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Microbiological and total volatile basic nitrogen (TVB-N) quality changes of groupers during ice storage

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Abstract
This study was conducted to monitor the total mesophilic and psychrotrophic aerobic bacterial counts, pH and TVB-N value of ten species of wild groupers stored in ice for 18 days. The total mesophilic and psychrotrophic aerobic bacterial counts and the TVB-N value of all the ten wild species of groupers were significantly increased ($p < 0.05$) during the storage. The pH values and TVB-N content of the groupers muscles during the 18 days of storage in ice were ranged from 6.4 to 7.2 and from 21.0 to 45.4 mg/100 g respectively.
The total mesophilic and psychrotrophic aerobic bacterial counts of the grouper species stored in ice were ranged from 4.20 to 7.67 log cfu/g, and from 4.41 to 7.88 log cfu/g respectively. Strong and positive correlation was exhibited between TVB-N and the total mesophilic and psychrotrophic aerobic bacterial counts (r =0.949 - 0.989), (r =0.963 - 0.994), respectively. This suggested that the total mesophilic and psychrotrophic aerobic bacterial counts and the TVB-N methods which have been used in this study may be considered as a useful indicators of the freshness quality of groupers muscle during ice storage.

Keywords: mesophilic, psychrotrophic, TVB-N, grouper, ice storage

Introduction

Groupers (Serranidae: Epinephelinae) are widely distributed in tropical and subtropical seas throughout the world (Craig *et al*., 2011) [1]. They are one of the most commercially important groups of coral reef fishes and considered to be a highly desirable fish in the world, particularly in Asia (Heemstra and Randall, 1993) [2].
Fish and seafood products are considered to be one of the most important sources in human nutrition, which often contain relatively high levels of protein, essential vitamins, minerals and rich in omega-3 fatty acids (Simopoulos, 2002) [3]. However, fish and seafood products are classified as one of the most highly perishable food products, due to their high levels of moisture and nutrients.
The quality of fish and seafood products are a major concern to industry and consumers. The quality of seafood begins to deteriorate immediately after capture and approximately 10% of the global seafood harvest is lost due to spoilage yearly (Svanevik and Lunestad, 2011) [4]. However, the quality and shelf life of fresh fish depends on several factors, such as the intrinsic factors of the animals, the initial microflora, seasonal period, the fishing method, storage conditions, handling, processing and temperature (Erkan, 2007; Chotimarkorn, 2014) [5, 6].
Microbial growth and metabolic activity are responsible for fish and seafood products spoilage. Freshness is one of the most important criterions when evaluating fish quality. The microbiology (total mesophilic and psychrotrophic aerobic bacterial counts) and the TVB-N methods have been widely used to evaluate the freshness quality of fish and seafood products. Additionally, TVB-N is considered to be one of the main chemical parameters correlated with the microbial growth of spoilage microorganisms (Gram and Huss, 1996; Gram and Dalgaard, 2002) [7, 8].
Among various preservation techniques, ice storage is one of the most widely methods used of fish preservation, providing an easy and cost effective method to control microbe-caused food spoilage and prolong shelf life, particularly during transportation. Although, global demand for groupers has increased substantially in recent decades. However, very few studies have investigated the quality and shelf life of groupers species, in the worldwide; in particular for brown spotted grouper (*Epinephelus chlorostigma*) (Jeyasekaran *et al*., 2008) [9], giant grouper (*E. lanceolatus*) (Hsiao and Chang, 2017) [10], orange-spotted grouper (*E. coioides*) (Sharifian *et al*., 2014) [11], spotted coral grouper (*Plectropomus maculatus*) (Surti *et al*., 2001) [12], white grouper (*E. aeneus*) (Özogul *et al*., 2008) [13], wire-netting reef cod (*E. merra*) (Jeyasekaran *et al*., 2005) [14], and yellow grouper (*E. awoara*) (Li *et al*., 2011) [15].
Nevertheless, there are no data available on the bacterial load and the TVB-N in wild groupers in Saudi Arabia. Therefore, the objective of the current study were to investigate the total mesophilic and psychrotrophic aerobic bacterial counts, pH and the TVB-N of ten species of wild grouper stored in ice of a period of 18 days.

Materials and Methods

Samples collection, preparation and storage of groupers

Groupers were selected for this study because they are considered to be a highly desirable fish in Saudi Arabia. A total of 120 fish specimens representing ten wild species of grouper used in this study were obtained from a local seafood market, in Riyadh, Saudi Arabia (Table 1). The samples were transported to the laboratory within 30 min in iced styrofoam boxes. Upon arrival at the laboratory, ungutted fish were wrapped individually in a septic sealed plastic bag and stored in ice up to 18 day. During storage, 3 random samples were taken on day 0, 6, 12 and 18 of storage from each of the 10 species for pH, the TVB-N and microbiological examination. On each sampling day, first, the external surfaces of the fish was dry thoroughly with paper towels and disinfecting the same area with 70% ethanol, and then grouper muscles were aseptically taken aseptically from the dorsal anterior area (from both right and left sides) of each fish separately, using sterilized scalpels and forceps.

Table 1: Total length and weight of groupers samples

Common name	Scientific name	Length	Weight
Vermillion hind	*Cephalopholis oligosticta*	29.5cm	425.1g
Tomato grouper	*Cephalopholis sonnerati*	45.8cm	1926.3g
Ariolated grouper	*Epinephelus areolatus*	36.9cm	532.2g
Orange-spotted grouper	*Epinephelus coioides*	46cm	1423.8g
Dotted grouper	*Epinephelus epistictus*	47.7cm	1478.3g
White blotched grouper	*Epinephelus multinotatas*	51.9cm	2031.5g
Smallscaled grouper	*Epinephelus polylepis*	58.7cm	2730.3g
Coral trout	*Plectropomus areolatus*	53cm	2453.83g
Roving coral grouper	*Plectropomus pessuliferus*	56.7cm	2840.1g
Moontail Seabass	*Variola louti*	56.2cm	2209.3g

Microbiological analysis of groupers muscles

For microbiological analysis, 10g of individual grouper muscle was homogenised in 90 ml of sterile physiological saline solution (0.85% NaCl), for 1 min in a Waring Blender (IUL Instruments, Spain). A series of 10-fold dilutions of the homogenate were prepared up to 10^{-5} in sterile 0.1% peptone water. For the total mesophilic and psychrotrophic aerobic bacterial enumeration, 0.1 ml samples of serial dilutions of muscle homogenates were spread onto the surface of tryptone soya agar plates (TSA, Oxoid, UK). Plates were incubated at 30 °C for 48 h and 10 °C for 10 days for mesophilic bacteria counts and psychrotrophic bacteria counts, respectively. All analyses were performed in triplicate.

Determination of pH value of groupers muscles

pH value was measured at room temperature on 5 g of homogenate of grouper muscle with 50 ml of distilled water (1:10, w:v) using a digital pH-meter (WTW Multiline P4, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

Determination of total volatile basic nitrogen (TVB-N) of groupers muscles

The TVB-N content of groupers muscles were determined by steam distillation according to the method described by Antonacopoulos and Vyncke [16], on 10 g of homogenate of grouper muscle with 250 ml of distilled water.

Statistical analysis

Data are expressed as mean ± SD. Statistical significant was determined using one-way analysis of variance (ANOVA). Correlation between the total mesophilic and psychrotrophic aerobic bacterial counts and the TVB-N during storage time was assessed using Pearson's correlation analysis.

Results and discussion

Microbiological analyses of groupers muscles

Changes in total mesophilic counts (TMC) and total psychrotrophic counts (TPC) of 10 wild species of groupers muscles during 18 days of iced storage are presented in Tables 2 and 3. Initial total mesophilic and psychrotrophic aerobic bacterial counts in groupers muscles were ranged from 4.20 to 4.64 log cfu/g and from 4.41 to 4.96 log cfu/g respectively. A high initial mesophilic and psychrotrophic aerobic bacterial counts were observed in brown spotted grouper (*E. chlorostigma*) with values of 10^6 and 10^5 cfu/g, respectively (Jeyasekaran *et al*., 2008) [9], and in wire-netting reef cod (*E. merra*) with counts of 10^5 cfu/g (Jeyasekaran *et al*., 2005) [14]. Similar to values reported by other authors for the muscle of Atlantic horse mackerel (*Trachurus trachurus*) (Alfaro *et al*., 2013) [17], Atlantic mackerel (*Scomber scombrus*) (Albertos *et al*., 2017; De Alba *et al*., 2019) [18, 19], haddock (*Melanogrammus aeglefinus*) (Karim *et al*., 2011) [20], herring (*Clupea harengus*) (Karim *et al*., 2011; Albertos *et al*., 2019) [20, 21], sardine (*Sardina pilchardus*) (Nuñez-Flores *et al*., 2013) [22], sea bass (*Dicentrarchus labrax*) (Papadopoulos *et al*., 2003) [23], gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) (Viji *et al*., 2015) [24]. In contrast, initial low bacterial counts were observed in white grouper (*E.*

aeneus) (10^3 cfu/g) (Özogul *et al*., 2008) [13], and in orange-spotted grouper (*E. coioides*) (10^2 cfu/g) (Sharifian *et al*., 2014) [11].

The higher initial total mesophilic and psychrotrophic aerobic bacterial counts could have resulted from contamination during transportation, handling, processing, storage, and/or elevated temperature during transportation and processing. However, these values were well below $5x10^5$ cfu/g^{-1} set by ICMSF standard (ICMSF, 1986) [25] as a measure of a good quality of food product.

The total mesophilic and psychrotrophic aerobic bacterial counts were significantly increased ($p < 0.05$) with time of storage for all grouper species. Higher correlation coefficients were observed between the total counts of mesophilic and psychrotrophic aerobic bacteria and storage time of all the 10 species of wild groupers ranged from (r = 0.980 - 0.999) to (r = 0.975 - 0.999) respectively. A comparable pattern of the increase in bacterial has been previously reported in wire-netting reef cod (*E. merra*) (Jeyasekaran *et al*., 2005) [14], wild white grouper (*E. aeneus*) (Özogul *et al*. 2008) [13], yellow grouper (*E. awoara*) (Li *et al*., 2011) [15], orange-spotted grouper (*E. coioides*) (Sharifian *et al*., 2014) [11], and in giant grouper (*E. lanceolatus*) (Hsiao and Chang, 2017) [10].

According to ICMSF [25] 7.0 log cfu/g is regarded as the maximum acceptable limit for freshwater and marine species that suitable for human consumption. At the end of iced storage (18 days), the total counts of mesophilic and psychrotrophic aerobic bacteria, in groupers muscles were ranged from 7.04 to 7.67 log cfu/g and from 7.18 to 7.88 log cfu/g respectively. A comparable pattern of mesophilic and psychrotrophic values were determined in the refrigerated orange-spotted grouper muscle after 14 and 10 days respectively, (Sharifian *et al*., 2014) [11]. Özogul *et al*. [13] observed that the total counts in the iced storage of white grouper muscle reached 7.15 log cfu/g on day 8 and towards the end of storage with 9.61 log cfu/g on day 22.

Furthermore, the total aerobic psychrotrophic bacterial counts were slightly higher than the total aerobic mesophilic bacterial counts over the storage period in all the 10 species of wild groupers used in this study, which were consistent with data reported previously in different fish species, such as sea bream and sea bass muscles (Kilinc *et al*., 2007) [26], Atlantic mackerel fillets (Albertos *et al*., 2017) [18], Atlantic herring (Albertos *et al*., 2019) [21], and greenback grey mullet muscle (Kuvei *et al*., 2019) [27]. These results suggest that psychrotrophic is the predominant microflora in fish (Albertos *et al*., 2019; Nuñez-Flores *et al*., 2013) [18, 22].

Table 2: Changes in mesophilic bacteria count of groupers muscle during ice storage

Fish species	**Storage period (Day)**				
	0	**6**	**12**	**18**	**r**
Cephalopholis oligosticta	4.36 ± 0.17	5.72 ± 0.43	6.43 ± 0.14	7.04 ± 0.14	0.980
Cephalopholis sonnerati	4.20 ± 0.41	5.87 ± 0.92	6.57 ± 0.82	7.67 ± 0.21	0.986
Epinephelus areolatus	4.54 ± 0.41	5.54 ± 0.41	6.23 ± 0.04	7.34 ± 0.24	0.996
Epinephelus coioides	4.64 ± 0.35	5.15 ± 0.61	6.38 ± 0.02	7.52 ± 0.85	0.986
Epinephelus epistictus	4.38 ± 0.02	5.36 ± 0.17	6.15 ± 0.61	7.26 ± 0.53	0.998
Epinephelus multinotatas	4.58 ± 0.98	5.36 ± 0.17	6.34 ± 0.24	7.20 ± 0.41	0.999
Epinephelus polylepis	4.51 ± 0.51	5.48 ± 0.71	6.11 ± 0.39	7.26 ± 0.53	0.995
Plectropomus areolatus	4.41 ± 0.49	5.46 ± 0.24	6.11 ± 0.39	7.08 ± 0.92	0.996
Plectropomus pessuliferus	4.59 ± 0.11	5.68 ± 0.12	6.18 ± 0.61	7.23 ± 0.04	0.991
Variola louti	4.43 ± 0.14	5.59 ± 0.11	6.20 ± 0.41	7.15 ± 0.61	0.994

Mean ± SD standard deviation.
r Correlation value

Table 3: Changes in psychrotophic bacteria count of groupers muscle during ice storage

Fish species	**Storage period (Day)**				
	0	**6**	**12**	**18**	**r**
Cephalopholis oligosticta	4.41 ± 0.49	5.85 ± 0.73	6.74 ± 0.04	7.23 ± 0.04	0.975
Cephalopholis sonnerati	4.52 ± 0.51	5.95 ± 0.42	6.79 ± 0.93	7.88 ± 0.51	0.995
Epinephelus areolatus	4.62 ± 0.32	5.61 ± 0.28	6.45 ± 0.72	7.41 ± 0.49	0.999
Epinephelus coioides	4.69 ± 0.89	5.38 ± 0.02	6.64 ± 0.35	7.79 ± 0.24	0.993
Epinephelus epistictus	4.66 ± 0.28	5.52 ± 0.85	6.34 ± 0.24	7.36 ± 0.17	0.999
Epinephelus multinotatas	4.69 ± 0.02	5.50 ± 0.51	6.76 ± 0.34	7.73 ± 0.24	0.997
Epinephelus polylepis	4.61 ± 0.28	5.59 ± 0.11	6.20 ± 0.41	7.28 ± 0.88	0.995
Plectropomus areolatus	4.69 ± 0.02	5.71 ± 0.76	6.20 ± 0.41	7.18 ± 0.61	0.992
Plectropomus pessuliferus	4.96 ± 0.38	5.98 ± 0.23	6.26 ±0.53	7.28 ± 0.88	0.979
Variola louti	4.74 ± 0.82	5.89 ± 0.21	6.58 ± 0.98	7.46 ± 0.24	0.995

Mean ± SD standard deviation.
r Correlation value

The changes of pH value of groupers muscles

The changes in pH values of groupers muscles during 18 days of iced storage are presented in Table 4. The initial pH values of all the 10 wild species of groupers stored in ice were ranged from 6.4 to 6.9. These results are in agreement with those of previously observed for other groupers species, such as wire-netting reef cod (*E. merra*) (Jeyasekaran *et al*., 2005) [14], brown spotted grouper (*E. chlorostigma*) (Jeyasekaran *et al*. 2008) [9], yellow grouper (*E. awoara*) (Li *et al*., 2011) [15], orange-spotted grouper (*E. coioides*) (Sharifian *et al*., 2014) [11], and giant grouper (*E. lanceolatus*) (Hsiao and Chang, 2017) [10]. The values of pH obtained in the present study were remained below the level of 7 in *C. sonnerati*, *E. epistictus*, *E. multinotatas*, *E. polylepis*, *P. pessuliferus* and *V. louti*, and showed no significant difference ($p < 0.05$) during the storage periods. A similar result has been previously reported in brown spotted grouper (*E. chlorostigma*) (Jeyasekaran *et al*., 2008) [9] and in vacuum packed yellow grouper (*E. awoara*) (Li *et al*., 2011) [15]. In contrast, the pH value was significantly increased ($p < 0.05$) and reached above the level of 7 during storage period in the rest of other groupers species, *C. oligosticta*, *E. areolatus*, *E. coioides* and *P. areolatus*. A comparable pattern of the increase in the pH value has been reported in orange-spotted grouper (*E. coioides*) during refrigerated storage period (Sharifian *et al*., 2014) [11] and in iced wire-netting reef cod (*E. merra*) (Jeyasekaran *et al*., 2005) [14]. These results revealed that the pH analyses could not be used as reliable indicators for the groupers quality during iced storage, which in agreement with previous study in yellow grouper (*E. awoara*) fillets stored under vacuum packaging at 0°C (Li *et al*., 2011) [15].

Table 4: Change in pH of groupers muscle during ice storage

Fish species	Storage period (Day)				
	0	**6**	**12**	**18**	**r**
Cephalopholis oligosticta	6.9	7.0	7.2	7.2	0.947
Cephalopholis sonnerati	6.6	6.6	6.6	6.7	0.775
Epinephelus areolatus	6.9	7.0	7.2	7.2	0.947
Epinephelus coioides	6.5	6.9	7.2	7.2	0.935
Epinephelus epistictus	6.4	6.4	6.5	6.5	0.894
Epinephelus multinotatas	6.6	6.7	6.7	6.9	0.923
Epinephelus polylepis	6.4	6.5	6.5	6.5	0.775
Plectropomus areolatus	6.7	7.0	7.2	7.2	0.929
Plectropomus pessuliferus	6.5	6.5	6.6	6.6	0.894
Variola louti	6.5	6.5	6.6	6.6	0.894

r Correlation value

Total Volatile Basic Nitrogen (TVB-N) of groupers muscles

The changes in TVB-N values of groupers muscles during 18 days of iced storage are presented in Table 5. The initial TVB-N value of all the 10 species of wild groupers muscles stored in ice was ranged from 21.0 to 26.9 mg/100 g. A high initial TVB-N were observed in a variety of fish, such as gutted and ungutted iced sea bass (*Dicentrarchus labrax*) (Papadopoulos *et al*., 2003) [23], iced whole and filleted rainbow trout (*Oncorhynchus mykiss*) (Chytiri *et al*., 2004) [28], refrigerated catfish fillet (*Ictalurus punctatus*) (Bonillaa *et al*., 2019) [29], vacuum packed haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) (Karim *et al*., 2011) [20]. However, our result revealed that the initial TVB-N value was higher than previously reported among various species of groupers such as spotted coral grouper (*P. maculatus*) with values of 10. 40 mg/100 g (Surti *et al*., 2001) [12], wire-netting reef cod (*E. merra*) with values of 4. 29 mg/100 g (Jeyasekaran *et al*., 2005) [14], brown spotted grouper (*E. chlorostigma*) with values of 16. 67 mg/100 g (Jeyasekaran *et al*., 2008) [9], wild white grouper (*E. aeneus*) with values of 15.4 mg/100 g (Özogul *et al*., 2008) [13], and orange-spotted grouper (*E. coioides*) with values of 13.10 mg/100 g (Sharifian *et al*., 2014)[11]. According to Connell [30] and Huss [31], the TVB-N level in freshly caught fish is generally range from 5 to 20 mg N/100g muscle. The slightly higher initial level of TVB-N in groupers muscle was in the upper acceptability limit for fresh fish, its increase in fish may be due to the activity of spoilage bacteria and endogenous enzymes (Kyrana *et al*., 1997; Chomnawang *et al*., 2007) [32, 33].

The TVB-N value of all the 10 species of wild groupers stored in ice were significantly increased ($p < 0.05$) during the storage period. Higher correlation coefficients were observed between TVB-N and storage time of all the 10 species of wild groupers ranged from ($r = 0.973$) to ($r = 0. 0.999$). The increase of TVB-N value has been previously reported in spotted coral grouper (*P. maculatus*) (Surti *et al*., 2001) [12], wire-netting reef cod (*E. merra*) (Jeyasekaran *et al*., 2005) [14], wild white grouper (*E. aeneus*) (Özogul *et al*., 2008) [13], yellow grouper (*E. awoara*) (Li *et al*., 2011) [15], orange-spotted grouper (*E. coioides*) (Sharifian *et al*., 2014) [11], and in giant grouper (*E. lanceolatus*) (Hsiao and Chang, 2017) [10].

A level of 30-35 mg /100g TVB-N in muscle is generally considered to be the acceptable limit for cold-water fish stored in ice (Connell, 1995) [30]. In the present study, the TVB-N value of all the 10 species of wild groupers muscles was exceeded the above limit of 30 mg/100g approximately 12 days of storage. In some studies among various species of groupers, TVB-N reached values of about 33.60 mg/100 g at day 14 in refrigerated orange-spotted grouper fillets (Sharifian *et al*., 2014) [11], 35.60 mg/100 g at day 19 in iced wire-netting reef cod (Jeyasekaran *et al*., 2005) [14] and 37.2 mg/100 g at day 20 in chilled spotted coral grouper (Surti *et al*., 2001) [12].

Based on these criteria, and agreement with (Hsiao and Chang, 2017) [10] that the groupers should not be stored than 12 days at ice. Moreover, the maximum periods of grouper consumption established by different authors are 10 days for refrigerated orange-spotted grouper fillets (Sharifian *et al*., 2014)[11] and 16 days for iced wild white grouper (Özogul *et al*., 2008) [13]. Meanwhile in some studies it never reached values above 12.94 mg/100 g even after 15 days storage of yellow grouper fillets stored under vacuum packaging at 0°C (Li *et al*., 2011) [15].

At the end of iced storage the TVB-N value of all the 10 species of wild groupers muscles were ranged from 35.5 to 45.4 mg/100 g. A similar patterns of TVB-N values were observed in iced wire-netting reef cod (35.60 mg/100 g) at day 19 (Jeyasekaran *et al*., 2005) [14], chilled spotted coral grouper (42.7 mg/100 g) at day 22 (Surti *et al*., 2001) [12], wild white grouper (50.5 mg/100 g) at day 19 (Özogul *et al*., 2008) [13] and chilled vacuum-packed giant grouper (75 mg/100 g) at day 15 (Hsiao and Chang, 2017)[10].

Our results revealed that the TVB-N could be a useful indicator for the groupers quality during iced storage. This statement is in agreement with previous studies proposed by Hsiao and Chang [10] for chilled vacuum-packed giant grouper (*E. lanceolatus*) fillets, Sharifian *et al*. [11] for refrigerated orange-spotted grouper (*E. coioides*) fillets and by Surti *et al*. [12] for chilled spotted coral grouper (*P. maculatus*) fillets.

The correlation between the total mesophilic and psychrotrophic aerobic bacterial counts and the TVB-N content of 10 species of wild groupers during storage in ice was assessed with the Pearson coefficient. Interestingly, strong and positive correlation coefficients were observed between TVB-N content and mesophilic and psychrotrophic bacterial counts (r =0.949 - 0.990), (r =0.963 - 0.994), respectively.

Table 5: Change in TVB-N content of groupers muscle during ice storage

Fish species	Storage period (Day)				
	0	6	12	18	r
Cephalopholis oligosticta	21.7	26.6	32.4	38.5	0.999
Cephalopholis sonnerati	25.2	27.1	31.3	35.5	0.987
Epinephelus areolatus	24.9	28.6	34.4	45.1	0.973
Epinephelus coioides	21.3	27.8	33.8	44.8	0.989
Epinephelus epistictus	26.9	29.8	37.9	45.4	0.983
Epinephelus multinotatas	26.8	29.6	38.5	44.8	0.983
Epinephelus polylepis	25.6	28.9	34.3	41.5	0.987
Plectropomus areolatus	21.0	25.5	31.2	35.3	0.998
Plectropomus pessuliferus	25.8	29.7	35.4	43.9	0.985
Variola louti	25.5	29.4	35.3	42.6	0.991

r Correlation value

Conclusion

The study focused on the pH, TVB-N content and the total mesophilic and psychrotrophic aerobic bacterial counts of 10 species of wild groupers stored in ice for 18 days. The results of this study showed that the total mesophilic and psychrotrophic aerobic bacterial counts and the TVB-N value of all the 10 species of wild groupers stored in ice were significantly increased ($p < 0.05$) during the storage period. Based on the results, the total mesophilic and psychrotrophic aerobic bacterial counts and the TVB-N methods used in this study displayed very strong correlations with storage time, and they may be considered as a useful indicators of the freshness quality of groupers muscle during ice storage. Further investigation is required to identify the bacterial communities in iced groupers muscle.

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References

1. Craig MT, Sadovy de Mitcheson YJ, Heemstra PC. Groupers of the World - A Field and Market Guide. National Inquiry Services Centre (NISC), Grahamstown, South Africa, 2011, 403.
2. Heemstra PC, Randall JE. Groupers of the world. FAO Fisheries synopsis no. FAO, Rome, 1993, 16(125).
3. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharma,2002:56:365-379.
4. Svanevik CS, Lunestad BT. Characterisation of the microbiota of Atlantic mackerel (*Scomber scombrus*). Int. J. Food Microbiol,2011:151:164-170.
5. Erkan N. Sensory, chemical, and microbiological attributes of sea bream (*Sparus aurata*): Effect of washing and ice storage. Int. J. Food Proper,2007:10:421-434.
6. Chotimarkorn C. Quality changes of anchovy (*Stolephorus heterolobus*) under refrigerated storage of different practical industrial methods in Thailand. J. Food Sci. Technol,2014:51:285-293.
7. Gram L, Huss H. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol,1996:33:589-595.
8. Gram L, Dalgaard P. Fish spoilage bacteria – Problems and solutions. Curr. Opin. Biotechnol,2002:13:262-266.

9. Jeyasekaran G, Anandaraj R, Ganesan P, Jeya Shakila R, Sukumar D. Microbiological and biochemical quality of grouper (*Epinephelus chlorostigma*) stored in dry ice and water ice. Int. J. Food Sci. Technol,2008:43:145-153.
10. Hsiao HI, Chang JN. Developing a microbial time–temperature indicator to monitor total volatile basic nitrogen change in chilled vacuum-packed grouper fillets. J. Food Process. Preser,2017:41:1-9.
11. Sharifian S, Alizadeh E, Mortazavi MS, Shahriari Moghadam M. Effects of refrigerated storage on the microstructure and quality of Grouper (*Epinephelus coioides*) fillets. J. Food Sci. Technol,2014:51:929-935. doi:10.1007/s13197-011-0589-4.
12. Surti T, Taylor A, Ma'ruf F. The effect of storage and tropical ambient temperature on the quality and shelf life of grouper (*plecteropomus maculatus*). Int. J. Food Sci. Technol,2001:36:517-522.
13. Özogul F, Özogul Y, Kuley E. Nucleotide degradation and biogenic amine formation of wild white grouper (*Epinephelus aeneus*) stored in ice and at chill temperature (4°C). Food Chem,2008:108:933-941.
14. Jeyasekaran G, Maeswari K, Ganesan P, Jeya Shakila R, Sukumar D. Quality changes in ice stored tropical wire-netting reef cod (*Epineohelus merra*). J. Food Process. Preserv,2005:29:165-182.
15. Li X, Li J, Zhu J, Wang Y, Fu L, Xuan W. Postmortem changes in yellow grouper (*Epinephelus awoara*) fillets stored under vacuum packaging at 0°C. Food Chem,2011:126:896-901.
16. Antonacopoulos N, Vyncke W. Determination of volatile basic nitrogen in fish: A third collaborative study by Western European Fish Technologists' Association (WEFTA). Zeitschrift fuer Lebensmittel Untersuchung und Forschung,1989:189:309-316.
17. Alfaro B, Hernandez I, Balino-Zuazo L, Barranco A. Quality changes of Atlantic horse mackerel fillets (*Trachurus trachurus*) packed in a modified atmosphere at different storage temperatures. J. Sci. Food Agric,2013:93:2179-2187.
18. Albertos I, Martín-Diana AB, Cullen PJ, Tiwari BK, Ojha SK, Bourke P, *et al*. Effects of dielectric barrier discharge (DBD) generated plasma on microbial reduction and quality parameters of fresh mackerel (*Scomber scombrus*) fillets. Innov. Food Sci. Emerg. Technol,2017:44:117-122.
19. De Alba M, Pérez-Andrés JM, Harrison SM, Brunton NP, Burgess CM, Tiwari BK. High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets. Innov. Food Sci. Emerg. Technol,2019:55:80-87.
20. Karim N, Kennedy T, Linton M, Watson S, Gault N, Patterson M. Effect of high pressure processing on the quality of herring (*Clupea harengus*) and haddock (*Melanogrammus aeglefinus*) stored on ice. Food Control,2011:22:476-484.
21. Albertos I, Martín-Diana AB, Cullen PJ, Tiwari BK, Ojha SK, Bourke P, *et al*. Shelf-life extension of herring (*Clupea harengus*) using in-package atmospheric plasma technology. Innov. Food Sci. Emerg. Technol,2019:53:85-91.
22. Nuñez-Flores R, Castro AX, López-Caballero ME, Montero P, Gómez-Guillén MC. Functional stability of gelatin-lignosulphonate films and their feasibility to preserve sardine fillets during chilled storage in combination with high pressure treatment. Innov. Food Sci. Emerg. Technol,2013:19:95-103.
23. Papadopoulos V, Chouliara I, Badeka A, Savvaidis IN, Kontominas MG. Effect of gutting on microbiological, chemical, and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. Food Microbiol,2003:20:411-420.
24. Viji P, Tanuja S, Ninan G, Lalitha KV, Zynudheen AA, Binsi PK, *et al*. Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. J. Food sci. technol,2015:52:3312-3321. doi:10.1007/s13197-014-1358-y
25. ICMSF (International Commission on Microbiological Specifications for Foods). Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications 2nd ed. University of Toronto Press, Toronto, 1986.
26. Kilinc B, Cakli S, Cadun A, Dinces T, Tolasa S. Comparison of effects of slurry ice and flake ice pretreatments on the quality of aquacultured sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) stored at 4°C. Food Chem,2007:104:1611-1617.
27. Kuvei FG, Khodanazary A, Zamani I. Quality index method (QIM) sensory scheme for gutted greenback grey mullet *Chelon subviridis* and its shelf life determination. Int. J. Food Proper,2019:22:618-629.
28. Chytiri S, Chouliara I, Savvaidis IN, Kontominas MG. Microbiological, chemical, and sensory assessment of iced whole and filleted aquacultured rainbow trout. Food Microbiol,2004:21:157-165.
29. Bonillaa F, Chouljenkoa A, Linb A, Youngb BM, Goribidanurb TS, Blakeb JC, *et al*. Chitosan and water-soluble chitosan effects on refrigerated catfish fillet quality. Food Biosci,2019:31:100426. doi: 10.1016/j.fbio.2019.100426
30. Connell JJ. Control of Fish Quality, 4th edn. London: Fishing News Books Limited, 1995.
31. Huss H. Fresh Fish: Quality and quality changes. Rome: Food and Agriculture Organization (FAO) of the United Nations, 1988.
32. Kyrana VR, Lougovois VP, Valsamis DS. Assessment of shelf-life of maricultured gilthead sea bream (*Sparus aurata*) stored in ice. Int. J. Food Sci. Technol,1997:32:339-347.
33. Chomnawang C, Nantachai K, Yongsawatdigul J, Thawornchinsombut S, Tungkawachara S. Chemical and biochemical changes of hybrid catfish fillet stored at 4C and its gel properties. Food Chem,2007:103:420-427.