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Hypoxanthine Levels, Chemical Studies And Bacteria Flora Count Of Frozen/Thawed Market Simulated Chub Mackerel (*Scomber Japonicus*) Under Cold Storage Temperature Conditions

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Summary

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 (22) fresh samples of average weight of 260gm were used for the study. Two pieces were exposed for 12 hours to thaw and defroze biweekly to simulate market conditions before taken them for further chemical analysis and microbiological assessment. Initial proximate analyses 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 fortnightly.

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), trimethylamine (TMA from 29.62 to 39.20mg/100gm fish), free fatty acid (% FFA from 1.74 to 2.32%) over the assessment period. The hypoxanthine level also increased considerably from 28.24mg/100gm fish to 37.54mg/100gm 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 Cfugm) – 14.70 Cfugm) in the 12th week with 29.36 Cfugm overall count recorded for the 12 weeks.

A total of 10 bacteria specie were detected in the study with *Lactococcus acidophilus* showing the highest prevalence of 6.14 Cfugm fish) also showing its presence from the 0 week (1.82 Cfugm fish) to 1.60cfugm fish recorded in the 12th week. Second to it is *Pseudomonas aureginosa* (3.50cfugm) detected from (8th – 12th week), while the third bacteria prevalent was *Clostridium welchii* with 2.72 Cfugm 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 subtilis* (2.40 cfu/gm), *Proteus morgani* (2.40 Cfu/gm); *Escherichia coli* (2.36 Cfu/gm fish); *Bacillus cereus* (2.10 cfu/gm), *Micrococcus acidiphilus* (1.50 Cfu/gm), *Staphylococcus aureus* (1.30 Cfu/gm) and lastly *Streptococcus faecium* (1.10 Cfu/gm).

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).

Keywords:- *Scomber japonicas*- Hypoxanthine levels- Organoleptic assessment- Market simulated- Shelflife.

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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 (Trimethylamine) and TVB (Total Volatile Bases), it increases in most species soon after death and in the early days of storage (9,8,7). 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 < 4µm/gm fish.

The Chub Mackerel (*Scomber japonicus*), family Scombridae is primarily a coastal pelagic species. 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 (2). According to (7) 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 fish. Other methods are 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:

- (i) Contamination of the raw material at the fishing ground.
- (ii) Use of unclean equipments, including fish boxes during handling.
- (iii) High level of unhygienic conditions in the processing factories
- (iv) 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

- (i) To determine the hypoxanthine levels in the Chub Mackerel (*Scomber japonicus*) over a period of time and its acceptable limit.

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- (ii) 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).
- (iii) 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 (– 40C). Twenty-two (22) fresh samples of the Chub Mackerel of average weight of 260gm were used for the study. Two pieces were exposed for 12 hours to thaw/defroze biweekly in order to simulate market conditions before taken them for further chemical analysis and microbiological assessment. Initial and final proximate analysis were carried out on the fresh fish according to (1). The chemical parameters analysed are Hypoxanthine levels, trimethylamine (TMA) (4), 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.

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 (6).

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

Parameters employed by the judges are as follows:-

- (i) Appearance or external characteristics, (ii) Texture refers to the degree of loss of elasticity of the flesh, (iii) Odour, (iv) Colour, (v) Taste.

For the cooked fish, the sample were filleted and steamed for 25 minutes. Taste panelists were then asked to rinse their mouth before tasting, so as to avoid any bias in the result.

The scores were based on; (i) Taste. (ii) Flavour

Microbiological Assessment/Analysis

Microbial count = Number of colonies x
Dilution factor.
Data Analysis

The statistical tools used to analyse the result obtained are; Correlation Analysis and Simple Linear Regression Analysis according (13).

Results

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.

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

Table 1 showed 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 -40C. Ten (10) bacteria species were detected in the samples under cold storage at – 40C. Out of the 10 bacteria species identified (a) *Lactococcus acidophilus* (b) *Proteus morganii* (c) *Pseudomonas aureginosa* and (d) *Clostridium welchii* had the highest number of occurrence with 6.14, 5.24, 3.50 and 2.72 x 10⁴ Cfugm respectively as shown in Table 3. 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 x 10⁴ Cfugm) to (14.70 x 10⁴ Cfugm) in the 12th week, with an overall Total Viable Count (TVC) of 29.36 Cfugm.

Results further revealed that a positive linear correlation exist for all the parameters with significant difference (P < 0.05) for PV, TMA, FFA, Hx and total viable 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.

TABLE 1: Organoleptic Assessment of The Fresh and Cooked Samples of The Chub Mackerel

Parameters determination	Samples And Length of Storage Period In Weeks						
	AB 0	A ₁ 2	A ₂ 4	A ₃ 6	A ₄ 8	A ₅ 10	A ₆ 12
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- Excellent
- 2- Very good
- 3- Good
- 4- Satisfactory
- 5- Fairly satisfactory
- 6- Fair
- 7- Poor
- AB- Baseline
- A₁ – A₆ – Stored sample

TABLE 2: Biochemical Assessment Of Cold Stored Sample Of The Chub Mackerel (*Scomber Japonicus*), At -4°C Length Of Storage Period In Weeks

Parameters determined	Weeks						
	0	2	4	6	8	10	12
Peroxide value (PV)meq/100gm fish	26.40	27.80	28.90	30.10	30.30	31.20	34.60
Trimethylamine (TMA) mg/100gm fish	29.62	30.10	31.60	33.20	34.60	36.70	39.20
Free fatty acid (FFA) %	1.74	1.82	1.88	1.93	1.98	2.14	2.32
Hypoxanthine (Hx) mg/100gm fish	28.24	29.46	30.72	31.86	32.65	34.26	37.54

TABLE 3: 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

Isolated organisms (bacteria flora) (cfu/gm (10 ⁴))	0	4	8	12	Overall total variable Count (TVC)
	AB	A ₁	A ₂	A ₃	
<i>Bacillus subtilis</i>	-	-	1.50	0.90	2.40
<i>Bacillus cereus</i>	-	-	-	2.10	2.10
<i>Clostridium welchii</i>	-	1.52	-	1.20	2.72
<i>Escherichia coli</i>	-	0.96	1.00	1.40	2.36
<i>Lactococcus acidophilus</i>	1.82	1.62	1.10	1.60	6.14
<i>Proteus morgani</i>	-	1.24	2.10	1.95	2.40
<i>Pseudomonas aureginosa</i>	-	-	1.80	1.70	3.50
<i>Micrococcus acidophilus</i>	-	-	-	1.50	1.50
<i>Staphylococcus aureus</i>	-	-	-	1.30	1.30
<i>Streptococcus faecium</i>	-	-	-	1.10	1.10
Total Viable Count	1.82	5.34	7.50	14.70	29.36

Discussion

From the proximate composition result 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 (5) and (12) findings.

The Chub Mackerel (*Scomber japonicus*) is a fatty fish thereby making it to spoil fast. This is further confirmed by the higher values of Hypoxanthine (Hx) (37.54mg/100gm fish), Peroxide value (PV) (34.60Meq/Kg), Trimethylamine (TMA) (39.20mg/100gm

fish), Free Fatty Acid (FFA) (2.32%) and Total Viable Count (TVC) (14.70 x 10⁴ Cfugm) recorded at the end of the 12 week study. These findings are in line with (10), (3), (4), and (11) as reported in their studies for the fish to be in good condition.

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 the work of (6).

A total of 10 bacteria species were detected in the study, with *Lactococcus acidophilus* showing the highest prevalence of 6.14 Cfugm and also showing its

presence from the 0 week (1.82 Cfugm) to the 12th week (1.60 Cfugm). Second to it is *Pseudomonas aureginosa* (3.50 Cfugm) detected from (8th – 12th week), while the third bacteria prevalent was *Clostridium welchii* with 2.72 Cfugm 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 subtilis* (2.40 Cfugm), *Proteus morganii* (2.40 Cfugm), *Escherichia coli* (2.36 Cfugm), *Bacillus cereus* (2.10 Cfugm), *Micrococcus acidiphilus* (1.50 Cfugm), *Staphylococcus aureus* (1.30 Cfugm) and lastly *Streptococcus faecium* (1.10 Cfugm).

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/shelf 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 *Clostridium 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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