# Quality of Yellowfin Tuna during Storage in Liquid Ice and Crushed Block Ice

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## Abstract:
This study aimed to compare the quality of yellowfin tuna, cooled and stored in two different media, namely liquid ice and traditional crushed block ice. Sensory evaluation by quality index method (QIM) and control methods, as well as total volatile basic nitrogen (TVB-N) determination were conducted for fish samples over the course of storage. It was observed that tuna chilled and stored in liquid ice (containing 3.5% NaCl and 48% initial ice mass) at a fish to ice ratio of 1/2 w/w was the best to retain its freshness and shelf life (27 days); fish refrigerated in liquid ice and preserved in crushed ice (fish/crushed ice= 1/1.2 w/w) had a storage life of 15 days; while the one with traditional icing resulted in the worst quality and the shortest shelf life of 6 days. The study confirmed the advantage of liquid ice in fast cooling and super chilled storage of seafood.

## Keywords:
Yellowfin tuna, sensory, total volatile basic nitrogen, liquid ice, crushed ice

## 1. Introduction
According to BusinessWire (BusinessWire, 2021), global tuna fish market will grow from US$ 26.1 billion in 2020 to US$ 31.7 billion by 2027. Yellowfin tuna (Thunnus albacares), an important commercial tuna fish species, has been found in all warm waters of the world, apart from the Mediterranean. The global production of yellowfin tuna was 1.5 million metric tons (MT) in 2018, accounting for US$4.4 billion in dock value and nearly US$16 billion as final value (Rattle, 2020).

As for Vietnam, tuna industry is among the most important ones, which bring high value to the seafood exportation of the country. The total tuna export of Vietnam in 2020 and in the first four and a half months of 2021 were nearly $US 649 million (VASEP, 2021b) and over $US 186 million (VASEP, 2021a), respectively. Yellowfin tuna has mainly been caught by long-lining and/or hand-lining with artificial light (since 2011), the later shortens catching time to 2/3 but causes higher quality degradation compared to the earlier (Tran Viet Hung, 2020). Recent application of electric pulse generators to shock the fish before landing on-board has improved tuna quality (Nguyen Trong Luong et al., 2019). However, inadequate equipment, improper handling and preservation techniques and practices have resulted in 20-21% post-harvest losses (Nguyen Trong Luong et al., 2021). It is a common procedure that tuna is cooled and stored by crushed block ice in one step, which results in slow chilling and very low sashimi-grade yield (Nguyen Trong Luong et al., 2021).

Liquid ice, a water-binary system of ice particles and an aqueous (salt) solution to decrease its freezing point and achieve subzero temperatures (Piñeiro et al., 2004), has shown advantageous over traditional ice in term of faster cooling rate and better-quality retention of seafood.

This study aimed to investigate the effect of various cooling and storage media, namely liquid ice and/or crushed block ice, on the quality of yellowfin tuna.

## 2. Materials and Methods

### 2.1. Materials, Handling, and Sampling

Three yellowfin tunas (Thunnus albacares) of 30 kg up, caught off-shore of Central areas of Vietnam (FAO 71-TB 2) on May 1st, 2020. On-board fish were bled, gutted, then chilled and stored in different media: the first one was cooled...
and stored in liquid ice (containing 3.5% NaCl and 48% initial ice fraction) with a fish to ice ratio of 1/2 (w/w); the second tuna chilled in the same type of liquid ice with a fish to ice ratio of 1/2 (w/w) to the core temperature around 0 °C in 12 h and then stored in crushed block ice (fish/ice = 1/1.2 w/w); the last fish was chilled and stored in crushed block ice (fish/ice = 1/1.2 w/w). The fishing vessel returned to Hon Ro harbor, Nha Trang city, Khanh Hoa province, Vietnam on May 5th, 2020, when studied tunas were unloaded from the boat, loaded on a refrigerated truck, covered with crushed ice, and transported to the laboratories of Nha Trang University, which is about 10 km away.

At the laboratory, tunas were put into insulated containers. Regarding fish 1, liquid ice was added (fish/ice = 1/2 w/w) to the tub, while fish 2 and 3 were surrounded with crushed ice (fish/ice = 1/1.2 w/w), making sure that there was ice at the bottom of the holder, in the fish gill and belly cavities, around and at least 10 cm above the fish. Each day, ice was checked and added/changed if needed to keep the temperature of the media around or below 0°C.

Samples were taken every 1-3 days for TVB-N content determination and organoleptic analysis. To monitor the temperatures, DS1922L-F5 iButton® loggers (Maxim Integrated Products, Inc., CA) were placed in the media, as well as in the fish flesh (at the thickest part). Temperatures were recorded at 15-minute intervals.

2.2. Sensory Assessment of Chilled Tuna

Three judges participated in the assessment. They are faculties at Nha Trang University, experienced with the sensory evaluation of other aquatic products, chosen and trained according to ISO 8586: 2012 (ISO, 2012).

Sensory evaluation was conducted by the QIM scheme for chilled yellowfin tuna (N. T. T. Mai et al., 2021), control sheet 1 (N. T. T. Mai et al., 2021), and control sheet 2 (Nóbrega et al., 2014), which has been used for grading bigeye tuna. All samples were coded with randomized three digits.

The QIM protocol for tuna composes of 6 attributes, namely color of whole fish, odor of whole fish and flesh, eyes, appearance of whole fish, flesh texture, and flesh color with their maximal scores of 1, 3, 3, 2, 3, and 3, respectively, which accumulate to a total score or quality index (QI) of 15.

Control sheet 1 contains 4 quality indicators, which are color, odor, meat texture, and flavor, their detailed description, and corresponding score from 1 to 9 to express decreasing level of freshness. Flavor is only check for doubt samples. Score 4 of any of the first three indicators is considered as acceptable limit.

Control protocol 2 comprises of 4 quality parameters, which are meat quality, freshness, texture, and fat. They are rated with codes (1, 2+, 2, and 2- for the first parameter; B+, B, B-, and C for the next two attributes; and FFF, FF, F, and no F for the last descriptor), classifying fish into 4 groups from very fresh to not fresh and/or from very fat to no fat. Grades 2- or C are regarded as rejection limit for human consumption (Nóbrega et al., 2014).

2.3. Determination of TVB-N Content in Yellowfin Tuna Flesh

The TVB-N concentration, analyzed according to EC 2074/2005 (EC, 2005), with some modification based on Malle & Tao (Malle & Tao, 1987) and the National Standard of Vietnam (TCVN 9215:2012, 2012), was calculated using the following formula:

\[ \text{TVB-N} = \frac{a \cdot V_1 \cdot V_2}{(m \cdot V_3)} \]

Where:
- \( a \) is mg N corresponding to 1 mL of standard HCl solution, \( a = 0.7 \) mg/mL for 0.05 M HCl solution.
- \( m \) is the sample weight (g).
- \( V_1 \) is the titration volume (mL) of the HCl solution.
- \( V_2 = 100 \) mL, volume of the filter.
- \( V_3 = 50 \) mL, volume of the filtrate taken for every distillation.

2.4. Data Analysis

Independent sample t-test or analysis of variance (ANOVA) with post hoc Tukey were used to compare two means or more, respectively, with a significant level of 0.05, by SPSS software version 20.0 (SPSS, Chicago, IL, USA). Microsoft® Excel for Mac version 16.49 was applied for computing means and standard deviations, and for building graphs. PanelCheck software V1.4.2 was implemented for principal component analysis (PCA) on the sensory dataset to study the main variance.

To enabling the PCA of the control sheet 2 dataset, corresponding numerical scores were assigned to the rated codes as shown in Table 1.

| Code  | Assigned Score | Code  | Assigned Score |
|-------|--------------|-------|--------------|
| 1     | 1            | B+    | 1            |
| 2+    | 2            | B     | 2            |
| 2     | 3            | B-    | 3            |
| 2-    | 4            | C     | 4            |

Table 1: Assignment of Numerical Scores to Rated Scores of Controls Sensory Data
3. Results and Discussion

3.1. Sensory Changes of Yellowfin Tuna Chilled and Stored in Different Media

Figure 1 shows the results of quality index (QI) changes of yellowfin tuna cooled and preserved by different methods, which are refrigerated and stored by liquid ice of 3.5% salt, 48% initial ice crystal, and initial temperature of -4.0°C for 30 days (tuna LI-LI); chilled in similar liquid ice and kept in crushed block ice for 30 days (tuna LI-CI); cooled and stored in crushed block ice for 12 days (fish CI-CI). The rate of QI growth was highest for tuna CI-CI (0.5118 score per day) with significant index alteration (p < 0.05) every three sampling days. Meanwhile, QI inceasement of fish LI-LI was the slowest (0.1677 score/day) and a clear QI change (p < 0.05) was only observed at days 21 and 27. The QI of tuna LI-CI exhibited a medium speed of variation (1.125 score daily) with sharp elevation (p < 0.05) at days 9, 15, 18, and 27. By the end of the storage time, the QI of tunas stored in crushed ice (fish CI-CI and LI-CI) approach its maximum scale of 15, while that of tuna chilled and stored in liquid ice (tuna LI-LI) remained below 9, accounting for less than 60% of the greatest level. According to Sykes (Sykes et al., 2009), acceptable QI should be 3/4 of the scale, which means that for yellowfin tuna the rejection limit was around a QI of 11. Based on that, the shelf life of tunas LI-LI, LI-CI, and CI-CI were > 30 days, < 18 days, and < 12 days, respectively. All these findings revealed the advantage of liquid ice in remaining the freshness of yellowfin tuna over traditional crushed ice.

![Figure 1: Quality Index (QI) of Yellowfin Tuna Chilled and Stored In Crushed Block Ice, Liquid Ice and /Or Crushed Block Ice.](image)

Linear relationship between QI and chilled storage time ($R^2 > 0.78$) (Figure 1) made the QIM for fresh yellowfin tuna a useful and convenient tool to quickly assess the quality and estimate the remaining shelf life of the seafood. The results were in agreement with the developed QIM protocols for other aquatic species and products (Martinsdottir et al., 2001).

Figure 2 shows attribute sensory scores based on scoresheet 1 of yellowfin tuna cooled and preserved in different ice types at various stage of store. Scores of each sensory attribute of all three tunas were indifferent (p > 0.05) at the beginning of storage (day 0) (Figure 2 (1)), indicating that all the studied fish had similar initial sensory quality. However, from day 6 onward, color, odor, and meat texture of tuna treated and stored in crushed ice (CI-CI) become significantly worse (p < 0.05) than those cooled in liquid ice (tunas LI-LI and LI-CI) (Figure 2 (2)-(3)). At day 6, odor and meat texture scores of the fish CI-CI exceeded the acceptable limit of 4, revealing a 6-day shelf life of this tuna.

After 12 days, the three tunas exhibited different (p < 0.05) levels of freshness (Figure 2 (3)), the best was LI-LI and the worst was CI-CI. This is also the time when there was some disagreement of the panel in rating the fish freshness, some judges scored the attributes of LI-CI below 4, but other graded them above the acceptable limit of 4.

Tuna cooled in liquid ice and stored in crushed ice (LI-CI) and the one chilled and preserved in liquid ice (LI-LI) clearly became unfit for human consumption at day 15 (Figure 2 (4)) and day 27 (Figure 2 (5)), respectively, when the fish sensory attributes were marked above 4. In general, although the fish had similar initial quality, but tuna refrigerated and kept in liquid ice presented the best quality over time, while fish treated and preserved by traditional crushed ice showed the worst freshness retention.
Compared to terrestrial animal flesh, fish muscle has higher activity of cathepsin enzymes and lower connecting tissue content, which explains faster rate of Z-discs and myosin-actin junction weakening, leading to the depletion of firmness and springiness, and softening of fish meat during chilled preservation (Lougovois & Kyrana, 2005). According to Guizani (Guizani et al., 2005), tuna stored at 0°C remained safe for 17 days, however, its freshness maintained only for 12 days. It was found that tuna fillets preserved at 0°C exhibited an acceptability for consumption for over 9 days; and that storage time and temperature significantly influenced sensory quality the product (Du et al., 2001).

| Sample  | Storage time (days) | Rated code | Texture | Freshness | Meat quality |
|---------|---------------------|------------|---------|-----------|--------------|
| LI-LI   | 27                  | C          | B       | 1         | LI-LI        |
| LI-LI   | 27                  | B-         | B       | 1         | LI-LI        |
| LI-LI   | 27                  | B          | B       | 1         | LI-LI        |
| CI-CI   | 6                   | B-         | B-      | 2+        | CI-CI        |
| CI-CI   | 6                   | B-         | B-      | 2+        | CI-CI        |
| CI-CI   | 6                   | B-         | B-      | 2+        | CI-CI        |
| LI-CI   | 15                  | B          | B       | 2         | LI-CI        |
| LI-CI   | 15                  | B          | B       | 2         | LI-CI        |
| LI-CI   | 15                  | B          | B       | 2         | LI-CI        |

Table 2: Sensory Rejection Time off 30 Kg Up Tuna in Various Media Based on Control Scheme 2
LI-LI = Tuna Chilled and Stored in Liquid Ice; LI-CI = Tuna Chilled by Liquid Ice and Stored in Crushed Ice; CI-CI = Tuna Chilled and Stored In Crushed Ice
2- Or C: Rejection Limits

For the Same Attribute, Different Letters Indicate Significant Differences (P < 0.05) in Mean Values of Sensory Scores between Storage Days

Figure 2: Sensory Scores Based on Control Sheet 1 of Yellowfin Tuna Chilled and Stored By Liquid Ice and/Or Crushed Block Ice
LI-LI = Tuna Chilled and Stored In Liquid Ice; LI-CI = Tuna Chilled by Liquid Ice and Stored in Crushed Ice, CI-CI = Tuna Chilled and Stored in Crushed Ice

Rejection Time of 30 Kg Up Tuna in Various Media Based on Control Scheme 2
2- Or C: Rejection Limits
Table 2 illustrate the rejection time of fish cooled and stored in different ice media. Tuna chilled and preserved in crushed ice (CI-CI) became unsuitable for human use just after 6 days, which was 4.5 times shorter than the fish cooled and stored in liquid ice (LI-LI, 27 days). Regarding tuna cooled in slurry ice and preserved in crushed ice (LI-CI), at day 15 its freshness was rated as C which signified unacceptable for consumption. The sensory outcomes of control method 2 were consistent with those of the control method 1.

The sensory results could be explained by the fact that binary ice systems of liquid ice (initial temperature -4.0°C), cooled down the fish faster than traditional ice. In addition, liquid ice maintained lower temperature compared to crushed ice during storage. This help to better slow down the microbial growth and enzyme activities. The findings are consistent with previous studies on skipjack tuna, which was obviously springier and chewier (p < 0.05), and fresher in slurry ice than in flake ice (Zhang et al., 2015).

The outputs of principal component analysis on the dataset of QIM method, control methods 1 and 2 of tuna chilled and stored in liquid ice and/or in crushed block ice are shown in Figure 3. It can be observed that the first principal component (PC1) explained 79.8% of the sample variance, and the second principal component (PC2) described another 19.3%. Fresh samples of all treatments located in the left-hand side of PC1, while those of more prolong storage moved further to the right of this PC. As time elapsed, representatives of different cooling and storage media formed distinctive groups on the scores plot. "Fresh" group included samples of tuna chilled and stored in crushed ice (CI-CI) at days 0-3; tuna chilled in liquid ice-stored in crushed ice (LI-CI) and fish chilled and preserved in liquid ice (LI-LI) at days 0-6. These confirm the advantage of liquid ice over traditional crushed ice in maintaining the quality of fish due to its faster cooling effect and lower storage temperature.

Figure 3: PCA Scores of 6 QIM Attributes, QI, 3 Attributes of Control Sheet 1, and 3 Attributes of Control Sheet 2 of Tuna Chilled and Stored by Liquid Ice and/or Crushed Block Ice LI-LI = Tuna Chilled and Stored in Liquid Ice; LI-CI = Tuna Chilled by Liquid Ice and Stored in Crushed Ice, CI-CI = Tuna Chilled and Stored in Crushed Ice; D = Day Of Storage, Two-Digit Number = Storage Day

3.2. Changes of TVB-N content in yellowfin tuna chilled and stored by different media

Results of TVB-N changes of ventral and dorsal flesh of yellowfin tuna, cooled and preserved by different methods, are shown in Figure 4. The concentrations of TVB-N in tuna chilled and stored in liquid ice (LI-LI fish) started from around 18 mg% (in both dorsal and ventral parts) and remained low at about 21 mg% over 30 days of storage (Figure 4 (1)), while those of tuna treated and kept in crushed ice (fish CI-CI) initiated at lower level (9 mg%), developed faster and ended up at approximately 19 mg% (Figure 4 (2)). The levels of TVB-N in fish chilled by liquid ice and stored in crushed ice (tuna LI-CI) were 15.50 and 16.47 mg% in the back and belly meat, correspondently, at day 0; and raised to 41.51 and 33.81 mg%, respectively, at day 30 (Figure 4 (3)). The increase of TVB-N in tuna LI-CI was more rapid compared to tuna LI-LI, but slower than that of tuna CI-CI.

Enzymes and microflora can quickly break down proteins and non-protein nitrogen-containing compounds to form histamine, tyramine, trimethylamine (TMA), dimethylamine (DMA), methylamine, trimaine, NH₃, putrescine, cadaverine, and other volatile basic nitrogen substances, the so-called TVB-N (Altissimi et al., 2018). General mechanism of TVB-N formation is as follows: protein → shorter chain polypeptides → tripeptides and dipeptides → Amino acids → TBV-N. Ammonia is formulated as a result of bacterial deamination of proteins, peptides and amino acids; or/and of autolytic breakdown of adenine monophosphate in chilled seafood (Huss, 1995). Reduction of trimethylamine oxide (TMAO) to TMA is often because of bacterial activities during chilled storage. As chilled storage time elapsed, the load of...
microorganisms increases, the deterioration process occurs more intensively, resulting in higher level of TVB-N accumulation (N. Mai & Huynh, 2017; N. T. T. Mai et al., 2011). In the other hand, in frozen products of some fish species, such as cod, where there is the present of muscle TMAO-ase or TMAO demethylase, TMAO is normally broken into DMA and formaldehyde, which cross links with muscle proteins, causing their toughening and lowering their water holding capacity (Etienne, 2005).

The TVB-N content in yellowfin tuna fillets increased significantly (p < 0.05) from 9.42 to 29.14 mg% during 7 storage days at 4°C (Wang et al., 2018). A level of 35 mg% TVB-N was proposed as an allowable limit for yellowfin tuna (Jinadasa, 2014), based on which fish chilled in liquid ice and stored in crushed ice (tuna LI-CI) became unsuitable at day 30, while other two tunas (LI-LI and CI-CI) maintained their TVB-N content below the rejection level after 30 and 12 days, respectively. The pace of TVB-N growth was in good agreement with the sensory declining trend.

![Figure 4: Changes in TVB-N Content of Tuna Chilled and Stored by Liquid Ice and /Or Crushed Block Ice](image)

**LI-LI = Tuna Chilled and Stored In Liquid Ice; LI-CI = Tuna Chilled by Liquid Ice and Stored in Crushed Ice; CI-CI = Tuna Chilled and Stored In Crushed Ice**

*On The Same Curve, Different Letters Indicate the Significant Difference (P <0.05) in TVB-N Content between Storage Days*

4. **Conclusions**

Among the studied chilling and storage media, liquid ice (3.5% NaCl and 48% initial ice concentration) showed the best preservation effect for yellowfin tuna quality in term of organoleptic and TVB-N indicators. The second most effective method was refrigeration by liquid ice and storage in crushed ice. Traditional icing was the worst option. The findings support the strength of liquid ice compared to other media in fish handling and storage to maintain product freshness and shelf life.

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