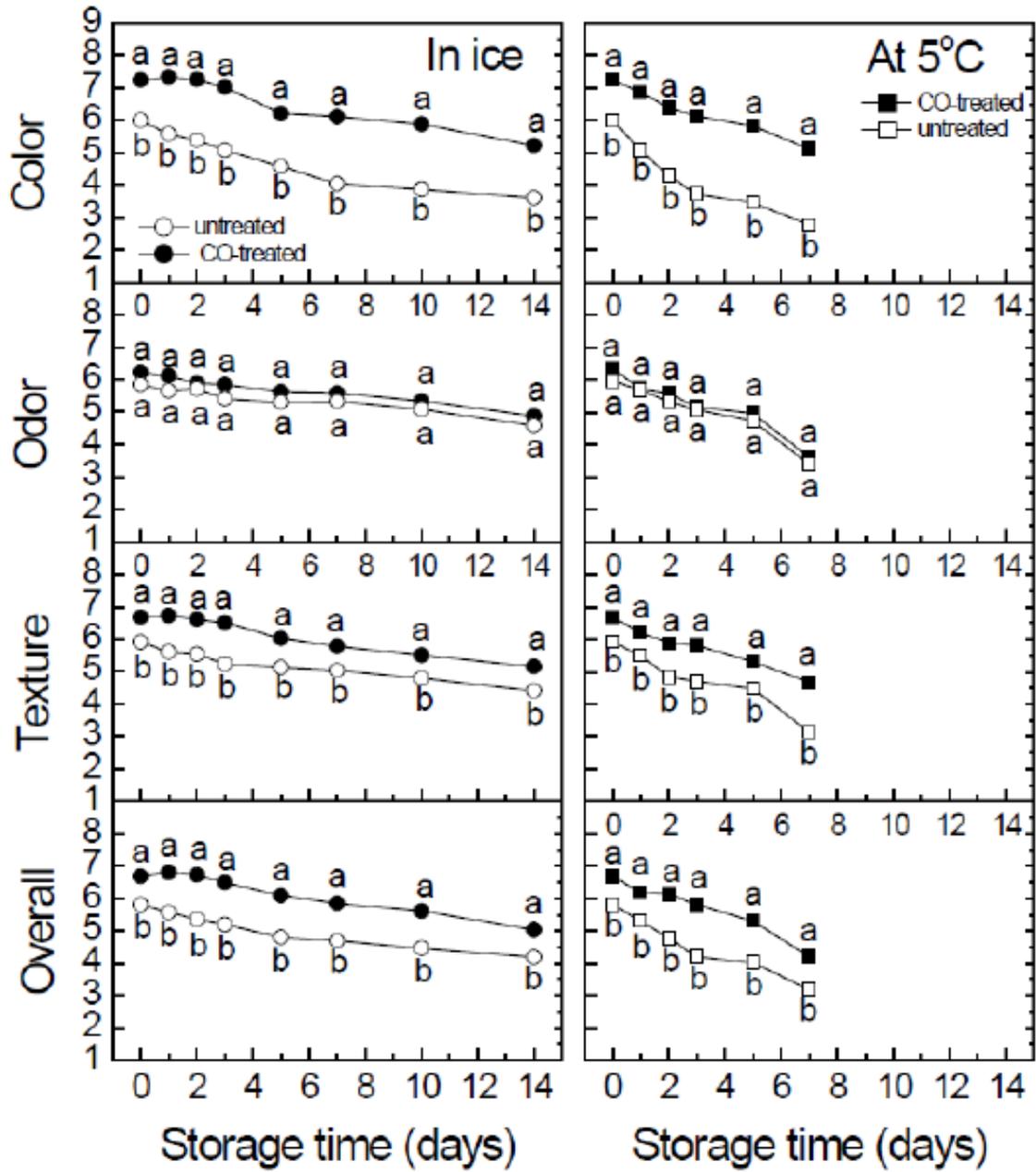


Figure 2. Sensory scores for freshness grading test and acceptance test of CO-treated (CO) and untreated (UN) frozen-thawed tilapia fillets during storage in ice and at 5°C. Data are mean values of 52-54 panelists. For freshness grading test, a 9-point scale was used: score 9 = extremely good; 5 = limit of reject to purchase; 1 = extremely bad. Means in the same storage time sharing different letters (a,b) are significantly different ($P < 0.05$)

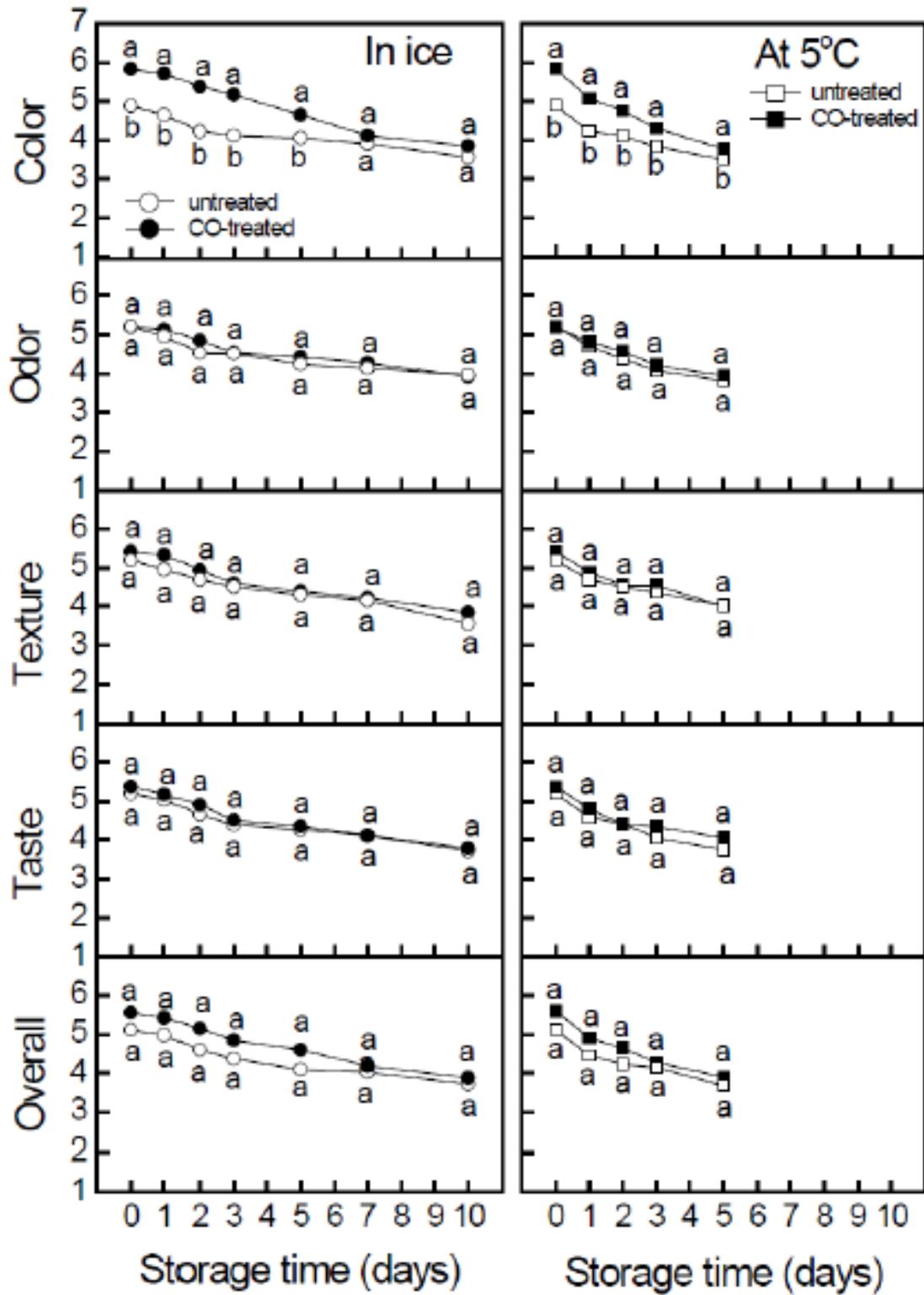


Figure 3. Sensory scores for freshness grading test and acceptance test of CO-treated (CO) and untreated (UN) frozen-thawed tilapia fillets during storage in ice and at 5°C. Data are mean values of 52-54 panelists. For acceptance test, a 7-point hedonic scale was used: score 7 = like very much; 4 = neither like nor dislike; 1 = dislike very much. Means in the same storage time sharing different letters (a,b) are significantly different ($P < 0.05$)

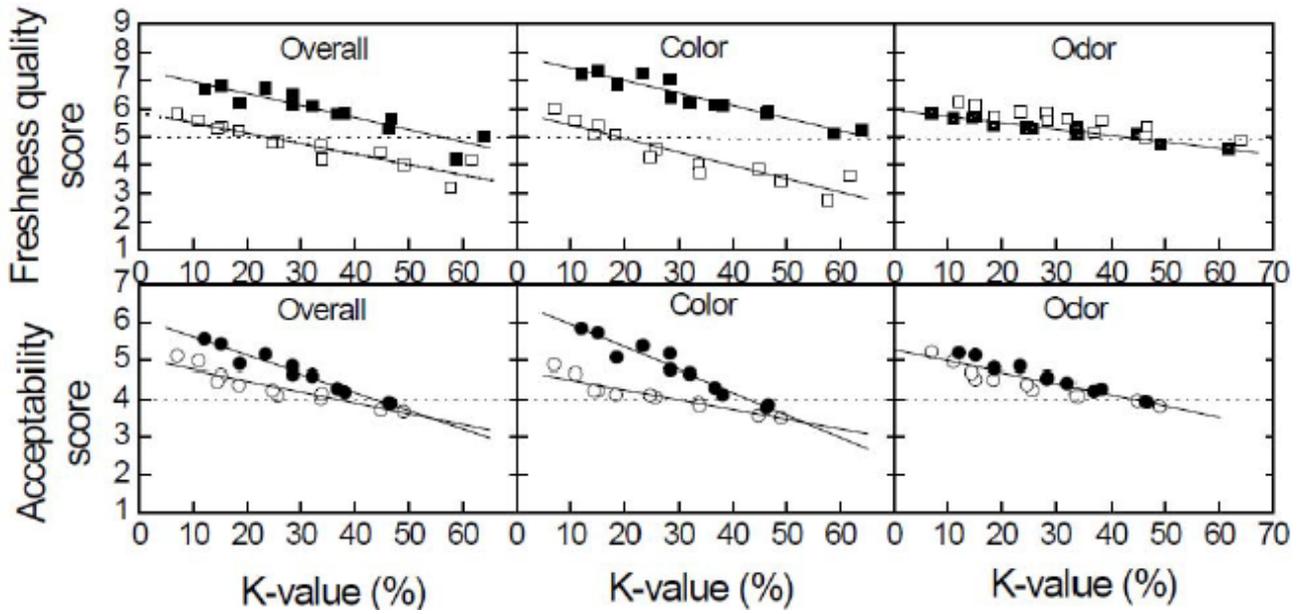


Figure 4. K-value correlation with overall, color and odor quality scores (upper), and with overall, color and odor acceptability scores (lower). Regression coefficients were $-0.94 \sim -0.98$ for overall freshness quality and acceptability scores, $-0.93 \sim -0.97$ for color quality and color acceptability scores, and $-0.83 \sim -0.91$ for odor quality and odor acceptability scores, respectively

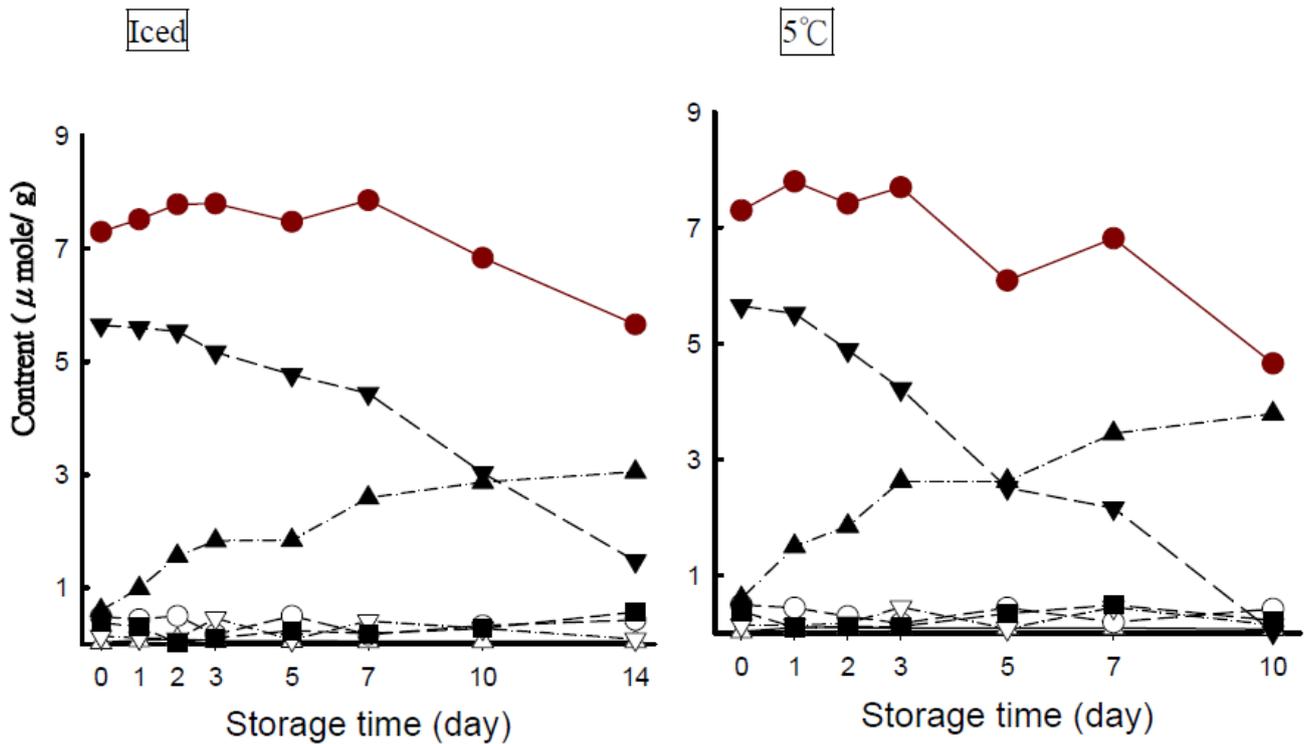


Figure 5. Changes in level of ATP and its related compounds in CO treated tilapia muscle during storage at different temperatures. Δ : ATP, \circ : ADP, ∇ : AMP, \blacktriangledown : IMP, \blacksquare : inosine, \blacktriangle : hypoxanthine, \bullet : total

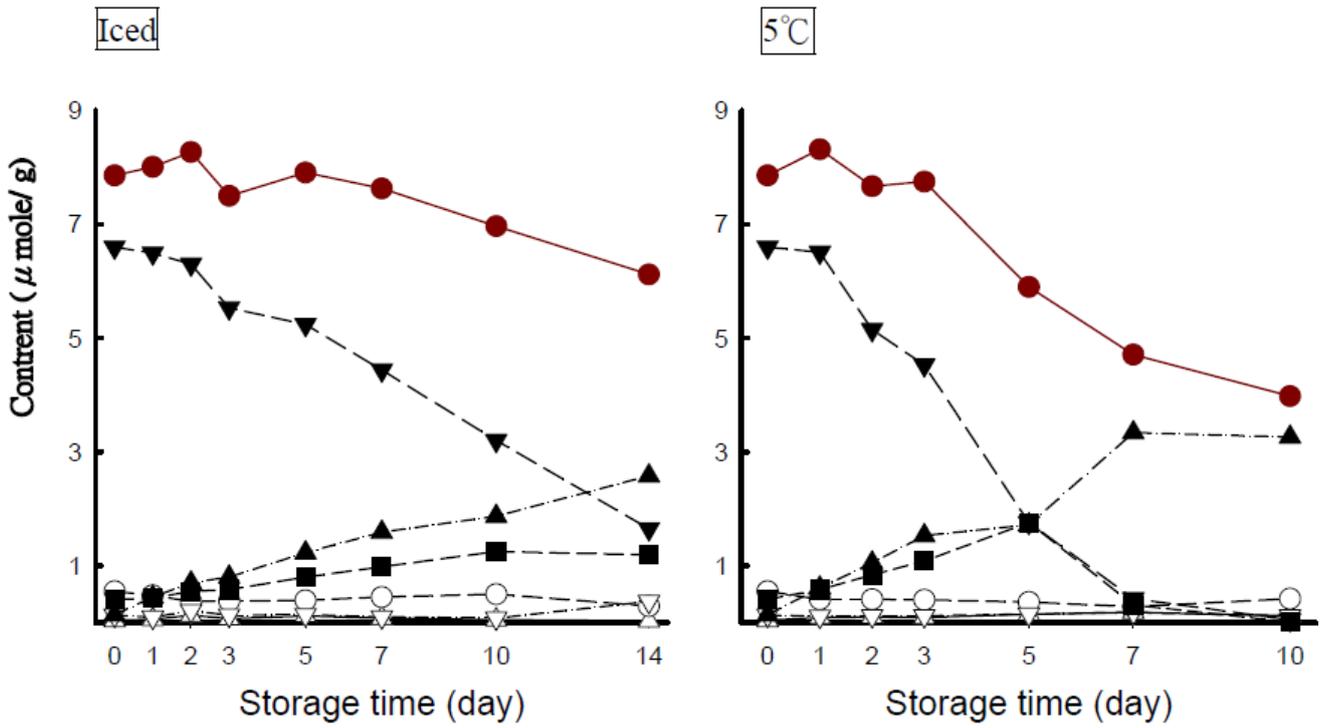


Figure 6. Changes in level of ATP and its related compounds in tilapia muscle during storage at different temperatures without CO treatment. Δ : ATP, \circ : ADP, ∇ : AMP, \blacktriangledown : IMP, \blacksquare : inosine, \blacktriangle : hypoxanthine, \bullet : total