Journal of Microbiology, Biotechnology and Food Sciences

Amegovu et al. 2012 : 2 (2) 653-666

REGULAR ARTICLE

NUCLEOTIDE DEGRADATION PRODUCTS, TOTAL VOLATILE BASIC NITROGEN, SENSORY AND MICROBIOLOGICAL QUALITY OF NILE PERCH (*LATES NILOTICUS*) FILLETS UNDER CHILLED STORAGE

Andrew Kiri Amegovu[1], Mohammed Luyima Sserunjogi[2], Patrick Ogwok[*], Vincent Makokha[3]

Addresses: Patrick Ogwok, PhD, Kyambogo University, Faculty of Science, Department of Food Processing Technology, P.O. Box 1, Kampala, Uganda

[1]Eastcoast Europa, Madrid, Spain

[2]Makerere University, School of Food Technology, Bioengineering and Nutrition, Department of Food Technology and Nutrition, P.O. Box 7062, Kampala, Uganda

[3]Uganda Industrial Research Institute, Chemistry Laboratory, P.O. Box 7086, Kampala, Uganda

*Corresponding author: Email: ogwokp@yahoo.com, Cell Phone: +256772647968

ABSTRACT

Degradation products of adenosine nucleotide and total volatile basic nitrogen (TVBN) concentration provide means of ascertaining freshness of commercial fish products. A complementary sensory analysis has also been adopted by export markets for assessing the quality of fresh fish. Nucleotide breakdown products and TVBN was determined in fresh fillets from beach seined and gill netted Nile perch, a highly commercialized freshwater fish from Lake Victoria (Uganda), under chilled storage. Microbiological and sensory qualities were also evaluated. Total plate and *Pseudomonas* spp. counts positively correlated with TVBN. Basing on sensory, microbiological and biochemical attributes of the fillets, shelf-life of gill netted Nile perch was lower (13 days) than that of the beach seined (17 days). Fillets of beach seined Nile perch have a better keeping quality than that of the gill netted.

Keywords: Nile perch, nucleotides, TVBN, microbiological, sensory, quality

INTRODUCTION

Fish is a highly perishable product limiting its utilization in fresh forms (**Mukundan *et al*., 1986; Ababouch *et al*., 1991**). Reactions of enzymes, particularly on proteins, greatly impact on the quality of fresh fish since it occur in the early period of storage (**Mukundan *et al*., 1986**). Bacterial action on fish constituents, especially on non-protein nitrogenous compounds, occur mostly in advanced spoilage making it a less vital quality indicator for estimating freshness **(Ababouch *et al*., 1991)**. Chemical products resulting from enzymatic reactions during post-mortem deteriorative changes in fish tissue provide a means of assessing the quality of fresh fish. This is advantageous when breakdown products accurately reproduce the sensory characteristics of the fresh fish product.

Sensory characteristics of stored fresh fish are demonstrated to correlate with adenine nucleotide degradation products. Hypoxanthine (Hx), among adenine nucleotide degradation products, is a widely used index for examining freshness in mainly Hx forming fish species **(Lakshmanam & Gopakumar, 1999)**. For inosine (HxR) forming fish species, deterioration of quality is accurately assessed by computing Ino-index **(Lakshmanam & Gopakumar, 1999)**. A combination of Hx and HxR, expressed as a percentage of total nucleotide degradation products (K-value), has also been proposed as a fairly acceptable approach for assessing the quality of fresh fish **(Lakshmanam & Gopakumar, 1999)**. Recent study, however, demonstrates a poor correlation between K-value and freshness for fish species with low amounts of nucleotide on degradation. It is generally recommended that a combination of nucleotide breakdown products, sensory characteristics, and K-value are employed for estimating the quality of fresh fish **(Liu *et al*., 2010)**.

Exports of fresh fish products to competitive markets with developed quality management systems require mandatory quality check. However, indicators for freshness vary widely depending on fish species, capture method, and environment. Multifaceted approaches have nowadays been adopted by developed markets using chemical indices complemented with sensory tests for clearing fresh fish and processed fish products. However, in most developing countries, quality assessment of fresh fish is still limited to sensory and microbiological parameters. Fresh fillets of beach seined and gill netted Nile perch, the most commercialized freshwater fish from Lake Victoria (Uganda), stored under chilled conditions were assessed for TVBN and nucleotide breakdown products. Microbiology and sensory quality were also evaluated and correlated with the chemical indicators.

MATERIALS AND METHODS

Twenty five (25) samples each of gill netted (mean weight 3.5 ± 0.4 kg) and beach seined (mean weight 3.8 ± 0.3 kg) Nile perch were obtained from Ggaba landing site located on the shores of Lake Victoria (Uganda). Fish were immediately packed, according to capture category, between ice layers and then covered with a black nylon polyethylene film. Anesthetized samples contained in cold boxes were immediately beheaded, gutted, filleted, skinned and washed with distilled water. Skinless fillets of beach seined and gill netted Nile perch were stored in sterile polystyrene boxes and covered with a transparent sterile polyethylene film of 30 microns in thickness. Samples were stored in a refrigerator maintained at 0 °C. Fillets were randomly obtained for sensory, chemical and microbiological analyses.

Sensory Analysis

Sensory characteristics of fresh Nile perch fillets were analyzed using a modified method of European Union **(EU 103/76, 1976)** scheme. Ten trained and experienced panelists from Byansi Fish Processing Plant (Uganda) evaluated the samples for freshness based on appearance of the fillets, firmness, colour, odour and gaping. Fresh Nile perch fillets were presented together with three samples under storage to every panellist. Each attribute was graded using a five point metric scale. One (1) represented exteremely spoilt, two (2) putrid, three (3) poor (objectionable), four (4) fair (acceptable) and five (5) good (fresh).

Nucleotide break-down products

Adenosine-triphosphate (ATP), adenosine-diphosphate (ADP), adenosine-monophosphate (AMP), inosine-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) were determined using a High Performance Liquid Chromatography (HPLC) according to the method described by **Ryder (1985)**. Ten grams (10 g) of fish muscle were homogenized and extracted using a 0.6 N perchloric acid (50 mL). The acid extract was centrifuged at 3000 g for 10 min and the supernatant separated. The mixture was added to 0.2 M potassium hydrogen phosphate (KH_2PO_4) (10 mL). The buffer solution was then added and neutralized to pH 7.0 by adding 0.51 N potassium hydroxide (KOH). Final extract was held in ice for one hour and thereafter filtered using Whatman filter paper (# 125 mm) to eliminate

the precipitated potassium chlorate ($KClO_4$). The neutral extract was stored at -30 °C and analyzed within two weeks for nucleotide breakdown products. A hypersil (ODS) 4.6 mm slurred in isopropanol was used to pack a 20 x 0.46 cm guard column using hexane as pumping solvent. The columns were coupled using 15 cm x 4.6 mm id tubing. The HPLC system comprised of a pump, detector (450 UV) and an injector (U6K). The analytical parameters had chromatographic speed of 0.5; flow rate, 1.0 mL per min; sample injection, 10 µL; column, STR ODS-II 15 cm x 4.6 mm id; solvent system, acetonitrile and 0.1 M KH_2PO_4 at pH 7 in the ratio of 1:9; detection wavelength of 254 nm. The nucleotide (ATP, ADP, AMP, INO and Hx) standards were obtained from Sigma (Sigma-Aldrich, UK).

Total volatile basic nitrogen (TVBN)

Nile perch fillets were analyzed for TVBN according to the procedure described by **Malle and Tao (1987)**. Ten grams (10 g) of fish muscle were homogenized and extracted using a 0.6 N perchloric acid (PCA) (50 mL). The PCA-fish extract was carried out using Kjeldahl steam distillation with 30 % (w/v) aqueous sodium hydroxide solution and 4 % (v/v) aqueous boric acid. The TVBN distillate was titrated against 0.01 N hydrochloric acid and expressed as mg nitrogen (N) per 100 g of fish muscle.

Microbiological analysis

Total plate count (TPC) and *Pseudomonas* spp. were enumerated according to the procedure of **Harrigan and MaCance (1986)**. Ten grams (10 g) of fish muscle were homogenized with 90 mL of sterile physiological saline using a blender (Patterson Scientific, Blender 800E, USA) run for two minutes. Homogenized muscles were serially diluted with saline. For each dilution, 0.1 mL was transferred onto the surface of a plate count agar (PCA, Oxoid, Unipath Ltd. Basingstoke, Hampshire, UK) in duplicate and incubated at 30 °C for 2 days. *Pseudomonas* spp. was enumerated using Cetrimide-Fusidin-Cephaloridine (CFC) agar (Sigma, Germany) after 3 days incubation at 22 °C.

Data Analysis

Data was analyzed using statistical computer software of SPSS version 11.5 (SPSS, Chicago, IL). Correlations were calculated using parametric Pearson's method employing SAS computer programme.

RESULTS AND DISCUSSION

Sensory quality

Beach seined and gill netted Nile perch fillets reached rejection points on different days during chilled storage (Table 1). Quality deterioration was characterized by off-odour, white strips, discoloration from bright red to brown, and softening of the muscle. Both types of fillets maintained a maximum score of five until day nine without signs of gaping. Gaping was evident on the eleventh day of storage for gill netted Nile perch and fifteenth for the beach seined. Rejection points occurred respectively on the thirteenth and seventeenth day when gapes were found all over the fish fillets. Fillets were rejected when firmness was rated as soft, colour as brown or red, the smell as bitter and gapping found all over. Based on sensory assessment, chilled tilapia fillets stored at 0 °C were rejected between 9 to 12 days lower than that of Nile perch **(Verma *et al.*, 1983; Liu *et al.*, 2010).** A similar sensory rejection point of 12 days have also been reported in Rain bow trout under iced storage **(Özogul & Özogul, 2000)**. Nile perch captured by beach seining presents a comparable keeping quality to good keeping fish species.

Table 1 Sensory characteristics of fresh fillets under chilled storage from beach seined and gill netted Nile perch from Lake Victoria

Days	Capture type	Colour	Texture	Odour	Gaping	Score
1	Gill netted	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
	Beach seined	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
3	Gill netted	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
	Beach seined	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
5	Gill netted	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
	Beach seined	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
7	Gill netted	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
	Beach seined	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
9	Gill netted	Dark red	Finger-pressed concave and elastic	Weak broth	Signs of gape	4
	Beach seined	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
11	Gill netted	Dark red	Finger-pressed concave and elastic	Weak broth	Pronounced gape near head part	4
	Beach seined	Red	Stiff without finger-pressed concave	Fresh sea weedy	None	5
13	Gill netted	Brown red	Soft	Bitter	Gapes with signs of white strips	3
	Beach seined	Dark red	Finger-pressed concave and elastic	Weak broth	Signs of gape	4
15	Gill netted	Brown-green	Very soft	Putrid	Gapes with signs of white strips	2
	Beach seined	Dark red	Finger-pressed concave and elastic	Weak broth	Pronounced gape near head part	4
17	Gill netted	Greenish	Very soft	Putrid	Gapes with signs of white strips	2
	Beach seined	Brown red	Soft	Bitter	Gapes with signs of white strips	3

Chemical changes in Nile perch fillets during chilled storage

Total volatile basic nitrogen (TVBN)

Levels of total volatile basic nitrogen (TVBN) in fresh fillets increased from 10 to 58 mg N per 100 g of muscle in gill netted Nile perch and 11 to 45 mg N per 100 g of muscle in beach seined Nile perch (Fig. 1). A high correlation (R=0.87 and R=0.93 for gill netted and beach seined Nile perch, respectively, occurred between storage time and TVBN concentration indicative of quality deterioration (Fig. 1). A positive correlation also occurred between TVBN and total plate count (TPC) (R=0.77) and *Pseudomonas* spp. (R=0.73). Sensory rejection of gill netted Nile perch fillet occurred at TVBN level 21 mg N per 100 g on thirteenth day while sensory rejection for the beach seined fish fillet occurred at TVBN level 25 mg N per 100 g on seventeenth day.

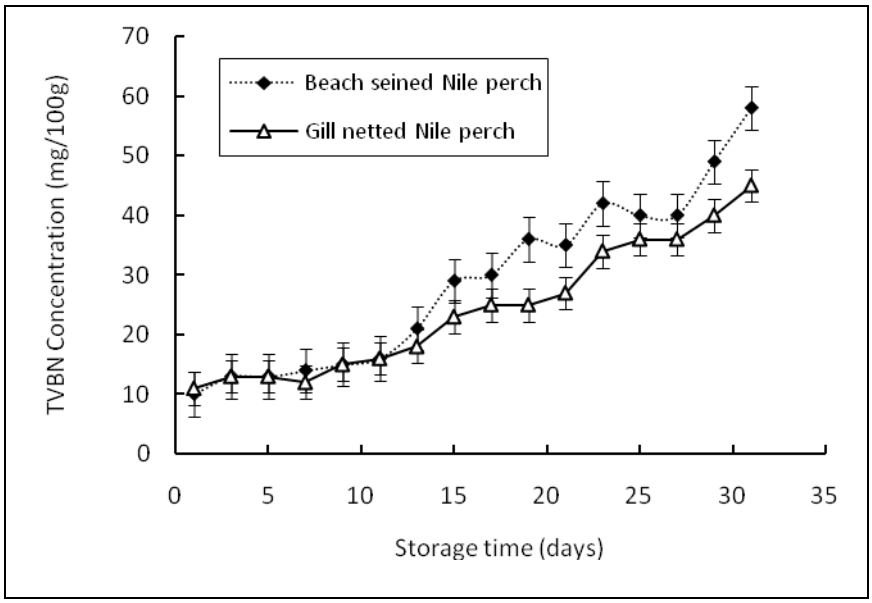

Figure 1 Changes in total volatile base nitrogen compounds during chilled storage of fresh fillets of beach seined and gill netted Nile perch. Error bars represent standard deviations for five determinations

Production of volatile basic nitrogen compounds in Nile perch fillets is a result of autolysis occurring at start of storage **(Mukundan *et al*., 1986)** and microbial metabolism that occurred during the later storage time **(Ababouch *et al*., 1991)**. According to **Huss (1995) and Connell (1995)**, TVBN amounts within 30 and 35 mg N per 100 g of muscles is considered acceptable for fresh fish stored at 0 °C. Trends in accumulation of TVBN in Nile

perch fillets during storage is comparable to that reported in chilled European hake which was 23 mg N per 100 g at rejection **(Perez-Villarreal & Howgate, 1987)**. A stringent use of TVBN for ascertaining fish quality has been shown to vary between 10 and 25 mg N per 100 g depending on fish species and fishing method **(Luten, *et al.*, 1997)**. Limits of TVBN from 25 to 35 mg N per 100 g of muscles have also been proposed for rejecting commercial fresh whole fish and processed fish products **(Connell, 1995; Dalgaard, 2000)**. Rejection of fresh fish products based on TVBN concentrations are derived based on sensory acceptability and bacterial counts **(Pons-Sanchez-Cascado *et al.*, 2006; Liu *et al.*, 2010)**. For most markets, TVBN is a determinant of quality of fresh fish because of its close relationship with sensory score and bacterial counts. This data demonstrates the suitability of TVBN for assessing freshness of Nile perch fillets under chilled storage albeit requires a complementary sensory and microbiological data. Nile perch fillets with TVBN concentrations above 25 mg N per 100 g of muscles could be considered for rejection.

Nucleotide breakdown products

Nucleotide degradation in fresh Nile perch fillets involved substantial loss of IMP and increase in Hx. Variation in IMP and Hx concentrations did not show important differences with capture method (Figure 2). IMP degraded into Hx during chilled storage. Degradation of IMP directly to Hx agrees with findings of **Gram (1990)** caused by the active nucleotide phosphorylase system. At sensory rejection, fillets of gill netted Nile perch contained IMP concentration of 0.06 μmol g^{-1} and Hx of 0.16 μmol g^{-1}. The concentrations of IMP and Hx (0.04 μmol g^{-1} and 0.16 μmol g^{-1}, respectively) in fillets from beach seined Nile perch were similar to that of gill netted at sensory rejection. Sensory quality of fresh fish is shown to correlate with IMP, a major compound responsible for flavour characteristics of most fish species **(Ozugol *et al.*, 2006; Liu *et al.*, 2010)**. It has been demonstrated that build up of Hx resulting from IMP breakdown is responsible for the bitter off-flavour developed in fish tissue **(Liu *et al.*, 2010)**. Basing on sensory scores, Nile perch fillets may be considered spoiled at IMP amount of 0.16 μmol g^{-1} and Hx of 0.04 μmol g^{-1} regardless of the capture method.

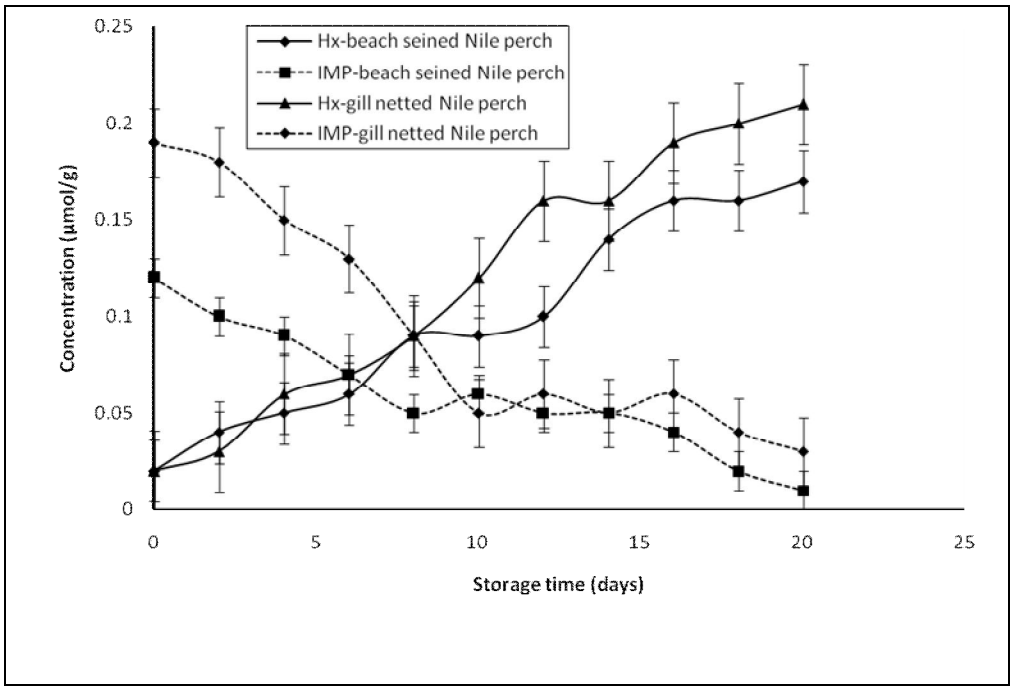

Figure 2 Nucleotide breakdown products in fresh fillets of beach seined and gill netted Nile perch under chilled storage. Error bars represent standard deviations for five determinations

Higher nucleotide products, ATP and ADP, were generally not observed in Nile perch. In gill netted fish, ATP and ADP were below quantifiable amounts all through storage. Notable ATP amounts were found in beach seined Nile perch fillets on the second and fourth day of storage (1.8 and 0.8 µmol g^{-1}, respectively). The amount of ADP was 0.03 µmol g^{-1} on the sixteenth day in the beach seined. Levels of AMP in fillets were in negligible concentration (≤ 0.01 µmol g^{-1}) due to its rapid depletion. Inosine was in very low amounts (0.13 µmol g^{-1} in fillets from gill netted and 0.19 µmol g^{-1} in that from beach seined Nile perch) during storage quantifiable only on day ten. Fish caught by gill netting experiences fast depletion of the energy reserves in form of ATP and ADP as it struggles. This study indicates that higher nucleotide products actually occur in Nile perch but are fast depleted as the fish struggles during capture. Fish capture method may have a strong influence on the early nucleotide profile of the fillet.

Microbiological quality of fresh fillets

Fresh fish under chilled storage spoils as a result of *Pseudomonas* spp. and the number at rejection is estimated between 8 to 9 log CFU g^{-1} (**Luten *et al.*, 1997**). The counts of *Pseudomonas* spp. in Nile perch fillets increased during storage (Figure 3). Fillets were

rejected when the number of *Pseudomonas* spp. was 4 log CFU g^{-1} for gill netted Nile perch and 4.5 log CFU g^{-1} for the beach seined. Total plate counts at rejection were 7.9 log CFU g^{-1} for beach seined Nile perch and 6 log CFU g^{-1} for the gill netted. This is similar to that reported by **Gram (1990)** for temperate fish (7 to 10 log CFU g^{-1}) under chilled storage. The shelflife of fresh Nile perch fillets obtained by gill netting can be estimated between 11 to 12 days based on the maximum microbial load limit of 7 log CFU g^{-1} recommended for freshwater fish (**ICMSF, 1986**). This coincides with sensory rejection period that occurred on the thirteenth day of storage. On the basis of microbial load, the shelflife of fillets from beach seined Nile perch is estimated between 14 and 17 since rejection was on day 18 (Figure 3). This estimate is well comparable to information provided by sensory scores that presented a rejection point at the seventeenth day. Hence, Nile perch fillets from gill netted fish spoiled faster in comparison to the beach seined.

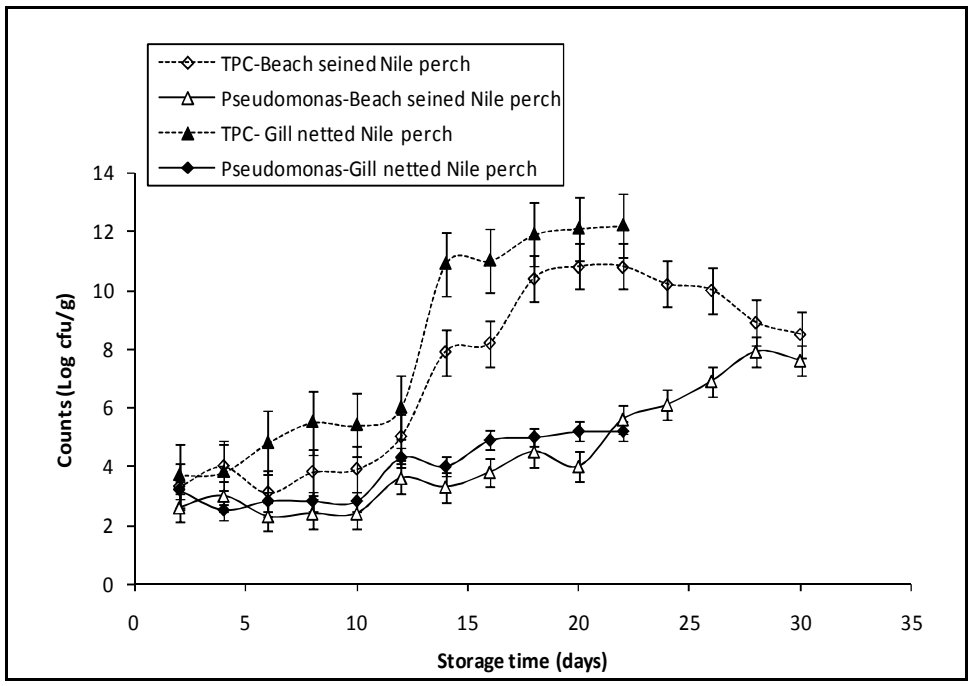

Figure 3 Total plate and *Pseudomonas* spp. counts in fresh fillets of beach seined and gill netted Nile perch under chilled storage. Error bars represent standard deviations for five determinations

Chemical and microbiological values of Nile perch fillets at sensory rejection

Nile perch fillets were rejected when considered to be objectionable or bad at sensory score of three (Table 2). The TVBN, Hx and IMP amounts in fillets of gill netted Nile perch

at rejection point were 21 mg/100g, 0.16 µmol g^{-1} and 0.06 µmol g^{-1}, respectively. Total plate and *Pseudomonas* spp. counts of fillets from gill netted Nile perch were 6.0 log CFU g^{-1} and 4.0 log CFU g^{-1} respectively, at sensory score of three. At rejection point, the amounts of TVBN, Hx and IMP in fillets of beach seined Nile perch were respectively, 25 mg/100g, 0.16 µmol g^{-1} and 0.04 µmol g^{-1}. Fillets of beach seined Nile perch had a TPC of 7.9 log CFU g^{-1} and *Pseudomonas* count of 4.5 log CFU g^{-1}. Hence, chemical and microbiological qualities of fillets followed similar trends for both gill netted and beach seined Nile perch (Table 2). It is recommendable that sensory, microbiological and chemical assessments are conducted to ascertain quality of Nile perch fillets.

Table 2 Chemical and microbiological quality of Nile perch fillets under chilled storage at different sensory score

Days	TPC (log CFU g^{-1})		*Pseudomonas* (log CFU g^{-1})		Hx (µmol g^{-1})		IMP (µmol g^{-1})		TVBN (mg/100g)		Sensory Score	
	Gill netted	Beach seined	Gill netted	Beach seined	Gill netted	Beach seined	Gill netted	Beach seined	Gill netted	Beach seined	Gill netted	Beach seined
0					0.02	0.02	0.19	0.12				
1									10	11	5	5
2	3.7	3.3	3.2	2.6	0.03	0.04	0.18	0.1				
3									13	13	5	5
4	3.8	4.0	2.5	3.0	0.06	0.05	0.15	0.09				
5									13	13	5	5
6	4.8	3.1	2.8	2.3	0.07	0.06	0.13	0.07				
7									14	13	5	5
8	5.5	3.8	2.8	2.4	0.09	0.09	0.09	0.05				
9									15	14	4	5
10	5.4	3.9	2.8	2.4	0.12	0.09	0.05	0.06				
11									16	15	4	5
12	6.0*	5.0	4.3	3.6	0.16*	0.1	0.06*	0.05				
13									21*	18	3*	4
14	10.9	7.9*	4.0*	3.3	0.16	0.14	0.05	0.05				
15									29	23	2	4
16	11.0	8.2	4.9	3.8	0.19	0.16*	0.06	0.04*				
17									30	25*	2	3*
18	11.9	10.4	5.0	4.5*	0.2	0.16	0.04	0.02				
19												
20	12.1	10.8	5.2	4.0	0.21	0.17	0.03	0.01				

Legend: *Values of quality parameters at sensory rejection. Hx: hypoxanthine, IMP: inosine-monophosphate, TVBN: total volatile basic nitrogen.

CONCLUSIONS

Spoilage of fresh Nile perch during chilled storage was influenced by bacterial action. Total volatile basic nitrogen (TVBN), a metabolite resulting from microbial degradation of muscle proteins, and *Pseudomonas* counts, are key quality indicators for predicting freshness of Nile perch fillets. Deterioration of Nile perch fillets, in addition, is dependent on capture method given that gill netted Nile perch had lower shelf-life than the beach seined.

Acknowledgement: This work was funded by Belgian Technical Cooperation (BTC) and World Bank. We thank Dick Nyeko, Executive Secretary, Lake Victoria Fisheries Organization, East Africa, for the technical support.

REFERENCES

ABABOUCH, L. – AFILAL, M.E. – BENABDELJELIL, H. – BUSTA, F.F. 1991. Quantitative changes in bacteria, amino acids and biogenic amines in sardine (*Sardina pilchardus*) stored at ambient temperature (25-28°C) and in ice. In *International Journal of Food Science and Technology*, vol. 26, 1991, p. 297-306.

CONNELL, J. J. 1995. *Control of fish quality*, 4th ed. London: In Fishing News Books Limited.

DALGAARD, P. 2000. *Fresh and lightly preserved seafood*. In: Shelf-Life Evaluation of Foods. 2nd ed. Edited by Man CMD, Jones AA. London: Aspen Publishers, Inc. p. 110-139.

EUROPEAN COUNCIL REGULATION (EEC) No 103/76 of 19 January 1976 laying down common marketing standards for certain fresh or chilled fish. In *Official Journal of the European Communities*, L20/29.

GRAM, L. C. – WEDELL-NEERGAARD, C. – HUSS, H. H. 1990. The bacteriology of spoiling Lake Victoria Nile perch (*Lates niloticus*). In *International Journal of Food Microbiology*, vol. 10, 1990, p. 303-316.

HARRIGAN & McCANCE, 1990. *Methods in Food and Diary Microbiology*. Academic Press Limited San Diego, CA.

HUSS, H. H. 1995. *Quality and Quality Changes in Fresh Fish.* Food Agricultural Organization (FAO). Fisheries Technical Paper number 348. Rome, Italy.

ICMSF, 1986. International Commission on Microbiological Specification for Foods: Microorganisms in foods. *Sampling for microbiological analysis: Principles and specific applications*, 2nd Ed. International Commission on Microbiological Specifications for Foods. Blackwell Scientific Publications.

LAKSHMANAM, P.T. – GOPAKUMAR, K. 1999. K-value and index for estimating fish freshness and quality. In *Current Science*, vol. 76, no. 3, p. 400-404.

LIU, S. – FAN, W. – ZHONG, S. – MA, C. –, LI, P. –ZHOU, K. – PENG, Z. – ZHU, M. 2010. Quality evaluation of tray-packed tilapia fillets stored at 0°C based on sensory, microbiological, biochemical and physical attributes. In *African Journal of Biotechnology*, vol. 9, no. 5, p. 692-701.

LUTEN, J. B. - BORRESEN T. - OIHLENSCHLAGER, J. 1997. *Seafood from producer to consumer, integrated approach to quality*. Development in Food Science 38. Proceedings of the international seafood conference on the occasion of the 25th anniversary of the West European Fish Technologists Association (WEFTA), The Netherlands.

MALLE P. - TAO S. 1987. Rapid quantitative determination of trimethylamine using steam distillation. In *Journal of Food Protection*, vol. 50, p. 756-760.

MUKUNDAN, M. K. - ANTONY, P.D., - NAIR, M.R. 1986. A review on autolysis in fish. In *Fisheries Research*, vol. 4, p. 259-269.

OZOGUL, F. - OZOGUL, Y. 2000. Comparisons of methods used for determination of volatile basic nitrogen (TVB-N) in Rainbow trout (Oncorhyncus mykiss). In *Turkish Journal of Zoology*, vol. 24, p. 113-120.

PÉREZ-VILLARREAL, B. - HOWGATE P. 1987. Spoilage of European Hake (Merluccus Merluccius) in ice. In *Journal of the Science of Food and Agriculture*, vol. 41, p. 335-350.

PONS-SA´NCHEZ-CASCADO, S., VIDAL-CAROU, M.C., NUNES, M.L., & VECIANA-NOGUE´S, M.T. 2006. Sensory analysis to assess the freshness of Mediterranean anchovies (Engraulis encrasicholus) stored in ice. In *Food Control*, vol. 17, p. 564–569.

RYDER, J.M. 1985. Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. In *Journal of Agricultural and Food Chemistry,* vol. 33, p. 678-680.

VERMA, P.R.G. - MATHEN, C. - THOMAS, F. 1983. Quality changes and shelf-life of Pearl Spot, Mullet and Tilapia during storage at ambient temperature and in ice. In *Journal of Food Science and Technology,* vol. 20, p. 219-222.