Influence of frozen storage time and thawing methods on the microflora of thawed Nile tilapia fillets

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Abstract. This study aimed to find out the influence of frozen storage time and thawing methods on the counts of coliforms, E. coli, Pseudomonas spp., and total viable counts (TVC) of thawed de-skinned Nile tilapia (Oreochromis niloticus) fillets. Individual quick frozen fillets of size 120-170 g, packed in polyamide packs (2 fillets/pack), were stored at -18 ± 2 °C. Samples were taken for thawing experiments every month (months 0, 1, 2, … and 8). Four different defreezing environments, namely cold air (7 ± 1 °C), air at ambient temperature (30 ± 1 °C), cold water (8 ± 1 °C), and water at normal temperature (25 ± 1 °C), were investigated. All experiments were triplicated. No E. coli was detected in all thawed samples. No differences (p > 0.05) were observed in coliforms, E. coli, Pseudomonas spp., and TVC of the newly stored samples (month 0) after thawing by the four studied methods. No significant effects (p > 0.05) of storage time (months 1, 2, and 3) and thawing methods on the coliforms and Pseudomonas quantity of tilapia fillets were found. Thawing by cold air showed to be the most suitable mean, which resulted in significantly lower TVC (p < 0.05) compared to defrosting in ambient air or in water at normal temperature. Meanwhile, air at ambient temperature showed to be the worst medium, giving the highest TVC in the fillets after thawing. The results supported a slow thawing method (e.g. by cold air) for this fillet product or similar ones, which are processed and frozen pre-rigor mortis.

Keywords: frozen storage time; microflora; Nile tilapia; thawing methods.

1. Introduction
Tilapia is known as a low-fat and high-quality protein fish species. Global production of tilapia and other cichlids was over 5.88 million tons (including 4.130 million tons of Nile tilapia) from aquaculture and 0.837 million tons (including 0.279 million tons of Nile tilapia) from capture in 2017 [1]. Top producers of tilapia in 2017 were China, Indonesia, Egypt, Brazil, the Philippines, Bangladesh, Vietnam, and Thailand [2]. Total farmed tilapia sales in 2018 exceeded 12 billion USD, and expected to be 9 million tons in volume and over 25 billion USD in value in 2028 [2]. Tilapia fillets, produced from alive farmed fish, have gained popularity with significantly increased price worldwide [2]. Fish fillets can be processed and purchased in fresh form, but are commonly distributed firstly in frozen form, which may be then subjected to thawing and marketing as chilled/fresh products at the end of the supply chain (e.g. at retailers) [3].
As common practices, farmed tilapia are put into processing when the fish is still alive, and with a quick rate of production, individually quick frozen (IQF) fish fillets are often in pre-rigor stage when they come to frozen storage. This means that care must be taken during the later thawing process, when rigor mortis can take place (thaw rigor [4]) in order to keep the product quality, minimize drip loss and microbial growth. Though there have been some suggestions that defreezing might help to reduce bacterial load [4], due to a so called osmotic shock; however, that decrease trend has not been proved yet. Thawing methods can be divided into two main principal types, conventional methods use heat transfer via product boundary layers (e.g. convection, conduction, condensation, or radiation with air or water media), while the newer concept generate heat within the product (e.g. high voltage electric field (HVEF) thawing, Ohmic thawing, microwave thawing and radio frequency systems) [5], [6]. Both types have their own strengths and weaknesses; however, the latter types have not been widely implemented in large-scale [5], mainly due to higher investment costs and more sophisticate operation, and in some methods because of inhomogeneous/uneven product heating [6].

This study was aimed to find out the influence of frozen storage time and some common conventional thawing methods, namely cold air (7 ± 1 °C), air at ambient temperature (30 ± 1 °C), , cold water (8 ± 1 °C), and water at normal temperature (25 ± 1 °C), , on the counts of hygiene indicators (e.g. coliforms and E. coli), and spoilage organisms (e.g. Pseudomonas spp. and psychrotrophic total viable counts (TVC)) of thawed de-skinned Nile tilapia (Oreochromis niloticus) fillets.

2. Materials and methods

2.1. Materials
De-skinned farmed Nile tilapia (Oreochromis niloticus) fillets (120-170 g/fillet) were bought in IQF form from a processing company in Mekong Delta, Vietnam. Frozen fillets, on the day of processing, was packed in 30-kg insulated boxes from expanded polystyrene, topped with frozen gel mats and tightly covered with lids. Fish boxes were moved to the laboratories in Nha Trang city within 16 h by car. Fillets, after arriving at the laboratories, were packed in polyamide (PA) packs (2 fillets/pack) and stored at -18 ± 2 °C for further experiments.

2.2. Methods

2.2.1. Sampling, handling & replication
Samples were taken for thawing experiments every month (months 0, 1, 2, … 8). Month 0 was considered month of beginning the cold storage at -18 ± 2 °C at the laboratories.

Fish fillets in PA packs were placed in the thawing media. The defreezing process was carried out until the fillet temperature was around 0-1 °C and there was no ice crystal observed on the product surface.

Four different thawing environments were investigated:

- **Method I: Cold air (7 ± 1 °C).** The thawing was done in a refrigerator with controlled temperature. The method could be classified as thawing in still air [5, 6];
- **Method II: Air/Still air at ambient temperature (30 ± 1 °C);**
- **Method III: Cold water (8 ± 1 °C).** Tap water and crushed ice were mixed to prepare a water medium of desired temperature before deeping the fish packs for thawing. Medium temperature was checked and ice was added to maintain the temperature within the required range throughout the process.
- **Method IV: Water at normal temperature (30 ± 1 °C).**

Digital penetration probe thermometers (TFA Dostmann, Germany) were used to check the defreezing media and product temperatures. Loggers DS1922L-F5 iButton® (Maxim Integrated Products, Inc., CA) were applied to monitor the temperatures of fish fillets and thawing media at 10-min intervals.
All experiments were triplicated, for each replicate 6 fillets were used (for microbiological, sensory and chemical analyses, the latter’s results are not reported in this paper).

2.2.2. Microbial analyses
Plate count agar (Merck, Germany) was used to determine TVC. Enumeration of presumptive pseudomonads was performed using Pseudomonas Agar Base (Merck, Germany) with Cephaloridine Fucidin Cetrimide selective Agar Supplement (Merck, Germany). Spread-plating was used for both media. Plates were incubated at 19 ± 1 °C for 5 days.

Coliforms were grown on Eosin methylene blue (EMB) Agar [7] (HiMedia, India), while *E.coli* was on Violet Red Bile Agar (VRB-A) [8] (HiMedia, India). The samples were incubated at 35 ± 1 °C for 24 h.

Microbial counts were reported as average values of colony-forming units per gram (CFU/g).

2.2.3. Sensory evaluation
Quality index method (QIM) using a modified scheme for tilapia fillets [9] was used to assess the quality of thawed fillets as a comparison to microbiological methods. The scheme is composed of 6 attributes, namely Colour and Mucus of the skin side, Odour, Colour, Texture and Stickiness of the flesh (fillet side), with the total score or quality index (QI) of 13. Lower scores and QI indicate better sensory quality of the product.

Two defrozen fillets of each sample time from each thawing method were used at every evaluation. Three-digit random numbers were attached to every single fillet for coding purpose. Three panellists, which are staffs of Nha Trang University, familiar with tilapia QIM scheme performed the analysis.

2.2.4. Data Analysis
Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) was implemented to calculate means and standard deviations and to build graphs.

One-way ANOVA (analysis of variance) and post hoc Tukey’s test were conducted with the software SPSS version 17.0 (SPSS, Chicago, Il, USA) to compare means at a 0.05 statistical significance level.

3. Results and discussion

3.1. Influence of thawing methods on the microflora counts of thawed fillets
No *E. coli* was detected in all thawed samples, which signaled good hygiene conditions of the frozen products and defreezing process. The absence of *E. coli* is understandable since low temperatures below 4 ± 1 °C were found to be not favorable for the indicated bacteria [10].

The average counts and their standard deviations of Coliforms, *Pseudomonas* spp., and TVC of fillets, thawed by 4 different methods: I - by cold air (7 ± 1 °C); II - by air at ambient temperature; III - by cold water (8 ± 1 °C); and IV - by water at normal temperature, are shown in Figure 1 and Figure 2, for every cold storage month, from month 0 to month 8. In general, Coliforms counts in the fillets, thawed by the 4 studied media, were not significantly different (p > 0.05) (Figure 1 and Figure 2), except for the 6th month samples, where Coliforms in the fillets thawed by ambient air were found with significantly higher counts (p < 0.05) compared to those by other media (Figure 2).

Thawing in air at ambient temperature (method II) showed to be the worst scenario to defreeze tilapia fillets, since it resulted in heavier TVC and *Pseudomonas* spp. loads in the thawed product, which became obvious (p < 0.05) since month 4th of storage (Figure 1 and Figure 2). This can be explained by the fact that high temperature handling facilitates bacterial growth in food [11] and seafood, e.g. the cases of de-skinned Pangasius fillets [12], tropical shrimp (*Penaeus notialis*) [13], and turbot (*Psetta maxima*) [14].

Thawing in water at normal temperature also resulted in distinct numbers of TVC (p < 0.05) in defrozen samples (of 7th month storage).
3.2. Influence of frozen storage time on the microflora counts of thawed fillets

To better demonstrate the effect of cold storage time on bacterial loads in thawed fillets, the average counts and their standard deviations of Coliforms, *Pseudomonas* spp., and TVC are shown for each of the thawing medium in Figure 3-6. No cold storage time effect was found for Coliforms in fillets thawed by the 4 studied media; for *Pseudomonas* spp. in fillets thawed by cold water (methods III, Figure 5); and for TVC in fish thawed using water (methods III and IV, Figure 5 and 6).

For the thawing in cold air (method I), it can be observed from Figure 3 that no storage time effect on *Pseudomonads* counts was observed. TVC was affected with the pattern similar to “chilled storage”: growth, stationary, and death phases (Figure 3). For the defreezing in ambient air (method II), the numbers of *Pseudomonas* spp. and TVC significantly dropped ($p < 0.05$) after 3 months and 6 months, respectively (Figure 4). It seems that there might be a combination effect of frozen storage time and thawing methods, that needs further investigation.
Figure 3. Microbial counts of tilapia fillets, thawed by method I in cold air (7 ± 1 °C), after 0-8 months of cold storage (at -18 ± 2 °C). For the same type of bacteria/line, different letters show significant differences (p < 0.05) of certain microflora mean counts between months of cold storage.

Figure 4. Microbial counts of tilapia fillets, thawed by method II in air at ambient temperature, after 0-8 months of cold storage (at -18 ± 2 °C). For the same type of bacteria/line, different letters show significant differences (p < 0.05) of certain microflora mean counts between months of cold storage.

Figure 5. Microbial counts of tilapia fillets, thawed by method III in cold water (8 ± 1 °C), after 0-8 months of cold storage (at -18 ± 2 °C). For the same type of bacteria/line, different letters show significant differences (p < 0.05) of certain microflora mean counts between months of cold storage.
3.3. Influence of cold storage time and thawing methods on the sensory quality of thawed fillets, in comparison with the microbial counts

The sensory quality of thawed fillets were assessed with the modified QIM scheme for de-skinned tilapia fillets. Scores of organoleptic attributes and quality index (QI) are shown for samples, defrosted by 4 different methods, after being stored at -18 ± 2 °C for e.g. 0, 1, 2, and 5 months (Figure 7-10).

The sensory quality of 0 month storage fish fillets, thawed by the 4 methods, were not differed (p > 0.5) from each other (Figure 7). However, the effect of thawing media became obvious after 1 month of cold storage, e.g. the colour of fillets, which were thawed in normal water was significantly changed (p < 0.05) compared to those by other methods (Figure 8). Similar, after 2 months of storage, the scores of colour and odour attributes and the quality index QI of the fillets defrosted in water at normal temperature increased significantly (p < 0.05), in comparison with the other 3 media (figure 9). Longer storage (e.g. for 5 months) showed clear influence of thawing temperature on the sensory quality of fish (Figure 10). The results indicated that thawing at normal/ambient temperature (both by air and water) reduced significantly (p < 0.05) the sensory quality of thawed fillets. Thawing by cold air seemed to be the most suitable method for tilapia fillets with the lowest QIM score. Again, sensory results show that there was possible interaction effects of the two factors, i.e. cold storage time and thawing methods, on the defrosten product quality. Similarly, Çankıriligil and Erbay (2016) [15] found that thawing of frozen Black Sea trout (Salmo trutta labrax) fillets in a refrigerator (+4 °C in 12 h) is more reliable, regarding preserving the product colour, than defrosting in room temperature (25 °C for 2 h), or in water (15 °C for 20 h), or in microwave (for 6 min). Genç, Esteves, Aníbal, Diler (2015) [16] also indicated that thawing meagre fillets in the refrigerator (+4 °C for 6 h) provided better fish quality as opposed to other methods, e.g. in the air at ambient temperature (+16 °C for 3.5 h), in water (+16 °C for 5 min), and in microwave oven (for 15 min at ca. 90 W). Cold air defreezing (5-6 °C for 5 h), rather than water thawing (25 °C for 30 min), and in microwave (1-2 min), was also recommended for pink shrimp as the first method provided better shrimp quality [17].
Figure 7. Quality index method (QIM) sensory scores of tilapia fillets, thawed by 4 different methods: I - by cold air (7 ± 1 °C); II - by air at ambient temperature; III - by cold water (8 ± 1 °C); and IV - by water at normal temperature, after no cold storage.

Figure 8. Quality index method (QIM) sensory scores of tilapia fillets, thawed by 4 different methods: I - by cold air (7 ± 1 °C); II - by air at ambient temperature; III - by cold water (8 ± 1 °C); and IV - by water at normal temperature, after 1 month of cold storage (at -18 ± 2 °C). For the same attribute/column, different capital letters show significant differences (p < 0.05) of certain attribute/index mean scores between thawing media.
Figure 9. Quality index method (QIM) sensory scores of tilapia fillets, thawed by 4 different methods: I - by cold air (7 ± 1 °C); II - by air at ambient temperature; III - by cold water (8 ± 1 °C); and IV - by water at normal temperature, after 2 months of cold storage (at -18 ± 2 °C). For the same attribute/column, different capital letters show significant differences (p < 0.05) of certain attribute/index mean scores between thawing media.

Figure 10. Quality index method (QIM) sensory scores of tilapia fillets, thawed by 4 different methods: I - by cold air (7 ± 1 °C); II - by air at ambient temperature; III - by cold water (8 ± 1 °C); and IV - by water at normal temperature, after 5 months of cold storage (at -18 ± 2 °C). For the same attribute/column, different capital letters show significant differences (p < 0.05) of certain attribute/index mean scores between thawing media.
Combining the findings on microbial and sensory indicators, slow defreezing in cold air is recommended for tilapia fillets to control microbial load and avoid negative effect of rigor during this process. The suggestions are supported by good thawing practices, if pre-rigor seafood stored in frozen stage for less than 8 weeks, then it needs a controlled slow thawing as a high temperature process can cause very strong rigor mortis, leading to fillet gaping and loss of drip [4]. Furthermore, water based thawing methods are not recommended for fish fillets since they can cause unacceptable soft texture and reduce product quality [6].

It should be noticed that each thawing method could be suitable for certain product, for instance, for eel water thawing (at 14 °C for 1.5 h) had the best quality-preserving effects on its physical, chemical, and microbiological quality, compared to thawing in air at 4 °C for 4 h, air at 15 °C for 2 h, and microwave oven for 5 min [18]. Microwave (160 W, 7 min) and water thawing (12.1 ± 0.8°C, 1.5 h) gave better quality of defrosted whole bonito (Sarda sarda), than thawing in the refrigerator (at 4 ± 2°C, 22 h) [19]. Thawing frozen headed and gutted pre-rigor Atlantic cod (Gadus morhua) by either water with and without air circulation, or contact defrosting in a converted plate freezer might produce fillets with acceptable quality and safety [20].

4. Conclusions
Defreezing at normal temperature (by air or water) resulted in significantly higher counts of TVC and Pseudomonas spp. No frozen storage time effect was found on Coliform counts of thawed fillets, defrozen by different methods. A slow defrosting by cold air (7 ± 1 °C) showed to be the most suitable method for this fillet product or similar ones, which are processed and frozen pre-rigor mortis. Thawed fillet quality might be influenced by an interaction between frozen storage time and thawing methods, which requires further research.

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