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# Hypoxanthine Levels, Chemical Studies and Bacteria Flora/Count of Frozen/Thawed Market Simulated Chub Mackerel (*Scomber japonicus*) under Cold Sotrage Temperature Conditions

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**Abstract:** Hypoxanthine levels, chemical studies, organoleptic assessment and bacteria flora/count were studied for a 12 week period on market simulated fresh samples of the Chub Mackerel (*Scomber japonicus*) in order to assess its keeping quality and shelflife under cold storage conditions of-4°C. Twenty-two fresh samples of average weight of 260 g were used for the study. Two pieces were exposed for 12 h to thaw and defroze biweekly to simulate market conditions before taken them for further chemical analysis and microbiological assessment. Initial proximate analysis were carried out on the fresh fish and also final proximate analysis at the end of the 12 week experiment. The chemical parameters analysed are Hypoxanthine levels, Trimethylamine (TMA), Peroxide Value (PV), Free Fatty Acid (FFA) (fortnightly) and bacteria identification and count (monthly). Organoleptic assessment was also carried out on the fresh and cooked fish samples forthnightly. The final proximate analysis showed increase (74.36%) in the moisture content compared to (69.25%) the initial. There were decreases observed in crude fibre (2.42% as against 2.78%), ether extract (fat) (16.89% as against 17.38%) and ash (2.86% as against 3.18%), but increase in crude protein content from 19.65 to 21.34%. The other chemical parameters assessed increased e.g peroxide value (PV from 26.40 to 34.60 Meq $kg^{-1}$), Trimethylamine (TMA from 29.62 to 39.20 100 $g^{-1}$ fish), free fatty acid (% FFA from 1.74 to 2.32%) over the assessment period. The hypoxanthine level also increased considerably from 28.24 mg 100 $g^{-1}$ fish to 37.54 mg 100 $g^{-1}$ fish at the end of the experiment (hence the increasing bitter taste with length of study). The organoleptic assessment also ranged from very good (2.0) to just fair (6.0) quality at the end of the 12 week study. The number of bacteria identified also increased with storage time. The overall bacteria total viable count varied from 0 week (1.82 Cfu $g^{-1}$) - 14.70 Cfu $g^{-1}$) in the 12th week with 29.36 Cfu $g^{-1}$ overall count recorded for the 12 weeks. A total of 10 bacteria specie were detected in the study with *Lactoccocus acidophilus* showing the highest prevalence of 6.14 Cfu $g^{-1}$ fish) also showing its presence from the 0 week (1.82 Cfu $g^{-1}$ fish) to 1.60 cfu $g^{-1}$ fish recorded in the 12th week. Second to it is *Pseudomonas aureginosa* (3.50cfu $g^{-1}$) detected from (8th-12th week), while the third bacteria prevalent was *Clostridium welchii* with 2.72 Cfu $g^{-1}$ detected at the end of the 4th week and 12th week of study. The other bacteria species detected (arranging them in their order of prevalence) include *Bacillus substilis* (2.40 cfu $g^{-1}$), *Proteus morganii* (2.40 Cfu $g^{-1}$); *Eschericha coli* (2.36 Cfu $g^{-1}$ fish); *Bacillus cereus* (2.10 Cfu $g^{-1}$), *Micrococcus acidiophilus* (1.50 Cfu $g^{-1}$), *Staphylococcus aureus* (1.30 Cfu $g^{-1}$) and lastly *Streptococcus faecium* (1.10 Cfu $g^{-1}$). All parameters measured showed drastic rises in their values as from the 8th week to the 12th week. Hence the limit of acceptability and shelflife of the market simulated Chub Mackerel (*Scomber japonicus*) under cold storage conditions of - 4°C is 8 weeks (2 months).

**Key words:** Chub mackerel (*Scomber japonicus*), hypoxanthine levels, organoleptic, chemical, bacteria assessment, frozen/thawed, market simulated, shelflife

## INTRODUCTION

Hypoxanthine (Hx) is a normal constituent of fish flesh though present in very low concentrations in live fish. It is the end product of a series of enzymatic reactions going on in the fish flesh namely:

ATP → ADP → AMP → IMP → Inosine- HxR → Hx

ATP-Adenosine Triphosphate, ADP-Adenosine Diphosphate, AMP-Adenosine Monophosphate, IMP-Inosine Monophosphate.

Unlike TMA (Trimethy lamine → and TVB (Total Volatile Bases), it increases in most species soon after death and in the early days of storage (Howgate, 1965; 1982; Howgate *et al*. 1972). It can be used to discriminate between batches of fresh fish. Hx concentration increases with storage time and it is more

variable between species than TMA or TVB. Generally, the measurement of hypoxanthine is a better index of freshness and gives a better indication of spoilage over a wide range of qualities than TMA or TVB. It is applicable to a wide range of species and products in which the limit of acceptance has been restricted to $\leq 4$ μm $g^{-1}$ fish.

The Chub Mackerel (*Scomber japonicus*), family Scombridae is primarily a coastal pelagic species, to a lesser extent epipelagic or mesopelagic over the continental slope, occurring from the surface to about 250 or 300 metres depth. Seasonal migrations may be very extended, the fish in the northern hemisphere moving further northward with increased summer temperatures, and southwards for overwintering and spawning. The reverse pattern generally applies to the populations in the southern hemisphere. Schooling by size is well developed and initiates at approximately 3 cm. Schools of adults are the most compact and structured.

Spawning most often occurs at water temperatures of 15°C to 20°C, which results in different spawning seasons by regions. Spawning occurs in several batches of about 250 to 300 eggs per 9 of fish with the total number of eggs per female ranging from approximately 100,000 to 400,000. The Chub Mackerel is believed to be in good competition with the species it schools with, such as the Eastern Pacific Bonta (*Sarda chiliensis*) the Jack Mackerel (*Trachurus symmetricus*) and others. Its feeding is opportunistic and non-selective, the diet of adults ranging from Copepods and other crustaceans to fish and squid. Its predators include tunas, billfishes, white seabass (*Cynoscion nobilis*) yellowtail (*Seriola lalandi*) and other fishes, as well as sealions, sharks and pelicans.

Several chemical tests indirectly related to bacterial activity have been often employed for assessing freshness or levels of spoilage in fish and other seafood products (Botta, 1995). The proposed tests have been used to establish quantities of different spoilage compounds. According to Howgate (1982) only three have stood the test of time as reliable i.e., determination of Trimethylamine (TMA), Total Volatile Bases (TVB) and Hypoxanthine (Hx). The first two are related to bacterial activity and the third is the end product of a series of enzymatic reactions in the fish flesh. There are other methods such as Peroxide Value (PV), Thiobarbituric Acid (TBA), Iodine Value (IV) and Anisine (A), which measure rancidity in fish and fish products.

The maximum contamination of freshly caught fish that could reduce its quality may originate from the following:

- Contamination of the raw material at the fishing ground.
- Use of polluted water in washing the fish
- Use of unclean equipments, including fish boxes during handling.
- High level of unhygienic conditions in the processing factories
- Lack of personal hygiene among the fish handlers.

In markets or during hawking process, buyers may contaminate the fish in the process of pricing and selection, which has no limit as to what portion or amount of fish touched or fiddled with. It is therefore the aims and objectives of this study.

- To determine the hypoxanthine levels in the Chub Mackerel (*Scomber japonicus*) over a period of time and its acceptable limit.
- Also to assess spoilage rate and shelf life of the fish through the monitoring of Free Fatty Acid (FFA) level, Trimethylamine (TMA) level and Peroxide Value (PV).
- Identification of bacteria flora/count on a monthly basis throughout the experimental period.

## MATERIALS AND METHODS

Hypoxanthine levels, chemical studies, organoleptic assessment and bacteria flora/count were studied for a 12 week period on market simulated fresh samples of the Chub Mackerel (*Scomber japonicus*) in order to assess its keeping quality and shelf life under cold storage conditions of - 4°C in a deep freezer.

Twentytwo fresh samples of the Chub Mackerel of average weight of 260 g were used for the study. Two pieces were exposed for 12 h to thaw and defroze biweekly in order to simulate market conditions before taken them for further chemical analysis and microbiological assessment. Initial proximate analysis were carried out on the fresh fish and also final proximate analysis at the end of the 12 week experiment according to AOAC (1990) methods.

The chemical parameters analysed are Hypoxanthine levels, Trimethylamine (TMA), Peroxide Value (PV), Free Fatty Acid (FFA) and organoleptic assessment of fresh, cooked and uncooked fish were carried out fortnightly; while bacteria identification and count were done monthly.

**Chemical test carried out on the fish**

**Determination of hypoxanthine:** Two gram of crushed fish sample was weighed into a 250 mL beaker, 1 g of active carbon (active charcoal), 100 g of distilled water and 5 mL of Carrez solution I and II were added and mixed for

30 min. The mixture was filtered through a Whatman No. 2 filter paper. Five milli liter of Clear colourless filtrate was pipetted into 15 mL test tube, 5 mL of 4 - DMAB solution added, mixed and placed in the water bath at 20°C. The absorbance of mixture was taken after colour development on a Spectronic 21D Spectrometer at a wavelength of 460 nm. Standard Hypoxanthine of range 2 ppm-10 ppm were also treated as sample and absorbance taken at the same wavelength.

$$\text{Concentration of Hypoxanthine in mg } 100\text{ g}^{-1} = \frac{\text{Absorbance of sample} \times \text{Gradient factor of standard} \times \text{Dilution factor}}{\text{Weight of Sample}}$$

**Determination of peroxide value of fish:** Two gram of crushed fish sample was weighed into a 250 mL beaker. Twenty milli liter of Chloroform and 10 mL of glacial acetic acid were added to the fish sample in the beaker and mixed. The mixture was filtered into 250 mL conical flask. 1 mL of 5% aqueous saturated Potassium Iodide (KI) solution was added and shaken thoroughly. The homogenous mixture was placed on the hot plate to boil for 30 seconds. Add 25 mL distilled water, shake, add 1 mL starch and titrate the hot mixture against 0.002M.

A blank determination was carried out at the same time. Peroxide value is the number of mLs of $Na_2S_2O_3$ (0.002M) used for the titration.

$$\text{PV (Meq kg}^{-1}) = \frac{\text{Titre value of sample} = \text{Titre value of blank} \times \text{M Na2S203} \times 103\text{ Meq kg}^{-1}}{\text{Weight of the Sample}}$$

**Determination of Trimethylamine (TMA):** Hundred gram of crushed fish sample was weighed into a 500 mL beaker. 300 mL of 5% TCA (Tricarboxylic acid) was added and macerated, homogenized to obtain a clear extract.

This test was carried out using a semi-micro distillation procedure (Burt *et al*., 1976). Five milli liter of the extract was pipetted into the Markham distillation apparatus, five milli liter of 2M NaoH was added and steam distilled into a 100 mL conical flask containing 15 mL of 0.01M HCl. One milli liter of 1% resolic acid indicator added and titrated against 0.01M Na0H to obtain a pale pink end point to get $T.V_1$ (Titre value). Add 1 mL of 16% formaldehyde (neutralized) to the mixture in the titration flask to liberate excess acid. Titrate the excess acid with 0.01M Na0H to obtain $T.V_2$.

$$\text{Trimethylamine Nitrogen} = \frac{14(300+\text{Weight of sample taken}) \times \text{T.V2 mg}}{\frac{100\text{ g}}{1\text{ fish}}} \quad 500$$

$T.V_2$ = Titre value of the liberated or excess acid.

**Determination of free fatty acid:** Two gram of crushed fish sample was dissolved in a 50 mL solvent ether and alcohol (1:1). The mixture was thoroughly shaken to dissolve the fish content. The mixture was then titrated against 0.1m NaoH using 1% phenolphthalein as indicator to obtain a faint pink colour at the end point. % Free Fatty Acid was calculated using the following formula.

$$\% \text{ FFA} = \frac{\text{Titre Value of Sample} \times 4.0}{\text{Weight of Sample used}} \times \frac{1}{2}$$

**Organoleptic assessment:** This assessment was based on the scoring system, which involved the measurement of certain parameters on graded scores. Therefore, a five man panel was briefly trained on the organoleptic or sensory system scales ranging from 1 to 6 based on the determination characteristics (Emokpae, 1979).

For uncooked fish, a whole round fish was placed on a clean table for grading.

Parameters employed by the judges are as follows;-

- Appearance or external characteristics which includes work pigmentation of the skin, it means, shape of the eyes, the eye tint, colour of the gill, rigidity of the abdominal wall and colour of the flesh.
- Texture refers to the degree of loss of elasticity of the flesh.
- Odour.
- Colour.
- Taste. For the cooked fish

The fish sample were filleted and steamed for 25 min and presented to the taste panel on plates. The panelists were asked to rinse their mouth before tasting, so as to avoid any bias in the result.

The scores were based on the following

- Taste.
- Flavour

**Microbiological assessment/analysis:** One gram of the grinded fish sample was dissolved in sterile peptone water. Specimen bottles and sterile test tubes were further sterilized before putting samples into them.

Pipette 9 mL of peptone water into each of the test tubes, add 1 mL of test sample to the first tube via 1 mL pipette and label as $10^{-1}$. Take another 1 mL of the first dilution using another sterilized pipette from the first dilution and add it to the second sterilized tube and label as $10^{-2}$. Another 3rd pipette is used to prepare 1 in 1,000 of $10^{-3}$ dilution as above. Dilution of $10^{-4}$,$10^{-5}$ etc can be done similarly depending on the probable bacteria content. Mix the contents of the final dilution tube and discard 1 mL into the disinfectant jar. Fifty micro liter of the last dilution test tube was pipetted into a 15 mL Mackonkey Agar and allowed to air dry in a pour plate (sterilized plate). The plate were incubated at 37°C for 24 h and the number of colony developed are counted using January Colony Counter.

Microbial count = Number of colonies×Dilution factor.

**Data analysis:** The statistical tools used to analyse the result obtained are (according to Steel and Torrie, 1960).

- Correlation analysis of the parameters
- Simple linear regression analysis

As shown in Table 1, higher final proximate composition values were recorded for crude protein (21.34%) and moisture content (63.36%) than it is for the initial (19.65%) and 60.25% respectively, while lower values were recorded for the final ash (2.80%), fat (10.02%) and crude fibre (2.42%) compared to the initial values 3.06, 14.14 and 2.78%, respectively.

Table 2 shows the organoleptic assessment of the fresh sample to be very good, (score 2 on the average) and just fair (score 6) at the end of the 12th week storage period at -4°C

Table 1: Initial and final proximate composition of the chub mackerel (*Scomber japonicus*)

| Parameters (%) | Initial (%) | Final (%) |
|---|---|---|
| Moisture | 60.25 | 63.36 |
| Etheractract (fat) | 17.14 | 10.02 |
| Crude Protein (CP) | 19.65 | 21.34 |
| Crude Fibre (CF) | 2.78 | 2.42 |
| Ash | 3.06 | 2.8 |
| NFE | 0.12 | 0.06 |

Table 2: Organoleptic assessment of the fresh and cooked samples of the chub mackerel

| | Length of storage period in weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| Samples | AB 0 | $A_1$2 | $A_2$ 4 | $A_3$ 6 | $A_4$ 8 | $A_5$ 10 | $A_6$12 |
| Parameters determination | | | | | | | |
| Taste | 2 | 4 | 5 | 5 | 4 | 5 | 7 |
| Odour | 2 | 3 | 4 | 3 | 4 | 5 | 6 |
| Appearance | 1 | 5 | 4 | 5 | 4 | 3 | 6 |
| Colour | 2 | 3 | 4 | 4 | 4 | 4 | 5 |

Key, 1- Excellent5- Fairly satisfactory, 2- Very good6- Fair, 3- Good7- Poor, 4- SatisfactoryAB- Baseline, $A_1$-$A_6$-Stored sample

Table 3: Biochemical assessment of cold stored sample of the chub mackerel (*Scomber japonicus*), at -4°c length of storage period in weeks

| Parameters determined | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
|---|---|---|---|---|---|---|---|
| Peroxide Value (PV) meg 100g$^{-1}$ fish | 26.4 | 27.8 | 28.9 | 30.1 | 30.3 | 31.2 | 34.6 |
| Trimethylamine (TMA) mg 100g$^{-1}$ fish | 29.62 | 30.1 | 31.6 | 33.2 | 34.6 | 36.7 | 39.2 |
| Free Fatty Acid (FFA) % | 1.74 | 1.82 | 1.88 | 1.93 | 1.98 | 2.14 | 2.32 |
| Hypoxanthine (Hx) mg 100 g$^{-1}$ fish | 28.24 | 29.46 | 30.72 | 31.86 | 32.65 | 34.26 | 37.54 |

Table 4: Isolated organisms (bacteria flora) and their counts for samples of the chub mackerel (*Scomber japonicus*) under cold storage medium at -4°c length of storage (4 weeks interval)

| Isolated organisms (bacteria flora) (cfu g$^{-1}$ ($10^4$) | 0 AB | 4 $A_1$ | 8 $A_2$ | 12 $A_3$ | Overal total variable Count (TVC) |
|---|---|---|---|---|---|
| Bacillus substilis | | | 1.50 | 0.90 | 2.40 |
| Bacillus cereus | | | | 2.10 | 2.10 |
| Clostridium welchii | | 1.52 | | 1.20 | 2.72 |
| Eschericha coli | | 0.96 | 1.00 | 1.40 | 2.36 |
| Lactococcus acidophilus | 1.82 | 1.62 | 1.10 | 1.60 | 6.14 |
| Proteus morganii | | 1.24 | 2.10 | 1.95 | 2.40 |
| Pseudomonas aureginosa | | | 1.80 | 1.70 | 3.50 |
| Micrococcus aciodophilus | | | | 1.50 | 1.50 |
| Staphylococcus aureus | | | | 1.30 | 1.30 |
| Streptococcus faecuim | | | | 1.10 | 1.10 |
| Total Viable Count | 1.82 | 5.34 | 7.50 | 14.70 | 29.36 |

Table 5: Correlation coeficient betsween the biochemical and microbial assessments with period of storage for the chub mackerel

| Dependent variable | Correlation Coefficient (R) | Decision ($p<0.5$) or ($p<0.05$) |
|---|---|---|
| Peroxide Value (PV) | 0.961 + ve | Significant ($p<0.05$) |
| Trimethylamine (TMA) | 0.985 + VE | Significant ($p<0.05$) |
| Free Fatty Acid (FFA) | 0.963+ ve | Significant ($p<0.05$) |
| Hypoxanthine (Hx) | 0.975 + ve | Significant ($p<0.05$) |
| Total Viable Count (TVC) | 0.940 + ve | Significant ($p<0.05$) |

Hypoxanthine values of the samples showed a progressive increase from 0 day to 12 weeks with ranges from 28.24 to 37.54 mg 100 g$^{-1}$ fish. A similar trend was observed for peroxide value with range of 26.40-34.60 meg kg$^{-1}$ Trimethylamine (TMA) values ranging from 29.62-39.20 mg 100 g$^{-1}$ fish and free fatty acid values ranging from 1.74-2.32%.

Ten bacteria species were detected in the samples under cold storage at-4°C. Out of the 10 bacteria species identified *Lactococeus acidophilus Proteus morganii Pseudomonas aureginosa* and *Clostridium welchii* had the highest number of occurrence with 6.14, 5.24, 3.50 and $2.72\times10^4$ Cfu g$^{-1}$, respectively as shown in Table 4. It was noticed that microbial build up rises with increase in the length of storage period. The Total Viable Count (TVC) showed an increase from 0 day ($1.82\times10^4$ Cfu g.$^{-1}$) to ($14.70\times10^4$ Cfu g$^{-1}$) in the 12th week, with an overall Total Viable Count (TVC) of 29.36 Cfu g$^{-1}$.

From Table 5, it is shown that a positive linear correlation exist for all the parameters with significant difference ($p<0.05$) for PV, TMA, FFA, Hx and total viable

Table 6: Shelflife prediction equation for the chub mackerel (*Scomber japonicus*) regression analysis for dependent variable

| Dependent variable | Independent variable | Prediction equation | $R^2$ | S.E. |
|---|---|---|---|---|
| PV | Period of storage | PV = 26.38+0.585 wks | 0.92 | 0.802 |
| TMA | Period of storage | TMA = 28.759+0.803 wks | 0.972 | 0.646 |
| FFA | Period of storage | FFA = 1.707+0.443 wks | 0.928 | 0.058 |
| Hx | Period of storage | Hx = 27.879+0.704 wks | 0.950 | 0.757 |
| TVC | Period of storage | TVC = 1.250+1.830 wks | 0.870 | 2.189 |

count. This also indicates that the parameters increase with increase in storage period where R = 0.961, 0.985, 0.693 and 0.975 for PV, TMA, FFA and Hx, respectively and for Total Viable Count, R = 0.940.

A regression analysis was carried out to predict the shelflife of *Scomber japonicus* as shown in Table 6. The relationship among PV, TMA, FFA, Hx and period of storage shows that the prediction equation is linear and can be expressed as Y = a + bx.

Y = Dependent variable (PV, TMA, FFA, Hx and TVC). a, b = beta values, x = period of storage PV in Meq $Kg^{-1}$, TMA in mg 100 $g^{-1}$ fish, FFA in % Hx in mg 100 $g^{-1}$ fish and TVC in Cfu $g^{-1}$ fish.

## DISCUSSION

From the proximate composition result in Table 1 much moisture is absorbed in the final analysis and also with increased crude protein which compensated for the lower values of fat (10.02%), crude fibre (2.42%) and Ash (2.80%) recorded in the final analysis. Moisture increased from 19.65 to 21.34% while other parameters decreased. Fat decreased from 14.14 to 10.02%, crude fibre from 2.78 to 2.42% and ash from 3.06% to 2.80%. This is in line with Bligh and Dyer (1959) and Stansby (1992) findings.

The Chub Mackerel (*Scromber japonicus*) is a fatty fish thereby making it to spoil fast. This is further confirmed by the higher values of Hypoxanthine (Hx) (37.54 mg 100 $g^{-1}$ fish), Peroxide Value (PV) (34.60 Meq $Kg^{-1}$), Trimethylamine (TMA) (39.20 mg 100 $g^{-1}$ fish), Free Fatty Acid (FFA) (2.32%) and Total Viable Count (TVC) ($14.70\times10^4$ Cfu $g^{-1}$) recorded at the end of the 12 week study. This findings is in line with Oyelese and Adejwnu (1998). Burt (1977), Burt *et al.* (1976), Shewan and Murray (1979) reports in their studies. For the fish to be in good condition must be at its optimum.

However, the cold storage medium does not totally halt spoilage, because all the measured parameters including hypoxanthine levels increased with storage period. Hence the fish loses its taste/bitter taste especially increasing at a faster rate towards the end of the 12 weeks. Thus the fish becomes unfit for consumption as confirmed by the organoleptic assessment results which puts the average score at the end of the 12 weeks study at score 6.0 which indicates just fair meaning the fish is virtually spoilt. This is also in line with Emokpae (1979) findings in his studies.

A total of 10 bacteria species were detected in the study, with *Lactococcus acidophilus* showing the highest prevalence of 6.14 Cfu $g^{-1}$ and also showing its presence from the 0 week (1.82 Cfu $g^{-1}$) to the 12th week (1.60 Cfu $g^{-1}$). Second to it is *Pseudomonas aureginosa* (3.50 Cfu $g^{-1}$) detected from (8-12th week), while the 3rd bacteria prevalent was *Clostridium welchii* with 2.72 Cfu $g^{-1}$ detected at the end of the 4th week and 12th week of study.

The other bacteria species detected (arranging them in their order of prevalence) include *Bacillus substilis* (2.40 Cfu $g^{-1}$), *Proteus morganii* (2.40 Cfu $g^{-1}$), *Eschericha coli* (2.36 Cfu $g^{-1}$), *Bacillus cereus* (2.10 Cfu $g^{-1}$), *Micrococcus acidiphilus (1.50* Cfu $g^{-1}$), *Staphylococcus aureus* (1.30 Cfu $g^{-1}$) and lastly *Streptococcus faecium* (1.10 Cfu $g^{-1}$).

All parameters measured showed drastic rises in their values as from the 8th week to the 12th week, during which significant increases in spoilage characteristics were detected. Hence the limit of acceptability/shell life of the Chub Mackerel (*Scomber japonicus*) should not exceed 8 weeks (2 months).

## CONCLUSION

The study revealed that hypoxanthine like other biochemical parameters (Peroxide Value (PV), Trimethylamine (TMA) and Free Fatty Acid (FFA) and Total viable bacteria count affects spoilage rate. The most pathogenic bacteria specie isolated is *Lactococcus acidophilus,* followed by *Pseudomonas aureginosa* and thirdly *Clsotriiium welchii* out of the 10 bacteria species isolated in the study. The rapid sudden drastic increases of all measured parameters as from the end of the 8th week to 12th week, puts the limit of acceptability and shelflife of the Chub Mackerel (*Scomber japonicus*) at 8 weeks i.e., (2 months).

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