Modified atmosphere packaging and quality of fresh Cape hake (*Merluccius capensis*) fish fillets

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Summary

Fresh ready-to-cook fish fillets are prone to rapid loss of freshness and other quality attributes, as well as accelerated growth of spoilage micro-organisms under sub-optimal storage conditions. Cape hake (*Merluccius capensis*) is an important seafood in South Africa; however, rapid loss of quality and eventual spoilage is a problem limiting the economic potential. Thus, the aim of this study was to investigate the effects of active (40% CO_2 + 30% O_2 + 30% N_2) and passive (0.039% CO_2 + 20.95% O_2 + 78% N_2) modified atmosphere packaging (MAP) under different storage temperatures (0°C, 4°C, and 8°C) on the quality attributes of Cape hake (*Merluccius capensis*) fish fillets. This was achieved by investigating the effects of MAP (with or without absorbent pads) and storage temperature on quality attributes (microbial, physicochemical and sensory), changes in composition and concentration of volatile compounds (VOCs) and shelf life of Cape hake fillets.

Modified atmosphere packaging, storage temperature and the use of absorbent pads had a significant ($p < 0.05$) impacts on physicochemical properties of Cape hake fillets during refrigerated storage. Highest storage temperature (8°C) led to accelerated deterioration of packaged Cape hake fillets. Generally, active MAP better maintained the quality attributes of Cape hake than passive MAP at 0°C and 4°C. Headspace gas composition of O_2 and CO_2 were significantly influenced by the storage time, temperature, MAP conditions and their interactions ($p < 0.05$). Irrespective of storage temperature, active-MA packaged fillets had lower pH values in comparison to fillets stored under passive-MAP. Drip loss was higher in active-MA fillets packaged without absorbent pad. Passive-MAP fillets did not show any drip loss. Absorbent pad was used to add value to MAP storage as MAP resulted in drip. The use of absorbent pad combined with low storage temperature maintained the firmness of hake fillets, across all temperatures. The interaction of MAP, absorbent pad and storage temperature had a significant effect on the aerobic mesophillic bacteria counts. Based on the aerobic mesophillic bacteria count fillets stored under active-MAP at 0°C (5.2 log cfu/g) was limited to day 12, while the fillet stored under passive-MAP at 0°C (log cfu/g) was limited to greater than day 3. Overall sensory acceptability of fillets decreased with increase in storage temperature across all treatments. Additionally, MAP had a significant ($p < 0.05$) impact on sensory attributes such as appearance and odour acceptability, with active-MA packaged fillets stored at 0°C having highest overall acceptability. A total of 16 volatiles were identified in Cape hake fillets, including 4 primary VOCs and 12 secondary VOCs. The VOCs associated with spoilage include tri-methylamine (TMA) (ammonia like), esters (sickeningly

sweet) and sulphur group (putrid). MAP had a significant ($p< 0.05$) influence on volatile composition and concentration. Active-MA packaged fillets performed better during storage and had lower TMA value of 0.85% on day 12 in comparison with 7.22% under passive-MAP on day 6 at 0°C. The results obtained demonstrated that changes in volatile compounds were significantly ($p< 0.05$) influenced by storage duration, temperature and MAP. The development of high levels of VOCs and off-odour corresponded with high aerobic mesophillic bacteria count (≥ 5.5 log cfu/g). Based on these developments the storage life of Cape hake fillets packaged under active-MAP with absorbent pad and stored at 0°C was limited to 12 d, while the passive-MAP (control) fillets stored at 0°C was limited to 3 d. The use of active-MAP, in combination with absorbent pads and 0°C storage in addition to good hygienic practices, was effective in maintaining the postharvest quality of Cape hake fish fillets and led to higher shelf life.

Opsomming

Vars, gereed-vir-kook vis filette is geneig om gou hulle varsheid en ander gehalte kenmerke te verloor en die vervoegde groei van mikro-organismes wat bederf tydens stoor veroorsaak, vind plaas. Kaapse stokvis (*Merluccius capensis*) is in Suid-Afrika 'n gewilde seekos maar die ekonomiese potensiaal daarvan word deur die feit dat dit so gou in gehalte afneem en bederf, beperk. Dus word daar met hierdie studie gepoog om die effek van aktiewe (40% CO_2 + 30% O_2 + 30% N_2) en passiewe (0.039% CO_2 + 20.95% O_2 + 78% N_2) aangepasde verpakking (MAP) onder verskillende stoortemperature (0°C, 4°C, en 8°C) op die gehalte kenmerke van Kaapse stokvis (*Merluccius capensis*) filette te ondersoek. Dit is gedoen deur om die effek van MAP (met of sonder kussinkies) en stoortemperatuur op die gehalte kenmerke (mikrobies, fisiochemies en sensories) asook veranderinge in komposisie en konsentrasie van vlugtige samestellings (VOCs) en die raklewe van Kaapse stokvis filette te ondersoek.

Aangepasde atmosfeer verpakking, stoortemperatuur en die gebruik van absorberende kussinkies het 'n groot impak ($p < 0.05$) op die fisiochemiese kenmerke van Kaapse stokvis tydens stoor in yskaste gehad. Hoë stoortemperature (8°C) het aanleiding gegee tot die vinnige bederf van verpakte Kaapse stokvis filette. Oor die algemeen het aktiewe MAP die gehalte van die Kaapse stokvis filette teen 0°C and 4°C beter bewaar. Die komposisie van O_2 en CO_2 is heelwat deur stoortyd, temperatuur, MA toestande en die interaksies tussen bogenoemde, beïnvloed ($p< 0.05$). By alle temperature het aktiewe MA verpakte filette laer pH waardes getoon in vergeleke met filette wat in onder passiewe MA verpak is. Die drupverlies was hoër in aktiewe MA filette verpak sonder absorberende kussinkies. Passiewe MAP filette het nie enige drupverlies getoon nie. Absorberende kussinkies is gebruik om waarde by te voeg tot MAP stoor aaangesien MAP gelei het tot drup. By alle temperature het die gebruik van absorberende kussinkies tesame met lae stoortemperature bygedra tot die behoud van fermheid. Die interaksie van MAP, absorberende kussinkies, en stoortemperatuur het 'n groot effek gehad op die aerobiese mesofiliese bakterië telling. Weens die aerobiese mesofiliese bakterië telling is stoor van filette onder aktiewe MAP teen 0°C (5.2 log cfu/g) beperk tot dag 12, terwyl filette gestoor onder passiewe MAP teen 0°C (log cfu/g) beperk is tot dag 3. Oor die algemeen het die sensoriese aanneemlikheid van filette sonder inasgneming van die behandeling, verklein met 'n toename in stoortemperature. MAP het ook 'n groot impak op die

sensoriese kenmerke soos voorkoms, reuk, en aktiewe MA verpakte fillets gestoor teen 0°C is oor die algemeen die aanneemlikste. ’n Totaal van 16 vlugtige substanse is in Kaapse stokvis identifiseer. Dit het vier primêre VOCs en 12 sekondêre VOCs ingesluit. Die VOCs wat met bederf assosieer word, sluit tri-metilamien (TMA) (soos ammoniak), esters (soet) en die swael groep (smetterig) in. MAP het ’n groot ($p < 0.05$) invloed op die vlugtige komposisie en konsentrasie. Aktiewe MA verpakte filette het beter tydens stoor presteer en het ’n laer TMA waarde van 0.85% op dag 12 gehad, in vergelyking met 7.22 % onder passiewe MAP op dag 6 teen 0°C. Die resultate toon dat veranderinge in vlugtige samestellings grootliks beïnvloed word ($p < 0.05$) deur stoortyd, temperature en MAP. Die ontwikkeling van hoë vlakke van VOCs, slegte reuke en verlies aan varsheid gaan tesame met hoë aerobiese mesofiliese bakterië telling ($\geq$ 5.5 log cfu/g). Gegrond op hierdie tellings is die stoorleeftyd van Kaapse stokvis filette beperk tot dag 12, terwyl passiewe MAP (die kontrole) filette gestoor teen 0°C, beperk is tot dag 3. Om op te som, die gebruik van aktiewe MAP tesame met absorberende kussinkies en 0°C stoor asook goeie higiëniese praktyk, kon die na-oes gehalte van Kaapse stokvis filette behou en het gelei tot ’n langer rakleeftyd.

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CHAPTER 1

INTRODUCTION

Background

Global changes in consumer lifestyle marked by increasing demand for nutritional and healthy food products have spurred the continuing rise in demand for fresh and ready-to-cook (RTC) fish and fishery products (De Silva & Yamao, 2006; Opara, 2009; Speranza *et al*., 2009). In the food industry, attempts to satisfy this rising demand has led to the production of RTC fish and fishery products such as steak, fillets, burgers, and squid rings (De Silva & Yamao, 2006; Speranza *et al*., 2009). This rising demand for RTC fresh instead of frozen fish requires the use of preservation procedures that can add value and reduce postharvest losses (Trondsen *et al*., 2003).

South Africa has a long coastline of approximately 3623 km (Figure 1) with the the fisheries industry being an important sector in the economy, and in 2009 approximately US$ 714 million was generated from landed fisheries (DAFF, 2012a).

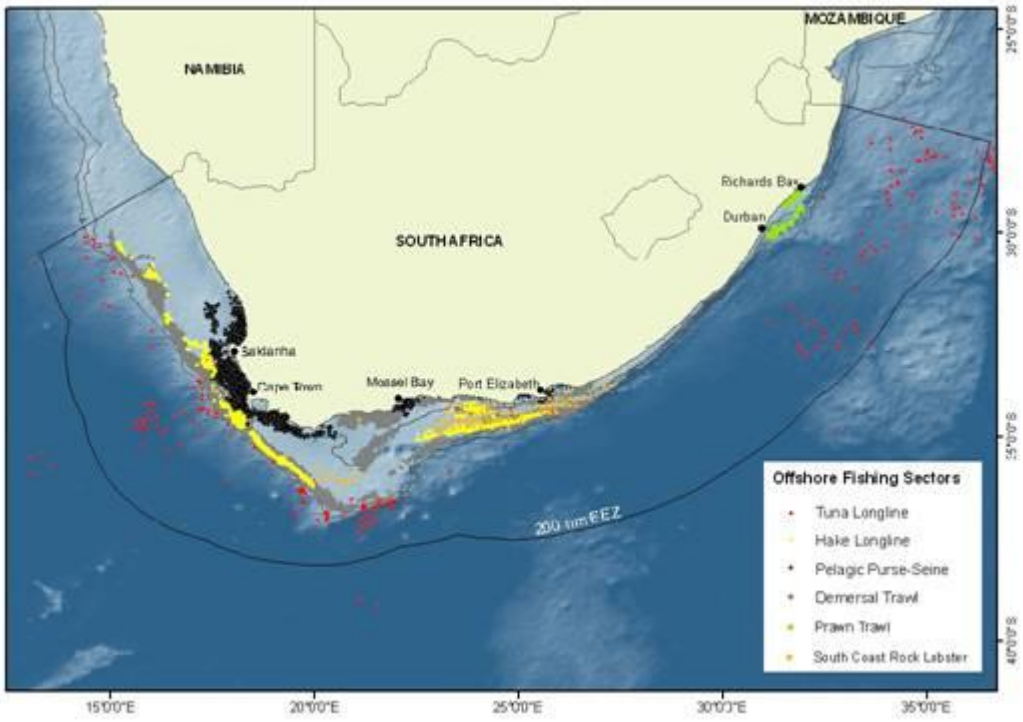

Figure 1 South African coastline indicating offshore fisheries sectors. Source (FAO, 2010)

Also commercial fisheries landed 551,794 metric tonnes in 2007 with an income of US$ 71 million from deep-sea hake (Feike, 2008; DAFF, 2012a). In South Africa, demersal fishery is considered the most economically viable in the fishery sector and is mostly dominated by the Cape hakes, *Merluccius paradoxus* (Deep-water hake) and *M. capensis* (Shallow-water hake). Hake belongs to the order Gadiformes which includes other species such as cod and haddock. It belongs to the family Phycidae, dwells at depths of 200-350 m, and can grow up to 1 m in length with average weights of 0.5-3.6 kg. Hake fish and fishery products constitute the major South African fish exports to Spain, Australia and USA, accounting for about 80% of the total economic contribution of the fish industry to the economy (DAFF, 2012b).

Fish provides about 17% of the global annual protein intake and only about 60% of palatable fish is utilized by end-users while the remainder is either converted or lost (Domingo *et al*., 2007; Ahmed, 2008; FAO, 2014). Postharvest loss due to decay is estimated to be between 10-12 million tonnes annually which accounts for about 10% of global capture and culture fish (Ruckes, 2003; Ahmed, 2008; FAO, 2014). Researchers have offered numerous solutions to these challenges and have developed systems to maintain the wholesomeness and shelf life of fish and fishery products (Dondero *et al*., 2004; Bellagha *et al*., 2007).

Fresh fish and fishery products have high water activity ($a_w \geq 0.95$) and post mortem pH of about 5.2 (Choubert & Baccaunaud, 2006; Jezek & Buchtova, 2007; Jezek, 2012). This condition is favourable for the growth of micro-organisms, which leads to eventual spoilage (Ocano-Higuerra *et al.*, 2006). Freshness of fish can be quantified based on changes in sensory, microbial, physical and chemical composition (Triqui & Bouchriti, 2003; Opara *et al*., 2009). Changes in nutritional quality attributes may arise as a result of degradation of enzymes in the fish muscle, and this degradation process is promoted by enzymes present in the microbial flora (FAO, 2001; FAO, 2005).

Bacterial infestation of fish muscle triggers a continuous breakdown of carbohydrates, nucleotides, amino acids and other non-protein nitrogen (NPN) (Gram & Huss, 2000). This results in the release of volatile compounds such as tri-methyl amine (TMA), aldehydes, ketones, esters, hypoxanthine, and low molecular weight sulphur complexes, which contribute to the characteristic odour of spoiled fish (Gram & Dalgaard, 2002: Triqui & Bouchriti, 2003).

Modified atmospheres packaging (MAP), combined with optimum cold storage, offers the possibility to extend shelf life and maintain quality of food products such as fish and

fishery products, meat, cheese, and fresh or fresh-cut fruit and vegetables (Sivertsvik *et al*., 2002; Caleb *et al*., 2012). MAP can be generated inside a package by the interaction of the natural process of produce respiration and film permeability to attain the desired gas composition over time or by flushing into the package the desired gases (Charles *et al*., 2003; Farber *et al*., 2003). Spoilage of fish and shellfish results from changes caused by three major mechanisms; namely, the breakdown of tissue by own enzymes (autolysis of cells), growth of micro-organisms, and oxidative reactions (Ghaly *et al*., 2010). Modified atmospheres packaging has been used successfully to enhance shelf life of fish by limiting the growth of microbes and lipid oxidation, and is driven largely by the bacteriostatic effects of CO_2. These are evidenced by studies undertaken by Arashisar *et al*. (2004) on Rainbow trout involving high amounts of CO_2, and which showed that high CO_2 delays the growth of aerobic microbes, with 100% CO_2 attaining psychrotrophic microbial count of 10^7 cfu/g on the 10th day and mesophillic microbial count of 10^5 cfu/g on the 14th day of storage. On the other hand, lower amounts of CO_2 (20%) has been used to inhibit microbial growth for Carp also increased shelf life from 3 to 7 days (Jezek & Buchtova, 2007). These studies demonstrate the potential of MAP for extending the shelf life and maintaining postharvest quality of fresh and RTC fish and fishery products.

Problem statement

In South Africa, Cape hake is mainly marketed as frozen or fresh fish due to short shelf life. Despite success in exports to countries such as Spain, Australia and USA trade to such countries is threatened due to quality and shelf life issues (FAO, 2005; DAFF, 2012b). Also research on the application of novel postharvest technologies such as MAP to extend shelf life and maintain postharvest quality of Cape hake in South Africa is limited. Studies using MAP have been restricted to species located in other countries such as Mediterranean hake (Ordonez *et al*., 2000) and European hake (Del Nobile *et al*., 2009). Hence, understanding the effect of MAP and storage conditions on quality of RTC Cape hake will assist the fish industry in selecting relevant packaging parameters for handling the produce.

Rationale and motivation

Modified atmosphere packaging combined with cold storage has been reported to enhance

the shelf life of fresh produce including fishery products (Ruiz-Capillas & Moral, 2001; Pantazi *et al*., 2008; Caleb *et al*., 2012). Furthermore, MAP offers the possibility to inhibit spoilage micro-organisms (Farber *et al*., 2003), and extend shelf life of seafood by several days in contrast to normal air storage (Pantazi *et al*., 2008). In addition, MAP reduces decay and enhances storage life of fresh food without the use of additives and preservatives (Pintado & Malcata, 2000; Torrieri *et al*., 2006). The capability of MAP to enhance storage life of muscle meat, especially seafood, and whey cheese has been reported (Pintado & Malcata, 2000). Although, RTC fresh fish and fishery products offer value addition to the fishing industry food safety should be given due consideration as lack of adherence to good hygienic practices and cross contamination could lead to proliferation of microbial pathogens during processing (Sivertsvik *et al*., 2002; Speranza *et al*., 2009; Mol *et al*., 2014). The use of MAP for various produce has offered value by reducing aerobic bacteria in fishery products significantly, led to better colour profile, better firmness, maintained proximate composition, maintained freshness attributes such as sensory and volatile profiles and extended shelf life (Sivertsvik *et al*., 2002; Farber *et al*., 2003; Speranza *et al*., 2009; Caleb *et al*., 2012; Mol *et al*., 2014).

In contrast MAP offers the possibility of four times the shelf life of air packaging (Pantazi *et al*., 2008; Del Nobile *et al*., 2009; Jezek & Buchtova, 2007). Hence, for the South African fish processing industry to remain competitive and maintain fresh fish attributes, it is important to identify suitable MAP and refrigerated storage parameters to provide shelf stable and safe fresh fish. The aim of this study was to investigate the effects of MAP, absorbent pads and storage temperature on the quality attributes of Cape hake (*Merluccius capensis*) fish fillet. This was achieved by accomplishing the following specific objectives:

a) evaluating the effects of storage temperature and active MAP on quality attributes (microbial, physicochemical and sensory) and shelf life of Cape hake
b) evaluating the effects of MAP and absorbent pads on quality parameters of Cape hake
c) evaluate the effects of storage temperature, absorbent pads and active MAP on changes in volatile composition of Cape hake.

The research chapters in this thesis were written in scientific paper format and hence the duplication of many parts of the materials and methods were inevitable.

References

Ahmed, A.A. (2008). Postharvest losses of fish in developing countries. *Nutrition and Health*, **19**, 273-287.

Arashisar, S., Hisar, O., Kaya, M. & Yanik, T. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. *International Journal of Food Microbiology*, **97**, 209-214.

Bellagha, S., Sahli, A., Farhat, A., Kechaou, N. & Glenza, A. (2007). Studies on salting and drying of sardine (*Sardinella aurita*): Experimental kinetics and modelling. *Journal of Food Engineering*, **78**, 947-952.

Caleb, O.J., Mahajan, P.V., Fahad, A.A. & Opara, U.L. (2012). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences - A review. *Food and Bioprocess Technology*, **6**, 303-329.

Charles, F., Sanchez, J. & Gontard, N. (2003). Active modified atmosphere packaging of fresh fruits and vegetables: Modelling with tomatoes and oxygen absorber. *Journal of Food Science*, **68**, 1736-1742.

Choubert, G. & Baccaunaud, M. (2006). Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss*) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere. *LWT-Food Science and Technology*, **39**, 1203-1213.

De Silva, D.A.M. & Yamao M. (2006). Regional preferences in the Japanese Seafood consumption: An empirical analysis of consumer purchasing behaviour on domestic versus imported seafood. *Japan Journal of the Regional Fisheries Society*, **46**, 83-104.

Del Nobile, M.A., Corbo, M.R., Speranza, B., Sinigaglia, M., Conte, A. & Caroprese, M. (2009). Combined effect of modified atmosphere packaging and active compounds on fresh blue fish burger. *International Journal of Food Microbiology*, **135**, 281-287.

Department of Agriculture, Forestry and Fisheries, DAFF (2012a).Current state of extension and advisory services in South African fisheries. Cape Town.

Department of Agriculture, Forestry and Fisheries, DAFF (2012b). Status of the South African marine fishery resources, 2012. Cape Town.

Domingo, J.L., Bocio, A., Falco, G. & Llobet, J.M. (2007). Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology*, **230**, 219-226.

Dondero, M., Cisternas, F., Carvajal, L. & Simpson, R. (2004). Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. *Food Chemistry*, **87**, 543-550.

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H. & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Review in Food Science and Food Safety*, **2**, 142-160.

Feike. 2008. *Fishinc*. A guide to South African Commercial Fishing Industry.

Food and Agricultural Organisation, FAO (2001). Fisheries and Aquaculture topics. Quality and safety of fish and fish products. Topics Fact Sheets. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 27 September 2001. [Cited 3 June 2013]. http://www.fao.org/fishery/topic/1514/en.

Food and Agricultural Organisation, FAO (2005). Fishery country profile-The republic of South Africa. FID/CP/ZAF, November 2005.

Food and Agricultural Organisation, FAO 2010-2014. Fishery and Aquaculture Country Profiles. South Africa (2010). Country Profile Fact Sheets. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 1 May 2010. [Cited 20 September 2014]. http://www.fao.org/fishery/facp/ZAF/en.

Food and Agricultural Organisation, FAO, (2014). World review of fisheries and aquaculture part 1. http://www.fao.org/docrep/013/i1820e/i1820e.

Ghaly, A.E., Dave, D., Budge, S. & Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques review. *American Journal of Applied Sciences*, **7**, 859-877.

Gram, L. & Huss, H.H. (2000). Fresh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Foods*, edn 1. (edited by B.M. Lund, A.C. Baird-Parker & G.W. Gould). Pp. 472-506. London: Chapman & Hall.

Gram, L. & Dalgaard, P. (2002). Fish spoilage bacteria: problems and solutions. *Current Opinion in Biotechnology*. **13**, 262-266.

Jezek, F. & Buchtova, H. (2007). Physical and chemical changes in fresh chilled muscle tissue of common carp (*Cyprinus carpio L.*) packed in a modified atmosphere. *Acta Veterinaria Brno*, **76**, *83-92.*

Jezek, F. (2012). Effect of Modified atmosphere packaging on the course of physical & chemical changes in chilled muscle of silver carp. (*Hypophthalmichthys molitrix*). *Polish Journal of Veterinary Science*, **15**, 439-445.

Mol, S., Ucok Alakavuk, D. & Ulusoy, S. (2014). Effects of modified atmosphere packaging on some quality attributes of a ready-to-eat salmon sushi. *Iranian Journal of Fisheries Sciences*, 13, 394-406.

Ocano-Higuerra, V.M., Maeda-Martinez, A.N., Lugo-Sanchez, M.E. & Pacheco-Aguilar, R. (2006). The effect of transportation on the physiological condition and free amino acid composition of *Nodipecten subnoddosus* adductor muscle. *Journal of Shellfish Research,* **25**(1), 297-298. C/O Dr. S E. Shumway, University of Connecticut, 1080 Shennecossett road, Groton, CT 06340 USA: Nationall Shell Fisheries Association.

Opara, U. L., Al-Jufaili, S.M. & Rahman, S.M. (2009). Postharvest handling and preservation of fresh fish and seafood. In: *Handbook of food preservation*. (edited by M. S. Rahman). Pp. 152-172. Boca Raton: CRC Press.

Ordonez, J.A., Lopez-Galvez, D.E., Fernandez, M., Hierro, E. & de la Hoz, L. (2000). Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, **80**, 1831-1840.

Pantazi, D., Papavergou, A., Pournis, N., Kontominas, M.G. & Sawaidis I.N. (2008). Shelf-life of chilled fresh Mediterranean swordfish (*Xiphias gladius*) stored under various packaging conditions: microbiological, biochemical and sensory attributes. *Food Microbiology,* **25**, 136-143.

Pintado, M.E. & Malcata, F.X. (2000). The effect of modified atmosphere packaging on the microbial ecology in Roqueijao- A Portuguese whey cheese. *Journal of Food Processing and Preservation* **24**, 107-124.

Ruckes, E. (2003). World fish trade, demand, forecasts and regulatory framework. The Expert Consultation on International Fish Trade, Rio de Janeiro, Brazil 3-5 December.

Ruiz-Capillas, C & Moral, A (2001). Chilled bulk storage of gutted hake (*Merluccius merluccius*) in carbon dioxide and oxygen enriched controlled atmospheres. *Food Chemistry* **74**, 317-325.

Sivertsvik, M. Jeksrud, W.K. & Rosnes, J.T. (2002). A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology*, **37**, 107-127.

Speranza, B., Corbo, M., Conte, A., Sinigaglia, M. & Del Nobile, M. (2009). Microbiological and sensorial quality assessment of ready-to-cook seafood products packaged under modified atmosphere. *Journal of Food Science*, **74**, M473-M478.

Torrieri, E., Cavella, S., Villani, F. & Masi, P. (2006). Influence of modified atmosphere packaging on the chilled shelf-life of gutted farmed bass (*Dicentrarchus labrax*). *Journal of Food Engineering*, **77**, 1078–1086.

Triqui, R. & Bouchriti, N. (2003). Freshness assessments of Moroccan sardine (*Sardina pilchardus*): Comparison of overall sensory changes to instrumentally derived volatiles. *Journal of Agricultural and Food Chemistry*, **51**, 7540-7546.

Trondsen, T., Scholderer, J., Lund, E. & Eggen. A.E. (2003). Perceived barriers to consumption of fish among Norwegian women. *Appetite*, **41**, 301–314.

CHAPTER 2

LITERATURE REVIEW ON MODIFIED ATMOSPHERE PACKAGING OF FISH AND FISHERY PRODUCTS

Background

According to FAO (2014), postharvest loss in fish due to decay is estimated to be about 10 – 12 million tonnes annually, representing approximately 10% of global capture and cultured fish. The major factors that escalate the incidence of losses include non-adherence to good management practices (GMP), inefficient postharvest handling practices, poor packaging and limited application of cold chain technologies to extend storage and shelf life of fresh produce (Godfray *et al*., 2010; Opara, 2010; Opara & Mditshwa, 2013).

Fish spoilage occurs through autolysis of fish tissue, bacterial infestation and oxidation (Ghaly *et al*., 2010). The use of efficient packaging minimises fish decay and reduces wastage (Verghese *et al*., 2013; Opara & Mditshwa, 2013), and offers a number of other benefits such as improved fish distribution, enhanced safety and shelf life of fresh ready-to-cook fish and fishery products (Triqui & Bouchriti, 2003; Speranza *et al*., 2009). Reduction of postharvest losses through the application of novel packaging has led to increased sales of pre-packaged fish (Verghese *et al*., 2013). Additionally, improved fish packaging systems have allowed food processors to respond and satisfy global changes in consumer lifestyles and the increased demand for nutritional and healthy food products (De Silva & Yamao, 2006; Speranza *et al*., 2009). Packaging systems used in fish handling include normal air/overwrap packaging which are used for short term packaging and vending, vacuum packaging, bulk-gas flushing, and modified atmospheres packaging (MAP) (Kerry *et al*., 2006). An example of normal air packaging involves sealing food with nylon overwrap in normal gas concentration of air (78% nitrogen**,** 21% oxygen, 0.01% of carbon dioxide). This system has a lot of disadvantages because it does not provide good barrier to moisture, oxygen or light, and hence, accelerates spoilage of the fresh produce (Sivertsvik *et al*., 2002). In vacuum packaging, the food product is packaged in a container with reduced oxygen permeability and the air is completely displaced before the container is made airtight (Oguzhan *et al*., 2013). Bulk packaging with gas flushing involves the use of 100% CO_2 packed in ice for extended periods (Kerry *et al*., 2006).

Modified atmospheres packaging is an active or passive dynamic process of changing the normal gas composition (0.039% CO_2 + 20.95% O_2 + 78% N_2) inside a package to desirable

equilibrium levels of CO_2 and O_2 (Mastromatteo *et al*., 2010; Mahajan *et al*., 2014), thereby maintaining the freshness of perishable products (Ghaly *et al*., 2010; Masniyom, 2011). Modified atmosphere packaging is widely used for a variety of products but the optimum gas blend depends on package film permeability, product physiology and storage temperature (Caleb *et al*., 2013; Wani *et al*., 2014). Since dead fish does not respire, knowledge of the interplay between the film package and the respiration of micro-biota is vital. Carbon dioxide has been established to have bacteriostatic and fungistatic properties (Arashisar *et al*., 2004) and is used in reducing the respiration of micro-biota, thereby enhancing the storage life of fish product (Choubert & Baccaunaud, 2006; Yesudhason *et al*., 2014). A review on MAP and shelf life of fish and fishery products was published by Sivertsvik *et al*. (2002), focusing on the significance of microbial growth and activities and product safety. The objectives of the current review are to discuss the effects of MAP on quality and shelf life of fish and fishery products under MAP, including the microbiological hazards associated with MA-packaged fresh fish.

Functions of packaging

Packaging plays a vital role in industrial growth as evidenced by its global market worth of about US$530 million, comprised of food (41%), beverages (14%), pharmaceuticals (4%), industry and transport (21%), cosmetics (3%) and others (17%) (WPO, 2008; Muncke, 2009; Opara & Mditshwa, 2013). End user packaging follows this trend, with wrapping and paper (38%), film (18%) and elastic plastics (12%), metallic substances (19%), glass (8%) and extra items (5%) (WPO, 2008; Muncke, 2009). Packaging materials account for about 15% of total variable costs in the food packaging industry (Esse, 2002).

Food packaging has been described as a procedure which manages food safely for distribution so as to ensure that quality food is supplied to the end user (Coles, 2003). The primary functions of food packaging are protection, containment and communication, while the secondary roles include tracking, providing suitability and interference indicators (Yam *et al*., 2005; Brody, 2008). The goal is to ensure that food products are presented in a cost efficient way to meet both industrial requirement and satisfy consumer demand for fresh quality, balanced with food safety increasing demand for sustainability (Mangaraj *et al*., 2009). The protection function has been described as safety from biological, chemical or physical outer action, slowing down of product decline and enhancing storage life of the product (Robertson, 2010).

Biological safety is achieved when the package hinders microbes, insects, rodents and other animals thus averting decay and disease outbreaks. Secondly, it enables product consistency (Marsh & Bugusu, 2007). Chemical safety is achieved when the packaged product structure is restrained from contact with moisture, light or gases by altering the environment or by using films that have the desired absorbance properties. Restraining packages include glass and metal which completely restrains and plastics, which have selective restraining abilities such as toughness, porosity to O_2, CO_2 or water vapour and flexibility. In addition, these packages weigh less, are reusable and budget friendly, and are equipped with closure devices such as tin foils to ease filling and emptying (Robertson, 2010). Physical safety is achieved through defence from knocks and impacts when the product is being delivered. The physical obstacles such as cardboard act as defensive mechanisms to forestall product damage during shipping and long distance delivery.

Product containment via packaging helps to prevent waste due to leakages, thereby ensuring that the product cannot be consciously discarded. Food wastage can be reduced through the use of packaging which provides adequate containment (Brody *et al*., 2008). A product can be contained in three levels of packaging, namely primary, secondary and tertiary packaging, which together, facilitate transport and distribution. A primary package is one which the product is in contact with the package e.g. glass bottles, metal cans, plastic pouches and trays which eases the transportation of products while a secondary package indicates that the package contains a number of primary packages e.g. a corrugated case which serves as distribution mover which is designed for use in retail outlets for displaying primary packages however, a tertiary package is one that contains a number of secondary packages e.g. a shipping container that contains a number of secondary corrugated cases which are transported on ships (Coles, 2003). Furthermore packaging communicates or disseminates marketing and nutritional information, thereby enhancing product presentation (Marsh & Bugusu, 2007). A package serves as the face of the product and it functions in this capacity as it is the only visible part of the product, end users rely on this information in order to decide whether to purchase a product or not. The goal of communication is to influence end user decisions. Consequently, products have been branded to increase end user appeal, boost sales and provide information (Kotler & Keller, 2006).

The extended functions of communication include tracking. This provides adequate information about the source of food and enables monitoring as it moves from harvesting, handling and delivery to end-users (Folinas *et al*., 2006). Golan *et al*. (2004) suggested that tracking aims atenabling track-back of food sources for safety and wholesomeness checks, branding and selling of foods with refined values and enhanced delivery. Tracking is

achieved through signs tagged on the product such as barcodes and radio frequency identification, therefore, the source and channel which a particular decayed product passed through can be identified and necessary steps can be taken to ensure that hazards are averted (Marsh & Bugusu, 2007). Tracking also functions as an anti-theft tool and helps in stock taking (Ozdemir & Floros, 2004).

Other innovative roles of packaging include suitability and interference proofs. Package suitability takes into consideration consumer convenience and strikes a balance with environmental sustainability (Brody *et al*., 2008). It offers end users the choice of microwave safe packages, re-sealable pouches and dispensing regulators which are user friendly. These add value and reduce postharvest losses (Brody *et al*., 2001). In recent times however, end-user demands has resulted in packaging which present wholesome ready-to-cook (RTC), fresh foods which are have satisfied criteria of suitability and enhanced storage life (Lagaron *et al*., 2004).

Interference indicators are tools that have been tagged on packages to deter deliberate interference with products. Indicators alert the end-user of the possibility of loss of freshness if the product has been tampered with although this cannot stop packages from being interfered with (Marsh & Bugusu, 2007). These indicators include banding, holograms, special membranes or breakaway closures. Currently, some of the advantages in active packaging such as vocal recordings, touch sensors and odour release are used as indicators of interference in food packages (Landau, 2007).

Food packaging materials

Storage life of a product is influenced by the packaging material that encloses it and the package is chosen based on the premise that it retains the product value (Marsh & Bugusu, 2007). Items such as glass, metals (tin, aluminium, foils, laminates), plastics and cartons have long been used but advances have seen the introduction of items which match function and aesthetics (Brody *et al*., 2008). In the U.S. items used in packaging are regulated by the Food and Drugs Administration (FDA) under section 409 of the federal Food, Drug, and Cosmetic Act and are referred to as ‘food contact substances’. Similarly they are also designated as ‘food contact substances’ in South Africa and Europe and are regulated by Department of Health (DoH), South Africa and stipulated under framework regulation EC 231/2012 in Europe (EC, 2012).

In packaging of fresh fish and fishery products, the requirements are such that the package must lower oxidation of fats, lead to decreased vapour losses, exclude drips and afford fewer chemical and microbial deterioration opportunities. This places a restriction on the use of some of the food contact substances (Byett *et al*., 2002a). For instance glass allows fat oxidation therefore it cannot be used for packaging fresh fish (Byett *et al*., 2002a). Although paper and paperboard products are a cheap option in the packaging of RTC fish its use is limited due to; deep concern over damage of forests in several countries, challenges on disposal of toxic substances generated during bleaching process, high water usage and pollution of water bodies through wastes generated from paper and paperboard mills, and finally its use in packaging is considered a waste of resources (Kirwan, 2003). In the case of metals, the limitations of chemical deterioration opportunities have been alleviated by coating tin free steel (TFS) with chromium and laminating it with poly ethylene terephthalate (PET). This packaging material does not allow contact between the RTC fish and the metal (Gupta & Nagar, 2010). The studies on the suitability of TFS in the packaging of RTC fish under heat treatment indicated that, its water retention ability was 500mL, it could tolerate an internal air pressure of 30 psi for 15 seconds and did not get distended or leak via its dual seam region The study also stated that at temperatures of 121.1°C and pressures of 15 psi normally used in RTC processing there was no deformation or overpressure of cans when processed (Pushparajan *et al*., 2013).

The flexibility of plastics makes them highly useful in the packaging of food because they are easy to impress images on, weigh less, are tough, not costly and can be heat sealed (Byett *et al*., 2002b). Several plastics such as polyolefin, polyester, polyvinyl chloride, polyvinyl-diene-chloride, polystyrene, polyamide, and ethylene vinyl alcohol have been used in food packaging based on their properties the frequently used ones are polyolefin and polyesters (Lau & Wong, 2000; Abdel-Barry, 2003). Plastics can be used in the form of laminates and co-extrusions and the process involves blending two or more films for lamination or molten films for co-extrusion (Hernandez *et al*., 2000). Table 1 summarises the properties of key polymeric films taking into consideration the benefits and limitations.

Table 1 Properties of key polymeric films used in MAP

Polymeric films	**Characteristics**	
	Benefits	**Limitations**
Ethylene vinyl acetate (EVA)	Exceptional clarity Heat-sealable Very good pasting attributes	Poor gas barrier Poor moisture barrier
Ethylene vinyl alcohol (EVOH)	Serves as a good oxygen barrier Exceptional barrier to gases and odour	Moisture sensitive barrier
Polyamide (nylon-6)	Performs well at high temperature Resilient Highly resistant to chemicals Moderate oxygen barrier, excellent odour and flavour barrier Mechanical and thermal properties compares with PET	Poor water vapour barrier
Polyvinyl chloride (PVC)	Heat-sealable Strong and transparent Highly resistant to chemicals, greases and oils Good barrier to gases but moderately bars water vapour	
Polyethylene	Good moisture barrier Heat-sealable Permeable to gases Chemically resistant Durable and flexible Performs well at low temperatures	LLDPE; heat sensitive HDPE has poor transparency
Polypropylene	Responds well to heat sealing Exceptional grease resistance Harder, denser and more translucent than polyethylene Higher gas and water vapour barrier Highly chemically resistant	
Polyvinylidene chloride (PVDC)	Good resistance to greases and chemicals Heat-sealable Beneficial in hot filling, and low temperature storage Less permeable to gases, water vapour and odours	Low permeability barrier
Polystyrene	Exceptionally translucent High tensile strength	Poor barrier to gas and water vapour
Polyesters (PET/PEN)	Highly resistant to degradation by heat, mineral oil and chemicals Displays exceptionally durable and mechanical properties Exceptionally translucent Suitable barrier to gases, water vapour and odours	

Source: (Page *et al*., 2003; Marsh & Bugusu 2007; Mangaraj *et al*., 2009; Caleb *et al*., 2012)

Polyesters are polymers condensed from the ester monomers and the available forms are polyethylene terephthalate (PET), polycarbonate, and polyethylene naphthalate (PEN). Polyethylene terephthalate is used to generate packaging for fizzy drinks (Van-Willige *et al*., 2002), beverages and bottled water. In the food industry, apart from being used to generate containers, PET is used to make semi-rigid sheets for thermoform trays and thin-oriented films (Galdi *et al.*, 2008). The melting point of PET is 260°C and it does not shrink beneath 100°C; therefore, it is valuable for use in 'steam sterilisation, microwave cooking or radiation ovens'. Its use in bottling mineral waters and fizzy drinks makes it a valuable plastic. Polycarbonates however, are generated by polymerization of a sodium salt of bisphenol acid with carbonyl dichloride. Polycarbonates, are used in food, medical and skin packaging although, they constitute a latent health threat because they catalyse the release of bisphenol A (Marsh & Bugusu, 2007). Polycarbonates are not used in fish packaging due to their latent health threats. Polyethylene naphthalate is generated from a combination of dimethyl naphthalene di-carboxylate and ethylene glycol polymers; it has high glass transition temperature, it greatly hinders CO_2, O_2 and moisture in contrast to PET and functions better at elevated heating conditions. Additionally, it is used in making beer bottles due to its odour and flavour rescinding features although it is four times more expensive than PET (Kirwan & Strawbridge, 2003; Marsh & Bugusu, 2007). Polyethylene naphthalate is used in refillable alcoholic and non-alcoholic beverages, sterilised infant feeders, sport drinks, juices and dry foods in elastic packages. Its relevance in the packaging of food has been restricted because it is more costly than PET (Kirwan & Strawbridge, 2003).

Deterioration of fish and fishery product

About one quarter of farming and fishery produce is lost annually as a result of biochemical decline and deterioration (Baird-Parker, 2000). Deterioration in fresh fish has been categorised into sensory, microbial, physical and chemical action (Triqui & Bouchriti, 2003; Pereira de Abreu *et al*., 2010; Amos 2007) reported that approximately 30% of 'landed' fisheries are lost through the action of deterioration micro-organisms. The mechanism of fresh fish deterioration in air has been outlined through the following steps. The micro-bacteria present in the fish's ecosystem initiate degradation of amino acids and non-protein nitrogen. This further initiates preferential development of microbes that aggressively cleave amino acids through oxidation. This is followed by control of protein enzyme release by *Pseudomonas* spp. These microbes then hydrolyse protein through amino acid conscription

on the fish muscle, resulting in the biosynthesis of ammonia and volatile fatty acids; which leads to the generation of sulphurous metabolites (Sivertsvik *et al*., 2002).

Degradation of fresh fish occurs swiftly and the course of decay (rigor mortis) commences barely a few hours after fish is landed at room temperatures especially in tropical environments (Berkel *et al*., 2004). Rigor mortis is an activity that leads to a loss of fish's elastic nature as a result of toughening of the tissues of muscle meat which occurs after a few hours of its demise (FAO, 2001). It has been observed that in the course of fish degradation, several constituents in the fish muscle are broken down and different compounds are such as tri-methyl amines (TMA), thio-barbituric acid reactive substances (TBARs), total volatile base nitrogen (TVBN), hypoxanthine and biogenic amines are generated. This newly generated compounds accounts for aroma, taste and consistency modification in fish tissue (Ghaly *et al*., 2010).

Decay

The factors that influence the action and growth of decay microbes are the state of the fish and conditions such as $a_{w,}$ atmosphere, pH, temperature and interactions amongst the microbes. Some patterns of such collaborative activities among fishes are antagonism, metabiosis and cell to cell communication according to Gram *et al*. (2002). Antagonism is instigated by the desire for iron facilitated by 'siderophore production' and the dominance of less aggressive microbes. Metabiosis involves a modification of microbial decay protocol due to nutrient provision from other microbes. Cell to cell communication is typified by Gram-negative bacteria's capacity to synchronise the articulation of some 'phenotypic attributes' by interacting with other microbes (Gram *et al*., 2002).

Fish decays through three fundamental ways, namely, autolysis of fish tissue by its own enzyme, bacterial progression, and oxidation (Ghaly *et al*., 2010). Enzymes degeneration in the flesh of fresh fish consistency initiates decline in fish quality, but this does not lead to the release of faecal stinky aromas (FAO, 2005). This initial breakdown puts a constraint on the storage potential and wholesomeness of fresh fish even when only small amounts of bacteria are present (FAO, 2005). Subsequently, enzymes located in the gut of the fish cause a wide range of changes such as spongy tissues, bursting of the abdomen and depletion of body fluids (FAO, 2001). Studies have shown that enzymes degradation of protein promotes sensory decline and changes during postharvest handling of fresh fish (Engvang & Nielsen,

2001). Furthermore, unsuitable postharvest handling of fresh fish leads to break down of proteins by protein enzymes (cathepsins).

The autolysis of fish muscle proteins yields peptides and free amino acids which can also promote deterioration of fresh fish flesh due to the presence of microorganisms and generation of non-volatile low molecular weight organic compounds known as biogenic amines (FAO, 2005). During autolysis of fresh fish the fish substrate is degraded and this has been described briefly as: glycolytic enzymes degrading glycogen to generate lactic acid which leads to a decrease in pH, nucleotide enzymes degrade adenosine tri phosphate (ATP), adenosine di phosphate (ADP), adenosine mono phosphate (AMP), inosine and inosine - mono- phosphate (IMP) which generates hypoxanthine, chymotrypsin, trypsin, carboxy-peptidases degrade peptides and proteins to induce belly bursting, calpain degrades myofibrillar proteins to soften fish, collagenases degrade connective tissue to induce tissue softening and gaping, and tri-methylamine oxide (TMAO) demethylase degrades TMAO to tri-methyl amine (TMA) to generate formaldehyde (FAO, 2005; Ghaly *et al*., 2010).

Another index that triggers fresh fish decay during storage is lipid oxidation (Ghaly *et al*., 2010). Lipid oxidation occurs due to elevated levels of polyunsaturated fatty acids in fish and this leads to a decline in freshness (Munasinghe *et al*., 2005; Kristinsson *et al*., 2006). Lipid oxidation process involves the action of free radicals and occurs in three steps namely initiation, propagation and termination. The initiation process entails generation of lipid free radicals by the catalytic action of metallic ions, heat and the subsequent reaction with oxygen to yield peroxy radicals. During the propagation step, peroxy radicals bond with other lipids to yield hydro-peroxides while the termination step entails numerous free radicals bonding to form non-radicals (Monahan, 2000; Zhong *et al*., 2007; Ghaly *et al*., 2010; Dave & Ghaly, 2011). Lipid oxidation is categorized into two groups namely enzymatic and non-enzymatic (FAO, 2005; Ghaly *et al*., 2010). Enzymatic oxidation occurs when lipid oxidising enzymes (namely lipases) aid in fragmenting glycerides to yield fatty acids which accounts for the sourness in decaying and decayed fish (Ghaly *et al*., 2010). Non-enzymatic lipid oxidation of fish involves the generation of hydro-peroxides from haematin complexes. Studies have shown that, deoxygenated haemoglobin in its enhanced oxidative state facilitates lipid oxidation in herrings (Undeland *et al*., 2005). Furthermore, when metabolites of lipid oxidation and protein react a yellow pigment is formed which indicates decayed fish (Masniyom, 2011). Thanonkaew *et al*. (2006) suggested that sourness leads to altered colours in *Sepia pharaonis* when frozen. Other studies on *Lates calcarifer*, *Oreochromis mossambicus* and *O*. *niloticus* indicated that, 'off-odour' were also associated with lipid oxidation when stored in ice for 15 days (Thiansilakul *et al*., 2010). Aryee *et al*.

(2007) stated that enzymes present in the fish muscle such as phospholipase and tri-acyl lipase and microbes initiate breakdown of lipids in stored fish. Other enzymes such as lipoxygenase and peroxidase oxygenate and convert lipids to hydro-peroxides which lead to auto-oxidation of fatty acids (Masniyom, 2011). Additionally, lipolysis is also influenced by O_2, light strength, temperature and water activity. For instance, high O_2 concentration serves to intensify rancidity which leads to fishy and off-odours (Chaijan *et al*., 2006).

Microbial infestation is another source of decay evidence of which is slimy clusters, modifications in texture, off-odours and off-flavours (Gram *et al.*, 2002). Studies have shown that habitat, natural microbial colony, postharvest handling methods and storage conditions determines rate of fish decay (Sivertsvik *et al*., 2002; Masniyom, 2011). It has however being noted that not all microbes cause decay and only a limited number cause unpleasant off-odours and lead to decay in fish and fishery products and these microbes are termed specific spoilage organisms (SSOs) (Gram & Dalgaard, 2002). Several microbes have limited ability to endure harsh environmental conditions, hence a microbial dynamics occur around the fish product. In fishes that are not well preserved, Gram-negative bacteria such as *Vibrio* spp. that are prone to ferment fish tissues cause decay. In chilled fishes, Gram-negative bacteria that survive low temperature such as *Pseudomonas* spp. and *Shewanella* spp. cause decay (Gram & Huss, 2000). For fishes living in tropical marine habitats the main decay organisms are *Bacillus*, *Brochothrix thermosphacta*, *Clostridium*, *Cornynebacterium*, *Micrococcus*, *Staphylococcus* spp. and *Streptococcus* (Al Bulushi *et al*., 2010). While in temperate aquatic habitats, psychrotrophic, aerobic or facultative anaerobic Gram-negative bacteria such as *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Moraxella*, *Pseudomonas*, *Shewanella putrefaciens*, and *Vibrio* are the main decay organisms (Sivertsvik *et al*., 2002).

An in depth understanding of the microbial ecology, SSOs and decay metabolites in fish is thus important in order to minimise losses after fish has been landed (Gram *et al*., 2002). This would lead to the development of postharvest handling and storage techniques suitable for fish and fishery products. For instance, packaging in elevated amounts of CO_2 inhibits aerobic bacteria that affect the gills but does not hinder lactic acid bacteria (LAB) and *Photobacterium phosphoreum* (Dalgaard, 2000). Salting combined with refrigerated vacuum storage of smoked fish leads to hindrance of respiratory Gram-negative bacteria (Jorgensen *et al*., 2000). However, this condition favours LAB (*Lactobacillus* and *Carnobacterium)*, Gram-negative bacteria such as *P*. *phosphoreum* and psychtrotrophic enterobacteriaceae that are prone to fermentation (Jorgensen *et al*., 2000; Gram & Dalgaard, 2002).

Fish contains high amounts of amino acids, tri-methyl amine oxide (TMAO) and small amounts of carbohydrates (Masniyom, 2011) Specific spoilage organisms in fish and fishery products initiate microbial decay resulting in the formation of metabolites such as ammonia, volatile bases, hypoxanthine, organic acids and biogenic amines (Jorgensen *et al*., 2000). Additionally, tri-methyl amine (TMA) is generated by anaerobic microbes that can reduce TMAO (Joffraud *et al*., 2001). In fresh water, fish use TMAO to log water into their tissues while marine fishes use it to control osmotic pressure while the micro-biota around the fish use the TMA generated to acquire energy (Gram & Dalgaard, 2002). TMA has been established as the fishy or ammonia like smell in decayed fish and is used as a microbial index in the decline of fish freshness (Sivertsvik *et al*., 2002). Specific spoilage organisms that can lead to a reduction of TMAO to TMA include microbes such as *Aeromonas* spp., *Shewanella putrefaciens*, *P. phosphoreum*, psychro-tolerant Enterobacteriaceae and *Vibrio* spp. (Gram & Dalgaard, 2002).

Several indices applied to detect decay in fresh fish involve decay metabolites which measure total volatile base nitrogen (TVBN), TMA, hypoxanthine, biogenic amines and quotients linking metabolites of adenosine tri-phosphate (ATP), referred to as K values (Dalgaard, 2000). K value is a bio-chemical index that measures fish freshness based on nucleotide catabolites formed during the degradation of phosphorylated compounds such as ATP, ADP, AMP, inosine and IMP by nucleotide enzymes present in fish tissues (Ghaly *et al*., 2010). The K-value is quantified as the percentage (%) of the ratio of the sum of hypoxanthine (Hyp) and inosine (Ino) to the total amount of nucleotides (Verachia *et al*., 2013):

K= [Ino + Hyp/ (ATP + ADP + AMP + IMP + Ino + Hyp)] x100

The K value is however not a suitable quality indicator for fish freshness in MA packaged fish and fishery products (Ordonez *et al*., 2000; Yesudhason *et al*., 2009).

Degradation of nutrients

Nutrients in fish flesh are dependent on flesh variety, reproduction type and species however, they consists of nutrients such as protein, lipid, water, carbohydrate, mineral, organic compounds, and nucleic acids which makes it prone to spontaneous decay when compared to animal flesh (Masniyom, 2011). Studies have shown that specific proteinases are responsible for breakdown of fish muscle including cathepsins D, B and L which cause flesh pliability, carboxy-peptidases and trypsin which cause abdominal rupture, the calpains

which stimulates molt-pliability in crustaceans and collagenases which cause gaping of fillets (FAO, 2001; Godisken *et al*., 2009). Collagen breakdown has been linked to modification of seafood muscle consistency for cod and prawn (Hernandez-Herrero *et al*., 2003; Sriket *et al*., 2010). In lipid breakdown the vast amounts of poly unsaturated fatty acids, organic compounds containing haemoglobin and mineral ions are oxidised after fish and fishery products are landed. The breakdown products hydro-peroxides are known to generate alcohols and organic compounds with few carbon atoms which result in the alteration of nutritional and physical attributes such as firmness, taste, aroma and colour of the fish (Masniyom, 2011). Unpleasant smells have been observed in ice stored sea bass and red tilapia after 15 days of storage due to lipid breakdown (Thiansilakul *et al*., 2010).

Microbial ecology

Factors leading to microbial proliferation in fish and fishery products may be classified into natural fish attributes and attributes of the fish habitat (Gram & Dalgaard, 2002). Literature evidence suggests that not all microbial growth on fish leads to unacceptable fish attributes and only a few are linked with the bulk of decay in fresh fish and these are termed specific spoilage organisms (SSOs). The microbes linked with decay in fresh fish are detailed within Table 2.

Table 2 Decay microbes that acts as SSOs in fresh fish

Decay microbe	Characteristics	Associated volatile compounds	What they produce	Target fish type	Reference
Pseudomonas spp.	Gram-negative rods, aerobic have motile polar flagella, are oxidase-positive, catalase-positive and obligate respiratory microbes	Psychrophilic, species-specific typified by fruity, oniony and faecal aromas because they produce ketones, aldehydes, esters and hydrogen sulphide compounds that do not contain sulphur such as methyl sulphide	Pigments, lipolytic and proteolytic enzymes that alter fresh and frozen fish quality	Iced freshwater fish	Gram *et al*., 2002; Vogel *et al*., 2005; Arias, 2009; Dave & Ghaly 2011
Shewanella putrefaciens	Gram-negative, facultative anaerobes are motile, micro-aerophiles or anaerobes, are ferric-reductase, grows in the absence of oxygen using alternative terminal electron acceptors (ATECs)	Fishy off and sulphide like aroma because they produce TMA, H_2S, CH_3SH, $(CH3)_2S$, hypoxanthine	They reduce TMAO to TMA, cysteine generates H_2S, methionine generates CH_3SH and $(CH3)_2S$, inosine generates hypoxanthine and other compounds attributed to this species to induce decay	Iced temperate-water marine fish species	Vogel *et al*., 2005; Arias, 2009; Ghaly *et al*., 2010; Dave & Ghaly 2011
Photobacterium phosphoreum	Gram-negative, have motile polar rods are psychrophilic and chemoorganotrophic, they grow in anaerobic conditions, and emits a blue-green light at 490nm	Fishy-off aroma because they produce TMA and hypoxanthine	They reduce TMAO to TMA, inosine generates hypoxanthine and other compounds attributed to this species to induce decay	Iced temperate-water marine fish species	Gram & Dalgaard, 2002; Arias, 2009; Dave & Ghaly 2011
Brochothrix thermosphacta	Gram-positive facultative anaerobe, it is psychrophilic and salt tolerant	Cheesy and sour off-odours because they generate acetoin-di-acetyl and 3-methylbutanols	Acetoin-di-acetyl and 3-methylbutanol generated induces decay	Psychrophilic spoilage organism of meat but growing evidence in MAP spoilage of fish	Gram & Dalgaard, 2002; Arias, 2009; Dave & Ghaly 2011; Gribble & Brightwell 2013

Overview of fish packaging

Food production in industrialised and non-industrialised nations suffers loss and wastes of about 30% to 40%, although non-industrialised nations incur more of these losses (Godfray *et al*., 2010; Opara & Mditshwa, 2013). The major factors that contribute to losses in non-industrialised nations are non-adherence to good management practices (GMP), and limited postharvest expertise and refrigeration structures (Opara & Mditshwa, 2013). Landed fish that has decayed and cannot be eaten has been classified into those which are lost due to inadequate postharvest handling practices and wasted fish, which occur in the course of distribution, selling and household consumption (Parfitt *et al*., 2010). Efficient packaging that addresses these three areas of fish decay will reduce waste of fish (Verghese *et al*., 2013). Fish packaging systems have led to a number of benefits such as improved fish distribution that enhances safety and shelf life for fresh RTC fish and fishery products (Triqui & Bouchriti, 2003; Speranza *et al*., 2009). Fish packaging system also aids in salvaging excess landings this leads to increased sales of pre-packaged fish and implementation of novel packaging expertise (Verghese *et al*., 2013). Furthermore, intelligent packaging uses tools which monitor and provide information on freshness attributes, temperature abuse and gas leaks, thus decreasing fish waste (Yam *et al*., 2005; Restuccia *et al*., 2010).

Normal air packaging involves packaging food in a normal gas concentration of 78% nitrogen**,** 21% oxygen, 0.01% of carbon dioxide - with traces of water vapour, argon, and other components present in air, sealing with nylon overwrap. This system has a lot of disadvantages because the overwrap package does not serve as a good hindrance to moisture, oxygen or light and accelerates spoilage (Sivertsvik *et al*., 2002). In vacuum packaging the food is packaged in a container with reduced oxygen penetration ability and the air is completely displaced before the container is made airtight (Oguzhan *et al*., 2013). Bulk gas flushing are systems that involve use of 100% CO_2 gas volumes in frozen states for extended periods (Kerry *et al*., 2006).

Modified atmosphere packaging allows the freshness of ‘perishing products’ to be enhanced by reducing the product’s natural deterioration pattern such as autolysis of fish tissue by its enzyme, bacterial progression, and oxidation (Ghaly *et al*., 2010; Masniyom, 2011). Various products have been packaged with modified atmosphers but the gas blend is subject to the film package used, product kind and temperature at which it is stored. Since dead fish do not respire the interface of the film package with the respiration of micro-biota is vital.

Fundamental principle of MAP

Modified atmospheres packaging is an active or passive dynamic process of changing the normal gas composition (0.039% CO_2 + 20.95% O_2 + 78% N_2) inside a package to desirable equilibrium levels of CO_2 and O_2 (Mastromatteo *et al*., 2010; Mahajan *et al*., 2014). Passive-MAP is generated in-situ relying on the interplay of film permeability and fresh produce physiological processes (mainly respiration) in order to change the original atmospheric gas composition inside the package (Mangaraj *et al*., 2009; Sandhya, 2010). On the other hand, active-MAP is achieved by the exclusion or addition of desired gas component(s) or by the use of substances that attract or remove known gas component/s to achieve desired gas composition levels in the package containing the produce (Velu *et al*., 2013). Equilibrium MAP is obtained when product respiration rate equals the diffusion rate of gases (Mangaraj *et al*., 2009). Factors affecting the success MAP are summarized in Fig. 1.

MAP has been applied to harvested fish which do not respire, and plays a role in maintaining the freshness, nutritional and microbial quality of fresh fish. It reduces the respiration rate of microbes, thereby slowing their growth and delays tissue enzymatic decay (Sivertsvik *et al.*, 2002; Masniyom, 2011).

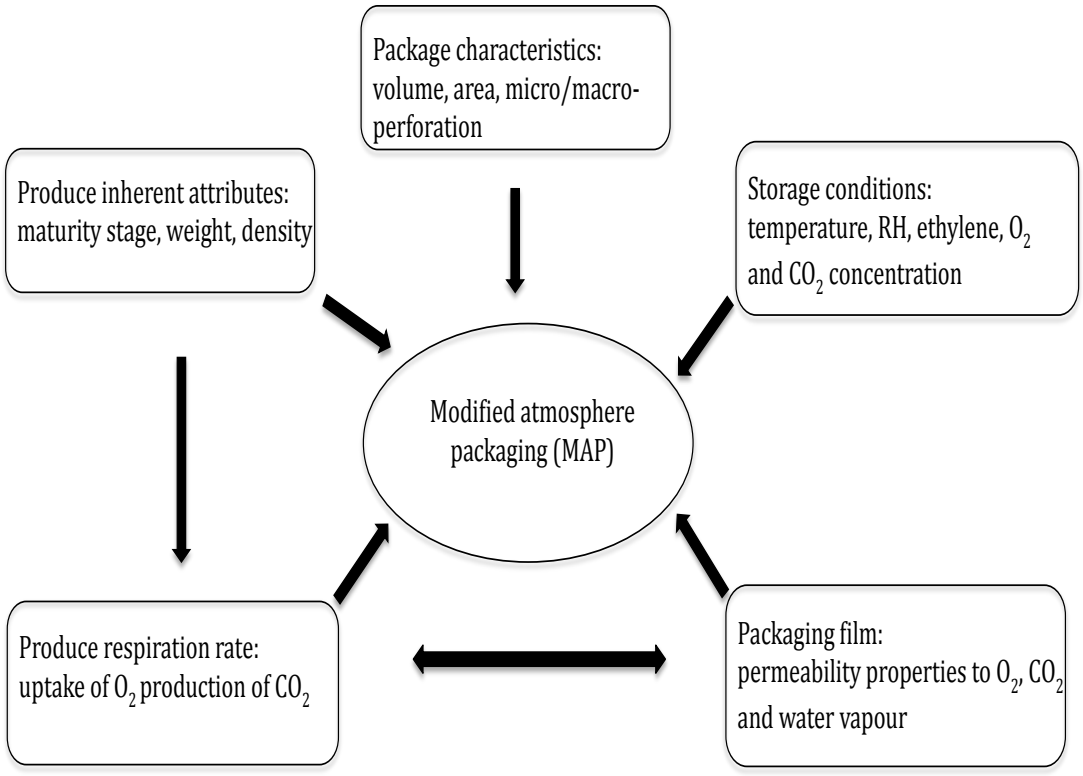

Figure. 1 Factors influencing the success of modified atmosphere packaging design (Adapted from Mahajan *et al*., 2014)

Role of gases used in MAP of fish

Common gases used in MAP of fresh fish and fish products include oxygen, nitrogen, carbon dioxide, carbon monoxide and noble gases (Gumus *et al*., 2011; Masniyom, 2011; Jezek and Buchtova, 2007). In MAP of fish and fish products, the product type and packaging material play a very important role in determining the blend of gases used (McMillin, 2008). For fatty fish such as salmon, trout and tuna, higher levels of CO_2 (> 70%) are used to take advantage of its bacteriostatic action, while for lean fish such as cod, hake and haddock the CO_2 levels required are lower (Arashisar *et al*., 2004; Speranza *et al*., 2009). High levels of CO_2 are used to delay the growth of aerobic microorganisms due to the high amount of fat in the fish muscle while, lower levels of CO_2 are needed for fish that have lower amount of fat. For example, gas compositions of 40% CO_2 + 30%N_2 + 30% O_2 and 90% CO_2 + 7.5%N_2 + 2.5% O_2 have been recommended for fish with low and high fat contents, respectively (Arashisar *et al*., 2004; Pantazi *et al*., 2008). The remaining part of this section briefly outlines the specific applications of the different types of gases in fish MAP.

Oxygen

Oxygen (O_2) is a colourless and odourless gas which dissolves readily in water with increasing solubility at lower temperatures (Mullan & McDowell, 2003). The presence of O_2 facilitates respiration of micro-organisms around the fish. Therefore, most packaging materials used in MAP act as barriers that prevent or restrict the access of O_2 to the product, thereby delaying the deterioration of the fish product (Bradenburg & Zagory, 2009). Presence of O_2 also promotes the growth aerobic microbes but inhibits anaerobic microbes, and influences oxidation of lipids in fish and fishery products (Cutter, 2002). The oxidation of lipids leads to fish rancidity and stimulates the generation of low molecular weight aldehydes, ketones, alcohols and carboxylic acids which causes deterioration in the quality of fish products (Ghaly *et al*., 2010). Therefore, in MA packaging of fish which have high lipid contents, O_2 concentration is kept at low level ($\leq$ 5%) to minimize oxidative rancidity and inhibit growth of anaerobic bacteria (Arashisar *et al*., 2004).

The presence of O_2 in MAP of fish and fish products also helps to prevent bacterial decay induced by the reduction of tri-methyl amine oxide (TMAO) to tri-methyl amine (TMA) and stops the 'fishy' smell aroma (Sivertsvik *et al*., 2002). However, when O_2 level is critically low inside the package, anaerobic pathogenic microorganisms such as *C. perfringens, C. botulinum and L. monocytogenes* might be stimulated (Farber *et al*., 2003). Studies with O_2

concentration of 2.5% using MA packaged rainbow fillets (95% CO_2 + 7.5% N_2 + 2.5% O_2) at 4°C revealed that psychrotrophic and mesophillic bacteria counts of 10^7 and 10^6 cfu/g were attained on day 8 and 13, respectively, while similar levels of bacterial count occurred on day 6 with air storage (Arashisar *et al*., 2004).

Nitrogen

Nitrogen (N_2) is a non-combustible gas that dissolves minimally in water and food elements. It is a tasteless, colourless, odourless and non-reactive gas that retards development of aerobic bacteria and impedes oxidative rancidity (Sandhya, 2010; Masniyom, 2011). Due to its low solubility in lipids and water, N_2 is used as a barrier to gases that have higher affinity to react with the product such as O_2 gas used in MAP of fish and fishery products (Bradenburg & Zagory, 2009), and to prevent package collapse as it serves to maintain equilibrium in the package as the volume of CO_2 decreases during storage (Velu *et al*., 2013).

High concentration of N_2 in packaged fish product helps to delay oxidation and to curb the progression of aerobic spoilage microbes (Velu *et al*., 2013). For example, the use of elevated levels of N_2 at 100% in MAP with active film (α tocopherol 0.5 %), helped guard against haemoglobin and lipid oxidation and increased storage life of fresh blue-fin tuna fillets from 2 to 18 days when stored at 3°C (Torrieri *et al*., 2011). Nitrogen levels of about 30% in MA packaged swordfish (40% CO_2 + 30% N_2 + 30% O_2) showed that it acted as a barrier gas to other reactive substances such as O_2 (Pantazi *et al*., 2008). Furthermore, the presence of N_2 as a barrier in conjunction with the presence of CO_2 increased the shelf life of seer steaks from 12 to 21 days when used in a ratio of 40:60 at 2°C (Yesudhason *et al*., 2009) and 30 to 60 days when used in a ratio of 50:50 at 4°C for Rainbow trout (Angis & Oguzhan, 2013).

Carbon dioxide

Carbon dioxide (CO_2) is colourless and in elevated amounts, has a faintly sharp aroma and becomes acidic in the presence of water (Sandhya, 2010). It is readily soaked-up by foods that have elevated water activity due to its high affinity to dissolve in water at low temperatures (Bradenburg & Zagory, 2009). It dissolves easily in lipids and water to form carbonic acid which lowers the pH of surrounding food products, thereby inhibiting microbial development by extending the lag phase (Bingol & Ergun, 2011; Sandhya, 2010; Yesudhason *et al*., 2014). Furthermore, the benefit of being able to dissolve in water is enhanced at temperatures lower

than 10°C; therefore, MAP in combination with lower temperatures has been used to enhance the storage life of fish and fishery products (Masniyom, 2011; Velu *et al*., 2013).

Furthermore, CO_2 serves as a preservative due to its bacteriostatic and fungistatic action on fish (Masniyom, 2011). The bacteriostatic action is influenced by the baseline microbial load, growth phase of microorganisms, gas concentration, storage temperature and nutritional components in the fish (Cutter, 2002). Action of CO_2 on microorganisms involves adjusting the role of the cell wall of the fish, obstructing the action of fish enzymes, saturating the bacterial cell wall when the CO_2 diffuses within the cell which results in pH variations and modification of the chemical and physical nature of fish's proteins (Sivertsvik *et al*., 2002; Soccol & Oetterer, 2003; Ghaly *et al*., 2010).

Studies of the action of CO_2 on fish microbial ecology have yielded diverse results for lactic acid bacteria (LAB). It leads to increased levels of LAB, although anaerobic growth is inhibited with a 10 - 20% gas volume. However, for gas volumes below 50% hazardous microbes such as *Clostridium perfringens*, *C. botulinum* and *Listeria monocytogenes* are not fully inhibited and this leads to safety concerns (Amanatidou *et al*., 2000; Farber *et al.*, 2003). Therefore, further studies need to be undertaken to understand how gases used in MAP balanced with optimum packaging material can delay the development of these hazardous microbes and enhance the storage life of fish and fishery products (Farber *et al.*, 2003).

Noble gases

Noble gases are chemically unreactive and they comprise of helium (He), argon (Ar), xenon (Xe) and neon (Ne) (Mullan & McDowell, 2003). They have a function similar to N_2 in MAP as they prevent gas barrier failure, delay development of aerobic bacteria and impede oxidative rancidity (Gimenez *et al.*, 2002). There is limited research on the use of noble gases in MAP of fish. Studies showed that green tomatoes at full maturity stage packaged in a controlled atmosphere (CA) of 3 kPa O_2 in Ar experienced delayed ripening in contrast to those packaged in 3 kPa N_2. However, fruit and vegetables stored in Ar, CA and MAP packages did not show better organoleptic properties than those that contained N_2. Additionally, when Ar is substituted with N_2 the cost of MAP process is higher (Zhang *et al.*, 2008; Bradenburg & Zagory, 2009). Studies in which Ar was replaced by N_2 suggest that blends with Ar successfully delayed total volatile base generation in comparison with N_2 although no substantial variation existed when packages containing the two gases where put under scientific scrutiny. The study concluded that, the most effective gas blend was 50%

CO_2+ 10% O_2+ 40% N_2 / Ar which resulted in storage life enhancement of up to 17 days at 1 ± 1°C for filleted rainbow trout (Gimenez *et al.*, 2002).

Ruffine *et al.*, (2010) investigated the mechanism of action of noble gases in MAP and suggested that noble gases form ice-like crystals called clathrate hydrates at temperatures and pressures varying from 253 K to 473 K and 0.1 MPa to 60 Mpa, respectively. These clathrate hydrate molecules are then confined inside water molecule enclosures where they attain stability through bonds from van der Waals forces (Ruffine *et al*., 2010). Consequently, the clathrate hydrates formed regulate the water activity (a_w) of the produce (Caleb *et al*., 2013). This would have a huge implication in the MAP of fish and fishery products with high $a_w \geq 0.95$ (Velu *et al*., 2013), as the water activity would be affected thereby, delaying the growth of microbes (Masniyom, 2011).

Carbon monoxide

Carbon monoxide (CO) is a colourless, odourless and tasteless gas. It is a highly poisonous gas and human contacts at about 200 ppm causes headache while higher levels lead to human mortality (Mullan & McDowell, 2003). It has been employed in maintaining the colour of packaged muscle foods such as tuna (Balaban *et al*., 2005). Studies suggest that the mechanism of CO activity on microbes involves cytochrome oxidase deactivation which results in obstruction of oxidative deterioration of the target product (Cutter, 2002).

A study involving gas treatment of 4% CO+ 10% O_2 + 20% CO_2 + 66% N_2 and irradiation levels of 1-2 KGy was conducted on fresh tuna (Balaban *et al*., 2005), which showed that radiation did not alter tuna pigmentation while CO maintained red colour in tuna. The impact of several gas treatments on aerobic bacteria in yellow-fin tuna steaks was studied by Kristinsson *et al*. (2008). The authors observed that CO treatment forms an unchanging clear cherry-red compound called carboxy-myoglobin and this compound does not change colour even when vacuum packed. The use of CO to mask colour quality has led to rising anxiety because carboxy-myoglobin cannot be oxidised even in anaerobic states as, tuna fish retains its colour even when the fish freshness has deteriorated (Olson, 2006). Currently, there is no law allowing its use in South Africa but its use has been prohibited in Japan (Huang *et al*., 2006).

Effects of MAP on quality and shelf life of fresh fish

Over the last four decades, there has been advancement in MAP technology for packaging and distribution of fresh fish and fish products. Table 3 presents a summary of scientific articles published over the last decade on the application of MAP and packaging materials. In one study, the effect of MA composition of 50% CO_2 + 45% N_2 + 5% O_2 and air combined with thyme essential oil pre-treatment was investigated on Mediterranean Swordfish (*Xiphias gladius*) fillets stored at 4°C for 18 days (Kykkidou *et al*., 2009). Results obtained showed that package head space gas atmosphere was not significantly affected throughout the storage period. Similarly, product pH (6.8 - 7.0) was not significantly influenced by thyme essential oil despite the high level of CO_2. However, chemical analysis showed that fish products treated with thyme essential oil and packaged with MAP had lower total volatile base nitrogen (TVB-N) than products stored in normal air alone.

Another study by Yesudhason *et al*. (2010) investigated the effects of MA gas composition (70% CO_2 + 30% O_2) and potassium sorbate treatment on quality of seer fish steak stored at 2°C for 32 days. Results obtained showed that TMA-N levels increased by 98 % from 0.63 mg N/100 g to 28 mg N/100 g for air stored products after 14 days of storage. Similarly, TMA-N content of products under MAP increased significantly ($p< 0.05$) from 0.63 mg N/100 g to 2.7 mg N/100 g after 8 days in storage and remained constant till day 14, after which it increased to 5.5mg N/100 g after 25 days in storage.

Table 3 Overview of selected research articles on the effects of modified atmosphere packaging on the shelf life of fresh fish and fishery products

Type of fishery product	Temp. °C	CO_2	N_2	O_2	Air/Vac.	Polymeric film(s)	Shelf life (days)	Reference
Rainbow trout fillets	4	90	7.5	2.5	Vac.; Air	OP*; EVA; PE	4 – 14	Arashisar *et al*. (2004)
Sardines	4	60	40	0	Vac.; Air	Nylon-PE pouches	3 – 15	Ozogul *et al*. (2004)
Bass	3	50	20	30	Air	PA; EVA; PE bags	<7- >9	Torrieri *et al*. (2006)
Eel	0	40	30	30	Vac.; Air	Suprovac PA bags	11 – 18	Arkoudelos *et al*. (2007)
Chub mackerel	4	70	25	5	Vac.; Air	HBP film bags	9 – 12	Erkan *et al*. (2007)
Farmed halibut	4	50	0	50	Air	HDP	13 – 19	Hovda *et al*. (2007)
Carp	4	20	0	80	Air	PA; PE	3 – 7	Jezek & Buchtova (2007)
Chub mackerel	3 and 6	50	50	0	Vac.; Air	Suprovac PA bags	7 – 12	Stamatis & Arkoudelos (2007)
Mussels	3	60	20	20	Air	PA; LDP	5 – 11	Goulas (2008)
Mediterranean Swordfish	4	40	30	30	Air	LDP-; PA-pouches	7 – 12	Pantazi *et al*. (2008)
Fresh Cod Loins	- 0.9	50	45	5	Air	LDP bags	9 - 21	Wang *et al*. (2008)
Carp	4	30	70	0	Air	PA; PE	3 – 8	Hudecova *et al*. (2009)
		20	0	80				
Mussels	3	60	20	20	Air	PA; LDP	5 – 11	Goulas (2008)
Mediterranean Swordfish	4	40	30	30	Air	LDP-; PA-pouches	7 – 12	Pantazi *et al*. (2008)
Fresh Cod Loins	- 0.9	50	45	5	Air	LDP bags	9 - 21	Wang *et al*. (2008)
Carp	4	30	70	0	Air	PA; PE	3 – 8	Hudecova *et al*. (2009)
		20	0	80				
Abalone muscle	2	40	30	30	Air	PVC with PA	3 – 11	Siripatrawan *et al*. (2009)
Chub mackerel fillets	4	95	0	5	_	PE bags	6	Speranza *et al*. (2009)
Eviscerated Cuttlefish	4	40	30	30		HBP; PE bags	10	Speranza *et al*. (2009)
Hake	4	95	0	5		PE bags	10	Speranza *et al*. (2009)
Yellow gurnard	4	95	0	5		HBP; PE bags	10	Speranza *et al*. (2009)
Seer steaks	0-2	60	40	0	Air	HDP laminated with PE	12 – 21	Yesuadhason *et al*. (2009)
Atlantic Salmon fillets	2	60	40	0	_	PA pouches; PE	28	Fernandez *et al*. (2010)

Table 3 Continued

Type of fishery product	Temp. °C	CO_2	N_2	O_2	Air/Vac.	Polymeric film(s)	Shelf life (days)	Reference
Shrimps	3	5.1	92.9	2	Air	PVC; lacquer; PE	2 – 5	Arvanitoyannis *et al*. (2011)
Gilthead Sea bream	4 and 8	60	40	0	_	PET; PE; EVA; PE bags	6 – 18	Campus *et al*. (2011)
Green mussel	4	80	10	10	Air	Vacuum bags	6 – 12	Masniyom *et al*. (2011)
Indian mackerel	5	100	0	0	Air	Nylon-PE and HDP bag	4 – 11	Chong *et al.* (2013)
Nile tilapia	1	50	50	0	Air	PE film	11 – 23	Cyprian *et al.* (2013)

In addition, thio-barbituric acid concentration in fresh fish steak increased significantly from 0.037 mg malon-di-aldehyde/kg to 3.6, 3.3 and 4.2 mg malon-di-aldehyde/kg of fish in air, and psMAP samples respectively on day 14, 25 and 32. The study indicated that MAP and the use of pre-treatment maintained desired quality (Yesudhason *et al.,* 2010). Summary on the combined effect of MAP and pre-treatments on the shelf life of fresh fish is presented in Table 4.

Studies on mackerel (*Scomber japonicus*) and hake (*Merluccius merluccius*) stored at 4°C for 28 d showed that the use of MAP and active compounds (what compounds) maintained nutritional quality (Del Nobile *et al*., 2009). The study also showed that the ratio of n6/n3 poly unsaturated fatty acids (PUFAs) in mackerel and hake from Italy was 0.35 and 0.20, respectively (Del Nobile *et al*., 2009), which are lower than the maximum recommended threshold of 0.45 (DoH, 1994; Del Nobile *et al*., 2009). Studies have shown that PUFAs enhance the nutritional attributes and counter disease conditions in humans (Moreira *et al*., 2001; Del Nobile *et al*., 2009).

Influence of MAP on microbial quality of Mediterranean swordfish fillets was investigated by Kykkidou *et al*. (2009) using MA composition of 50% CO_2 + 45% N_2 + 5% O_2 and air combined with thyme essential oil (EO) stored at 4°C for 18 d. It was shown that MAP and EO-MAP delayed microbial growth and inhibited the exponential growth phase (< 7 log cfu/g) for total viable count. Additionally, *Pseudomonads* and hydrogen sulphide-producing bacteria were inhibited compared to air stored samples (Kykkidou *et al*., 2009). Similarly, Del Nobile *et al*. (2009) reported that air stored mackerel and hake at 4°C for 28 d, had the highest aerobic plate count (APC) (6.37 x 10^2 cfu/g) while MAP 50% O_2 + 50% CO_2 (4.92 ± 0.22 x 10^2 cfu/g). Furthermore, the trend for hydrogen sulphide-producing bacteria (HSPB) and psychro-tolerant and heat labile aerobic bacteria (PHAB) showed that MAP and active compound MAP helped in reducing growth of these microbes compared with air storage (Del Nobile *et al*., 2009). These results were in agreement with studies conducted by Speranza *et al*. (2009) using hake fillets, which showed that MAP1 (30% O_2 + 40% CO_2 + 30% N_2) and MAP2 (5% O_2 + 95% CO_2) without antimicrobial compounds played a role in inhibiting the growth of microbes. This is in agreement with literature which suggests that when O_2 levels are reduced and CO_2 levels are increased strong bacteriostatic effects are exerted on aerobic microbes and thus Gram-negative bacteria such as *Pseudomonas* spp. and *Shewanella* spp. are hindered (Gram & Huss, 2000; Sivertsvik *et al*., 2002).

MAP has been reported to play a significant role on sensory attributes of fish. Speranza *et al*. (2009) stated that, the overall sensory quality of hake fish in packaged in air decreased

Table 4 The effects of modified atmosphere packaging and pre-treatments on the shelf life of fresh fish and fishery products

Type of fishery product	Temp.(°C)	CO_2	N_2	O_2	Air/Vac.	Polymeric film(s)	Pre-treatment(s)	Shelf life (days)	Reference
Atlantic Salmon	5	50	0	50	Vac.	Polyester; PE	150 MPa	13 – 18	Amanatidou *et al*. (2000)
Sea bream	4	40	30	30	Air	LDP*; PA	Salt; Oregano oil	15 – 33	Goulas & Kontominas (2007)
Farmed Atlantic cod	1.3	60	0	40	Vac.	HDP; EVA	CO_2 emitter; citric acid	7 - 21®	Hansen *et al*. (2007)
Grouper	2	80	20	0	Air	PE; EVA; HDP bags	Potassium Sorbate	7 – 24	Siah & Ariff (2007)
Cod fillets	0	37	0	63	No	HDP semi rigid trays	Filleted	14	Sivertsvik, (2007)
Red claw crayfish	2	80	10	10	Air	PP tray; PE	Pre-cooked; peeled	7 – 21	Chen & Xiong (2008)
Mediterranean Swordfish fillets	4	50	45	5	Air	LDP; PA	Thyme essential oil 0.1% v/w	8 – 15	Kykkidou *et al*. (2009)
Cod loins	0 and -2	50	45	5	Air	Cryovac film	Super chilling	14 – 21	Lauzon *et al.* (2009)
Blue mackerel & hake	4	95	0	5	_	Nylon; PE bags	Essential oils	23	Del Nobile *et al*. (2009)
Striped catfish slices	4	35	60	5	Air	LDP bag	10 mg tannic acid	9 – 15	Maqsood & Benjakul (2010)
Gilthead Sea bream fillets	0	50	50	0	Air	HDP pouches	Nisin; NaCl (5%)	10 – 48	Tsironi & Taoukis (2010)
Seer steaks	2	70	0	30	Air	HDP	Potassium sorbate	12 – 30	Yesuadhason *et al*. (2010)
Blue Fin Tuna	3	0	0	100	Air	LDP	α tocopherol (0.5%)	2 – 18	Torrieri *et al*. (2011)
Atlantic Bonito	2	80	20	0	Air	Plastic bag	Salting	31;8*	Caglak *et al.* (2012)
Rainbow trout fillets	4	50	50	0	Vac.	PA and PE bags	Thyme; Essential oil	9 – 18	Angis & Oguzhan (2013)
Rainbow trout fillets	4	50	50	0	Vac.	PE; PA bags	Salting and smoking	30 – 60	Oguzhan *et al.* (2013)

*Abbreviations: Oriented polyamide (OP); Ethylene vinyl alcohol (EVA); Polyethylene (PE); Polyamide (PA); Vacuum (Vac.); Low density polythene (LDP); High barrier plastic (HBP); High density Polyethylene (HDP); Polyvinylidene chloride (PVC).

more rapidly than that under MAP 30% O_2 + 40% CO_2 +30% N_2 and 5% O_2 + 95% CO_2 samples. The preceding review showed that the use of MAP contributes effectively in slowing down the loss of sensory quality of the hake fish. The appearance, texture, odour and overall acceptance of seer fish packed under air, MAP and passive-MAP and stored at 2°C for 32 days was investigated by Yesudhason *et al.* (2010). Air packaged steaks displayed the lowest score (3.4) compared to MAP and MAP pre-treated with potassium sorbate on day 14 ($P < 0.05$). The study concluded that, there was a significant extension ($P < 0.05$) in shelf life due to treatment with MAP and potassium sorbate (Yesudhason *et al*., 2010).

Microbiological hazards associated with MA-packaged fresh fish and fishery products

MA-packaged fresh fish and fishery can be grouped into ready-to-cook (RTC) and ready-to-eat (RTE) products. Ready-to-cook fish would be cooked to temperatures that kill all microbes before eaten. The latter are more hazardous than the former (Sivertsvik *et al*., 2002). Fresh fish stored under MAP conditions with high amounts of CO_2 can retain freshness attributes for a longer period. Due to the ability of CO_2 can retain freshness attributes for a longer period, due to the ability of CO_2 to retard enzyme action and growth of micro-organisms (Sivertsvik, 2007). However, the capability of pathogenic micro-organisms to grow under this gas conditions heightens the microbial hazard and safety concerns. Pathogenic microbes that act on fresh fish are summarized in Table 5.

Pathogenic microbes are classified as indigenous (V*ibrio* spp., *L. monocytogenes*, *C. botulinum*) and non–indigenous due to faecal or cross contamination during processing such as *Salmonella* spp., pathogenic *Escherichia coli*, *Bacillus cereus* and *Stapylococcus aureus* (Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly, 2011). Studies have shown that 100% concentration of CO_2 in MAP conditions lowered the growth of *L. monocytogenes* on RTE shrimps packaged at 3°C in contrast to air or vacuum packaged shrimps (Rutherford *et al*., 2007). The lag phase of *L. monocytogenes* was prolonged and growth of pathogenic *E. coli* O157 was inhibited when sea bass was stored under MAP conditions (80% CO_2 + 10% O_2 + 10% N_2) and pyrophosphate (Masniyom *et al*., 2006).

Non-proteolytic and psychrotrophic species of *C. botulinum* type E due to its toxin, is of critical importance to consumer safety (Ravi-Sankar *et al*., 2008). The synthesis of *C. botulinum* type E toxin was inhibited in flounder fillets stored at 4°C under MAP (100% CO_2), vacuum packaging for 35 days. However, there was toxin production in flounder fillets stored at 10°C under MAP and VP on day 20 and 25, respectively, before decay was

established on day 35 (Arritt *et al*., 2007). Thus, one of the critical factors in enhancing the freshness and safety in fresh-cut salmon is storage temperature. Nevertheless, *C. botulinum* growth and the production of toxin in retail packs starts before decay is noticed and longer storage duration of salmon under MAP conditions and temperature abuse can lead to endangering the wellbeing of consumers due to ingestion of *C. botulinum* toxins leads to botulism in humans (Peck *et al*., 2008). This was confirmed by a study where a selective medium of buffered peptone, yeast, glucose, starch broth inoculated with non-proteolytic *C. botulinum* type E and B spores was evaluated (Gibson *et al*., 2000). The study indicated that 100% CO_2 MA delayed growth of pathogenic non-proteolytic *C. botulinum* type E and B spores at 5°C (Gibson *et al*., 2000; Sivertsvik *et al*., 2002).

In another study, grouper (*Epinephelus* spp.) fillets dipped in 1% potassium sorbate for one min prior to storage in MAP (80% CO_2 + 20% N_2) and stored at 2°C for 27 days, had a longer shelf life (24 d) in comparison to air (7 d) stored fillets (Siah & Ariff, 2007). The use of potassium sorbate protected fresh fish from the action of pathogens and hinders the growth of TMA generating bacteria (Siah & Ariff, 2007). Although packages with high CO_2 environments prolong the lag phase of *L. monocytogenes* nonetheless, MAP and cold storage on its own does not regulate the growth of pathogens on muscle foods (Sivertsvik *et al*., 2002). Therefore, in order to eliminate microbial hazards and sustain overall quality assurance of RTE or RTC fish application of hurdle technologies such as pre-treatments coupled with MAP, adequate hygienic practices and good management practices are necessary (Sivertsvik *et al*., 2002; FAO, 2005; Ababouch, 2006).

Table 5 Potential pathogenic microbes that infest fresh fish and fishery products

Microbe	Characteristics	Remarks	Reference
Clostridium botulinum	Gram positive, spore generating rods, requires low oxygen	Causes botulism due to the nerve toxin generated	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011
Listeria monocytogenes	Gram positive, short rods, are facultative and psychrotrophic	(a) Causes listeriosis; (b) Abundant in the environment through faeces, water, sewage, and silos	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011
Staphylococcus aureus	Gram positive, generates cocci, are facultative and mesophillic	(a) Linked with human diarrhoeal illnesses; (b) Found on skin, hair, faeces, water and clothes	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011
Vibrio parahaemolyticus	Gram negative, marine microbe are facultative and mesophillic	(a) Cause human gastroenteritis; (b) *V. parahaemolyticus* O3:K6 accounts for recent outbreaks (c) proper cooking increases food safety	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011
Salmonella spp.	Gram negative, facultative and mesophillic	(a) Causes salmonellosis; (b) Abundant in water, sewage, soil, domestic animals and humans	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011
Escherichia coli	Gram negative, generates rods are facultative and mesophillic	(a) Linked with human diarrhoeal illnesses but lethal in children; (b) Abundant in water, sewage, soil, domestic animals and humans	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011
Aeromonas	Gram negative, facultative and psychrotrophic bacteria	(a) Cause human gastroenteritis in healthy humans and septicaemia in individuals with impaired immune systems; (b) Sources include fresh and marine waters, estuaries, aquatic and domestic animals	Gram *et al.*, 2002; Arias, 2009; Dave & Ghaly 2011

Advancements in MAP and future applications in the fishing industry

Smart packaging offers interplay between the package and product that confers's intelligence appropriate to function and use of product itself (Kerry *et al*., 2006; McMillin, 2008). Obvious modifications occurs as a result of interplay between product and package which reflects on the indicators, such as change in colours or diffusion of a dye in a particular direction, which correlates with microbial decay and release of volatiles (Yam *et al*., 2005). End users can therefore monitor freshness and muscle meat shelf life (Pacquit *et al.*, 2007). Smart packaging of food can be classified into active and intelligent packaging (Yam *et al*., 2005; Sandhya, 2010).

Active packaging can also be described as the integration of certain pre-treatments into a package whether inside the food, in the blend of gases used or inside the material used for packaging so as to enhance the storage-period and quality of the food (Ozdemir & Floros, 2004; Kerry *et al*., 2006). In fulfilling the roles of MAP some pre-treatments known as freshness enhancers are used. These pre-treatments maintain diet attributes, reduce the infestation of hazardous and decay microbes, and assist the food in retaining freshness attributes (Kerry *et al*., 2006). The major types of substances used in active packaging of fisheries are O_2 scavengers, CO_2 scavengers and emitters, moisture scavengers and antimicrobials (Brody *et al*., 2008).

Oxidation process in fish results in degradation of nutritional components, induce development of off flavours and undesirable colour changes. Oxygen scavengers offer a cheaper alternative to vacuum packaging or gas flushing (Ozdemir & Floros, 2004). The use of O_2 scavenging sachets on fresh pork sausages at varying gas compositions in modified atmosphere stored at 2°C for 20 days has been reported (Martinez *et al*., 2006). They observed that the levels of aerobic psychrotrophes diminished and the physical and sensory attributes was enhanced with the use of O_2 scavenging sachets. Oxygen scavengers exist as cards, closure-liners; concentrate films, pouch, and tags. They have also been introduced into packaging film to eliminate unintentional breakage and to encourage end-user acceptance (Suppakul *et al*., 2003).

Packaging may be designed with chemicals such as zeolite, which regulate CO_2 release for products that need CO_2 until equilibrium is achieved (Lee *et al*., 2001). Studies on muscle meat showed that a CO_2 range of 10% to 80% in a package enhances its bacteriostatic effect (Kerry *et al*., 2006). Therefore the inclusion of CO_2 discharging sachets is beneficial in such

systems (Kerry *et al*., 2006). Chemicals that are water absorbers have been incorporated into compounds such as CaO which reacts with CO_2 in the package to avert rupturing of the packages and has being applied to lengthen the storage period of some muscle foods (Ahvenainen, 2003). In foods that are affected by excess CO_2, packaging that can exhaust the CO_2 generated have been created and used beneficially to delay food decay (Brody *et al*., 2001).

Moisture scavengers are used in products that are affected by condensation. The roles of these are to eliminate the water vapour condensation on the film or drips from frozen products which can accelerate the growth of microbes (Lopez-Rubio *et al*., 2004). These substances are chosen based on the fact that they do not react with the packaging material or cause colour changes in the product (Brody *et al*., 2001; Lopez-Rubio *et al*., 2004). Substances such as silica gel, natural clay and molecular sieves have been used successfully, to regulate build-up of in-package condensations that hinder the passage of water vapour (Lopez-Rubio *et al*., 2004). The devices used to regulate and forage moisture in fish and muscle foods include pads, superabsorbent polymeric laminate films and sachets (Ozdemir & Floros, 2004).

In the last decade, anti-microbial packaging has been used to control the growth of bacteria in fresh fish through the interplay of films with constituents that hinder bacterial growth (Han, 2000; Cooksey, 2001). Incorporation of anti-microbial agents into the films with gradual release of these substances when needed, offers innovative approach to packaging (Quintavalla & Vicini, 2002). Acid salts, acid anhydrides, alcohol, antioxidants, antibiotics, bacteriocins, chelating agents, enzymes, fungicides, metals, organic acids, plant volatiles, plant and spice extracts, phenolics, polysaccharides, probiotics, sanitizing agents and sterilizing gases could be used as antimicrobials. Bacteriocins, enzymes, fungicides, organic acids and its salts, triclosan and silver zeolites have been incorporated into films used in packaging muscle foods to hinder microbes (Quintavalla & Vicini, 2002). Anti-microbial packaging with nisin and poly-lactic acid have been shown to be effective in inhibiting food born pathogen such as *L. monocytogenes*, *E. coli* O157:H7 and *S. enteritidis* present in food beverages (Jin & Zhang, 2008). Rosemary extract incorporated into polypropylene film inhibited metmyoglobin and lipid oxidation and maintained the quality of meat cuts (Nerin *et al*., 2006). The use of 0.8% oregano essential oil with 100 g/L NaCl at 8°C for 1 hour in conjunction with MAP was shown to maintain sensory attributes of sea bream fillets 33 of storage at 4°C (Goulas & Kontominas, 2007).

Intelligent packaging can be tailored into two approaches which quantify the atmosphere around the product and the other which assesses product freshness attributes. The second approach involves the interaction of the package with the product or its headspace and this affords an opportunity to determine freshness attributes in packaged products (Restuccia *et al*., 2010). Tools used in intelligent packaging include time-temperature indicators (TTI), gas leakage indicators, freshness indicators, toxin indicators, biosensors, and radio frequency identification (RFID) (Han *et al*., 2005; Stauffer, 2005; Yam *et al*., 2005; Restuccia *et al*., 2010).

Time temperature indicators exists as labels affixed on packaged products helping to furnish records of temperature throughout the period between harvest and delivery, giving alerts on temperature abuse helping to appraise the storage life of fresh foods (Yam *et al*., 2005). These tools employ different methods such as the strengthening of directional movement of dye incorporated in the pack. Marketable TTIs includes critical temperature indicators, partial history indicators, and full history indicators (Singh, 2000). Current TTIs centre on microchips which review the temperature periodically to establish the length at which the product can be stored rather than old TTIs which were centred on biochemical reactions (Yam *et al*., 2005). In assessing freshness attributes, gas leakage indicators are vital and they are described as tools that give warnings when there are modifications in the gas levels of a package (Kerry *et al*., 2006). The gases in a fish package can be modified due to the atmosphere within the package, film permeability and microbial activity which can lead to the release of gas by decay microbes (Yam *et al*., 2005). These indicators exist as labels or as prints on films and studies show that the oxygen indicators can recognize when package seals are broken and decay of pre-cooked muscle foods stored under MAP conditions starts (Smiddy *et al*., 2002).

Conclusion

Modified atmosphere packaging (MAP), combined with optimum cold storage, has been successfully used to enhance the storage potential of fish and fishery products, while maintaining the organoleptic attributes. Research has shown that the lag phase of microbes endogenous to fish and fishery products was extended resulting in the inhibition of decay metabolites, thus delaying decay.

Optimized quality and safety assurance tools would guarantee consumer confidence in MA-packaged fresh fish products. The combination of MAP with active and intelligent

packaging, hurdles and good managing practices will go a long way in meeting increased consumer demand for fresh and value added fish products such as ready-to-cook fish. Applications of active packaging technologies such as O_2 and CO_2 scavengers and emitters, moisture scavengers and antimicrobials which inhibit the major decay mechanisms in MAP offer considerable promise. Intelligent packaging technologies involving that of TTI's, gas leakage indicators, freshness indicators, toxin indicators, biosensors, and RFIDs which communicate freshness attributes of the fish to the consumer present novel opportunities to influence purchase behaviour with the potential to reduce food wastage. These innovations will assist food processors in adding value, ensure food safety in fish and fishery products, enhance nutritional and health benefits, address conservation issues, and extend the storage potential and organoleptic properties of fresh fish.

References

Ababouch, L. (2006). Assuring fish safety and quality in international fish trade. *Marine Pollution Bulletin*, **53**, 561–568.

Abdel-Bary E.M. ((2003). Hand book of plastic films. Rapra Technology Ltd., Shawbury, Shrewbury, Shropshri, SY4 4NR, UK.

Ahvenainen, R. (2003). Active and intelligent packaging: an introduction. In: *Novel food packaging techniques* (edited by R. Ahvenainen). Pp. 5–21 Cambridge, UK: Woodhead Publishing Ltd.

Al Bulushi, I.M., Poole, S.E., Barlow, R., Deeth, H.C. & Dykes, G.A. (2010). Speculation of Gram-positive bacteria in fresh and ambient-stored sub-tropical marine fish. *International Journal of Food Microbiology*, **138**, 32-38.

Amanatidou, A., Schluter, O., Lemkau, K., Gorris, L.G.M., Smid, E.J. & Knorr, D. (2000). Effect of combined application of high pressure treatment and modified atmospheres on the shelf life of fresh Atlantic salmon. *Innovative Food Science Emerging Technologies*, **1**, 87-98.

Amos, B. (2007). Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement. United Nations University, Uganda. http://www.unuftp.is/static/fellows/document/amos 06prf.pdf.

Angis, S. & Oguzhan, P. (2013). Effect of thyme essential oil and packaging treatments on chemical and microbiological properties of fresh rainbow trout (*Oncorhynchus mykiss*) fillets during storage at refrigerator temperatures. *African Journal of Microbiology Research*, **7**, 1136-1143.

Arashisar, S., Hisar, O., Kaya, M. & Yanik, T. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. *International Journal of Food Microbiology*, **97**, 209-214.

Arias, C. (2009). Microbiology handbook of fish and seafood In: *Chilled and frozen raw fish* (edited by R. Fernandes) Pp. 1-27. Leatherhead Publishing, a division of Leatherhead Food International Ltd Randalls Road, Leatherhead, Surrey KT22 7RY, UK and Royal Society of Chemistry Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK.

Arkoudelos, J., Stamatis, N. & Samaras, F. (2007). Quality attributes of farmed eel (*Anguilla anguilla*) stored under air, vacuum and modified atmosphere packaging at 0°C. *Food Microbiology*, **24**, 728-735.

Arritt, F.M., Eifert, J.D., Jahncke, M.L., Pierson, M.D. & Williams, R.S. (2007). Effect of modified atmosphere packaging on toxin production by *Clostridium botulinum* in raw aqua-cultured summer flounder (*Paralichthys dentatus*). *Journal of Food Protection*, **70**, 1159-1164.

Arvanitoyannis, I. S., Vasiliki, K., Bouletis, A. D. & Papaloucas, C. (2011). Study of changes in physicochemical and microbiological characteristics of shrimps (*Melicertus kerathurus)* stored under modified atmosphere packaging. *Anaerobe*, **17**, 292-294.

Aryee, A.N.A., Simpson, B.K. & Villalonga, R. (2007). Lipase fraction from the viscera of grey mullet (*Mugil cephalus*) isolation, partial purification and some biochemical characteristics. *Enzyme and Microbial Technology*, **40**, 394-402.

Baird-Parker, T.C. (2000). The production of microbiologically safe and stable foods. In: *The microbiological safety and quality of food*, (edited by B.M. Lund and T.C. Baird-Parker) Pp. 3-18. Aspen Publishers Inc., Gaithersburg, MD., USA., ISBN: 0834213230.

Balaban, M.O., Kristinsson, H.G. & Otwell, W.S. (2005). Evaluation of colour parameters in a machine vision analysis of carbon monoxide treated fresh tuna. *Journal of Aquatic Food Product Technology*, **14**, 5-24.

Berkel, B.M., Boogaard, B.V. & Heijnen, C. (2004). Agrodok 12: Preservation of fish and meat. *Agromisa Foundation*, Wageningen, The Netherlands. Pp. 78-80, ISBN: 90-72746-01-9.

Bingol, E.B. & Ergun, O. (2011). Effects of modified atmosphere packaging on the microbiological quality and shelf life of ostrich meat. *Meat Science*, **88**, 774-785.

Bradenburg, J. S. & Zagory, D. (2009). Modified and controlled atmosphere for the storage of, transportation and packaging of horticultural commodities. In: *Modified and controlled atmosphere packaging technology and applications* (edited by E.M.Yahia). Pp. 73-77.

Brody, A, Strupinsky, E.R. & Kline L.R. (2001). Odour removers. In: *Active packaging for food applications*. (edited by A. Brody, E.R. Strupinsky and l. R. Kline). Pp. 107–117 Lancaster, Pa.: Technomic Publishing Company, Inc.

Brody, A. (2008). Packaging by the numbers. *Journal of Food Technology*, **62**, 89–91.

Byett, J., Bruyns, O., Glover, T., Oakley, B., Smithard, E. & Tinkler, R. (2002a). A handbook of packaging technology. In: *Food Packaging of Fish.* (edited by J. Byett, B. Oakley and A. Oakley) Pp. 582-589. Institute of Packaging, South Africa, the Golden Era Group.

Byett, J., Bruyns, O., Glover, T., Oakley, B., Smithard, E. & Tinkler, R. (2002b). A handbook of packaging technology. In: *Plastic Packaging materials.* (edited by J. Byett, B. Oakley and A. Oakley) Pp. 242-263. Institute of Packaging, South Africa, the Golden Era Group.

Caglak, E., Cakli, S. & Kilinc B. (2012). Effect of modified atmosphere packaging on quality and shelf-life of salted bonito (*Sarda sarda*). *Journal of Aquatic Food Product Technology*, **21**), 206–221.

Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology*, **5**, 15-30.

Caleb, O.J., Mahajan, P.V., Al-Said, F.A.J. & Opara, U.L. (2013). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences—.a review. *Food and Bioprocess Technology*, **6**, 303-329.

Campus, M., Bonaglini, E., Cappuccinelli, R., Cristina Porcu, M., Tonelli, R. & Roggio, T. (2011). Effect of modified atmosphere packaging on quality index method (QIM) scores of farmed gilthead sea-bream (*Sparus aurata* L.) at low and abused temperatures. *Journal of Food Science*, **76**, 185-191.

Chaijan, M., Benjakul, S., Visessanguan, W. & Faustman, C. (2006). Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. *Food Chemistry*, **99**, 83-91.

Chen, G. & Xiong, Y.L. (2008). Shelf-stability enhancement of precooked red claw crayfish (*Cherax quadricarinatus*) tails by modified $CO_2/O_2/N_2$ gas packaging. *LWT Food Science and Technology*, **41**, 1431–1436

Chong, C.Y., Fatimah, A.B., Russly, A.R., Jamilah, B., Selvi, V. & Maryam, S. (2013). Effects of different levels of CO_2 on biochemical changes and relationships among different quality indices in Indian mackerel (*Rastrelliger kanagurta*). *International Food Research Journal*, **20**, 383-389.

Choubert, G. & Baccaunaud, M. (2006). Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss W.*) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere. *LWT Food Science and Technology*, **39**, 1203-1213.

Coles, R. (2003). Plastic in food packaging. In: *Food packaging technology* (edited by R. Coles, D. Mcdowell and M.I. Kirwan). Pp. 1–31. Blackwell Publishing, CRC Press, London, UK.

Cooksey, K. (2001). Antimicrobial food packaging materials. *Additives for Polymers*. Pp. 6–10.

Cutter, C.N. (2002). Microbial control by packaging: a review. *Critical reviews in food science and nutrition*, **42**, 151-161.

Cyprian, O., Lauzon, H.L., Johannsson, R., Sveinsdottir, k., Arason, S. & Martinsdottir, E. (2013). Shelf life of air and modified atmosphere-packaged fresh tilapia (*Oreochromis niloticus*) fillets stored under chilled and super-chilled conditions. *Food Science & Nutrition*; **1**, 130–140.

Dalgaard P. (2000). Fresh and lightly preserved seafood. In: *Shelf-Life Evaluation of Foods*, (edited by C.M.D. Man and A.A. Jones). Pp. 110-139. London: Aspen Publishers, Inc.

Dave, D., & Ghaly, A. E. (2011). Meat Spoilage Mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Sciences*, **6**, 486.-510

De Silva, D.A.M. & Yamao M. (2006). Regional preferences in the Japanese Seafood consumption: An empirical analysis of consumer purchasing behaviour on domestic versus imported seafood, *Japan Journal of the Regional Fisheries Society*, **46**, 83-104.

Del Nobile, M.A., Corbo, M.R., Speranza, B., Sinigaglia, M., Conte, A. & Caroprese, M. (2009). Combined effect of modified atmosphere packaging and active compounds on fresh blue fish burger. *International Journal of Food Microbiology*, **135**, 281-287.

DoH. (1994). Department of Health. Nutritional aspects of cardiovascular disease. Report of the cardiovascular review group committee on medical aspects of food policy. In: *Report on health and social subjects*, vol. 46. HMSO, London.

Engvang, K. & Nielsen, H.H. (2001). Proteolysis in fresh and cold-smoked salmon during cold storage: effects of storage time and smoking process. *Journal of Food Biochemistry*, **25**, 379-395.

Erkan, N., Ozden, O. & Inugur, M. (2007). The effects of modified atmosphere and vacuum packaging on quality of chub mackerel. International *Journal of Food Science and Technology*, **42**, 1297–1304.

Esse, R. (2002). Flexible packaging end-use market analysis. Linthicum, Md.: Flexible Packaging Association.

EC (European Commission). (2012). Commission Regulation (EC) No 231/2012 of 9th March 2012 laying down specifications for food additives listed in annexes II and III Regulation (EC) No 1333/2008 of the European Parliament and of the Council. *Official Journal European Union League*, **83**:1–295.

Food and Agricultural Organisation, FAO (2001). Fisheries and Aquaculture topics. Quality and safety of fish and fish products. Topics Fact Sheets. In: *FAO Fisheries and*

Aquaculture Department [online]. Rome. Updated 27 September 2001. [Cited 3 June 2013]. http://www.fao.org/fishery/topic/1514/en.

Food and Agricultural Organisation, FAO (2005). Fisheries and Aquaculture topics. Post-harvest changes in fish. Topics Fact Sheets. Text by Lahsen Ababouch. In: *FAO Fisheries and Aquaculture Department [online]*. Rome. Updated 27 May 2005. [Cited 3 June 2013]. http://www.fao.org/fishery/topic/12320/en.

Food and Agricultural Organisation, FAO. (2014). Post-harvest changes in fish. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations.

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H. & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Review in Food Science and Food Safety*, **2**, 142-160.

Fernandez, K., Aspe, E. & Roeckel, M. (2010). Scaling up parameters for shelf-life extension of Atlantic salmon (*Salmo salar*) fillets using super chilling and modified atmosphere packaging. *Food Control*, **21**, 857–862.

Folinas, D., Manikas, I. & Manos, B. (2006). Traceability data management for food chains. *British Food Journal*, **108**, 622-633.

Galdi, M.R., Nicolais, V., Di, M. & Incarnato, L. (2008). Production of active poly ethylene films: evaluation of scavenging activity. *Packaging Technology and Science*, **21**, 57–268.

Ghaly, A.E., Dave, D., Budge, S. & Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: review. *American Journal of Applied Sciences*, **7**, 859-877.

Gibson, A.M., Ellis-Brownlee, R.C.L., Cahill, M. E., Szabo, E.A., Fletcher, G.C. & Bremer, P. J. (2000). The effect of 100% CO on the growth of non-proteolytic Clostridium botulinum at chill temperatures. *International Journal of Food Microbiology*, **54**, 39-48.

Gimenez, B., Roncales, P & Beltran, J.A. (2002). Modified atmosphere packaging of filleted rainbow trout. *Journal of the Science of Food and Agriculture*, **82**, 1154-1159.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. & Toulmin, C (2010). Food Security: the challenge of feeding 9 billion people. *Science*, **327**, 812-818 [DOI: 10.1126/science.1185383].

Godiksen, H., Morzel, M., Hyldig, G. & Jessen, F. (2009). Contribution of cathepsins B, L and D to muscle protein profiles correlated with texture in rainbow trout (*Oncorhynchus mykiss*). *Food Chemistry*, **113**, 889-896.

Golan, E., Krissoff, B., Kuchler, F., Calvin, L., Nelson, K. & Price, G. (2004). Traceability in the U.S. food supply: economic theory and industry studies. *Agricultural Economic Report* **830**, 48.Washington, DC: Economic Research Service, U.S. Department of Agriculture.

Goulas, A.E. & Kontominas, M.G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food Chemistry*, **100**, 287-296.

Goulas, A.E. (2008). Combined effect of chill storage and modified atmosphere packaging on mussels (*Mytilus galloprovincialis*) preservation. *Packaging Technology and Science*, **21**, 247–255

Gram, L. & Dalgaard, P. (2002). Fish spoilage bacteria: problems and solutions. *Current Opinion in Biotechnology*, **13**, 262-266.

Gram, L. & Huss, H.H. (2000). Fresh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Foods*, edition 1. (edited by B.M. Lund, A.C. Baird-Parker, and G.W. Gould). Pp. 472-506. London: Chapman & Hall.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B. & Givskov, M. (2002). Food spoilage interactions between food spoilage bacteria. *International Journal of Food Microbiology*, **78**, 79– 97.

Gribble, A. & Brightwell, G. (2013). Spoilage characteristics of *Brochothrix thermosphacta* and *B. campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. *Meat science*, **94**, 361-368

Gumus, B., Balaban, M. O. & Unlusayın, M. (2011). Machine vision applications to aquatic foods: a review. *Turkish Journal of Fisheries and Aquatic Sciences*, **11**, 171-181.

Gupta, V. & Nagar, R. (2010). Effect of cooking, fermentation, de-hulling and utensils on antioxidants present in pearl millet *rabadi*—a traditional fermented food. *Journal of Food Science and Technology*, **47**, 73-76

Han, J. H. (2000). Antimicrobial food packaging. *Food Technology*, **54**, 56–65.

Han, J. H., Ho, C. H. L. & Rodrigues, E. T. (2005). Intelligent packaging. In: *Innovations in food packaging* (edited by J. H. Han). Oxford: Elsevier Academic Press.

Hansen, A.A., Morkore, T., Rudi, K. Olsen, E. & Eie, T. (2007) Quality changes during refrigerated storage of modified atmosphere packaged pre-rigor fillets of farmed Atlantic cod (*Gadus morhua L.*) using traditional modified atmospheres packaging, CO_2 emitter, and vacuum. *Journal of Food Microbiology and Safety*, **72**, M423-M430.

Hernandez, R.J., Selke, S.E.M. & Culture, J.D. (2000) *Plastics packaging: properties, processing, applications, and regulations*. Hanser, Munich, Germany.

Hernandez-Herrero, M.M., Duflos, G., Malle, P. & Bouquelet, S. (2003). Collagenase activity and protein hydrolysis as related to spoilage of iced cod (*Gadus morhua*). *Food Research International*, **36**, 141-147.

Hovda, M.B., Sivertsvik, M., Tore-Lunestad, B., Lorentzen, G. & Rosnes, J. T. (2007). Characterisation of the dominant bacterial population in modified atmosphere packaged farmed halibut (*Hippoglossus hippoglossus*) based on 16S rDNA-DGGE. *Food microbiology*, **24**, 362-371

Huang, Y., Shiau, C., Hung, Y. & Hwang, D. (2006). Change of hygienic quality and freshness in tuna treated with electrolyzed water and carbon monoxide gas during refrigerated and frozen storage. *Journal of Food Science*, **71** M127-M133.

Hudecova, K., Buchtova, H. & Steinhauserova, I. (2009). The effects of modified atmosphere packaging on the microbiological properties of fresh common carp (*Cyprinus carpio Ls.). ACTA Veterinaria Brno* **79** 93–100.

Jezek, F. & Buchtova, H. (2007). Physical and chemical changes in fresh chilled muscle tissue of common carp (*Cyprinus carpio L.*) packed in a modified atmosphere. *Acta Veterinaria Brno*, **76**, *83-92.*

Jin, T. & Zhang, H. (2008). Biodegradable poly-lactic acid polymer with nisin for use in antimicrobial food packaging. *Journal of Food Science*, **73**, M127–M134.

Joffraud, J.J., Leroi, F., Roy, C. & Berdague, J.L. (2001). Characterization of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon**.** *International Journal of Food Microbiology*, **66**, 175-184.

Jorgensen, L.V., Huss, H.H. & Dalgaard, P. (2000). The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon. *Journal of Applied Microbiology*, **89**, 920-934.

Kerry, J.P., O'Grady, M.N. & Hogan S.A. (2006). Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review. *Meat Science,* **74**, 13–130.

Kirwan, M.J. (2003). Paper and paperboard packaging. In: *Food packaging technology* (edited by R. Coles, D. McDowell, M.J. Kirwan). Pp. 241–281. London, U.K.: Blackwell Publishing, CRC Press.

Kirwan, M.J. & Strawbridge, J. W. (2003). Plastics in food packaging. In: *Food packaging technology* (edited by R. Coles, D. McDowell, M.J. Kirwan). Pp. 174–240. London, U.K.: Blackwell Publishing, CRC Press.

Kotler, P. & Keller, K. (2006). Marketing management, p.729. 12th ed. Upper Saddle River, N.J. Pearson.

Kristinsson, H.G., Balaban, M.O. & Otwell, W.S. (2006). The influence of carbon monoxide and filtered wood smoke on fish muscle. In: *Modified Atmospheric Processing and Packaging of Fish - Filtered Smokes, Carbon Monoxide and Reduced Oxygen Packaging* (edited by W.S. Otwell, H.G. Kristinsson & M.O. Balaban). Pp. 29-52. Iowa, USA: Blackwell Publishing.

Kristinsson, H.G., Crynen, S. & Yaqiz, Y. (2008). Effect of a filtered smoke treatment compared to various gas treatments on aerobic bacteria in yellow-fin tuna steaks. *Food Science and Technology,* **41**, 746-750.

Kykkidou, S., Giatrakou, V., Papavergou, A., Kontominas, M.G. & Savvaidis, I.N. (2009). Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4 °C. *Food Chemistry,* **115**, 169–175.

Lagaron, J.M., Catala, R. & Gavara, R. (2004). Structural characteristics defining high barrier properties in polymeric materials. *Materials Science and Technology*, **20**, 1–7.

Landau S. (2007). The future of flavour and odour release. In: *the future of caps and closures latest innovations and new applications for caps and closures*. Intertech Pira conference. 20–21 June 2007, Atlanta, Ga.

Lau, O.W. & Wong, S.K. (2000). Contamination in food from packaging materials. *Journal of Chromatography A*. **882**, 255–270.

Lauzon, H. L., Magnusson, H. Sveinsdottir, K., Gudjonsdottir, M. & Martinsdottir, E. (2009). Effect of brining, modified atmosphere packaging, and super-chilling on the shelf-life of cod (*Gadus morhua*) loins. *Journal of Food Science*, **74**, 258-267.

Lee, D.S., Shin, D.H., Lee, D.U., Kim, J.C. & Cheigh, H.S. (2001). The use of carbon dioxide absorbents to control pressure build-up and volume expansion of Kimchi packages. *Journal of Food Eng*ineering, **48**, 183–188.

Lopez-Rubio, A., Almenar, E., Hernandez-Munoz, P., Lagaron, J.M., Catala, R. & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International*, **20**, 357-387.

Mahajan, P.V., Caleb, O.J., Singh, Z., Watkins, C.B. & Geyer, M. (2014). Postharvest treatments of fresh produce. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, **372**, 20130309.

Mangaraj, S., Goswami, T.K. & Mahajan, P.V. (2009). Applications of plastic films for modified atmosphere packaging of fruits and vegetables: A review. *Food Engineering Reviews*, **1**, 133-158.

Marsh, K. & Bugusu, B. (2007). Food packaging roles, materials, and environmental issues. *Journal of Food Science*, **72**, R39–R54.

Martınez, L., Djenane, D., Cilla, I., Beltran, J.A. & Roncales, P. (2006). Effect of varying oxygen concentrations on the shelf-life of fresh pork sausages packaged in modified atmosphere. *Food Chemistry*, **94**, 219–225.

Masniyom, P., Benjakul, S. & Visessanguan, W. (2006). Synergistic antimicrobial effect of pyrophosphate on *Listeria monocytogenes* and *Escherichia coli* O157 in modified atmosphere packaged and refrigerated sea bass slices. *LWT-Food Science and Technology*, **39**, 302-307.

Masniyom, P. (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Songklanakarin Journal of Science and Technology*, **33**, 181-192.

Masniyom, P., Benjama, O. & Maneesri, J. (2011). Extending the shelf-life of refrigerated green mussel (*Perna viridis*) under modified atmosphere packaging. *Sonklanakarin Journal of Science and Technology*, **33**, 181-192.

Maqsood, S. & Benjakul, S. (2010). Synergistic effect of tannic acid and modified atmospheric packaging on the prevention of lipid oxidation and quality losses of refrigerated striped catfish slices. *Food Chemistry*, **121**, 29–38.

Mastromatteo, M., Conte, A. & Del Nobile M.A. (2010). Combined use of modified atmosphere packaging and natural compounds for food preservation. *Food Engineering Review*, **2**, 28–38.

McMillin, K.W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat Science*, **80**, 43-65.

Monahan, F.J. (2000). Oxidation of lipids in muscle foods: fundamental and applied concerns. In: *Antioxidants in Muscle Foods* (edited by E.A. Decker, C. Faustman & C.J. Lopez-Bote). Pp.3-23. New York: Wiley-Interscience.

Moreira, A.B., Visentainer, J. V., De Souza, N. E. & Matsushita, M. (2001). Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. *Journal of Food Composition and Analysis* **14**, 565–574.

Mullan, M. & McDowell, D. (2003). Gases used in Modified atmosphere packaging. : *Food packaging technology* (edited by R. Coles, D. McDowell, M.J. Kirwan). Pp. 304–306. London, U.K.: Blackwell Publishing, CRC Press

Munasinghe, D.M.S., Ohkubo, T. & Sakai, T. (2005). The lipid peroxidation induced changes of protein in refrigerated yellowtail minced meat. *Fisheries Science*, **71**(2), 462-464.

Muncke, J. (2009). Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source? *Science of the Total Environment*, **407**, 4549–4559.

Nerin, C., Tovar, L., Djenane, D., Camo, J., Salafranca, J., Beltran, J.A. & Roncales, P. (2006). Stabilization of beef meat by a new active packaging containing natural antioxidants. *Journal of Agricultural and Food Chemistry*, **54**, 7840–7846.

Oguzhan, P., Kaban, G. & Atamanalp, M. (2013). The effects of modified atmosphere packaging on microbiological, chemical properties of hot smoked Rainbow Trout (*Oncorhynchus mykiss*) fillets. *Journal of Microbiology Research*, **1**, 16-22.

Olson, B.E. (2006). Commercial aspects of filtered wood smoke technology compared to carbon monoxide gassing of seafood products. In: *Modified Atmospheric Processing and Packaging of Fish - Filtered Smokes, Carbon Monoxide and Reduced Oxygen Packaging* (edited by W.S. Otwell, H.G. Kristinsson & M.O. Balaban). Pp.15-28. Iowa, USA: Blackwell Publishing.

Opara, U.L. 2010. Editorial: High incidence of postharvest food losses is worsening global food and nutrition security. *International Journal of Postharvest Technology Innovation*, **2**, 1-3.

Opara U.L & Mditshwa, A. (2013). A review on the role of packaging in securing food system: adding value to food products and reducing losses and waste. *African Journal of Agricultural Research*, **8**, 2621-2630.

Ordonez, J.A., Lopez-Galvez, D.E., Fernandez, M., Hierro, E. & de la Hoz, L. (2000). Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, **80**, 1831-1840.

Ozdemir, M. & Floros, J.D. (2004). Active food packaging technologies. *Critical Reviews in Food Science and Nutrition*, **44**, 185-193.

Ozogul, F., Polat, A. & Ozogul, Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). *Food Chemistry*, **85**, 49-57.

Pacquit, A., Frisby, J., Diamond, D., Lau, K. T., Farrell, A., Quilty, B. & Diamond, D. (2007). Development of a smart packaging for the monitoring of fish spoilage. *Chemistry*, **102**, 466-470.

Page, B., Edwards, M. & May N. (2003). Metal cans. In: *Food packaging technology* (edited by R. Coles, D. McDowell and M. J. Kirwan. Pp. 121–151. London, United Kingdom: Blackwell Publishing, CRC Press.

Pantazi, D., Papavergou, A., Pournis, N., Kontominas, M.G. & Savvaidis, I.N. (2008). Shelf-life of chilled fresh Mediterranean swordfish (*Xiphias gladius*) stored under various packaging conditions: microbiological, biochemical and sensory attributes. *Food Microbiology*, **25**, 136-143.

Parfitt, J., Barthel, M. & Macnaughton, S. (2010). Food waste within food supply chains: quantification and potential for change to 2050. *Philosophical Transactions of the Royal Society of Biological Sciences*, **365**, 3065-3081.

Peck, M.W., Goodburn, K.E., Betts, R.P. & Stringer, S.C. (2008). Assessment of the potential for growth and neurotoxin formation by non-proteolytic Clostridium botulinum in short shelf-life commercial foods designed to be stored chilled. *Trend in Food Science and Technology*, **19**, 207-216.

Pereira de Abreu, D.A., Paseiro-Losada, P., Maroto, J. & Cruz, J.M. (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar L.*). *Food Research International*, **43**, 1277-1282.

Pushparajan, N., Varadharajan, D. & Soundarapandian, P. (2013). Feasibility of fish and shellfish curries stored in indigenous polymer coated tin free steel cans. *Journal of Aquatic Research Development*, **4**, 2

Quintavalla, S. & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science*, **62**, 373–380.

Ravi-Sankar, C.N., Lalitha, K.V., Jose, L., Manju, S. & Gopal, T.K.S. (2008). Effect of packaging atmosphere on the microbial attributes of pearl-spot (*Etroplus suratensis Bloch*) stored at 0-2°C. *Food Microbiology*, **25**, 518-528.

Restuccia, D., Spizzirri, U. G., Parisi, O.I., Cirillo, G., Curcio, M., Iemma, F. & Picci, N. (2010). New EU regulation aspects and global market of active and intelligent packaging for food industry applications. *Food Control*, **21**, 1425-1435.

Robertson, G.L. (Ed.). (2010). Food Packaging and Shelf Life: A Practical Guide. In: *Role of Food Packaging* (edited by G.L. Robertson). Pp. 1–4 CRC Press, Boca Raton, Fl.

Ruffine, L., Donval, J.P., Charlou, J.L., Cremiere, A. & Zehnder, B.H. (2010). Experimental study of gas hydrate formation and destabilisation using a novel high-pressure apparatus. *Marine and Petroleum Geology*, **27**, 1157-1165.

Rutherford, T.J., Marshell, D.L., Andrews, L.S., Coggins, P.C., Schilling, M.W. & Gerard, P. (2007). Combined effect of packaging atmosphere and storage temperature on growth of *Listeria monocytogenes* on ready-to-eat shrimp. *Food Microbiology*, **24**, 703-710.

Sandhya, (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *Food Science and Technology*, **43**, 381-392.

Siah, W.M. & Ariff, W.M. (2007). Effect of modified atmosphere packaging and potassium sorbate on chemical, microbiological and sensorial properties of grouper (*Epinephelus* sp.) fillets. *Journal of Tropical Agriculture and Food Science*, **35**, 237-243.

Singh R.P. (2000). Scientific principles of shelf-life evaluation. In: *shelf-life evaluation of food*, (edited by D. Man & A. Jones) Pp. 3-22. 2nd edition, Gaithersburg, Md., Aspen Publishers.

Siripatrawan, U., Sanguandeekul, R. & Narakaew, V. (2009). An alternative freshness index method for modified atmosphere packaged abalone using an artificial neural network. *LWT - Food Science and Technology*, **42**, 343–349.

Sivertsvik, M. Jeksrud, W. K. & Rosnes, J.T. (2002). A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology*, **37**, 107-127.

Sivertsvik, M. (2007). The optimized modified atmosphere for packaging of pre- rigor filleted farmed cod (*Gadus morhua*) is 63ml/100ml oxygen and 37ml/100ml carbon dioxide. *LWT-Food Science and Technology*, **40**, 430-438.

Smiddy, M., Fitzgerald, M., Kerry, J.P., Papkovsky, D.B., O'sullivan, C.K. & Guilbault, G.G. (2002). Use of oxygen sensors to non-destructively measure the oxygen content in modified atmosphere and vacuum packed beef: impact of oxygen content on lipid oxidation. *Meat Science*, **61**, 285-290.

Soccol, M.C.H. & Oetterer, M. (2003). Use of modified atmosphere in seafood preservation. *Brazilian Archives of Biology and Technology*, **46**, 569-580.

Speranza, B., Corbo, M., Conte, A., Sinigaglia, M. & Del Nobile, M. (2009). Microbiological and sensorial quality assessment of ready-to-cook seafood products packaged under modified atmosphere. *Journal of Food Science*, **74**, M473–M478.

Sriket, C., Benjakul, S. & Visessangguan, W. (2010). Post-mortem changes of muscle from fresh water prawn (*Macrobrachium rosenbergii*) as influenced by spawning stages. *LWT Food Science and Technology*, **43**, 608-616.

Stamatis, N. & Arkoudelos, J. (2007). Quality assessment of *Scomber colias japonicus* under modified atmosphere and vacuum packaging. *Food Control*, **18**, 292–300.

Stauffer, J.E. (2005). Radio frequency identification. *Cereal Food World*, **50**, 86–87.

Suppakul, P., Miltz, J., Sonneveld, K. & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*, **68**, 408–420.

Thanonkaew, A., Benjakul, S., Vissessanguan, W. & Decker, E.A. (2006). The effect of metal ions on lipid oxidation, colour and physicochemical properties of cuttlefish (*Sepia pharaonis*) subjected to multiple freeze-thaw cycles. *Food Chemistry*, **95**, 591-599.

Thiansilakul, Y., Benjakul, S. & Richards, M. P. (2010). Changes in heme proteins and lipids associated with off-odour of sea bass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus* and *O. niloticus*) during iced storage. *Food Chemistry*, **121**, 1109-1119.

Torrieri, E., Cavella, S., Villani, F. & Masi, P. (2006). Influence of modified atmosphere packaging on the chilled shelf-life of gutted farmed bass (*Dicentrarchus labrax*). *Journal of Food Engineering*, **77**, 1078–1086.

Torrieri, E., Carlino, P.A., Cavella, S., Fogliano, V., Attianese, I., Buonocore, G.G. & Masi, P. (2011). Effect of modified atmosphere and active packaging on the shelf-life of fresh blue-fin tuna fillets. *Journal of Food Engineering*, **105**, 429-435.

Triqui, R. & Bouchriti, N. (2003). Freshness assessments of Moroccan Sardine (*Sardina pilchardus*): Comparison of overall sensory changes to instrumentally derived volatiles. *Journal of Agricultural Food Chemistry*, **51**, 7540-7546.

Tsironi, T. N. & Taoukis, P. S. (2010). Modelling microbial spoilage and quality of gilthead sea-bream fillets: combined effect of osmotic pre-treatment, modified atmosphere packaging, and nisin on shelf life. *Journal of Food Science*, **75**, 243-251.

Undeland, I., Hall, G., Wendin, K., Gangby, I. & Rutgersson, A. (2005). Preventing lipid oxidation during recovery of functional proteins from herring (*Clupea harengus*) fillets by an acid solubilisation process. *Journal of Agricultural and Food Chemistry*, **53**, 5625-5634

Van-Willige R.W.G., Linssen J.P.H., Meinders M.B.J., Van der Steger H.J. & Voragen A.G.I. (2002). Influence of flavour absorption on oxygen permeation through low density polyethylene, polypropylene, polyvinyl chloride and polyethylene terephthalate plastic food packaging. *Food Additives Contamination*, **19**, 303–313.

Velu, S., Abu Bakar, F., Mahyudin, N.A., Saari, N. & Zaman, M.Z. (2013). Effect of modified atmosphere packaging on microbial flora changes in fishery products. *International Food Research Journal*, **20**, 17-26.

Verachia, W., Lazzarino, G., Niven, B. & Bremer, P. J. (2013). The effect of holding live sea urchins (*Evechinus chloroticus*) in air prior to gonad removal on gonad adenine nucleotide profiles during storage at 4˚ C. *Food Chemistry*, **141**, 841-846.

Verghese, K., Lewis, H., Lockrey, S. & Williams, H. (2013). FINAL REPORT: The role of packaging in minimising food waste in the supply chain of the future.

Vogel, B.F., Venkateswaran, K., Satomi, M. & Gram. L. (2005). Identification of *Shewanella baltica* as the most important hydrogen sulphide-producing species during iced storage of Danish marine fish. *Journal of Applied and Environmental Microbiology*, **71**, 6689-6697.

Wang, T., Sveinsdottir, K., Magnusson, H & Martinsdottir, E. (2008). Combined application of modified atmosphere packaging and super-chilled storage to extend the shelf-life of fresh cod (*Gadus morhua*) loins. *Journal of Food Science*, **73**, 11–19.

Wani, A.A., Singh, P., Gul, K., Wani, M.H. & Langowski, H.C. (2014). Sweet cherry (*Prunus avium*): Critical factors affecting the composition and shelf life. *Food Packaging and Shelf life*, **1**, 86-99.

World Packaging Organization (WPO), (2008). Market statistics and future trends in global packaging. World Packaging Organisation/PIRA International Limited. http:// www.worldpackaging.org/publications/documents/market-statistics.pdf (Accessed 18.07.2013).

Yam, K.L., Takhistov, P.T. & Miltz, J. (2005). Intelligent packaging: Concepts and applications. *Journal of Food Science*, **70**, 1–10.

Yesudhason, P., Gopal, T.K.S., Ravi Shankar, C.N., Lalitha, K.V. & Kumar, K.N.A. (2009). Effect of modified atmosphere packaging on chemical, textural, microbiological and sensory quality of seer fish (*Scomberomorus commerson*) steaks packaged in

thermo-formed trays at 0–2ºC. *Journal of Food Processing and Preservation* **33**, 777-797.

Yesudhason, P., Gopal, T.K.S., Ravi Shankar, C.N., Lalitha, K.V. & Kumar, K.N.A. (2010). Effect of potassium sorbate and modified atmosphere packaging on the shelf-life extension of seer fish (*Scomberomorus commerson*) steaks during iced storage. *Journal of Food Biochemistry*, **34**, 399–424.

Yesudhason, P., Lalitha, K. V., Gopal, T. K. S. & Ravi Shankar, C.N. (2014). Retention of shelf life and microbial quality of seer fish stored in modified atmosphere packaging and acetate pre-treatment. *Journal of Food Packaging and Shelf life*, **1**, 123-130.

Zhang, M., Zhan, Z. G., Wang, S. J. & Tang, J.M. (2008). Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT-Food Science and Technology*, **41**, 686-691.

Zhong, Y., Madhujith, T., Mahfouz, N. & Shahidi, F. (2007). Compositional characteristics of muscle and visceral oil from steelhead trout and their oxidative stability. *Food Chemistry*, **104**, 602-608.

CHAPTER 3

EFFECTS OF MODIFIED ATMOSPHERE PACKAGING, STORAGE TEMPERATURE AND ABSORBENT PADS ON THE QUALITY OF FRESH CAPE HAKE FISH FILLETS

Summary

Fresh ready-to-cook fish fillets are susceptible to loss of freshness and accelerated microbial spoilage under abuse temperature and packaging conditions. This study investigated the effects of modified atmosphere packaging (MAP), storage temperature and the use of absorbent pad (PAD) on the quality attributes (nutrient and gas composition, pH, colour, drip loss, texture, microbial growth and sensory) of Cape hake (*Merluccius capensis*) fish fillets. Fresh Cape hake fillets were packaged under active-modified atmosphere (40% CO_2 + 30% O_2 + 30% N_2) or passive-modified atmosphere (0.039% CO_2 + 20.95% O_2 + 78% N_2), with and without absorbent pad, and stored at 0°C, 4°C and 8°C (to mimic abuse temperature). The control fresh fillets were stored under passive-MAP without absorbent pads at 0°C, 4°C and 8°C. Headspace O_2 gas composition continuously decreased below critical limits under passive-MAP with increase in storage temperature. Similarly, O_2 levels decreased under active-MAP but did not reach critical levels with the lowest being 9.5% at 0°C. The interaction of storage temperature and modified atmosphere had a significant effect on quality attributes of Cape hake fillets. Drip loss was higher in active-MAP packaged fillets without PAD (0.64%) than passive-MAP packaged fillets without PAD (0.27%). Drip loss was significantly reduced by the use of absorbent pad ($p< 0.05$). Firmness, colour and pH were better maintained under active-MAP compared to passive-MAP at lowest temperature 0°C. Firmness (work of shear) of active-MA packaged Cape hake fillets on day 12 at 0°C and 4°C was 527 N/s and 506 N/s, respectively. Cape hake fillets packaged under active-MAP at 0°C had longer shelf life (12 days) than control passive-MAP fillets stored at 0°C (> 3 days). Due to the development of off-odour, loss of freshness, and higher aerobic mesophillic bacteria count (> 5.5 log cfu/g) as well as unfavourable changes in overall sensory attributes (appearance and odour). This shows the potential for active-MAP at 0°C, in conjunction with

high grade fillets, to deliver safe, shelf stable fish when good hygienic practices are used during packaging.

Introduction

Cape hake is a lean fish, rich in poly-unsaturated fatty acids (PUFAs) such as omega-3 fatty acids, eicosapentaenoic (EPA) acid and docosahexaenoic acid (Moreira *et al*., 2001). These fatty acids have been reported to increase fish nutritional value and guard against disease conditions in humans (Del Nobile *et al*., 2009). Poly-unsaturated fatty acids have been reported to lower blood pressure and cholesterol levels in humans (Kris-Etherton *et al*., 2002; Trondsen *et al*., 2003; Domingo *et al*., 2007). Fish has also been regarded as a healthy alternative to fatty meat products due to its high protein content which makes it beneficial in preventing the incidence of non-communicable diseases such as heart attacks and strokes (Domingo *et al*., 2007; Del Nobile *et al*., 2009).

Fish provides about 17% of the world's annual protein intake and only about 60% of palatable fish is utilized by end-users, thus the remainder is either converted into animal feed or lost (Ahmed, 2008). Postharvest loss due to decay is ~ 10 - 12 million tonnes annually and this is estimated as 10% of the total global captured and cultured fish (Ruckes, 2003; Ahmed, 2008; FAO, 2014). Numerous postharvest solutions have been proposed by researchers to maintain the quality of fish after harvest. These include salting, drying, smoking, cold storage and frying (Dondero *et al*., 2004; Bellagha *et al*., 2007).

Due to shift in consumer lifestyles globally, there is an increase in the demand for fresh ready-to-cook (RTC) fish and fishery products (Trondsen *et al*., 2003; De Silva & Yamao, 2006; Speranza *et al*., 2009). Modified atmospheres packaging (MAP) combined with optimum cold storage offers the possibility to extend shelf life and maintain quality for fish and fishery products (Ordonez *et al*., 2000; Erkan *et al*., 2007; Sivertsvik, 2007; Del Nobile *et al*., 2009; Lauzon *et al*., 2009; Yesudhason *et al*., 2009; Caglak *et al*., 2012; Angis & Oguzhan, 2013). Erkan *et al*. (2007) reported that active-modified atmospheres packaging: 70% CO_2 + 5% O_2 + 25% N_2) and vacuum packaging (VP) extended the shelf life of chub mackerel stored at 4°C for 12 and 9 days, respectively. Lauzon *et al*. (2009) indicted that, aerobic mesophillic bacteria was lower in cod fillets stored at -2°C in contrast to those stored at 0°C and MA packaged fillets had a shelf life of about 15d at 0°C and 21d at -2°C Furthermore, the use of active-MAP (A = 40% CO_2 + 30% O_2 + 30%; B = 50% CO_2 + 50% O_2 and C = 95% CO_2 + 0% O_2) at 4°C combined with natural preservatives on blue fish burger (mackerel and hake) led to lower aerobic mesophillic bacteria in fillets when compared to air packed fillets at day 28 (Del Nobile *et al*., 2009). Currently, there is no information on the combined effects of MAP, absorbent pads and storage temperature on the quality attributes of Cape hake fish. Thus, this study investigated the effects of MAP, combined with

absorbent pad and storage temperatures, on the physicochemical, microbiological and sensory quality attributes of Cape hake fish.

Materials and methods

Preparation of fish samples and packaging

Fresh Cape hake fillets (average weight of 200 g) were purchased from a local retail market in Stellenbosch, South Africa. Fillets were iced with appropriate quantity of ice (with 1:3 parts w/w flake/ice) and packed in sterile padded polystyrene box. The fillets were collected approximately 18 h after cutting and transported in an air-conditioned and ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University, South Africa within 15 min. On arrival, the fish fillets were kept on ice and packaged into the following treatments: active-MAP (40% CO_2 + 30% O_2 + 30% N_2) with and without (control) absorbent pad; passive-MAP (0.039% CO_2 + 20.95% O_2 + 78% N_2) with and without (control) absorbent pad.

All fish samples were packaged in polyethylene terephthalate (PET) trays with dimension 280 X 190 mm (Zibo containers (Pty.) Ltd., Kuilsrivier, South Africa) and heat sealed with bi-axially oriented polyester film (O_2 permeability: 75 cm^3 at 23°C, 70% RH bar^{-1}; water vapour permeability: 2 g d^{-1} at 38°C, 90% RH) from Knilam Packaging (Pty.) Ltd., South Africa. A total of 380 packages were used in this study. Modified atmosphere packaging was performed using a Multivac packaging machine (Multivac Traysealer T100, Sepp Hagenuller GmbH & Co.KG, Germany) and food grade gases from Air products Pty., South Africa and absorbent pads (Dri-Fresh®, Sirane Ltd., Shropshire, UK). The absorbent pads were made of an inert hygroscopic material according to manufacturer's specification. Packaged fillets were then stored at 0°C, 4°C and 8°C ± 0.5°C, for 15 d. Sample for analyses were taken on days 0, 3, 6, 9, 12 and 15 from each respective storage temperature. On each sampling time, three packs of fish from each lot were taken from each treatment and storage temperature. Analysis of microbiological and chemical parameters was terminated on the days sensory spoilage was observed (attributes like flesh colour and off-odour) for packages stored at 8°C and under passive-MA.

Headspace gas analysis and temperature

Before the packages were opened, changes in CO_2 and O_2 levels in the headspace of the tray was measured using a gas analyser with 0.5% accuracy (Checkmate 3, PBI Dansensor, Ringstead, Denmark) and reported as percentage (%) of the atmosphere composition inside the package. Three packages were measured for each treatment at sampling day. After the gas composition analyses, packs were unwrapped for further analysis of fillet quality attributes.

Physicochemical analysis

pH

Fish samples from each package treatment was homogenized in milliQ water (Millipak® express 40 filter unit, Merck KGaA, Darmstadt, Germany) and dilution was used to measure the pH using a Crison pH meter Basic 20+ (Crison, Barcelona, Spain). The pH meter was standardised using buffer solutions of pH 9.02 ± 0.01, 7.00 ± 0.01 and 4.01 ± 0.01 at 25ºC as described by Erkan *et al*. (2007).

Colour

Colour attributes of fillets were measured with a colorimeter (Chroma Meter CR-400, Minolta corp. Osaka, Japan). In order to make representative measurements, approximately five measures were taken on each fillet. The CIELAB parameters were assessed; L^* (describing lightness/brightness), a^* (describing the balance of green to red), and b^* (describing blue to yellow). Total colour difference (ΔE) was calculated based on the magnitude of colour difference between baseline sample and other sampling days using equation (Eqn.) 1:

$$\Delta E = \sqrt{(a - a_o^*)^2 + (b - b_o^*)^2 + (L - L_o^*)^2} \quad (1)$$

Where a_o^*, b_o^* and L_o^* are the baseline values for unpackaged fresh Cape hake fillets at day 0, and a^*, b^* and L^* are the values for fillets packaged under each treatment at each sampling day (Pathare *et al*., 2013).

Firmness measurements

Firmness of each fillet was determined by using texture profile analyser fitted with Warner Bratzler Blade-set (HDP/BS) (TA-XT Plus, Stable Micro Systems, Surrey, England). A test speed of 1.5 mm s^{-1} and distance of 30 mm was used for the study. Firmness was expressed as the work of shear (N/s) based on force required to shear through the fish muscle and connective tissues (Chen & Opara, 2013a: Chen & Opara, 2013b; Cheng *et al*., 2014).

Drip loss

Fish sample was carefully removed from the tray leaving behind the drip. Tray containing the drip was then weighed in order to obtain the weight of the drip loss. Individual package trays were weighed prior to packaging. Thus, drip loss was calculated based on difference between the gross weight of the package with drip and the known weight of the tray and expressed as a percentage loss based on the initial sample weigh as shown in Eqn. 2:

$$\%DL = \frac{W_i - W_s}{W_i} \; x \; 100 \qquad (2)$$

where DL is the drip loss (%), Wi is the gross weight of package and drip (g) and Ws is the known weight of tray (g). A conversion factor of 1 g = 1 ml was used to covert DL in weight (g) to volume (mL) (Goulas & Kontominas, 2007).

Microbial quality analysis

Approximately 1g of fish sample was taken from randomly selected packaged fillets for each treatment on the sampling d and mashed under aseptic conditions using mortar and pestle. Mashed sample was then diluted in test tubes containing 10 mL of sterile physiological saline solution (PSS) (0.85 g NaCl in 100 mL distilled water). Serial dilutions up to four-fold were prepared by adding 1 mL of homogenate sample to 9 mL PSS and vortex each dilution. In order to enumerate microbial load 1 mL of each, the dilutions were plated in triplicate onto appropriate media using the pour plate method (Da Silva *et al*., 2012).

Aerobic mesophillic bacteria was counted using plate count agar (PCA) method 4833; (SANS, 2007). The plates were incubated upside down at 37°C for 48 h (Hara-Kudo, *et al*., 2003). For the screening of *Escherichia coli* violet red bile glucose (VRBG) agar was used, plates were incubated upside down at 37°C for 24 h (method 21528-2; ISO, 2004). Presence

of *Vibrio parahaemolyticus* in fish fillet was investigated using a combination of thio-sulphate bile salt sucrose agar (TCBS) and HiChrome *vibrio* agar, method 21872-1 (ISO/TS, 2007). HiChrome *vibrio* Agar was used because colour development by *Vibrio* species is not affected by the presence of colonies of other bacteria as the amount of colour developed depends on the reaction of bacterial ß-galactosidase with the substrate contained in the media. The plates were then incubated upside down at 37°C for 24 h (Hara-Kudo *et al*., 2003). After incubation, plates with 30 - 300 colonies were counted. The results were transformed into Log colony forming unit (log CFU g^{-1}).

Proximate analysis

Proximate analysis was carried out only on day 0 and at the end of 12 and 15 days of refrigerated storage. Crude protein analysis was carried out using a modification of AOAC method 981.10 and AOAC 960.52 micro-kjeldahl methods (AOAC, 2007). Approximately 2 g of fish sample was digested with H_2SO_4 using the heating digestion unit (VELP Scientifica DKL, Italy). The digested sample was then distilled with distillation unit (UDK 129, VELP Scientifica, Italy) using NaOH, Boric acid and 5 drops of Bromo-cresol green indicator after cooling. The distillate was then titrated with 0.2 M HCl until the end point was reached i.e. when colour changes from green to pink. The titre volume of acid used in the titration was documented. A blank was set without Cape hake fillets as control. The percentage of protein content was calculated according to Eqn. 3:

$$\%\ Nitrogen = Normality\ of\ HCL\ x \frac{corrected\ acid\ vol.(ml)nx}{g\ of sample} x \frac{14gN}{mol} x100 \qquad (3)$$

Percentage protein is calculated by multiplying % nitrogen with a conversion factor of 6.25. The unit of normality is mol/100mL. Corrected acid volume = (mL standard acid for sample) – (mL standard for blank).

Fat content was analysed using AOAC method (2000). The solvent extraction unit (SER 148, VELP Scientifica, Italy) was used for fat extraction. About 3 g of the sample was extracted with the solvent extraction unit using petroleum ether (B.P. 60°C) as the extracting solvent.

The moisture content of fish fillets was measured using AOAC Official Method 934.01 (AOAC, 2002a). Clean, empty porcelain crucible were dried for 2 h at 100°C. The crucibles were then allowed to cool in desiccators for 30 min – 2 h. Approximately 2.5 g of sample was weighed into the dried crucibles and heated to 105°C for 24 h in a Pro-Lab oven and incubator (OTE 160L Lab tech. Separation Scientific, South Africa). The crucibles were

allowed to cool down for 30 min and then weighed. This was then expressed as a percentage of sample weight (AOAC, 2002a). Ash content was then determined by continuing with dried sampled obtained from moisture loss analysis and the samples were ashed using Muffle Furnace (LEF 115 P-1 Lab tech. Separation Scientific, South Africa). Crucibles containing moisture free fish samples were placed in the furnace and incubated at 500°C for 6 h. The crucibles were placed in a desiccator and allowed to cool overnight. The crucibles were then weighed accurately and expressed as a percentage of sample weight (AOAC, 2002b).

Sensory analysis

Consumer sensory analyses were based on hedonic scale using untrained sensory panels (Ivanov *et al*., 2009). The panel consisted of 10 untrained researchers who were familiar with fresh fish. The preferences and evaluation of the quality of Cape hake were determined immediately after opening the containers with chilled fish. The appearance, odour and overall acceptability of the Cape fillets were scored on five point hedonic scale: 1 = dislike a lot, 5 = like a lot. The average score for each parameter was calculated and was presented as the sensory scores appearance, odour and overall acceptability.

Statistical analysis

Statistical analysis was carried out using Statistica software (Statistica version 11, StatSoft Inc., Tulsa, USA). Factorial analysis of variance (ANOVA) at 95% confidence interval was used to evaluate the combined effect of MAP, storage temperature and absorbent pad on the quality of packaged Cape hake fillets. When there was statistical significance, of main factors and the interaction between them, the Fischer LSD multiple range Post-hoc-tests was used to determine the significant differences. In order to establish correlation trends between physicochemical, microbial and sensory quality of the examined Cape hake fillets data were processed according to principal component analysis (PCA) using XLSTAT software Version 2012.4.01 (Addinsoft, France). Significant correlation coefficients were classified as “strong, “moderate” and “weak” corresponding to $r > 0.7$, $r > 0.5$ - < 0.7 and $r < 0.5$, respectively.

Results and Discussion

Headspace gas composition

Level of O_2 decreased continuously across all treatments, with passive-MA packaged fillets reaching critical levels of 2.7% at the end of storage life (Fig. 1). In active-MA packaged fillets (Fig. 1a, b), the levels of O_2 decreased during storage; however, the levels did not reach critical limits and the lowest level was 9.5% at 0°C. Similar results were obtained for cod fillets package with gas composition (37% CO_2 + 63% O_2) and stored at 0°C for 14 d (Sivertsvik, 2007). The author reported that this decrease was associated with utilisation of O_2 by microbes and biochemical interactions such as oxidation of lipids in the fillets packed (Sivertsvik, 2007). High levels of O_2 assisted in inhibiting the development of anaerobic environments and preventing the occurrence of pathogenic anaerobes, such as non-proteolytic *Clostridium botulinum* (Masniyom, 2011).

Furthermore, in this study an initial decrease in CO_2 levels was observed for fillets stored under active-MAP across all temperatures. The decrease was higher at 0°C compared with 4°C and 8°C on day 3 (Fig. 1a, b). After day 3 there was a subsequent increase in CO_2 levels, the highest increase was observed at 4°C on day 12 with a value of 40.2% (Fig. 1a, b). The initial drop in CO_2 levels during first days of storage for MA stored fillets could be attributed to dissolution of CO_2 in muscle fluids of Cape hake fillets (Ruiz-Capillas & Moral, 2001; Sivertsvik *et al*., 2004; Torrieri *et al*., 2006). This trend confirms that CO_2 becomes more soluble in water at lower temperatures (Sivertsvik *et al*., 2002). Related results were reported by Cyprian *et al*. (2013) in their study on Nile tilapia packaged in 50% CO_2 + 50% N_2 and 100% air and stored at -1°C and 1°C for 27 and 20 days, respectively. The authors stated that, CO_2 levels dropped initially during storage until day 3 although it stabilized later with fillets packed under MA (Cyprian *et al.*, 2013). Similarly, CO_2 levels in fresh cod loins packaged with gas composition (50% CO_2 + 5% O_2 + 45% N_2) and stored at 1.5°C and -0.9°C for 21 days, decreased rapidly in the first few days of storage then latter increased. The rate of decrease was higher at lower temperatures (Wang *et al*., 2008).

Subsequently, as storage time increased the levels of CO_2 increased in both MAP conditions. The increase in CO_2 levels is associated with the action of spoilage microbes and enzymes (Wang *et al.*, 20008).

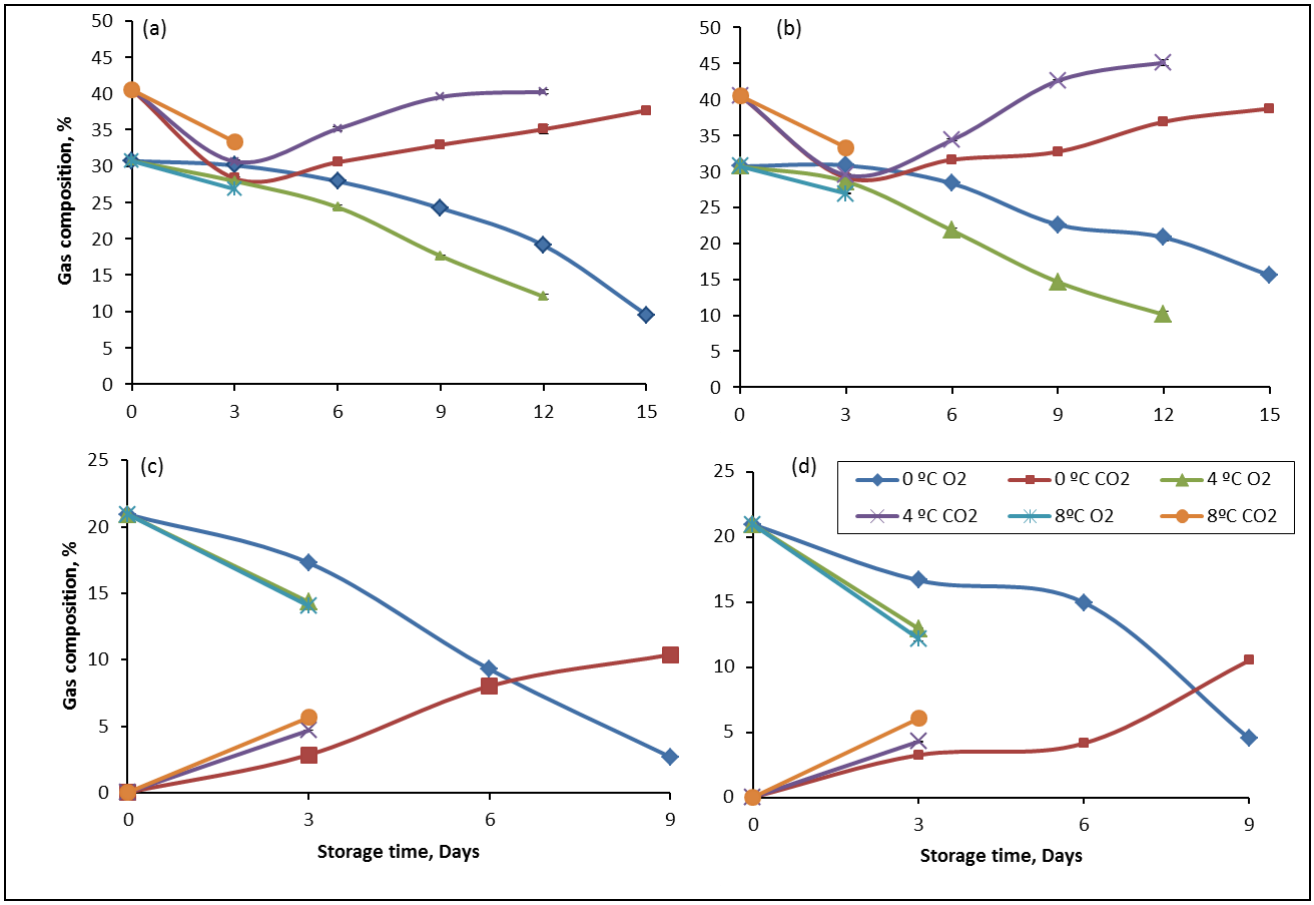

Figure 1 Effects of absorbent pad, packaging, temperature (0°C, 4°C and 8°C) and time on oxygen and carbon dioxide gas composition during storage (a) MAP no PAD, (b) MAP plus PAD, (c) PMAP no PAD and (d) PMAP plus PAD respectively; while MAP = active-MAP, PMAP = passive-MAP.

Proximate composition

There were no significant changes in proximate composition of active-MA packaged fillets at 0°C after 15 days in storage in comparison to day 0. In contrast, proximate composition of fillets stored under passive-MAP decreased due to deterioration in fish flesh (results not shown). Fat, protein, moisture and ash content values ranged from 0.29 - 0.286%, 17.22 - 17.21%, 82.95 - 82.88% and 4.90 - 4.80%, respectively, for active-MAP stored fillets after storage. This agrees with findings reported by Del Nobile *et al.* (2009) for blue fish burger consisting of fresh hake and mackerel fillets stored under three different gas compositions 40% CO_2 + 30% O_2 + 30% N_2, 50% CO_2 + 50 % O_2 and 5% O_2 +95% CO_2 for 28 days at 4°C. The authors observed that the different gas compositions had no significant effect on the

fat, moisture and protein content of hake and mackerel fillets. Results obtained were also in agreement with findings on chub mackerel fish packaged with 50% CO_2 + 50% N_2 and stored at 3°C and 6°C for 15 days. Proximate composition was not influenced by MA (Stamatis & Arkoudelos, 2007).

Nevertheless, the proximate composition obtained in this study differed from those reported for hake fish from the Galician Sea, Spain stored at -10°C and -30°C for 40 weeks. The values obtained were moisture (80.2%), ash (1.17%), total protein (18.7%) and fat (0.9%) (Careche *et al*., 2002). The difference in proximate composition may be explained based on the fact that chemical composition of marine fish are dependent on catching period, fish diet, habitat, fish size, deviations due to season and gender and other environmental conditions (Pacheco-Aguilar *et al*., 2000).

Physicochemical analysis

pH

Average pH at the end of storage was 6.3 and 7.1 for active-MAP and passive-MAP, respectively (Fig. 2 and Table 1). A significant increase in pH was observed for all treatments during the storage period ($p < 0.05$). Temperature, packaging and duration had a significant impact on pH of fillets (Table 1). Lower values of pH were observed in active-MA packaged fillets stored at 0°C (6.18) in comparison to 4°C (6.43) (Table 1). However, pH was higher in active-MA packs without PAD than those with PAD and this was consistent for all temperature conditions (Fig. 3). The pH values of fillets stored at 0°C on day 15 for active-MA package without PAD was 6.23, with PAD was 6.13 (Table 1). In contrast, passive-MA packaged fillets had pH values of 7.96 and 7.94 for no PAD and plus PAD fillets respectively; on day 9 at 0°C (Table 1). In addition, the fillets stored under active-MAP, exhibited an initial slow increase in pH at the start of the storage period. This was due to dissolved CO_2 in the liquid phase of the muscle tissue giving rise to non-dissociated carbonic acid as observed by other researchers (Ordonez *et al*., 2000; Sivertsvik, 2007: Caglak *et al*., 2012).

There was a subsequent increase in pH as storage progressed in fillets stored at 0°C and 4°C under active-MAP. At the end of storage, pH was higher in fillets packed under passive-MA than active-MA. These results corroborate the report on active-MA packaged ready-to-eat hake, chub mackerel, yellow gurnard and cuttle fillets stored at 4°C for 14 days. Lower pH

was observed under active-MA in comparison to passive-MA (Speranza *et al.*, 2009). Thus, based on the role of pH in limiting microbial growth, gas composition of 40% CO_2 + 30% O_2 + 30% N_2 at 0°C is ideal in storing Cape hake fillets due to the observed lowest differences of pH during the trials.

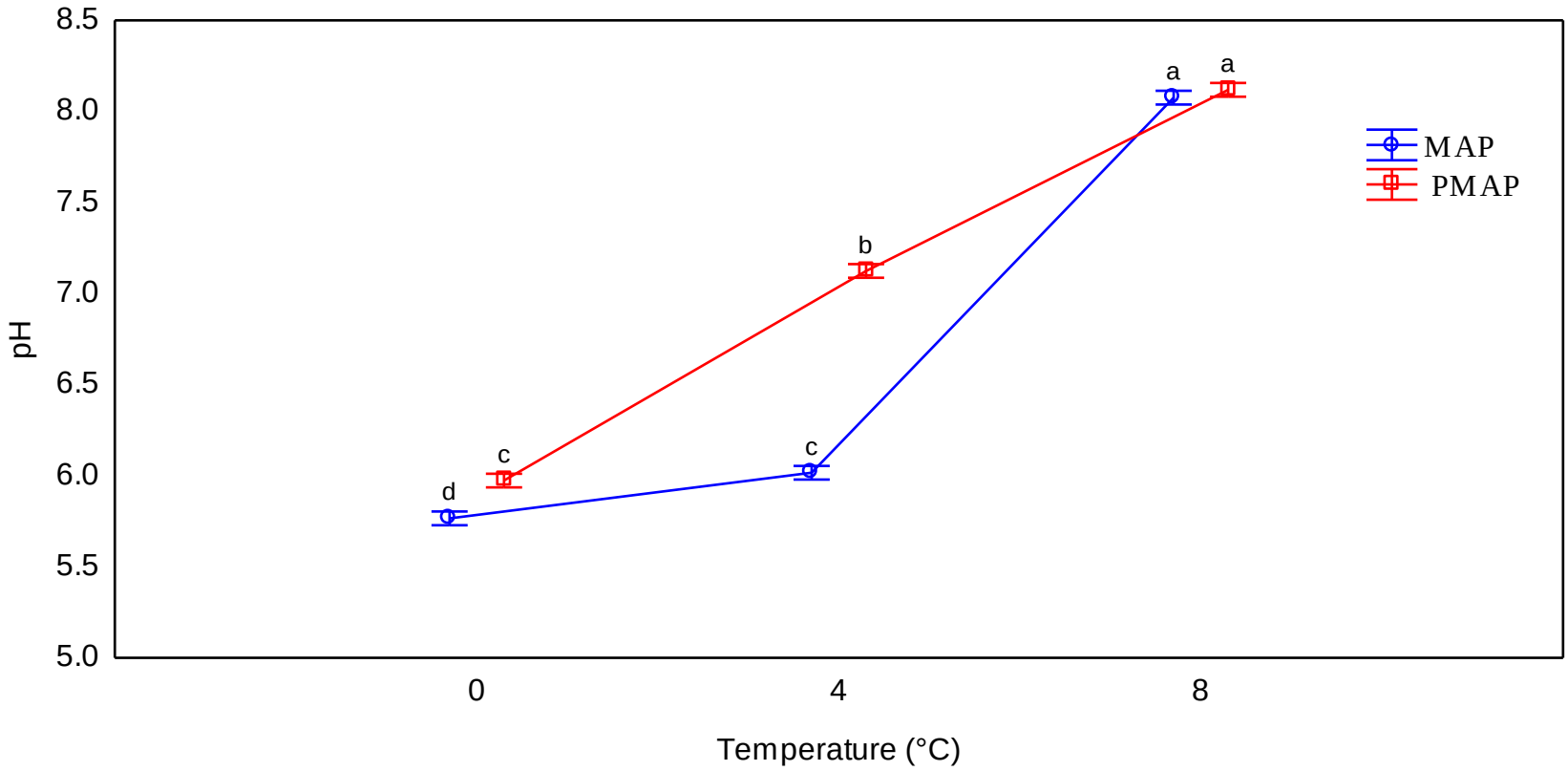

Figure 2 Effects of active and passive modified atmosphere packaging and storage temperatures (0°C, 4°C and 8°C) on the change in pH on day 3. Different letters represents mean values that are significantly different ($p < 0.0001$). MAP: active-MA and PMAP: passive-MA

Table 1 Effects of storage temperatures (0°C, 4°C and 8°C), duration (d), modified atmosphere packaging with/without absorbent pad on pH of Cape hake fillets

STORAGE DAYS	TEMP. (ºC)	MAP – PAD	MAP + PAD	PMAP - PAD	PMAP + PAD
0		5.7 ± 0.013^{n}	5.7 ± 0.013^{n}	5.7 ± 0.013^{n}	5.7 ± 0.013^{n}
3	0	5.8 ± 0.004^{m}	5.7 ± 0.006^{n}	6.1 ± 0.004^{j}	5.9 ± 0.004^{l}
	4	6.1 ± 0.009^{j}	5.9 ± 0.006^{l}	7.2 ± 0.006^{e}	7.1 ± 0.003^{f}
	8	7.4 ± 0.004^{d}	7.1 ± 0.005^{f}	8.2 ± 0.005^{a}	8.1 ± 0.004^{b}
6	0	6.0 ± 0.006^{k}	6.0 ± 0.006^{k}	7.9 ± 0.013^{c}	7.9 ± 0.013^{c}
	4	6.4 ± 0.006^{g}	6.3 ± 0.006^{h}	nd	nd
9	0	6.2 ± 0.010^{l}	6.1 ± 0.003^{j}	nd	nd
	4	6.4 ± 0.012^{g}	6.3 ± 0.005^{h}	nd	nd
12	0	6.2 ± 0.004^{i}	6.1 ± 0.004^{j}	nd	nd
	4	6.4 ± 0.005^{g}	6.3 ± 0.006^{h}	nd	nd
15	0	6.2 ± 0.004^{i}	6.1 ± 0.004^{j}	nd	nd

nd = Not determined due to sensory rejection; All values rounded off to one significant figure; Different letters indicate significant difference in pH values ($p < 0.05$); MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when sensory spoilage and critical bacterial counts was reached thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well. Also sensory spoilage was observed in fillets stored under PMAP at 0°C by day therefore drip loss analysis was stopped on day 6

Drip loss

Drip loss increased significantly with storage time for Cape hake fillets stored under active-MA as well as for those under passive-MA without absorbent pad. Absorbent pad was effective in preventing accumulation of drips in other treatments (Fig. 3). Furthermore, the interaction of storage temperature, duration and absorbent pad had a significant influence on drip loss in fillets packaged under active-MA (Fig. 4). Drip loss was highest at the lowest temperature as follow: at 0°C (1.39%), 4°C (1.16%) and 8°C (1.14%). This might be due to the increased solubility of CO_2 at lower temperatures, resulting in lower pH, which leads to loss of water holding capacity in fish tissue and release of drips (Ordonez *et al*., 2000; Sivertsvik, 2007).

Similar results were obtained by Fletcher *et al*. (2004) who studied the impact of two different gas mixtures (100% CO_2 and 40% CO_2+ 60% N_2) on the quality of fresh King salmon stored at 0°C for 90 days. The authors stated that, drip loss increased with storage time and higher drip loss was reported in the 100% CO_2 packages (Fletcher *et al*., 2004). This was also corroborated by an investigation on the effect of MAP (37% CO_2 + 63% O_2) on cod fillets stored at 0°C for 14 days. Presence of CO_2 in the MA-packaged cod fillets resulted in formation of drips and the higher the levels of CO_2 in a package the higher the drips (Sivertsvik, 2007). Studies have shown that, dissolved CO_2 in fish tissues leads to reduced pH in MA-packaged fillets. This initiates drip loss which might be due to tissue pliability and loss of fluid retaining ability (Soccol & Oetterer, 2003; Ayala *et al*., 2010; Masniyom, 2011). The presence of drip loss in packaged fillets reduces the freshness quality. Therefore, the use of absorbent pads assists in improving fillet quality.

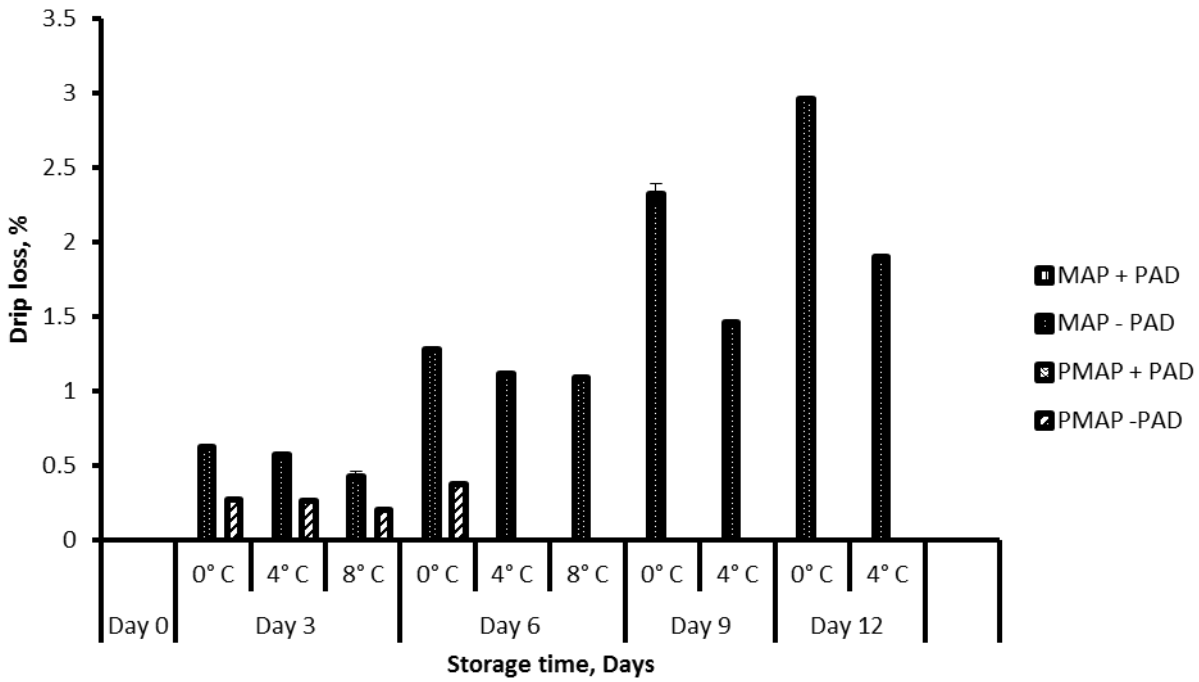

Figure 3 Effects of storage temperatures (0°C, 4°C and 8°C), duration (d), modified atmosphere packaging with/without absorbent pad on drip loss of Cape hake fillets. Different letters indicate significant difference in pH values ($p < 0.001$).

MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when sensory spoilage was observed thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well. Also sensory spoilage was observed in fillets stored under PMAP at 0°C by day therefore drip loss analysis was stopped on day 6

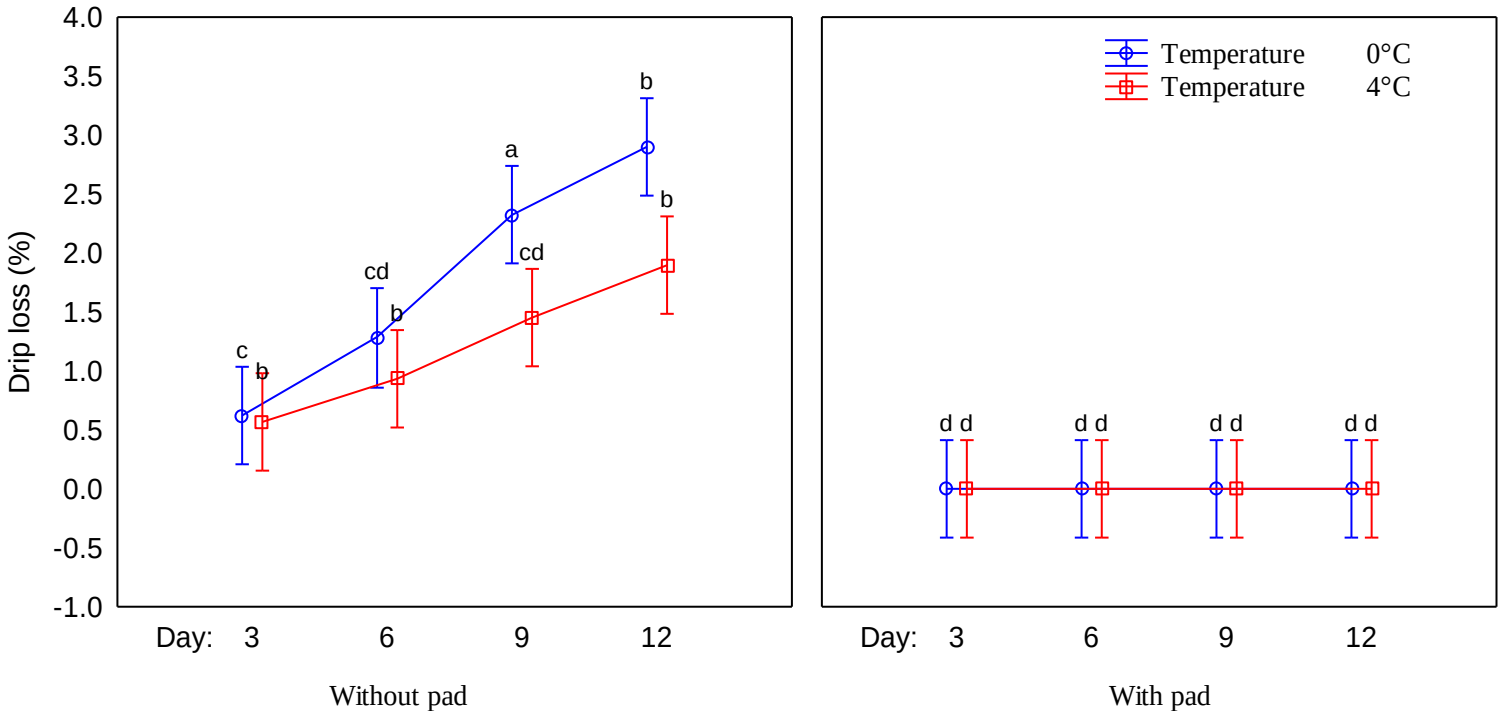

Figure 4 Effect of the interaction of storage temperature (0°C, 4°C and 8°C), with or without absorbent pad and storage duration (d) on drip loss for active-MA packaged fillets, different letters indicate significant difference in pH values ($p < 0.001$).

MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad

Fillet firmness

Firmness of fresh Cape hake fillet at day 0 based on the measurement of the work of shear was 838.9 ± 34.1 N/s, and this decreased over time during storage across all treatments. Fillets stored under active-MAP maintained better firmness than those under passive-MAP across all treatments. The interaction of modified atmosphere gas composition (in packages) and storage temperature had a significant effect ($p < 0.001$) on firmness of the hake fillets (Fig.5 and Table 2). Passive-MA packaged fillets stored at 8°C had lowest firmness values of 512.7 ± 0.7 N/s by day 3. As storage time progressed the firmness of hake fillets reduced, but the firmness at lower temperatures was better across all treatments. This agrees with studies by Roth *et al*. (2009), that higher temperatures and time causes stored fish fillets to lose their firmness. This is because degradation of fish muscles was better delayed under active-MAP at lower temperatures than under passive-MAP at 0°C, 4°C and 8°C. Similar effect was observed for Atlantic salmon stored at -3.6°C and -1.4°C for 34 days. Firmness decreased with storage duration and lower temperature better helped to maintain firmness in stored salmon fillets (Duun & Rustad, 2008).

The use of absorbent pad had a significant impact on the firmness, across all temperatures. Active-MAP with absorbent pad maintained better firmness at 0°C than other temperature conditions during storage (Table 2). This could be attributed to the ability of the absorbent pad to better absorb drips in the pack and delay the degradation of fish muscles which results in tissue pliability and hastens loss of fish freshness (Ayala *et al*., 2010). Firmness is a vital attribute for fresh or ready-to-cook (RTC) fish, soft fillets are however a drawback in marketing of fresh RTC fish (Hultmann & Rustad, 2004). Therefore, the use of absorbent pads offers value addition to improve fillet quality.

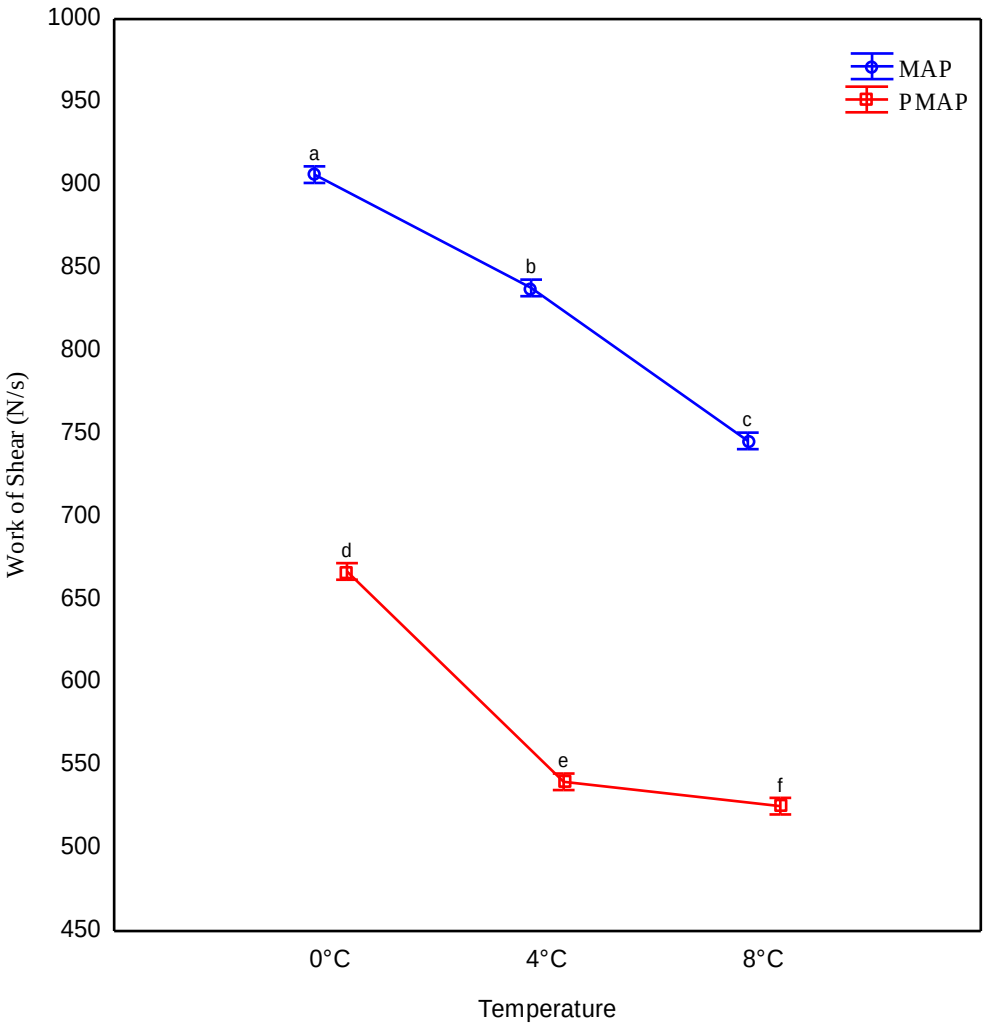

Figure 5 Effect of temperature and packaging on firmness (work of shear (N/s)) on day 3, with absorbent pad. Different letters indicates significant difference in work of shear values **(***p* < 0.001). MAP active-MAP, PMAP passive-MAP

Table 2 Effect of packaging with/without absorbent pad, temperature (0°C, 4°C and 8°C) and storage time on firmness (work of shear (N/s)) of Cape hake fillet

STORAGE DAYS	TEMP. (ºC)	MAP – PAD	MAP + PAD	PMAP - PAD	PMAP + PAD
0		838.9± 3.79^{a}	838.9± 3.79^{a}	838.9± 3.79^{a}	838.9± 3.79^{a}
3	0	814.0 ± 0.06^{b}	819.4 ± 1.22^{ab}	649.7± 2.30^{f}	675.3 ± 2.54^{e}
	4	807.7 ± 2.31^{c}	812.1 ± 0.06^{c}	517.1 ± 0.52^{l}	563.4 ± 1.16^{h}
	8	741.2 ± 0.06^{d}	749.2 ± 1.97^{d}	512.7 ± 0.71^{m}	539.3 ± 0.94^{j}
6	0	809.7 ± 0.45^{c}	814.4 ± 1.29^{bc}	524.0 ± 0.37^{k}	538.3 ± 0.26^{j}
	4	799.4 ± 4.62^{c}	809.0 ± 1.28^{c}	nd	nd
9	0	665.9 ± 2.02^{e}	673.0 ± 0.02^{e}	nd	nd
	4	635.8 ± 1.32^{g}	648.5 ± 2.77^{f}	nd	nd
12	0	527.2 ± 1.85^{j}	543.3 ± 2.39^{i}	nd	nd
	4	506.4 ± 0.72^{n}	523.8 ± 0.54^{k}	nd	nd

nd = Not determined due to sensory rejection; All values rounded off to one significant figure; Different letters indicate significant difference in pH values ($p < 0.05$); MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when sensory spoilage was observed thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well. Also sensory spoilage was observed in fillets stored under PMAP at 0°C by day therefore drip loss analysis was stopped on day 6

Colour measurement

Total colour difference (TCD) of fillets is presented in (Fig. 6 and Table 3). Temperature and duration led to significantly increases in TCDs during storage of fillets ($p < 0.05$). In addition, fillets stored under active-MAP had lower TCDs than those under passive-MAP (control), with fillets stored under active-MAP at 0°C having the lowest TCD of 5.9 on day 12 (Fig. 6). The change in fillets TCD is consistent with results obtained for Atlantic salmon stored at -1.4°C and 3.6°C for 34 days (Duun & Rustad, 2008). The authors reported that, temperature and duration are vital indices in determining colour changes in Atlantic salmon fillets (Dunn & Rustad, 2008). Similarly, Regost *et al*. (2004) investigared the influence of essential oils and cold storage at 4°C and 20°C on muscle quality attributes of Atlantic salmon. The colour of Atlantic salmon was shown to be dependent on both storage duration and temperature (Regost *et al*., 2004).

Oxidation of haemoglobin pigments is a major source of colour change during storage of fish fillets (Mancini & Hunt, 2005; Sone *et al*., 2012). Chaijan *et al*. (2004) reported on pigment and colour differences in tissues of mackerel and sardine stored at 4°C for 15 days. It was observed that, levels of pigment and heme iron reduced while, non-heme iron levels improved through iced storage (Chaijan *et al*., 2004). Thus, fish fillet colour is best maintained at lower temperatures. This corroborated our results that lower temperature and active-MAP lowered the TCDs of fillets during storage.

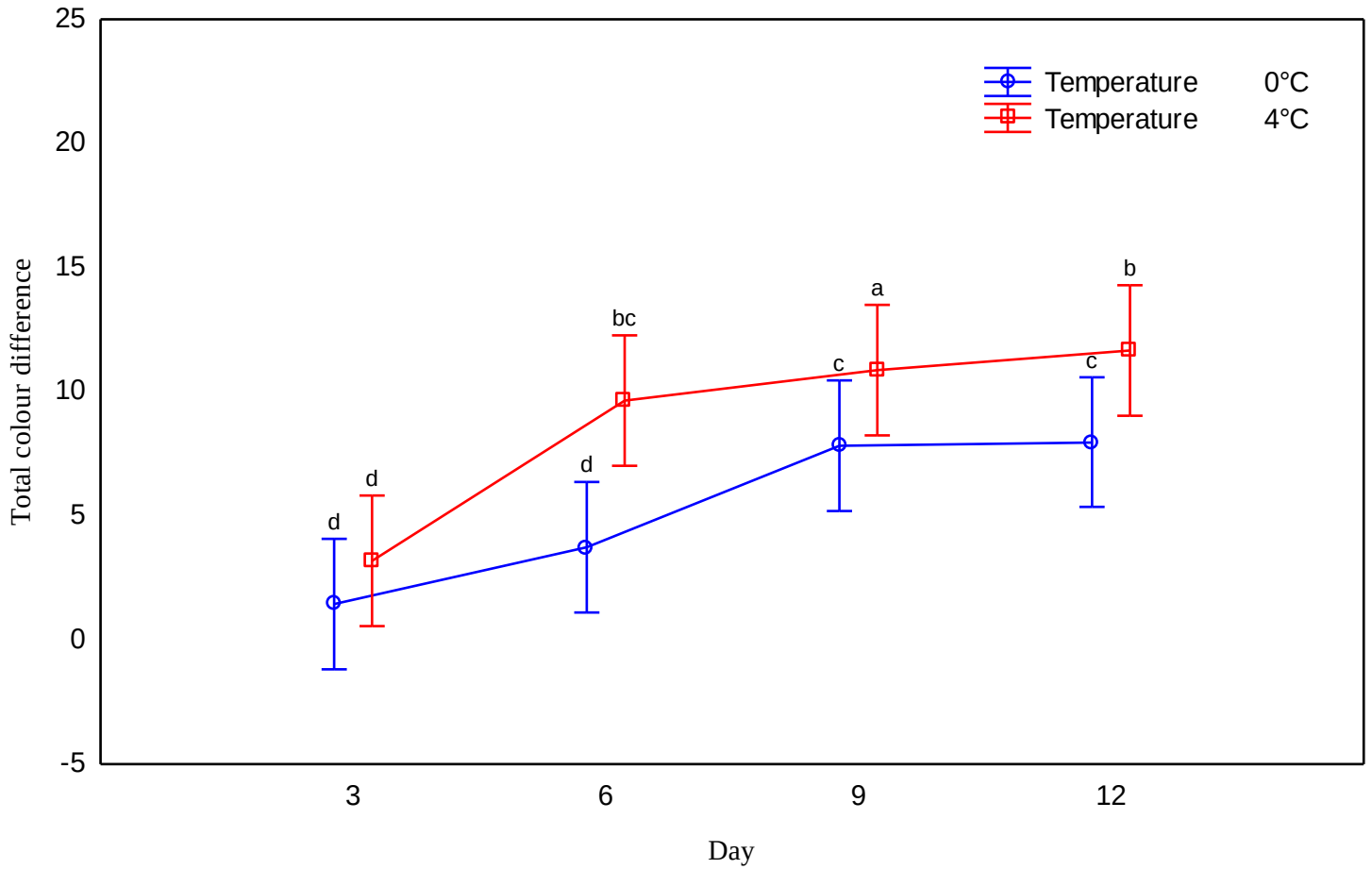

Figure 6 Effect of modified atmosphere packaging, temperature (0°C and 4°C) and duration (d) on total colour difference of stored Cape hake fillets Different letters indicate significant difference in work of shear values ($p < 0.02$).

Table 3 Effects of packaging with/without absorbent pad (MAP + PAD, MAP – PAD, PMAP + PAD and PMAP – PAD), temperature (0°C, 4°C and 8°C) and storage time (3-12 d) on total colour difference of Cape hake fillet

STORAGE DAYS	TEMP. (ºC)	MAP – PAD	MAP + PAD	PMAP - PAD	PMAP + PAD
3	0	1.0 ± 0.06^{n}	0.9 ± 0.07^{n}	1.9 ± 0.044^{l}	1.8 ± 0.044^{m}
	4	2.6 ± 0.15^{k}	2.6 ± 0.16^{j}	5.1 ± 0.49^{g}	2.9 ± 0.18^{i}
	8	5.7 ± 0.21^{f}	3.6 ± 0.37^{h}	5.8 ± 0.72^{e}	5.8 ± 0.25^{e}
6	0	4.8 ± 0.14^{g}	2.8 ± 0.09^{i}	8.3 ± 0.46^{b}	5.9 ± 0.41^{f}
	4	8.9 ± 0.48^{d}	5.5 ± 0.20^{e}	nd	nd
9	0	5.8 ± 0.35^{e}	3.1 ± 0.25^{i}	nd	nd
	4	9.3 ± 0.32^{b}	5.6 ± 0.42^{f}	nd	nd
12	0	5.9 ± 0.17^{ef}	3.1 ± 0.05i	nd	nd
	4	9.9 ± 0.42^{a}	5.7 ± 0.42^{e}	nd	nd

nd = Not determined due to sensory rejection; All values rounded off to one significant figure; Different letters indicate significant difference in pH values ($p < 0.05$); MAP-PAD: active-MA without pad; MAP + PAD: active-MA with pad, PMAP - PAD: passive-MA without pad and PMAP + PAD: passive-MA with pad.

Sampling was stopped on days when sensory spoilage was observed thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well. Also sensory spoilage was observed in fillets stored under PMAP at 0°C by day therefore drip loss analysis was stopped on day 6

Microbiology quality

Fillets used in this study had initial good microbial quality as evidenced by low initial aerobic mesophillic bacteria (1.2 log cfu/g) and the absence of *Vibrio* spp and *E. coli*. Lowest aerobic mesophillic bacteria were observed in fillets stored under active-MAP, for fillets stored at 0°C had the least counts of 5.2 log cfu/g on day 12. The interaction of MAP, absorbent pad and storage temperature had a significant effect on the aerobic mesophillic bacteria counts (Fig. 7). In fillets stored at 4°C and 8°C under passive-MA, aerobic mesophillic bacteria count reached the critical limits of < 5.5 log cfu/g by day 3 (DOH, 2001; HPA, 2009), while fillets stored at 0°C had lower counts. For fillets under active-MAP storage at 0°C and 4°C, did not exceed the critical limit until after day 12 and 9, respectively (Table 4). These results agreed with the findings of Ordonez *et al*. (2000), for MAP conditions (40% CO_2 + 60% air, 20% CO_2 + 80% air and 100% passive-MAP) for hake fillets stored at 2°C for 12 days. They reported that, AMCs exceeded the microbial limits faster in fillets stored under 100% passive-MAP than active MAP stored fillets and MAP (40% CO_2 + 60% air) was more efficient in hindering microbes (Ordonez *et al*., 2000). This highlights the importance of maintaining optimum cold chain for packaged fresh RTC fish products. As abusive handling temperature would result in a shorter shelf life and compromise safety.

Furthermore, the use of pad led to lower microbial loads in fillets stored at 0°C and 4°C for active-MAP ($p < 0.001$), but the use of pad was only effective at 0°C for fillets stored under passive-MAP (Fig 7 and Table 4). This is could be attributed to the ability of the absorbent pad to better absorb drip loss in the package, which limits the exudates on which micro-organisms strives. Also, the lower microbial growth observed at 0°C during storage might be due to increased solubility of CO_2 at lower temperatures, which increases acidity and inhibits microbial growth. This correlates with results obtained during pH analyses in this study which showed a slow increase in pH at 0°C compared with 4°C. Similar results were reported by Ordonez *et al.* (2000) for hake steaks and Stamatis & Arkoudelos. (2007) for chub mackerel, they stated that aerobic mesophillic bacteria were better hindered when slow increase in pH occurred (Ordonez *et al.*, 2000; Stamatis & Arkoudelos, 2007). Research findings have indicated that, CO_2 hinders growth of psychotropic, aerobic and Gram-negative microbes and slows down deterioration of fresh RTC fish fillets (Stamatis & Arkoudelos, 2007).

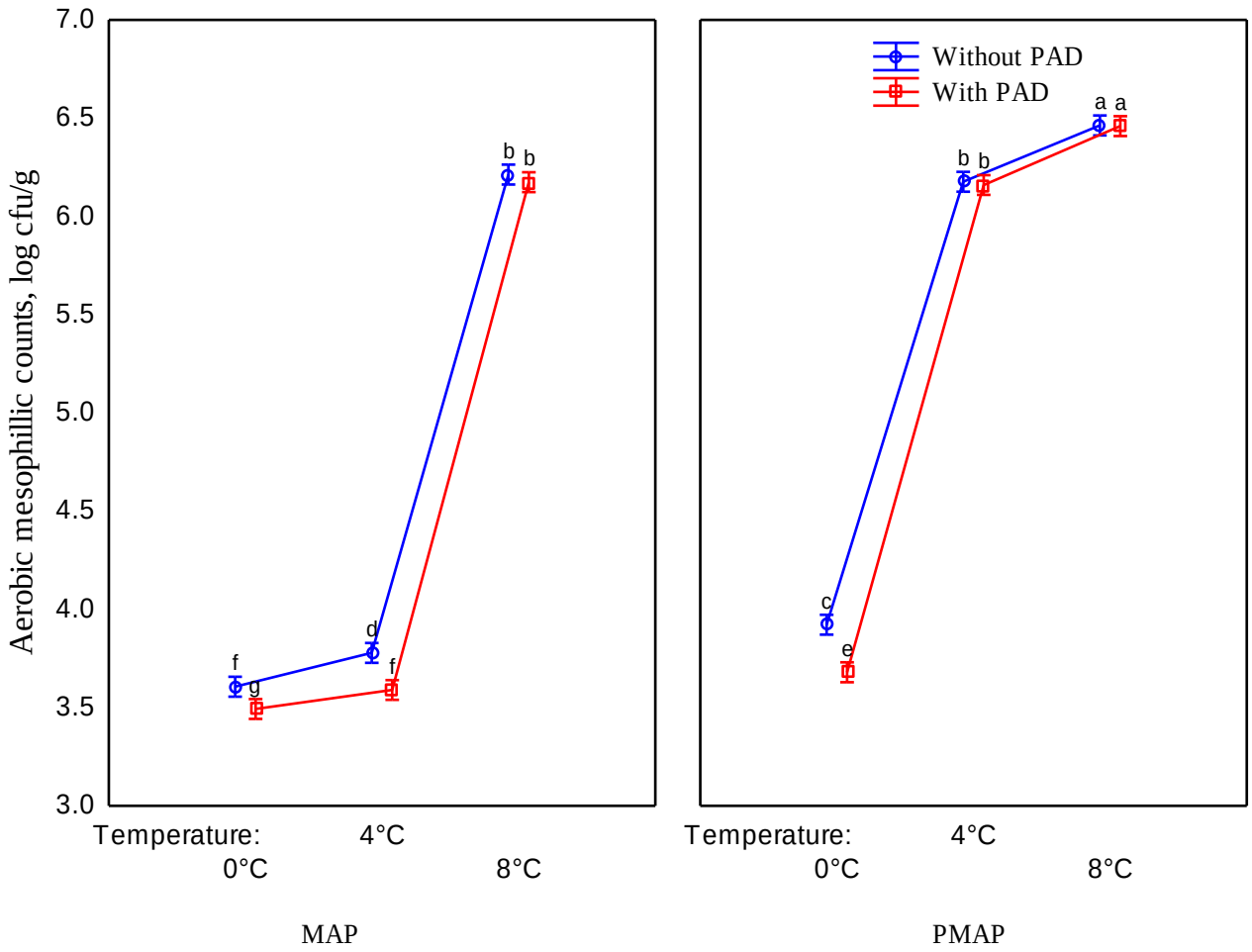

Figure 7 Effects of temperature (0°C, 4°C and 8°C), packaging with/without absorbent pad on bacterial growth of fillets on day 3: Different letters indicate significant difference in aerobic mesophillic count ($p < 0.001$). MAP; active-MA, PMAP; passive-MA

Table 4 Effects of packaging with/without absorbent pad, temperature (0°C, 4°C and 8°C) and storage time (d) on bacterial growth of Cape hake fillet

STORAGE DAYS	TEMP. (°C)	MAP – PAD	MAP + PAD	PMAP - PAD	PMAP + PAD
0 (Harvest)		1.2 ± 0.13^{j}	1.2 ± 0.13^{j}	1.2 ± 0.13^{j}	1.2 ± 0.13^{j}
3	0	3.6 ± 0.07^{i}	3.5 ± 0.05^{i}	3.9 ± 0.05^{g}	3.7 ± 0.09^{h}
	4	3.8 ± 0.04^{h}	3.6 ± 0.01^{h}	6.2 ± 0.09^{c}	6.2 ± 0.03^{c}
	8	6.2 ± 0.07^{c}	6.2 ± 0.02^{c}	6.5 ± 0.01^{b}	6.5 ± 0.01^{b}
6	0	4.5 ± 0.04^{f}	4.5 ± 0.04^{f}	7.1 ± 0.1^{a}	6.9 ± 0.03^{a}
	4	4.8 ± 0.03^{e}	4.8 ± 0.03^{e}	nd	nd
9	0	5.0 ± 0.03^{e}	4.9 ± 0.03^{e}	nd	nd
	4	6.0 ± 0.03^{d}	5.9 ± 0.03^{d}	nd	nd
12	0	5.2 ± 0.03^{e}	5.1 ± 0.03^{e}	nd	nd
	4	7.2 ± 0.03^{a}	7.2 ± 0.03^{a}	nd	nd

nd = Not determined due to sensory rejection; All values rounded off to one significant figure; Different letters indicate significant difference in pH values ($p < 0.05$); MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when sensory spoilage was observed thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6

Previous studies have established that active-MAP lowers microbial loads by extending the lag phase of bacteria (Arashisar *et al*., 2004; Sivertsvik *et al*., 2004; Lauzon *et al*., 2009). This effect was attributed to the dissolution of CO_2, which leads to formation of carbonic acid, the un-dissociated form of carbonic acid (bicarbonate ion) that changes cell permeability and hinders metabolic processes of microbes (Masniyom, 2011). Hence, the use of active-MAP combined with absorbent pad under optimum cold storage can assist in extending the shelf life and maintain microbial safety of RTC hake fillets.

In this study, *E. coli* and *Vibrio* spp. growths were not detected throughout the duration of storage and across all treatments. These microbes are indicators for spoilage and pathogenic microbes, respectively. This indicates that during this study adequate hygiene levels were maintained and there was no cross contamination from faecal material. Thus, for the success of any postharvest treatments on fresh or minimally processed fish products. Good agricultural practices (GAPs), hazard analysis and critical control points (HACCP) along the processing and packaging should be strictly adhered to.

Sensory analysis

Sensory scores for packaged Cape hake fillets are summarised in Table 5. Overall, acceptability of fillets decreased with increase in storage temperature across all treatments. Additionally, MA had a significant impact on the sensory attributes evaluated ($p < 0.05$), with active-MA packaged fillets stored at 0°C having highest scores for overall acceptability in comparison to fillets packaged under passive-MA. Temperature and time significantly influenced appearance quality of fillets ($p < 0.05$). Similar to the findings from measured fillet drip loss, sensory scores of drip loss (watery discharge) was higher in active-MA packed fillets without pad than in passive-MA packages without pad ($p < 0.05$). The freshness scores decreased significantly as temperature and storage time increased ($p < 0.05$). By day 12, active-MA packaged fillets stored at 0°C, had higher freshness scores than fillets stored at 4°C.

The interaction of MA, storage temperature and duration had significant effects on the overall acceptability of the fillets ($p < 0.05$). On day 6 of storage, the overall acceptability scores for passive-MA packaged fillets without pad was lower (2.1) than that of passive-MAP with absorbent pad (2.54) for fillets stored at 0°C. This low score observed correlates with the

aerobic mesophillic counts which were higher than the microbial limits of < 5.5 log cfu/g by day 6. Thus, based on sensory evaluation and microbial data the shelf life of passive-MA packaged Cape hake fillets stored at 0°C was limited to < 6, while 4°C and 8°C were limited to < 3 days. On the other hand, fillets under active-MAP with pads stored at 0°C had a higher overall acceptability score (3.02) than, those stored at 4°C (1.76) on day 12. The sensory score obtained for active-MA packaged fillets was consistent with the aerobic mesophillic counts reported on day 12. Aerobic mesophillic counts for active-MA packaged fillets stored at 4°C exceeded the microbial limits of 5.5 log cfu/g by day 9, but those of 0°C were within the microbial limits. Therefore, based on sensory attributes and microbial load the shelf life of active-MA packaged Cape hake fillets store at 4°C is limited to about 9 days. The shelf life reported for active-MA packaged Cape hake fillets store at 4°C in this study is shorter than the shelf life of 10 d reported by Speranza *et al*. (2009) for hake fillets stored at 4°C. This could be due to the difference in fish habitat, season when fish was harvested as well as the postharvest treatment.

However, for active-MA packaged Cape hake fillets store at 0°C, shelf life and overall acceptability were maximum after 12 d. Lauzon *et al*. (2009) obtained similar results in their study of unsalted cod fillets stored at 0°C and −2°C in MAP ($CO_2/O_2/N_2$:50/5/45). The authors also found that AMCs were below 5.5 log cfu/g on day 12 but increased to 7.2 log cfu/g on day 15 for fillets stored at 0°C. Based on these findings Lauzon *et al*. (2009) concluded that produce shelf life was 14 to 15 d at 0°C and 21 d at −2°C. The difference between shelf life at 0°C and our findings could be attributed to the microbial limits of < 5.5 log cfu/g (DOH, 2001; HPA, 2009) used in our study, the geographical location or due to the fact that cod was used in the study. This study is consistent with literature as lower temperatures lead to greater dissolution of CO_2 in fish tissues. (Ruiz-Capillas & Moral 2001; Sivertsvik *et al* 2004; Torrieri *et al*., 2006). Furthermore, the higher CO_2 levels led to increase in bacteriostatic ability at 0°C (Sivertsvik, 2007; Lauzon *et al*., 2009).

Table 5 Consumer perception scores of packaged Cape hake fillets during storage

Storage (days)	TEMP. (ºC)	Treatment	Appearance		Odour			Overall acceptability
			Colour	Watery discharge	Firmness	Fish freshness	Not fermenting	
3	0	MAP – PAD	4.70 ± 0.48^{b}	2.50 ± 0.53^{g}	4.40 ± 0.52^{b}	4 80 ± 0.42^{b}	4. 50 ± 0.53^{b}	4.18 ± 0.05^{b}
		MAP + PAD	5.00 ± 0.01^{a}	5.00 ± 0.01^{a}	5.00 ± 0.01^{a}	4.90 ± 0.32^{a}	5 00± 0.01^{a}	4.98 ± 0.14^{a}
		PMAP – PAD	3.90 ± 0.32^{d}	4.10 ± 0.32^{c}	2.20 ± 0.63^{e}	3.40 ± 0.52^{e}	3.20 ± 0.42^{e}	3.36 ± 0.14^{d}
		PMAP + PAD	4.50 ± 0.53^{c}	4.80 ± 0.42^{b}	3.40 ± 0.52^{c}	4.30 ± 0.48^{c}	3.60 ± 0.52^{c}	4.12 ± 0.04^{c}
	4	MAP – PAD	2.60 ± 0.70^{f}	1.70 ± 0.48^{i}	2.50 ± 0.53^{d}	2 80± 0.63^{d}	3.00± 0.47^{f}	2.52 ± 0.10^{f}
		MAP + PAD	2.90 ± 0.57^{e}	3.90 ± 0.57^{d}	2.50 ± 0.53^{d}	3.80 ± 0.63^{d}	3 50± 0.53^{d}	3.32 ± 0.04^{e}
		PMAP – PAD	1.50 ± 0.53^{h}	2.50 ± 0.85^{g}	1.60 ± 0.52^{h}	2.70 ± 0.48	2.50 ± 0.53^{h}	2.16 ± 0.15^{h}
		PMAP + PAD	1.40 ± 0.70^{i}	2.30 ± 0.48 ^{h}	2.00 ± 0.67^{f}	3.00 ± 0.67^{f}	2.80 ± 0.42^{g}	2.30 ± 0.13^{g}
	8	MAP – PAD	1.00 ± 0.01^{k}	1.60 ± 0.32^{j}	1.50 ± 0.71^{i}	1.20 ± 0.42^{h}	1.00 ± 0.32^{j}	1.26 ± 0.25^{l}
		MAP + PAD	1.90 ± 0.91^{g}	2.30 ± 0.82 ^{h}	1.80 ± 0.79 ^{g}	1.40 ± 0.52^{g}	1 00± 0.32^{j}	1.70 ± 0.30^{i}
		PMAP – PAD	1.00 ± 0.01^{k}	2.20 ± 0.63^{f}	1.00 ± 0.67^{k}	1.00 ± 0.01^{j}	1.00 ± 0.01^{j}	1.24 ± 0.36^{k}
		PMAP + PAD	1.30 ± 0.82^{j}	3.30 ± 0.48^{e}	1.20 ± 0.42^{j}	1.10 ± 0.32^{i}	1.10 ± 0.32^{i}	1.60 ± 0.21^{j}
6	0	MAP – PAD	3.70 ± 0.48^{b}	2.10 ± 0.74^{e}	3.70 ± 0.67^{b}	4 50 ± 0.53^{b}	4. 70 ± 0.48^{b}	3.74 ± 0.12^{b}
		MAP + PAD	4.10 ± 0.57^{a}	4.80 ± 0.42^{a}	4.70 ± 0.48^{a}	4.60 ± 0.52^{a}	4 80± 0.42^{a}	4.60 ± 0.06^{a}
		PMAP – PAD	2.80 ± 0.67^{d}	3.30 ± 0.67^{c}	1.40 ± 0.52^{f}	1.80 ± 0.42^{f}	1.70 ± 0.68^{f}	2.20 ± 0.11^{f}
		PMAP + PAD	3.30 ± 0.48^{c}	4.10 ± 0.57^{b}	2.20 ± 0.42^{d}	2.20 ± 0.42^{e}	1.80 ± 0.42^{e}	2.72 ± 0.06^{d}
	4	MAP – PAD	2.40 ± 0.52^{e}	1.70 ± 0.68^{f}	2.10 ± 0.57^{e}	2 40± 0.52^{d}	2.60± 0.52^{d}	2.24± 0.07^{e}
		MAP + PAD	2.40 ± 0.52^{e}	3.20 ± 0.42^{d}	2.30 ± 0.48^{c}	3 20± 0.42^{c}	2.90± 0.32^{c}	2.80 ± 0.08^{c}

Data are means of scores of 10 panellists; Superscript alphabets are significant differences between each packaged fillet; Different letters indicate significant difference in pH values ($p < 0.05$); MAP-pad: active-MA without pad; MAP + pad: active-MA with pad, PMAP - pad: passive-MA without pad and PMAP + pad: passive-MA with pad. Sampling was stopped on days when sensory spoilage was observed thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6

Table 5 (Continued)

Storage (days)	TEMP. (°C)	Treatment	Appearance		Odour			Overall acceptability
			Colour	Watery discharge	Firmness	Fish freshness	Not fermenting	
12	0	MAP – PAD	2.30 ± 0.48[b]	1.50 ± 0.85[c]	2.60± 0.52[b]	2 90 ± 0.74[b]	2.80 ± 0.42[b]	2.42± 0.18[b]
		MAP + PAD	2.60 ± 0.52[a]	3.10 ± 0.32[d]	3.20 ± 0.42[a]	3.10 ± 0.32[a]	3 10± 0.32[a]	3.02 ± 0.09[a]
	4	MAP – PAD	1.00 ± 0.01[d]	1.00 ± 0.01[e]	1.10± 0.03[d]	1 40 ± 0.52[d]	1.30 ± 0.48[d]	1.16± 0.25[d]
		MAP + PAD	1.20 ± 0.42[c]	2.40 ± 0.70[b]	1.30 ± 0.70[c]	2 00± 0.01[c]	1.90± 0.32[c]	1.76 ± 0.26[c]

Data are means of scores of 10 panellists; Superscript alphabets are significant differences between each packaged fillet; Different letters indicate significant difference in pH values ($p < 0.05$); MAP-pad: active-MA without pad; MAP + pad: active-MA with pad, PMAP - pad: passive-MA without pad and PMAP + pad: passive-MA with pad.

Sampling was stopped on days when sensory spoilage was observed thus, sampling for fillets stored under MAP at 8°C was stopped on day 3, similarly, sampling for fillets stored under PMAP at 4°C and 8°C were stopped on day 3 as well, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6

Correlation between quality indices

Pearson correlation was conducted to explore the relationships between the investigated quality attributes of Cape hake fish (Table 6). Interesting significant relationships with varying correlation coefficients were obtained. A weakly negative relationship (r = -0.40) between work of shear and pH suggests that fillets stored in a weakly acidic (pH 5.6 - 6.5) medium had better firmness quality than those stored in a basic (pH 7 - 8) medium (Table 6). Results also showed that acidic medium (low pH) could influence the colour of fillets as evident by the positive correlation (r = 0.43) between pH and total colour difference of fish fillets (Table 6). Although the relationship between CO_2 levels and exudates was moderately positive (r = 0.52), with the strong negative correlation (r = -0.78) between CO_2 levels and aerobic mesophillic counts, it is reasonable to hypothesize that CO_2 levels could minimise the effects of microbes on fish fillet. Another interesting relationship was the moderately negative correlation between work of shear and aerobic mesophillic counts (r = -0.62) suggesting that fillet firmness would be maintained when microbial activity was minimal. This was also buttressed by the moderately positive correlations between work of shear and all the sensory attributes such as work of shear-colour (r = 0.54), work of shear-freshness (r = 0.51), work of shear-fresh fish odour (r = 0.51), and work of shear-overall acceptability (r = 0.52) (Table 6). It is also noteworthy that pH showed negative correlations with most of the investigated sensory attributes such as with colour (r = -0.43), firmness (r = -0.39), freshness (r = -0.48), fresh fish odour (r = -0.45), and overall acceptability (r = -0.42) (Table 6). This association suggests the importance of low pH in stored fish fillet in maintaining the colour, firmness, freshness, fresh fish odour and overall acceptability of Cape hake fillets.

Furthermore, strong negative correlations between aerobic mesophillic counts AMCs and the sensory scores for colour (r = -0.70), firmness (r = -0.72), freshness (r = -0.80), fresh fish odour (r = -0.77), and overall acceptability (r = -0.73) were observed (Table 6). The relationships imply that minimal microbial activity is also essential in maintaining the colour, firmness, freshness, fresh fish odour and overall acceptability quality attributes which are desirable in Cape hake fillets. This is validated by strong positive correlations observed between freshness and firmness (r = 0.89), fresh fish odour and firmness (r = 0.90), fresh fish odour and freshness (r = 0.97), overall acceptability and firmness (r = 0.92), overall acceptability and freshness (r = 0.95) and overall acceptability and fresh fish odour (r = 0.92).

Table 6 Pearson correlation coefficient matrix between quality indicators measured in Cape hake fillets during storage

Variables	pH	WS	DL	TCD	O_2	CO_2	AMC	Colour	Exudate	Firmness	Freshness	Fresh odour	OA
pH	**1**												
WS	**-0.40**	**1**											
Drip loss	0.19	-0.194	**1**										
TCD	**0.43**	-0.34	0.22	**1**									
O_2	-0.27	**0.77**	0.07	-0.36	**1**								
CO_2	-0.25	0.17	0.29	**0.37**	0.36	**1**							
AMC	**0.52**	**-0.62**	0.07	**0.61**	**-0.77**	**-0.78**	**1**						
Colour	**-0.43**	**0.54**	-0.27	**-0.78**	**0.44**	-0.24	**-0.70**	**1**					
Exudate	-0.08	0.23	**0.46**	**-0.56**	0.02	**0.47**	-0.22	**0.59**	**1**				
Firmness	**-0.39**	**0.48**	-0.09	**-0.66**	**0.62**	0.09	**-0.72**	**0.82**	**0.42**	**1**			
Freshness	**-0.48**	**0.51**	-0.17	**-0.68**	**-0.60**	0.10	**-0.80**	**0.86**	**0.40**	**0.89**	**1**		
Fresh odour	**-0.45**	**0.51**	-0.15	**-0.65**	**0.61**	0.15	**-0.77**	**0.82**	0.32	**0.90**	**0.97**	**1**	
OA	**-0.42**	**0.52**	-0.27	**-0.76**	**0.52**	-0.09	**-0.73**	**0.94**	**0.63**	**0.92**	**0.95**	**0.920**	**1**

Correlation values in **bold** are significant at $p < 0.05$.

WS = Work of shear, DL = Drip loss, TCD = Total colour difference, AMC = Aerobic mesophillic count, OA = Overall acceptance.

Conclusion

Headspace gas composition of O_2 and CO_2 were significantly influenced by the storage time, temperature, packaging conditions and their interactions. Level of O_2 decreased continuously across all treatments, and passive-MA packaged fillets reached about 2.7% on 9th day of storage. These results highlight the need for research to select appropriate packaging materials with desirable gas permeability properties in the design of MAP systems for RTC fish products.

Storage temperature, the use of absorbent pads, and MA had significant impacts on changes in physicochemical properties of Cape hake fillets during storage ($p < 0.05$). Active-MA packaged fillets had lower pH values in comparison to fillets stored under passive-MA, across all temperatures. Drip loss was higher in fillets packaged without absorbent pad. The use of pad, combined with low storage temperature, had a significant impact on the firmness of hake fillets, across all temperatures ($p < 0.05$). Cape hake fillets stored at 0°C maintained better firmness than those at higher storage temperatures. Based on measured physicochemical, microbial and sensory attributes, active-MAP (40% CO_2 + 30% O_2 + 30% N_2) combined with low temperature (0°C) extended the shelf life and maintained quality attributes of Cape hake fillets up to 12 d. These findings provide a useful guide for the fresh fish processing industry, towards improving the postharvest handling, packaging and storage of Cape hake fillets and other fish products. The use of high grade fillets, optimum processing and storage temperature regime, good agricultural practices, good hygienic practices, and correct gas composition is needed to achieve the best results with MAP.

References

Angis S. & Oguzhan P. (2013). Effect of thyme essential oil and packaging treatments on chemical and microbiological properties of fresh rainbow trout (*Oncorhynchus mykiss*) fillets during storage at refrigerator temperatures. *African Journal of Microbiology Research*, **7**, 1136-1143.

Ahmed, A.A. (2008). Postharvest losses of fish in developing countries. *Nutrition and Health*, **19**, 273-287.

AOAC International, (2000). (Association of Official Analytical Chemists) Official Methods of Analysis (17th edition), AOAC International, Arlington, VA (2000).

AOAC International, (2002a). Official methods of analysis (17th edition), Arlington, Virginia, USA: Association of Official Analytical Chemists Inc. Loss on drying (moisture) at 95 – 100°C for feeds. AOAC Official Method 934.01.

AOAC International, (2002b). Official methods of analysis (17th edition), Arlington, Virginia, USA: Association of Official Analytical Chemists Inc. Ash of Animal Feed. AOAC Official Method 942.05.

AOAC International, (2007). Official Methods of Analysis. 18th edition, 2005; Current through revision 2, 2007 (On line). Method 960.52 (Micro-Kjeldahl method) and Method 981.10 (crude protein in meat and meat products). The Association of Official Analytical Chemists, Gaithersburg, MD.

Arashisar, S., Hisar, O., Kaya, M. & Yanik, T. 2004. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. *International Journal of Food Microbiology*, **97**, 209-214.

Ayala M.D., Santaella, M., Martınez, C., Periago, M.J., Blanco, A., Vazquez, J.M. & Albors, O.L. (2010). Muscle tissue structural changes and texture development in sea bream (*Sparus aurata*) during post-mortem storage. *LWT Food Science Technology*, **43**, 465–475.

Bellagha, S., Sahli, A., Farhat, A., Kechaou, N. & Glenza, A. (2007). Studies on salting and drying of sardine (*Sardinella aurita*): Experimental kinetics and modelling. *Journal of Food Engineering*, **78**, 947-952.

Caglak, E., Cakli, S. & Kilinc B. (2012). Effect of modified atmosphere packaging on quality and shelf-life of salted bonito (*Sarda sarda*). *Journal of Aquatic Food Product Technology*, **21**, 206–221.

Careche, M., Del Mazo, M.L. & Fernandez-Martín, F. (2002). Extractability and thermal stability of frozen hake (*Merluccius merluccius*) fillets stored at − 10°C and − 30°C. *Journal of the Science of Food and Agriculture*, **82**, 1791-1799.

Chaijan, M., Benjakul, S., Visessanguan, W. & Faustman, C. (2005). Changes of pigments and colour in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. *Food Chemistry*, **93**, 607-617.

Chen, L. & Opara, U. L. (2013a). Texture measurement approaches in fresh and processed foods—A review. *Food Research International*, 51, 823-835.

Chen, L. & Opara, U. L. (2013b). Approaches to analysis and modeling texture in fresh and processed foods–A review. *Journal of Food Engineering*, 119, 497-507.

Cheng, J., Sun, D. W., Zeng, X. A. & Liu, D. (2014). Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: A review. *Critical Reviews in Food Science and Nutrition*, **13**, 52-61.

Cyprian, O., Oduor-Odote, P., Lauzon, H., Martinsdottir, E. & Arason, S. (2013). Microbiological quality and shelf life of fresh packaged tilapia fillets stored under different chill temperatures. *Journal of Microbiology, Biotechnology and Food Sciences*, **2**, 2431-2455.

Da Silva, N., Taniwaki, M.H., Junqueira, V.C., Silveira, N., Do Nascimento, M.D.S. & Gomes, R.A.R. (2012). Microbiological examination methods of food and water. A laboratory manual. Washington, USA: CRC Press.

De Silva, D.A.M. & Yamao M. (2006). Regional preferences in the Japanese Seafood consumption: An empirical analysis of consumer purchasing behaviour on domestic versus imported seafood. *Japan, Journal of the Regional Fisheries Society*, **46**, 83-104.

Del Nobile, M.A., Corbo, M.R., Speranza, B., Sinigaglia, M., Conte, A. & Caroprese, M. (2009). Combined effect of modified atmosphere packaging and active compounds on fresh blue fish burger. *International Journal of Food Microbiology*, **135**, 281-287.

DOH - Department of Health (2001). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. South Africa: Department of Health Directorate: Food Control.

Domingo, J. L., Bocio, A., Falco, G. & Llobet, J.M. (2007). Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology*, **230**, 219-226.

Dondero, M., Cisternas, F., Carvajal, L. & Simpson, R. (2004). Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. *Food chemistry*, **87**, 543-550.

Duun, A.S. & Rustad, T. (2008). Quality of super-chilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at− 1.4 and− 3.6° C. *Food Chemistry*, **106**, 122-131.

Erkan, N., Ozden, Ö. & Inugur, M. (2007). The effects of modified atmosphere and vacuum packaging on quality of chub mackerel. *International Journal of Food Science and Technology*, **42**, 1297-1304.

Fletcher, G.C., Summers, G., Corrigan, V.K., Johanson, M.R. & Hedderley, D. (2004) Optimizing gas mixtures for modified atmosphere packaging of fresh king salmon (*Oncorhynchus tshawytscha*), *Journal of Aquatic Food Product Technology*, **13**, 5-28, DOI: 10.1300/J030v13n04_02.

Food and Agricultural Organisation, FAO, (2014). World review of fisheries and aquaculture part 1. http://www.fao.org/docrep/013/i1820e/i1820e.

Goulas, A.E. & Kontominas, M.G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (Sparus aurata): Biochemical and sensory attributes. *Food Chemistry*, **100**, 287-296.

Hara-Kudo, Y., Sugiyama, K., Nishibuchi, M., Chowdhury, A., Yatsuyanagi, J., Ohtomo, Y. & Kumagai, S. (2003). Prevalence of pandemic thermo stable direct haemolysin-producing *Vibrio parahaemolyticus* O3: K6 in seafood and the coastal environment in Japan. *Journal of Applied and Environmental Microbiology*, **69**, 3883-3891.

HPA – Health protection agency, (2009). Guidelines for assessing the microbiological safety of ready-to-eat foods. Health Protection Agency, London: UK Government Printers; 2009.

Hultmann, L. & Rustad, T. (2004). Iced storage of Atlantic salmon (*Salmo salar*) - effects on endogenous enzymes and their impact on muscle proteins and texture. *Food Chemistry*, **87**, 31-41.

ISO, (2004) International Organization for Standardization. Microbiology of food and animal feeding stuffs horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 2: Colony count method. ISO 21528-2, 1st ed., 08-15-2004. International Organization for Standardization, Geneva,

ISO/TS 21872-1: (2007). Microbiology of food and animal feeding stuffs. Horizontal method for the detection of potentially entero-pathogenic *Vibrio* spp. Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholera.*

Ivanov, G., Balev, D., Nikolov, H. & Dragoev, S. (2009). Improvement of chilled salmon sensory quality by pulverisation with natural di-hydro-quercetin solutions. *Bulgarian Journal of Agricultural Sciences*, **15**, 154-162.

Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, **106**, 2747-2757.

Lauzon, H.L., Magnusson, H. Sveinsdottir, K., Gudjonsdottir, M. & Martinsdottir, E. (2009). Effect of brining, modified atmosphere packaging, and super-chilling on the shelf-life of cod (*Gadus morhua*) loins. *Journal of Food Science*, **74**, 258-267.

Mancini, R.A. & Hunt, M.C. (2005). Current research in meat colour. *Meat Science* **71**, 100-121.

Masniyom, P. (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Sonklanakarin Journal of Science and Technology*, **33**, 181-192.

Moreira, A.B., Visentainer, J.V., de Souza, N.E. & Matsushita, M. (2001). Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. *Journal of Food Composition and Analysis*, **14**, 565–574.

Ordonez, J.A., Lopez-Galvez, D.E., Fernandez, M., Hierro, E. & de la Hoz, L. (2000). Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, **80**, 1831-1840.

Pacheco-Aguilar, R., Lugo-Sanchez, M.E. & Robles-Burgueno, M.R. (2000). Post-mortem biochemical and functional characteristic of Monterey sardine muscle stored at 0°C. *Journal of Food Science*, **65**, 40–47.

Pathare, P.B., Opara, U.L. & Al-Said, F.A.J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology*, 6, 36-60.

Regost, C., Jakobsen, J.V. & Rora, A.M.B. (2004). Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Research International*, **37**, 259-271.

Roth, B., Birkeland, S. & Oyarzun, F. (2009). Stunning, pre slaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on fresh and smoked fillets. *Aquaculture*, **289**, 350-356.

Ruiz-Capillas, C & Moral, A. (2001). Chilled bulk storage of gutted hake (*Merluccius merluccius*,) in carbon dioxide and oxygen enriched controlled atmospheres. *Food Chemistry* **74**, 317-325.

SANS (2007). Method 4833. Second Edition. Microbiology: General guidance for the enumeration of microorganisms - Colony count technique at 30°C, Pretoria: South African Bureau of Standards, Pretoria, South Africa.

Sivertsvik, M., Jeksrud, W.K. & Rosnes, J.T. (2002). A review of modified atmosphere packaging of fish and fishery products–significance of microbial growth, activities and safety. *International Journal of Food Science & Technology*, **37**, 107-127.

Sivertsvik M, Rosnes J.T. & Jeksrud W.K. (2004). Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 2: raw fish fillets. *Journal of Food Engineering*, **63**, 451–458.

Sivertsvik, M. (2007). The optimized modified atmosphere for packaging of pre- rigor filleted farmed cod (*Gadus morhua*) is 63ml/100ml oxygen and 37ml/100ml carbon dioxide. *LWT-Food Science and Technology*, **40**, 430-438.

Soccol, M.H. & Oetterer, M. (2003). Use of modified atmosphere in seafood preservation. *Brazilian Archives of Biology and Technology*, **46**(4), 569-580.

Sone, I., Olsen, R.L. & Heia, K. (2012) Spectral changes of Atlantic salmon (*Salmo salar*) muscle during cold storage as affected by the oxidation state of heme. *Journal of Agricultural and Food Chemistry*, **60**(38), 9719–9726. Available at http://dx.doi.org/10.1021/jf302505y.

Speranza, B., Corbo, M., Conte, A., Sinigaglia, M. & Del Nobile, M. (2009). Microbiological and sensorial quality assessment of ready-to-cook seafood products packaged under modified atmosphere. *Journal of Food Science,* **74**, M473–M478.

Stamatis, N. & Arkoudelos, J. (2007). Quality assessment of *Scomber colias japonicas* under modified atmosphere and vacuum packaging. *Food Control*, **18**, 292-300.

Torrieri, E., Cavella, S., Villani, F. & Masi, P. (2006). Influence of modified atmosphere packaging on the chilled shelf-life of gutted farmed bass (*Dicentrarchus labrax*). *Journal of Food Engineering*, **77**, 1078–1086.

Trondsen, T., Scholderer, J., Lund, E. & Eggen, A.E. (2003). Perceived barriers to consumption of fish among Norwegian women. *Appetite* **41**, 301–314.

Wang, T., Sveinsdottir, K., Magnusson, H. & Martinsdottir, E. (2008). Combined application of modified atmosphere packaging and super-chilled storage to extend the shelf life of fresh cod (*Gadus morhua*) loins. *Journal of Food Science*, **73**, 11–19.

Yesudhason, P., Gopal, T.K.S., Ravi Shankar, C.N., Lalitha, K. V. & Kumar, K. N. A. (2009). Effect of modified atmosphere packaging on chemical, textural, microbiological and sensory quality of seer fish (*Scomberomorus commerson*) steaks packaged in thermo-formed trays at 0–2ºC. *Journal of Food Processing and Preservation* **33**, 777-797.

CHAPTER 4

CHANGES IN VOLATILE COMPOSITION AND CONCENTRATION OF CAPE HAKE FILLETS AS AFFECTED BY MODIFIED ATMOSPHERE PACKAGING, STORAGE TEMPERATURE AND DURATION

Summary

This study investigated the effects of modified atmosphere packaging (MAP) and storage temperature (0°C and 4°C) on the volatile compounds (VOCs) of Cape hake (*Merluccius capensis*) fish fillets as predictor of shelf life and quality. Fresh Cape hake fillets were packaged under active modified atmosphere (40% CO_2 + 30% O_2 + 30% N_2) and passive modified atmosphere (0.039% CO_2 + 20.95% O_2 + 78% N_2) with or without absorbent pad and stored at 0°C and 4°C for 12 d. Results obtained demonstrated that changes in VOCs and concentration were significantly ($p < 0.05$) influenced by MAP, storage temperature and duration. A total of 16 volatiles were identified in the packaged Cape hake fillets: 4 primary VOCs and 12 secondary VOCs. Spoilage VOCs identified include tri-methylamine (TMA) (ammonia like), esters (sickeningly sweet) and sulphur group (putrid). These VOCs increased continuously in concentration during storage. Modified atmosphere packaging had a significant influence on the change in volatile composition and concentration. Active-MA packaged fillets performed better and had lower TMA values of 0.31% at 0°C on day 12 in comparison to 7.22 % at 0°C under passive on day 6. Ethyl acetate was detected in passive-MA packaged fillets stored at 0°C on day 3 and the levels increased to 3.26% on day 6, while active-MA packaged fillets maintained freshness. Cape hake fillets packaged under active-MAP at 0°C had longer shelf life (12 d) than control passive-MAP fillets stored at 0°C (> 3d), based on the development of higher levels of VOCs, off-odour, loss of freshness and higher aerobic mesophillic bacteria count (5.5 log cfu/g) as observed in this study. Esters and sulphur related compounds in conjunction with TMA could therefore be relied upon as good spoilage markers for active-MA packaged marine fish fillets.

Introduction

Fish provides about 17% of the world's annual protein intake besides, it is also a healthy alternative to meat due to the presence of poly-unsaturated fatty acids which have been reported to increase fish nutritional value, guard against disease conditions, such as high blood pressure and cholesterol levels in humans (Moreira *et al*., 2001; Kris-Etherton *et al*., 2002; Trondsen *et al*., 2003; Domingo *et al*., 2007; Del Nobile *et al*., 2009; FAO, 2014). Fresh fish is highly perishable due to its high water activity (aw $\geq$ 0.95) and post mortem pH level of about 5.2 (Choubert & Baccaunaud, 2006; Jezek & Buchtova, 2007; Jezek, 2012). The quality of fresh fish and fishery products has been assessed through different procedures such as measuring chemical, microbial, physical and sensory attributes (Zhang *et al*., 2010). Although sensory attributes are vital in determining fish wholesomeness, other fast and unbiased ways to support recommendations of sensory panels are needed (Triqui & Bouchriti, 2003; Zhang *et al*., 2010).

Change in quality attributes of fresh fish arises as a result of fish tissue degradation as well as changes in volatile compounds (VOCs) that evolve from the fish. Studies have shown that spoilage occurs due to bacteria infestation in fish muscles which triggers a continuous breakdown of glycogens, nucleotides, amino acids and other non-protein nitrogen (Gram & Huss, 2000). Spoilage results in the release of volatile compounds such as tri-methyl amine (TMA), aldehydes, ketones, esters, hypoxanthine, and low molecular weight sulphur complexes, which contributes to the characteristic odour of spoilt fish (Gram & Dalgaard, 2002; Triqui & Bouchriti, 2003). Specific VOCs have been identified as vital descriptors of the aroma and freshness of fresh fish and fishery products (Olafsdottir & Jonsdottir, 2010).

Changes in VOCs which occur before deterioration in fish quality have been reported (Zhang *et al*., 2010). Other researchers have noted that these volatile compounds can be used to assess changes in freshness of packaged fresh fish fillets during storage (Triqui & Bouchriti, 2003; Summo *et al*., 2010; Zhang *et al*., 2010; Giogios *et al*., 2013; Boscaino *et al*., 2014). For example, Giogios *et al*. (2013) reported that commercial fresh Mediterranean seafood, comprising seven types of fish such as sand-smelt, picarel, hake, pilchard, bogue, anchovy and crustaceans including striped-mullet, squid, shrimp and mussel, contained a total of 298 VOCs. Amongst these types of fish, pilchard had higher number of VOCs, while mussels had the highest concentration of VOCs. Furthermore, levels of alcohols in pilchards were high; amines and sulphur VOCs were present in shrimps, while aldehydes, furans, pyridine, pyrazines and pyrrols were detected in mussels at elevated levels (Giogios *et al*.,

2013). In a more recent study, Boscaino *et al*. (2014) compared the effects of vacuum packaging (VP) and home refrigeration at 4°C on quality attributes of rainbow trouts for 6 days and found that VP maintained quality attributes of trout better than refrigerated storage by controlling oxidation and decay. Also VP controlled foul aromas and resulted in better flesh firmness with enhanced fish flesh colour and restricted odour development by day 6 (Boscaino *et al*., 2014).

Numerous researchers have reported the benefits of modified atmosphere packaging (MAP) in extending shelf life of fillets of different fish species (Ordonez *et al*., 2000; Erkan *et al*., 2007; Sivertsvik, 2007; Del Nobile *et al*., 2009; Lauzon *et al*., 2009; Yesudhason *et al*., 2009; Caglak *et al*., 2012; Angis & Oguzhan, 2013). Lauzon *et al*. (2009) found that fillets in MAP (50% CO_2 + 5% O_2 + 45%) had higher shelf life of about 15 d at 0°C and 21 d at -2°C, compared with 11d at 0°C and 14 d at −2°C in normal air packaging. Similarly, a study investigating the role of active-MAP [(A) = 40% CO_2 + 30% O_2 + 30% (B) = 50% CO_2 + 50% O_2 and (C) = 95% CO_2 + 0% O_2], combined with a mixture of 3 natural preservatives thymol, lemon extract and grapefruit seed extract on blue fish burger (mackerel and hake) stored at 4°C for 28 d was reported by Del Nobile *et al*. (2009) who found lower aerobic mesophillic bacteria counts in active-MAP fillets.

In summary, none of these previous studies has considered the changes in VOCs as a potential descriptor of postharvest quality of packaged fresh fish fillets under MAP. Hence, the objective of this study was to investigate the effects of MAP, combined with absorbent pad and storage temperature, on the changes in volatile compounds and concentration of Cape hake fish fillets.

Materials and methods

Preparation of fish samples and packaging

Fresh Cape hake fillets (average weight of 200 g) were purchased from a local retail outlet in Stellenbosch, South Africa. Fillets were iced with appropriate quantity of ice (with 1:3 parts w/w flake/ice) and packed in sterile padded polystyrene box. Fillets were collected approximately 18 h after preparation and conveyed to the postharvest laboratory within 15 min. On arrival, the fish fillets were kept on ice and packaged into the following treatments: active-MA (40% CO_2 + 30% O_2 + 30% N_2) without absorbent pad; (40% CO_2 + 30% O_2 +

30% N_2) with absorbent pad; passive-MA (0.039% CO_2 + 20.95% O_2 + 78% N_2) (PMAP) with absorbent pad; and, (0.039% + 20.95% + 78%) without absorbent pad (as control).

All fish samples were packaged in polyethylene terephthalate (PET) trays with dimension 280 X 190 mm (Zibo containers (Pty.) Ltd., Kuilsrivier, South Africa) and heat-sealed with bi-axially oriented polyester film (O_2 permeability: 75 cm^3 at 23°C, 70% RH bar^{-1}; water vapour permeability: 2 g d^{-1} at 38°C, 90% RH (Knilam Packaging (Pty.) Ltd., South Africa). A total of 380 packages were used in this study. Modified atmosphere packaging was performed using a Multivac packaging machine (Multivac Traysealer T100, Sepp Hagenuller GmbH & Co.KG, Germany) and food graded gases (Air products Pty., South Africa) and absorbent pads (Dri-Fresh®, -Sirane Ltd., Shropshire, UK). The absorbent pads were made of an inert hygroscopic material according to manufacturer's specification. Packaged fillets were then stored for 15 d at 0°C and 4°C. On each sampling day, three packs of fish were taken for each treatment and temperature. Sampling was done on days 0, 3, 6, 9 and 12.

Extraction of volatile compounds and chromatographic analysis

For each packaged fish fillets approximately 7 g of fish fillets was taken and placed in a 20 mL solid phase micro-extraction (SPME) vial containing 3 g of NaCl (Caleb *et al*., 2013). To each SPME vial with fish sample, 5 mL of double distilled water and 50 µL of 3-Octanol (internal standard) was added and the vial was gently vortexed to mix the contents. Trapped volatile compounds (VOCs) in the vial headspaces were then extracted on a SPME fiber. The vials were permitted to equilibrate for 2 min at 50°C in the CTC auto sampler incubator (with agitation speed of 250 rpm). A 50/30 µm fibre coated with divinylbenzene/-carboxen/-poly-dimethylsiloxane was inserted (10 mm deep) into the vial headspace and exposed for 10 min at 50°C (Caleb *et al*., 2013). For desorption of VOCs, the fibre coating was injected into the gas chromatography-mass spectrometry (GC-MS) for 2 min in splitless mode. The fibre was inserted in a fibre conditioning station for 15 min between samples for cleaning to prevent cross-contamination (Caleb *et al*., 2013). Temperature of the gas chromatography (GC) injection port was maintained at 250°C. The oven temperature program was as follows: the oven temperature was maintained at 70°C for 1 min, and then ramped up to 142°C at the rate of 3°C/min; then finally ramped up to 240°C at the rate of 5°C/min, and kept at that temperature for 3 min (Duflos *et al*., 2010; Caleb *et al*., 2013).

Separation of VOCs was performed using GC (Agilent 6890 N, Agilent, Palo Alto, CA), coupled with mass spectrometer detector (Agilent 5975 MS, Agilent, Palo Alto, CA) (Caleb *et al*., 2013). The GC-MS system was equipped with a polar DB-FFAP column (Model number: J&W 122-3263), with nominal length of 60 m; 250 µm internal diameter; and 0.5 µm film thickness. Analyses were carried out using helium as carrier gas with at a constant flow rate of 1.9 mL/min (Caleb *et al*., 2013). The MS data was collected on MSD operated in full scan mode and the ion source and quadrupole temperatures were maintained at 230°C and 150°C, respectively. The MSD transfer line was held at 280°C. The individual peaks were categorised by comparing their retention times (RT) and with mass spectral library (NIST, ver. 2.0) and certified with the database Flavornet in conjunction with those published in literature (Acree & Arn, 2004; Jonsdottir *et al*., 2008; Duflos *et al*., 2010; Moreira *et al*., 2013).

Microbial quality analysis

Approximately 1g of fish sample was taken from randomly selected packaged fillets for each treatment on the sampling d and mashed under aseptic conditions using mortar and pestle. Mashed sample was then diluted in test tubes containing 10 mL of sterile physiological saline solution (PSS) (0.85g NaCl in 100 mL distilled water). Serial dilutions up to four-fold were prepared by adding 1 mL of homogenate sample to 9 mL PSS and vortex each dilution. In order to enumerate microbial load 1 mL of each the dilutions were plated in triplicate onto appropriate media using the pour plate method (Da Silva *et al*., 2012).

Aerobic mesophillic bacteria was counted using plate count agar (PCA); method 4833 (SANS, 2007). The plates were then incubated upside down at 37°C for 48 h (Hara-Kudo, *et al*., 2003). After incubation, plates with 30 - 300 colonies were counted. The results were transformed into Log colony forming unit (log CFU g^{-1}).

Statistical Analysis

Statistical analysis was carried out using Statistica software (Statistica version 11, StatSoft Inc., Tulsa, USA). Analysis of variance (ANOVA) at 95% confidence interval was used to evaluate the effect of modified atmosphere packaging (MAP) on the volatile quality of packaged Cape hake fillets. The variation between the mean values was examined according

to Fischer least significant difference (LSD) Multiple Range tests. In order to establish correlation trends between, microbial and volatile quality of the examined Cape hake fillets data were processed using principal component analysis (PCA) using XLSTAT software Version 2012.4.01 (Addinsoft, France). Significant correlation coefficients were classified as strong, and moderate corresponding to r > 0.7and r > 0.5 - < 0.7, respectively.

Results and Discussion

Microbiology analysis

Fillets used in this study had good microbial quality as evidenced by low initial aerobic mesophillic bacteria (1.2 log cfu/g) on fresh samples. Lowest aerobic mesophillic bacteria were observed in fillets packaged under active-MAP and stored at 0°C while, passive-MAP stored fillets stored at 0°C had the highest (7.13 log cfu/g by day 6) without absorbent pad (Table1). In fillets stored at 0°C under passive-MA, aerobic mesophillic bacteria count reached the critical limits of < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009). For fillets under active-MAP storage at 0°C, AMCs did not exceed the critical limit until after day 12 and at 4°C reached 6.0 and 7.2 log cfu/g for 9 and 12 d, respectively (Table 1). These results agreed with the findings of Ordonez *et al*. (2000), for MAP conditions (40% CO_2 + 60% air, 20% CO_2 + 80% air and 100% passive - MAP) for hake fillets stored at 2°C for 12 d. They reported that, AMCs exceeded the microbial limits faster in fillets stored under 100% passive MAP fillets than active MAP stored fillets and MAP (40% CO_2 + 60% air) was more efficient in hindering microbes. This highlights the value addition that MAP gives in maintaining shelf life and quality of packaged fresh ready-to-cook fish products.

Furthermore, the use of absorbent pads had a significant impact on microbial load of fillets (p < 0.005). Lower microbial counts were observed for active-MA packaged fillets with absorbent pads stored at 0°C and 4°C, but the use of pad was only effective at 0°C for passive-MA packed fillets (Table 1). The lower microbial growth observed at 0°C during storage might be due to increased solubility of CO_2 at lower temperatures, which increases the acidity of fish tissue and inhibits microbial growth. Similar results were reported by Ordonez *et al.* (2000) for hake steaks, and for chub mackerel Stamatis & Arkoudelos, (2007). The authors stated that aerobic mesophillic bacteria were best inhibited at lower storage temperatures due to increased solubility of CO_2.

Table 1 Effects of packaging with/without absorbent pad, temperature (0°C and 4°C) and storage time (d) on bacterial growth of Cape hake fillet

STORAGE DAYS	TEMP. (ºC)	MAP – PAD	MAP + PAD	PMAP – PAD	PMAP + PAD
0		1.2 ± 0.13^{j}	1.2 ± 0.13^{j}	1.2 ± 0.13^{j}	1.2 ± 0.13^{j}
3	0	3.6 ± 0.07^{i}	3.5 ± 0.05^{i}	3.9 ± 0.05^{g}	3.7 ± 0.09^{h}
	4	3.8 ± 0.04^{h}	3.6 ± 0.01^{h}	6.2 ± 0.09^{c}	6.2 ± 0.03^{c}
6	0	4.5 ± 0.04^{f}	4.5 ± 0.04^{f}	7.1 ± 0.1^{a}	6.9 ± 0.03^{a}
	4	4.8 ± 0.03^{e}	4.8 ± 0.03^{e}	nd	nd
9	0	5.0 ± 0.03^{e}	4.9 ± 0.03^{e}	nd	nd
	4	6.0 ± 0.03^{d}	5.9 ± 0.03^{d}	nd	nd
12	0	5.2 ± 0.03^{e}	5.1 ± 0.03^{e}	nd	nd
	4	7.2 ± 0.03^{a}	7.2 ± 0.03^{a}	nd	nd

nd = Not determined due to sensory rejection. Different letters indicate significant difference in pH values ($p < 0.05$) according to Fischer least significant difference (LSD) Multiple Range tests; MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when bacterial growth exceeded microbial limit of < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009), thus, sampling for fillets stored under PMAP at 4°C was stopped on day 3, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6

Research findings have indicated that CO_2 retards the growth of psychotropic, aerobic and Gram-negative microbes, and slows down deterioration of fresh RTC fish fillets (Stamatis & Arkoudelos, 2007). Delayed deterioration in active-MAP stored fillets because dissolved CO_2 leads to formation of carbonic acid, the un-dissociated form of carbonic acid (bicarbonate ion) changes cell permeability and hinders metabolic processes of microbes (Masniyom, 2011). Therefore active-MAP combined with absorbent pad at optimum cold storage (0°C) can assist in extending the shelf life and maintain microbial safety of RTC hake fillets.

Volatile composition

A total of 16 VOCs were identified from MA-packaged Cape hake fillets, this includes 4 primary VOCs which were identified on fresh fish sample and 12 secondary VOCs which evolved with storage duration (Table 2-3 and Fig 1). The volatile compounds found in Cape hake fish fillets were grouped into 6 chemical classes namely: (a) alcohols; (b) Ketones; (c) organic acid; (d) amines; (e) esters; and (f) sulphur containing compounds (Table 2). Similar classes of VOCs have been reported for fish such as cod gilthead sea bream, sand-smelt, picarel, hake, pilchard, bogue, anchovy and fishery products such as squid, shrimp and mussel (Olafsdottir *et al.*, 2005; Zhang *et al.*, 2010; Giogios *et al.*, 2013; Boscaino *et al.*, 2014). Temperature, modified atmosphere and storage duration had a significant impact ($p < 0.05$) on change in composition and the relative abundance of VOCs in packaged fillets. For example there was an initial increase in the levels of ethyl alcohol across all treatments, but, this decreased as storage progressed (Table 3). These results were consistent with other reports on the volatile composition in cod fish (Olafsdottir *et al.*, 2005). The authors stated that, although the levels of ethyl alcohol increased initially there was a subsequent decrease as storage progressed and concluded that, the volatile quality of cod fish filets was affected by temperature and storage time (Olafsdottir *et al.*, 2005). Also, the initial high levels in ethyl alcohol levels could be as a result of fermentation of glycogen and its breakdown by lactic acid bacteria (Spaziani *et al.*, 2009).

Table 2 Volatile compounds identified from MA-packaged Cape hake fillets

Compound	**RT* (mins)**	**Quality**	**Aroma descriptor** ***
Alcohol			
**Ethyl alcohol	4.73	91	Sweet
**3-methyl-1-butanol	10.4	86	Malt
**2-ethyl-1-Hexanol	20.9	83	Sweet
1,2-Butanediol	7.74	83	Unknown
Phenyl ethyl Alcohol	35.1383	97	Spicy
Butylated hydroxy toluene	34.8562	95	Spicy, phenolic
Ketone			
**3-Octanone	12.3639	83	Earthy, mushroom
2,3- butanedione	5.3933	53	Butter, cheese
4- hydroxy-4 methyl-2 pentanone	16.5687	78	Unknown
Organic acid			
Acetic acid	19.92	58	Sour
Amine			
Tri-methylamine	3.7916	72	Fish like
Ester			
Ethyl acetate	4.349	72	Pineapple
Butanoic acid, ethyl ester	6.3173	91	Fruity, banana
Sulphur			
1-Propanol, 3-(methyl thio)-	29.7057	99	Unknown
Dimethyl sulphide	3.566	94	Cabbage
Dimethyl disulphide	7.3122	94	Onion, putrid

*RT = retention time; (MS software, NIST version 2.0); ** = primary volatile compounds; *** (Acree & Arn, 2004; Jonsdottir *et al*., 2008; Duflos *et al*., 2010; Moreira *et al*., 2013)

Table 3 Common volatiles identified from MA-packaged Cape hake fillets using gas chromatography mass spectra analysis showing freshness and spoilage indicator markers expressed as % peak areas indicating sampling days 0, 6 and 12

Common volatiles	RT* (Min)	Day 0	Day 6					Day 12				
			0°C MAP – PAD	0°C MAP + PAD	0°C PMAP – PAD	0°C PMAP + PAD	4°C MAP – PAD	4°C MAP + PAD	0°C MAP – PAD	0°C MAP + PAD	4°C MAP – PAD	4°C MAP + PAD
Ethyl alcohol	4.73	0.6 ± 0.10^{t}	2.3 ± 0.12m^{e}	2.2 ± 0.12^{n}	3.6 ± 0.12^{c}	3.2 ± 0.12^{f}	3.0 ± 0.12^{j}	2.9 ± 0.12^{k}	1.1 ± 0.12^{r}	0.7 ± 0.12^{s}	2.2 ± 0.12^{n}	2.1 ± 0.12^{o}
3- methyl butanol	10.4	0.1 ± 0.03^{q}	0.3 ± 0.04^{l}	0.2 ± 0.01^{n}	1.0 ± 0.03^{c}	0.9 ± 0.02^{d}	0.5 ± 0.01^{h}	0.5 ± 0.01^{h}	0.7 ± 0.01^{f}	0.5 ± 0.01^{h}	1.3 ± 0.03^{a}	1.1 ± 0.01^{b}
2-ethyl hexanol	20.9	0.3 ± 0.06^{a}	nd	Nd	0.2 ± 0.02^{b}	0.2 ± 0.02^{c}	nd	Nd	nd	nd	nd	nd
3-octanone	12.36	0.2 ± 0.01^{s}	0.4± 0.01^{n}	0.3 ± 0.02^{p}	1.0 ± 0.02^{a}	0.9 ± 0.02^{b}	0.5 ± 0.02^{l}	0.4 ± 0.01^{h}	0.8 ± 0.01^{d}	0.55 ± 0.02^{i}	0.88 ± 0.01^{c}	0.8 ± 0.02^{d}
Tri-methylamine	3.79	nd	nd	Nd	7.2 ± 0.07^{a}	5.4 ± 0.05^{b}	nd	Nd	0.3 ± 0.03^{k}	0.1 ± 0.03^{l}	2.4 ± 0.14^{g}	1.8 ± 0.13^{h}
Ethyl Acetate	4.35	nd	nd	Nd	3.3 ± 0.01^{a}	3.1± 0.01^{b}	nd	Nd	nd	nd	1.2 ± 0.01^{c}	1.2 ± 0.01^{d}
Butanoic acid Ester	6.32	nd	nd	Nd	0.8± 0.002^{a}	0.6 ± 0.001^{b}	nd	Nd	nd	nd	0.3 ± 0.001^{c}	0.2 ± 0.001^{d}
Acetic acid	19.92	nd	nd	Nd	0.4 ± 0.002^{a}	0.4± 0.002^{b}	nd	Nd	nd	nd	0.2± 0.007^{c}	0.1 ± 0.002^{d}
3-methyl thio-1-Propanol	29.71	nd	nd	Nd	0.5± 0.04^{a}	0.4± 0.03^{b}	nd	Nd	nd	nd	0.02 ± 0.0004^{c}	0.01 ± 0.0004^{b}
Dimethyl sulphide	3.57	nd	nd	Nd	2.9± 0.04^{a}	2.2± 0.03^{b}	nd	Nd	nd	nd	0.7 ± 0.013^{a}	0.6 ± 0.002^{b}
Dimethyl disulphide	7.31	nd	nd	Nd	0.2 ± 0.004^{a}	0.1 ± 0.004^{b}	nd	Nd	nd	nd	0.03 ± 0.0004^{a}	0.01 ± 0.0004b
1,2-Butane-diol	7.74	nd	nd	Nd	0.1 ± 0.002^{a}	0.06 ± 0.002^{b}	nd	Nd	nd	nd	0.04± 0.002^{c}	0.01 ± 0.002^{d}
Phenyl ethyl Alcohol	35.14	nd	nd	Nd	0.6 ± 0.04^{a}	0.5 ± 0.03^{b}	nd	Nd	0.02 ± 4X10^{-4f}	0.01 ± 3 X10^{-3g}	0.10 ± 3X10^{-4d}	0.06 ± 2X10^{-4e}
Butylated hydroxy toluene	34.86	nd	nd	Nd	0.3 ± 0.04^{a}	0.2 ± 0.03^{b}	0.1 ± 0.03^{c}	0.07 ± 0.01^{d}	nd	nd	0.2 ± 0.003^{c}	0.09 ± 0.002^{d}
2,3- butanedione	5.39	nd	nd	Nd	0.4± 0.004^{a}	0.3± 0.004^{b}	nd	nd	nd	nd	nd	nd
4-hydroxy-4methl-2-pentanone	16.57	nd	nd	Nd	0.02± 0.0024^{a}	0.02± 0.0022^{b}	nd	nd	nd	nd	0.001± 3X10^{-4c}	0.0009± 1X10^{-5d}

Peak areas are means of two GC-MS runs and approximated to one decimal places except when values are very low; nd = not detected. Different letters are significant differences between each packaged fillet; MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when bacterial growth exceeded microbial limits < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009), thus, sampling for fillets stored under PMAP at 4°C was stopped on day 3, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6. Annex shows data for days o, 3, 6, 9 and 12 (Table 3a).

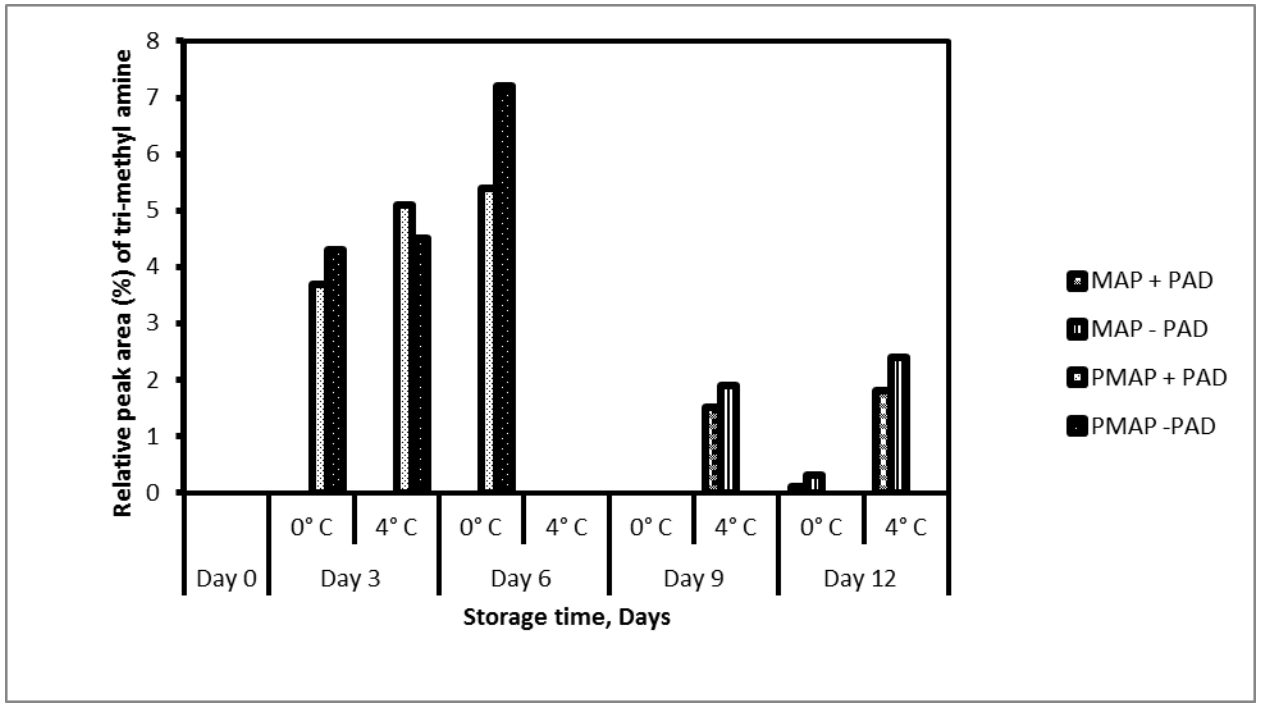

Figure 1 Effects of packaging, temperature (0°C and 4°C) and storage time on tri-methyl amine volatile quality of Cape hake fillet. Different letters indicate significant difference in pH values ($P < 0.05$), according to Fischer least significant difference (LSD) Multiple Range tests.

MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad

Sampling was stopped on days when bacterial growth exceeded microbial limits < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009), thus, sampling for fillets stored under PMAP at 4°C was stopped on day 3, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6

An increase in VOCs was observed as storage temperature increased from 0°C to 4°C on each measurement d ($p < 0.05$). For instance, lower concentrations of 3-mehyl-1-butanol were observed in active-MAP stored fillets at 0°C when compared with 4°C the values were 0.33% and 0.54%, respectively on day 6 (Table 3). Similar results were reported by Edirisinghe *et al*. (2007) in their study which investigated VOCs in yellow fin tuna at 0°C and 30°C using SPME and GC–MS. The authors found that the levels of 3-methyl-2-butanol were higher in tuna stored at 30°C than 0°C. Furthermore, 3-methyl-2-butanol was detected after 24-36 h storage in fillets stored at 30°C and on day 16 at 0°C (Edirisinghe *et al*., 2007). Similarly, Olafsdottir *et al*. (2006) investigated the effects of storage temperature on decay microbes using electronic nose in haddock fillets packaged in expanded polystyrene boxes and stored at 0°C, 7°C and 15°C. The results showed that decay alcohols, aldehydes, esters amine and sulphur volatiles were detected between day 2 and 3 at 15°C, between day 2 and 7 for fillets stored at 7°C, and days 4 and 9 for fillets stored at 0°C. Furthermore, the concentrations of these decay volatiles were highest in fillets stored at 15°C and lowest at 0°C ($p < 0.05$) (Olafsdottir *et al*., 2006).

Active-MAP maintained the evolution and composition of VOCs better than passive-MAP (Table 3 and Fig. 1). Production of TMA was significantly lower in active-MA packaged fillets throughout the storage period and across temperatures (Table 3 and Fig. 1). In passive-MAP fillets, TMA were detected on day 3 and increased throughout storage, ranging from 0.25% in fillets stored in active-MAP at 0°C on day 12 to 7.22% in fillets packaged in passive-MAP at 0°C on day 6. Similar results were reported by Alasalvar *et al*. (2005) in their study comparing the VOCs in farmed and wild sea bream stored at 2°C and 4°C for 23 days. The authors reported that TMA levels increased in both fish fillets during storage and suggested that this could be as result of bacterial degradation of marine fish fillets. Other studies have suggested that TMA may be used as index of microbial deterioration because Gram negative microbes acquire energy by reducing tri-methyl amine oxide to TMA, thereby creating fishy odour at low levels and ammonia like odours at higher levels (Gram & Dalgaard, 2002; Masniyom, 2011). High levels of TMA in fish lead to spoilage, due to short shelf life and low fillet quality (Olafsdottir *et al*., 2005).

Other spoilage indicator volatile groups identified in the present study include acetic acid, phenyl ethyl alcohol, butylated hydroxy toluene, butane 1, 2-diol, esters and sulphur (Table 3). Acetic acid was detected in passive-MA packaged fillets stored at 0°C on day 3, and this compound increased continuously with the storage duration the value at day 6 was 0.35%

(Table 3). Studies have indicated that formation of organic acids might be due to bacterial fermentation of amino acids as well as lipid oxidation which could result in acid production (Alasalvar *et al*., 2005). Acetic acid and other organic acids were identified as the source of the unpleasant odours during storage of herring at 6°C for 8 days (Aro *et al*., 2003; Alasalvar *et al*., 2005).

Similarly, butane 1, 2-diol increased from 0.07% to 0.1% in passive-MA packaged fillets as at time of sensory rejection on day 6 (Table 3). Likewise, esters were detected in passive-MA packaged fillets on day 3 for fillets stored at 0°C and the values for ethyl acetate was 3.26% on day 6 when they were rejected (Table 3). In contrast these VOCs were not detected in active-MA fillets stored at 0°C at end of storage as active–MAP and lower temperature helped to maintain volatile quality. Furthermore, the presence of esters could be as a result of microbial activity. Similar observation were reported in a study that characterised the volatiles in chilled cod fillets stored at 0.5°C, using GC and electronic nose (Olafsdottir *et al.*, 2005). The authors reported that, esters and sulphur compounds were detected at spoilage stage on day 17 (Olafsdottir *et al.*, 2005). In addition, di-methyl sulphur were detected in passive-MA packaged fillets stored at 0°C on day 6 (2.9%) while, sulphur spoilage volatiles were not detected in active-MA packaged fillets stored at 0°C at the end of storage (Table 3). Similar results were obtained with studies on the VOCs of cod using GC and electronic nose at 0°C and abuse temperatures (Olafsdottir *et al*., 2005).

Level of VOCs was higher in packages without pad than those with pad and this was consistent for all temperature conditions (Table 3). For instance, the concentration of 3-methyl-1-butanol in packages without pad was higher than those with pad. Active MA-packaged fillet without pad was 0.74%, while packages with pad had concentrations of 0.54% at 0°C. Absorbent pads helped to reduce the levels of secondary VOCs such as esters and sulphur volatiles during storage across all treatments. For example, the levels of ethyl acetate in passive-MAP without pad were 3.26%, while packages with pad had concentrations of 3.09% at 0°C. In the same vein, dimethyl sulphide levels in passive-MAP without pad were 2.86%, while package with pad had concentrations of 2.22% at 0°C. Similar results were obtained by Olafsdottir *et al*. (2005) in a study which classified VOCs present in frozen cod fillets using GC and electronic nose stored in expanded polystyrene boxes at 0.5°C. The authors proposed that the dimethyl sulphide, dimethyl disulphide and other sulphur VOCs were decay markers in cod and where the origin of putrid odours however, improper storage protocols could lead to the evolution of dimethyl trisulphide in fresh cod (Olafsdottir *et al*.,

2005; Olafsdottir & Jonsdottir, 2010). Sulphur VOCs are formed when sulphur containing amino acids in fish such as cysteine and methionine are degraded by microbes and further oxidation leads to formation of dimethyl sulphide and dimethyl disulphide (Olafsdottir & Jonsdottir, 2010). Furthermore, Hansen *et al.* (2007) investigated chemical and microbial quality of dairy farm red smear cheese made from pasteurized and un-pasteurized milk studies. The authors reported that, late acidification of red smear cheese influenced the production of VOCs such as dimethyl sulphide and this could be as a result of higher microbial counts. Furthermore they concluded that, lower pH resulted in lower microbial counts in red smear cheese (Hansen *et al*., 2007). Results from this study is consistent with findings in literature that higher CO_2 levels better maintains volatile quality of muscle meat at 0°C and 4°C with 0°C being the optimum storage temperature (Summo *et al*., 2010).

Correlation between quality indices

Pearson correlation was conducted between aerobic mesophillic counts and selected spoilage indicators in the VOCs to identify spoilage markers that predict the shelf life and quality attributes of Cape hake fish (Table 4). Significant associations of interest with various correlation coefficients were obtained. Moderately positive relationships (r = 0.57) and (r = 0.66) were established between aerobic mesophillic counts (AMCs) and ethyl acetate and butanoic acid esters suggesting that the presence of moderate amounts of esters in the headspace of fish fillets could have been produced by the AMCs (Table 6). In addition, moderate association was established between AMCs and di-methyl sulphide (r = 0.68), in contrast, strong correlations were shown between AMCs and dimethyl disulphide (r = 0.73). This implies that sulphur containing volatiles could be good indicators of spoilage in Cape hake fish fillets. The use of SPME/GC/MS has led to the establishment of correlations between AMCs and dimethyl sulphide in fresh and iced fish such as, European sea bass, gilthead sea bream, cod and salmon, however, correlations between AMCs and dimethyl disulphide have only been established in red smear cheese (Hansen *et al*., 2007; Leduc *et al*., 2012).

Furthermore, moderate correlations where observed between AMCs and TMA (r = 0.50), implying that TMA might not be a good spoilage marker as spoilage might have occurred before TMA was detected in the headspace of packaged fillets. Nevertheless, strong positive

correlations were established between TMA and other VOCs the values were ethyl acetate (r = 0.84), butanoic acid ester (r = 0.88), di-methyl sulphide (r = 0.80) and dimethyl disulphide (r = 0.74). This suggests that TMA could be used in conjunction with other spoilage markers to predict shelf life and quality of Cape hake fish fillets during storage.

Table 4 Pearson correlation coefficient matrix between aerobic mesophillic counts selected spoilage quality indicators measured in Cape hake fillets during storage

Variables	AMC	EA	BE	DMS	DMDS	TMA
AMC	**1**					
EA	**0.565**	**1**				
BE	**0.661**	**0.901**	**1**			
DMS	**0.677**	**0.887**	**0.910**	**1**		
DMDS	**0.728**	**0.825**	**0.908**	**0.968**	**1**	
TMA	**0.496**	**0.843**	**0.878**	**0.796**	**0.738**	**1**

Correlation values in **bold** are significant at $p < 0.05$ using principal component analysis (PCA) using XLSTAT software Version 2012.4.01 (Addinsoft, France).

AMC = Aerobic mesophillic count, EA = Ethyl acetate, BE = Butanoic acid ester, DMS= Dimethyl sulphide, DMDS = Dimethyl disulphide, TMA= Tri-methyl amine.

Conclusion

This study investigated the effects of MAP with/without absorbent pad on the VOCs of packaged Cape hake fillets at 0°C and 4°C. The use of MAP with absorbent pad had a significant impact on changes in volatile compounds of Cape hake fillets at 0°C and 4°C during storage ($p < 0.05$). Comparison of the changes in the volatile compositions during storage and their correlations with AMCs led to the identification of spoilage compounds that could possibly be used to predict shelf life and quality indicators for packaged Cape hake fish fillets. Fillets in active-MAP packages performed better than passive-MA packaged fillets stored at 0°C as evidenced by lower amounts of TMA, ester group and sulphur group. Based on measured microbial and volatile attributes, active-MAP (40% CO_2 + 30% O_2 + 30% N_2) combined with low temperature storage (0°C) extended the shelf life and maintained quality attributes of Cape hake fillets for 12 d. These findings provide a useful guide in fresh fish processing towards predicting the shelf life and quality of Cape hake fillets and other fish products during storage. Rapid detection and monitoring of the concentrations of spoilage VOCs, such as esters in conjunction with tri-methyl amine, which are detected early during storage can provide warning signals (spoilage marker) on the quality and safety status of fresh Cape hake during postharvest handling and marketing fillet.

References

Acree, T. & Arn, H. (2004). Flavornet and Human Odour Space, Cornell University, <http://flavornet.com>.

Angis S. & Oguzhan P. (2013). Effect of thyme essential oil and packaging treatments on chemical and microbiological properties of fresh rainbow trout (*Oncorhynchus mykiss*) fillets during storage at refrigerator temperatures. *African Journal of Microbiology Research*, **7**, 1136-1143.

Alasalvar, C., Taylor, K.A. & Shahidi, F. (2005). Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis/gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, **53**, 2616-2622.

Aro, T., Tahvonen, R., Koskinen, L. & Kallio, H. (2003). Volatile compounds of Baltic herring analysed by dynamic headspace sampling–gas chromatography–mass spectrometry. *European Food Research and Technology*, **216**, 483-488.

Boscaino, F., Acierno, V., Saggese, P., Cozzolino, R., Motta, C.M. & Sorrentino, A. (2014). Effectiveness of vacuum devices for home storage of rainbow trouts from game fishing lakes. *African Journal of Science and Research*, **3**, 16-22.

Caglak, E., Cakli, S. & Kilinc B. (2012). Effect of modified atmosphere packaging on quality and shelf-life of salted bonito (*Sarda sarda*). *Journal of Aquatic Food Product Technology*, **21**, 206–221.

Caleb, O.J., Mahajan, P.V., Al-Said, F.A.J. & Opara, U.L. (2013). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences—.a review. *Food and Bioprocess Technology*, **6**, 303-329.

Choubert, G. & Baccaunaud, M. (2006). Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss W.*) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere. *LWT Food Science and Technology*, **39**), 1203-1213.

Da Silva, N., Taniwaki, M.H., Junqueira, V.C., Silveira, N., Do Nascimento, M.D.S. & Gomes, R.A.R. (2012). Microbiological examination methods of food and water. A laboratory manual. Washington, USA: CRC Press.

Del Nobile, M.A., Corbo, M.R., Speranza, B., Sinigaglia, M., Conte, A. & Caroprese, M. (2009). Combined effect of modified atmosphere packaging and active compounds on fresh blue fish burger. *International Journal of Food Microbiology*, **135**, 281-287.

DOH - Department of Health (2001). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. South Africa: Department of Health Directorate: Food Control.

Domingo, J.L., Bocio, A., Falco, G. & Llobet, J. M. (2007). Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology*, **230**, 219-226.

Duflos, G., Leduc, F., N'Guessan, A., Krzewinski, F., Kol, O. & Malle, P. (2010). Freshness characterisation of whiting (*Merlangius merlangus*) using an SPME/GC/MS method and a statistical multivariate approach. *Journal of the Science of Food and Agriculture*, **90**, 2568-2575.

Edirisinghe, R.K., Graffham, A.J. & Taylor, S.J. (2007). Characterisation of the volatiles of yellowfin tuna (*Thunnus albacares*) during storage by solid phase micro-extraction

and GC–MS and their relationship to fish quality parameters. *International Journal of Food Science & Technology*, **42**, 1139-1147.

Erkan, N., Ozden, O. & Inugur, M. (2007). The effects of modified atmosphere and vacuum packaging on quality of chub mackerel. *International Journal of Food Science and Technology*, **42**, 1297-1304.

Food and Agricultural Organisation, FAO, (2014). World review of fisheries and aquaculture part 1. http://www.fao.org/docrep/013/i1820e/i1820e.

Giogios, I., Kalogeropoulos, N. & Grigorakis, K. (2013). Volatile compounds of some popular Mediterranean seafood species. *Mediterranean Marine Science*, **14**, 343-352

Gram, L. & Dalgaard, P. (2002). Fish spoilage bacteria: problems and solutions. *Current Opinion in Biotechnology*, **13**, 262-266.

Gram, L. & Huss, H.H. (2000). Fresh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Foods*, edition 1. (edited by B.M. Lund, A.C. Baird-Parker, and G.W. Gould). Pp. 472-506. London: Chapman & Hall.

Hara-Kudo, Y., Sugiyama, K., Nishibuchi, M., Chowdhury, A., Yatsuyanagi, J., Ohtomo, Y. & Kumagai, S. (2003). Prevalence of pandemic thermo stable direct haemolysin-producing *Vibrio parahaemolyticus* O3: K6 in seafood and the coastal environment in Japan. *Journal of Applied and Environmental Microbiology*, **69**, 3883-3891.

Hansen, T.B., Larsen, L.B., Hammershoj, M., Nielsen, J.H., Hojgaard, K.S. & Knochel, S. (2007). Microbiological and chemical profiles of dairy farm red smear cheese made from pasteurized and un-pasteurized milk. *Food Research International.*

HPA – Health protection agency, (2009). Guidelines for assessing the microbiological safety of ready-to-eat foods. Health Protection Agency, London: UK Government Printers; 2009.

Jezek, F. & Buchtova, H. (2007). Physical and chemical changes in fresh chilled muscle tissue of common carp (*Cyprinus carpio L.*) packed in a modified atmosphere. *Acta Veterinaria Brno*, **76**, *83-92.*

Jezek, F. (2012). Effect of Modified atmosphere packaging on the course of physical & chemical changes in chilled muscle of silver carp. (*Hypophthalmichthys molitrix*). *Polish Journal of Veterinary Science*, **15**, 439-445.

Jonsdottir, R., Olafsdottir, G., Chanie, E. & Haugen, J. E. (2008). Volatile compounds suitable for rapid detection as quality indicators of cold smoked salmon (*Salmo salar*). *Journal of Food chemistry*, **109**, 184-195.

Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, **106**, 2747-2757.

Lauzon, H.L., Magnusson, H. Sveinsdottir, K., Gudjonsdottir, M. & Martinsdottir, E. (2009). Effect of brining, modified atmosphere packaging, and super-chilling on the shelf-life of cod (*Gadus morhua*) loins. *Journal of Food Science*, **74** 258-267.

Leduc, F., Krzewinski, F., Le Fur, B., N'Guessan, A., Malle, P., Kol, O. & Duflos, G. (2012). Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus* labrax), gilthead sea bream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS. *Journal of the Science of Food and Agriculture*, **92**, 2560-2568.

Masniyom, (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Sonklanakarin Journal of Science and Technology*, **33**, 181-192.

Moreira, A.B., Visentainer, J.V., de Souza, N.E. & Matsushita, M., (2001). Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. *Journal of Food Composition and Analysis*, **14**, 565–574.

Moreira, N., Valente, L. M. P., Castro-Cunha, M., Cunha, L. M. & Guedes de Pinho, P. (2013). Effect of storage time and heat processing on the volatile profile of Senegalese sole (*Solea senegalensis Kaup*, 1858) muscle. *Journal of Food Chemistry*, **138**, 2365-2373.

Olafsdottir, G., Jonsdottir, R., Lauzon, H.L., Luten, J. & Kristbergsson, K. (2005). Characterization of volatile compounds in chilled cod (*Gadus morhua*) fillets by gas chromatography and detection of quality indicators by an electronic nose. *Journal of Agriculture and Food Chemistry*, **53**, 10140-10147.

Olafsdottir, G., Lauzon, H. L., Martinsdottir, E. & Kristbergsson, K. (2006). Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. *International Journal of Food Microbiology*, **111**, 112-125.

Olafsdottir, G. & Jonsdottir, R. (2010). Volatile aroma compounds in fish. In: Handbook of seafood and seafood products analysis (edited by L. M. Nollet & F. Toldra). Pp. 97-117. Boca Raton CRC Press.

Ordonez, J.A., Lopez-Galvez, D.E., Fernandez, M., Hierro, E. & de la Hoz, L. (2000). Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, **80**, 1831-1840.

SANS (2007). Method 4833. Second Edition. Microbiology: General guidance for the enumeration of microorganisms - Colony count technique at 30°C, Pretoria: South African Bureau of Standards, Pretoria, South Africa.

Sivertsvik, M. (2007). The optimized modified atmosphere for packaging of pre- rigor filleted farmed cod (*Gadus morhua*) is 63ml/100ml oxygen and 37ml/100ml carbon dioxide. *LWT-Food Science and Technology*, **40**, 430-438.

Spaziani, M., Torre, M. D. & Stecchini, M. L. (2009). Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. *Meat Science*, **81**, 77-85.

Stamatis, N. & Arkoudelos, J. (2007). Quality assessment of *Scomber colias japonicas* under modified atmosphere and vacuum packaging. *Food Control*, **18**, 292-300.

Summo, C., Caponio, F., Tricarico, F., Pasqualone, A. & Gomes, T. (2010). Evolution of the volatile compounds of ripened sausages as a function of both storage time and composition of packaging atmosphere. *Meat Science*, **86**, 839-844.

Triqui, R. & Bouchriti, N. (2003). Freshness assessments of Moroccan Sardine (*Sardina pilchardus*): Comparison of overall sensory changes to instrumentally derived volatiles. *Journal of Agricultural Food Chemistry*, **51**, 7540-7546.

Trondsen, T., Scholderer, J., Lund, E. & Eggen. A.E. (2003). Perceived barriers to consumption of fish among Norwegian women. *Appetite*, **41**, 301–314.

Yesudhason, P., Gopal, T. K. S., Ravi Shankar, C. N., Lalitha, K.V. & Kumar, K. N. A. (2009). Effect of modified atmosphere packaging on chemical, textural, microbiological and sensory quality of seer fish (*Scomberomorus commerson*) steaks packaged in thermo-formed trays at 0–2ºC. *Journal of Food Processing and Preservation* **33**, 777-797.

Zhang, Z., Li, G., Luo, L. & Chen, G. (2010). Study on seafood volatile profile characteristics during storage and its potential use for freshness evaluation by headspace solid phase micro-extraction coupled with gas chromatography–mass spectrometry. *Analytica chimica acta*, **659**, 151-158.

Annex

Table 3a Common volatiles identified from MA-packaged Cape hake fillets using gas chromatography mass spectra analysis showing freshness and spoilage indicator markers expressed as % peak areas by sampling days 0, 3, 6, 9, and 12

Common volatiles	RT* (Min)	Day 0	Day 3							
			0°C MAP – PAD	0°C MAP + PAD	0°C PMAP – PAD	0°C PMAP + PAD	4°C MAP – PAD	4°C MAP + PAD	4°C PMAP – PAD	4°C PMAP + PAD
Ethyl alcohol	4.73	0.6 ± 0.10^{t}	3.3 ± 0.04^{h}	3.2 ± 0.01^{i}	4.9 ± 0.02^{a}	4.5 ± 0.02^{b}	3.5 ± 0.02^{f}	3.4 ± 0.01^{g}	3.8± 0.02^{c}	3.7± 0.02^{d}
3- methyl butanol	10.4	0.1 ± 0.03^{q}	0.15 ± 0.01^{o}	0.13 ± 0.01^{p}	0.72 ± 0.03^{e}	0.68 ± 0.03^{f}	0.33 ± 0.02^{k}	0.27 ± 0.02^{m}	0.54 ± 0.02^{g}	0.52 ± 0.02^{g}
2-ethyl hexanol	20.9	0.3 ± 0.06^{a}	nd	nd	0.04 ± 0.02^{b}	0.03 ± 0.01^{c}	nd	nd	nd	nd
3-octanone	12.36	0.2 ± 0.01^{s}	0.21 ± 0.041^{r}	0.21 ± 0.01^{r}	0.73 ± 0.01^{f}	0.64 ± 0.01^{h}	0.33 ± 0.01^{o}	0.26 ± 0.01^{q}	0.55 ± 0.01^{i}	0.52 ± 0.01^{k}
Tri-methylamine	3.79	nd	nd	nd	4.3 ± 0.03^{e}	3.7 ± 0.02^{f}	nd	nd	5.1 ± 0.01^{c}	4.5 ± 0.01^{d}
Ethyl Acetate	4.35	nd	nd	nd	1.14 ± 0.05^{e}	0.86 ± 0.03^{f}	nd	nd	nd	nd
Butanoic acid Ester	6.32	nd	nd	nd	0.32 ± 0.002^{e}	0.26 ± 0.001^{f}	nd	nd	nd	nd
Acetic acid	19.92	nd	nd	nd	0.14 ± 0.003^{e}	0.12 ± 0.002^{f}	nd	nd	nd	nd
3-methyl thio-1-Propanol	29.71	nd	nd	nd	0.04 ± 0.003^{e}	0.03 ± 0.002^{f}	nd	nd	nd	nd
Dimethyl sulphide	3.57	nd	nd	nd	0.2 ± 0.003^{e}	0.1 ± 0.002^{f}	nd	nd	nd	nd
Dimethyl disulphide	7.31	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,2-Butane-diol	7.74	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phenyl ethyl Alcohol	35.14	nd	nd	nd	0.2 ± 0.003^{c}	0.1 ± 0.002^{d}	nd	nd	nd	nd
Butylated hydroxy toluene	34.86	nd	nd	nd	0.02± 0.003^{f}	0.02± 0.002^{f}	nd	nd	nd	nd
2,3- butanedione	5.39	nd	nd	nd	0.01 ± 0.001^{c}	0.01± 0.001^{d}	nd	nd	nd	nd
4-hydroxy-4methl-2-pentanone	16.57	nd	nd	nd	nd	nd	nd	nd	nd	nd

Peak areas are means of two GC-MS runs and approximated to one decimal places except when values are very low; nd = not detected. Different letters are significant differences between each packaged fillet; MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when bacterial growth exceeded microbial limits < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009), thus, sampling for fillets stored under PMAP at 4°C was stopped on day 3, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6.

Table 3a: continued

Common volatiles	RT* (Min)	Day 6					
		0°C MAP – PAD	0°C MAP + PAD	0°C PMAP – PAD	0°C PMAP + PAD	4°C MAP – PAD	4°C MAP + PAD
Ethyl alcohol	4.73	2.3 ± 0.12^{m}	2.2 ± 0.12^{n}	3.6 ± 0.12^{e}	3.5 ± 0.12^{f}	3.0 ± 0.12h^{j}	2.9 ± 0.12^{k}
3- methyl butanol	10.4	0.3 ± 0.04^{l}	0.2 ± 0.01^{n}	1.0 ± 0.03^{c}	0.9 ± 0.02^{d}	0.5 ± 0.01^{h}	0.5 ± 0.01^{h}
2-ethyl hexanol	20.9	nd	nd	0.2 ± 0.02^{b}	0.2 ± 0.02^{c}	nd	nd
3-octanone	12.36	0.4± 0.01^{n}	0.3 ± 0.02^{p}	1.0 ± 0.02^{a}	0.9 ± 0.02^{b}	0.5 ± 0.02^{l}	0.4 ± 0.01^{n}
Tri-methylamine	3.79	nd	nd	7.2 ± 0.07^{a}	5.4 ± 0.05^{b}	nd	nd
Ethyl Acetate	4.35	nd	nd	3.3 ± 0.01^{a}	3.1± 0.01^{b}	nd	nd
Butanoic acid Ester	6.32	nd	nd	0.78 ± 0.002^{a}	0.59 ± 0.001^{b}	nd	nd
Acetic acid	19.92	nd	nd	0.4 ± 0.002^{a}	0.4± 0.002^{b}	nd	nd
3-methyl thio-1-Propanol	29.71	nd	nd	0.5± 0.04^{a}	0.4± 0.03^{b}	nd	nd
Dimethyl sulphide	3.57	nd	nd	2.9± 0.04^{a}	2.2± 0.03^{b}	nd	nd
Dimethyl disulphide	7.31	nd	nd	0.2 ± 0.004^{a}	0.1 ± 0.004^{b}	nd	nd
1,2-Butane-diol	7.74	nd	nd	0.1 ± 0.002^{a}	0.06 ± 0.002^{b}	nd	nd
Phenyl ethyl Alcohol	35.14	nd	nd	0.6 ± 0.04^{a}	0.5 ± 0.03^{b}	nd	nd
Butylated hydroxy toluene	34.86	nd	nd	0.3 ± 0.04^{a}	0.2 ± 0.03^{b}	0.1 ± 0.03^{c}	0.07 ± 0.01^{d}
2,3- butanedione	5.39	nd	nd	0.4± 0.004^{a}	0.3± 0.004^{b}	nd	nd
4-hydroxy-4methl-2-pentanone	16.57	nd	nd	0.02± 0.0024^{a}	0.02± 0.0022^{b}	nd	nd

Peak areas are means of two GC-MS runs and approximated to one decimal places except when values are very low; nd = not detected. Different letters are significant differences between each packaged fillet; MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when bacterial growth exceeded microbial limits < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009), thus, sampling for fillets stored under PMAP at 4°C was stopped on day 3, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6.

Table 3a: continued

Common volatiles	RT* (Min)	Day 9				Day 12			
		0°C MAP – PAD	0°C MAP + PAD	4°C MAP – PAD	4°C MAP + PAD	0°C MAP – PAD	0°C MAP + PAD	4°C MAP – PAD	4°C MAP + PAD
Ethyl alcohol	4.73	1.9 ± 0.02^{p}	1.8 ± 0.01^{q}	2.9 ± 0.12^{k}	2.8 ± 0.12^{l}	1.1 ± 0.12^{qr}	0.7 ± 0.12^{s}	2.2 ± 0.12^{n}	2.1 ± 0.12^{o}
3- methyl butanol	10.4	0.41 ± 0.02^{i}	0.36 ± 0.02^{j}	0.54 ± 0.02^{g}	0.50 ± 0.02^{h}	0.7 ± 0.01^{f}	0.5 ± 0.01^{h}	1.3 ± 0.03^{a}	1.1 ± 0.01^{b}
2-ethyl hexanol	20.9	nd	nd	nd	nd	nd	nd	nd	nd
3-octanone	12.36	0.54 ± 0.02^{j}	0.45 ± 0.01^{m}	0.76 ± 0.01^{e}	0.69 ± 0.01^{g}	0.8 ± 0.01^{d}	0.55 ± 0.02^{i}	0.88 ± 0.01^{c}	0.8 ± 0.02^{d}
Tri-methylamine	3.79	nd	nd	1.9 ± 0.03^{i}	1.5 ± 0.02^{j}	0.3 ± 0.03^{k}	0.1 ± 0.03^{l}	2.4 ± 0.14^{g}	1.8 ± 0.13^{h}
Ethyl Acetate	4.35	nd	nd	0.52 ± 0.03^{g}	0.46 ± 0.03^{h}	nd	nd	1.2 ± 0.01^{c}	1.2 ± 0.01^{d}
Butanoic acid Ester	6.32	nd	nd	0.15 ± 0.002^{g}	0.09 ± 0.001^{h}	nd	nd	0.27 ± 0.001^{c}	0.24 ± 0.001^{d}
Acetic acid	19.92	nd	nd	nd	nd	nd	nd	0.2 ± 0.007^{c}	0.1 ± 0.002^{d}
3-methyl thio-1-Propanol	29.71	nd	nd	nd	nd	nd	nd	0.02 ± 0.0004^{c}	0.01 ± 0.0004^{d}
Dimethyl sulphide	3.57	nd	nd	nd	nd	nd	nd	0.7 ± 0.013^{c}	0.6 ± 0.002^{d}
Dimethyl disulphide	7.31	nd	nd	nd	nd	nd	nd	0.03 ± 0.0004^{a}	0.01 ± 0.0004b
1,2-Butane-diol	7.74	nd	nd	nd	nd	nd	nd	0.04 ± 0.002^{c}	0.01 ± 0.002^{d}
Phenyl ethyl Alcohol	35.14	nd	nd	nd	nd	0.02 ± 0.0004^{f}	0.01 ± 0.0003^{g}	0.10 ± 0.0003^{d}	0.06 ± 0.0002^{e}
Butylated hydroxy toluene	34.86	nd	nd	nd	nd	nd	nd	0.2 ± 0.003^{b}	0.09 ± 0.002^{d}
2,3- butanedione	5.39	nd	nd	nd	nd	nd	nd	nd	nd
4-hydroxy-4methl-2-pentanone	16.57	nd	nd	nd	nd	nd	nd	0.001 ± 0.0003^{c}	0.00009 ± 0.00001^{d}

Peak areas are means of two GC-MS runs and approximated to one decimal places except when values are very low; nd = not detected. Different letters are significant differences between each packaged fillet; MAP - PAD: active-MA without absorbent pad; MAP + PAD: active-MA with absorbent pad, PMAP - PAD: passive-MA without absorbent pad and PMAP + PAD: passive-MA with absorbent pad.

Sampling was stopped on days when bacterial growth exceeded microbial limits < 5.5 log cfu/g by day 6 (DOH, 2001; HPA, 2009), thus, sampling for fillets stored under PMAP at 4°C was stopped on day 3, furthermore, sampling for fillets stored under PMAP at 0°C was stopped on day 6

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Introduction

Global changes in consumer lifestyle have led to a rise in demand and consumption of fresh ready-to-cook (RTC) fish due to the reported high nutritional and health benefits (Kris-Etherton *et al*., 2002; Trondsen *et al*., 2003; De Silva & Yamao, 2006; Domingo *et al*., 2007; FAO 2009; Speranza *et al*., 2009). According to the FAO (2014), postharvest fish loss due to decay is estimated to be 10 – 12 million tonnes annually and researchers have reported that fish decay is due to the high perishability which in turn is linked to the high water activity ($a_w \geq 0.95$) and post mortem pH (≥ 5.2) (Choubert & Baccaunaud, 2006; Jezek & Buchtova, 2012). Although numerous postharvest technological solutions such as salting, drying, smoking, cold storage and frying have been offered by researchers to maintain the postharvest quality of fish (Dondero *et al*., 2004; Bellagha *et al*., 2007), value addition has been minimal, particularly in developing countries. The application of improved and novel postharvest technique such as MAP can play an important role in reducing food losses and waste of fish and other fish products. Modified atmosphere packaging (MAP), in combination with optimum cold storage, offers the possibility to extend shelf life and maintain quality for fresh fish and fishery products (Ordonez *et al*., 2000; Erkan *et al*., 2007; Sivertsvik, 2007; Del Nobile *et al*., 2009; Lauzon *et al*., 2009; Opara, 2009; Yesudhason *et al*., 2009; Caglak *et al*., 2012; Angis & Oguzhan, 2013). Furthermore, MAP offers the possibility of inhibitng spoilage micro-organisms (Farber *et al*., 2003), and prolonging shelf life of seafood by several days in contrast to normal air storage (Pantazi *et al*., 2008) without the usage of additives and preservatives.

In South Africa, Cape hake falls under wild capture fisheries. Although Cape hake accounts for about 80% of the total economic contribution of the fish industry to the economy, the short shelf life and poor quality of the produce remain important challenges limiting the market potential (FAO, 2005; DAFF, 2012). Active-MAP has been demonstrated to prolong the shelf life of fish and fishery products, meat, cheese, and fresh or fresh-cut fruit and vegetables by up to four times compared with passive-MAP (Sivertsvik *et al*., 2002; Pantazi *et al*., 2008; Del Nobile *et al*., 2009; Caleb *et al*., 2012; Jezek & Buchtova, 2012; Cyprian *et al*., 2013) however, information on the application of active MAP on Cape hake is

lacking. The application of novel postharvest technologies such as active MAP to maintain product quality and provide shelf-stable and safe fresh fish and fishery products would also contribute towards ensuring the competitiveness of the South African fish processing industry. The aim of this study was to investigate the effects of MAP (active versus passive) on postharvest quality and shelf life of Cape hake (*Merluccius capensis*) fish fillets. To achieve this aim, the specific objectives were to: (a) evaluate the effects of active MAP and storage temperature on quality attributes (microbial, physicochemical and sensory) and shelf life of Cape hake, (b) study the effects of adding absorbent pads on the effectiveness of MAP on Cape hake quality, and (c) investigate the effects of active-MAP and storage temperature on volatile composition of Cape hake. The outputs of the study would provide new scientific information to assist in reducing fish food losses and waste, thereby contributing towards maintaining a sustainable and competitive hake fish industry in South Africa.

This dissertation was structured into the following five chapters:

- Chapter 1: Background, problem statement and rationale
- Chapter 2: Literature review on modified atmosphere packaging of fish and fishery products.
- Chapter 3: Effects of modified atmosphere packaging, storage temperatures and absorbent pads on quality of fresh Cape hake fish fillets.
- Chapter 4: Changes in volatile composition and concentration of Cape hake fillets as affected by modified atmosphere packaging, storage temperature and duration.
- Chapter 5: General discussion and conclusions.

Introduction and Literature review on modified atmospheres packaging of fish and fishery products (Chapter 1 and 2)

This chapter introduced the thesis and reviewed the literature on modified atmosphere packaging (MAP) in combination with cold storage of fresh fish and fishery products. The objective of the review was to discuss the successful use of MAP to enhance the storage period of fish and fishery products. Literature evidence showed that the application of MAP has been successful in enhancing the storability of fish and fishery products, while maintaining its organoleptic attributes. (Lauzon *et al*., 2009; Yesudhason *et al*., 2009; Caglak *et al*., 2012; Angis & Oguzhan, 2013). Furthermore, research findings have shown that the lag phase of microbes endogenous to fish and fishery products was extended, resulting in the inhibition of decay metabolites and thus delaying decay incidence (Sivertsvik *et al*., 2002;

Arashishar *et al*., 2004; Stamatis & Arkoudelos, 2007; Lauzon *et al*., 2009; Caglak *et al*., 2012).

The combination of MAP with active and intelligent packaging, hurdles and good managing practices has gone a long way in meeting increased consumer demand for fresh and value added fish products such as ready-to-cook fish. Applications of active packaging technologies such as O_2 and CO_2 scavengers and emitters, moisture scavengers, and antimicrobials which inhibit the major decay mechanisms in MAP offer promising prospects. For instance, intelligent packaging technologies involving time temperature indicators, gas leakage indicators, freshness indicators, toxin indicators, biosensors, and radio frequency identification tools which communicate freshness attributes of the fish to the consumer present novel opportunities to influence purchase behaviour with the potential to reduce food wastage. These technological innovations have assisted food processors in adding value, ensuring that fish is devoid of hazards, addressing nutritional and health benefits, conservation issues, lowering costs and enhancing the storage potential and organoleptic properties of fresh fish. Furthermore, this will result in meeting the global consumer demand for safe and fresh RTC fish and predict the microbial safety of fishery products.

This review indicated that various species of fish reacted to MAP in different ways depending on the conditions used. Therefore, experimental studies should be carried out on an indigenous South African fish species to ascertain information on quality attributes such as volatile, microbial, sensorial, chemical and physical under diverse states so as to facilitate a productive use of MAP.

Moreover, other problems that arise in MAP packaged fish and fishery products such as optimal polymeric films for fresh fish can be assessed. In addition when unhygienic practices and packaging temperatures are abused the enzymatic, respiratory and lipid oxidative activities are propagated and microbial decline of fresh fish is accelerated.

This places emphasis on maintaining good management practice and low temperatures so as to stem postharvest loss of fresh fish assure quality and reduce the effect of hazardous pathogens in ready-to-cook fresh fish. Literature has shown that, the use of active-MAP depends on the type of fish being packaged with fatty fish such as Rainbow trout the recommended gas composition is 90% CO_2 + 7.5% N_2 + 2.5% O_2 due to high amounts of fat in fish muscles (Arashishar *et al*., 2004). On the other hand in lean fish such as hake gas compositions of 40% CO_2 + 30% N_2 + 30% O_2 has been shown to extend shelf life optimally (Ordonez *et al*., 2000; Speranza *et al*., 2009). Fresh fish stored under MAP conditions with high amounts of CO_2 can retain freshness attributes for a longer period, due to the ability of carbon dioxide to retard enzyme action and growth of microorganisms (Sivertsvik, 2007).

However, the capability of pathogenic micro-organisms to grow under this gas conditions highlights the microbial hazard and safety concerns. Therefore, in order to eliminate microbial hazards and sustain overall quality assurance of ready-to-eat or ready-to-cook fish application of hurdle technologies such as pre-treatments coupled with MAP, value addition such as absorbent pads, adequate hygienic practices and good management practices are necessary (Sivertsvik *et al*., 2002; FAO, 2005; Ababouch, 2006). The use of high or low levels of gases in active-MAP does not present negative effect because food grade gases are used in packaging. In addition active-MAP enhances product organoleptic attributes four times that of passive-MAP (Erkan *et al*., 2007; Sivertsvik, 2007; Del Nobile *et al*., 2009; Lauzon *et al*., 2009; Opara, 2009; Yesudhason *et al*., 2009; Caglak *et al*., 2012; Angis & Oguzhan, 2013)

Effects of modified atmosphere packaging, storage temperature and absorbent pads on the quality of fresh Cape hake fish fillets (Chapter 3)

Average baseline concentrations of fat, protein, moisture and ash content were approximately 0.29%, 17.22%, 82.90 % and 4.9 %, respectively. Active-MAP and storage temperatures of 0°C and 4°C better helped to retain proximate compostion of Cape hake fillets by end of storage on day 12 and 9. On the other hand, proximate composition reduced by a higher rate in passive-MA packaged fillets by day 1 for fillets stored at 8°C and day 3 and 6 for fillets stored at 4°C and 0°C, respectively. Percentage difference in fat, protein, moisture and ash content for active-MAP fillets stored at 0°C was 1.4%, 0.06%, 0.08 % and 2.0 %, respectively but percentage difference in fat, protein, moisture and ash content for passive-MAP fillets stored at 0°C was 6.2%, 9.8%, 0.43 % and 12.0 %, respectively. Similarly, fillets packaged with active-MA at 8°C (abuse temperature) had a higher rate of reduction in proximate compostion by day 3. The values were 4.3%, 7.6%, 0.25 % and 8.35 %, respectively. Our results showed that active-MAP and storage at temperatures 0°C and 4°C better helped to maintain proximate quality but active-MAP fillets stored at 8°C (abuse temperature) nullified the benefits of acive-MAP. Similar findings were reported by Del Nobile *et al.* (2009) for blue fish burger consisting of fresh hake and mackerel fillets stored under three different gas compositions (MAP 1 40% CO_2 + 30% O_2 + 30% N_2, MAP 2 50% CO_2 + 50 % O_2 and MAP 35% O_2 +95% CO_2) and passive-MAP stored for 28 days at 4°C. The authors observed that the percentage difference in fat, protein, moisture and ash for passive-MAP stored fillets were 16.7%, 7.2%, 3.8% and 11.8% while MAP 1 stored fillets which was the same gas composition used in our study had 1.4%, 1.2%, 0.64% and 3.2%. They also stated that reduction in proximate composition was highest in MAP 3 and lowest in MAP 2 stored fillets.

This could be as a result of lower oxygen in MAP 3. They concluded that active-MAP storage did not result in significant losses in the fat, moisture and protein content of hake fillets. Therefore the use of optimal packaging conditions such as MAP in the storage of fresh Cape hake fillets is vital in maintaining quality and shelf life of Cape hake fillets.

Headspace gas composition of O_2 and CO_2 was significantly influenced by the MAP storage temperature, duration, and their interactions. Level of O_2 decreased continuously across all treatments, and the levels for passive MA-packaged fillets reached critical limits of about 2% at day 9. Oxygen levels decresed in all treatments across all temperatures however the decrease was higher in passive MA-packaged fillets at 0°C on day 9. For instance decrease in passive MA-packaged fillets at 0°C on day 9 was about 90% however decrease at 0°C in active-MA-packaged fillets was about 66% on day 15. This was as a result of the CO_2 which was able to inhibit growth of microbes in fillets stored in active-MA-packages in contrast to passive-packages. Furthermore, CO_2 levels initially decreased during the first few days of storage of fillets in active-MAP and this decrease was more at 0°C than at 4°C and 8°C. The decrease at 0°C, 4°C and 8°C were 35%, 20% and 12% the results obtained shows why fillets stored at 8°C (abuse temperature) deteriorated faster than fillets stored at other temperatures. This advocates the enhanced solubility of CO_2 at lower temperatures, as the benefits of a CO_2 enriched packaged are lost at abuse temperatures (Sivertsvik *et al*., 2002). Other authors corroborated our findings on active-MAP-stored fillets and stated that the initial decrease in CO_2 levels during storage was higher at lower temperatures (Sivertsvik *et al*., 2004; Torrieri *et al*., 2006; Wang *et al*., 2008; Cyprian *et al.*, 2013). In addition, it is well known that CO_2 hinders the growth of psychotropic, aerobic and Gram-negative microbes and slows down deterioration of fresh RTC fish fillets (Stamatis & Arkoudelos, 2007). Previous reports have also shown that the antimicrobial action of CO_2 is dependent on its increased solubility in water at lower temperatures and the extension of lag phase of bacteria (Sivertsvik *et al*., 2002; Arashishar *et al*., 2004; Speranza *et al*., 2009; Cyprian *et al*., 2013). The mechanism involves the formation of carbonic acid from the dissolved CO_2 and the bicarbonate ion changes cell permeability and hinders metabolic processes of microbes thereby extending the lag phase of the endogenous microbes (Sivertsvik *et al*., 2002; Arashishar *et al*., 2004; Stamatis & Arkoudelos, 2007; Lauzon *et al*., 2009; Caglak *et al*., 2012). This highlights the benefits of using active-MA in conjunction with low temperature storage in packaging fresh fish fillets as it enhances shelf life of fresh fish fillets about four times that of passive-MA by hindering the growth of microbes due to its bacteriostatic properties.

Modified atmospheres packaging, storage temperature and the use of absorbent pads, had significant impacts on physicochemical properties of Cape hake fillets during storage ($p < 0.05$). Active-MA packaged fillets had lower pH than fillets stored under passive-MA, across all temperatures. The percentage increase in pH in active-MAP fillets stored at 0°C, passive-MAP at 0°C and actve-MAP fillets stored at 8°C (abuse temperature) were 7%, 39% and 30%. The closeness in the percentage increase in pH between abuse temperature and passive-MAP stored fillets further buttresses our earlier results that abuse temperature negates the benefits of active-MAP. Also a slightly acidic medium which can be generated at 0°C and 4°C leads to extended shelf life as evidenced by higher shelf-life 12 and 9 days compared with < 3 d for fillets stored at abuse temperature and < 6 d in passive-MAP fillets stored at 0°C. Maintaining a slightly acidic medium helps in the preservation of fresh fish tissue (Sivertsvik *et al*., 2002; Arashishar *et al*., 2004; Stamatis & Arkoudelos, 2007; Lauzon *et al*., 2009; Caglak *et al*., 2012).

As storage time progressed, the firmness of hake fillets reduced but the firmness at lower temperatures was better across all treatments. The percentage reduction in firmness for active–MAP stored fillets were 0°C (37% on day 12), 4°C (40% on day 12) and 8°C (35% on day 3). Firmness of fillets under passive-MAP stored at 0°C, 4°C and 8°C was reduced by 38% on day 6, 38 % and 39% on day 3, respectively. Firmness reduced faster in passive-MAP than active-MAP packaged fillets. Also higher temperature accelerated loss of firmness and abuse temperature resulted in loss of active-MAP benefits. Similarly, Roth *et al*. (2009) reported that higher temperatures above 6°C in Atlantic salmon fillets resulted in accelerated reduction in firmness of stored fish fillets. In packaging of Cape hake fillets temperature is vital as abuse and higher temperature resulted in accelerated reduction in firmness.

The use of absorbent pad combined with low storage temperature had a significant ($p < 0.05$) impact on the firmness of hake fillets across all temperatures. This is in agreement with Hansen *et al*. (2009) who studied pre-rigor salmon fillets stored under MAP (60% CO_2 + 40% N_2) at 0.1°C for 28 d in thin-bedded honeycombed pads. The authors reported that the pads absorbed water drips, resulting in better texture of MAP-stored salmon fillets than fillets stored in regular air (Hansen *et al*., 2009). The main function of the absorbent pad is to eliminate water vapour condensation on the film or prevent drips forming inside the package which can accelerate the growth of microbes (Lopez-Rubio *et al*., 2004). Drip loss was higher in packaged fillets without absorbent pad while fillets stored at 0°C maintained better texture than those at higher storage temperatures (4°C and 8°C). This could be attributed to the ability of the absorbent pad to better absorb drips in the pack and delay the degradation of fish

muscles, which reduces tissue pliability and loss of fish freshness (Ayala *et al*., 2010). Given the importance of firmness in fresh and ready-to-cook (RTC) fish, the use of absorbent pads in this study offered value addition by improving fillet quality. Although this brings in some added costs the gains in firmness compensates for this as end-users would pay more for better quality products and increased shelf life.

The interaction between MAP, absorbent pad and storage temperature had a significant effect on the aerobic mesophillic bacteria counts. Lowest count of aerobic mesophillic bacteria was observed in fillets stored under active-MAP, while fillets stored at 0°C had the least count of 5.2 log cfu/g on day 12 compared to the control passive-MAP at 0°C and other stored fillets across all temperatures. In fillets stored at 4°C and 8°C under passive-MAP, the aerobic mesophillic bacteria count reached the critical limits of < 5.5 log cfu/g by day 3 and 1, respectively (DOH, 2001; HPA, 2009), while fillets stored at 0°C had lower counts and reached critical levels by day 6. For Fillets stored under active-MAP at 0°C, AMCs did not exceed the critical limit until after day 12 and at 4°C reached 6.0 and 7.2 log cfu/g for 9 and 12 d, respectively. These results agree with the findings of Ordonez *et al*. (2000), where MAP conditions (40% CO_2 + 60% air, 20% CO_2 + 80% air and 100% passive MAP) for Mediteranean hake fillets stored at 2°C for 12 d carried out in Spain. The authors reported that aerobic mesophillic bacteria count was above 5.5 log cfu/g after day 2 in fillets stored under passive-MAP while aerobic mesophillic bacteria count was below 5.5 log cfu/g after day 11 for active-MAP, (40% CO_2 + 30% O_2 + 30% N_2) fillets and concluded that active-MAP was more efficient in hindering microbes than passive-MAP (Ordonez *et al*., 2000). Similarly, Stamatis & Arkoudelos (2007) investigated the influence of normal air, vacuum and MAP gas composition (50% CO_2 + 50% N_2) on quality attributes of chub mackerel at 3°C and 6°C for 15 d. The authors reported that microbial count of fillets at 3°C were 6.6, 7.4 and 8.4 log cfu/g and 6.7, 7.8 and 8.7 log cfu/g at 6°C for MAP, VP and air storage, respectively. These findings highlight the importance of maintaining optimum cold chain in combination with applying postharvest techniques such as active-MAP to maintain the quality and safety of fresh ready-to-cook fish products. Also abusive handling temperature resulted in shorter shelf life and compromises product safety in spite of an efficient packaging solution.

Overall acceptability (odour and appearance) of fillets decreased with increase in storage temperature across all treatments, active-MAP stored fillets at 0°C were acceptable on day 12. Overall acceptability of fillets was influenced by temperature and duration. Overall acceptability scores for fillets stored in passive-MAP at 0°C, 4°C and 8°C was reduced by 56% day 6, 57% and 56% by day 3. Active-MAP fillets stored at 0°C and 4°C had there

overall acceptability reduced by 40% and 64% on day 12. The overall acceptability of abuse temperature stored fillets was 66% on day 3. This shows that Cape hake fillets stored in passive-MA are subject to quick loss of overall acceptability (odour and acceptability). Futhermore, the benefits of active-MAP on odour and acceptability were lost at abuse temperature. This highlights the benefits of the use of active-MA and low temperatures in maintaining the overall acceptability and quality of fresh cape hake fillets. Furthermore, the use of absorbent pad led to higher overall acceptability in all treatments. For instance active-MAP fillets at 0°C packaged with pad had the overall acceptability reduced by 40% while those stored without pad had theirs reduced by 48% on day 12. Similar results were obtained across all treatments. Absorbent pads were used in the packaging of active-MAP stored fillets due to the accumulation of drip loss during storage this has been reported by other researchers (Sivertsvik *et al*., 2002; Sivertsvik, 2007). The use of absorbent pad added value to the overall acceptability and better enhanced the odour and appearance of Cape hake fish fillets.

Comparison and correlation between quality indices

Fillets under passive-MAP stored at higher temperatures (4°C and 8°C) had lower overall acceptability 2.16 and 1.44 on day 3 while those stored at 0°C had an overall acceptability of 2.2 on day 6. Changes in physicochemical quality followed the same trend, resulting in shelf life of < 3 days at 4°C, about 1 day at 8°C, and 5 days at 0°C for packaged fillets. In comparison, fillets stored under active-MA better maintained organoleptic attributes than passive-MA stored fillets with the overall acceptability and corresponding maximum shelf life of 2.72 at 0°C (day 12), 1.46 at 4°C (day 12) and 1.68 at 8°C (day 3). The same trend was observed with physicochemical attributes as weakly negative relationship (r = -0.40) between firmness (work of shear) and pH suggests that fillets stored in a weakly acidic (pH 5.6 - 6.5) medium had better firmness quality than those stored in a basic (pH 7 - 8) medium. Result also showed that acidic medium (low pH) could influence the colour of fillets as evidenced by the positive correlation (r = 0.43) between pH and total colour difference of fish fillets (Table 6). Although the relationship between CO_2 levels and drip loss was moderately positive (r = 0.52), with strong negative correlation between CO_2 levels and aerobic mesophillic counts (r = -0.78), it is reasonable to hypothesize that CO_2 levels could minimise the effects of microbes on fish fillet. Another interesting relationship was the moderately negative correlation between work of shear and aerobic mesophillic counts (AMCs) (r = -0.62) suggesting that fillet texture would be maintained when microbial activity was minimal. This could be as a result of the presence of drips in the package which could lead to the proliferation of microbes, lead to degradation of fish muscles and result in tissue pliability

which hastens loss of fish freshness (Lopez-Rubio *et al*., 2004; Ayala *et al*., 2010). This was also buttressed by the moderately positive correlations between firmness (work of shear) and all the sensory attributes such as firmness-colour (r = 0.54), firmness -freshness (r = 0.51), firmness -fresh fish odour (r = 0.51), and firmness -overall acceptability (r = 0.52). Research findings by Hernandez-Herrero *et al*. (2003) established that pliability of fish muscle was dependent on changes that occur in collagen constituents present in cod while Sriket *et al*. (2010) stated that enzymatic activity on collagen constituents led to pliability during ice storage of prawn meat. Similarly, Pereira de Abreu *et al*. (2010) established that degradation in salmon fish muscles were due to activities of enzymes and microbes. Furthermore, Thanonkaew *et al*. (2006) indicated that colour changes in fish fillet could be as a result of catalysed enzyme oxidation of hematic compounds in fish fillet was catalysis is accelerated at high temperatures. Additionally, studies have shown that freshness quality degradation occurs due to bacteria infestation in fish muscles which triggers a continuous breakdown of glycogens, nucleotides, amino acids and other non-protein nitrogen (Gram & Huss, 2000). This results in the release of volatile compounds such as tri-methyl amine (TMA), aldehydes, ketones, esters, hypoxanthine, and low molecular weight sulphur complexes, which contributes to the characteristic odour of spoilt fish (Gram & Dalgaard, 2002; Triqui & Bouchriti, 2003). Consequently, the correlation established between data on firmness (work of shear) and physicochemical, microbial and sensory attributes in our findings is in agreement with research findings by other researchers (Fagan *et al*., 2004; Lauzon *et al*., 2009; Speranza *et al*., 2009).

Although there is considerable information in the literature on the application of MAP on various types of fish and fishery products such as Mediterranean hake, swordfish, salmon, tuna and prawn (Fagan *et al*., 2004; Pantazi *et al*., 2008; Speranza *et al*., 2009), no information is available on the effects of MAP and absorbent pad on the quality of fresh Cape hake fish fillets. This is surprising given the market potentials of Cape hake in the South African fishing industry. Knowledge gained in this study on physicochemical, microbial and sensory attributes of Cape hake needs to be applied towards the development of a reliable quality index by correlating the effect of MAP on the quality attributes of Cape hake with postharvest storage performance of packaged fillets.

Changes in volatile composition and concentration of Cape hake fillets as affected by modified atmosphere packaging, storage temperature and duration (Chapter 4)

In the second research chapter, the effects of active MAP (with or without absorbent pads) and storage temperature on volatile components of fresh Cape hake fillets stored at 0°C and 4°C was investigated as potential predictor of fillet quality and shelf life .

Storing Cape hake fillets at 0°C or 4°C in MAP with absorbent pad had a significant impact ($p < 0.05$) on the concentration of volatile compounds. Comparison of the changes in the volatile compositions during storage duration, as well as their correlation with aerobic mesophillic counts (AMCs) led to the identification of spoilage compounds that could possibly be used to predict shelf life and serve as quality indicators for packaged Cape hake fish fillets. Fillets stored at 0°C or 4°C maintained quality attributes better in active-MAP than in passive-MAP. For instance fillets stored in active-MAP at 0°C had lower concentrations of tri-methyl amine (TMA), no ethyl acetate, butanoic acid ester, di-methyl sulphide and dimethyl disulphide fillets while fillets stored in passive-MAP at 0°C had higher conmposition and concentration of VOCs. Moderate positive correlations were established between AMCs and spoilage markers such as TMA (r = 0.57), ethyl acetate (r = 0.57), butanoic acid ester(r = 0.66), dimethyl sulphide(r = 0.68), and dimethyl disulphide (0.73). This implies that TMA, esters and sulphur groups could be good indicators of spoilage in Cape hake fish fillets. Leduc *et al*. (2012) investigated the volatile compounds (VOCs) of freshly harvested European sea bass, gilthead sea bream, cod and salmon using solid phase micro-extraction /gas chromatography/ mass spectrometry (SPME/GC/MS) after iced storage at -20°C for 30 and 90 d in a study. The authors detected alcohols; ketones; organic acids; amines; esters; and sulphur containing compounds VOCs in stored fillets and concluded that dimethyl sulphide, 3-methylbutanal, ethyl acetate and 2-methylbutanal could be used to distinguish between fresh and frozen fish based on their concentrations. They further stated that the concentration of these classes of VOCs were higher in the fresh fillets but they were still present in frozen fish fillets, presumably because even though the action of microbes is hindered during iced storage, oxidation of lipids and enzymatic breakdown of poly unsaturated fatty acids still occur (Leduc *et al*., 2012).

Based on measured physicochemical, microbial, sensory and volatile attributes, fillets in stored in active-MAP (40% CO_2 + 30% O_2 + 30% N_2) better maintained organoleptic attributes than those in passive-MAP at all storage temperatures. The shelf life of fillets in

passive-MAP stored at 0°C, 4°C and 8°C were 3-X days, < 3 days and around 1 day, respectively. In contrast, the shelf life of fillets in active-MAP stored at 0°C, 4°C and 8°C were 12 d, 9 d and less than 3 d, respectively. The shelf life of South African Cape hake fillets stored at 4°C in active-MAP found in the present study was lower than the shelf life of 10 d reported by Speranza *et al*. (2009) in Italy for Mediteranean hake fillets packaged in MAP (40% CO_2 + 30% O_2 + 30% N_2) and stored at 4°C. This could be due to the difference in fish habitat, harvest season as well as the postharvest treatment. Our study investigated the effects of MAP on a species domiciled in South Africa and purchased at retail outlet while the study undertaken by Speranza *et al*. (2009) investigated a species domiciled in Italy purchased at a fish farm. Also the fillets used in their study were slaughtered by immersion in ice-cold water. Furthermore, the fillets were transported to their laboratory within 2 h of slaughter while our study investigated fillets that were collected approximately 18 h after cutting and conveyed to the postharvest laboratory within 15 min. Literature has shown that storage temperature and duration affects the organoleptic attributes of fish fillets (Sivertsvik *et al*., 2002; Ayala *et al*., 2010; Speranza *et al*., 2009). Lauzon *et al*. (2009) obtained similar results in a study on unsalted cod fillets stored at 0°C and −2°C under passive MAP (50% CO_2 + 5% O_2 +45%). The authors found that the AMCs were below 5.5 log cfu/g on day 12, but rose to 7.2 log cfu/g by day 15 for fillets stored at 0°C. Hence, the authors concluded that fillet shelf life was 14 to 15 d at 0°C and 21 d at −2°C (Lauzon *et al*., 2009). The difference in shelf-life observed by Lauzon *et al*. (2009) at 0°C with the results of the present study could be attributed to the time between landing and time of purchase, geographical location, differences in fish species or microbial limits < 5.5 log cfu/g used in our study (DOH, 2001; HPA, 2009). For instance in our study fillets were purchased and collected approximately 18 h after cutting and conveyed to the postharvest laboratory within 15 min from a retail outlet while the fillets used by Lauzon *et al*. (2009) in their study was caught by trawling and processed immediately on the fishing vessel. Also the species used in their study was cod fillets domiciled in north Iceland while the specie used in our study was Cape hake domiciled in South Africa. In addition the microbial limit used in their study was not disclosed but they reported that the AMCs were below 5.5 log cfu/g on day 12, but rose to 7.2 log cfu/g by day 15 for fillets stored at 0°C while our study used a microbial limit of < 5.5 log cfu/g (DOH, 2001; HPA, 2009; Lauzon *et al*., 2009). This study is consistent with literature evidence which states that storage in lower temperatures leads to greater dissolution of CO_2 in fish tissues (Ruiz-Capillas & Moral 2001; Sivertsvik *et al* 2004; Torrieri *et al*., 2006). Furthermore, higher CO_2 levels also lead to increase in bacteriostatic ability at 0°C (Sivertsvik, 2007; Lauzon *et al*., 2009). Results obtained from the present study indicate that active-MA packaged fillets stored at 0°C extends

shelf-life and better maintains organoleptic quality of Cape hake fillets. This agrees with those reported in literature that active-MAP and optimum cold storage, extends the shelf life and quality of fish and fishery products and results in reduced microbial load by extending the lag phase of aerobic bacteria in stored fish fillets (Sivertsvik *et al*., 2002; Arashisar *et al*., 2004; Sivertsvik *et al*., 2004; Stamatis & Arkoudelos, 2007; Lauzon *et al*., 2009).

It can be deduced that the overall quality of the investigated Cape hake fish was influenced by good agricultural practices and good hygienic practices, as observed in the low microbial levels before the application of MAP and storage. However, temperature, packaging, absorbent pad and storage duration had significant impacts on shelf life and quality attributes as demonstrated by evidence from microbial and GC-MS analyses. Significant correlations were established between aerobic mesophillic counts and selected spoilage indicators in the VOCs; for example, moderate correlations were observed between AMCs and TMA (r = 0.50). This suggests that TMA could be used, in conjunction with other spoilage markers such as esters and dimethyl sulphide, to predict shelf life and quality of Cape hake fish fillets during refrigerated storage. The observed correlations between aetobic mesophillic counts and VOCs indicate that VOCs could offer potential as predictor of quality and shelf life of Cape hake fillet in MAP. It is noted that the results reported in this thesis are only representative of the Cape hake fillets purchased in retail stores which are subject to landing time, time of purchase from the store and postharvest handling hence, the results can at this stage be used only as a guide. The approach could provide food scientists and exporters with a tool that would ensure high quality in consideration of long supply chains for packaged Cape hake fillets. Overall, this study has shown that the use of modified atmosphere offers a new technological tool to reduce postharvest losses, extend shelf life and add value to fresh cape hake fish in South Africa

General conclusions and future prospects

This thesis embodies the findings of a pilot research aimed at developing modified atmosphere packaging technology to assist in reducing postharvest losses and waste of Cape hake, thereby enhancing the competitiveness of the South African fish industry. The use of active MAP (40% CO_2 + 30% O_2 + 30% N_2), incorporation of absorbent pad in combination with refrigerated storage at 0°C offers the potential to extend the shelf life and maintain quality attributes of Cape hake fillets for 12 d. These findings could be used for the

formulation of new guidelines for the application of MAP to improve postharvest handling, packaging and storage of Cape hake fillets and other fish products in South Africa.

However, it is worth mentioning that the use of high grade fillets, optimum processing and storage temperature conditions, good agricultural practices, good hygienic practices, and correct gas composition are necessary to achieve the best results with modified atmosphere packaging. Further studies are required to extend this study to other types of fresh fish produced in South Africa. Additionally, mathematical models which offer considerable advantages to the fresh fish industry because they assist in achieving best possible product quality could be used to predict the quality of packaged Cape hake fillets. In our trials, GC-MS and GC-O were used to identify the VOCs based on resources available. However, future studies should investigate alternative methods that accurately quantify VOCs by GC–FID using internal standards.

References

Ababouch, L. (2006). Assuring fish safety and quality in international fish trade. *Marine Pollution Bulletin*, **53**, 561–568.

Angis S. & Oguzhan P. (2013). Effect of thyme essential oil and packaging treatments on chemical and microbiological properties of fresh rainbow trout (*Oncorhynchus mykiss*) fillets during storage at refrigerator temperatures. *African Journal of Microbiology Research*, **7**, 1136-1143.

Arashisar, S., Hisar, O., Kaya, M. & Yanik, T. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. *International Journal of Food Microbiology*, **97**, 209-214.

Ayala M.D., Santaella, M., Martınez, C., Periago, M.J., Blanco, A., Vazquez, J.M. & Albors, O.L. (2010). Muscle tissue structural changes and texture development in sea bream (*Sparus aurata*) during post-mortem storage. *LWT Food Science Technology*, **43**, 465–475.

Bellagha, S., Sahli, A., Farhat, A., Kechaou, N. & Glenza, A. (2007). Studies on salting and drying of sardine (*Sardinella aurita*): Experimental kinetics and modelling. *Journal of Food Engineering*, **78**, 947-952.

Caglak, E., Cakli, S. & Kilinc B. (2012). Effect of modified atmosphere packaging on quality and shelf-life of salted bonito (*Sarda sarda*). *Journal of Aquatic Food Product Technology*, **21**, 206–221.

Caleb, O.J., Opara, U.L. & Witthuhn, C.R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology*, **5**, 15-30.

Caleb, O. J., Mahajan, P. V., Al-Said, F. A. J. & Opara, U. L. (2013). Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences—.a review. *Food and Bioprocess Technology*, **6**, 303-329.

Choubert, G. & Baccaunaud, M. (2006). Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss W.*) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere. *LWT Food Science and Technology*, **39**, 1203-1213.

Cyprian, O., Oduor-Odote, P., Lauzon, H., Martinsdottir, E. & Arason, S. (2013). Microbiological quality and shelf life of fresh packaged tilapia fillets stored under different chill temperatures. *Journal of Microbiology, Biotechnology and Food Sciences*, **2**, 2431-2455.

Department of Agriculture, Forestry and Fisheries, DAFF (2012). Status of the South African marine fishery resources, 2012. Cape Town.

De Silva, D.A.M. & Yamao M. (2006). Regional preferences in the Japanese Seafood consumption: An empirical analysis of consumer purchasing behaviour on domestic versus imported seafood. *Japan, Journal of the Regional Fisheries Society*, **46**, 83-104.

Del Nobile, M.A., Corbo, M.R., Speranza, B., Sinigaglia, M., Conte, A. & Caroprese, M. (2009). Combined effect of modified atmosphere packaging and active compounds on fresh blue fish burger. *International Journal of Food Microbiology*, **135**, 281-287.

DOH - Department of Health (2001). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. South Africa: Department of Health Directorate: Food Control.

Domingo, J.L., Bocio, A., Falco, G. & Llobet, J.M. (2007). Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology, **230**, 219-226.

Dondero, M., Cisternas, F., Carvajal, L. & Simpson, R. (2004). Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. *Food Chemistry*, **87**, 543-550

Erkan, N., Ozden, Ö. & Inugur, M. (2007). The effects of modified atmosphere and vacuum packaging on quality of chub mackerel. *International Journal of Food Science and Technology*, **42**, 1297-1304.

Fagan, J.D., Gormley, T.R. & Ui Mhuircheartaigh, M.M. (2004). Effect of modified atmosphere packaging with freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. *Innovative Food Science & Emerging Technologies*, **5**, 205-214.

Farber, J.N., Harris, L.J., Parish, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H. & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 142-160.

Food and Agricultural Organisation, FAO (2005). Fishery country profile-The republic of South Africa. FID/CP/ZAF, November 2005.

Food and Agricultural Organisation, FAO (2009). Fisheries and Aquaculture topics. Utilization and trade. Topics Fact Sheets. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 28 September 2001. [Cited 24 September 2013]. http://www.fao.org/fishery/topic/2888/en.

Food and Agricultural Organisation, FAO. (2014). Post-harvest changes in fish. Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations.

Gram, L. & Huss, H.H. (2000). Fresh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Foods*, edn 1. (edited by B.M. Lund, A.C. Baird-Parker, G.W. Gould). Pp. 472-506. London: Chapman & Hall.

Gram, L. & Dalgaard, P. (2002). Fish spoilage bacteria: problems and solutions. *Current Opinion in Biotechnology*. **13**, 262-266.

Hansen, A.A., Mørkøre, T., Rudi, K., Langsrud, O., & Eie, T. (2009). The combined effect of super chilling and modified atmosphere packaging using CO_2 emitter on quality during chilled storage of pre-rigor salmon fillets (*Salmo salar*). *Journal of the Science of Food and Agriculture*, **89**, 1625-1633.

Hernandez-Herrero, M.M., Duflos, G., Malle, P. & Bouquelet, S. (2003). Collagenase activity and protein hydrolysis as related to spoilage of iced cod (*Gadus morhua*). *Food Research International*, **36**, 141-147.

HPA - Health Protection Agency, (2009). Guidelines for assessing the microbiological safety of ready-to-eat foods. Health Protection Agency, London: UK Government Printers; 2009.

Jezek, F. & Buchtova H. (2012). Effect of Modified atmosphere packaging on the course of physical & chemical changes in chilled muscle of silver carp. (*Hypophthalmichthys molitrix*). *Polish Journal of Veterinary Science*, **15**, 439-445.

Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, **106**, 2747-2757.

Lauzon, H.L., Magnusson, H. Sveinsdottir, K., Gudjonsdottir, M. & Martinsdottir, E. (2009). Effect of brining, modified atmosphere packaging, and super-chilling on the shelf-life of cod (*Gadus morhua*) loins. *Journal of Food Science*, **74**, 258-267.

Leduc, F., Krzewinski, F., Le Fur, B., N'Guessan, A., Malle, P., Kol, O. & Duflos, G. (2012). Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus* labrax), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS. *Journal of the Science of Food and Agriculture*, **92**, 2560-2568.

Lopez-Rubio, A., Almenar, E., Hernandez-Munoz, P., Lagaron, J. M., Catala, R. & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International*, **20**, 357-387.

Opara, U.L. (2009). Quality management: an industrial approach to produce handling. In: *Postharvest handling. A systems approach* (edited by W. J. Florkowski, S. E. Prussia, R. L. Shewfelt & B. Brueckner). Pp. 153-204. USA: Academic Press.

Ordonez, J.A., Lopez-Galvez, D.E., Fernandez, M., Hierro, E. & de la Hoz, L. (2000). Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, **80**, 1831-1840.

Pereira de Abreu, D.A., Paseiro-Losada, P., Maroto, J. & Cruz, J.M. (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from

barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar L.*). *Food Research International*, **43**, 1277-1282.

Pantazi, D., Papavergou, A., Pournis, N., Kontominas, M.G. & Sawaidis I.N. (2008). Shelf-life of chilled fresh Mediterranean swordfish (*Xiphias gladius*) stored under various packaging conditions: microbiological, biochemical and sensory attributes. *Journal of Food Microbiology*, **25**, 136-143.

Roth, B., Birkeland, S. & Oyarzun, F. (2009). Stunning, pre slaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on fresh and smoked fillets. *Journal of Aquaculture*, **289**, 350-356.

Ruiz-Capillas C & Moral A.l. (2001). Chilled bulk storage of gutted hake (*Merluccius merluccius, L.*) in carbon dioxide and oxygen enriched controlled atmospheres. *Food Chemistry* **74**, 317-325.

Sivertsvik, M., Jeksrud, W. K. & Rosnes, J. T. (2002). A review of modified atmosphere packaging of fish and fishery products–significance of microbial growth, activities and safety. *International Journal of Food Science & Technology*, **37**, 107-127.

Sivertsvik M, Rosnes J.T. & Jeksrud W.K. (2004). Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 2: raw fish fillets. *Journal of Food Engineering*, **63**, 451–458.

Sivertsvik, M. (2007). The optimized modified atmosphere for packaging of pre- rigor filleted farmed cod (*Gadus morhua*) is 63ml/100ml oxygen and 37ml/100ml carbon dioxide. *LWT-Food Science and Technology*, **40**, 430-438.

Speranza, B., Corbo, M., Conte, A., Sinigaglia, M. & Del Nobile, M. (2009). Microbiological and sensorial quality assessment of ready-to-cook seafood products packaged under modified atmosphere. *Journal of Food Science* **74**, 473–478.

Sriket, C., Benjakul, S. & Visessangguan, W. (2010). Post-mortem changes of muscle from fresh water prawn (*Macrobrachium rosenbergii*) as influenced by spawning stages. *Journal of Food Science and Technology*, **43**, 608-616.

Stamatis, N. & Arkoudelos, J. (2007). Quality assessment of *Scomber colias japonicas* under modified atmosphere and vacuum packaging. *Food Control*, **18**, 292-300.

Thanonkaew, A., Benjakul, S., Vissessanguan, W. & Decker, E.A. (2006). The effect of metal ions on lipid oxidation, colour and physicochemical properties of cuttlefish (*Sepia pharaonis*) subjected to multiple freeze-thaw cycles. *Food Chemistry*, **95**, 591-599.

Torrieri, E., Cavella, S., Villani, F. & Masi, P. (2006). Influence of modified atmosphere packaging on the chilled shelf-life of gutted farmed bass (*Dicentrarchus labrax*). *Journal of Food Engineering*, **77**, 1078–1086.

Triqui, R. & Bouchriti, N. (2003). Freshness assessments of Moroccan sardine (*Sardina pilchardus*): Comparison of overall sensory changes to instrumentally derived volatiles. *Journal of Agricultural and Food Chemistry*, **51**, 7540-7546.

Trondsen, T., Scholderer, J., Lund, E. & Eggen, A.E., 2003. Perceived barriers to consumption of fish among Norwegian women. *Appetite* 41, 301–314.

Wang, T., Sveinsdottir, K., Magnusson, H & Martinsdottir, E. (2008). Combined application of modified atmosphere packaging and super-chilled storage to extend the shelf-life of fresh cod (*Gadus morhua*) loins. *Journal of Food Science*, **73**, 11–19.

Yesudhason, P., Gopal, T. K. S., Ravishankar, C. N., Lalitha, K. V. & Kumar, K. N. A. (2009). Effect of modified atmosphere packaging on chemical, textural, microbiological and sensory quality of seer fish (*Scomberomorus commerson*) steaks packaged in thermo-formed trays at 0–2ºC. *Journal of Food Processing and Preservation* **33**, 777-797.