The United Nations University
FISHERIES TRAINING PROGRAMME

P.O. Box 1390, Skulagata 4

120 Reykjavik, Iceland **Final Project 2007**

ALTERNATIVE METHODS FOR FISH GRADING

Chebet Lillian, B.A.C.
Fisheries Training Institute,
P.O. Box, 124
Entebbe, Uganda
Tel: 256772953484
lilchebet@yahoo.com

Supervisors
Prof.Hjorleifur Einarsson, Ph.D.
University of Akureyri
hei@unak.is
and
Arnheidur Eythorsdottir, M.Sc.
University of Akureyri
arnh@unak.is

ABSTRACT

This study is aimed at screening for alternative methods for fish grading through choosing appropriate quality indicators and comparing alternative methods with sensory methods using the quality index method (QIM). Whole gutted cod fish samples were stored for 2, 3, 5, 6, 7, 8, 10 and 11 days from capture and were analysed within 24 hours at the University of Akureyri. Prior to the actual analysis, a pilot analysis was carried out to determine the application and precision of the application resazurin dye, ATP meter, and combur^{-3} strips to measure, microbial ATP expressed in relative light units (RLU), pH, protein, and glucose concentration in fish respectively in comparison with sensory methods for fish grading using QIM. Skin swab area of 100 cm^2 and gills swab, 10 mL of autoclaved water and 1 mL of resazurin were used. The results obtained indicated a wide variation in the different methods evaluated. RLU values indicated an inverse relationship with QI values, resazurin dye indicated a linear relationship with QI values, protein, glucose and pH test values indicated an inconsistent relationship with QI values and in some cases there was no relationship. However, conclusions and recommendations were made which are; QI values as expected exhibited a linear relationship with storage time; relative light units could be an appropriate method for grading fish but further research to identify appropriate volume (sample area) is recommended for better results. Resazurin dye could be a reliable and appropriate alternative method for grading fish both within and outside the laboratory, but calibration of resazurin dye colour change is highly recommended to enable estimation of the number of bacteria in the sample. pH, glucose and protein concentration seem not to be appropriate methods for grading fish due to inconsistent test values and perhaps other alternative indicators could be identified.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Fish quality is evaluated several times from harvest until finally the consumer judges the quality in his mouth. According to the findings of Alejandra *et al.* (1992), consumers consider quality the most important thing in making the choice between a variety of alternatives, although for others cost and parameters such as convenience are vital.

The outcome of these evaluations is often used for grading and classification and to decide appropriate operations following handling and processing steps. Different methods can be applied at different steps of the process depending on purpose, definition (of quality at that point) and suitability of different methods.

Methods can be subjective or objective in their nature and they can be fast or slow, destructive or non-destructive, online, at-line or of-line, expensive or cheap. It is clear that it is difficult to find a method that combines all requirements at the same time. From an industrial point of view, a rapid, objective, on-line and non-destructive method for quality determination would be of great benefit.

Sensory methods are widely used in the food industry to judge different quality attributes of raw materials, ingredients and final products. Sensory analysis has the advantages of being fast, measure several important attributes at the same time and relatively accurate in the hands of trained operators and often it is non-destructive.

On the other hand sensory methods can sometimes be subjective and can be difficult to calibrate between operators and even the results from one and the same operator can vary as a result of his or her condition and skills.

In the fish industry sensory based methods have been applied at several points in the handling and processing chain to sort and grade fish.

There has been an interest in finding alternative rapid methods for this task. The use of alternative rapid methods for fish grading in fish production does not only improve scientific interest or technical performance but can add value for both producers and consumers in the fish processing chain (Amerongen *et al.* 2007).

Alternative rapid methods for fish grading are also of significance to fish processing industries in determining the effectiveness of food safety measures, and meeting legal compliance with international standards as well as achieving logistical and operational goals in production.

Furthermore, with reference to the Food and Agricultural Organisation (FAO), the access by developing countries to food, including fish export markets worldwide, and particularly in the developed countries depends on their capability to meet the regulatory requirements of import countries. FAO technical assistance has enabled some developing countries adopt and implement comprehensive national food quality and safety standards based on the internationally recommended Codex Alimentarius Commission standards, guidelines and codes of practice which enables the production of better fish products acceptable to the consumers.

However, in all countries the fish industry bears the responsibility of meeting fish quality and safety regulatory requirements and one of the ways to ensure this is by using alternative rapid methods for fish grading. Currently, the quality index method (QIM) is the main and is commonly used method for fish grading world wide (QIM Eurofish 2005).

Whereas alternative rapid methods of fish evaluation are of international importance, they are of special interest in areas where fish can spoil very rapidly, e.g. in tropical countries like Uganda.

Further elucidation of these points is given below with a special reference to the situation around Lake Victoria and especially in Uganda.

1.1 Uganda fisheries profile

According to the Uganda Fisheries Department report (Export Promotion Board 2006) the Ugandan economy largely relies on the fishery contribution more particularly the earnings from fish exports. Fisheries export value ranks second to Uganda's traditional cash crops like coffee, cotton, tea, and tobacco.

With reference to the Uganda Export Promotion Board statistics (2006) coffee had the highest volume of 126,887 metric tonnes that earned US$ 189,830 whereas fish and fish products ranked second, with 36,461 metric tonnes of finished products that earned US$ 145,837

The total annual catch is approximated to 330,000 tonnes and the major commercial fish species are: "Mputa" or Nile perch *(Lates niloticus),* "Mukene cyprinid (*Rastrineobola argentea*) and "Ngege" a tilapia specie. Nile perch is estimated to constitute to 60% of the total annual catch, of which more than 80% is exported as chilled or frozen products.

Fishing is done using "planked" (open) boats, and a few motorised boats with gillnets, seines and long lines. Depending on the weather conditions and other factors, fish is delivered to fish processing plants a couple of days later the post harvest losses are relatively high (Lake Victoria Fisheries Organisation 2006).

With reference to the Uganda Fish Processors and Export Association (UFPEA) (2006), Uganda ensures the safety of its fish exports at all points in the production chain.

The Department of Fisheries Resources is the competent authority responsible for approval of HACCP manuals of all fish factories and certifying the safety and fitness for human consumption of all fish exports from Uganda, by carrying out tests on fish, water, and sediments of contaminants including heavy metals and pesticide residues. Sampling plans include periodic testing at internationally accredited laboratories in Uganda and the Netherlands.

In fish processing factories, UTPEA members have implemented the HACCP safety management system which includes:

- Organoleptic checks on fish performed throughout the processing chain.
- Microbiological and chemical testing done under strict scrutiny of the competent authority.
- Traceability systems are developed by the Fish Quality Assurance Managers Association, a branch of UTPEA which unites the Quality Managers of all member factories.

Identification of appropriate alternative rapid methods for fish grading would require adequate knowledge of fish spoilage processes and the resulting products (indicators) that can be measured.

1.2 Fish spoilage process and spoilage indicators

Fish spoilage is a sequential phenomenon that starts immediately after fish is captured and slaughtered. The combination of mechanical, autolytic, chemical and bacteriological changes leads to irreversible, undesirable fish quality changes.

Bremner (2002) defines fish spoilage as deteriorative changes in the sensory characteristics of a product such as appearance, flavour, odour and texture, which can also be used to indicate nutritional value, and safety.

1.2.1 Rigor mortis

When fish is slaughtered, blood circulation stops and consequently the supply of oxygen to facilitate the energy molecules ATP, required to enable muscle contraction and relaxation is inhibited.

In this event glycogen is broken down to enable production of energy in fish muscle and as the glycogen level declines the amount of ATP produced also declines.

Since the interaction of actin and myosin is triggered by myosin ATPase and calcium ions during muscle contraction requires ATP to fuel the reaction whose amount is already inhibited after fish slaughter, calcium ions leak into the muscles resulting in contraction (stiffening), a process referred to as rigor mortis. Stiffening continues for a couple of hours before they relax since there is no ATP to enable the muscles to relax again and operate as required.

The onset and end of rigor mortis is determined by fish holding temperature during handling (mechanical stress), fish size, and fish species. Small sized fish species, e.g. sardines and mackerel undergo rigor mortis earlier and faster than large fish species (Huss 1995).

The process of rigor mortis can lead to quality defects in fish flesh such as muscle damage/gaping, blood stain, loss of water holding capacity (WHC) and softening of fish flesh (Bremner 2002).

The resolution (end) of rigor mortis coincides with autolysis and the subsequent spoilage changes that include bacterial and chemical spoilage changes which finally deteriorate the quality of the fish, rendering it unpleasant or unsafe for consumption.

1.2.2 Autolytic spoilage

On slaughter of fish, enzymes in the gut and flesh, previously involved in metabolic activities instead catalyze autolysis (self digestion). Autolytic changes lead to decomposition of proteins, and other vital compounds that consequently eventually result in the softening of the fish flesh and unpleasant loose/mushy substances in the gut cavity (Bremner 2002).

1.2.3 Bacterial fish spoilage

Bacterial activity is the main cause of fish deterioration and particularly the specific spoilage bacteria (SSB).

In a healthy living fish, bacteria are present on the surface, gills and intestines but are not able to cause any spoilage because of the natural defensive mechanism in the fish. Autolytic changes ease bacterial entry into the flesh where they get good nutrition for growth by decomposing various fish components such as trimethylamine oxide (TMAO) and other non protein nitrogen molecules, lipids, amino acids among others, leading to production of undesirable off-odours in fish.

However, tropical water fish species exhibit a longer shelf life than temperate water fish. *Lates niloticus* at 25^{o}C spoils in 11-17 hours time with bacterial counts of 10^{8} org /gm with *Aeromonas* being the dominant bacteria (Huss 1992).

1.2.4 Chemical spoilage

Lipid hydrolysis and oxidation are the major deteriorative changes depending on the chemical composition of the fish.

In accordance with Huss *et al.* (1992), the primary stage of lipid oxidation leads to the production of hydro-peroxides associated with tasteless and brown, yellowish discolouration in fish tissues; further degradation of hydro peroxides result in the production of more volatile compounds; aldehydes, kentones and alcohols associated with strong, rancid odours.

Rancid odours are more associated with frozen or dried storage state fish products which usually occur rather late in the spoilage process. However, post mortem changes in fish are irreversible. A summary of these changes is shown in Table 1.

Table 1: Fish spoilage processes and resultant products (indicators) and period of occurrence (Bremner 2002, Huss *et al.* 1992, Huss 1995, Bourgeois *et al.* 1995, and Farid 1991).

Cause of deterioration	**Substrate**	**Product/spoilage indicator**	**Period of occurrence**
-Chemical activity	-Glycogen	1.Lactic acid(Low pH) 2. Flesh discoloration 3 Eh voltage lower.	Early stages in spoilage process
-Mechanical damage	Connective tissues	1.Gaping,.softened fish muscles 2.WHC lowers 3.Blood stained flesh	Early stages
-Autolytic activity	Nucleotide, ATP	1.K-Value	Early stages
-Bacterial activity	- Fish tissue	1. ATP 2 Soften fish muscle	Later stages
-Autolytic & bacterial activity	-Nucleotide, ATP,	1.Loss of fresh fish flavours & production of (Hx), a bitter flesh taste	Later stages
-Bacterial & autolytic activity	-Protein, peptides & amino acid	1.Ammonia (NH_3) 2. Hydrogen sulphide (H_2S) 3. Softening of fish flesh 4. Lipopolysachaarids (endotoxins) 5. Peroxidase 6.TVB-N	Later stages
-Bacterial activity	-Amines, TMAO	1. TMA- (Fishy off-odours) 2. DMA &FA (in frozen fish) 3. Ammonia	Later stages
-Bacterial activity	-Biogenic amines N-containing amino acid	1. Histamine	Later stages
-Chemical & bacterial activity	-Lipids (fish oils) (Lipid oxidation & hydrolysis)	1. Hydro peroxides 2.Soapy off-odours, (aldehydes, & ketones, alcohol) 3.Discoloration (yellowish browning) in fish flesh	Later stages

1.3 Methods for fish grading

Fish grading and analysis can be done by methods such as sensory evaluation, microbiological and chemical methods that can be rapid or slow but the principle of analysis in both slow and rapid methods is based on measurement of the products resulting from fish post mortem spoilage.

Fung (2002) defines "rapid methods" as miniaturised biochemical kits, antibody, DNA-based tests, which are modifications of conventional tests aimed at making analysis and detection faster, more convenient, sensitive, and more specific than conventional methods.

According to Bourgeois *et al.* (1995) the use of rapid methods in fish quality grading is based on the detection of physical or chemical signals which are indicative of the presence and activity of microorganisms even though they may not have a direct relationship with their possible harmfulness.

These signals include: cellular bodies, macromolecules, intracellular bio-molecules, excreted metabolites, physical parameters of the medium and indicators changing colour under microbial metabolic influence.

Various rapid methods can be used to measure these signals/parameters and among others they include electrical, chemical, physiological and immunological changes.

1.3.1 Traditional (conventional) methods for fish grading

1.3.1.1 Sensory analysis

As demonstrated by Meilgaard *et al*. (1991), a sensory method is a scientific discipline that the analyst uses to measure and interpret the characteristics of a product (food) following given described quality parameters (appearance, texture, odour and taste or flavour) on the prepared quality index (QI) scheme with 0-3 demerit score for each quality attribute.

With reference to QIM_eurofish (2005), QIM is an objective method used for evaluation of raw fish, based on specific attributes such as the eyes, skin, and gills using a scoring system from 0-3 with a description of each parameter written in a guideline.

The scores for all the attributes are added up to give an overall sensory score referred to as a quality index (QI), the lower the score, the fresher the fish. The QI increases linearly with the keeping time of fish in ice.

Sensory methods are commonly used for food grading today in food production including fish or everything else that can be used and it is considered to be more reliable particularly if it is done properly.

In accordance with Meilgaard *et al.* (1991) and Alejandra *et al.* (1992), the primary purpose of evaluation is to conduct valid and reliable tests, to provide data upon which informed decisions on the product can be made. However, the use of sensory

methods to grade fish can be limited by training, the physiological and psychological status of the analyst.

1.3.1.2 Measurement of total volatile bases (TVB)

As mentioned earlier, bacteria produce enzymes that catalyze decomposition of various fish components after slaughter that reduce compounds such as trimethylamine oxide (TMAO) to trimethylamine (TMA), and other non-protein nitrogen to total volatile basic nitrogen (TVB-N).

Accumulations of TMA and/or TVB-N are characterised by undesirable odours and flavours in fish and this can be used to indicate fish spoilage.

Determination of TVB-N and TMA in fish flesh can by measured by steam distillation of fish extract with subsequent titration against a strong acid, such as sulphuric acid (H_2SO_4) (Malle and Poumeyrol 1989).

However, according to Huss (1995), the use of TMA or TVB-N as indicative parameters of fish quality deterioration is limited to fish species, e.g. TMA is not common in some fish species and its accumulation shows up in the later stages of spoilage. TVB-N concentrations may be affected by the mode of handling during the analysis, and they are often destructive in application.

1.3.1.3 Microbiological analysis (plate count) method

Bacteria naturally exist on the skin, gills, and in the gut but they are not able to cause spoilage because of the natural defensive mechanism in healthy living fish. After slaughter of fish, bacteria find access into the fish flesh not only due to lost sterility, but because of the various mechanical and autolytic changes that rupture gut walls and soften fish flesh, making it easy for bacteria to access fish tissue. The activity of bacteria in the fish, especially the specific spoilage bacteria (SSB), including *(Shewanella, Photobacterium* or *Pseudomonas*) decomposes various fish components such as TMAO and produces undesirable odours, flavours and taste in the fish as well as being a health hazard to consumers (Bremner 2002).

With reference to Bourgeois *et al.* (1995), plate count methods are used to determine both total viable cells and specific spoilage bacteria in food products, (fish) and it involves inoculation and incubation of samples in petri-plates at appropriate temperature and subsequent colony counting.

The most important of these bacteria are those that have the ability to produce H_2S and/or reduce trimethylamine oxide (TMAO) to trimethylamine (TMA). Specific spoilage bacteria estimation can be useful in determining the quality and the remaining shelf life of fish products.

However, the plate count method requires a lengthy period to produce results and therefore it is more often used as fish quality monitoring measure.

.

1.3.2 Rapid methods for fish grading

1.3.2.1 Measure of fish electrical properties (Torry meter)

With reference to Huss (1995), electrical properties of fish skin and tissue change immediately after slaughter. Metabolic activities that enable microbial growth in fish convert various molecules that are electrically inert to ionized molecules which results in changes in the dielectric properties (conductance, capacitance) in the fish tissue.
Change in the electrical properties in fish can be used as an indicative sign of quality deterioration in fish and therefore provides a means of measuring post mortem changes or the level of spoilage by using the Torry meter.

The Torry meter is a compact device, equipped with a sensing unit with a pair of electrodes which passes an alternating current through the fish and as a result voltage is sensed in the inner pair. Both the current and voltage are converted electronically into digital numbers that are conveniently read on the scale in the range of 0-16 scores.

The Torry meter measures and indicates scores that can be correlated with spoilage level, as measured by sensory methods or by any of the non-sensory methods. The Torry meter measures up to 16 samples, giving the average sum score. According to the findings of Huss *et al.* (1992) the use of the Torry meter requires the following:

- An organoleptic chart for the fish species in question.
- Temperature of the fish should be between 0°C to 10°C; dryness or ice crystals on fish surface should be avoided to ensure accurate results.
- Only chilled whole fish or fillets with skin, but not frozen and thawed fish instead, the meter may be used to detect thawed fish that has previously been frozen.

Other alternative ways of measuring changes in electrical properties in fish is by using electrodes or RT-Freshness Grader.

The RT-Freshness Grader is a recent development of Rafagnataekni Electronics (1986) in Iceland. The sensing unit is based on the Torry meter and it determines changes in electric properties such as conductivity, capacitance of skin on fish utilising the same principle as the Torry meter. The RT-Freshness Grader reads scores ranging from 11-16 for fresh cod and the values decrease with decreasing freshness to 1-6 (score), and can be used in online processing efficiently. However, despite its efficiency, up to today it has not achieved any commercial use (Huss *et al.* 1992).

1.3.2.2 Luminometric test of ATP

Adenosine tri-phosphate (ATP) is a universal energy molecule in all living cells including bacteria. In the cell, energy is principally transferred by ATP from the producing mechanism (respiratory process/chain) to the energy-consuming mechanism/system where it is utilised in muscular contraction, biosynthesis and/or bioluminescence of the organism.

Bacteria naturally existing on the skin surface, in the gills and the surroundings of live fish grow and proliferate into the fish tissue after fish slaughter and since all cells

including bacteria utilise energy (ATP) for metabolic activities, microbial ATP can be used as an indicator of the presence of living organisms in a medium.

In accordance with Bourgeois *et al.* (1995) microbial ATP concentration in a medium/food can be measured by using a luminometer. The presence of luciferase luciferin (a firefly system), oxygen, and magnesium ions, ATP will facilitate the reaction to generate light. However, the amount of light generated in this reaction is proportional to the ATP in the sample and therefore the light units can be used to estimate the number of cells in the sample.

With reference to Huss *et al.* (1992), the use of adenosine tri-phosphate (ATP) concentration to determine the actual number of microorganism is questionable in that:

- ATP concentration is not only the function of the presence of microorganisms but also the physiological status, species and stress exposed to the fish prior to capture and product residual/somatic ATP may alter the estimation of bacterial counts.
- ATP concentration could be reduced with efficient cleaning up of all possible sources.
- ATP concentration only provides an order of magnitude for a composite population of microorganisms. Therefore, it appears to be suitable for measurement of monospecific organisms under uniform growth conditions, like in monitoring fermentation progress.

This method also poses difficulty in the extraction of ATP, although various other reagents are usable, like dimethyl sulfoxide.

The actual measurement of light emitted is a problem in that the fluorimeters used have varying range limits of light detection of 1 µg/ml (Bourgeois *et al.* 1995).

1.3.2.3 Texturometer reading

During capture and after slaughter of fish, the connective tissues are destroyed. This depends on the mechanical handling and fish temperature during rigor mortis. Deterioration in fish flesh texture has quality implications on the product by lowering the water holding capacity (WHC), increasing micronutrient leakage and mushy, damaged texture, which allows better access of bacteria into the product.

Texture can be measured using the texturometer, a device utilising a cylindrical probe that is exerted on the fish with force, *F* that increases the preset value, and the corresponding depth of the concavity produced can be determined and related to fish flesh texture and elasticity.

The higher the value, the greater the deterioration of fish texture and therefore the poorer the elasticity and fish quality.

However, according to Huss (1995), the use of the probe press device poses difficulty in calculating the actual texture or elasticity especially when the probe cylinder has no digital scale. The force exerted on the food product/fish is destructive; causes

deformity of the product. The use of this method has a relatively high cost implication and has not gained popularity in fish processing.

1.3.2.4 Measurements of fish skin and gills redox potential using resazurin dye

After fish slaughter, oxygen transport cells stop and the redox potential (expressed as E_h) in the fish drops from approximately +200 mV to -200 mV. The time for this to occur is from 1 or 2 days (Huss 1995).

Bacterial multiplication on the surface of fish after slaughter absorbs oxygen and excretes substances that have the affinity of lowering the redox potential which consequently influences the quality of fish.

With reference to Bourgeois *et al.* (1995) and Huss (1995), redox potential can be measured in several ways using either resazurin indicator (dye), methylene blue indicator, resazurin strips (filter paper impregnated with resazurin dye), or by using electrodes such as silver-chloride, calomel reference electrodes.

Resazurin dye and methylene blue have been used in the evaluation of milk quality through bacteriological assessment. The principle of the redox potential test involves adding a small quantity of resazurin indicator or methylene blue to the sample and the blue colour, in its oxidised form, is reduced (decoloured) by the bio-reduction activity of the viable cells present in the food.

The time required for decolouration of the indicator to occur indicates the level of bacterial activity in the medium and this is correlated to the quality of the sample.

Colour change in milk takes 2 to 3 hours to have the reaction completed, and the faster the greater the contamination level although different species of bacteria have varying influence on food spoilage.

According to Bourgeois *et al.* (1995), applying a similar procedure as in milk, resazurin has been used to estimate aerobic microflora or psychotropic flora in various food products like meat, marine food, and vegetables and valuable results have been achieved. However, an effort must be made to avoid masking the indicator colour change by coloured substances naturally present in meat and this can be done by preparation of a clear solution from the sample or by a surface sampling technique.

The amount of dye conversion can be determined spectrophotometrically, fluorometrically or by using visual observations.

In accordance with Atherton and Newlander (1977) and Bourgeois *et al.* (1995), an acceptable relationship of colour and quality of the milk are:

- Blue (no colour change): Excellent
- Blue to deep mauve: Good
- Deep mauve to deep pink: Fair
- Deep pink to whitish pink: Poor
- White: Bad.

However, one positive attribute of resazurin is the ability to measure the metabolic activity of living cells which are vital elements of quality deterioration of food product.

Oxidoreduction indicator (resazurin dye) is the main component, and this makes it simpler to use.

In measuring redox potential using resazurin dye there are a number of short comings involved:

- Inherent inhibition of bacteria by pesticide residues although the risk of occurrence seems to be minimal.
- The indirectness of this test may be a probable source of doubts on its validity though various studies have shown the comparative justifiability of this method.
- In addition to this, the use of resazurin dye for food grading has been adapted in milk processing rather than other food products.
- Oxygen or light presence may cause inaccuracy in redox potential measurement using resazurin dye however, caution should be taken to ensure that oxygen and light is avoided during the manipulation of the test (Bourgeois *et al.* 1995).

1.3.2.5 Glucose, pH, and protein analysis using combur^{-3} test strips

According to Bremner (2002), the pH of fish flesh when alive is 7.0, and after slaughter residual glycogen is broken down through glycolysis to pyruvic acid and lactic acid which lowers the pH to approximately 6.6-5.5 depending on the fish species. In this event, the flesh becomes more acidic although acidity of fish flesh may inhibit bacterial growth and their subsequent deteriorative effect on the product this alters the normal texture of the fish flesh, making it unreasonably and unacceptably firm and tough.

However, in some fish species, free sugars and phosphorylated sugars are mobilised by glycolysis and other reactions persist post rigor, particularly phosphates sugar that consequently precipitate in browning reactions which affects the quality of the product.

In live fish, various proteins play different and significant roles e.g. the myofibrillar protein responsible for muscle structure. After fish slaughter, protein's secondary, tertiary and quaternary structure is destroyed, and/or reduced to simple polypeptide chains by a number of factors including storage temperatures and slow freezing. In this event, the proteins do not only lose their ability to function as an enzyme but lose their water holding capacity resulting in excessive drip in the fish flesh especially when thawed, leaving the product appearance dull, spongy, unpalatable.

The pH value, protein and glucose change can be used as an indicative sign in fish quality deterioration and this can be measured by using Combur^{-3} test strips. A clear solution is prepared from the fish sample in a test tube, is drawn into a pastur pipette and dropped on the strip.

Excess solution on the strip should be shaken off and put against the colour chart after 10 minutes and the colour is read accordingly.

pH, glucose, and protein alternatively can be measured by placing the test strip directly into the fillet or in the minced fish flesh solution, transferred and placed against the colour chart

There are various rapid methods that can be used to measure parameters of spoilage but may vary in sensitivity and convenience or flexibility. Table 2 is a summary of examples of rapid methods and the corresponding analytes.

Table 2: : Examples of rapid methods for fish freshness grading including indicators and kits needed (Huss *et al.* 1992, Huss 1995, Bremner 2002, Bourgeois *et al.* 1995)

Indicator	**Test kit/Instrument**	**Principle/Method**
1. Electrolytes	-Torry meter -RT-Freshness Grader	1. Meter reading (Electrical conduct metric test)
	-Electrodes	2. Electrochemical test
2.Colour	-Colour meter	1. Colour reading
3. Microbial ATP	-Luminometer	Bioluminescence reaction • (ATP metric test)
4. Glucose	-Clinistix strips -Combur-3 strips	1. $^{14}CO_2$ 2. Spectrophotometric/ 3. Fluorimetric evaluation 4. Visual observation
5. pH	-Color-pHast strip -Combur-3 strips -Litmus paper	
	-Calomel Electrodes	5. Electrochemical test
6. Protein	-Combur-3 strips	1.Chromatographic test
7. Redox potential	-Resazurin dye or methylene blue -Resazurin strips	Bio-reduction cell activity 1. (Spectrometric evaluation) or 2. visual observation
	-Electrodes	3. Electrochemical test
8.Texture/Elasticity	-Texturometer	1. Probe press test
9. Lipopolysachaarids	-Limulus lysate test	1. Limulus test
13.Catalase/Peroxidase	-H_2O_2	Burble test

1.4 Problem statement

In Uganda, organoleptic (sensory) assessment is the main method used for fish grading.

Upon landing and reception of fish at factories, fish is graded by one or two persons using the prepared organoleptic scheme.

Production of acceptable quality fish products to a greater extent depends on the quality of the raw material.

There are no other alternative rapid methods in use to support the existing ones. This leaves a challenge to Uganda's fisheries industry, especially today where consumers are very particular in the quality of the product.

The outcome of grading fish using the organoleptic method, although grading is based on the prepared scheme, is likely to be affected by several factors like training, physiological, psychological status of the analyst and the environmental conditions where the analysis is done. As a result, fish of acceptable quality may be rejected and or fish of unacceptable quality can be passed on for processing.

In addition to this, chemical and plate count methods used for fish quality grading are not always reliable; the time the analysis requires to obtain results is lengthy and they are in inadequately facilitated in Uganda. There is, therefore, a need to find other possible alternative methods for fish quality grading.

This can be ensured by either improving the sensory evaluation scheme through better training, or by the use of alternative suitable methods. These methods must be fast, accurate and should require little training but still reflect the important quality attributes of fish.

This study is aimed at screening for alternative methods for fish grading in fish production in Uganda and more specifically it attempts to:

- Identify possible alternative rapid methods for fish grading.
- Compare sensory methods using QIM and rapid methods for fish grading.
- Identify alternative methods for fish quality grading which could be applicable to the Ugandan situation.

The main research questions tackled will be:

- What are the possible alternative rapid methods for fish grading?
- Which differences exist between QIM and these methods?
- Which rapid methods can be recommended for fish grading in processing plants and landing sites in Uganda?

2 MATERIALS AND METHODS

In an attempt to achieve the objectives of this study and answer the above research questions, fish samples were analysed using different alternative rapid methods in comparison with sensory methods using the QIM.

2.1 Samples

Fresh whole gutted fish were obtained from Brim fish processing factory and stored in ice for 2, 3, 5, 6, 7, 8, 10 and 11 days at the University of Akureyri and subsequent analyses were done within 24 hours accordingly.

2.2 Pilot analysis

Pilot analysis was carried out to establish the precision of using resazurin dye, ATP meter, and Combur^{-3} test strips in measuring fish redox potential, microbial ATP expressed as relative light units (RLU) and pH, protein and glucose levels respectively. Sensory evaluation (QIM) was done on all samples to make comparisons with the alternative methods.

From the pilot analysis results a skin swab area of 100 cm^2, and gills swab in 10 mL of autoclaved water to 1 mL of resazurin solution was established and used.

2.3 Sensory evaluation (QIM)

Sensory evaluation of all samples was done by a panellist using the QIM. Evaluation of the appearance, smell, shape and texture of all samples were given demerit scores (0-3) and recorded in the QI form shown in Appendix 1. These scores were added up to get a freshness score expressed as a QI value and comparisons were made with various alternative methods (QIM_eurofish 2005).

2.4 Microbial ATP test (relative light units)

Microbial ATP expressed as relative light units (RLU) was measured in all samples by taking replicate swabs on fish skin surface (100cm^2) and gills, separately using ultrasnap swab sticks and a meter reading in the ATP measuring device was done accordingly (Hygiena International 2007).

2.5 Microbiological analysis

To find out the relationship between RLU and colony forming units (CFU), comparison analysis was done using a microbiological method. Using sterilised swab sticks, replicate gill swabs and 100 cm^2 skin swabs were taken from all samples and dissolved in 10 mL of autoclaved water in glass tubes and a serial dilution of 1^1 to 10^{-5} was done. 50µL of all dilution prepared were inoculated in iron agar plates,using eddy spiral jet and incubated for 48 hours at 22^{o}C and colony forming units were counted using the Eddy counter.

2.6 Resazurin test

Resazurin solution was prepared from 0.011 g of resazurin powder in 200 mL of autoclaved water and stoppered to avoid oxygen entry.

Using sterilised swab sticks, replicate gill swabs and 100 cm^2 skin swabs were taken from all samples and dissolved in 10 mL of autoclaved water in glass tubes and stoppered accordingly.

1 mL of prepared resazurin dye was drawn using a pipette and added to this sample in a glass tube and colour change over time (5 hours) was recorded accordingly as demonstrated by Atherton and Newlander (1977).

2.7 pH, protein and glucose analysis

Using sterilised swab sticks, replicate gill swabs and 100 cm^2 skin swabs were taken from all samples and dissolved in 10 mL of autoclaved water in glass tubes and stoppered.

This solution was dropped on the Combur $^{-3}$ strip using a pastur pipette and after 10 minutes the test strips were placed against the colour chart and colour reactions were read recorded accordingly.

2.8 Data analysis

All data were consolidated and statistically analysed using the MS Excel programme.

The criteria of the choice of redox potential change, microbial ATP, pH, protein and glucose concentration was based on the fact that they are the prominent indicators of fish spoilage in the early spoilage process. Although they may not necessarily pose any serious safety problem to the consumer, they can be a gateway for major spoilage processes. Measurement of these indicators could, therefore, be important in grading fish (Bremner 2002).

On the other hand, the use of resazurin, ATP meter and Combur^{-3} strips to measure these indicators respective were readily available at the time of the study and could be used to measure fish quality indicators chosen.

3 RESULTS

3.1 Sensory evaluation

The purpose of this work was to identify possible alternative rapid methods for fish grading by comparing the commonly used sensory method, QIM with the alternative rapid methods analysed.

As shown in Figure 1, there was, as expected, a linear relationship between storage time of fish on ice and QI value. The QI value is 0 in very fresh fish and increases to 24 in totally spoiled fish. Also, as expected the QI value increased linearly during the storage time. This relationship was found by a linear regression to be: QI value = 1.0 + 1.4 days on ice (Figure 1).

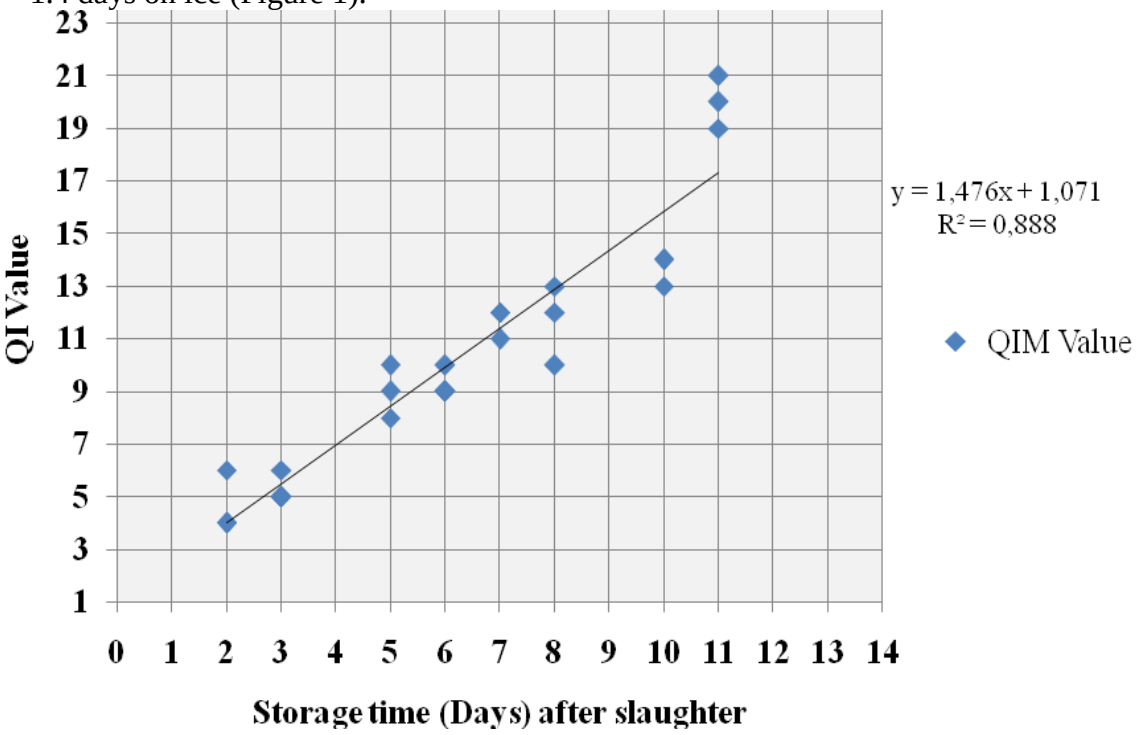

Figure 1: Relationship between storage days and quality index (QI) values in cod fish stored in ice.

3.2 ATP (RLU) measurements

The relationship between QI value and ATP content/concentration, expressed as relative light units (RLU) is shown in Figure 2. It was expected that RLU would increase as QI value increased. However RLU values obtained in all samples and at all sample sites were higher at lower QI values and lower at higher QI values.

The RLU obtained from the skin were slightly higher than those from the gills. All RLU measures from the skin samples were very high (>2000) and the highest RLUs were close to the upper limit of the meter 9999.

However, the RLUs from the gills ranged from 200 to approximately 8000.

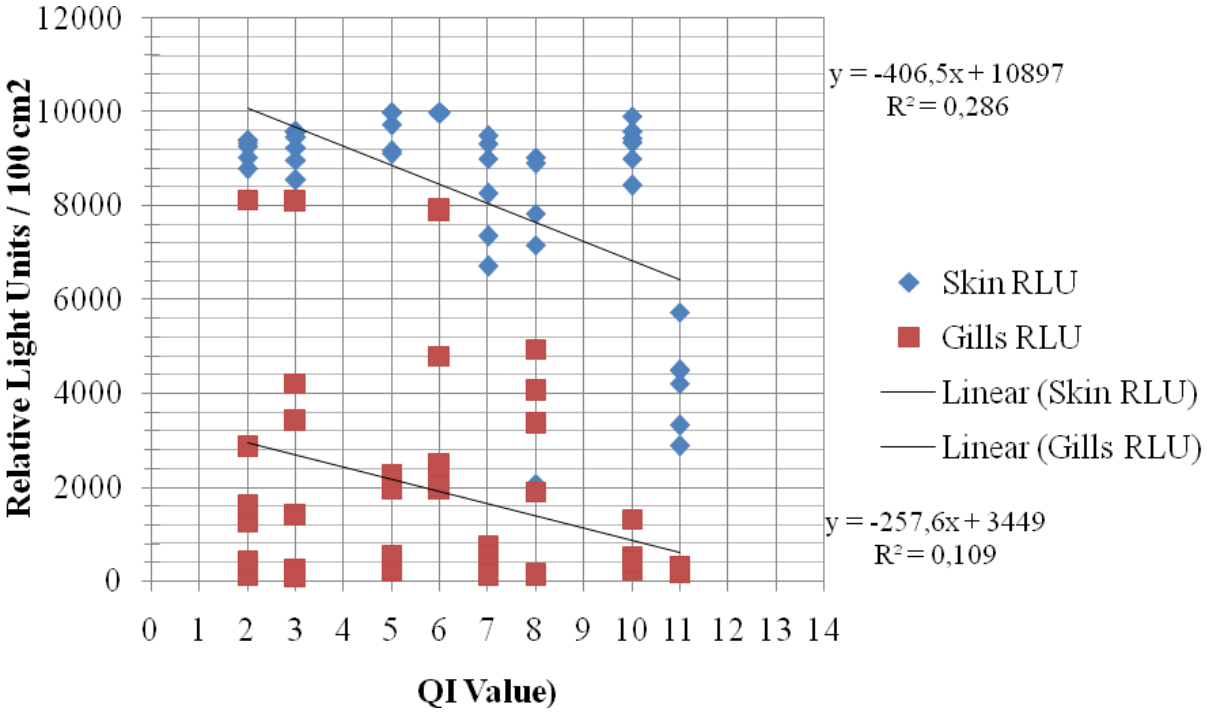

Figure 2: Relationship between QI values and RLU values/100 cm2 from cod skin and gill swab stored in ice for 2 to 11 days.

3.3 Bacterial numbers versus RLU

As RLU from samples taken from surfaces are usually good indicators of bacterial contamination the previously mentioned results were not expected. It was, therefore, decided to compare directly RLU and bacterial numbers obtained by serial dilution of swab samples. The results (Figure3) show, as expected, that an increase in bacterial numbers increases the RLU and on a log-log scale this relationship is linear. At approximately 1600 bacteria per mL (Log_{10}=3.2) the RLU was approximately 1.8 on the log_{10} scale. At CFU of 100,000 bacteria per mL (log_{10} =5) the RLU was approximately 3 on the log_{10} scale.

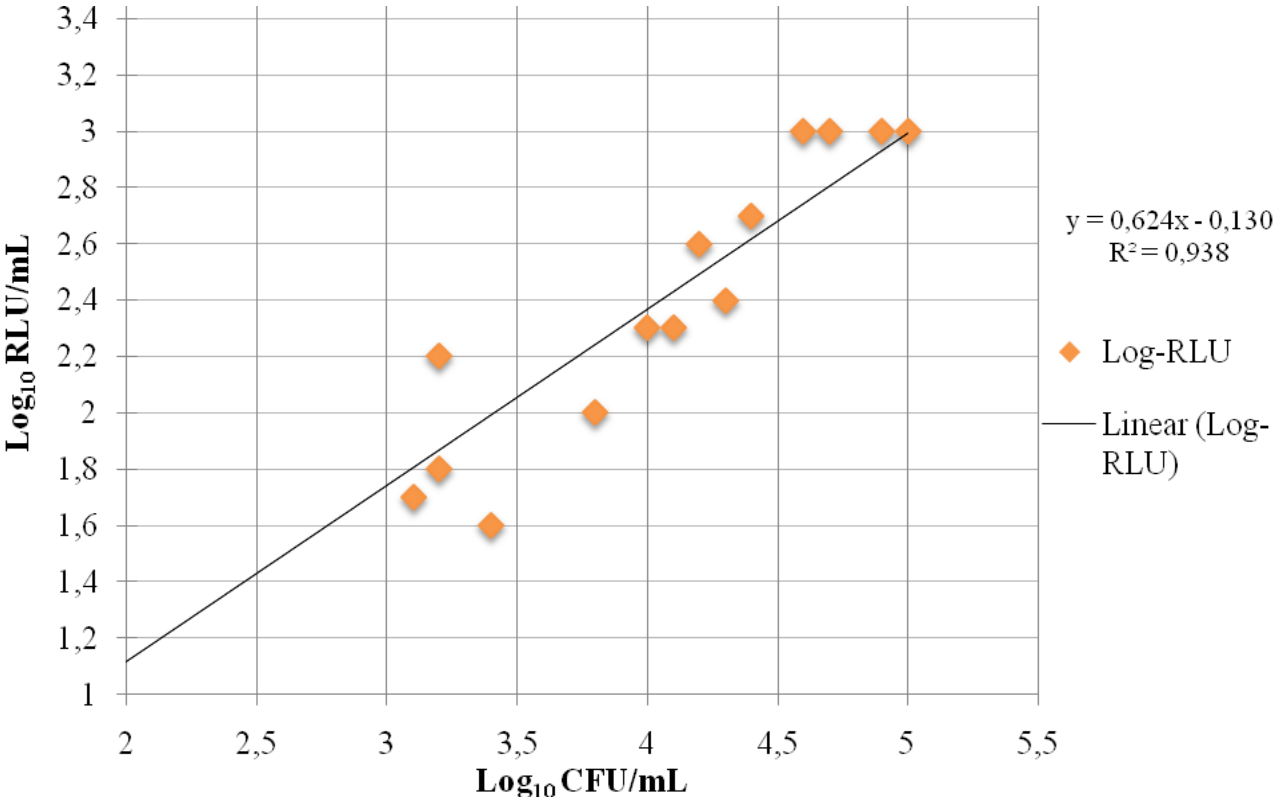

Figure 3: Correlation between colony forming units (CFU)/mL and relative RLU/mL in cod fish stored in ice for 2 to 11 days.

3.4 Dye (resazurin) reduction test

The relationship between QI value and the time it took for the colour of resazurin dye to change from blue (oxidised state) to light blue and pink (reduced state) was observed and the results shown in Figure 4. In fresh fish (low QI values) it took approximately 5 hours for the colour change to take place. However, in pretty spoiled fish (QI value >12) it took approximately 1 hour for the resazurin dye to change colour.

The initial colour change from dark blue to light blue took only 2 minutes in both the skin and gill samples.

However, there was a variation in time of 42 minutes in colour change at the end of the first experiment but the final colour change of the dye was reached at the same time duration stipulated for the reaction.

Similar time duration of resazurin dye colour change was observed in the subsequent analysis.

The time duration of decolouration of resazurin dye decreased with increases in the QI value as shown in Figure 4.

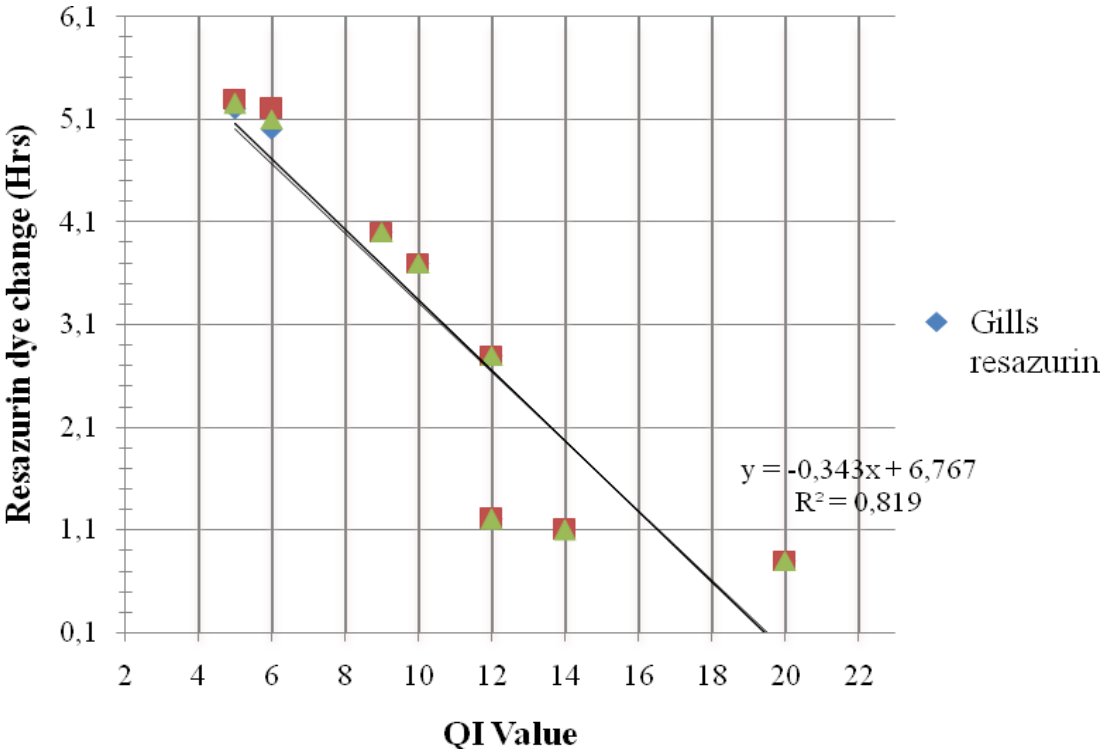

Figure 4: Relationship between QI and time for colour change of resazurin dye from blue to pink in cod fish samples stored on ice for 2 to 11 days.

3.5 Analysis of pH using Combur $^{-3}$strips

The relationship between QI value and pH is shown in Figure 5 indicated increasing pH values with increases in QI value. The range of pH values was between 0.1 and 0.3 in all samples.

Figure 5 also shows that the pH value range was wider between QI values of 10 and 20. However, the average higher pH value was 7 at QI value 20.

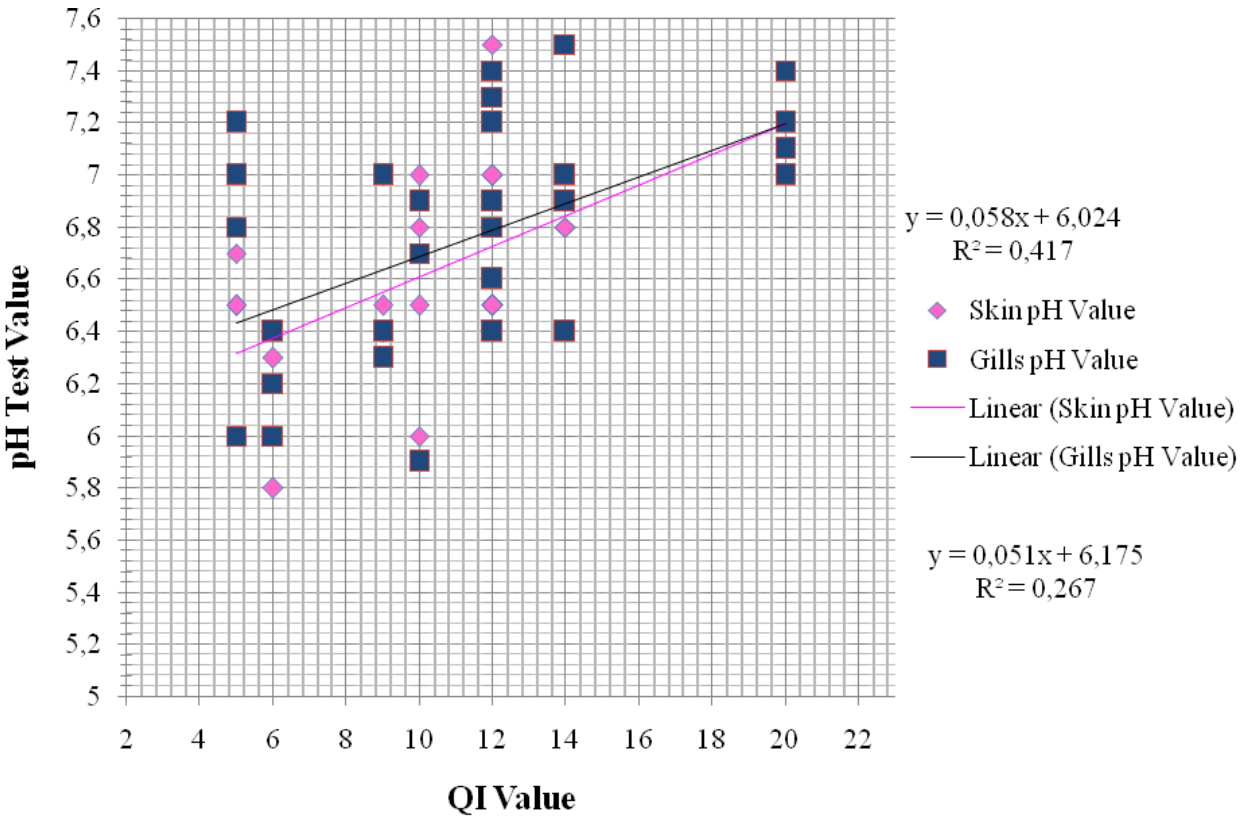

Figure 5: Correlation in quality index QI values and pH test values in cod fish stored in ice for 2 to 11days.

3.6 Protein analysis using Combur^{-3} strip

The protein concentration in the samples was between 0.5 and 1.3 units and there was no clear correlation between QI values and the protein concentration there was a wide inconsistence as shown in Figure 6.

The protein values were inconsistent with increase in the quality index values, apart from QI value 20 the protein concentration dropped to 0.5.

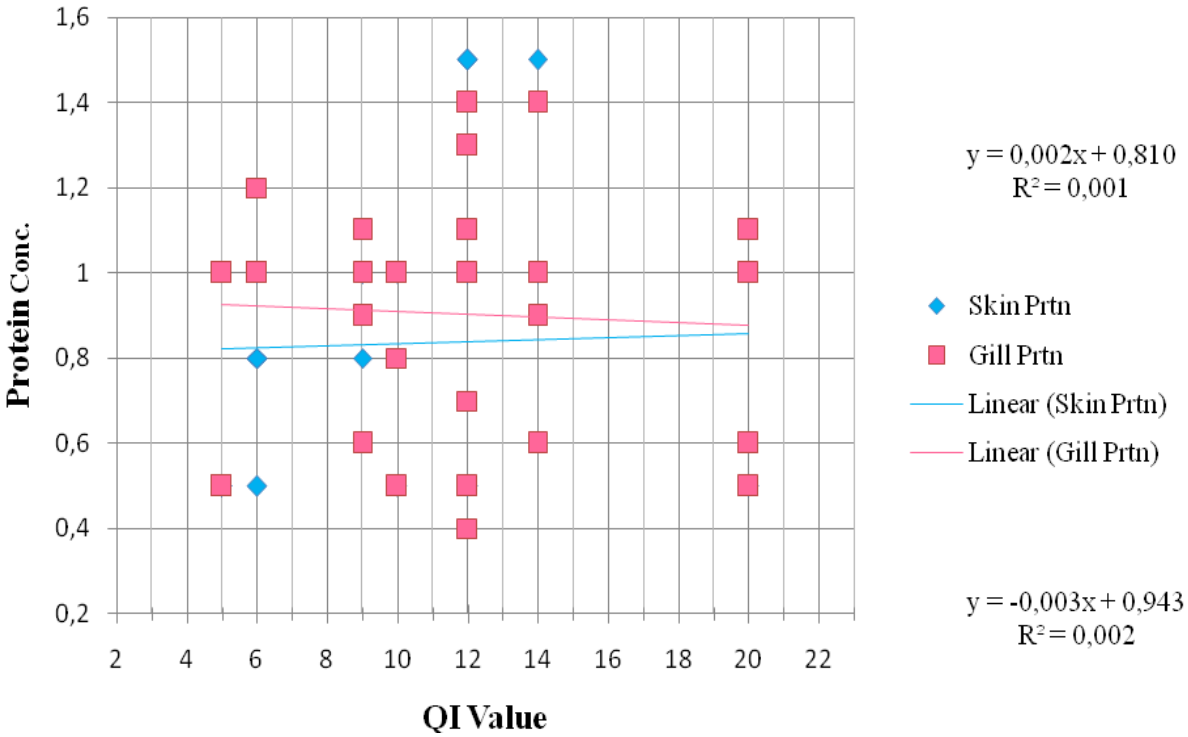

Figure 6: Correlation in QI and protein test values in cod fish stored in ice for 2 to 11 days.

3.7 Glucose analysis using Combur^{-3} strips

The glucose concentrations obtained were generally around zero (0), so no relationship with the QI was detected.

4 DISCUSSIONS

4.1 Sensory evaluation results

The QI value indicated a linear trend with increases in storage time (days) of cod fish as shown in Figure 1. However, obtaining results using QIM in this study was rapid.

This is generally in correlation with findings of QIM_eurofish (2005) who noted that higher QI value indicate less fresh fish and lower QI values indicate fresh fish and the QI value can be used to determine the shelf life of the fish product.

However, sensory evaluation requires proper training of the panellist to avoid biased evaluation. The psychological and physiological status of the panellist is vital (Meilgaard *et al*. 1991).

4.2 Microbial ATP test values expressed as RLU

As illustrated in Figure 2, the RLU values obtained decreased slightly with increases in QI values and this does not correspond to various studies carried out.

Huss (1995) pointed out that bacterial activity dominates the fish spoilage process after a couple of days of autolytic changes after fish slaughter.

Although specific spoilage bacteria (SSB) responsible for fish spoilage may not constitute a larger number in the total viable cells in the sample it is believed that the larger the number of the total viable cells, the higher the possibility of larger numbers of SSB.

In accordance with Bourgeois *et al.* (1995), the amount of light generated during bioluminescence reaction of luciferase luciferin is proportional to the ATP in the sample thus the biomass of the cells in the sample.

In this case, RLU values would be expected to increase with increasing QI values which increase with fish spoilage during storage (QIM_eurofish 2005).

However, the skin indicated a higher value of RLU as compared to the gills RLU value. With reference to Huss (1995), bacteria in fish gills are equally higher in this case therefore one would expect higer RLU values from the gills.

However, this could be attributed to the small sample size taken from the gills as pointed out by Hygiena International (2007) that the sample area of 100 cm^2 is recommended perhaps this could be the reason of RLU variation in the RLU in the sample sites.

Huss *et al.* (1992) pointed out the different possible sources that can alter the RLU can be efficient in cleaning up the possible sources reduces RLU measured.

Perhaps the decrease in number as indicated in the analysis could have been the function of melting ice that may have washed off parts of the bacteria during sample storage in ice for subsequent analysis.

The value of RLU obtained from the skin indicated values close to the top limit of the meter value and in this case determination of the maximum RLU value could be difficult in cases where the sample maximum RLU value went beyond the meter value.

However, this was taken care of by comparing the RLU test values with the colony forming unit values as shown in Figure 3.

4.3 Colony forming units and RLU analysis

The results obtained, as shown in Figure 3 indicate a linear relationship between RLU values and CFU values. RLU increased with increased CFU which seems to agree with findings of (Bourgeois *et al.* 1995) who pointed out that the amount of light emited (RLU) is proportional to the amount of microbial ATP in the sample and therefore RLU could be used to estimate the number of viable cells in the sample if an appropriate sample area could be identified.

4.4 Resazurin dye test

According to findings shown in Figure 4 the use of resazurin dye seems to be an appropriate rapid method for grading fish. The time of analysis took approximately 5 hours.

This is shown by the value of R^2, 0.819. Although the different sample sites of the individual fish samples, for example the gills, indicated a slight variation in the duration of resazurin dye decolouration, this would be expected in that fish gills harbour more bacteria.

In accordance with QIM_eurofish (2005), it is pointed out that no excessive emphasis is laid on a single attribute so a sample cannot be rejected on the basis of a single criterion in using QIM to evaluate fish freshness and therefore slight variation in the time taken for resazurin dye decolouration can be neglected.

The period of time resazurin dye took to undergo colour change from blue to pink decreased with increase in QI value.

This agrees with the findings of Atherton and Newlander (1977) and Bourgeois *et al.* (1995), who pointed out an acceptable relationship between colour and quality of milk using resazurin dye. In their findings, the colour range from blue, light blue to pink and white was used to indicate a descending order of acceptable relationship of colour with the quality of milk.

Similarly the time to identify spoiled fish (QI > 12) took approximately 1 hour. This correlates with the findings of Atherton and Newlander (1977) who noted that shorter time (hours) of resazurin decolouration indicates a higher degree of quality deterioration in milk although resazurin colour change did not reach the final colour as in milk. Perhaps this could have been attained with subsequent storage of the fish samples.

However, it is important to note that as much as resazurin dye could be a possible alternative rapid method for grading fish by indicating the presence and activity of bacteria in fish, it does not indicate the actual bacterial counts and more particularly organisms of public health which is vital in making informed decisions about the product.

Correlated with the findings of Bourgeois *et al.* (1995), the use of resazurin dye in food including fish quality evaluation exhibits difficulty in colour reading, sensitivity to oxygen. Similar short comings were encountered in this study and this may affect the accuracy of the analysis if precautions are not taken. On the other hand, the use of resazurin dye does not require training and can give accurate results if properly used.

4.5 pH test values in cod fish

From the findings in Figure 5, the pH test value range was between 6.2 and 7.1 and there was an increase with an increase in the QI value and the pH values obtained were not consistent.

This seems to agree with the findings of Bremner (2002) who noted that the pH range in most fish species is 6.2 and 6.6 and in other fish species pH can fall to as low as 5.5.

Although the pH range obtained from this study seems to correlate with the findings of Brenner (2002) and was obtained rapidly, within approximately 15 minutes, it appears to be quite difficult to use pH for fish grading because of the difference of pH ranges in fish species, health status of the fish at capture and the catching method (Huss 1995).

4.6 Protein concentration analysis in cod fish

The obtained protein values shown in Figure 6 were inconsistent and there was generally no definite relationship with the QI value although the time for analysis was rapid (approximately 15 minutes). The increase in the QI value seemed to have almost no effect on the protein concentration apart from at point QI 20 and Prtn T 0.5.

With reference to Bremner (2002), denaturation of protein has a considerable effect on the quality of fish and this protein denaturation occurs after fish slaughter especially at freezing or if excessively high or low temperatures are encountered during handling and processing.

In this regard, one would expect a considerable change in protein concentration test values with a change in the QI value. In this case, therefore, the protein test value may not be an appropriate method for grading fish although its denaturation has an implication for the quality of the product.

4.7 Glucose concentration analysis in cod fish

Glucose test values obtained in the analysis were generally at zero. There seems to be no correlation with QI values.

According to Bremner (2002) and Huss (1995), glucose is utilised in the production of energy in the post mortem muscle cell respiration and the residual glucose concentration depends on the stress exposed to the fish during capture and health status of the fish at slaughter.

In this case, therefore, the use of glucose test values for grading fish perhaps may not be the appropriate method although the time taken to carry out the analysis was rapid (approximately 15 minutes).

5 CONCLUSIONS AND RECOMMENDATIONS

Based on the findings from this study, QI value exhibits a linear relationship with storage time as expected, it was rapid as pointed out by QIM_Eurofish (2005) and could be used to determine the quality of fish and the remaining shelf life of a product in Uganda but a special quality index scheme would be required for the different fish species.

ATP could be an appropriate method for grading fish and could be applicable to the Ugandan fisheries situation. However, further research to find appropriate sample volumes is highly recommended to avoid obtaining values that are beyond the upper limit of the meter; but a fish skin swab would perhaps be recommended and not the gills.

Resazurin dye indicated the level of fish quality deterioration but estimation of the bacterial counts was not possible. This method could be recommended for fish grading in Uganda as it can be applicable inside and outside the laboratory and it takes about 1 hour to grade low quality fish accordingly.

However, calibration of this method is highly recommended to enable estimation of bacterial counts in the fish or food.

Furthermore, the method of colour reading requires improvement for accurate interpretations of colour changes.

Although the use of Combur^{-3} to measure protein level, glucose and pH concentration was quite convenient and rapid (approximately 10 minutes), in this study they seemed not to be appropriate indicators for fish grading due to the inconsistence in the test values and poor correlation with QI values. Perhaps other related attributes could be identified for use as indicators for fish grading.

ACKNOWLEDGEMENTS

The author is grateful to the UNU-FTP management, particularly to Dr. Tumi, Mr. Thor, Ms. Sigridur and Gudni for the valuable, educative programme and their dedication to the overall management of the programme.

To her supervisors, Prof. Hjorleifur Einarsson and Arnheidur Eythorsdottir for their valuable guidance, Gudny J. Johannsdottir for her technical guidance in the laboratory experiments and Lara Gudmundsdottir for her industrious work in coordinatorship

To the management of the Fisheries Training Institute and particularly the Ag. Principal, Ms Gertrude Abalo, for her support which enabled me to be away from duty and complete my studies successfully.

And most important is my son Jonah L. Kiprop for his patience in his tender infant stage while away from me.

LIST OF REFERENCES

Alejandra, M.G.V.Munoz, and B.T.Carr 1992. *Sensory Evaluation in Quality control*. Library of Congress Cataloguing in Publishing Data.

Amerongen, A.V, D. Barug, M. Lauwaars 2007. *Rapid Methods for food and feed quality determination. Wabeninggen* Academic Publishers, Netherlands.

Atherton, H.V. and J.A. Newlander 1977. Chemistry and Testing of Dairy Products.4th Edition A V I Westport, CT.

Bourgeois, C.M., J.C.Leveau, Stephen Davids and D.Y.C Fung 1995. *Microbiological Control for Foods and Agricultural Products.* VCH, Publishers, Inc.

Bremner, H.A. 2002. *Safety and Quality issues in Fish processing*. Wood head Publishing limited

Export Promotion Board 2006. Uganda Export statistics

Farid, E.A 1991. *Seafood safety; Safety committee on evaluation of the fishery products.* National Academy Press Washington D.C.

Fung, D. 2002. Vol.1 *Comprehensive reviews in food science and food safety, Rapid methods and automation in microbiology*

Huss, H.H., M. Jakobsen and J. Liston 1992. *Quality Assurance in the fish industry*. Elsevier Science Publishers B.V.

Huss, H.H 1995. *Quality and Quality changes in fresh fish*. FAO Fisheries Technical Paper 348. 195 pages. FAO. Rome. Italy.

Hygiena Internatonal (2007*) Directions for use of Ultrasnap™ ATP swab with system SURE II™ ATP Hygiene Monitoring De*vice. www.hygienausa.com

Lake Victoria Fisheries Organisation 2006. http://www.lvfo.org/index.php?option=displaypage&Itemid=103&op=page

Malle, P and M. Poumeyrol 1989. *A new chemical criterion for the quality control of fish*: trimethylamine / Total volatile Basic nitrogen (%), Journal of Food protein, Vol. 52 No6, p419-423.

Meilgaard, M., G.V Civille and B.T Carr 1991 *Sensory evaluation techniques* Press Inc, Boca Roton, FL.

Olafsdóttir, G., Martinsdóttir, E., Oehlenschlager, J., Dalgaard, P., Jensen, B., Undeland, Mackie, I. M., Henehan, G., Nielsen, J. and Nilsen, H. 1997 *Methods do evaluate fish freshness in research in industry. Trends in Food Science & Technology 8:258-265*

QIM Euro fish (2005) *QIM –your ideal tool for quality determination of fish freshness* www.qim_eurofish.com.

Uganda fish processors an exporters Association (UFPEA) report 2006

6 APPENDICES

6.1 Appendix 1

Table 3: Whole gutted cod fish Quality index (QI) form for sensory analysis (Huss 1992)

Quality parameter	Character	Demerit Score	Score
General appearance	Appearance of skin surface	0 -Very bright 1 - Bright 2 - Slightly dull 3- Dull	
	Stiffness	0- Rigor 1- Post rigor	
	Slime	0- Clear 1- Sl. Cloud 2- Cloudy 3- V. cloudy	
Eye	Clarity	0- Clear 1- Sl. Cloudy 2- Cloudy	
	Shape	0- Normal 1- Sl. Sunken 2- Sunken	
Gills	Colour	0- Characteristic red 1- Sl. Faded 2 Faded, discoloured	
	Smell	0- Fresh sea weedy/ Metallic 1-Fishy 2- Stale 3- Spoilt	
	Mucus	0- Absent 1- Moderate 2 Excessive	
Flesh colour	In open surface	0- Translucent 1- Grey 2- Yellow- brown	
Blood	In throat	0- Red 1- Dark red 2- Brown	
Characteristic sum			

6.2 Appendix 2

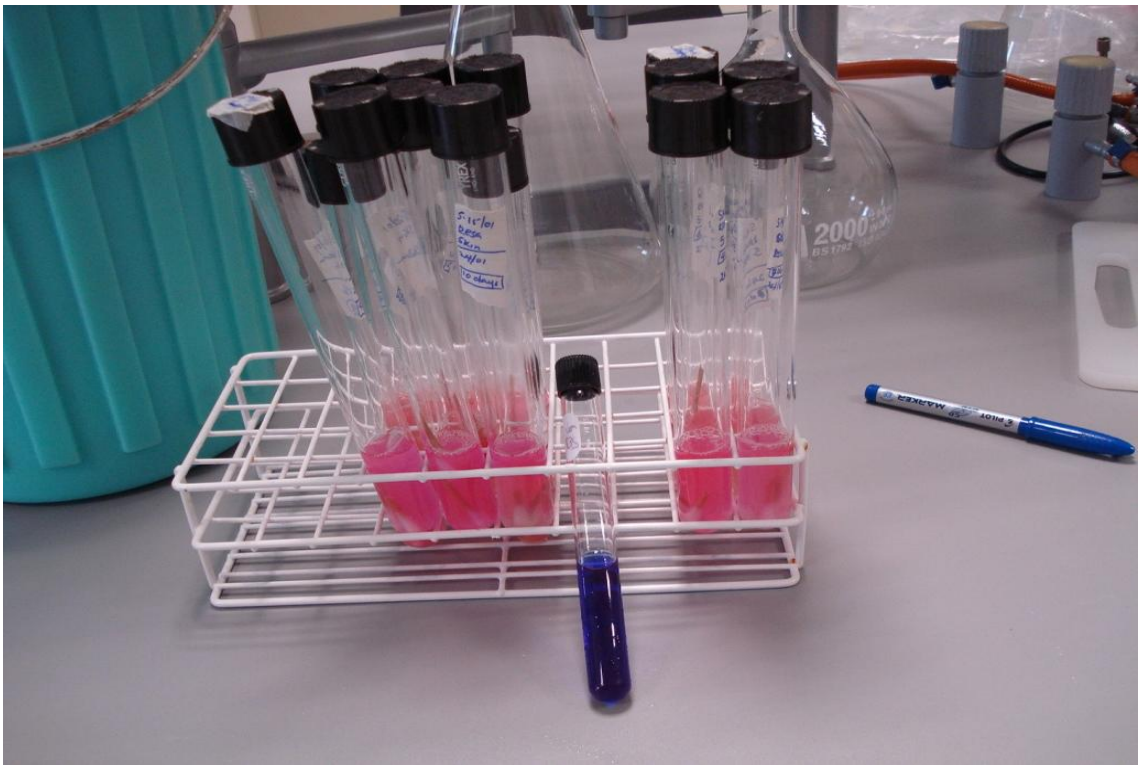

Figure 7: Experimental analysis of resazurin colour change in fish swab (11 days after slaughter) Indication of pretty spoiled fish (Pink colour).

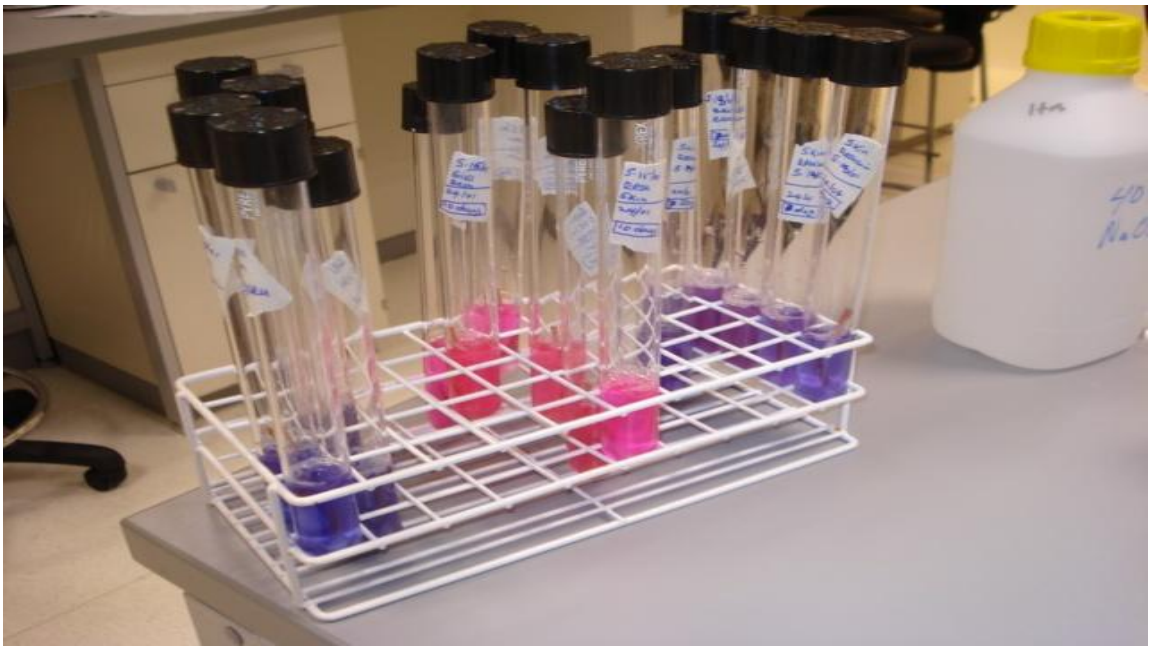

Figure 8: Experimental analysis of resazurin colour change in fish swab (10 days and 3 days after slaughter) indicating pink and blue colour respectively.