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EFFECT OF SALT AND OZONIZED-SLURRY ICE ON THE QUALITY INDICES OF TIGER GROUPER (*Epinephelus fuscoguttatus*)

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Abstract: Tiger grouper is a high value of live reef fish, which mainly exported to Hong Kong, Japan and Singapore. This study was carried out to investigate the quality indices (microbial and chemical) of tiger grouper (*Epinephalus fuscoguttatus*) stored for 20 days in slurry ice treated with 0.1 mg ozone l^{-1} (O_3) and 3.3% sodium chloride (NaCl). Slurry ice contains 60% ice and 40% water was used as control. All samples were analysed for total aerobic, psychrotrophic bacteria, total volatile based nitrogen (TVBN) and trimethylamine (TMA) at interval of 5 days during 20 days storage. The total bacteria count of tiger grouper treated in slurry ice with O_3 (SI+O_3) showed significantly (p<0.05) decreased to about 0.24 log units compared to controls. However, bacteria counts of tiger grouper treated in slurry ice with NaCl (SI+NaCl) were not significantly different from both the controls and SI+O_3 samples. Psychrotrophics bacteria counts were effectively reduced to 0.39 and 0.18 log units after treatment of SI+O_3 and SI+NaCl, respectively. TVBN and TMA contents during 20 days storage for all treatments did not exceed the acceptable limit of 35 mg N $100g^{-1}$ and 15 mg N $100g^{-1}$, respectively. No significant difference (p>0.05) was found between controls and treated samples for TVBN and TMA contents. The combination of the slurry ice and ozone may be recommended for chilling and storage of tiger grouper to improve its storage quality.

Keywords: Microbiological quality, total volatile based nitrogen (TVBN), trimethylamine (TMA), ozone, slurry ice.

Introduction

Fish is one of the most highly perishable food products because of their neutral pH and presence of autolytic enzymes (Jeyasekaran *et al.*, 2004). High temperature might increase the rate of fish spoilage and low temperatures will slow it down (Huss, 1995). In tropical country with high ambient temperature, growth of microorganisms and fish deteriorates happen very rapidly (Gandotra *et al.*, 2012). The faster a lower temperature is attained during fish chilling, the more effective spoilage activity is inhibited. Without a proper preservation, fish can be spoiled and leads to a decreasing quality. The quality of fish degrades due to complex process in which physical, chemical and microbiological forms of deteriorations are implicated (Sallam, 2007). The decomposition of trimethylamine oxide (TMAO) is due to bacterial spoilage and enzymatic activity in producing trimetylamine (TMA). TMA only appears 3 or 4 days after death, which the rate of production parallels to the pattern of bacteria proliferation (Howgate, 2010).

Chilling is one of the preservation techniques that helps to reduce the post-harvest losses. By inhibiting microorganism after caught during onboard, the quality and shelf life of fish can be extended. Therefore, consumers insist to have product that can maintain freshness and high quality during storage. To reach the demand of consumers, chilling process by using slurry ice and other combinations have been pursued. Slurry ice is a promising technique to preserve aquatic products in ice suspension at subzero temperature (Chapman, 1990; Harada, 1991). Ice slurries has provided several advantages such as lower temperature, faster chilling, lower physical damage to product and better heat exchange power. Slurry ice is also beneficial for direct contact chilling fish surface by allowing a better protection of the fish material from oxidation and dehydration events (Piñeiro *et al.,* 2004). An additional of sodium chloride (NaCl) during chilling process is common as freezing point depressant in seafood processing. Salt was one of the best methods for inhibiting the growth and survival of undesirable microorganisms.

Ozone is a bactericidal agent that benefits fish preservation due to its ability to reduce the microbial activity (Hubbs, 1991). Therefore, ozone has been successfully employed as a disinfectant for fresh water aquaculture systems and its application in improving the sensory quality and shelf life of fish (Kotter *et al.,* 1997; Kim *et al.,* 2000). Ozone does not produce significant toxic residues in the environment after the treatment (Kim *et al.,* 1999).

The combination of slurry ice with ozone treatment in fresh fish preservation is not used commercially in Malaysian seafood industry. Besides that, the study on the preservative effect of ozonated slurry ice on the grouper is also limited. The present study is undertaken to find out whether the application of slurry ice combination with ozone treatment is more effective than slurry ice alone in aspects of microbiological and chemical quality on tiger grouper.

Material and Methods

Sample Preparation

Thirty six tiger grouper were harvested from local farm. Alive fish specimens were sacrificed by immersion in ice and immediately transfer to hatchery. Three different treatments were subjected to the samples; slurry ice storage (ratio ice:water; 3:2) as the control; slurry ice mixture with 3.3% NaCl; and ozonated (0.1 mg l^{-1}) slurry ice. Ozonated slurry ice were prepared by subjected slurry ice (ice:water, 3:2) at constant concentrations of 0.1 mg ozone l^{-1} using ozone generator (Ozomax, OZO-4VTT) for 20 minutes. Fish fillets were then immersed in ozonated slurry ice for 2 hours. All samples were subsequently stored in chilled storage and maintained at 2±0.1ºC. The analyses were carried out on day 0, 5, 10, 15 and 20 for each treatments and controls. The analyses were performed in triplicate.

Microbiological Analysis

Bacterial counts were determined by using the serial dilution and spread plate method as described by Linton *et al*. (2003); Karim *et al*. (2011). For aerobic condition, the plates were placed in incubator 30ºC for 48 hours. Meanwhile for psychrotrophs condition, the plates were incubated in chilled storage at 7ºC for 10 days. Microbiological counts were expressed as log colony forming units per gram of samples (CFU g^{-1}). Microbial analyses were carried out on day 0, 5, 10, 15 and 20 for each treatment and controls.

Chemical Analysis

Total volatile based nitrogen (TVBN) and trimethylamine (TMA) was conducted to determine the chemical quality of fish using method published by Malle & Tao (1987) with a minor modification (Karim *et al*., 2011). 50±0.1g of fish muscle were homogenized with 100 ml of 7.5% aqueous trichloroacetic acid (TCA) solution using homogenizer (Ultra-Turrax T25 basic IKA Werke GmbH & Co, Selangor, Malaysia). The homogenate were centrifuged at 3000 rpm for 5 min and the supernatant liquid was then filtered through Whatman No 1 filter paper into the conical flask. Twenty five of each filtrate was transferred into Kjeldahl distillation tube followed by 5 ml of 10% sodium hydroxide solution. The receiving flask contains 15 ml of 4% boric acid and 15 drop of indicator (methyl red mix with bromocresol green). The distillation tube was attached in the Kjeldahl distillation apparatus and 50 ml of distillate were collected in the receiving flask. Each distillate was titrated against an aqueous 0.05 M sulphuric acid solution with a constant shaking until a pink colour persisted at 15 s. The same experimental procedure of TVBN was used for the TMA measurement (Malle & Poumeyrol, 1989). The only difference was the addition of 20 ml of 35% (v/v) formaldehyde to the distillation tube. The amount of TVBN and TMA were calculated from the volume of 0.05 M sulphuric acid (*n* ml) used for titration and the results were expressed in mg nitrogen 100 g^{-1} of sample.

$$\text{TVBN} = n \times 16.8 \text{ mg N } 100\ g^{-1}$$
$$\text{TMA} = n \times 16.8 \text{ mg N } 100\ g^{-1}$$

Statistical Analysis

Data were analysed statistically using SPSS Software (version 16.0). All measurements were performed in triplicate and the values were expressed as the mean ± standard deviation. The collected data were analyzed using one way ANOVA and hypothesis test were analyzed using Tukey Test at significance level 0.05. The variable responses to storage period and response curves were fitted for each treatment and replicates. An estimate of shelf-life were calculated from the response curve parameters. The microbiological shelf-life was taken as the time to reach 10^7 CFUg^{-1} (Erkan *et al*., 2010). TVBN and TMA data was also used to estimate shelf-life of tiger grouper by fitting exponential curves as the response curve. The chemical shelf life was taken at maximum limit of acceptability values of TVBN and TMA reach 35 mg N 100 g^{-1} and 15 mg N 100 g^{-1} (Connell & Shewan, 1980).

Results and Discussion

Microbiological Analysis

The means of total aerobic count of tiger grouper stored in the ozonated slurry ice (SI+O_3) (5.63±0.04 $\log_{10}$CFUg^{-1}) showed significantly ($p<0.05$) lower amount compared to the controls (5.88±0.05 $\log_{10}$CFUg^{-1}). However, the total bacteria count in samples treated in SI+O_3 showed no significant difference ($p>0.05$) compared to samples treated in SI+NaCl storage (5.75±0.05 $\log_{10}$CFUg^{-1}) (Fig. 1). With regards to the storage day, the total bacteria counts in SI+O_3 showed a significant lower ($p<0.05$) amount compared to samples treated in SI+NaCl at storage day of 5, 10 and 15 (Fig 2).

Meanwhile, the means of total psychrotrophic count for tiger grouper stored in the ozonated slurry ice (SI+O_3) were significantly ($p<0.05$) lower compared to the controls and samples treated in SI+NaCl (Fig. 1). Grouper treated in ozonated slurry ice (SI+O_3) significantly ($p<0.05$) showed lower amount compared to controls on day 5 and 10 with value of 4.53 $\log_{10}$CFUg^{-1} and 5.92 $\log_{10}$CFUg^{-1}, respectively (Fig 3). In addition, the psychrotrophic bacteria in samples treated in SI+O_3 were significantly lower ($p<0.05$) compared to samples treated in SI+NaCl at 10th and 20th day of storage (Fig 3).

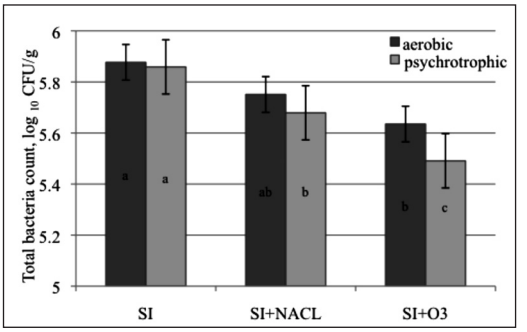

Figure 1: Comparison on the effects of different ice storage in tiger grouper for total aerobic and psychrotrophic count
SI, Slurry ice; SI+NaCl, Slurry ice with 3.3% sodium chloride; SI+O_3, Slurry ice with 0.1 mg ozone l^{-1}

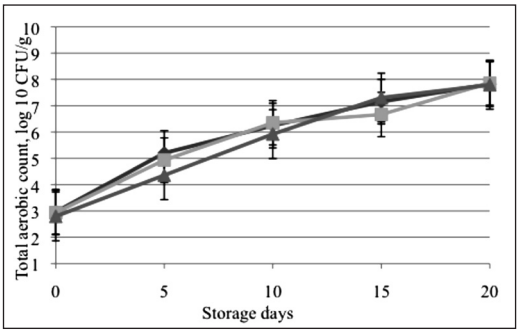

Figure 2: Changes in total aerobic bacteria count ($\log_{10}$CFUg^{-1}) of tiger grouper stored in three different ice storage during 20 days
♦, Slurry ice; ■ SI+NaCl; ▲, SI +O_3

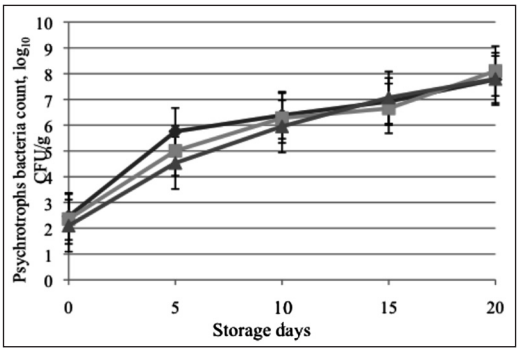

Figure 3: Changes in total psychrotrophic bacteria count ($\log_{10}$CFUg^{-1}) of tiger grouper stored in three different ice storage during 20 days
♦, Slurry ice; ■ SI+NaCl; ▲, SI +O_3

The initial bacteria counts in the study showed low amount for all three different ice storage. Newly caught fish have total bacteria of 2.0 $\log_{10}$CFUg^{-1}. The combined use of ozone and slurry ice induced a decline in the growth of bacteria. These findings meet an agreement of Campos *et al.* (2005) in sardines (*Sardina pilchardus*) treated at 0.17 mg ozone l^{-1}. The sardines show a reduction of being in the range of 1.5–2.5 log units below compared to control after 12 days. The combination of slurry ice and ozone also show a better microbiological control in surface and muscle of turbot compared to samples stored in the mixture of slurry ice and NaCl with reduction of 1.8 $\log_{10}$CFUg^{-1} after 21 days of storage (Campos *et al.,* 2006).

Chemical Analysis

The mean values of TVBN and TMA for tiger grouper showed no significant ($p<0.05$) difference in all treatments. However, ozonated slurry ice (SI+O_3) showed the lowest mean values of TVBN and TMA compared to the control and samples treated in the mixture of slurry ice with NaCl (SI+NaCl).

Similar result was obtained from previous study by Campos *et al*. (2006). The farmed turbot (*Psetta maxima*) treated in ozone-slurry ice (0.2 mg ozone l^{-1}) show no significant difference ($p>0.05$) of TVBN and TMA accumulation to the controls. However, TVBN and TMA contents showed the lowest amount in ozone-slurry ice storage compared to non-ozonized-slurry ice.

Estimation of Shelf Life

Huss *et al*, (1997) reported the number of spoilage bacterial counted at the point of rejection was from 7 to 9 $\log_{10}$ CFU g^{-1}. From the total aerobic count graph (Fig. 2), the shelf life or rejection point for slurry ice (controls) is on day 15 (Table 1). Similar result was also observed on tiger grouper treated with SI+NaCl and SI+O_3. However, with regards to the psychrotrophic bacteria count (Fig. 3), the shelf life of slurry ice alone had reached the limit of acceptability on day 14. Meanwhile, tiger grouper treated with both SI+NaCl and SI+O_3 prolonged the shelf life up to 15 days (Table 1).

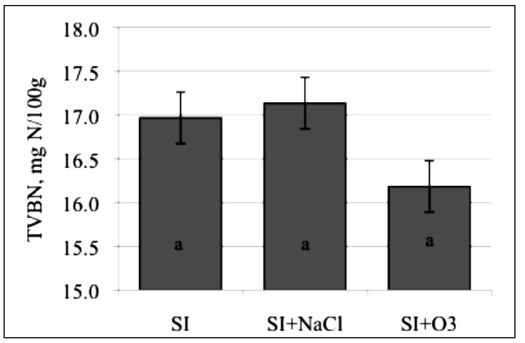

Figure 4: Effects different ice storage of tiger grouper on TVBN

SI, Slurry ice; SI+NaCl, Slurry ice with 3.3% sodium chloride; SI+O_3, Slurry ice with 0.1 mg ozone l^{-1}

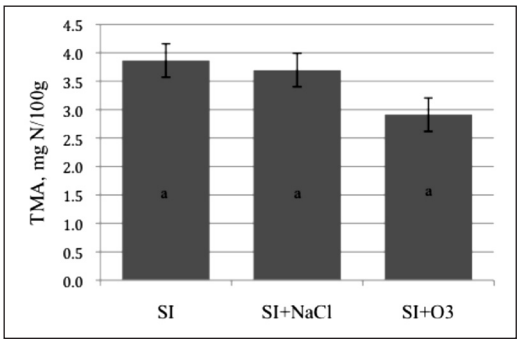

Figure 5: Effects different ice storage of tiger grouper on TMA

SI, Slurry ice; SI+NaCl, Slurry ice with 3.3% sodium chloride; SI+O_3, Slurry ice with 0.1 mg ozone l^{-1}

The estimation of shelf life were also analyzed as the time taken for TVBN and TMA values reach the limit of 35 mg N 100g^{-1} and 15 mg N 100g^{-1}, respectively (Karim *et al*., 2011). The shelf life of tiger grouper stored in slurry ice were spoiled at 51 and 67 days. Meanwhile, the tiger grouper treated with SI+NaCl recorded an extension of storage up to 43 and 59 days, respectively (Table 1). However, tiger grouper treated with SI+O_3 reached the limit of acceptability at 52 and 75 days of storage. The estimation of shelf life were based on the microbial spoilage due to the slower rate of chemical indicator of spoilage production (Karim *et al.,* 2011). Therefore, taken to this principle, the shelf life of tiger grouper were extended up to 15 days storage for both SI+NaCl and SI+O_3 treatments.

Table 1: The estimation shelf life on tiger grouper for microbiological criteria

Treatment	SI	SI+NaCl	SI+O_3
Total aerobic count	15	15	15
Total psychrotrophic counts	14	15	15
TVBN value	51	43	52
TMA value	67	59	75

Conclusion

Storage of tiger grouper in slurry ice with ozone treatment shows a potential practices for chilling storage due to the reduction of total aerobic and psychrotrophs bacteria and lower contents of TVBN and TMA during the study. The combination of the slurry ice and ozone can be recommended for chilling and storage of tiger grouper to improve the microbiological and chemical quality and reduce post harvest losses.

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