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Evaluation of total volatile bases and trimethylamine in hake (*Merluccius capensis*) fish preserved at low temperature in Vanderbijlpark, South Africa

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Abstract

Fish is one of the most highly perishable food product and its quality aspects are of significance to retailers and consumers. The purpose of this study was to investigate the change in quality of hake (*Merluccius capensis*) fish preserved at -14°C over a period of seven weeks in other to evaluate fish spoilage indicators. The fish quality parameters assessed were pH, trimethylamine nitrogen (TMA-N) and total volatile bases nitrogen (TVB-N). The data revealed that there was an increase in pH from 7.4 to 7.7 during the storage period. The concentration of TVB-N in the hake samples increased from 7.21 to 23.35 (± 0.036) mg-N/100g while that of TMA-N increased from (range: mean ± std) 3.18 - 3.34: 3.25 ± 0.068 to 13.90 – 15.01: 14.29± 0.493 mg-N/100 g within the seven weeks of preservation, with percent increase of 69.13% (TVB-N) and 77.26% (TMA-N) of the initial amount. The overall data exhibited a steady and consistent increase in the amount of TVB-N, TMA-N and pH in the flesh of hake fish preserved at the temperature and duration of the study. This steady increase in these chemical parameters is a significant potent warning to consumers to resist lengthy storage of fresh fish and its products before consumption. One-way analysis of variance was adopted to assess the validity of the parallel increase of TVB-N, TMA-N, pH with storage duration at low temperature and the analysis revealed no significant differences between increase in the three chemical parameters and storage time. In other words increase in TVB-N, TMA-N, pH content is directly proportional to the increase in storage time. This study have provided useful data to benefit South African retailers on storage and maintenance of TVB-N and TMA-N limits of acceptance in the consumption quality of hake to be sold to consumers and also for consumers on storage duration of hake in their refrigerators before consumption.

Keywords: Total Volatile Bases; Trimethylamine; Hake Fish; Food Quality; Food Measure; Food Properties

1. Introduction

Hake fish is South Africa's third most commercial fish species after and provides half of the value of all fisheries in South Africa and consumed by majority of South Africans. Hake is a member of the *merluccius* spp family, found on the continental shelf and slope to depths between 150 to over 1,000 metres (SFRI, 1994).

The South African Fishing industry currently contributes an estimated R6bn to South Africa's economy but only ranks 30th among fishing nations worldwide. The fishery is divided into an offshore sector targeting deep-water hake (*M. paradoxus)* and an inshore sector targeting shallow-water hake (*M. capensis*) with a total landings of hake fish amounted to 111,487 tonnes in 2011. 55% of the deep-water hake (*M. paradoxus)* catches are exported, while over 90% of the

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shallow-water hake (*M. capensis*) end up for local consumption (WWF Fisheries, 2012). 55% of demersal trawl catches are exported. Therefore hake plays a vital role in the national economy of South Africa and its contribution to export earning is rapidly increasing. The local market for the shallow-water hake (*M. capensis*) in South Africa is important because of its lucrative size, good taste and high market demand by consumers.

Fish is a significant part of human nourishment because they are an extraordinary source of protein, low saturated fat, vitamins and minerals and omega-3 fatty acids which are acknowledged to support good well-being (Kumarand Mukherge, 2011, Dural *et al*., 2007 and Nkpaa *et al*., 2016). Omega-3 fatty acids is a vital nutrient for brain development in human (Spencer *et al.,* 1971, Jaclyn *et al.,* 2010). Therefore fish consumption is an affirmative contribution to healthful diet.

However, fish is more perishable than other protein foods (Burgess and Shewan, 1970) and its freshness is the most important criteria for judging the quality (Rodriquez-Jerez *et al.,* 2004).

Putrefaction in dead fish tissue is a consequence of series of complicated deteriorative changes brought about by its own enzyme, by bacteria and by chemical action (Shewan, 1976, (Ali *et al.,* 2010).). According to Jones, (1954) the early reaction of spoilage is autolytic, while bacterial enzymes become gradually more active in the later stages. After death of fish, the oxygen supply in the tissue stops due to disruption of the circulatory system. At the early phase of post-mortem, the mitochondrial system also stop to function, this leads to a steady exhaustion of Adenosine triphosphate (ATP) by various ATPase actions. Subsequently, when the remaining supplies of creatine phosphate have been exhausted, anaerobic glycolysis continue to regenerate some ATP which results with the end product of lactate accumulation (Foegeding *et al.,* 1996).

Due to formation of lactic acid from glycogen by a series of enzymatic reaction in the tissue, the pH value decreases during the post-mortem changes of the fish muscle. When the pH reaches a critical value, certain critical enzymes, such as phosphofructokinase, are inhibited and glycolysis stops. A drop in the pH of the muscle triggers the release of proteolytic enzymes such as *cathepsin*, which has the capacity to metabolize trimethylamine oxide (TMAO) in fish muscle producing a wide variety of volatile compounds such as ammonia (NH_3), mono-methylamine (MMA), dimethylamine (DMA) and trimethylamine (TMA) resulting in off-flavors and odors. The chemical reduction of trimethylamine oxide (TMAO) in the fish muscle initiated by aerobic microbes may be expressed as

$$6(CH_3)_3N^+O^- \xrightarrow[\text{microbes}]{\text{aerobic}} (CH_3)_3N + (CH_3)_2NH + CH_3NH_2 + NH_3 + 3O_2 + N_2 + 15H_2$$

The total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile bases (TVB-N) nitrogen content of the fish, which is normally used as an estimate of spoilage. Total volatile nitrogen (TVB-N) has been widely used as an index for freshness of fish (Stansby *et al.,* 1944), while changes in TMA-N content and pH value can be used as a chemical method to indicate fish spoilage. These chemical changes in stored fish products lead to short shelf life and economic losses (Horsfall *et al.,* 2006). This deteriorative changes occurring in the fish muscle makes the fish to lose its freshness and its consumption may pose health risks to consumers. Quantification of these compounds can provide a measure of the progress of deterioration (Connell, 1995). The required TVB-N limit of acceptance in *Merluccius spp* family as stated by the law regulation is max 35 mg N/100 g (EC No.2074/2005, EU/EC 2008). Yusuf *et al*., (2010) discovered that, the increase in the amount of TVB-N is parallel with the increase in TMA-N during spoilage. It has been found experimentally by Oehlenschläger, (1997) that TMA-N remains on its initial level, inferior to 3 mg/100 g concentration during about 10 days for cod in iced storage, until the onset micro-bacterial action starts. After this first stage of apparent stability, TMA-N increases rapidly until the fish is spoiled. Oehlenschläger (1997) also found that, after the early days of ice storage, the TVB-N content increases with the scattering of the values mostly produced by spoilage bacteria of the muscle fish during iced storage.

An investigation of microbiological and chemical changes of Nile tilapia fillet in ice storage by Okeyo *et al*., (2009) were total volatile base nitrogen (TVB-N) and pH were measured at the interval of four days, reveals that the lower the pH of fish flesh, the slower the bacteria growth and vice versa. While using pH and TVB-N as parameters to determine the quality change of breaded Kilka fish during frozen storage of -18°C over a period of four months, Khanipour *et al.,* (2014) found no significant differences between the initial pH value and those at the storage period. It was further observed that changes in TVB-N of breaded Kilka fish during frozen storage increased as the storage time increased. The increasing of TVB-N value during storage is related to bacterial spoilage and activity of endogenous enzymes.

Despite the significance of pH, TVB-N and TMA-N as a measure of the degree of fish spoilage, information on these substances in the South African shallow-water hake (*M. capensis*)) is very scanty, which is the focus of this study.

Therefore, the aim of this study is to investigate chemical changes of hake during -14°C temperature storage and evaluate the effect of low temperature on pH, formation of TVB-N and TMA-N as indices of fish spoilage in South African.

2. Materials and Methods

2.1. The Hake Fishery

South Africa has a coastline in excess of 3 000 km and an Exclusive Economic Zone (EEZ) in excess of 1 million km^2 which contains a variety of fish species (SFRI 1994). The mainstay of the demersal catch consists of hake (*Merluccius capensis* and *M. paradoxus*) which occur on the south coast over the Agulhas Bank and are distributed on the west coast of South Africa. The distribution of each species is depth-dependent; *M. paradoxus* occurs in deep water while *M. capensis* is a shallow water species (Botha 1973, 1985) (Figure 1). The deep-sea fishery operates on the west coast, and in waters deeper than the 110 m isobath on the south coast, whereas a small inshore fishery operates over the shallower Agulhas Bank.

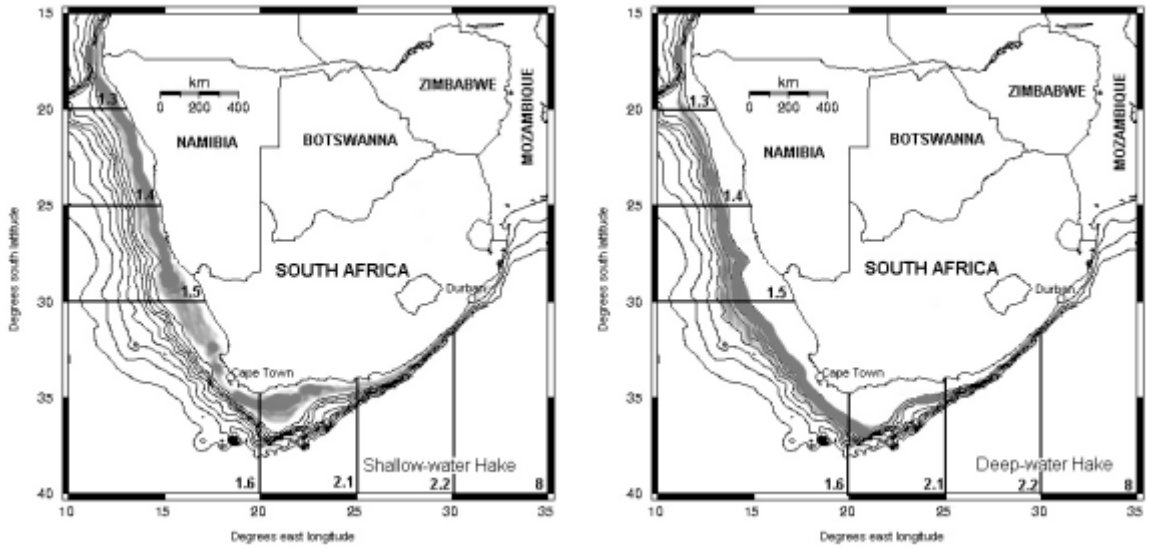

Figure 1 Distribution of *Merluccius capensis* (shallow-water cape hake off the coast of South Africa and Namibia. The ICSEAF divisions are also shown. (*Source:* Hutton and Sumaila, 2000)

2.2. Sample Collection

The hake fish caught at the south coast over the Agulhas Bank was collected at Fishery Landing Berth of the Container Terminal at the Cape Town Port. The samples were transported to VUT laboratory in insulated plastic boxes and in the most aseptic manner and immediately stored -14°C temperature in the refrigerator for subsequent morphological identification and chemical analyses.

2.3. Chemicals and Reagents

Distilled deionized water was used throughout this investigation. All chemicals used were analytical reagent grade. All standards and fish slurry were prepared in 7.5% trichloroacetic acid (TCA). Other reagents used include 10% NaOH, 4% boric acid solution, methyl red/methylene blue (2:1) indicator (Merck), 35% of formaldehyde (Merck), buffer 4 and buffer 7 (Merck) and 0.01 N Sulphuric acid (H_2SO_4) solution.

2.4. Sample preparation

A total of approximately, 1kg of each fresh hake (*Merluccius capensis**)*** with individual weights between 300-400g and total lengths between 45-55 cm were selected for the chemical quality analysis. Total of four individuals hake samples were studied separately at the interval of two, four, five and seven week storage period. A sample was allowed to thaw by placing on a ceramic tile in the laboratory at ambient temperature, then fleshy muscles around abdomen just below

the head and above the tail was carefully sliced and blended using a food processor to ensure homogeneity of the sample. Experiments were carried out in triplicate for each species.

2.5. Determination of pH of Flesh Muscle of in Hake (*Merluccius capensis)*

pH determination was achieved by the method of Huss *et al.,* (1995). Following this method, 5 g of homogenized fish sample was weighed and 50 ml of distilled water was added. The pH was measured using digital pH meter, by inserting the electrode into the sample solution. The pH meter was calibrated using pH 4 buffer and pH 7 buffer.

2.6. Determination of Total Volatile Bases (TVN) in Hake (*Merluccius capensis)*

Total Volatile Bases nitrogen (TVB-N) was determined by a slight modification of the method of Malle and Poumeryrol, (1989). 100 grams of the fish muscle were chopped and homogenized and mixed thoroughly with 200 ml of 7.5% aqueous Trichloroacetic acid (TCA) solution in a 500 ml beaker which extracted organic nitrogen bases that yielded ammonium chlorate precipitate. The pH was adjusted to 5.2 by addition of few drops of 2M HCl, followed by heating at 70°C and cooling to room temperature. After cooling, the digested solution was centrifuged at 3000 rpm for 5 min and the supernatant liquid was filtered using Whatman filter paper No1 to obtain clear extract. An aliquot of filtrate was pipetted and added to a Kjeldahl-type distillation tube, followed by addition of 30 ml 10% NaOH solution that reacts with ammonium salt to form ammonia gas. Distillation process then commenced and during the distillation, the ammonia gas released was trapped inside a receiving flask containing 25ml of 4% boric acid and few drops of mixed indicator (methyl red/methylene blue 2:1). The flask was placed under the condenser, where the end of the condenser was immersed inside the boric acid solution. Steam entrainment continued until a final volume of 200 ml was obtained in a flask. The colour change from dark grey to green was observed as the ammonia gas was trapped inside the receiving flask to yield ammonium borate complex. The ammonium borate complex solution was titrated against standardized 0.01 M H_2SO_4 to yield ammonium sulphate salt which purify nitrogen bases by altering their solubility to the endpoint indicated by green - grey colour change. TVB-N content was calculated as below and expressed as mg N/100 g sample.

$$\text{TVB (mg N/100mg)} = \frac{(V_T)(14\ \text{g/mol})(0.01\ \text{mol/L})(100)}{V_S}$$

Where: V_T = Volume of titre (H_2SO_4); V_S = Volume of sample (Digested aliquot) and 14 = Molecular weight of Nitrogen

2.7. Determination of Trimethyl -Amine-Nitrogen (TMA-N) in Hake (*Merluccius capensis)*

Trimethyl-amine nitrogen (TMA-N) was determined by a slight modification of method of Malle and Poumeryrol, (1989). 100 grams of the fish muscle were chopped and homogenized and mixed thoroughly with 200 ml of 7.5% aqueous Trichloroacetic acid (TCA) solution in a 500 ml beaker which extracted organic nitrogen bases that yielded ammonium chlorate precipitate. The pH was adjusted to 5.2 by addition of few drops of 2M HCl, followed by heating at 70°C and cooling to room temperature. After cooling, the digested solution was centrifuged at 3000 rpm for 5 min and the supernatant liquid was filtered using Whatman filter paper No1 to obtain clear extract. An aliquot of filtrate was pipetted and added to a Kjeldahl-type distillation tube, followed by addition of 30 ml 10% NaOH solution that reacts with ammonium salt to form ammonia gas. A 20 ml 35% of formaldehyde was added to the distillation tube, to mask the primary and secondary amines. Distillation process then commenced and during the distillation, the ammonia gas released was trapped inside a receiving flask containing 25ml of 4% boric acid and few drops of mixed indicator (methyl red/methylene blue 2:1). The flask was placed under the condenser, where the end of the condenser was immersed inside the boric acid solution. Steam entrainment continued until a final volume of 200 ml was obtained in a flask. The colour change from dark grey to green was observed as the ammonia gas was trapped inside the receiving flask to yield ammonium borate complex. The ammonium borate complex solution was titrated against standardized 0.01 M H_2SO_4 to yield ammonium sulphate salt which purify nitrogen bases by altering their solubility to the endpoint indicated by green - grey colour change. TMA was quantified using a calculation as shown in equation 3 and expressed as mg N/100 g sample.

$$\text{TMA (mg N/100mg)} = \frac{(V_T)(14\ \text{g/mol})(0.01\ \text{mol/L})(100)}{V_S}$$

Where: V_T = Volume of titre (H_2SO_4), V_S = Volume of sample (Digested aliquot, 14 = Molecular weight of Nitrogen

2.8. Statistical evaluation

The reliability of experimental data collected from this study were analysed using the Excel XP 2013 computer software maintain a significance level of 90%. The data were first subjected to a descriptive statistical analysis to compute the mean, standard deviation and errors. One-way analysis of variance (ANOVA) was also performed to test the significance difference in the levels of quality indicators in the hake fish during the seven storage periods. Finally correlations between the quality parameters during different storage periods were assessed.

3. Results and Discussion

Contaminations and subsequent decomposition of fish and marine products could occur when handled and treated un-hygienically. The processing and preservation of fresh fish were of utmost importance since fish is highly susceptible to deterioration immediately after harvest and to prevent economic losses (Okonta and Ekelemu, 2005). Lack of adequate fish handling, processing techniques and storage facilities contribute significantly to the low supply of fish to poor rural dwellers that form three quarters of the population in developing countries (Ayuba and Omeji, 2006). Fish harvesting, handling, processing, storage and distribution provide livelihood for millions of people as well as providing valuable foreign exchange earnings to many countries (Al-Jufaili and Opara, 2006). It had been noted that more than 20% of the processed fish were lost before reaching market. Fish processors identified the problems due to the following factors: delays in landing and processing of fish caught, inadequate processing, and poor handling prior to marketing. This report is in agreement with Regenstein and Regenstein (1991).

3.1. pH of Flesh Muscle of Hake (*Merluccius capensis)*

pH is one significant guide to define the quality of fresh fish. The pH of muscle tissue of live fish is close to neutrality (Huss, 1995, Pacheco-Aguilar *et al.,* 2000). In this investigation, the initial pH value (mean ± std) of fresh hake fish muscle was 6.79 ± 0.075). The storage of the hake fish for a period of seven (7) weeks gave a gradual and consistent increase in pH value of the fish muscle. At week two (2), the pH value was 6.57 ± 0.044 which increased to a value of 6.311 ± 0.021, 6.22 ± 0.041, and 5.79 ± 0.026. for weeks 4, 5, and 7respectively. The changes in pH value of the hake flesh muscle during storage at -14°C within the study storage periods are represented in Fig 3, indicating that, pH values increased with the increase in storage time.

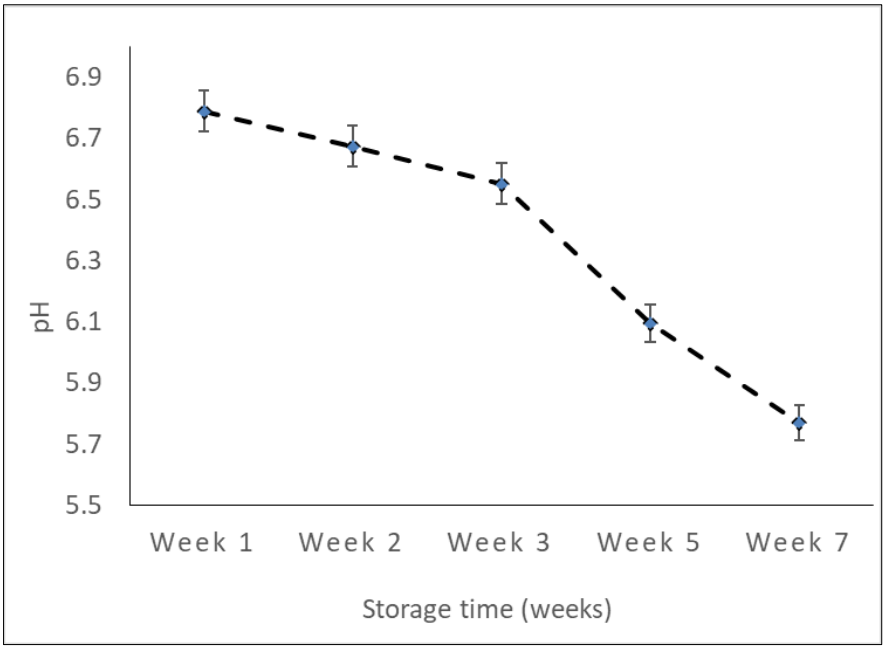

Figure 2 Changes in pH value of hake flesh muscle during storage at -14°C.

This value increased during storage at the temperature of -14°C, reaching a value of 7.7 at the end of the 7-week storage period. The increasing pH values could be associated with the production of basic components induced by the growth of bacteria (Simeonidou *et al*., 1997). Lower pH value is related to greater losses during further meat processing and high pH value is related to shorter shelf life but also better eating quality (Gregory *et al*., 1994). The pH changes are in agreement with the findings of Manthey *et al.,* (1988) and Ryder *et al.,* (1993). According to Bremner (2002), the pH

level of live fish is 7.0 and the post mortem pH varies from 6.0 to 7.1 was found to be sensorial acceptable (Erkan and Ozden, 2008). The increase in pH levels with regard to increase in volatile bases and accumulation of ammonia due to decomposition of nitrogenous compounds by the microbial activities. According Kyrana and co-workers (1997) the increase in pH values after initial period reflected the production of alkaline metabolites in bacterial degradation of the fish muscle. This acidic pH of the fish muscle has an ability to support the bacteria and formation of a wide variety of amine compounds resulting from the direct decarboxylation of amino acids. Most spoilage bacteria possessing decorboxylase activity in response to acidic pH presumably, so that the organism may raise the pH of the growth medium through the production of volatile basic compounds, such as ammonia through amino degradation (Galli *et al*., 1993). This leads to proteolysis and the anaerobic breakdown of protein or putrefaction, which releases foul-smelling amine compounds. In this event, the flesh becomes more alkaline through alkalinity of fish flesh may inhabit bacterial growth and their subsequent deteriorative effect on the product alter the normal texture of the fish flesh making it unreasonably and unacceptably firm and tough (Gould and Peters, 1971).

3.2. Total Volatile Base Nitrogen (TVB–N) in Hake (*Merluccius capensis)* stored at – 14°C

Total Volatile Base Nitrogen (TVB–N) is a measure of the total content of TMA, DMA, ammonia and other basic nitrogenous compounds. The levels of TVB–N in hake fish stored at –14°C at different length of storage time up to seven (7) weeks for the four samples are presented in Fig 3. The data revealed that the concentration of TVB-N in the hake samples ranged from 7.21 to 7.33 mg-N/100g with a mean and standard deviation of 7.23 and 0.050 mg-N/100g within the first two weeks of storage, while in the third week of storage, TVB-N level (range, mean ± std) has risen to 10.21 – 10.29, 10.24 ± 0.042 mg-N/100 g. Storage of hake fish up to five weeks at the temperature of -14°C increases the TVB-N concentrations to between 15.79 to 16.15 (16.04 ± 0.165) mg-N/100 g. By the end of the seen week storage study period, the concentration has increase by a margin of 30.87% from its initial concentration to a mean (± std) value of 23.35 (± 0.036) mg-N/100g. As depicted in the figure (Fig.3), the levels of the initial concentration of the hake fish muscle lower in all the samples are much lower than the others and a gradual increase in the levels of TVB-N was observed from the first week up till week seven at the low temperature storage condition.

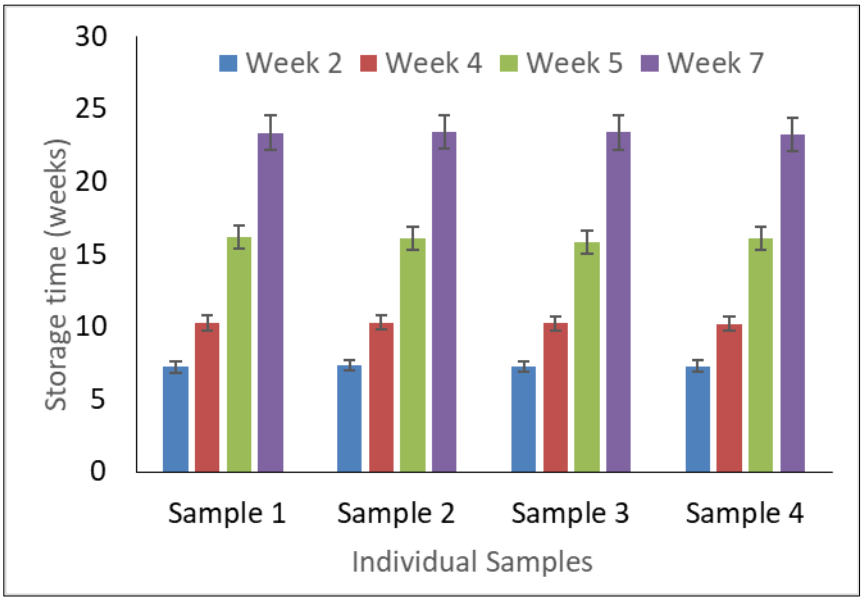

Figure 3 Changes in TVB (mg N/100g) in hake during storage at -14°C

At the end of the storage time (7-week), the data reveals a higher increase in TVB-N content from about 16 to 23 mg-N/100 g. The increase in TVB-N content is directly proportional to the increase in storage time. The initial low value of TVB-N is a sign of good quality of the fresh hake fish, while the slow and steady increases may be as a result of the action of autolytic enzymes and putrefaction microorganisms (Benjakul *et al*., 2003) that have initiated chemical conversion of the protein muscles of the fish.

The TVB-N contents of hake (*Merluccius capensis)* and the relationship between overall quality acceptances of good quality fish for human consumption was assessed using the TVB-N limit values for certain categories of fishery products ((EC) No.2074/2005, EU/EC 2008). According to the EC regulatory Law, the maximum specification of TVB-N required for fish to be unfit for consumption is 35 mg N/100 g. The TVB-N value obtained at the maximum time of seven-week

storage of the hake (*Merluccius capensis)* in this study ranged between 23.25 – 23.42 (23.35 ± 0.071) mg-N/100 g. This value of TVB-N found in hake stored at low temperature, was found to be within the required specification (max 35 mg-N/100 g), thus it is considered a good quality hake and fit for human consumption and considered spoiled and unfit for consumption when the value exceeds the law regulation by (EC) No.2074/2005, EU/EC 2008. The gradual and consistent increase in the level of TVB-N during storage indicates that prolonged storage of the hake fish before consumption should be discouraged.

Trymethylamine Nitrogen (TMA-N) is the best known compound produced during fish spoilage and it is mainly derived from bacterial breakdown of trimethylamine oxide (TMAO) which is an osmolyte naturally found in marine fish (Pedraso-Menabrito and Regenstein, 1990). Normally, fresh fishes are having 0.2 – 2.0 mg/100g TMA-N (Govindan, 1985). TMA does not increase much during the early stages of spoilage. Trimethylamine oxidase produce by spoilage organisms reduces trimethylamine oxide (TMAO) of fish flesh to trimethylamine (TMA-N), which is assumed to react with fish fats to produce the typical spoilage odor that are related to fish beyond their prime (Sikorski, *et al*., 1990; Triqui and Bouchriti, 2003). In this study the production of TMA-N in hake followed the similar pattern to TVB-N during the storage at -14°C, where a significant increase was observed with storage time (Fig. 6). The results indicate that formation of TMA-N increased from (range: mean ± std) 3.18 - 3.34: 3.25 ± 0.068 to 13.90 – 15.01: 14.29± 0.493 mg-N/100 g. The initial (2-week) low content of TMA-N of 3.25 (± 0.068) mg N/100 g could be considered as the most acceptable level of freshness of the hake fish. The TMA-N content of hake increased slightly during the 4 week of storage (3.19 – 4.23: 3.94 ± 0.50 mg-N/100 g). At the end of the study storage time of 7 weeks, TMA-N content has increased to about 77.26% of its initial amount. This higher increase in TMA-N content is directly proportional to the increase in storage time, which is a significant warning sign to consumers to resist lengthy storage before consumption. It can be seen from these results that increase in storage time even at low temperature reduces the acceptability of the fish for consumption. According to Malle and co-workers, (1986), TMA is produced during the chilled storage of fish as a consequence of the microbial degradation of trimethylamine oxide (TMAO).

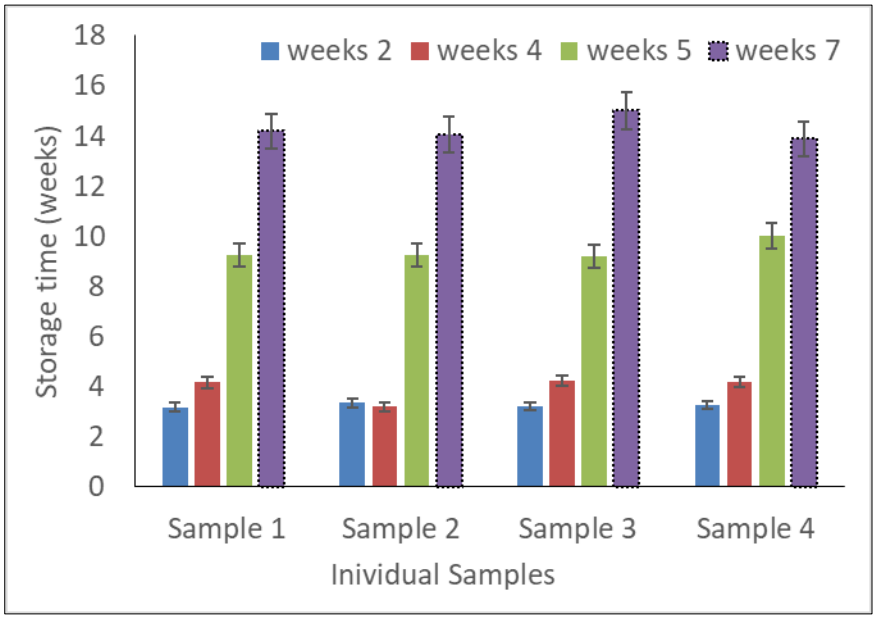

Figure 4 Changes in TMA (mg N/100 g) in hake during storage at -14°C

3.3. Comparative Evaluation of pH, TVB-N and TMA-N analyses Hake (M. spp)

pH, TVB-N and TMA-N are indices for evaluation of fish quality. These three chemical parameters are comparatively depicted in Fig 5 for Hake fish (insert) stored at lower temperature of -14°C over a period of seven weeks. The data showed a steady and consistent increase in the amount of TVB-N, TMA-N and pH respectively. According to Huss (1988) and supported by Khanipour and co-workers (2014), the stable increase in the amount of these chemical properties in fish muscles even at low temperatures are an evidential indices of quality deterioration.

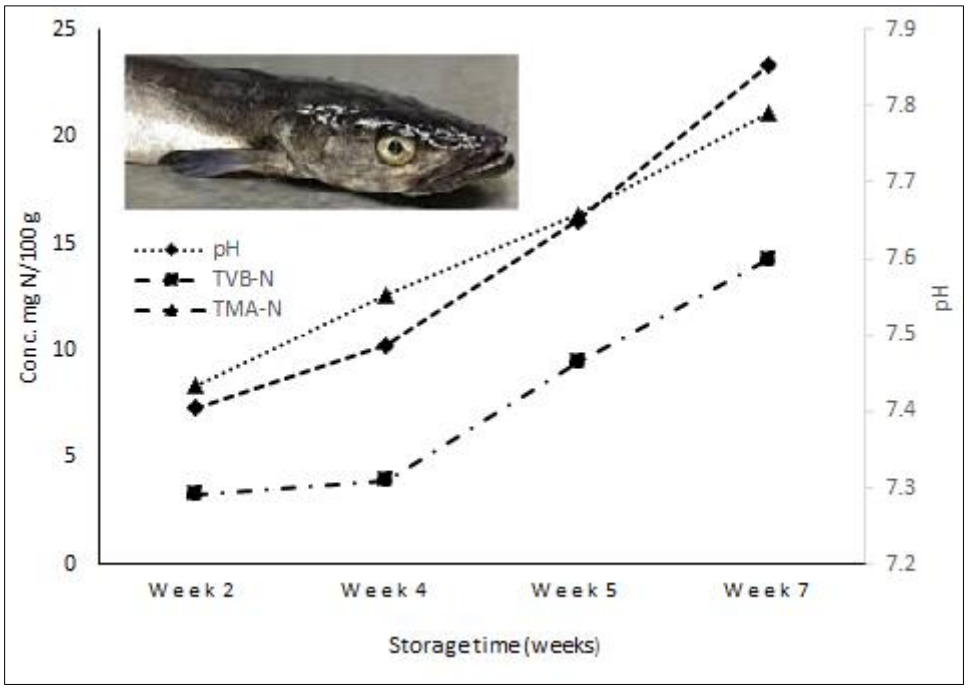

Figure 5 Comparative changes in the amount of pH, TVB and TMA in hake during storage at -14°C.

The increase in the amount of the three chemical parameters (pH, TVB and TMA) with change in storage period was evaluated by one-way analysis of variance (ANOVA). The internal structures of the analytical data of the three parameters were to were used to estimate the one-way analysis of variance (ANOVA) at α = 0.05 by considering the fish quality indices (pH, TVB-N, TMA-N as the objects and the storage period as the variable to formulate a null hypothesis, which states that the increase in amount of the three fish quality indices is equal to the seven weeks of storage time at the 0.05 significant level. The computed data from the one-way ANOVA (Table 1), result shows that, F_{exp} (3.22) < F_{crit}(4.01) meaning that there is no difference in the potential increase of the three fish quality parameters with storage time at the low temperature at the 0.05 significant level. A significance level of 0.05 indicates a 5% risk of concluding that a difference exists when there is no actual difference. To determine whether any of the differences between the means are statistically significant, the p-value obtained from the computation (Table 1) was compared with the significance level (0.05) used to assess the null hypothesis. Because the p-value (0.083), which is greater than the significance level of 0.05, (p > 0.05), the null hypothesis is accepted and the conclusion is that there is no significant differences between the increase in the amount of pH, TVB-N and TMA-N with storage time.

Table 1 One-Way Analysis of Variance (ANOVA) to compare increase in pH, TVB-N and TMA-N with storage time

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	149.4205	2	74.71025	3.221354	0.083202	4.102821
Within Groups	231.9219	10	23.19219			
Total	381.3424	12				

Pearson correlation matrix was also used to evaluate the behaviour of the three chemical fish quality parameters with each other and the data as shown in Table 2 revealed strong positive correlations > 0.95. This is a confirmation that, pH values increases, the levels of TVB-N and TMA-N may also increase.

Table 2 Correlation matrix of pH, TVB-N and TMA-N

	pH	**TVB-N**	**TMA-N**
pH	1		
TVB-N	0.97469	1	
TMA	0.95769	0.992053	1

Theses nonstop proportional increase in TVB-N, TMA-N, and pH content to increase in storage time may be adopted to regulate hake fish storage time for retailers and consumers in South Africa.

Consumer's greatest concern is the quality and safety of food they eat. The freshness of fish is very vital as positive medical advice of fish consumption continue to increase. In South Africa, storage temperature at retail outlets and individual homes for fresh fish and meat products are usually - 14 to -18^{o} C. These temperatures have the capacity to reduce chemical changes due to microbial actions but recent electricity load-shedding has the capacity to initiate faster chemical degradation of stored fresh protein products such as fish and meat. It is therefore recommended, that regulatory agencies should consider the incorporation of TVB-N and TMA-N parameters to determine fish and meat shelf-life. A study is recommended for fish freshness determination to be cooperated with the determination of TVB and TMA as indices for quality determination in fish.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to disclosed.

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