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Use of Electrolyzed Water Ice for Preserving Freshness of Pacific Saury (*Cololabis saira*)

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ABSTRACT

The effects of electrolyzed water ice (EW-ice), compared with traditional tap water ice (TW-ice), on the microbiological, chemical, and sensory quality of Pacific saury (*Cololabis saira*) stored for a period of up to 30 days at 4°C were evaluated. EW-ice with active chlorine at a concentration of 34 mg/kg was prepared from weak acidic electrolyzed water, whose pH, oxidation-reduction potential, and chlorine content were 5, 866 mV, and 47 mg/liter, respectively. Microbiological analysis showed that EW-ice, compared with TW-ice, markedly inhibited the growth of both aerobic and psychrotrophic bacteria in saury flesh during refrigerated storage, primarily because of the action of active chlorine. Chemical analysis revealed that EW-ice retarded the formation of volatile basic nitrogen and thiobarbituric acid–reactive substances and reduced the accumulation of alkaline compounds in the fish flesh in comparison with TW-ice. Sensory analysis confirmed that the freshness of saury was better preserved in EW-ice than in TW-ice and showed that the saury stored in EW-ice had a shelf life that was about 4 to 5 days longer than the fish stored in TW-ice.

The freshness of fish, which is a major concern to industry and consumers, diminishes during refrigerated storage, mainly because of microbiological activity *(5, 6).* Major spoilage microorganisms developed in fish during aerobic refrigerated storage consist typically of gram-negative rod-shaped psychrotrophic bacteria, such as *Shewanella putrefaciens, Pseudomonas, Alteromonas,* and *Flavobacterium* spp. *(6, 8).* To more effectively slow microbial growth and related biochemical reactions, different refrigeration systems, traditionally based on flaked ice, have been developed with refrigerated seawater, slurry ice, ozonized refrigerated water, and ozonized slurry ice *(5, 15, 18).*

Electrolyzed water (EW), generated from anodic electrolysis of a dilute sodium chloride solution, has been known to possess strong bactericidal activity *(10, 17).* The primary bactericidal components of typical acidic EW are active chlorine species, including dissolved chlorine gas (Cl_2), produced by electrolysis, and hypochlorous acid (HOCl), formed by the hydrolysis of Cl_2 *(14, 17).* As the pH of EW is raised above 5.0, HOCl is dissociated to form hypochlorite anions (OCl^-), a weaker bactericide *(14, 17).* Besides the active chlorine species, a highly positive oxidation-reduction potential, often higher than 1,000 mV, is known to be responsible for the outstanding bactericidal action of EW *(10).*

The ice of EW (EW-ice) has also been demonstrated to have bactericidal activity. Notable reductions in the populations of aerobic bacteria and pathogens, including *Listeria monocytogenes* and *Escherichia coli* O157:H7, have been examined on lettuce stored in ice prepared from an acidic EW of pH 2.5 to 2.6 *(11, 12).* This suggests that EW-ice can effectively preserve the freshness of fish during refrigerated storage by inhibiting microorganisms associated with fish spoilage. However, few empirical data have so far been reported on the practical advantages of the use of EW-ice for fish storage. The objective of this study was to evaluate the effects of storage in flake-type weak acidic EW-ice, compared with storage in the traditional flaked ice of tap water (TW-ice), on the microbiological, chemical, and sensory quality of Pacific saury (*Cololabis saira*), which is one of the most popular blue-backed fish species of high commercial value in the Far East countries.

MATERIALS AND METHODS

Preparation of EW-ice. Weak acidic EW was generated from the electrolysis of a dilute NaCl-HCl solution at 17 A and 7 V with a commercial nonmembrane-type EW generator (DIPS, e-SUENC Co., Ltd., Incheon, Korea) at a flow rate of 2.6 liters/min. The generated EW was continuously discharged into a commercial ice flaker (SLF 225, NTF Co., Milano, Italy) to produce EW-ice. TW-ice was also prepared with the same ice flaker as a control in our fish storage experiments. For examining changes in the physicochemical properties of EW during the freezing process, EW-ice was melted. EW-ice was fully packed in a 200-ml gastight glass bottle and melted in a water bath at 80°C for 10 min, and the residual ice was completely melted by shaking. The concentrations of total free available active chlorine in tap water, EW, and melted EW-ice were determined in milligrams per liter as Cl_2 by an iodometric method with a chlorine test kit (Hach, Ames, Iowa) at 16°C. The pH and oxidation-reduction potential of the

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TABLE 1. *Modified Tasmanian Food Research Unit scheme used for the sensory assessment of Pacific saury*

Parameter	Demerit points[a]: 0	1	2	3
Appearance	Very bright	Bright	Slightly dull	Dull
Skin	Firm	Soft		
Slime	Absent	Slightly slimy	Slimy	Very slimy
Stiffness	Prerigor	Rigor	Postrigor	
Clarity of eyes	Clear	Slightly cloudy	Cloudy	
Color of gills	Characteristic	Slightly faded	Very faded	
Color of belly	Opalescent	Grayish	Some yellow	Yellow
Smell	Sharply seaweedy	Fishy	Stale	Spoiled
Firmness of belly	Firm	Soft	Sunken	Burst

[a] Total demerit points ranged from 0 to 22.

three solutions were measured with a digital combination pH–oxidation-reduction potential meter (Inolab level 1, WTW Co., Weilheim, Germany) at 16°C.

Preparation of saury samples and storage conditions. Pacific saury specimens in a frozen state were obtained from a local fishery market and thawed with seawater at room temperature before the experiments. The length and weight of the specimens were in the range of 17 to 20 cm and 50 to 60 g, respectively. For our study, the whole fish specimens were surrounded by either EW-ice or TW-ice at a fish-to-ice weight ratio of 1:1 in polyethylene boxes, provided with outlets for water drainage, and stored in an isothermal room at 4°C. EW- and TW-ices were renewed every 2 days, and the stored specimens were sampled for analysis on days 0, 3, 6, 10, 13, 17, 24, and 30. Three different groups were investigated separately.

Microbiological analysis. Samples for bacterial enumeration were prepared according to Rodríguez et al. *(18)* with slight modifications. On each day of analysis, 25 g of fish flesh was taken from the anterior dorsal region of the saury specimens, suspended in 225 ml of 0.85% (wt/vol) NaCl solution, and homogenized for 1 min in a stomacher (Seward Medical, Norfolk, UK). The homogenate was serially diluted with 0.85% NaCl solution, and 1 ml of each dilution was inoculated on the surface of plate count agar (Difco, Becton Dickinson, Sparks, Md.). Aerobic and psychrotrophic bacterial counts were determined after incubating the agar plates at 30°C for 48 h and at 7°C for 10 days, respectively *(5).* Coliform counts were also determined on violet red bile agar (Merck, Darmstadt, Germany) after incubation at 30°C for 24 h.

Chemical analysis. The contents of moisture and ash in the fish flesh were determined according to the methods of the Association of Official Analytical Chemists *(3).* Total crude fat and protein were determined by the Soxhlet extraction method *(3)* and the Kjeldahl distillation method *(2),* respectively. Total volatile basic nitrogen (TVB-N) was determined by Conway's microdiffusion method *(7).* Briefly, 10 g of fish flesh was mixed with 90 ml of 20% (wt/vol) trichloroacetic acid for 2 min with a homogenizer (T25B, IKA Works Asia Sdn Bhd, Kuala Lumpur, Malaysia). The homogenate was centrifuged and filtered through filter paper (TY5C-070, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The filtrate (1 ml), appropriately diluted when necessary, and Conway indicator solution (1 ml) were pipetted into the outer and inner rings of the Conway unit, respectively, and then 1 ml of saturated K_2CO_3 solution was added to the outer solution. The Conway units were closed and incubated at 40°C for 120 min; then, the inner solutions were titrated with 0.02 N H_2SO_4 solution. Thiobarbituric acid (TBA) was determined according to Turner et al. *(20).* Briefly, 5 ml of the fish filtrate, prepared as described above, was mixed with 5 ml of a test solution containing 0.02 N 2-TBA (Sigma, St. Louis, Mo.). The mixture was heated in boiling water for 30 min and chilled in an ice bath for 10 min; then, its absorbance at 538 nm was measured with a spectrophotometer (UV-1601, Shimadzu Co., Kyoto, Japan). The values of TBA were expressed as milligrams of malonaldehyde (Acros Organics, Geel, Belgium) per kilogram of fish flesh. The pH of the fish flesh was also determined by measuring the pH of the fish homogenate prepared with 10 g of fish flesh in 40 ml of distilled water *(9, 13).*

Sensory analysis. Sensory analysis was assessed on the basis of the Tasmanian Food Research Unit scheme as modified by Alasalvar et al. *(1)* and Özogul et al. *(16),* with further slight modifications for saury. The modified scheme used in the current study is shown in Table 1. On each day of assessment, each of nine characteristic quality parameters, whose demerit points ranged from 0 to a maximum of 3, was evaluated by at least five panelists trained in assessing fish quality. The evaluated demerit points were summed and averaged to give overall sensory scores between a minimum of zero and a maximum of 22, where a higher score represented poorer quality.

Statistical analysis. The active chlorine concentration, pH, and oxidation-reduction potential of tap water, EW, and melted EW-ice were determined on each day of ice renewal ($n = 45$). The significance of the differences between the corresponding values of EW and melted EW-ice was accessed by the Student's t test ($P \leq 0.05$). All the storage experiments were performed in triplicate with two separated test groups ($n = 6$). The significance of the differences between the quality values obtained in the presence of EW-ice and TW-ice was determined by the Student's t test ($P \leq 0.05$). Triplicate proximate analysis was performed for each test group ($n = 18$). All data were expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Effect of freezing on physicochemical properties of EW. EW was generated at weak acidic pH (pH 5.1) because the fraction of HOCl, the strongest bactericidal component of EW, is highest at pH 5.0 *(17),* and weak acidic EW-ice was expected to have a less deteriorative effect on fish quality than the ice of traditional strong acidic EW. The active chlorine concentration of melted EW-ice was determined to be 34 mg/liter, which was lower than that of EW

TABLE 2. *Physicochemical properties of TW, EW, and melted EW-ice*[a,b]

Property	TW	EW	Melted EW-ice
Active chlorine (mg/liter as Cl_2)	0.2	47.2 ± 2.2 A	34.2 ± 4.1 B
ORP (mV)	406 ± 4	866 ± 19 A	962 ± 37 B
pH	6.9 ± 0.3	5.1 ± 0.3 A	4.9 ± 0.3 A

[a] All values are means of 45 replicates ± standard deviations. Different letters within a row indicate significant differences ($P \leq 0.05$) by the Student's t test.

[b] TW, tap water; EW, electrolyzed water; EW-ice, electrolyzed water ice; ORP, oxidation-reduction potential.

at 47 mg/liter (Table 2). This indicates that about 30% of the active chlorine in EW was lost during the formation of the EW-ice flakes in the current continuous EW-ice production system. The pH of melted EW-ice (pH 4.9) was lower than that of EW (pH 5.1), although the difference between the values was not statistically significant (Table 2). This is probably because of the adsorption of anions such as Cl^- and OCl^- on positively charged ice surfaces. Theoretically, active chlorine exists as nonvolatile HOCl and OCl^- at pH 5.1, while HOCl is in equilibrium with volatile Cl_2 at pH 4.9 *(17).* Therefore, the chlorine loss observed during the freezing process might be caused by the evaporation of Cl_2 as well as the exclusion of chlorine from the ice lattice. Interestingly, the oxidation-reduction potential of melted EW (960 mV) was higher than that of EW (870 mV), although the active chlorine concentration was lower in melted EW-ice (Table 2). This is probably because a large portion of Na^+ ions that are dissolved in EW, as well as other oxidation-reduction potential–reducing substances originating from tap water, is excluded from the ice lattice during the freezing process. The evaporation of electrochemically produced hydrogen gas from EW during freezing might also cause an increase of oxidation-reduction potential.

Microbiological analysis. The changes in the population of aerobes and psychrotrophes in the saury flesh stored in EW-ice were compared with the changes in TW-ice during 30 days of refrigerated storage (Fig. 1). As shown in the figures, the growth of both aerobes and psychrotrophes was much slower in the presence of EW-ice. Analysis showed that the counts of aerobes and psychrotrophes in the TW-ice batch reached levels of 5.0 and 5.2 log CFU/g, respectively, after 10 days of storage, while similar levels, 5.1 and 5.4 log CFU/g, respectively, were observed at day 17 when EW-ice was used ($P \leq 0.05$). In both TW- and EW-ice batches, very low coliform counts, below 1.0 log CFU/g (data not shown), were observed, and the growth of coliforms did not occur during storage. The results indicate that the EW-ice produced in the current system exhibits bactericidal activity against aerobic and psychrotrophic bacteria naturally occurring in Pacific saury during refrigerated storage. On each day of ice renewal, which was conducted every 2 days during the storage experiment, the concentrations of active chlorine remaining in

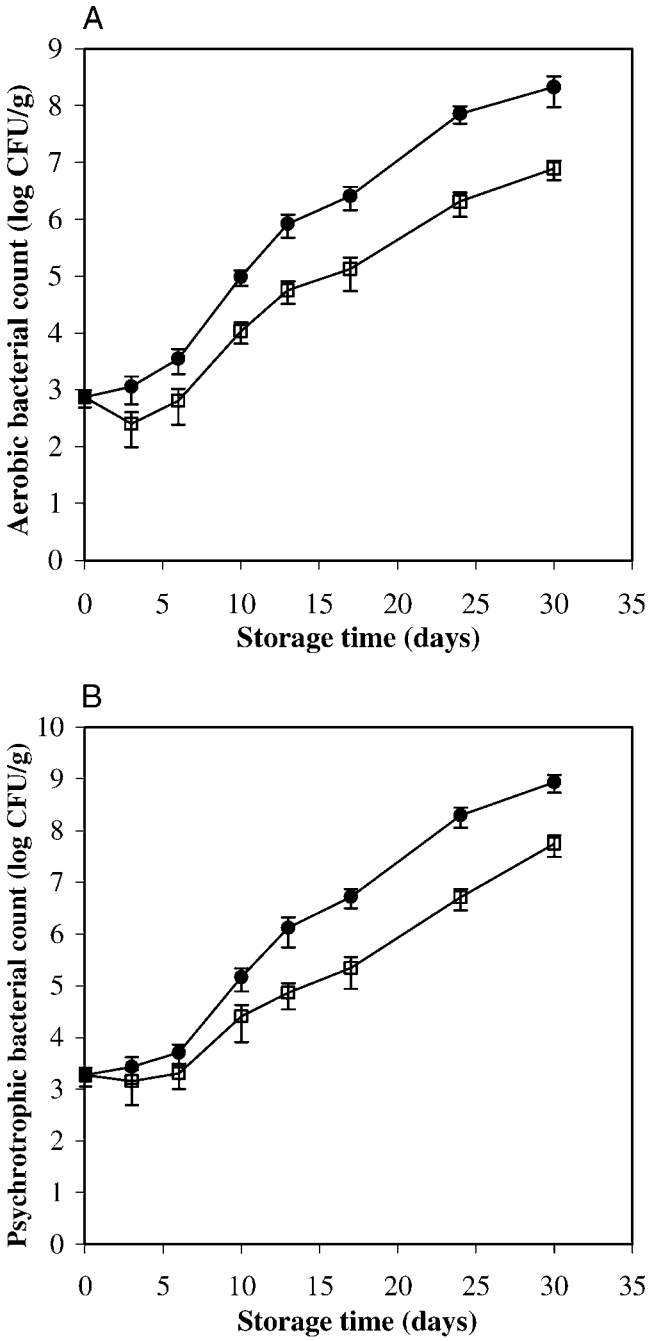

FIGURE 1. *Microbial populations in Pacific saury flesh during refrigerated storage in either tap water ice (TW-ice, ●) or electrolyzed water ice (EW-ice, □). (A) Total aerobes and (B) psychrotrophes. Values are log(mean ± SD),* n = 6.

residual EW-ice were determined. The concentrations were found to be less than 1.0 mg/liter on the basis of the melted ice, implying that the active chlorine entrapped within the EW-ice was almost completely released during the 2 days under the current storage conditions. The release is probably caused by the emission of chlorine gas (Cl_2), as reported by Koseki et al. *(11)* for acidic EW-ice. This observation suggests that not only the active chlorine freed during the melting of EW-ice but also the active chlorine released from EW-ice is primarily responsible for the bactericidal action of EW-ice.

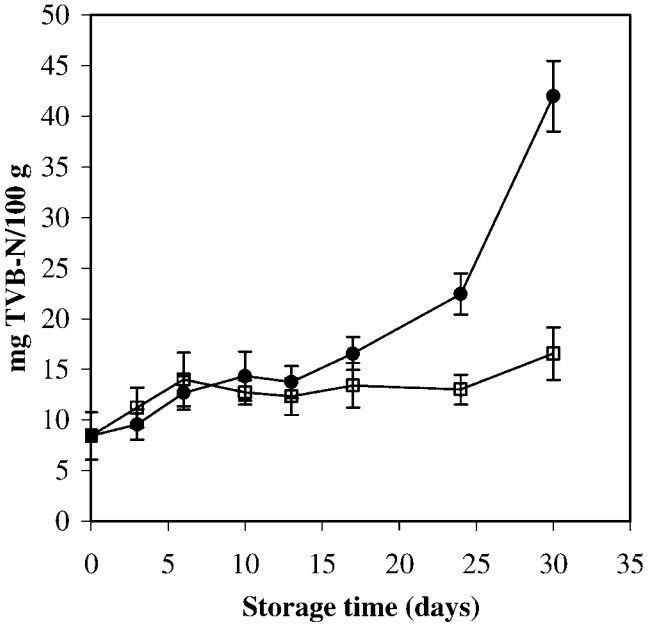

FIGURE 2. *Changes in the TVB-N content of Pacific saury flesh during refrigerated storage in either tap water ice (TW-ice, ●) or electrolyzed water ice (EW-ice, □). Values are mean ± SD,* n *= 6.*

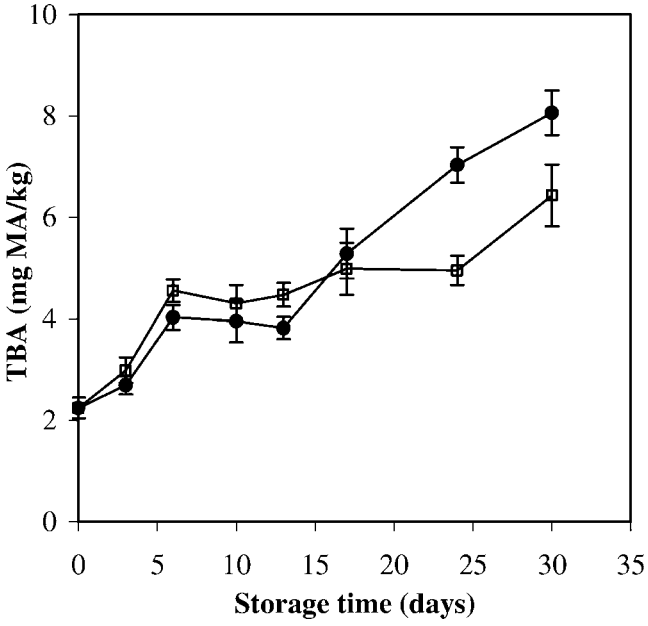

FIGURE 3. *Changes in TBA values of Pacific saury flesh during refrigerated storage in either tap water ice (TW-ice, ●) or electrolyzed water ice (EW-ice, □). Values are mean ± SD,* n *= 6.*

Chemical analysis. Proximate analysis of the saury specimens used in the current study showed that the amounts of moisture, crude fat, crude protein, and ash were 736.3 ± 30.3, 54.9 ± 8.4, 188.1 ± 10.2, and 16.7 ± 4.1 g/kg, respectively. The change in the TVB-N content of saury flesh during the refrigerated storage in EW-ice was compared with that observed during TW-ice storage (Fig. 2). Notable differences ($P \leq 0.05$) were not observed between the two storage systems up to 13 days of storage, during which time the TVB-N contents increased from 8.4 to 12.3 and from 8.4 to 13.7 mg/100 g of flesh in the EW- and TW-ice systems, respectively. After day 13, the content in the TW-ice batch sharply increased and reached 42.0 mg/100 g at the end of storage, while in the EW-ice batch, only a slight increase of TVB-N (up to 16.5 mg/100 g) was observed during the remaining storage period ($P \leq 0.05$ from day 24). This indicates that EW-ice suppresses the formation of volatile basic nitrogen in saury flesh during refrigerated storage in comparison with TW-ice. TVB-N is mainly generated by the bacterial decomposition of fish flesh *(6)*; therefore, the lower production of TVB-N in the presence of EW-ice may be accounted for by the retardation of bacterial growth caused by EW-ice. All the determined TVB-N values, except for the value at day 30 in the TW-ice batch, were lower than the levels of 25 to 35 mg/100 g, which are generally regarded as the limit of acceptability for many fish species, including sardine, trout, and sea bream *(5, 6, 13),* although the specimens were rejected by sensory analysis after certain periods of storage. This suggests that the TVB-N level is not a reliable indicator of freshness for Pacific saury stored in ice, as has also been reported for many other ice-stored fishes *(5, 6, 13, 19).*

The changes in the TBA value of the saury flesh stored in either EW- or TW-ice are shown in Figure 3. During the initial 13 days of storage, the TBA values determined in the EW-ice system, which increased from 2.2 to 4.5 mg of malonaldehyde per kg of flesh, were always slightly higher than the corresponding values in the TW-ice system, indicating that a bit higher degree of lipid oxidation occurred in the fish stored in EW-ice. This is probably due to the oxidative action of active chlorine entrapped in EW-ice. After day 13, however, the values in TW-ice batch sharply increased and became higher than those in EW-ice. From day 13 until the end of storage, the values in the TW-ice batch increased from 3.8 to 8.1 mg of malonaldehyde per kg, whereas a much smaller increase, from 4.5 to 6.4 mg of malonaldehyde per kg, was observed in the EW-ice batch ($P \leq 0.05$ from day 24). This indicates that in spite of its oxidative property, EW-ice suppresses the lipid oxidation in saury flesh during this storage period. The slurry ice containing ozone, another strong oxidative bacteriocide, was also reported to decrease the formation of TBA-reactive substances in turbot muscle during the final period of refrigerated storage in comparison with the control slurry ice *(4).* This is probably because bactericidal ices can retard the lipid oxidation by microbial action or the bacterial production of TBA-reactive compounds.

Comparative changes in pH values are shown in Figure 4. For saury stored in TW-ice, pH values increased from 6.15 to 7.33 over the whole period of storage. The increase of pH indicates the accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, mainly produced by the action of alkalinizing bacteria that are present in fish flesh *(4, 5, 13, 18).* Similar results have been also reported for other fish species, such as sardine, hake, and sea salmon, stored in flaked ice *(5, 9, 18).* In the EW-ice system, the pH values also steadily increased but

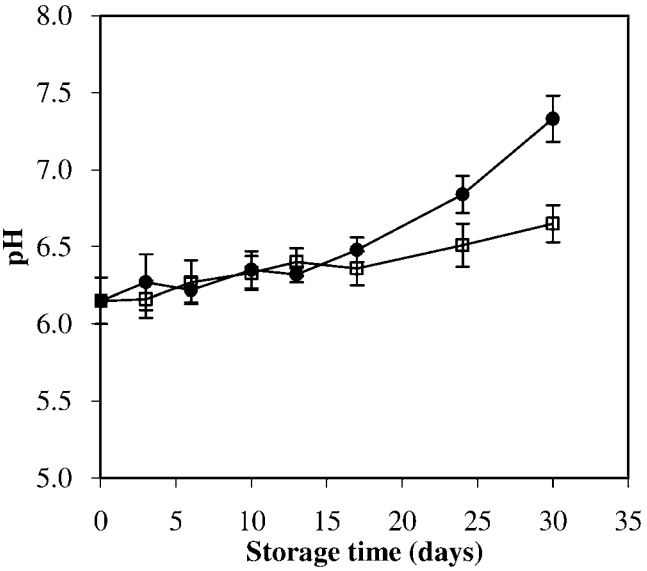

FIGURE 4. *Changes in pH values of Pacific saury flesh during refrigerated storage in either tap water ice (TW-ice, ●) or electrolyzed water ice (EW-ice, □). Values are mean ± SD,* n = 6.

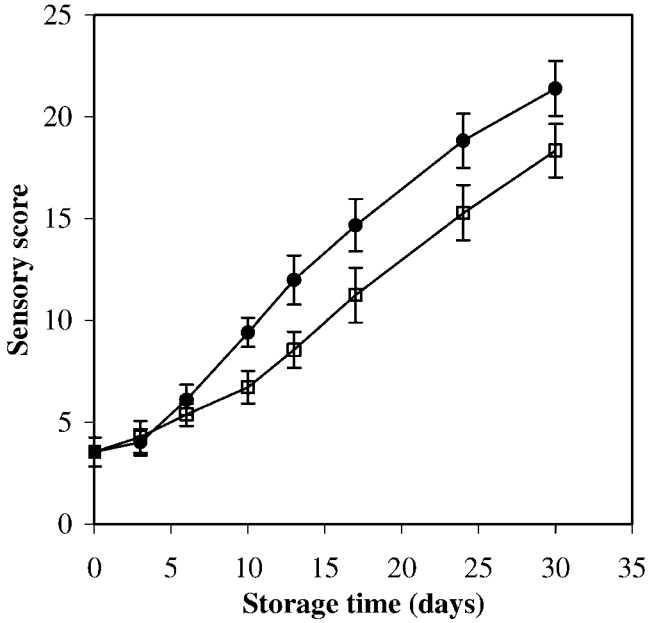

FIGURE 5. *Changes in sensory scores of Pacific saury during refrigerated storage in either tap water ice (TW-ice, ●) or electrolyzed water ice (EW-ice, □). Values are mean ± SD,* n = 6.

reached only 6.65 at the end of storage ($P \leq 0.05$ from day 24), indicating less accumulation of alkaline compounds in the fish flesh stored in this system than in the TW-ice system. This indication is supported by the results obtained in the current study; the bactericidal effect of EW-ice (Fig. 1) and a lower production of volatile basic nitrogen in the presence of EW-ice (Fig. 2).

Sensory analysis. The changes in the sensory score of Pacific saury during the 30 days of refrigerated storage in either TW-ice or EW-ice, determined on the basis of the modified Tasmanian Food Research Unit scheme (Table 1), are shown in Figure 5. In this scheme, the range of sensory scores is from 0 (best quality) to 22 (poorest quality), and a score of about 10 was considered the limit of acceptability by the panelists. In both batches, sensory scores increased with storage time. A linear increase of scores has been reported for sea bream stored in flaked ice *(1)*; however, the increase observed in this study was not quite linear. For saury stored in TW-ice, the scores reached 9.4 at day 10 and 12.0 at day 13. This indicates that the shelf life of saury stored in TW-ice is between 10 and 13 days, although notable changes in chemical properties, such as TVB-N, TBA, and pH, occurred after 13 days of storage. In contrast, scores of 8.6 and 11.2 were determined at days 13 and 17 when EW-ice was used. The results indicate that Pacific saury stored in EW-ice had about a 4- to 5-day longer shelf life than the fish stored in TW-ice under the current storage conditions ($P \leq 0.05$). Recent studies showed that the shelf lives of sardine and turbot stored in slurry ice were extended by 4 and 14 days, respectively, when ozone was combined with slurry ice *(4, 5)*. It should be stressed that a slight degree of discoloration was observed on fish skin in the presence of EW-ice during the initial 3 days of storage, although it did not greatly affect the overall sensorial quality.

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