Sensory, Microbiological and Chemical Assessment of Cod (*Gadus morhua*) Fillets during Chilled Storage as Influenced by Bleeding Methods

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Abstract—The effects of seawater and slurry ice bleeding methods on the sensory, microbiological and chemical quality changes of cod fillets during chilled storage were examined in this study. The results from sensory evaluation showed that slurry ice bleeding method prolonged the shelf life of cod fillets up to 13-14 days compared to 10-11 days for fish bled in seawater. Slurry ice bleeding method also led to a slower microbial growth and biochemical developments, resulting lower total plate count (TPC), H2S-producing bacteria count, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), free fatty acid (FFA) content and higher phospholipid content (PL) compared to those of samples bled in seawater. The results of principle component analysis revealed that TPC, H2S-producing bacteria, TVB-N, TMA and FFA were in significant correlation. They were also in negative correlation with sensory evaluation (Torry score), PL and water holding capacity (WHC).

Keywords—Bleeding method, chilled storage, microbial growth, sensory evaluation.

I. INTRODUCTION

ATLANTIC cod (*Gadus morhua*) is one of the most important commercial species in the fishery industry of Nordic countries. Cod has traditionally been sold as salted fish or frozen in form of whole fish or fillets. In recent years, the demand for fresh fillets of cod has been rapidly increasing [1]. However, the shelf life of fresh fish products is usually limited, mainly due to the microbial and enzymatic activities, leading to quality deterioration [2]. It is well-known that the shelf life of fish products is a function of many variables, including fish species, fishing ground, fishing and bleeding method and catching season as well as packaging method and storage temperature. The extension of shelf life of fresh fish products can allow transport products to distant markets, resulting in economic gain [3]. Therefore, certain techniques have been applied to extend the shelf life of fresh fish products such as chilled and superchilled storage, vacuum packaging and modified atmosphere packaging (MAP) of fish products or combination of these techniques [1], [4]-[6].

In general, bleeding of fish is carried out to remove most of the blood from the fish muscle [7]. Bleeding is thought to be one of the most important steps in the processing line of fish and has big influences on the overall quality of the final product. It has for example effect on the shelf life, taste and visual appearance of fresh fish products. If the fish has not been bled properly, spoilage occurs faster because residual blood in the fish muscle is an excellent nutrition for bacterial growth. The rupture of fine blood vessels in the fish flesh results also in bruises on the surface of fish fillets and is considered to be major quality defect. The amount of haemoglobin and myoglobin in the fish muscle is one of the main factors, affecting the development of undesirable discolouration of the fish flesh [8], [9] and unpleasant flavor [7]. Immediate bleeding of live fish can delay rigor-mortis state compared to the flesh of fish without bleeding during ice storage [10], [11]. Furthermore, it is well documented that haemoglobin and myoglobin of bloods have potential to accelerate lipid oxidation [9], mainly due to pseudo-lipoxygenase activities [12] and heme or non-heme released from haemoglobin and myoglobin autoxidation [9], [13]. Hence, choosing the most suitable bleeding method is important for assuring a good quality of the fish products and promoting economic gain [14].

Recently, there are different bleeding methods that have been applied depending on fish species such as asphyxiation, concussion, CO_2 anesthetization, electrical stunning and live-chilling [15]. Traditionally, cod is bled on-board by a deep throat cut through the main vein. Bleeding is then followed by gutting and washing in seawater before ice storage. From literature review, no information has been available regarding the effects of different bleeding methods on the quality changes of cod during chilled storage.

The present study was aimed at investigating the effects of bleeding method focused on the bleeding time and bleeding medium on the microbiological and chemical quality changes of cod fillets during chilled storage. The shelf life of cod fillets was also determined using sensory evaluation (Quantitative Descriptive Analysis (QDA) and Torry freshness score sheet).

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II. MATERIALS AND METHODS

A. Materials

The cod was caught by trawl by a commercial fishing vessel, trawler Sturlaugur H. Böðvarsson AK-10, in Eldeyjargrum (the South-West coast of Iceland), in November 2011. The average weight of the fish used in this study was 4.0 ±0.5kg. The fish was bled by four different bleeding methods as depicted in Table I and gutted on board, washed in seawater and stored in ice for 3 days before processing. The temperature of bleeding media was recorded using IButton Micro-T DS1922L temperature loggers (Dayton, OH, USA). The fish was beheaded, filleted and skinned in fish processing facility of HB Grandi in Reykjavik, Iceland. The fillets of four different groups were packaged in Styrofoam boxes with inner plastic bag and two bags of ice mats/packs (PromensTempra ehf., Hafnarfjordur, Iceland) on the top. The fish fillets were transported to the Matis laboratory in Reykjavik where the experiments were carried out.

TABLE I
BLEEDING MEDIUM AND BLEEDING TIME

Sample code	Bleeding medium	Bleeding time (min)
SW-5	Bleeding in seawater	5
SW-30	Bleeding in seawater	30
SI-5	Bleeding in slurry ice	5
SI-30	Bleeding in slurry ice	30

B. Storage and Sampling

The Styrofoam boxes of fish fillets were stored in a refrigerated cabinet at the temperature of -1 ±0.5°C. Samples were evaluated on the first, third, seventh, tenth and fourteenth day of storage. At each sampling point, three fillets of each group were collected for sensory evaluation, microbiological and chemical determination.

C. Sensory Evaluation

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel [16], and the Torry freshness score sheet [17] were used to assess cooked samples of cod. Eleven panel lists were trained according to the International Standards [18] for detection and recognition of odor, appearance, flavor and texture. The members of the panel were familiar with the QDA and Torry method and experienced in sensory analysis of cod. Each sensory attribute was evaluated and described the intensity using an unstructured scale (from 0 to 100%). The attributes were defined and described based on the previous studies [5], [6], [19] and are shown in Table II.

About 40g sample were taken from the loins and placed in aluminum boxes coded with three-digit random numbers. The samples were cooked in a steaming oven with air circulation (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100°C for 6min, and then served to the panel. Each panelist evaluated duplicates of each sample and maximum four samples per time. A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data collecting.

TABLE II
DESCRIPTION OF SENSORY ATTRIBUTES FOR COOKED SAMPLES OF COD

Sensory attribute	Short name	Description of attribute
Odor		
sweet	o-sweet	Sweet odor
shellfish, algae	o-shellfish	Shellfish, algae, characteristic fresh odor
vanilla/warm milk	o-vanilla	Vanilla, sweet heated milk
boiled potatoes	o-potatoes	Reminds of whole warm boiled potatoes
frozen storage	o-frozen	Freezer storage odor, refrigerator
table cloth	o-cloth	Reminds of a dirty, moist table cloth
rancid	o-rancid	Rancid odor
TMA	o-TMA	TMA odor, reminds of dried salted fish, amine
sour	o-sour	Sour odor, sour milk, spoilage sour, acetic acid
sulfur	o-sulfur	Sulfur, matchstick, boiled cabbage
Appearance		
color	a-color	Left end: light, white color. Right end: dark, yellowish, brownish, grey
heterogeneous	a-hetero	Left end: homogenous, even color. Right end: variegated, e.g. stains, spots, heterogeneous.
white precipitation	a-preci	White precipitation on the fish surface
Flavor		
salt	f-salt	Salty taste on tongue
metallic	f-metallic	Characteristic metallic flavor of fresh white lean fish (cod)
sweet	f-sweet	Characteristic sweet flavor of very fresh (boiled) white lean fish (cod)
fish oil	f-oil	Fresh fish oil, fresh liver (not rancid)
frozen storage	f-frozen	Freezer storage flavor, refrigerator, cardboard flavor
pungent	f-pungent	Pungent taste on tongue, bitter taste
rancid	f-rancid	Rancid flavor
sour	f-sour	Sour flavor, spoilage sour
TMA	f-TMA	TMA flavor, reminds of dried salted fish, amine
off-flavor	f-off	Strength of off-flavor (spoilage flavor/off-flavor)
Texture		
soft	t-soft	How firm or soft the fish is during the first bite
juicy	t-juicy	Left end: dry. Right end: juicy. Evaluated after chewing several times
tender	t-tender	Left end: tough. Right end: tender. Evaluated after chewing several times
mushy	t-mushy	Mushy texture, porridge
meaty	t-meaty	Meaty texture, meaty mouthfeel, crude muscle fibers
clammy	t-clammy	Clammy texture, tannin (dry red wine)
rubbery	t-rubbery	Rubbery texture, springy

D. Microbiological Analysis

Total plate count (TPC) and count for H2S-producing bacteria were evaluated according to the method of Gram, Trolle and Huss [20] with some modifications as described in Margeirsson, Magnusson, Sveinsdottir, Valtysdottir, Reynisson and Arason [19]. A 20g of minced flesh was mixed with 180g of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1min. After that, 1mL of 10-fold dilutions samples was transferred to the iron agar petri plates using pipettes and then petri plates were incubated at the temperature of 17°C for 5 days. The total colonies were

considered for total plate count and black colonies were considered for H_2S-forming bacteria count.

E. Chemical Determination

1. Water Content

Water content was determined as the weight loss during drying at 103 ±1°C for 4h according to ISO 6496 [21].

2. Water Holding Capacity (WHC)

Water holding capacity was determined by a centrifugation method [22]. The fish meat was coarsely minced in a Braun Mixer (Type 4262, Germany) for 10-15sec and 2g of the sample were weighed in the glass. Samples were centrifuged at 210xg for 5min at 4°C (Heraeus Biofuge Stratos Reconditioned 75005289R, Rotor 3335, DJB Labcare Limited, UK). Centrifugation loss of water was calculated as the difference in weight before and after centrifugation. The water holding capacity (expressed as percent WHC) was calculated as the ratio of remaining water compared to the water content in the sample before centrifugation.

3. Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine (TMA)

The method of Malle and Poumeyrol [23] was used for measuring total volatile basic nitrogen (TVB-N) and trimethylamine (TMA). TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. TMA was measured in trichloroacetic acid (TCA) extract by adding 20mL of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine.

4. Total Lipid Content (TL)

Lipids of the fish samples were extracted according to the Bligh and Dyer [24] method. The lipid content was determined gravimetrically and the results were expressed as a percentage of the weight of sample analyzed. The rest of extracts was used for determination of free fatty acid content and phospholipid content.

5. Free Fatty Acid Content (FFA)

Free fatty acid content was determined by the method of Lowry and Tinsley [25] with some modifications as described in Bernardez, Pastoriza, Sampedro, Herrera and Cabo [26], based on complex formation with cupric acetate-pyridine followed by absorbance reading at 715nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). The results were expressed as g FFA/100g lipids and calculated using a standard curve prepared from oleic acid.

6. Phospholipid Content (PL)

Phospholipid content of the fish muscle was determined on the lipid extraction [24] by using a spectrophotometric method [27], based on complex formation of phospholipid with ammonium ferrothiocyanate, followed by absorbance reading at 488nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). The results were expressed as a percentage of total lipid content and calculated using a standard curve prepared from phosphatidylcholine.

F. Statistical Analysis

The data sets obtained were analysed by General Linear Modelling (GLM) to investigate the effects of different bleeding methods on the microbiological and chemical quality of cod fillets during chilled storage. Means were compared by using ANOVA and Duncan's Multiple-Comparison Test using NCSS 2000 software (NCSS, Kaysville, Utah, USA). Significance of differences was defined at the 5% level ($p < 0.05$).

Principal Component Analysis (PCA) was performed on significant mean values of QDA sensory attributes and on all data obtained using Unscrambler® (Version 10.2, CAMO ASA, Trondheim, Norway). Full cross-validation with uncertainty test was performed in all validation models.

III. RESULTS AND DISCUSSION

A. Sensory Evaluation

PCA was performed on Quantitative Descriptive Analysis (QDA) attributes to investigate the main tendencies in the data and to assess the effects of different bleeding methods on the sensory quality of cod fillets during chilled storage. The first two components (PC1 and PC2) explained 90% of the total variation between the sample groups (Fig. 1). PC1 accounted for 82% of the total variation, mainly described the freshness/spoilage characteristics of samples during storage. The sample groups were located to the right side at the beginning of storage, but on the left side toward end of the storage period (Fig. 1 (a)). The freshness of samples was characterized by flavor and odor characteristics of fresh cod such as shellfish, sweet and vanilla odors and metallic and sweet flavors. Whereas, the spoilage sensory attributes were detected at the end of storage such as TMA and sulphur odors and TMA, sour, pungent and off-flavors as well as yellowish and brownish discoloration appearance (Fig. 1 (b)). These sensory attributes have been used as indicators for fish samples at the end of shelf life by several authors [3], [5], [6], [28]. It is interesting to see that the plots of fish samples bled by four different methods (SW-5, SW-30, SI-5 and SI-30) were located closed to each other during the first seven days of storage (Fig. 1 (a)), indicating the differences between the sample treatments were not significant. Nevertheless after storage for 14 days, the plots of samples bled in seawater (SW-5-D14 and SW-30-D14) were located far away from the plots of samples bled in slurry ice (SI-5-D14 and SI-30-D14). The samples bled in seawater were strongly described by the flavor and odor of TMA, sour and pungent as signs of spoilage. The results were in harmony with the results of microbial and chemical measurements (Figs. 3 and 5, respectively), where the TPC, H2S-producing bacteria count, TVB-N and TMA content of samples bled in seawater were significantly ($p<0.05$) higher than those of the samples bled in

slurry ice at the end of the storage period. Furthermore, PC2 primarily explained variation between samples with regard to the differences in texture attributes, accounting for 8% of total variation. Cod fillets appeared to be more tender and juicier at the beginning of storage. However, no significant (p>0.05) differences in texture attributes were found between the sample groups throughout the storage time. The sensory evaluation results of this study were in agreement with previous observations for shelf life study of fresh cod loins [5], [6], [19] and for shelf life study of fresh tilapia [4].
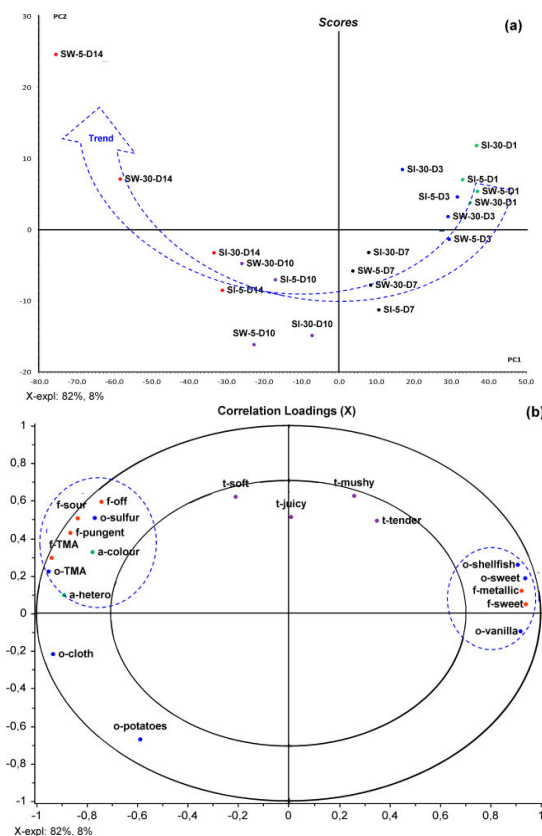

Fig. 1 Principle component analysis (PCA) of scores (a) and correlation loadings (b) for different sensory attributes of cooked cod samples. PC1 describes 82% of variation and PC2 describes 8%. Abbreviations: SW, seawater; SI, slurry ice; 5 and 30, bleeding time of 5 and 30 min; D1, D3, D7, D10 and D14, chilled storage for 1 day, 3 days, 7 days, 10 days and 14 days; f, flavor; o, odor; a, appearance and t, texture

The changes in Torry freshness score with storage time of four different sample groups are shown in Fig. 2. At the processing day, no difference in Torry score was observed between the sample treatments with the average value of 9. With storage time, the Torry score of the samples bled in slurry ice generally remained higher than that of the samples bled in seawater. Moreover, the results indicate the bleeding time (i.e. 5 and 30min) showed minor effect on the Torry score of the samples both bled in seawater and in slurry ice (Fig. 2). The average Torry score of 5.5 has been used as the limit for human consumption [29], [30]. According to this, the

maximum shelf life of SW-5 and SW-30 samples was 10-11 days, whereas the samples treated by slurry ice (SI-5 and SI-30) reached maximum shelf life of 13-14 days (Fig. 2). Similar findings have been reported by Margeirsson, Magnusson, Sveinsdottir, Valtysdottir, Reynisson and Arason [19] and Magnusson and Martinsdottir [31] who reported that the shelf life of cod fillets is about 10 to 12 days of storage. However, the shelf life of cod fillets is prolonged up to 17 to 21 days of storage if the fish fillets are packaged by MAP method in combination with superchilled storage [5], [6]. Cyprian, Lauzon, Johannsson, Sveinsdottir, Arason and Martinsdottir [4] demonstrated that the shelf life of tilapia fillets in air-packed, stored at -1 °C is 21 days. This might be attributed to the different fish species used, resulting in different biochemical composition that contributes effects on the shelf life of fish products.
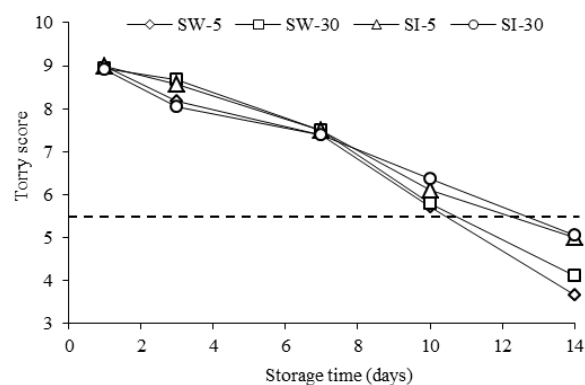

Fig. 2 Changes in Torry score of fish fillets during chilled storage as influenced by bleeding medium and bleeding time [SW-5 (◊), SW-30 (□), SI-5 (Δ) and SI-30 (○)]

B. Microbial Measurements

Generally, the prychrotrophic bacteria (TPC) and H2S-producing bacteria of fish sample in all treatments increased significantly (p<0.05) throughout the storage period (Fig. 3 (a) and (b), respectively). The initial TPC and H2S-producing bacteria count of all samples was satisfactory (TPC<log 5 CFU/g). At the end of the storage time, the SI-30 sample group had significantly (p<0.05) lower TPC and H2S-producing bacteria count compared to other groups. The values were in the range of log 6.5 to log 7.5 CFU/g for TPC (Fig. 3 (a) and log 6.0 to log 8.0 CFU/g for H2S-producing bacteria count (Fig. 3 (b)). The results were in agreement with those reported in previous publications [5], [6], [19]. Furthermore, the TPC and H2S-producing bacteria count of samples treated in seawater (SW-5 and SW-30) were higher compared to those of samples treated in slurry ice (SI-5 and SI30) during storage. This might be attributed to the difference in temperature of seawater and slurry ice used for bleeding of fish. Where, the temperature of seawater was higher than that of slurry ice (i.e. seawater of -0.8 ± 0.1°C versus slurry ice of -1.5 ± 0.1°C), leading to higher growth rate of bacteria in the samples treated in seawater.

It is well documented that H2S-producing bacteria such as *Shewanella putrefaciens* along with *Pseudomonas* spp. and *Photobacterium phosphoreum* are considered as main spoilage bacteria in fish [32], [33]. They are able to utilize trimethylamineoxide (TMAO) as the terminal electron acceptor in an anaerobic respiration, resulting in off-flavor and off-odor due to formation of trimethylamine (TMA) [34], [35]. Therefore, the results of microbial determination were strongly related to the results of sensory and TMA evaluation (Fig. 1 (b) and 5 (b), respectively).
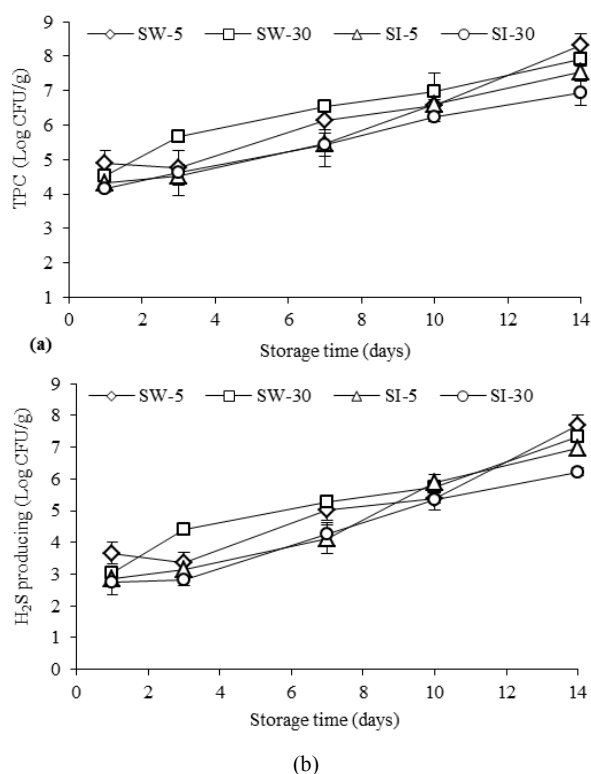

(a)

(b)

Fig. 3 Changes in TPC (a) and H$_2$S-producing bacteria (b) of cod fillets during chilled storage as affected by different bleeding methods [SW-5 (◊), SW-30 (□), SI-5 (Δ) and SI-30 (○)]

C. Chemical Measurements

1. Water Content and Water Holding Capacity (WHC)

As expected, the water content of all fish samples was in the range of 81.0 to 82.0% (Table III) and water holding capacity was varied from 85.0 to 90.0% (Fig. 4). The results were in agreement with previous observations [1], [19], [36]. Bleeding methods did not show any effects on the water content, but did on the other hand have an effect on WHC. Both fish samples bled in slurry ice (SI-5 and SI-30) had a slightly higher (p>0.05) WHC value compared to that of the fish samples bled in seawater throughout the storage time (Fig. 4). The water content of the fish muscle in all treatments was stable during storage; whereas the WHC decreased slightly. The decrease in WHC is thought to be mainly due to the biochemical changes in the fish muscle occurred during chilled storage such as protein denaturation [37], [38] and

lipid oxidation [39]. Furthermore, the microbial and enzymatic activities are also thought to have a negative effect on the WHC. This is more obvious to see that lower WHC content (Fig. 4) and higher total plate count (TPC) and higher H2S-producing bacteria count were observed in the samples bled in seawater (SW-5 and SW-30) (Fig. 3 (a) and (b), respectively).

TABLE III
CHANGES IN WATER CONTENT OF COD FILLETS

Sample	Storage time (days)				
	1	3	7	10	14
SW-5	81.9	81.5	81.7	81.9	82.1
SW-30	81.7	81.7	81.8	81.5	81.8
SI-5	81.6	81.5	81.7	81.9	81.5
SI-30	81.1	81.4	81.8	81.5	81.5

No significant difference was found in water content between the treatments at each sampling point and between storage times within the treatment.
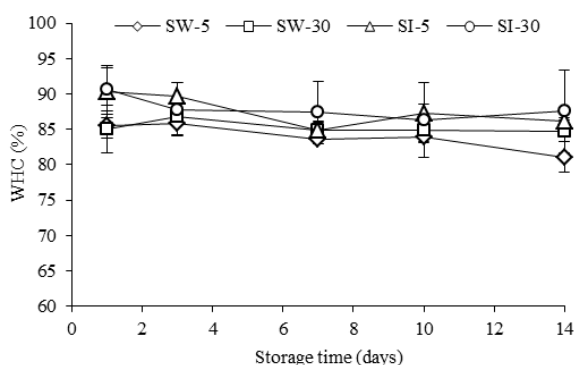

Fig. 4 Changes in WHC of cod fillets during chilled storage as a function of bleeding medium and bleeding time [SW-5 (◊), SW-30 (□), SI-5 (Δ) and SI-30 (○)]

2. Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine (TMA) determination

The total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) are the most widely used as biochemical indicators for assessment of shelf life of fresh fish products [40]. TVB-N includes trimethylamine, dimethylamine, ammonia and other volatile basic nitrogenous compounds. TMA is a pungent volatile amine often associated with the typical fishy odors and flavors of fish spoilage [41]. Generally, the TVB-N and TMA content of fish sample in all treatments increased slightly during the first seven days of storage and no significant (p<0.05) differences were observed between the treatments (Fig. 5 (a) and (b), respectively). During the subsequent storage time, the TVB-N and TMA content of samples treated in sweater (SW-5 and SW-30) increased significantly (p<0.05), whereas no significant increases were noted in the samples treated in slurry ice (SI-5 and SI-30). The TVB-N and TMA values of the SW-5 and SW-30 groups were significantly (p<0.05) higher compared to those of the SI-5 and SI-30 groups. Furthermore, the bleeding time (i.e. 5 and 30min) significantly affected the TVB-N and TMA contents of samples treated in seawater, but not for samples treated in slurry ice. This is believed to be attributed

to the higher microbial and endogenous enzyme activities in seawater treatments, mainly due to higher temperature and longer bleeding time. It is well-known that TMA is transformed from TMAO by bacteria [32], [33], [35] as well as bacterial enzyme TMA oxidase and endogenous enzyme [42]. At the end of the storage period, the TVB-N and TMA values were about 40mg N/100g and 32mg N/100g for seawater bleeding groups and about 20mg N/100g and 10mg N/100g for slurry ice bleeding groups, respectively. The results were in comparison with those noted in previous publications [5], [6], [19]. According the limitations for human consumption of TVB-N and TMA values (i.e. 30-35mg N/100g and 10-15mg N/100g, respectively), the fish bled in seawater were unfit for human consumption after storage for 10-11 days, but fish bled in slurry ice were still fit after storage for 14 days. This was in harmony with the sensory evaluation results (Torry score, Fig. 2).
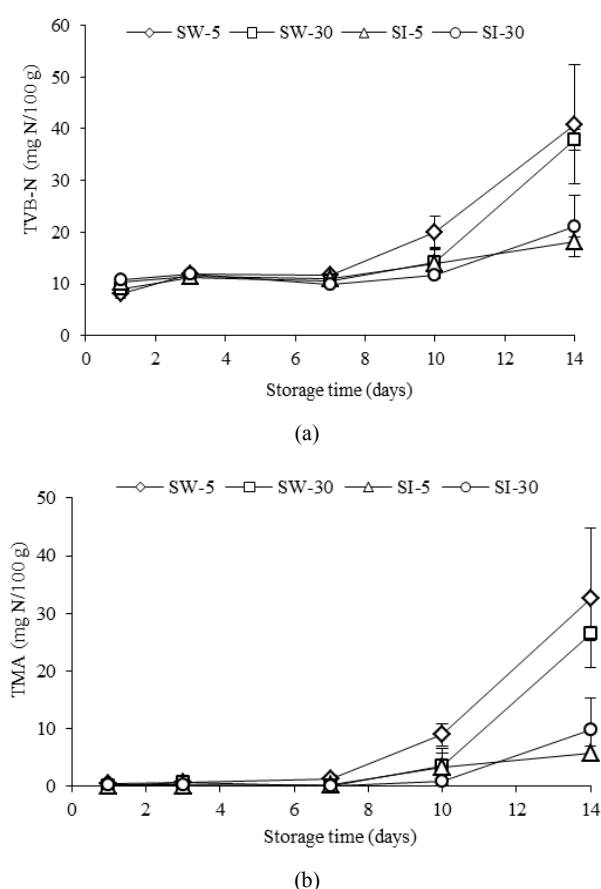

Fig. 5 Changes in TVB-N (a) and TMA (b) of cod fillets during chilled storage as affected by bleeding medium and bleeding time [SW-5 (◊), SW-30 (□), SI-5 (Δ) and SI-30 (○)]

3. Free Fatty Acid (FFA) and Phospholipid (PL) Determination

The changes in free fatty acid (FFA) and phospholipid (PL) content of fish samples during chilled storage as influenced by different bleeding methods are shown in Fig. 6 (a) and (b),

respectively. As expectation, the FFA content of fish samples in all treatments increased significantly (p<0.05) throughout the storage period, whereas the PL content of all samples dramatically (p<0.05) decreased. Generally, higher FFA content and lower PL content were noted in the samples treated in seawater (SW-5 and SW-30) compared to those of the samples treated in slurry ice (SI-5 and SI-30). Furthermore, the bleeding time seems to have a minor effect on the development of FFA and the reduction of PL.
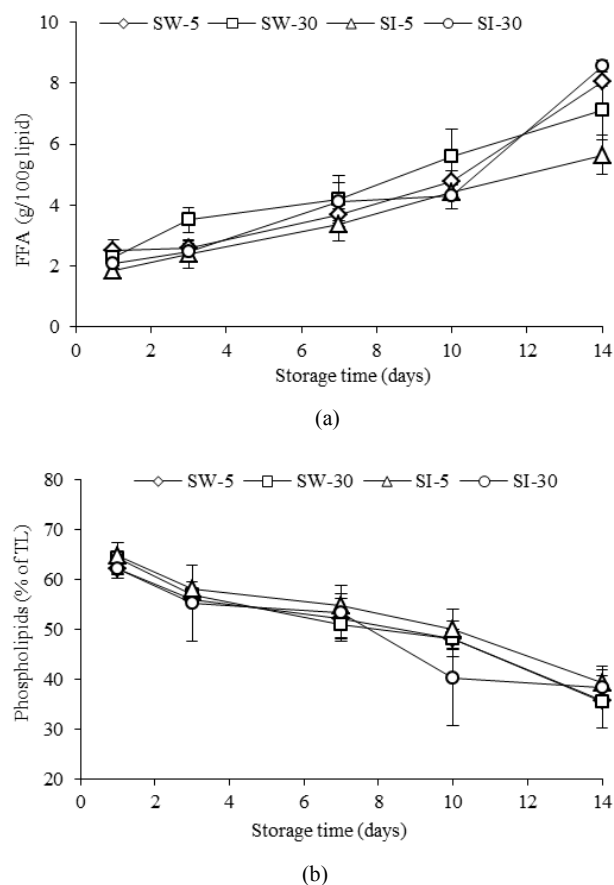

Fig. 6 Changes in FFA (a) and PL (b) of cod fillets during chilled storage as influenced by different bleeding methods [SW-5 (◊), SW-30 (□), SI-5 (Δ) and SI-30 (○)]

The results indicate that the rate of lipid hydrolysis in the fish samples treated in seawater was higher than that in the fish samples treated in slurry ice. It has been well demonstrated during storage of fish products, lipid in the fish muscle is hydrolyzed mainly caused by microbial enzyme activity such as lipases and phospholipase [43], [44] and non-microbial enzyme activity (i.e. natural lipase present in the fish muscle) as well as spontaneous lipid hydrolysis [45]. Therefore, higher microbial growth in the samples treated in seawater could be the main cause of higher lipid hydrolysis rate. In addition, higher temperature in the seawater may contribute an effect on lipid hydrolysis. Moreover, the decrease in PL content reveals that the lipid degradation

occurred during storage. This resulted in a decrease in lipid content in all samples (data not shown).
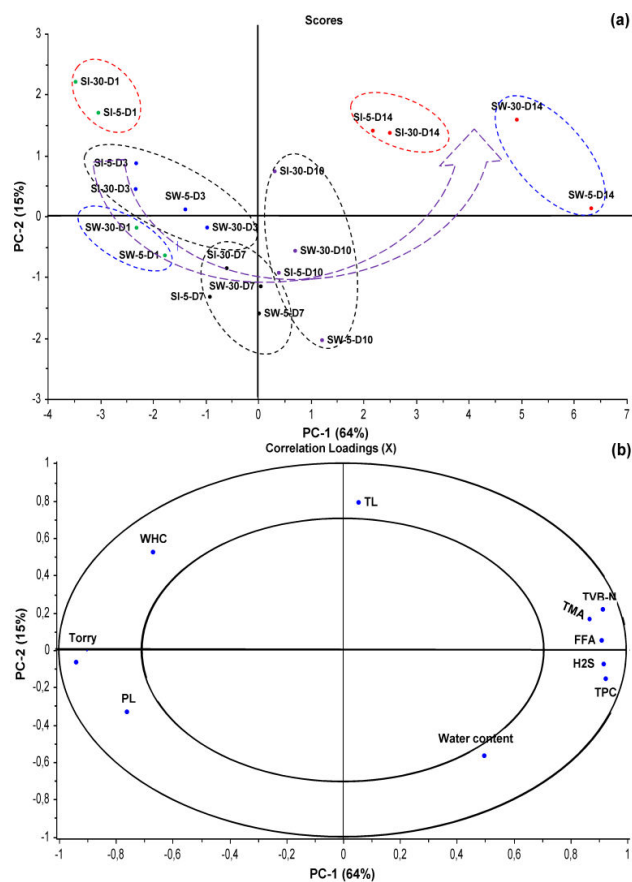
D. Multivariate Data Analysis

Fig. 7 Principle component analysis (PCA) of scores (a) and correlation loadings (b) for different quality measurements of cod fillets during chilled storage. PC1 describes 64% of variation and PC2 describes 15%. Abbreviations: SW, seawater; SI, slurry ice; 5 and 30, bleeding time of 5 and 30 min; D1, D3, D7, D10 and D14, chilled storage for 1 day, 3 days, 7 days, 10 days and 14 days

PCA was carried out in order to gain an overview of the similarities and differences among the variables. The scores and correlation loadings from the first two components can be seen in Fig. 7 (a) and (b), respectively. The PC1 described 64% of total variation, mainly due to the changes in quality of fish during storage. The PC2 accounted for 15% of total variation and described the differences between the bleeding methods. It is interesting to note that the biochemical changes in the fish muscle (i.e. TVB-N, TMA and FFA) were significantly correlated with the microbial growth (i.e. TPC and H2S-producing bacteria count). In addition, the microbial and biochemical changes were inversely correlated with the sensory evaluation (Torry score), PL and WHC content (Fig. 7 (b)).

IV. CONCLUSIONS

The results of the present study indicate that bleeding medium significantly affected the sensory, microbiological and chemical quality of cod fillets during chilled storage, but bleeding time did on the other hand show a minor effect. The seawater bleeding method resulted in a faster microbial (TPC and H2S-producing bacteria count) and biochemical (TVB-N, TMA, FFA and PL) development compared to slurry ice bleeding method, leading to a shorter shelf life (10-11 days for seawater bleeding versus 13-14 days for slurry ice bleeding). At the end of the storage period, the samples treated in slurry ice for 30min (SI-30) remained the highest quality. This revealed that alternative bleeding method (in slurry ice) is a better method compared to traditional one (in seawater) in terms of remaining better quality and longer shelf life. Furthermore, the results also indicate that the microbial growth has positive correlation with biochemical changes and negative correlation with sensory changes.

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