MICROBIOLOGICAL QUALITY AND SHELF LIFE OF FRESH PACKAGED TILAPIA FILLETS STORED UNDER DIFFERENT CHILL TEMPERATURES

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) farmed in recirculation aquaculture system (RAS) was filleted and packaged in 100% air and 50% CO_2: 50% N_2 modified atmosphere (MA) prior to storage at 1°C and -1°C for up to 27 days. Fillets were sampled regularly and analysed for headspace gas composition, sensory and microbial changes. Shelf life varied with apparent relation to storage temperature, package atmosphere and microflora. Pseudomonads were reported as the main spoilage organisms in tilapia fillets during chilled storage conditions. Sensory analysis of cooked samples as well as microbial growth indicated fillets packaged in 100% air had a shelf life of 13-15 days during storage at 1°C and 20 days at -1°C. At the end of shelf life in 100% air packaged groups, TVC and pseudomonads counts reached log 7 colony-forming units g^{-1} in flesh. Whereas in 50% CO_2: 50% N_2 packaged fillets, the lag phase and generation time of bacteria was extended and recorded counts of < log 4 colony-forming units g^{-1} up to 27 days of storage at both 1°C and -1°C. However, 50% CO_2 : 50% N_2

conditions restricted fillets shelf life to 23 days based on sensorial changes mainly fillets colour characteristics.

Keywords: Shelf life, tilapia fillets, microbial, modified atmosphere

INTRODUCTION

The intensive farming of tilapia, *Oreochromis* sp. is rapidly expanding and tilapias (including all species) are the second most widely farmed fish in the world with annual production exceeding 2 million tons in 2005 (**FAO, 2007**). On world market, fishery products are among the most internationally traded food categories, in the sense that they have been widely crossing boarders (**FAO 2006a; Moller, 2007**). Moreover, safety rules and regulations imposed by the importing countries have continued to be more challenging to the exporting countries (**FAO, 2006b**). Freshness is one of the most important aspects of fish because consumers have a strong tendency to select very fresh fish (**Luten and Martinsdóttir, 1997; Ross, 2000**).

The extension of shelf life of fresh fish and fishery products is of importance when it comes to transporting products to distant markets. Shelf life of fishery products is usually limited by microbial activities that are influenced most importantly by storage temperature (**Huss, 1994; Simpson *et al*., 2003**). Bacterial changes are considered the most important cause of fish spoilage (**Gram and Dalgaard, 2002**). This is because spoilage is often a result of off-odors and off-flavours caused by bacterial metabolism (**Gram *et al*., 1990**). During fish storage, a characteristic flora develops but only a part of this flora contributes to spoilage (**Huss, 1994**). At chilled storage (0-5°C), *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas* spp. and *Pseudomonas* spp. have been reported to be the main spoilage microorganisms (**Liston, 1992; Gram *et al*., 1990; Gram *et al.*, 1987**).

Typical shelf life under chilled storage conditions ranges from 6 to 20 days (**Martinsdó ttir *et al.*, 2001; Cyprian *et al*., 2008**) depending on species, harvest location and season, and can result in heavy economic loss (**Reddy *et al*., 1995; Sivertsvik *et al*., 2002**). This has made packaging an integral part of the food industry. In addition to preservation function, packaing has several other important roles to play in delivering safe, wholesome and attractive foods to the market (**Kilcast and Subramaniam, 2000**).

Modified atmosphere packaging (MAP) of fishery products has been shown to inhibit the normal spoilage flora and increase shelf life significantly at cool temperatures (**Sivertsvik

et al., **2002**). The application of this technology to foods has become increasingly more available in recent years as food manufacturers have attempted to meet consumer demands (**Sveinsdottir *et al.*, 2010**). Studies have shown MAP to perform an effective synergy with superchilling in prolonging the shelf life of fresh cod (*Gadus morhua*) loins (**Wang *et al.*, 2008**) and Atlantic salmon (*Salmo salar*) fillets (**Duun and Rustad, 2008; Sivertsvik *et al.*, 2003**). **Duun and Rustad** (**2008**) reported superchilled salmon fillets stored at -2 °C in combination with MAP to have maintained good quality with negligible microbial growth for more than 24 days based on both sensory and microbial analyses. on the other hand, ice chilled reference fillets maintained good quality up to 17 days.

The present study aimed at investigating the effect of MA-packaging of fresh tilapia fillets during storage at different chilled condition on the sensory and microbiological changes.

MATERIALS AND METHODS

The study was carried out at MATIS Reykjavik-Iceland in two phases referred to as pre-trial and main trial.

Pre-trial: Selection of gas composition and main spoilage organisms (SSO)

To establish the appropriate gas mixture to MA-package tilapia fillets, forty fish (un-starved) weighing between 450g and 700g were harvested from recirculating aquaculture system (RAS) and filleted on the same day pre-rigor. Fillets were divided into 4 groups of 20 fillets each; A (air), B (50% CO_2: 50% N_2), C (70% CO_2: 30% N_2) and D (90% CO_2: 10% N_2). In all groups, four fillets were placed on a foam tray with a built-in absorption mat (expanded polystyrene, Linstar E 39-34, LINPAC packaging, West Yorkshire, UK) and put in a labeled high-barrier film bag (40PA/70LDPE, 250 mm × 400 mm × 0.120 mm, Plastprent Iceland). Packs for B, C and D groups were evacuated and packaged under respective MAs, whereas air packs (A) were left open. All the packs were stored chilled at 2°C up to 10 days. Samples were analysed for headspace gases and drip on days 3 and 10, and microbial load on day 10.

To find out the appropriate media and main flora developing in spoiling air- and MA-packaged tilapia fillets (A, B, C and D), various media were evaluated. On the sampling d 10, two packs per group were used. Fillets were sampled aseptically and analysed in duplicate per

group. Fillets were minced, assessing 2 pooled fillets for each sample. Minced flesh (20 g) was mixed with 180 g of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Total viable counts (TVC) were enumerated by spread-plating of aliquots on modified Long and Hammer´s agar (mLH) and Iron Agar (**Gram *et al.*, 1987**) both containing 1% NaCl (no overlay), with aerobic incubation at 17˚c for 4-5 days. H_2S-producers were also counted. Presumptive pseudomonad counts (22˚c, 3-4 days) were obtained using the modified Cephaloridine Fucidin Cetrimide (CFC) agar (**Stanbridge and Board, 1994**). Pseudomonas Agar Base (Oxoid) with CFC Selective Agar Supplement (Oxoid) was used. Pink colonies were counted. *Vibrio* counts were evaluated on Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS, Difco), following incubation at 17°C for 4-5 days.

Nitrite-Actidione-Polymyxin (NAP) agar was used in comparison to MRS-S agar for counts of lactic acid bacteria (LAB) at 22˚c for 4 days under anaerobic conditions. LAB confirmation was done by assessing presence of catalase-negative colonies using 3% H_2O_2. Counts of *Brochothrix thermosphacta* were determined on STAA selective agar (CM0881 with SR0162, Oxoid) following aerobic incubation at 22°C for 4 days and colony confirmation done by catalase and oxidase tests, assessing 5 to 10 colonies per sample. Catalase-positive and oxidase-negative colonies were regarded as *Brochothrix thermosphacta*. In all analysis spread-plating was used and detection limit was 20 colony forming units (CFU)/g. Counts of *Photobacterium phosphoreum* (Pp) were estimated by the PPDM-Malthus conductance method (**Dalgaard *et al.* 1997**).

Main trial

Three hundred and forty tilapia (approximately 6 months old) weighing between 450 and 700 g were harvested, bled and transported iced to Matis laboratory within three hours. 12 fish were hand filleted on arrival (pre-rigor) and used for sensory and microbiological analyses. The rest were hand filleted the following day (post-rigor) and fillets kept chilled at 0°C during packaging. Four fillets weighing 250-290 g (altogether) were placed individually with 'skin side' (red-colored) up on a foam tray with a built-in absorption mat. Foam trays with fillets were put into high-barrier film bags and divided into four groups; T1, T2, T3 and T4 as elaborated in Table 1.

Table 1 Definition of tilapia fillets sample groups

Treatment codes	Atmosphere in packages	Storage temperature	Filleted and de-skinned	Sampling days (d)
Control (CO)	Not packaged	Iced	Pre-rigor	0
T1	100% Air	1°C	Post-rigor	2, 6, 9, 13, 16 and 20
T2	100% Air	-1°C	Post-rigor	2, 6, 9, 13, 16 and 20
T3	50% CO_2 : 50% N_2	1°C	Post-rigor	2, 6, 9, 13, 16, 20, 23 and 27
T4	50% CO_2 : 50% N_2	-1°C	Post-rigor	2, 6, 9, 13, 16, 20, 23 and 27

d=Storage time calculated from day of filleting (packaging), 1 day from harvesting and storage at 1°C

Packaging

In T1 and T2, each bag was sealed with 100% air using a Vacuum Packaging Machine (HENKOVAC Heavy duty 2000, Hertogenbosch, The Netherlands) equipped with a built-in vacuum pump and gas flushing modes. In T3 and T4 the bags were packaged under headspace of 50% CO_2: 50% N_2. CO_2 and N_2 was mixed using gas mixer MAP Mix 9000 (PBI-Dansensor, Ringsted, Denmark). The gas volume-to-product (G/P) ratio was approximated 5:1. Prior to sample packaging, gas composition was confirmed by packaging dummy packs and their headspace gas composition analysed using an oxygen and carbon dioxide analyzer (CheckMate 9900 Analyzer, PBI-Dansensor, Ringsted, Denmark).

After packaging, T1 and T3 packages were stored at 1°C whereas T2 and T4 were stored at -1°C throughout the study. Sampling was done twice a week until the end of shelf life. At each sampling, five packs from each treatment (group) were analysed for headspace gas composition. Thereafter, two packs were used for microbiological analysis and three for sensory evaluation per treatment.

Headspace gas composition

Gas composition in 100% air and 50% CO_2: 50% N_2 MA packs was determined upon packaging and on every sampling day, using oxygen and carbon dioxide analyzer. The gas concentration measurement was an average for 5 packs per treatments (n = 5).

Sensory evaluation of raw and cooked fillets

Evaluation of raw fillets was done using Quality Index Method (QIM) scheme developed for deskinned tilapia fillets (**Cyprian et al., in press**). The QIM scheme was based on significant, well-defined characteristics of appearance, odor, and texture attributes changing through storage time. A score from 0–1, 0–2, or 0–3 points was given for changes occurring on each attributes. Every sampling day, three fillets were taken randomly from each group and placed on a clean table at room temperature and under white fluorescent light. Each fillet was blind coded with a number consisting of three digits. Ten trained panellists individually evaluated changes in colour, mucus, texture and odour. They had all been trained according to international standards (**ISO 8586, 1993**); including detection and recognition of tastes and odours, use of scales and, in the development and use of descriptors.

Sensory evaluation of the cooked tilapia fillets was performed in parallel to the QIM evaluation. Fillet loins were cut into pieces of about 4-5 cm long and 3-4 cm wide. The pieces were placed in aluminium boxes coded with three digit random numbers and cooked in a preheated electric oven Convostar (Convotherm GmbH, Eglfing, German) with circulation air and steam at 95-100 °C for six min. Ten trained panellists used descriptive analysis (**Stone and Sidel, 1985**) to describe the intensity of sensory attributes for cooked tilapia fillets (Table 2) on an unstructured scale from 0 to 100%. Each panellist evaluated duplicates of samples in a random order for each group. A computerised system (FIZZ, Version 2.0, 1994-200, Biosystèemes, France) was used for data recording.

Table 2 Some sensory vocabulary for cooked tilapia (*Oreochromis niloticus*)

Short name	Sensory attribute	Description of attribute
Odour		
O-Boilpot	Boiled potatoes	Whole newly-boiled potatoes
O-Boilmilk	Boiled milk	Hot milk, fruity odour
O-Earthy	Earth	Fresh earth
O-Musty	Musty	Moldy
O-Rancid	Rancid	Rancidity
Flavour		
F-A.char	Arctic char	Arctic char, new trout
F-Sweet	Sweet	Typical sweet flavour of fresh fish
F-Metallic	Metallic	Metallic flavour
F-Earthy	Earthy	Fresh earth
F-Musty	Musty	Moldy flavour
F-Sour	Sour	Sour taste, spoilage sour
F-Pungent	Pungent	Pungent flavour, bitter
F-Rancid	Rancid	Rancidity, cod liver oil, reminds of paint or solvent/thinner.
F-Spoilage	Spoilage	spoilage, queasy sweet flavour

Microbiological analysis

Two packs of four fillets were analysed in duplicate upon filleting and per treatment on subsequent sampling as storage progressed. Analysis of specific microflora in air- and 50% CO_2: 50% N_2 MA-packaged tilapia fillets was as elaborated under pre-trial section, except that aliquots were plated only on Iron agar and MRS-S agar for TVC and LAB respectively, evaluations twice a week. For pseudomonads, counts were obtained on CFC medium (twice a week) whereas counts of *Photobacterium phosphoreum* (Pp) were evaluated once a week using a RT-PCR method as described earlier.

Data analysis

The mean values of QI, QDA attributes scores and microbial counts changes were plotted separately against storage time using Microsoft excel (2007). Correlation analysis of QIM data was performed in the SPSS statistic software (Version 8.0 Alibre Inc, Texas, USA). Analysis of variance (ANOVA) carried out on the results were performed in the statistical program NCSS 2000 (NCSS, Utah, USA). The program (ANOVA) calculates multiple comparisons using Duncan's test to determine if sample means are significantly different at $P \leq 0.05$.

RESULTS

Pre-trial: Selection of gas and main spoilage organisms

Headspace gas and drip loss

Headspace carbon dioxide concentration seemed to decrease during early storage (up to day 3) but thereafter stabilized in MA packaged groups. Conversely, oxygen level increased during storage from about 0% at the beginning to almost 2% on day 10 (Figure I).
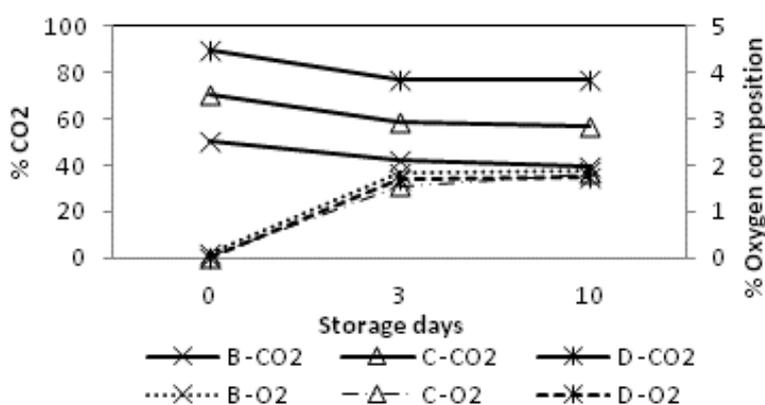

Figure 1 Gas changes in the headspace of tilapia fillets packaged under different gas mixtures

Microbiological analyses

Figure 2 shows the microbial profile of air- and MA-packaged tilapia fillets in different CO_2 levels. Similar patterns were observed for the microflora developing on TVC media, mLH and IA. TVC decreased considerably in presence of CO_2. All presumed specific spoilage organisms enumerated were present except H_2S-producers and *Vibrio* spp. Lactic acid bacteria (LAB) counts were tenfold higher on MRS-S than NAP agar. Pseudomonads were apparently dominating the microflora in air-stored fillets, while found at lower levels in MAP fillets. Among the presumed SSO investigated, *Brochothrix thermosphacta* ranked second in importance followed by LAB while *Photobacterium phosphoreum* was just at the detection level after 10 days at 2°C. Few SSO tolerated 70% or higher CO_2 concentration.
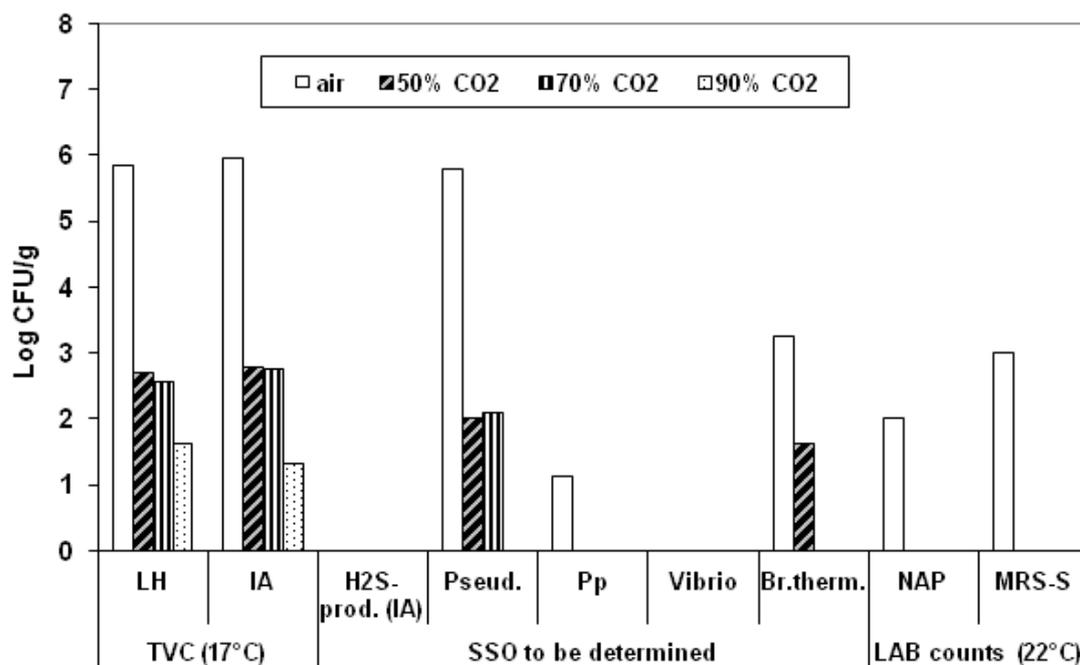
Figure 2 Microbial load in tilapia fillets air- and MA-packaged stored 10 days at 2°C. LH=Long and Hammer's Agar; IA=Iron Agar; NAP=Nitrite-Actidione-Polymyxin agar; SSO=specific spoilage organisms

Main trial

The average ambient and fillets contact temperature in the cold chamber was 1.0 ± 0.5°C (T1 and T3) and -1.0 ± 0.5°C (T2 and T4) throughout the storage time (23 and 27 days for air and MAP groups accordingly).

Headspace gas composition

On packaging, 100% air (T1 and T2) had approximately 21% O_2 and 0% CO_2 (comparable to 20.95 ambient O_2) as shown in Figure 3 A. There was a decline in mean O_2 levels with storage time contrary to CO_2 in 100% air packaged treatments. In T1 O_2 and CO_2 levels reached an equilibrium point of around 10% between d16 and d20 and thereafter, CO_2 surpassed O_2 attaining a maximum level of about 15% on d23 when O_2 was around 0.5%. A significant decline (p <0.05) in O_2 level of 13.5% to 1.5% was observed between d16 and d20. Although O_2 levels in T2 decreased with storage time, the lowest concentration reached was 17.2% when CO_2 was 4.2% on d23. Figure 3 B shows the initial headspace of 50%CO_2:

50%N_2 atmosphere packs (T3 and T4) contained low levels of O_2 (<1%). After day 2 of storage oxygen levels increased to 2.5% and thereafter, its concentration in the headspace gradually decreased as storage progressed. Initial CO_2 levels in headspace were 50% T3 and T4). On the day 2, its concentration had reduced to around 39%. Thereafter, the concentration in headspace (CO_2) remained on average at 39% in both groups during later storage days.
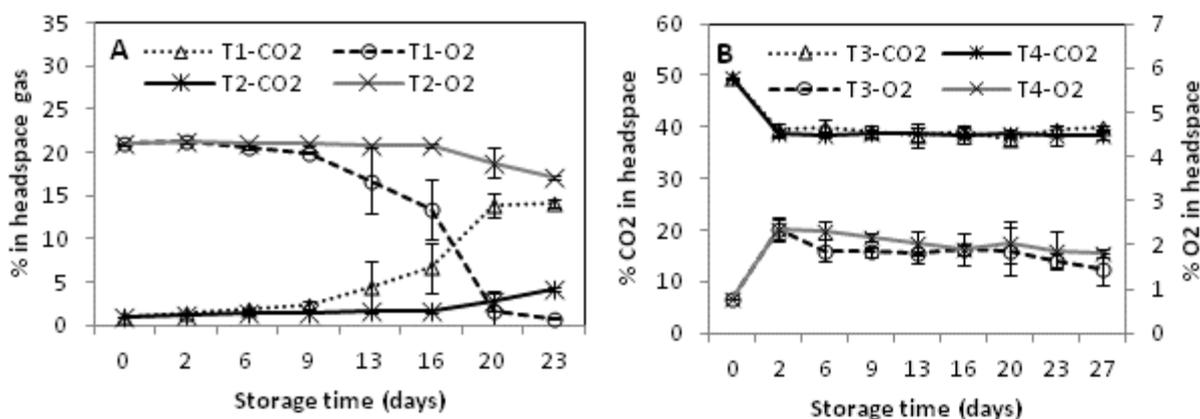

Figure 3 Gas changes in headspace of 100% air (A) and MAP (B) packs of tilapia fillets during storage (average score of 5 packs)

Sensory evaluation

The average scores for the fillets colour quality parameters increased with storage time (Figure 4), but differently for the four groups. 50%CO_2: 50%N_2 packaged (T3 and T4) fillets recorded significantly ($P \leq 0.05$) higher scores throughout the storage time. At the beginning of storage time d0, colour parameters (colour skin side, flesh colour loin and colour belly flap) was approximately zero. However, on subsequent assessment on d2, T3 and T4 groups received higher scores whereas counterparts' T1 and T2 recorded scores close to d0. The score for T1, T3 and T4 increased consistently reaching almost the maximum at the end of storage time whereas, T2 recorded less than 1 of the possible 2 score.
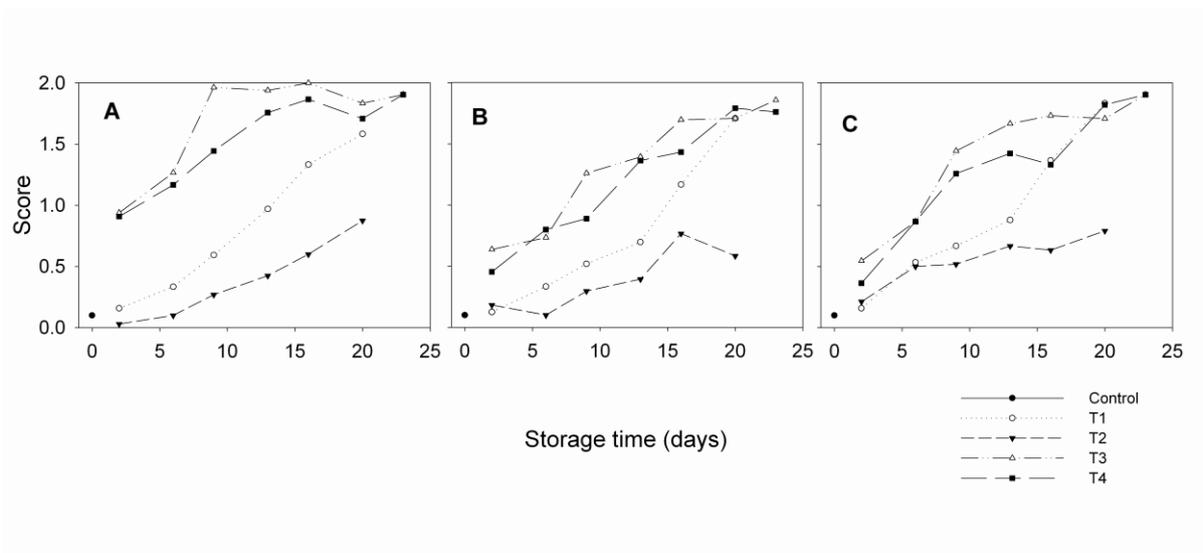
Figure 4 Averages scores (3 fillets) of colour changes (colour related parameters) as assessed by QIM scheme for deskinned tilapia fillets during storage at 1 ˚C and -1 ˚C. A: flesh colour from skin side; B: flesh colour from loin side; and C: colour of belly flap

Average scores for most freshness indicator attributes (odour (Boilpot and Boilmilk) and flavour (A.char and Sweet)) for cooked tilapia fillets did not change significantly with storage time varying between 40 and 20 except scores for flavour A. char and sweet that were significantly different (P<0.05) from other days on d 16 and d 20 (Table 3). In contrast to freshness attributes, spoilage indicator attributes scores (musty, rancid and spoilage) were not important in the groups at the beginning of storage (score of <10) except musty. The attributes however became prominent as storage time progressed especially after d9, but differently by treatments. Scores for rancid and spoilage flavour rose to around 20 on d13 in T1. On d9, T1 was significantly different (P<0.05) from other groups in flavour rancid and thereafter in all spoilage indicator attributes (musty, rancid and spoilage).

Table 3 Mean sensory scores (scale 0-100) of odour and flavour attributes for cooked tilapia fillets (average scores of 2 taste portion)

Storage time (d)	Group	Odor				Flavor			
		Boilpot	Boilmilk	Musty	Rancid	A.char	Sweet	Rancid	Spoilage
0	Co	33	28	23	3	33	33	4	3
2	T1	31	27	28	2	37	36	2	2
	T2	30	27	25	2	37	35	2	2
	T3	27	22	24	3	34	35	2	2
	T4	28	28	23	2	36	35	2	2
	P-value	*0.442*	*0.649*	*0.905*	*0.699*	*0.737*	*0.999*	*0.997*	*0.990*
6	T1	33	22	26	9	34	30	7	7
	T2	35	23	26	4	33	33	6	4
	T3	33	27	20	3	34	33	4	3
	T4	34	20	25	6	32	31	7	5
	P-value	*0.953*	*0.342*	*0.252*	*0.080*	*0.937*	*0.765*	*0.483*	*0.580*
9	T1	32	26	27	8	32	25	12[a]	6
	T2	33	25	24	5	38	30	6[b]	3
	T3	31	27	23	6	36	29	4[b]	6
	T4	29	26	25	5	38	27	4[b]	1
	P-value	*0.597*	*0.95*	*0.586*	*0.554*	*0.075*	*0.479*	***0.020***	*0.130*
13	T1	32	21	31[a]	15[a]	30	22	24[a]	20[a]
	T2	30	21	30[a]	4[b]	32	24	5[b]	9[b]
	T3	27	22	25[b]	5[b]	24	25	7[b]	5[b]
	T4	27	21	24[b]	6[b]	20	23	12[b]	8[b]
	P-value	*0.401*	*0.997*	***0.004***	***<0.001***	*0.254*	*0.804*	***<0.001***	***0.002***
16	T1	38	24	32 [a]	20[a]	27[a]	22[a]	22[a]	26[a]
	T2	37	26	24 [b]	12[a]	34[b]	23[a]	13[b]	12[b]
	T3	36	28	25 [b]	8[b]	36[b]	30[b]	10[b]	8[b]
	T4	35	29	27 [b]	7[b]	34[b]	29[b]	10[b]	7[b]
	P-value	*0.759*	*0.428*	***0.028***	***0.014***	***0.002***	***0.006***	***0.024***	***<0.001***
20	T1	35	26	32 [a]	25[a]	28	14[a]	25[a]	36[a]
	T2	40	30	26 [b]	14[b]	34	24[b]	18	18[b]
	T3	37	25	28 [b]	14[b]	33	22[b]	18	19[b]
	T4	36	26	28 [b]	10[b]	32	25[b]	12[b]	12[b]
	P-value	*0.520*	*0.603*	***0.048***	***0.004***	*0.075*	***0.006***	***0.049***	***<0.001***
23	T3	35	24	29	13	26	21	18	14
	T4	33	22	34	14	28	20	19	18
	P-value	*0.538*	*0.639*	*0.377*	*0.893*	*0.588*	*0.432*	*0.472*	*0.256*

Data within the same column in respect to storage time (d) with different letters are significantly ($p<0.05$) different

Microbiological analyses

Microbial profile of air and MA packaged fillets was generally good at the beginning of storage (d0) with total viable counts (TVC) and presumptive pseudomonads counts being somewhat above the limit of detection (log 1.3 colony-forming units g^{-1}), whereas H_2S-producers, *Brochothrix thermosphacta* and *Lactobacillus* counts were not detected.

TVC and Pseudomonads

Figure 5 shows growth curves for TVC and pseudomonads in 100% air- and 50%CO_2: 50%N_2 -packaged tilapia fillets stored at 1˚C and -1˚C. TVC and pseudomonads counts increased more rapidly in air than 50% CO_2: 50% N_2 packs. More so, higher growth was evidenced in storage at 1˚C than at -1°C. Counts of T1 reached log 8.6 colony-forming units g^{-1} and log 8.4 colony-forming units g^{-1} for TVC and pseudomonads, respectively on d16. However, the counts reduced slightly on the successive sampling d20. In T2, TVC and pseudomonads growth was moderate, reaching log 7 colony-forming units g^{-1} at end of storage time d20. An extended lag phase of TVC and pseudomonads was observed in T3 and T4 at both storage temperatures. TVC counts reached log 4 colony-forming units g^{-1}, though earlier in T3 (d14) than T4 (d28). *Pseudomonas* spp. counts in T4 (superchilled) fell below the detection limit after d9 but were detected towards the end of storage time whereas, slow growth in T3 (chilled) occurred from d16 after a lag phase.
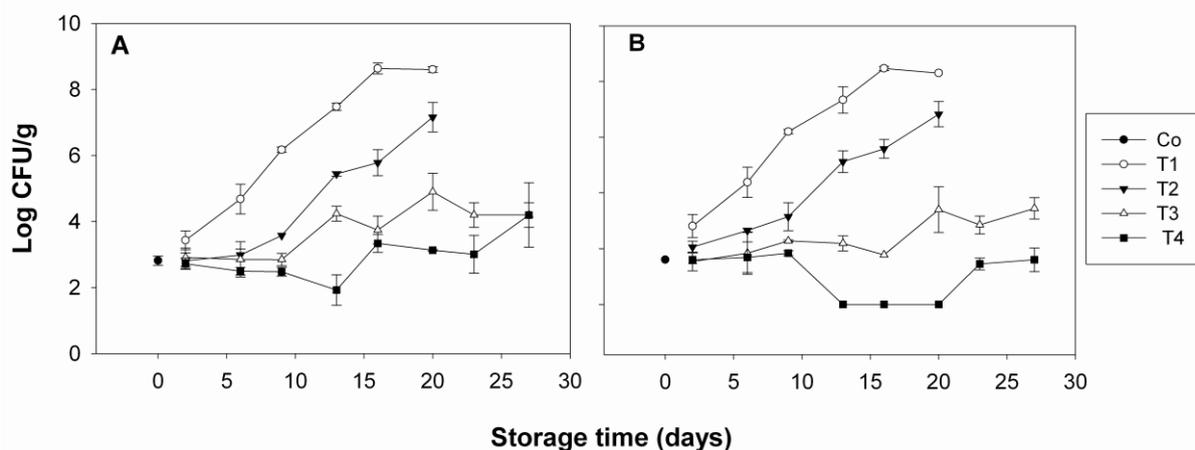
Figure 5 Total psychrotrophic viable counts (A) and presumptive pseudomonads (B) in packaged tilapia fillets during storage (average score of 2 packs)

H$_2$S- producers

Counts of H$_2$S-producing bacteria varied considerably within the groups and storage time (Figure 6). The effects on growth of H$_2$S-producers was more evidenced with storage temperature than atmospheric condition with T1 and T3 (100% air and 50% CO$_2$: 50% N$_2$ chilled) recording counts of log 3.1 and <1.3 colony-forming units g^{-1}, respectively, on d20 and d27, at end of storage time for 100% air and 50% CO$_2$: 50% N$_2$ packaged. Low H$_2$S-producers' counts (< log 4 colony-forming units g^{-1}) were observed in the treatments at the end of storage time compared to other specific spoilage organisms evaluated. T2 and T4 (superchilled) counts were at or below the detection limit during the whole storage period.
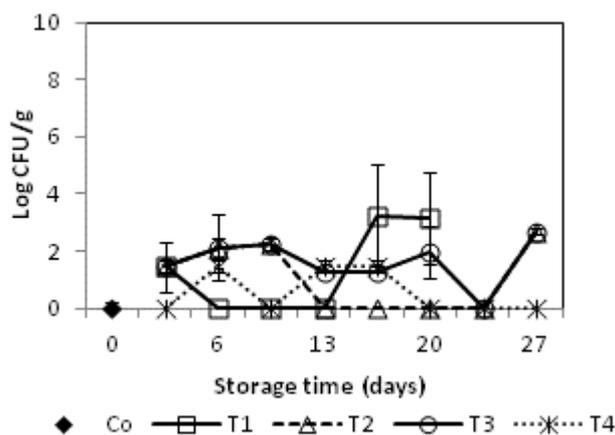

Figure 6 H$_2$S-producing bacteria in packaged tilapia fillets during storage (average score 2 packs)

Brochothrix thermosphacta and Lactobacillus spp.

Brochothrix thermosphacta and lactobacilli developed significantly (p≤0.05) faster between day 2 and 6 at 1°C in 100% air packaged T1 compared to other groups, attaining log 6 colony-forming units g^{-1} on d16 (Figure 7) after which no further growth was observed in the group. *Brochothrix thermosphacta* and lactobacilli counts in 100% air-packaged T2 were log 5 and log 4 colony-forming units g^{-1} respectively on d20. In 50% CO_2: 50% N_2 packages, a lag phase was evidenced for both bacterial groups, up to 6-9 d for T3 (chilled) and 13 d for T4 (superchilled), after which growth occurred. Levels of lactobacilli reached in MAP groups towards the end of storage were tenfold higher than those observed for *Brochothrix thermosphacta*. As noted with other bacterial groups, *Brochothrix thermosphacta* and lactobacilli counts were higher in fillets stored at 1 °C than at -1 °C with respect to atmosphere conditions.
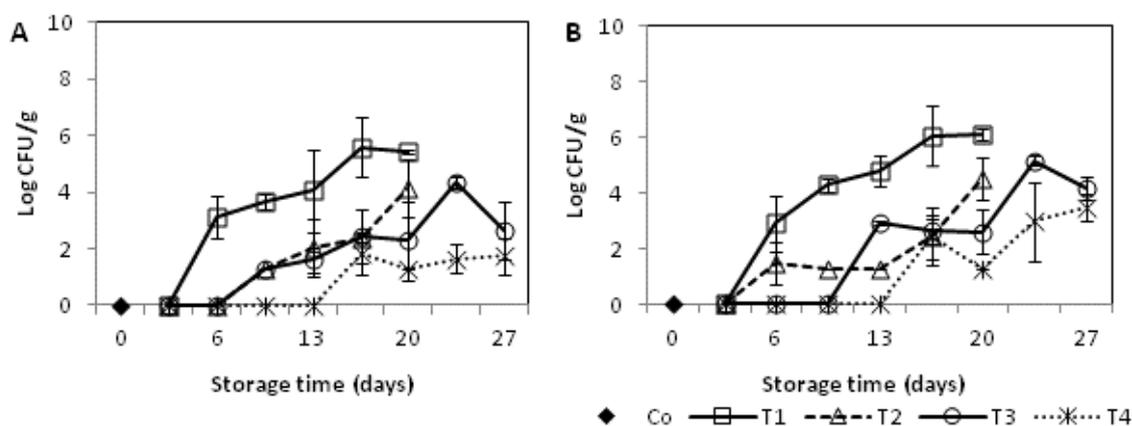

Figure 7 Counts of Brochothrix (A) and Lactobacillus in packaged tilapia fillets during storage (average score of 2 packs)

Photobacterium phosphoreum

Counts of *Photobacterium phosphoreum* were estimated using a newly developed RT-PCR method. As shown in Table 4, only few samples contained *Photobacterium phosphoreum* and at low levels (< log 3 colony-forming units g^{-1}). Furthermore, the numbers did not reflect changes with respect to storage time but were only reported in 100% air packaged fillets (T1 and T2).

Table 4 Counts (log CFU/g) of *Photobacterium phosphoreum* in tilapia fillets during storage at 1°C and -1°C as enumerated by real time PCR (average score of 2 packs)

Storage time (d)	6	13	20
Sample group			
T1	2.62	0.01	ND
T2	ND	0.35	ND
T3	ND	ND	ND
T4	ND	ND	ND

Initial count of product (control) was log 0.47/g; ND= not detected

DISCUSSION

Selection of gas composition (MA) and main spoilage organisms

CO_2 composition in the packages reduced during storage because of its high solubility in water and fat (**Reddy and Armstrong, 1992; Sivertsvik *et. al.*, 2002**). In terms of quality parameters MA packaged with 50% CO_2: 50% N_2 (B) showed good characteristics when cooked, were less dry and had less exudate compared to other combinations of MA-packing (data not provided).

Bacterial growth in tilapia fillets packaged in 50% CO_2 was not different to 70% CO_2 for all microbial groups evaluated except *Brochothrix thermosphacta*. It's therefore worth noting that microbiologically 90% CO_2 packaged fillets offered better results. However, on considering sensory and other quality parameters as aforementioned, the appropriate MA for storage of tilapia fillets was 50% CO_2: 50% N_2 (B). For TVC and pseudomonads that seem to be the main specific spoilage organism in tilapia, approximately log 4/g lower counts were recorded in 50% CO_2 packaged compared to 100% air stored. For that reason, the quality of the fillets remained good under such atmosphere and thus used for MA packaging in the main study. The pre-trial results enabled a focused study due to establishment of optimized culture media and packaging conditions (air and 50% CO_2: 50% N_2).

Main study

Headspace gas composition

Oxygen levels in all the treatments (packs) showed similar trends of decline with storage time as those reported elsewhere (**Reddy *et. al.,* 1995; Hovda *et. al.,* 2007**). The largest decline occurred in 100% air packaged samples stored at 1°C (T1) which corresponded with the occurrence of high bacterial numbers (> log 8 colony-forming units g^{-1} for TVC and pseudomonads) suggesting that the oxygen was being consumed during microbial metabolism. In contrast oxygen did not decrease significantly in 100% air packaged stored at -1°C (T2) which in relation to the preceding statement was due to low bacterial numbers (< log 4 colony-forming units g^{-1}) at the end of storage time. On the other hand CO_2 composition in headspace of T1 and T2 (100% air packaged) increased as time progressed since CO_2 was being released as secondary product of metabolism (**Hovda *et. al.,* 2007**).

Initial oxygen levels in the headspace of 50% CO_2: 50% N_2 packaged fillets (T3 and T4) were low, but rose on subsequent sampling d2. The initial increase in O_2 as reported by **Reddy *et al*., (1995)** was as a result of oxygen leaching from the foam tray, soak pad, and fillet into the package headspace. Thereafter oxygen decreased gradually as time progressed, in packages stored at 1°C the decrease was more rapid than at -1°C as microbial and post-mortem metabolic activity increased. However, O_2 did not get to below the initial 1% as less microbial growth was reported in T3 and T4 groups. On the other hand, CO_2 reduction from 50% at packaging to around 40% during early storage days was allied to dissolving into fillets due to its high solubility in water and fat (**Soccol and Oetterer, 2003**). At later storage days, CO_2 showed an insignificant upward trend especially in T3 which may have been due to an increase in microbial activity.

Sensory analysis

At the beginning of storage time, colour related attributes were scored low and characterised by freshness attributes; 'dark red colour on the skin side and light appearance with trace of red colour or hue on backbone side'. The scores however, increased as colour faded soon after packaging for T3 and T4. It was characterised by red brown and light brown on the skin and backbone sides accordingly. This is in accordance with how the QIM scheme are constructed, where fish evaluated shortly after catch should be scored low and

subsequently increase with storage time reaching close to maximum score at the end of shelf life (**Martinsdóttir *et al*., 2001**). Skin discolouration of some MA-treated fish samples has previously been reported (**Lauzon et al., 2002**) due to denaturation of muscle and pigment proteins. The discoloration process was slower in 100% air packages as fillets retained the dark red colour characteristics longer, especially with storage at -1°C. This could be explained by the maintenance of the oxymyoglobin pigment in the muscle in presence of oxygen, being more soluble under superchilled conditions (**Sivertsvik et al., 2003; Ando et al., 2005, 2004**).

End of shelf life is usually determined when spoilage-related sensory attributes become evident and most panellists detect them. The average score of above 20 (on the scale 0 to 100) for these spoilage attributes has been applied previously as an indication that fish sample is approaching the end of shelf life (**Cyprian et al., 2008; Bonilla et al., 2007; Magnusson et al., 2006; Sveinsdottir et al., 2002**). 100% air-packaged fillets stored at 1°C (T1) showed first spoilage characteristics on d13. On d16 and d20, groups T2, T3 and T4 also showed signs of spoilage. However, the scores for spoilage attributes were below 20 on d23 for groups T3 and T4. On the other hand, a part of the panel could not taste T1 samples due to strong spoilage odour indicating that the group (T1) was no longer fit for human consumption on that day. Similar observation was noted by **Sveinsdottir et al., (2002)** who reported salmon to be unfit for consumption when part of the panel did not taste samples due to spoilage odour. T2 and T3 recorded spoilage attributes scores close to 20 on d20 and d23 respectively implying the groups could be approaching the end of shelf life considered at an average score of above 20.

Microbiological analyses

During pre-trial, pseudomonads were identified as the main specific spoilage organisms in tilapia. This is in agreement with **Huss (1994)** who reported *Pseudomonas* spp. to be the specific spoilage bacteria of iced stored tropical freshwater fish. TVC maximum limit of log 8 colony-forming units g^{-1} has previously been used in tilapia as limit for human consumption (**Waliszewski and Avalos, 2001; Reddy *et al.*, 1995**). **Reddy *et al.* (1995)** reported total aerobic, anaerobic and coliform counts to have reached a maximum level of log 8.0 colony-forming units g^{-1} on the day of tilapia fillets spoilage. In this study, TVC and *Pseudomonas* spp. reached > log 8 CFU (log 8.6 and log 8.4 respectively) for 100% air packaged during storage at 1°C (T1) on d16 indicating that they were past consumption limit. However on subsequent evaluation d20, same or lower counts for TVC and *Pseudomonas*

spp. were reported in T1 mainly due to change in headspace gas composition whereby the exponential aerobic growth resulted to high oxygen consumption and an increase in CO_2 a secondary product of metabolism whose accumulation restricts further growth (**Adams and Moss, 2008**). In T2, TVC and *Pseudomonas* spp. counts were within consumption limit (log 7 colony-forming units g^{-1}) at the end of storage time d20 using log 8 colony-forming units g^{-1} as rejection limit for tilapia consumption. The lower counts evidenced in air packaged during storage at -1°C compared to 1°C is due to effective delay of bacterial growth during storage at superchilling conditions (**Huss, 1995, Church, 1998**).

The extended lag phase in 50% CO_2: 50% N_2 packaged groups (T3 and T4) during early storage days and low aerobic counts indicates that counts were not observed to reach human consumption limit. The delayed growth and lower counts of aerobic microorganisms can be attributed mainly to CO_2 inhibition (**Sivertsvik et al., 2002**), the presence of low level O_2 (**Reddy and Armstrong, 1992**) and low storage temperature (**Huss, 1995**) used in the present study. Low counts of H_2S-producing bacteria recorded throughout the study could partly be due to absence of *Shewanella putrefaciens* (main H_2S-producer) in tropical freshwater fish since the organism is typical of marine waters (**Sivertsvik et al., 2002**). It has also been reported that growth of microbial association during food spoilage depends not only on the imposed environmental conditions, as is well known, but also on microbial interaction. **Gram and Melchiorsen (1992)** reported *Pseudomonas* spp. to inhibit the growth of *Shewanella putrefaciens* due to the ability of the former to produce siderophores, and this interaction could have been a factor overriding the development of spoilage flora since higher counts of *Pseudomonas* spp. were reported in the study.

The facultative anaerobe *Brochothrix thermosphacta* and the strictly fermentative lactobacilli showed unstable growth trends in all groups except T1. Growth of these organisms in T1 might be attributed to the fact that different anaerobic microorganisms show different sensitivity levels to oxygen (**Soccol et al., 2005**). The lag phase extension for *Brochothrix thermosphacta* and lactobacilli was however observed to be a function of storage temperature and atmosphere. Low *Photobacterium phosphoreum* reported in tilapia fillets air and 50% CO_2: 50% N_2 packaged may be because the organism is typical of marine waters. **Dalgaard et al. (1997)** did not detect the presence of *P. phosphoreum* in fresh water fish, despite finding great growth ($> 10^7$ colony-forming units g^{-1}) of this microorganism in marine species. Correlating microbial results for various groups, it can be deduced that T2; T3 and T4 were within microbial allowable consumption limits on d20 and d27 accordingly, whereas T1 surpassed the limit of log 7 colony-forming units g^{-1} on d16.

Shelf life

Descriptive analysis can be useful in shelf life studies, as it provides information about maximum shelf life (**Cyprian et al., 2008; Bonilla et al., 2007**). The results from descriptive analysis and microbiological analysis indicated that 100% air-packaged tilapia fillets had a shelf life of 13 - 15 days when stored at 1 °C (T1) in contrast to about 20 days when stored at -1 °C (T2). On the other hand, 50% CO_2: 50% N_2 -packaged fillets on d23 were within consumption limits as evaluated with descriptive analysis (< 20 for spoilage attributes score) and microbiologically suitable for consumption up to d27 on which sensory evaluations were not conducted. Nevertheless, the sample groups (T3 and T4) were rejected based on appearance of raw fillets caused by changes in colour as well as mucus and texture. This is because colour of the fresh meat is an important indicator of quality and a major factor in influencing retail purchase decisions. **Reddy et al., (1995)** reported MA-packaged (75% CO_2 : 25 N_2) tilapia fillets stored at 4°C to have shelf life of > 25 days based on microbial evaluation, which is longer than the findings from the present study despite storage at low temperature. This could be attributed mainly to emphasis placed on sensory results in the current study.

Tilapia fillets seems to have longer shelf life than fillets from most temperate species; cod fillets; 8 days in ice (**Bonilla *et al*., 2007**), vacuum packed cod fillets; 9-10 days at 0°C (**Gram *et al*., 1987**), and MA and vacuum packaged snapper (*C. auratus*) fillets; 9 days at -1°C (Scott, *et al*., 1986). This is because when fish is held under chilled conditions, the fish from warm waters keep longer than fish from cold waters (**Gram *et al*., 1990**). This is apparently in relation to the relative proportions of psychrotrophic bacteria on fish from waters of different temperatures. Cold waters tend to favor proliferation of high numbers of psychrotrophs on fish, which in turn enhances spoilage at chilled condition and ultimately shortens the shelf life of fish (**Karugi *et al*., 2004**).

CONCLUSIONS AND RECOMMENDATIONS

Modified atmosphere packaging with 50% CO_2: 50% N_2 performed an effective synergy with superchilling in extending microbiological quality of fillets as microbial growth were suppressed compared to counterparts refrigerated and air packaged. On the other hand, 50% CO_2: 50% N_2 in headspace impacted negatively on colour characteristics which limit its application in extending the shelf life of fresh tilapia fillets. Therefore, based upon sensory

evaluation and microbial profile the maximum storage time was 13 -15 days for 100% air packaged during storage at 1°C (T1) and 20 days for superchilled (T2).

The present study was done under well controlled conditions (chilled and superchilled) therefore, the results are more specific than they would be in most commercial situations where conditions can be variable. The data on shelf life reported should be used with discretion and in most cases assumed to represent maximum values.

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