Effects of salting and drying on quality attributes of snoek (*Thyrsites atun*)

by

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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 single author thereof (except to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March, 2015

Abstract

Snoek (*Thyrsites atun*) is an important commercial fish species in South Africa, particularly in the Western Cape province. Snoek is mainly sold as fresh fish with excess fish being processed into dried salted fish. Although fishing is regulated by the various government agencies, the processing chain of snoek is largely unmonitored and unstandardized which has resulted in variable snoek quality becoming available to the consumer.

Therefore, the aim of this study was to assess the quality (proximate, biochemical composition and microbiological safety) of salted and dried snoek products both experimentally and through market sampling. This was achieved by investigating two lines of research: 1) Establishing the quality of locally sold salted and dried snoek from informal vendors and 2) Investigating the effects of different levels of salting (%) and drying (relative humidity and temperature) conditions on snoek meat quality. Product quality was assessed through the analysis of proximate composition, fatty acid composition, physico-chemical attributes, water activity, moisture loss, salt content and microbial safety and oxidative stability.

Moisture, protein, lipid, and ash contents of snoek varied substantially between vendors, thereby providing products with variable quality to consumers. The water activity and TBARS (thiobarbituric acid reactive substances) of snoek from all vendors were higher than the reported threshold for good quality dried fish, indicating high levels of lipid oxidation. The level of TBARS and water activity varied between 9.58-19.83 mg MDA.Kg^{-1}, and 0.75-0.85, respectively. This is undesirable in dried fish products as high water activity predisposes the products to spoilage. These findings indicate that the salted and dried snoek retailed in the Western Cape province require further drying to reduce the water activity in order to ultimately prolong the shelf life of the product. However, low histamine, high salt, low microbial counts and the absence of pathogens (*Salmonella* spp. and *Escherichia coli*) were observed which suggests that the products were wholesome at time of sale.

An assessment of the effects of salting and drying on water activity, moisture loss, and salt content of Cape snoek was carried out. The treatments included 0% salt (unsalted) and salting at 20% salt concentration and salt saturation; the treated snoek portions were dried at 30°C/40% RH (relative humidity), 40°C/40% RH and 40°C/50% RH. During the salting process higher moisture loss resulted in high weight loss and low water activity in snoek portions salted to saturation compared to 20% (w/w). Once oven drying commenced the unsalted snoek portions had significantly higher moisture loss, higher weight loss but higher water activity than the salted groups. Lower salt content (12.8%), higher product yield and

higher water activity were observed in snoek treated with 20% salt compared to those treated with saturated salt (23.6% salt). Low water activity and a salt content above 6% inhibits microbial growth and prevents spoilage of salted dried fish; therefore, the salt content of the salted snoek (12.8% for 20% salted).after the drying process is adequate for preventing microbial spoilage.

A study examining the drying kinetics of snoek determined the moisture ratio, drying rate and effective moisture diffusivity of salted (20% w/w) and unsalted snoek dried at different drying environments (30°C/40% RH; 40°C/40% RH; 40°C/50% RH). A number of mathematical models (Lewis/Newton, Page, Henderson, Pabis and asymptotic logarithmic) were tested to predict the drying kinetics of snoek where the most effective predictive model was the Page model (r^2 = 0.9999; mean square error = 6.6 x 10^{-6}). The Page model showed that salting at 20% and drying at 40°C/40% RH was the best treatment with the highest effective moisture diffusivity (1.54 x 10^{-5}) among the salt treatments, which is an indication of high drying rate. Therefore, the Page model can be used to optimise the drying process and design of dryers for snoek processing. The drying conditions, various treatments and their interactions (p ≤ 0.05) significantly influenced the drying rate and moisture content of snoek portions. Low effective moisture diffusivity and drying rate were observed in salted portions, emphasising the effect of salting on drying of snoek.

This study has provided baseline information on the quality of locally sold dried snoek and established that salting at 20% and drying at 40°C/40% RH is adequate for the production of consistent quality salted dried snoek. Further research is needed to study the effects of salting and drying on the nutritional quality and shelf life of snoek.

Opsomming

Snoek (*Thyrsites atun*) is 'n belangrike visspesie wat in die kuswaters van die Wes-Kaap Provinsie van Suid-Afrika geoes word. Snoek word na vangs tipies vars verkoop, met oorskot vis wat verwerk en as 'n gesoute gedroogde produk verkoop word. Ten spyte van die amptelike regulering van die oes en verwerking van snoek, vind die verwerking van snoek onder toestande plaas wat nie beheer word nie en ook nie gestandaardiseerd is nie, wat gevolglik lei tot 'n variasie in produkgehalte.

Die doel van hierdie studie was dus om eerstens die gehalte van gesoute en gedroogde visprodukte deur 'n marksteekproefneming te bepaal en om hierdie inligting dan te gebruik vir die ontwikkeling van protokolle vir die versouting en droging van snoek om uiteindelike produkkwaliteit te optimaliseer. In die tweede deel van die studie is die invloed van verskillende drogingsprotokolle, wat in terme van relatiewe humiditeit (RH) en temperatuur verskil, op produkgehalte ondersoek. Gehalte parameters wat in hierdie deel van die studie geëvalueer is, het die proksimale komponente (d.i. vog-, proteïen-, vet- en mineraal inhoud), vetsuursamestelling, fisies-chemiese eienskappe, mate van water aktiwiteit, vogverlies, sout-inhoud en mikrobiese veiligheid en stabiliteit, ingesluit.

Die vog, proteïen-, vet- en mineraal parameters van gedroogde snoek soos bepaal in monsters versamel van verskillende verskaffers, het aansienlik gewissel. Die water aktiwiteit en tiobarbituriese suur stowwe (TBARS) in snoek monsters verkry vanaf verskaffers, was hoër as die aanbevole vlakke vir gedroogde vis. 'n Hoë mate van water aktiwiteit en TBARS is ongewens in gedroogde visprodukte, omrede 'n hoë mate van water aktiwiteit snoekvleis maklik laat bederf, terwyl 'n hoë TBARS waarde 'n aanduiding is van 'n groter waarskynlikheid dat lipied oksidasie kan voorkom. Die bevindinge van die eerste deel van die studie het aangedui dat gesoute gedroogde snoek as 'n handelsproduk in die Kaapse Skiereiland omgewing, aan langer drogings- en versoutingstye onderwerp moet word om die vestiging van organismes wat die vleis kan bederf te voorkom, wat op sy beurt sal kan bydra daartoe om die rakleeftyd van snoek te verleng. Daar moet egter genoem word dat die monsters verkry vanaf die verskaffers gekenmerk was deur lae histamien vlakke, 'n hoë soutinhoud, lae mikrobiese tellings en die afwesigheid van patogene (*Salmonella* spp. en *Escherichia coli*), wat gesamentlik daarop dui dat die produkte met verkoop daarvan, geskik was vir gebruik.

In die tweede deel van die studie is wisselvallige resultate verkry vir die verskillende drogings- en soutingsprotokolle. Snoek monsters wat tot versadigspunt versout is, het 'n groter hoeveelheid vog en dus gewig verloor, asook 'n lae graad van water aktiwiteit getoon, in vergelyking met monsters versout teen 20% (w/w). Met die aanvangs van die oond

drogingsproses, het ongesoute snoek monsters aansienlik meer vog en gewig verloor, met dié monsters wat kenmerkend 'n hoër vlak van die water aktiwiteit as die onderskeie versoutingsbehandelings gehad het. Die waardes aangeteken vir water aktiwiteit (0.68 vs. 0.64) en sout konsentrasie (13% vs. 24%) vir die monsters wat onderskeidelik met 'n 20% sout (w/w) en tot versadigspunt versout is, voldoen aan die nodige vereistes om mikrobiese groei te inhibeer en dus te voorkom dat gesoute gedroogde snoek produkte maklik bederf.

Die derde deel van die studie het die drogingskinetika (d.i. afdrogingstempo, vog verhouding, en effektiewe vog diffusiwiteit) van versoute (20%, w/w) en ongesoute snoek monsters, gedroog volgens drie drogingsprotokolle (d.i. 30°C en 40% RH; 40°C en 40% RH, 40°C en 50% RH), ondersoek. Die Page model het die drogingskinetika van die snoek monsters die beste voorspel, met 'n koëffisiënt van bepaling van 0.9999 en 'n gemiddelde vierkante fout van 6,6 x 10^{-6}. Die Page model het aangedui dat versouting by 20% (w/w) en 'n drogingsprotokol van 40°C en 40% RH, die hoogste effektiewe vog diffusiwiteit (1.54 x 10^{-5}) tot gevolg gehad het. Die Page model kan dus potensieel gebruik word om die drogingsproses asook die ontwerp van die drogingsoonde gebruik in die verwerking van snoek monsters, te optimaliseer.

Hierdie studie bied basislyn inligting oor die gehalte van plaaslik gedroogde snoek en ander gedroogde vis produkte, met die protokol van versouting teen 20% (w/w) en droging by 40°C en 40% RH, wat aanvaarbaar gevind is vir die lewering van snoek produkte van konstante gehalte. Toekomstige studies word benodig om die effek van versouting en droging op die voedingswaarde en raklewe van snoek vleis te ondersoek.

Acknowledgements

Dedications

This work is dedicated to my husband Akinwunmi and children Oluwafunmibi, Oluwafunmiso and Oluwafunmilade.

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Chapter 1: General Introduction

Fish is an important source of nutrients such as protein, vitamins, minerals and fats; particularly essential polyunsaturated omega 3 and 6 fatty acids (Ikem & Egiebor, 2005; Sikorski & Kolodziejska, 2002; Ahmed, 2008). The consumption of fish in developing countries is relatively high and accounts for about 71% of global fish consumption (Knap, 2011) and can provide a large portion of the dietary protein necessary for normal anatomical function (Usydus *et al.*, 2009). Therefore, it is essential that consistently high quality fish is available in order to meet consumer nutritional needs; however, this is often not the case due to high incidence of postharvest losses and poor quality products (Diei-Ouadi & Mgawe, 2011).

Fish undergo rapid degradation during postharvest handling which can alter the nutritional contents which subsequently affects the nutritional uptake by consumers (Uçak *et al.*, 2011). In addition, partial or complete spoilage can result in large economic losses (Ahmed, 2008; Kabahenda *et al.*, 2009; Diei-Ouadi & Mgawe, 2011; Foline *et al.*, 2011). During postharvest handling fish immunity decreases and microorganisms present on the skin of the fish can multiply and migrate into the fish flesh (Farag, 2012). Activities of microorganisms, enzymes and lipid oxidation are responsible for fish deterioration and spoilage (Uçak *et al.*, 2011). The activities of microorganisms are responsible for global loss of about 4-5 million tons of trawled fish per annum (Ghaly *et al.*, 2010). The activities of specific spoilage organisms present on the surface of the fish such as *Pseudomonas fluorescences* which thrives under aerobic conditions, *Aeromonas hydrophila, Shewanella* spp*.* and *Flavobacterium* spp*.,* which grow under refrigeration conditions, and *Vibrio* spp. These microorganisms spoil unpreserved fish, which results in development of amines, biogenic amines, organic acids, sulphides, aldehydes and ketones with the production of off flavours (Mohan *et al.*, 2010; Iturriaga *et al.*, 2012). Enzymes such as lipases and proteases, which are present in the muscle and intestinal tract, and oxidation of polyunsaturated fatty acids also contribute considerably to fish deterioration and spoilage. A number of factors such as harvesting practices (unhygienic practices on boat, dirty fish holdings and baskets, boats, inappropriate fish cleaning, throwing and stepping on the fish), handling (infrastructure, transportation, market facilities, environmental conditions and storage facilities) and processing (gutting, salting, drying and smoking) procedures can affect the shelf life of fish; where inadequate techniques and processes can accelerate fish spoilage (Opara *et al.*, 2004; Oyelese, 2012). Fish deterioration and spoilage can be delayed by the use of good harvesting techniques, preliminary processing (deheading and gutting), good sanitary procedures on board fishing vessels and use of an adequate cold

chain during the processing, transportation and storage of the fish (Borderías & Sánchez-Alonso, 2010). The use of plant extracts or chemical preservatives, modified atmospheric packaging, vacuum packaging and irradiation can all be used to delay fish spoilage (Reza *et al.*, 2009; Ghaly *et al.*, 2010). However, cold storage is the most common method (Mol *et al.*, 2010) used in the developed countries to delay spoilage but lack of adequate cold storage facilities makes salting and drying the most used method for fish preservation in the developing countries (Bellagha *et al.*, 2007). In order to maintain freshness, fish is stored in ice immediately after harvest and where there is no cold storage, the excess fish is processed to prevent wastage (Al Ghabshi *et al.*, 2012; Darvishi *et al.*, 2013). Fish processing is used as a tool to maintain fish quality, prevent enzyme and bacteriological activities thereby increasing shelf life and mitigating postharvest losses (Andrés *et al.*, 2005; Barat *et al.*, 2006; Fuentes *et al.*, 2007). Traditional fish processing methods include salting, drying, smoking and fermentation (Thorarinsdottir *et al.*, 2004; Riebroy *et al.*, 2008). The use of these processing methods varies between countries and regions and also within the same region depending on local demand and the product desired (Chukwu & Mohammed, 2009). Two basic methods are employed in fish salting: wet (brine) and dry also known as Kench salting (Gudjónsdóttir *et al.*, 2011) which involves either covering the fish with layers of salt (dry salting) or dipping the fish in a salt solution (brining/wet salting) at a low temperature, usually 3-5°C for a selected period of time (Gallart-Jornet *et al.*, 2007; Martínez-Alvarez & Gómez-Guillén, 2013). New processing techniques used in fish salting include vacuum-tumbling, vacuum-sealing in plastic bags with salt and injection of salt into the fish muscle (Jittinandana *et al.*, 2002). Subsequent to salting, fish is traditionally sun dried to further improve storage and preservation (Albarracín *et al.*, 2011). Traditional fish drying is conducted by hanging (scaffolds and bamboo racks) or laying fish on a surface (nets laid on the ground) to dry in the sun for 2-6+ days (Galib & Samad, 2009; Samad *et al.*, 2009; Nooralabettu, 2008). Sun drying is not consistent resulting in poor quality and lack of uniformity. Mechanical dryers such as solar dryers, heat pump dryers, ovens, super-heated steam and freeze dryers are used industrially in order to overcome problems associated with traditional sun drying (Wang *et al.*, 2011). Mechanical drying allows control of processing conditions such as temperature, velocity, relative humidity and drying time which results in consistent and good quality products (Sobukola & Olatunde, 2011).

In South Africa, snoek (*Thyrsites atun*) is responsible for more than 50% of the total marine line fish landed in the Western Cape province and is a commonly consumed fish by residents of coastal (low income) communities (Isaacs, 2013). The snoek market is largely informal with huge potential for growth; however, lack of adequate cold chain and standardised processing procedures results in inconsistent product quality and therefore market restrictions (Isaacs, 2013). Although most snoek is sold fresh, a large portion of the

catch is further processed (salting and sun drying) to facilitate later selling of unsold fresh produce (Isaacs, 2013). Snoek with a high incidence of pap snoek (*Kudoa thyrsites* parasite) is considered of low quality and processing may be employed to disguise the quality related issue which is more visible when fresh. The salting method involves sprinkling small quantities of food grade sodium chloride on whole headed, gutted and cleaned fish and is subsequently hung on outdoor racks to dry (muscle becomes yellow as a result of exposure to sunlight). Due to inconsistent processing techniques and unhygienic processing conditions, the traditional salting and drying processes used for snoek can result in contaminated products due to microbial, pest and insect infestation during and after processing (Sobukola & Olatunde, 2011). Where salt concentration is inconsistent and uneven, product quality can vary, resulting in the production of products with high, low and uneven salt concentration. The consumption of over salted fish can lead to hypertension and gastric cancer (Tsugane, 2005; Gwak & Eun, 2010) while under salting can also have potential hazards where the storage stability of the product can be compromised. Due to the unstandardized and largely unregulated processed snoek market and the limited literature available on the subject, the overall aim of this research was to evaluate the effect of salting and drying on the quality of snoek. In order to achieve this aim, specific objectives were designed as follows:

- Establish the quality (proximate, biochemical composition and microbiological safety) of salted and dried snoek sold by road side vendors in the Western Cape province.
- Evaluate the effect of salting at different salt concentrations and drying conditions on moisture loss, water activity and final salt content of snoek.
- Evaluate the drying dynamics of salted and unsalted snoek portions dried under diverse drying conditions.

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Chapter 2: Literature Review

2.1. Introduction

The fisheries sector contributes to food security in many developing countries through the provision of a high protein food as well as direct and indirect employment (FAO, 2012). The contribution of developing countries in Africa, Asia, and Latin America to the fisheries sector is substantial and together, they produce more than 50% of the world's fish supply during the 1990s (Delgado *et al.*, 2003). Global fish production grew to 154 million tonnes in 2011 out of which 131 million tonnes was used for human consumption with the developing countries supplying over 50% of the global fish and fisheries products (FAO, 2012).

Fish and fish products provide about 3 billion people with about 20% of their animal protein intake (FAO, 2012). Approximately 60% of people in developing countries rely on fish for more than 30% of their animal protein intake, while 80% of people in most developed countries obtain less than 20% of their animal protein from fish (FAO, 2005). Overall, fish is a good source of micronutrients, minerals, proteins, low saturated fatty acids and is rich in essential polyunsaturated fatty acids (PUFAs); particularly omega 3 fatty acids such as eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3) (Sikorski & Kolodziejska, 2002; Ikem & Egiebor, 2005). A considerable body of literature exists which discuss the health benefits of a diet high in DHA and EPA which includes but is not limited to prevention and treatment of cardiovascular diseases, heart attack, lowering of plasma triglycerides in people with type II diabetes, atherosclerosis, thrombosis, inflammatory bowel diseases, bronchial asthma, sudden cardiac death, stroke, mental disorders and cancer (Ruxton *et al.*, 2004; Sierra *et al.*, 2008). In addition, fish oils are also important for normal cellular function and the development of the retina and brain (Sierra *et al.*, 2008). Both DHA and EPA are essential fatty acids and therefore cannot be synthesised within the human body and must be consumed (Arterburn *et al.*, 2006; Sierra *et al.*, 2008) further highlighting the importance of fish in the human diet. Fatty fish offer greater cardio-protection because they provide more DHA and EPA than lean fish (Ruxton *et al.*, 2004). Oily fish such as tuna (*Thunnus spp.*), trout (*Salmo trutta*), sardines (*Sardina pilchardus*), mackerel (*Scromber scombrus*) and salmon (*Salmo salar*) are recommended for consumption because they have high concentrations of essential fatty acids and low levels of mercury. These species are safe for consumption with minimal risk of exposure to heavy metal contaminants in fish (Saravannan *et al.*, 2008).

Although fish composition varies spatially, temporally and between species, fish meat generally contains about 56-83% (w/w) water, 8-25% proteins, 0.5-30% fat and 0.6-1.5% mineral compounds (Opara *et al.*, 2004). The chemical composition of some fish species particular to Republic of South Africa and other countries are presented in Table 2.1. In

addition to these, fish is also a good source of water soluble vitamins and also rich in fat soluble vitamin A and D (Huda *et al.*, 2010). It is noted that one serving of sea food meets the human daily requirement of B vitamins and that the biological value of fish is higher than that of red meat (Opara *et al.*, 2004). Although the fish nutritional value is high, postharvest handling processes, storage and preservation can significantly reduce the nutritional value (Benjakul *et al.*, 2005).

In southern Africa, fish from the oceans are readily consumed and of importance are the species caught by local fisherman and sold in the informal market (Isaacs, 2013). However, very little information exists on their nutritional profile and on the changes that occur during the storage of these fish. One such species is the snoek (*Thyrsites atun*). Although snoek are normally sold fresh into the informal market, when caught in abundance they are salted and sun dried prior to storage. The effect of these preservation techniques and subsequent storage on the nutritional quality needs to be quantified.

Table 2.1 Chemical composition (%) of some species of fish (FAO, 1995)

Lean	Fatty	Species	Water	Fat	Protein
Cod		*Gadus morhua*	78-83	0.1-0.9	15-19.0
	Redfish	*Sebastes sp.*	73-79	3.2-8.1	16.8-19.7
	Herring	*Clupea harengus*	60-80	0.4-22.0	16-19.0
	Mackerel	*Scomber scombrus*	56-74	1.0-23.5	16-20.0
Hake		*Merluccius merluccius*	80	0.4-1.0	17.8-18.6
Sole		*Solea solea*	78	1.8	18.8
	Albacore	*Thunnus alalunga*	59-72	4.3-16.1	21-27.0

2.2. Overview of biology, distribution and fisheries of Snoek (*Thyrsites atun*)

Snoek (*Thyrsites atun*) belongs to the snake mackerel family (Gempylidae) and is locally known as Cape snoek in South Africa, barracouta in New Zealand and Australia and sierra in Chile (FAO, 2011). It is the same species in all these countries (Froese *et al.*, 2013). Snoek is a coastal pelagic fish found in the southern hemisphere (Fig. 2.1). Adult snoek are long, thin and perch-like in colour and can grow up to 200 cm in length and 9 kg in weight (Griffiths, 2003; Nepgen, 1979; Sierra *et al.*, 2008).

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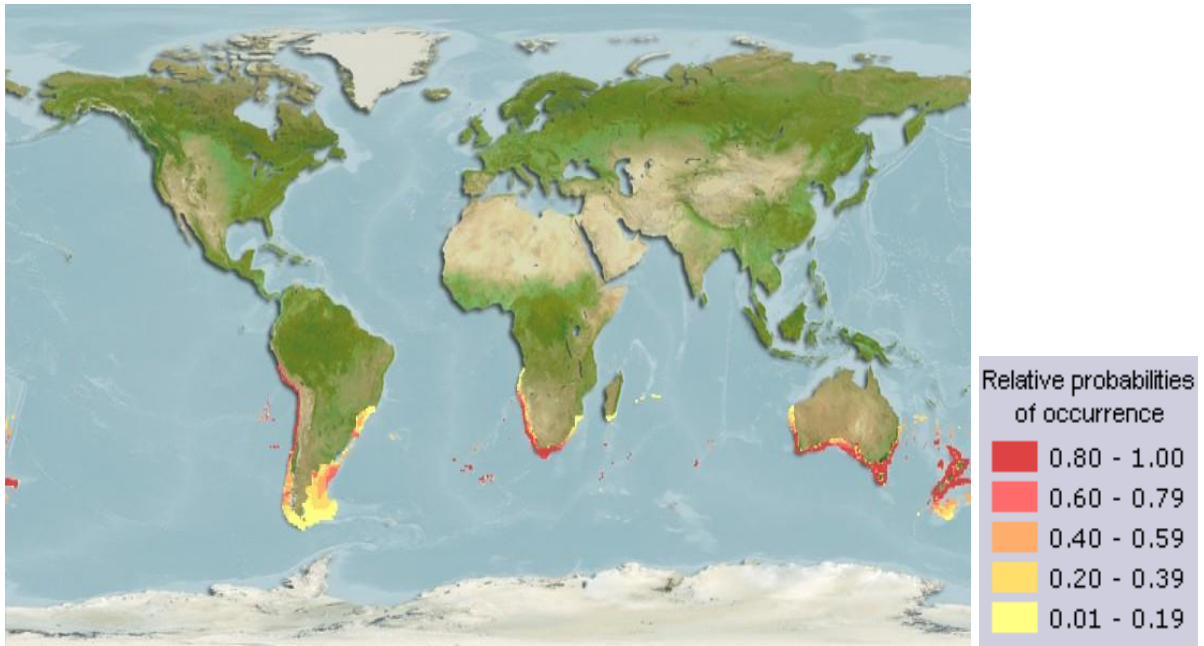
Figure 2.1. Native Distribution and abundance of *Thyrsites atun* (Snoek) (FishBase, 2013).

In South Africa snoek is found between the Cunene River and Cape Agulhas in the Benguela ecosystem (Griffiths, 2003). Although adult snoek are resident in South Africa all year round it usually undergoes an offshore spawning migration in winter and spring (Griffiths, 2003). A number of distinct stocks have been observed throughout the snoek distribution. To date three stocks have been identified off New Zealand and 3-5 stocks were reported off Australia. However, severe knowledge gaps remain in our understanding of South African snoek population dynamics as no stock structure analysis has yet been conducted (Griffiths, 2003). South African snoek fisheries had been in existence since 1800s but it currently runs as an informal sector operated by small-scale fishermen (Isaacs, 2013). Snoek is a target species for line fishers and few trawl vessels and it forms more than 50% of landed line fish in the Cape Peninsula (Isaacs, 2013). South African catch, landings and imports (Tonnes) of snoek in 2010 is summarised in Table 2.2. Snoek plays a vital role in the livelihood, cultural practices and as a protein source, of the poor people in the Cape Peninsula (Isaac, 2013). The Primary Snoek fishing areas around the Cape Peninsula are shown in Figure 2.2 (Isaacs, 2013).

Table 2.2 Snoek catch, landings and imports (Tonnes) in 2010 (Isaacs, 2013; DAFF, 2012)

Sources of snoek	Landing and imports
Line fish	6,638,139
Deep sea hake trawl	3,650,270
Hake longline	3,491
Inshore trawl	709
New Zealand barracouta (imports)	5,690,968
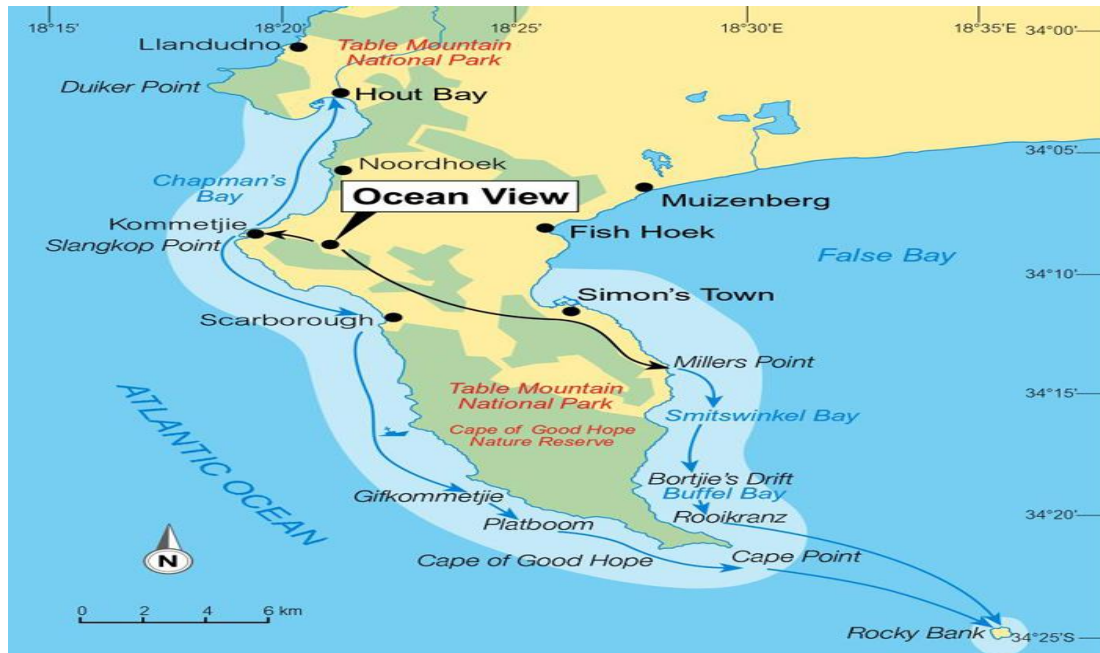
Fig. 2.2 Primary Snoek fishing areas around the Cape peninsula (Isaacs, 2013).

2.3. Postharvest loss of fish

The high water and protein content of fish render it sensitive to rapid degradation with improper handling, storage and preserving techniques further enhancing perishability leading to unnecessary wastage (Masniyom *et al.*, 2011). Postharvest losses occur when fish are discarded because it is harmful and unfit for consumption as a result of deterioration or spoilage. Postharvest losses occur in most fish distribution chains throughout the world which results in direct financial loss to the fishermen and reduces the overall fish quantity available to the consumer (Diei-Quadi & Mgawe, 2011). Postharvest losses may be quantitative or qualitative. Quantitative loss occurs when fish are thrown away as a result of physical damage, decay or are partially consumed by animals. Qualitative loss in fish or fish products occurs when the fish has unwholesome quality attributes and it is no longer acceptable to the end user (Ward & Jeffries, 2000). Although a number of researchers have conducted surveys to assess the postharvest loss of fish, quantification is difficult due to inadequate data (Opara *et al.*, 2004).

2.4. Food spoilage

Food spoilage occurs when the original nutritional value, texture and flavour of the food is destroyed, and becomes harmful, unpleasant and unsuitable for consumption (Aberoumand, 2010; Sivertsvik *et al.*, 2002). Physiological process continues after harvest/post mortem in agricultural produce such as vegetables, fruits and animal products which causes deterioration immediately postharvest (Abbas *et al.*, 2009). Chemical, enzymatic or microbial activities commonly cause deterioration and spoilage of food products (Pereira de Abreu *et al.*, 2010). Approximately 25% of global food production and 30% of total landed fish are lost to microbial activities (Gram & Dalgaard, 2002; Ghaly *et al.*, 2010). Overall, enzymatic and microbial spoilage are responsible for losses of 4-5 million tonnes of trawled fish and shrimp annually mainly due to improper post catch handling and storage (Ghaly *et al.*, 2010).

2.5. Phases of spoilage in fish

Once harvested, fish have reduced protection from external factors which can enhance deterioration. High ambient postharvest temperatures predispose fish to rapid spoilage and can also result in gaping (separation of myotomes) which occurs when the muscle structure disintegrates. Gaping is species specific and is irreversible when the temperature rises above 30°C. Round fish such as cod, haddock and salmon gape more than flat fish, while other species such as catfish do not gape (Borderías & Sánchez-Alonso, 2011). Improper or poor handling practices during and after harvest likewise accelerates the rate of spoilage. Dirty boats, equipment, fish boxes and baskets, inappropriate fish washing and processing as well as physically damaging the fish (throwing and/or stepping on the fish) all contribute towards spoilage (Oyelese, 2012). Poor handling practices accelerate the onset of rigor mortis, autolysis, increases gaping, which cause changes in skin colour and increases protein solubilisation resulting in drip loss (Borderías & Sánchez-Alonso, 2011). In addition, the fishing method used can also affect the fish meat quality. Trawled fish are subjected to stress as they spend hours struggling in the net and spoil faster than line caught fish (Borderías & Sánchez-Alonso, 2011). In total three main activities contribute to fish spoilage (Ghaly *et al.*, 2010):

- Enzymatic activities,
- Microbial activities and
- Oxidative rancidity

2.5.1. Spoilage due to enzymatic activities or autolysis

Rigor mortis precedes autolysis and commences within 24 hours after death of fish (Ghaly *et al.*, 2010; Lougovois & Kyrana, 2005). When meat is exposed to high ambient temperatures

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post-mortem and/or stress ante mortem, the development of rigor mortis occurs more quickly. Rigor mortis occurs when anaerobic respiration takes place within the muscle tissue after death resulting in the interruption of adenosine triphosphate (ATP) synthesis (Bahuaud *et al.*, 2010; Mellefont *et al.*, 2003). Consequently, glycogen is converted to lactic acid, the pH drops and the muscle becomes rigid, hardened, shortened, loses transparency and elasticity.   During rigor mortis there is conversion of di- and tri-phosphopyridine and adenosinediphosphate (ADP) and ATP to di- or mono- phosphoridine, inosine, hypoxanthine, xanthine, uric acid, formaldehyde and other products (Hamada-Sato *et al.*, 2005; Mohan *et al.*, 2009) resulting in spoilage and the development of off flavours (Lougovois & Kyrana, 2005; Ghaly *et al.*, 2010). This usually lasts for one day after which the muscle softens. Autolysis is the degradation of muscle after death due to the activities of enzymes present within the muscle and viscera of the animal (Ghaly *et al.*, 2010). Two diverse processes are responsible for autolysis in fish, which include;

(1). Activities of digestive enzymes or self-digestion - Digestive enzymes within the gut digest the food in the intestine resulting in a breakdown of the muscle (Abbas *et al.*, 2009).

(2). Activities of proteolytic enzymes in the fish muscle, such as cathepsins and calpains play an important role in autolysis of fish and mammals bringing about muscle degradation and softening (Bahuaud *et al.*, 2010). Autolysis results in breakdown of the muscle structure, rendering it tender and flabby 1-2 days post-harvest.   Post mortem changes in fish are represented by the following chart:

Catch → Fish → Rigor motis → Dissolution of rigor → Autolysis → Spoilage
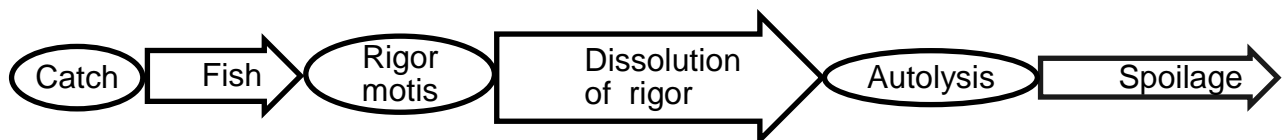
(Hamada-Sato *et al.*, 2005).

2.5.2. Spoilage due to microbial activities in fish

Microbial activities play an important role in fish spoilage and produce hydrogen sulphide (H_2S) and other metabolites such as amines, putrescine, histamine, cadaverine, organic acids, aldehydes and ketones which cause foul and off flavours (Emborg *et al.*, 2005). When fish is harvested it contains the micro floral of the water from where it was harvested (Gram & Dalgaard, 2002). However, only a few specific organisms (SSOs) within the micro floral are responsible for spoiling fish.   Gram-negative microorganisms such as *Pseudomonas, Aeromonas, Moraxella, Vibrio, Flavobacterium* and *Cytophaga spp.* are predominantly found in cold water fish, while mesophilic Gram-positive microorganisms such as *Micrococcus* and *Bacillus* are predominant in warm water fish (Al Bulushi *et al.*, 2010).

Gram-negative microorganisms such as *Pseudomonas* spp., *Shewanella putrifaciens,* and *Aeromonas* spp.are implicated in spoilage of chilled fish under aerobic conditions (Ravi Sankar *et al.*, 2008) while fermentative microorganisms such as *Vibrionacaea* spp. are responsible for the spoilage of unpreserved fish (Gram *et al.*, 2002). *Pseudomonas* and *Shewanella* spp. were involved in the spoilage of iced cod chilled sea bream (Parlapani *et al.*, 2012), farmed turbot stored at 0, 5, 10 and 15 $^{\circ}$C (Nuin *et al.*, 2008) and appeared to be responsible for sweet, fruity spoilage odours (Ólafsdóttir *et al.*, 2006). Spoilage of heavily salted and dried fish is caused by the growth of filamentous fungi or insect infestation while yeasts may grow in heavily wet-salted fish (e.g. barrel-salted herring). Spore forming microbes like *Clostridium* or *Bacillus* can grow in mildly heat treated fish especially when the salt content is low (Rosnes *et al.*, 2011).

Pathogens found in fish and sea products include spores of *Clostridium botulinum, Listeria monocytogen* and *Vibrio parahaemolyticus.* These organisms survive salting and smoking, and have been found to cause food poisoning. For example, *V. parahaemolyticus* was reported to have caused food poisoning in Japan (Opara *et al.*, 2004), and *Shewanella, putrifaciens, Aeromonas* spp.*, Enterobacteriacceae, P. phosphoreum* and *Vibrio* spp*.* are responsible for the reduction of Trimethylamine oxide (TMAO) to Trimethylamine (TMA) producing an ammonia like off flavour in fish (Joffraud *et al.*, 2001; Jørgensen *et al.*, 2001). A number of spoilage metabolites such as TMA, total volatile nitrogen (TVN), and hypoxanthine levels are used as quality indices to determine fish spoilage. In addition, K-Values which describe the ratio between ATP degradation by products and biogenic amines are regularly used as a quality index to assess fish spoilage (Dalgaard, 2000). Volatile compounds produced as a result of spoilage by microorganisms in fish are summarized in Table 2.3.

Table 2.3 Selected volatile compounds produced by microorganisms in spoiling fish and their sources (Opara *et al.*, 2004)

Compound	Probable Source	Associated Microbe(s)	References
Hydrogen Sulphide	Cystein	*Shewanella Putrifaciens, Vibrionacaea*	Abbas *et al.*, (2009); Ghaly *et al.*, (2010)
Dimethyl Sulphide	Methionine	*Shewanella Putrifaciens*	Ghaly *et al.*, (2010)
Methyl Mercaptan	Methionine	*Shewanella Putrifaciens*	Ghaly *et al.*, (2010)
Acidic Propionic, Butyric and Hexanoic acid esters	Glysine, Leucine, Serine	*Aerobic spoilers*	Ghaly *et al.*, (2010)
Trimethylamine	Trimethylamineoxide	*Shewanella Putrifaciens, Photobacterium phosphoreum, Vibrionacaea, Flavobacteria.*	Lakshmanan *et al.*, (2000); Abbas *et al.*, (2009); Ghaly *et al.*, (2010)
Ammonia	Urea, various Amino Acids	*Aerobic spoilers*	Ghaly *et al.*, (2010)
Ketones, aldehydes, non- hydrogen sulphide products		*Pseudomonas spp.*	Abbas *et al.*, (2009)
Histamine	Histidine	*Morganlla morganii, Klebsiella pneumoniae, Hafnia alvei*	Lakshmanan et al., (2000); Auerswald et al., (2006)

2.5.3. Oxidative rancidity in fish

Oxidative rancidity also known as lipid oxidation occurs when the double bonds of polyunsaturated fatty acids are inundated with oxygen in the presence of catalysts such as iron, salts, and heat to form peroxides, aldehydes, ketones and epoxides (Faustman *et al.*, 2010). This usually involves three steps known as initiation, propagation and termination (Ghaly *et al.*, 2010). Lipid oxidation has been reported to occur in a number of meat products such as pork, beef, chicken, fish, fats and oils when they are exposed to light and/or extreme temperatures during handling, processing and storage (Pazos *et al.*, 2010; Utrera & Estévez, 2013). Apart from these external factors, intrinsic factors such as muscle composition also influence oxidation of meat (Chaijan *et al.*, 2006). Lipid oxidation is a key cause of spoilage in pelagic fish species such as mackerel, salmon and herring (Maestre *et al.*, 2011). Higher proportions of polyunsaturated fatty acids and the pro-oxidant nature of haemoglobin predispose fish muscle to oxidation (Maestre *et al.*, 2011). Oxidative spoilage may be enzymatic or non-enzymatic. Enzymatic lipid oxidation is caused by the activities of enzymes; lipases which hydrolyse glycerides from free fatty acids in a process known as lipolysis and proteolytic enzymes which break down proteins (Wang *et al.*, 2011a). Proteins are degraded into amino acids and peptides which are further deaminated into methyl

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mercaptan, dimethylsulfide and hydrogen sulphide (Lougovois, 2005). Oxidative enzymes are either endogenous or from psychrotropic microorganisms. Non enzymatic lipid oxidation is the reaction between free fatty acids formed during hydrolysis and sarcoplasmic and myofibrillar proteins. The addition of acids as preservatives typically increases lipid oxidation by deoxygenating haemoglobin (Undeland *et al.*, 2005). Some of the post-mortem changes in fish muscle can be associated with lipid oxidation, which include an increase in ATP by-product hypoxanthine, changes in xanthine dehyrdoxygenase to xanthineoxidase, increase in low molecular weight transition metals, conversion of heme Fe II pigments to Fe III and softening of the muscle. These changes frequently cause development of rancid flavours in stored fish products (Opara *et al.*, 2004; Richards *et al.*, 2007).

2.6. Histamine in fish

Histamine is a biogenic amine found mostly in seafoods and fermented foods such as salami, cheese and wine (Bjornsdottir *et al.*, 2009) and is an indication of spoilage (Yesudhason *et al.*, 2013). It is formed as a result of decarboxylation of the free amino acid histidine by the activity of decarboxylase enzymes produced by gram-negative bacteria such as *Vibrio*, *Photobacterium, Klebsiella, Morganella* and others (Auerswald *et al.*, 2006). Fish from the Scomberesocidae and Scombridae families have a high concentration of histidine (free amino acid) in their muscle which is converted to histamine and are implicated in scrombroid food poisoning (Hungerford, 2010). Processing methods such as canning, cooking, drying, smoking etc. cannot eliminate histamine once it is produced (Lehane & Olley, 2000).

Histamine poisoning is one of the most reported seafood poisoning in many countries with high incidences in the United States, Japan and the United Kingdom (Yesudhason *et al.*, 2013) and fewer occurrences reported in Canada, New Zealand, France, Germany, Norway, Australia, Indonesia, Sweden, Sri Lanka, Czechoslovakia, Netherlands, South Africa and Egypt (Yesudhason *et al.*, 2013). Occurrence of histamine food poisoning linked to cold smoked-tuna was reported in Denmark in 2004 (Emborg & Dalgaard, 2006). Furthermore, histamine contents above the recommended limit of 50 mg/100 g were reported to have caused food poisoning in June, 2007, in Kaohsiung city, southern Taiwan (Chen *et al.*, 2010). Due to the relatively high incidence of histamine poisoning, safety limits regarding histamine consumption have been set in a number of countries however, the acceptable limit can vary. For instance the histamine acceptable limit in Australia is 200 ppm (Australian food safety code, 2001), in Europe it is 100 ppm (EC, 2003), USA it is 50 ppm (FDA, 1998) and in South Africa it is 100 ppm (South African Bureau of Standards, 2001) (Auerswald *et al.*, 2006). When fish and fish products contain histamine levels higher than the required limit (≥500-1000 mg/kg), they are classified as toxic. Ingestion of

histamine at this toxic level results in scrombroid or histamine poisoning. The symptoms of histamine poisoning include development of a rash, headache, sweating, nausea, vomiting, diarrhoea, stomach pain, dizziness, swelling of the tongue and face (Emborg *et al.*, 2005).

2.7. Effect of storage on shelf life of fish.

A number of storage systems are currently employed within the fisheries industry which includes cold storage, ambient temperature storage after processing (smoking and drying hot smoked and dried fish products), vacuum packaging, modified atmosphere packaging (MAP), canning, etc. The use of the various packaging methods reduces some of the organisms responsible for unattractive characteristics which render them unacceptable for human consumption (FAO, 2005). In order for fish and fish products to remain nutritious and safe during storage the water activity must be reduced to a level that will prevent enzyme activity and microbial growth. Bacteria growth is favoured at water activities between 0.85-0.96, while moulds and yeast will grow between 0.65-0.90 (Mellefont *et al.*, 2003). Therefore, reducing water activity to less than 0.6 will prevent both bacterial and mould growth thereby reducing the incidence of spoilage (Aberoumand, 2010). The pH at which the fish is stored is also important, as all microorganisms have minimum, optimum, and maximum pH at which they thrive (Ghaly *et al.*, 2010). For instance activities of proteolytic enzymes were retarded in fish stored at 0°C and at a pH of 5 (Ghaly *et al.*, 2010). Similarly, shelf life of frozen silver carp was increased to 25-30 days by coating with chitosan, a polymer formed by deacetylation of chitin found in the exoskeleton of arthropods or cell walls of fungi and yeast. It is biodegradable, biocompatible and non-toxic (Jayakumar *et al.*, 2010; Sogias *et al.*, 2008) and it inhibited activities of endogenous proteases by lowering the pH (Fan *et al.*, 2009). The pH at which fish is stored also has an influence on lipid oxidation and development of fishy flavour during prolonged storage (Maqsood & Benjakul, 2011). Freshly harvested fish have a pH close to neutral (pH = 7). Increase in fish muscle pH is an indication of decomposition (Kilincceker *et al.*, 2009).

The storage temperature (short and long term) of fish is a critical element to the storage stability of the product. Similarly to water activity and pH, microorganisms have a minimum, optimum and maximum growing temperature (Ghaly *et al.*, 2010). Storage temperatures of between 0-5°C can reduce spoilage of fish such as cod (*Gadus morhua*) and ray fish (*Actinopterygii* spp.) for up to 10 and 15 days, respectively (Opara *et al.*, 2004; Ocaño-Higuera *et al.*, 2011). However, lower storage temperatures of -10°C (or below) can further improve preservation and reduce microbial growth when storing fish for long periods (Ghaly *et al.*, 2010), however there is an additional energy cost to the use of these low temperatures. Cold storage renders bacteria inactive and slows down chemical change due to reduced enzyme activities (Abbas *et al.*, 2009). Arannilewa *et al.* (2006) reported on the

effect of frozen period on the chemical, microbiology and sensory quality of frozen tilapia fish (*Sarotherodun galiaenus*), a commercial species in West Africa. They observed that the overall protein and fat content decreased during cold storage. Additionally, autolytic reactions can still occur and cause the development of off flavours and eventual spoilage of the fish.

Modified atmospheric packaging (MAP) is also used in the storage of fresh meat products such as fresh fish (Table 4), while its effects on shelf life of various food products have been reviewed extensively. Sivertsvik *et al.* (2002) reported a 30-60% increase in the shelf life of fresh fish stored using MAP with high CO_2 level. Additionally, MAP was reported to suppress the growth of *L. monocytogens, Salmonella* spp. and *E. coli* at 70-100% CO_2, when compared to air and vacuum packaging (VP) (Masniyom *et al.*, 2011). However, high levels of CO_2 did not inhibit microbial growth in sea bass when a combination of MAP and pyrophosphate was used (Masniyom *et al.*, 2011).

Masniyom *et al.* (2011) observed that MAP retarded microbial growth and spoilage in fish. Ghaly *et al.* (2010) also concluded that although freezing, is the best way to store fish, it is only a temporary method of preservation for fresh or minimally processed fish (cold smoked fish and other intermediate moisture fish products). Consequently, a combination of certain processing methods such as salting, brining, drying or smoking, and the addition of preservatives with MAP and freezing is preferable for maintaining good fish quality for prolonged storage times (FAO, 2005; Ghaly *et al.*, 2010; Masniyom *et al.*, 2011).

Table 2.4 Shelf life extension of fish and fishery products under MAP

Fish and fishery product	Storage temp. (^{0}C)	MAP condition $CO_2:O_2:N_2$	Shelf life (days)	References
Mediterranean swordfish	4	40:30:30	12	Pantazi *et al.* (2008)
Pearlspot	2	60:40:00	10	Ravi-Sankar *et al.* (2008)
Cod	-0.9	50:05:45	21	Wang *et al.* (2008)
Sea bass	4	60:10:30	13	Kostaki *et al.* (2009)
Atlantic salmon	2	90:00:10	22	Fernández *et al.* (2009)
Mediterranean swordfish	4	50:05:45	13	Kykkidou *et al.* (2009)
Atlantic salmon	1.2	60:00:40	15	Hansen *et al.*(2009)
Sea bass	4	60:00:40	18	Provincial *et al.* (2010)

2.8. Fish Processing Techniques

2.8.1. Gutting and beheading

When fish are not consumed fresh they must undergo preliminary processing operations prior to packaging and storage. Preliminary operations in fish processing include beheading and gutting which should be done immediately to prevent autolytic spoilage (Borderías & Sánchez-Alonso, 2011). Digestive enzymes and bacteria present in fish guts can cause spoilage therefore the head and the guts are removed in order to reduce microbial load and

prevent autolytic spoilage (Bensid *et al.*, 2014).   Potential parasitic gutting increases bacterial contamination of the fish (López-Caballero *et al.*, 2002).   Therefore migration from the stomach into the muscle is also reduced by gutting (Borderías & Sánchez-Alonso, 2011; Tejada *et al.,* 2013) while improper handling during, care must be taken to prevent cross contamination (Erkan & Özden, 2006; Erkan, 2007).   These activities are either done manually or mechanically where the methods are usually determined by the size of the enterprise. The effects of gutting and non-gutting on shelf life of fish have been studied with varying results (López-Caballero *et al.*, 2002; Papadopoulos *et al.*, 2003; Baixas-Nogueras *et al.*, 2009; Erkan 2007;).   No significant difference in bacteria counts was found between whole and gutted orange roughy (*Hoplostenthus atlanticus*), though a slight increase in shelf life was observed in gutted fish.   On the other hand, the evisceration of Atlantic croacker (*Micropogon undulates*), grey trout (*Cynoscon regalis*) and Atlantic mackerel (*Scomber scombrus*) in combination with a pressure wash significantly reduced the microbial load (Borderías & Sánchez-Alonso, 2011).

Fish are beheaded immediately after harvest in order to remove the gills because fish are usually not bled which results in formation of blood clot in the gills (Borderías & Sánchez-Alonso, 2011).   This causes rapid growth of spoilage organisms, especially *Pseudomonas* spp., which increases gradually in the gills and spreads within the tissues thereby causing autolytic spoilage (Pacquit *et al.*, 2006).

2.8.2. Salting

Salting was the only widely available method for preserving fish up until the 19th Century at which point alternative methods such as canning, cold storage, slurry ice, vacuum packaging, modified atmospheric packaging, use of hydrostatic pressure, irradiation, edible films and coatings become available (Jain & Pathare, 2007; Reza *et al.*, 2009).   Nonetheless salting is still a popular method for preserving food, particularly in communities which do not have cold storage facilities.   Salting as a process of preservation has been studied in a number of fish species, including sardine, Atlantic cod (*Gadus morhua*), salmon (*Salmo salar*), Bombay duck (*Harpodon neherius*), European sea bass (*Dicentrarchus labrax*) and milk fish (C*hanon chanon*) (Andrés *et al.*, 2005; Bellagha *et al.*, 2007; Fuentes *et al.*, 2007; Gallart-Jornet *et al.*, 2007).   The authors reported that the rate of salt uptake by fish muscle depends on a number of factors which could be classified into intrinsic and extrinsic factors (Sannaveerappa *et al.*, 2004; Thorarinsdottir *et al.*, 2002; Thorarinsdottir *et al.*, 2004). Factors influencing rate of salt uptake in fish are shown in Table 2.5. During the salting process, there is osmotic movement of solute from the salting medium into the fish muscle and water subsequently diffuses out of the muscle (Barat *et al.*, 2003). Consequently, there is decrease in water activity of the fish making little or no water available for microbial

activities (Barat *et al.,* 2003). Fish salting can therefore reduce/slow down the rate of spoilage as chloride ions from the salt also inhibit the growth of some microorganisms (Leroi *et al.*, 2000; Goulas & Kontominas, 2007; Al Bulushi *et al.*, 2010).

Table 2.5 Factors influencing rate of salt uptake by fish muscle during salting

Intrinsic Factor	Extrinsic Factor
Flesh thickness	Temperature
Freshness	Type of salt
Fat content	Salting method
Species	Salt or brine concentration
Size/weight	Duration of salting
Physiological state	Fish-salt ratio

There are two major salting methods used in fish processing, dry and wet salting, whilst, pickling is a combination of both the dry and wet techniques (Bellagha *et al.*, 2007). Dry salting is also referred to as Kench salting, where fish are piled up in layers on a drainage rack. Salt is stacked extensively in between the fish to prevent fish on fish contact, while the entire pile is covered with salt (Barat *et al.*, 2003; Oliveira *et al.*, 2012). As the fish take up salt, water diffuses out and the brine produced is allowed to flow away. Dry salting is done in a cool room (temperature below 10°C) for about 6 to 30 days, depending on the degree of curing desired (Thorarinsdottir *et al.*, 2004; Gallart-Jornet *et al.*, 2007). Pickling also involves dry salting but instead of draining the water which diffuses out of the fish, the fish is allowed to stay in the resultant high concentration brine for several days (Brás & Costa, 2010). Wet salting, also known as brining is prepared by dissolving pure sodium chloride in water. It is a process whereby fish are submerged in brine at a low temperature (below 10°C) for a predetermined period (Oliveira *et al.*, 2012). The amount of sodium chloride used to obtain the desired brine concentration is measured and dissolved in an appropriate volume of water. However, the concentration used depends on the desired product (Jittinandana *et al.*, 2002). Brine concentration plays an important role in texture development, it influences inter and intra molecular bonds of the muscle protein bringing about change in structure, denaturation of proteins and water holding capacity of the muscle (Thorarinsdottir *et al.*, 2004). Barat *et al.* (2003) and Guizani *et al.* (2008) reported that increasing the brine concentration above 9-10% (w/w) brings about loss of water and shrinking of the muscle due to the formation of protein-protein bonds. Such protein denaturation reduces the water holding capacity of the cells, which reduces the water content and hardens the fish muscle. Conversely, a low brine concentration causes swelling of the muscle due to an increase in water holding capacity and resulting in a softening of the fish muscle (Gallart-Jornet *et al.,* 2007; Oliveira *et al.*, 2012). Therefore determining the optimal salt concentration is crucial for the production of high quality fish products. Furthermore, the fat content of fish can also influence the quantity of salt absorbed (Gallart-

Jornet *et al.*, 2007). Fat is hydrophobic in nature and poses a physical barrier between salt and water thereby reducing salt uptake (Gallart-Jornet *et al.*, 2007). A comparative study examining the effect of salting on weight of lean (cod) and fatty (salmon) fish found that lean fish absorbed more salt and therefore lost more weight when compared to the fatty fish (Gallart-Jornet *et al.*, 2007). Fish brining as compared to dry salting has been reported to reduce process time (Barat *et al.*, 2002; 2003; Thorarinsdottir *et al.*, 2004) and produce products with high weight yield, salt uptake, and prevent oxidation due to lack of aerial exposure (Oliveira *et al.*, 2012). New innovations in fish salting including vacuum–tumbling, salt injection and vacuum osmotic dehydration; techniques often used to increase the yield, reduce brining time, facilitate the standardisation of salt concentration and reduce the loss of water soluble constituents of the muscle (Lauritzsen *et al.*, 2004; Yanar *et al.*, 2006; Larsen & Elvevoll, 2008).

Salting is usually carried out before the fish is sun dried, smoked or marinated (Sobukola & Olatunde, 2011). Hwang *et al.* (2012) substantiated the preservative effect of salting and reported that aerobic plate counts, total coliform, water activity, moisture contents, total volatile basic nitrogen (TVBN) and thiobarbituric acid (TBARS) decreased with increased salt concentrations in milkfish. Guizani *et al.* (2008) also reported lower counts for total aerobic bacteria and *Staphylococci* in osmo-air-dried shark meat stored at room temperature for two months. These reports show that salting could be used to extend the shelf life of fish without compromising its nutritional quality.

Salting can also influence the extent of lipid oxidation particularly during cold storage which can negatively affect the meat quality. High brine concentrations have been shown to increase peroxide formation in salted horse marckerel (*Trachurus trachurus*) during frozen storage (Aubourg & Ugliano, 2002) as well as to increase the peroxide values (PV) and thiobarbituric acid (TBARS) values of hot smoked tilapia (Yanar, 2007). Nonetheless, the degree of lipid oxidation, PV, TBARS and TVBN did not exceed the recommended level for safe consumption (Yanar, 2007). Therefore, salting is considered an overall safe method of preserving fish.

Different types of salt exist namely; mine, solar, rock, vacuum processed and refined salts (Oliveira *et al.*, 2012) all of which can vary in composition and subsequently influence the quality (smell, taste, texture, colour) of salted fish (Rodrigues *et al.*, 2005). Refined salt is made up of almost pure sodium chloride while all others contain a mixture of sodium chloride, calcium sulphate, magnesium sulphate, nitrite, polyphosphate, chloride and other impurities such as gypsum, shale, dolomite and quartz (Qadir *et al.*, 2005). Often pure sodium chloride is used for salting fish as it produces a desirable flavour and texture and is safe to use and consume. Although, magnesium, calcium and potassium salts can enhance the colour and texture of salted cod (Lauritzsen *et al.*, 2004; Rodrigues *et al.*, 2005),

potassium salt could produce undesirable characteristic such as bitter taste if not properly applied while calcium salt at 0.15-0.35% has been found to be satisfactory for fish salting.

2.8.3. Drying

Dried products are processed using either natural or artificially created air currents. The air flows over the surface of the fish causing diffusion of water from the interior muscle to the surface of the fish, which then evaporates (Bellagha *et al.*, 2007; Shi *et al.*, 2008). The preservative effect of drying is based on the reduction of water activity of the fish in order to reduce microbial activities and therefore prevent spoilage (Shitanda & Wanjala, 2006; Al-Harahsheh *et al.*, 2009). The quality of the final product is dependent on the air temperature used and the pre-treatment given to the fish prior to drying (Bellagha *et al.*, 2007). Fish drying has other advantages apart from preserving and enhancing the flavour and aroma of the fish. Drying reduces the weight and volume of fish making transportation more efficient and reduces the packaging and storage cost (Augustus *et al.*, 2002; Al-Harahsheh *et al.*, 2009) and is generally more cost effective than other preserving techniques such as canning, cold storage, vacuum packaging, etc. (Jain & Pathare 2007; Reza *et al.*, 2009). Traditional fish drying is achieved by laying the fish on sand/rocks at the beach, hanging on racks of scaffold in the sun (Nooralabettu, 2008), or by using convection air drying technique (Wang *et al.*, 2011b). However, insect infestation, contamination from insects and rodents, soil particles and losses due to spoilage, fungi and microbial growth are difficult to control using traditional drying methods (Jain & Pathare, 2007). Drying takes a week or more, and the long drying duration can result in spoilage of fish, especially during unfavourable weather conditions (Reza *et al.*, 2009). Additionally, improper handling of fish during traditional drying results in products with poor quality (Nooralabettu, 2008; Wang *et al.*, 2011b). Due to the inconsistent quality of sun dried fish, artificial drying has become more popular with the use of artificial dryers. These driers use convectional hot air (oven drying, solar drying and vacuum freeze drying) under controlled conditions (temperature, air velocity, relative humidity and drying time) (Bala & Mondol, 2001). Use of artificial dryers has led to improved product quality, reduced postharvest losses, and reduced insect infestation and contamination (Reza *et al.*, 2009). A combination of both salting and drying can result in the production of a high quality product with water activity low enough to be microbiologically safe (Mol *et al.*, 2010).

2.8.4. Effect of salting and drying on the nutritional profile of fish

Salting and drying play important role in preservative and development of sensory characteristics of fish products but they generally affect the final quality of the products (Tokur, 2007). During drying chemical, physical and biochemical reactions take place which

denature protein and thus increase its digestibility (Wu & Mao, 2008). However, heat labile, polyunsaturated fatty acids, physical (colour, texture) and chemical (vitamins and minerals) compounds are lost while some undergo modifications (Kilic, 2009; Perera, 2005). Nutrient loss during drying depends on temperature, drying time and species of fish (Wu & Mao, 2008; Kilic, 2009). Wu and Mao (2008) observed decrease in fat content, saturated and monounsaturated fatty acids while polyunsaturated fatty acids (by 23.8%) and protein content increased after drying. They concluded that there was no negative effect of drying process on the amino acids composition of grass carp (*Ctenopharyngodon idellus*) fillets dried at 180°C for 90 minutes. Chukwu and Mohammed (2009) also presented similar result for catfish dried at 120°C for 30 minutes in an electric oven. Colour change is one of the physical changes that occur in salted and dried products. Maillard reaction is responsible for browning of fish muscles during drying (Vega-Gálvez *et al.*, 2011). This is a non-enzymatic browning reaction between amines, amino acids, reducing sugars and muscle proteins to form high molecular weight carbonyl compounds known as melanoidins which are brown compounds (Zamora & Hidalgo, 2005). Loss of colour in salted and dried fish is as a result of moisture loss during salting and drying (Brás & Costa, 2010). Moisture removal from the fish muscle reduces light scattering and causes loss of transparency and subsequent increase lightness (Lauritzsen *et al.*, 2004; Steinhauser *et al.*, 2006; Brás & Costa, 2010; Wang *et al.*, 2011b). Wang *et al.* (2011b) reported that the redness (a) and yellowness (b) which is an indication of browning was significantly higher in dried silver carp (*Hypophthalmichthys molitrix*) than in the fresh fish. Drying causes collapse of structure which causes changes in shape and loss of tenderness (Kilic, 2008; Wang *et al.*, 2011b). Salting enhances lightness of the colour of the fish muscle depending on the type of salt used. Magnesium and calcium ions result in whitening of fish muscle (Brás & Costa, 2010). Additionally, proteins are denatured during salting, and these result in loss of salt soluble proteins, especially high molecular weight proteins. Sannaveerappa *et al.* (2004) observed that salting reduced the amino acids, non-protein nitrogen and sulphydryl groups in salted milkfish and that more protein was lost during brining than in the dry salting. Salting has an influence on the oxidation of poly-unsaturated fatty acids which are predominant in fish muscle. Aubourg and Ugliano (2002) observed that increasing salt content resulted in oxidative rancidity in frozen horse mackerel. However, the use of low temperature and low salt concentration during salting and drying of fish have been reported to reduce nutrient degradation and enhance product quality (Kilic, 2009).

2.9. Conclusion

The fisheries sector is important to the global economy but is currently faced with numerous issues such as those relating to management, regulatory, safety, sustainability and

postharvest loss due to fish spoilage. Various fish processing and preservation techniques have been developed to combat postharvest loss ensuring that this high protein food source is available both in and out of season, enabling year round availability for the local communities which rely on them. Within South Africa the snoek fisheries operates as an informal small-scale sector with little or no regulation relating to handling, storage and preservation. Unsold fresh snoek is often salted and sun-dried for selling at a later stage. However, no standardised salting and drying procedure currently exists leading to a lack of uniformity in the quality of processed snoek. Nonetheless, the development of standardised postharvest handling and preservation methods may enhance the potential for the processed snoek sector within South Africa thereby decreasing postharvest losses, ensuring food security and enhancing access to high quality value-added snoek products.

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Chapter 3: Evaluation of quality of commercially available salted dried snoek (*Thyrsites atun*) in the Western Cape province, South Africa

Abstract

This study investigated the quality attributes of salted and dried snoek (*Thyrsites atun*) purchased from four local commercial vendors in the Western Cape, South Africa. Fish quality was assessed through the analysis of proximate composition, physicochemical attributes, and microbial safety and stability. Substantial variation was observed in the proximate, biochemical composition and microbiological safety (total viable count, *Escherichia coli* and *Salmonella* spp.) of the salted dried snoek. Product moisture, protein, lipid and ash content varied between 33.4-49.3%, 26.7-39.4%, 7.0-21.0% and 11.3-16.6%, respectively while water activity (0.75-0.85) and TBARS (thiobarbituric acid reactive substances) (9.58 mg/MDA/kg-19.83 mg/MDA/kg) were higher than the recommended threshold for dried fish. Low histamine (<2.5 ppm), high salt (6.73-8.87%), low microbial counts (3.12 log CFU/g-3.4 log CFU/g) and the absence of pathogens (*Salmonella* spp. and *Escherichia coli*) were observed. Collectively, these results suggest that the products were wholesome and that the salted and dried snoek retailed in the Cape Peninsula require further drying and/or salting to help minimise the presence of spoilage organisms and ultimately extend the shelf life of the product.

3.1 Introduction

The fisheries sector accounts for 1% of the annual South African gross domestic product (GDP) (FAO, 2005) and generates about ZAR 4-5 billion revenue per annum providing employment for an estimated 27 700 people (FAO, 2010). South African fisheries are comprised of commercial, subsistence and recreational sectors such as line fish, West Coast lobster, abalone, beach seine and gill net fishing (Stewart *et al.*, 2010). Snoek (*Thyrsites atun*) is traditionally part of the line fisheries sector in South Africa and constitutes more than 50% of the total landed line fish in the Western Cape province South Africa (Isaacs, 2013) which translates to 2 765-6 839 tonnes/year (2001 to 2010) (DAFF, 2012).

The snoek fishery sector is largely an informal market operated by small-scale fishers in the Western Cape and is sold locally, either as a fresh, smoked or salted-dried product. Due to the informal nature of the snoek trade there is currently no estimated value for the processed snoek industry (salted and dried) due to lack of reported sales and associated documentation (Isaacs, 2013). Although not a substantial contributor towards the overall state GDP the snoek fishery provides local employment both directly (fishers and middle man whom sell to the public) and indirectly (fish processors) to coastal communities (Béné *et al.*, 2010). The dried snoek sector has remained significantly underdeveloped when compared to countries such as Iceland and Norway which have a well-developed salted and

dried fish industry. Combined, both countries produced 330 000 tonnes ($US 2 billion) of salted dried Atlantic cod in 2005 and this industry is continuing to grow (Sobukola & Olatunde, 2011). Therefore, with strategic planning, management and the development of standard operating procedures (SOP's) in cold chain systems, hygiene and processing (salting, drying and smoking), the potential to grow the snoek processing sector in South Africa is enormous (Britz presentation)

Fish are a nutritive food source which contain high amino acid and polyunsaturated-fatty acid concentrations making it an overall healthier choice compared to traditionally consumed terrestrial animals such as goat, chicken or beef (Ogundiran *et al.*, 2014). The high nutritional value and health benefits associated with fish have been recognised internationally and currently two servings of fish are recommended for consumption per week (García-Arias *et al.*, 2003). In spite of the nutritional and health benefits of fish it is highly perishable and prone to rapid quality loss (Immaculate *et al.*, 2013). Adding salt to fish replaces the water in the muscle, thus reducing the amount of moisture available for mould growth, as well as microbial and enzyme activities, which can cause spoilage characteristics such as off-flavour and odour (spoilage) (Gallart-Jornet *et al.*, 2007; Mujumdar & Law, 2010). In addition, salting and drying of fish can prolong shelf life thereby ensuring availability throughout the year (Tawari & Abowei, 2011) whilst careful handling and prompt preservation (freezing, salting and drying) can reduce the fish perishability, increase shelf life, prevent wastage and reduce economic losses (Darvishi *et al.*, 2013). Despite the introduction of cold storage units, salted and dried fish has remained popular in developed and developing countries largely due to the characteristic flavour and texture which develop during processing (Guizani *et al.*, 2008; Aas *et al.*, 2010). However, the salting and drying process is more of a necessity in developing countries due to limited cold storage facilities available to the general public (Reza *et al.*, 2009).

The salting and drying techniques employed can vary between the producers (Chukwu & Mohammed, 2009) where improper processing can result in poor quality fish products which are undesirable and unsafe for consumption (Guizani *et al.*, 2008). Prolonged drying time and the inability to control salt concentration and its even distribution may favour microbial growth and subsequently product degradation (Wang *et al.*, 2011), whilst exposure to direct sunlight could lead to loss of light sensitive nutrients (photo oxidation) such as thiamin, niacin, and riboflavin resulting in off-flavour (Perera, 2005). In addition, where fish is open air dried contamination by insects, rodents and dust is common and can result in a lack of uniformity in product quality (Kituu *et al.*, 2010; Wang *et al.*, 2011). Within the Western Cape province, South Africa, salted dried snoek is processed by deheading, and cutting along the spine to remove the gut. The gutted fish is rinsed with water to remove blood debris and dirt. The fillet is fleshed into three pieces which is salted overnight with a handful

of salt for a whole fish as there is no standard procedure. The salted fish is left to dry out in the sun and wind until it is dried.

Snoek forms an important part of the daily diet of coastal communities in the Western Cape province, South Africa (Isaacs, 2013). However, the quality of snoek can vary post catch due to inconsistencies in handling, storage and preservation techniques. Whilst some vendors may take care in handling and processing their produce, others may employ substandard strategies which can lead to a lesser quality salted dried fish. Determining the quality of dried snoek sold by vendors will enhance our understanding of what the current standard is in this unregulated market. Therefore, the main aim of this study was to establish the quality of salted dried snoek sold by road side vendors in the Western Cape province.

3.2. Materials and methods

3.2.1. Sampling

Salted dried snoek were purchased from three spatially distinct vendors within the Western Cape province [Strand (A and B), Philippi and Saldanha; Fig. 3.1]. Ten fish were sampled from Strand A, Strand B and Philippi while five fish were sampled from Saldanha, due to scarcity of dried snoek at the sampling time. Strand A and Strand B samples were attained from the same vendor where fish were collected three months apart (Table 3.1). Due to the temporal difference in sampling Strand A and Strand B, both batches were deemed distinct batches and are hereafter referred as separate vendors. Therefore, a total of four sites/vendors were sampled. The sampling dates and number of fish sampled per site is presented in Table 3.1. All fish were wrapped in clean plastic bags and placed in plastic crates during transportation. The fish were cut into two equal parts along the line of symmetry. Each half section was homogenised, vacuum parked and stored at -18 ⁰C until analysed.

Table 3.1 Sampling site, sampling date and number of snoek (*T. atun*) sampled within the Western Cape province, South Africa

Sample site	Date collected	Number of fish
Strand A	11th September, 2013	10
Strand B	18th December. 2013	10
Phillipi	27th November, 2013	10
Saldanha	12th December, 2013	5

Figure 3.1 Map outlining the sampling sites [Phillipi, Strand (A & B) and Saldanha] located within the Western Cape province, South Africa.

All homogenised samples were thawed at 4ºC over 24 hours prior to chemical analyses. A total of ten fresh snoek were also assessed for proximate composition to facilitate a comparison between locally sold fresh and salted dried snoek. The fresh samples were obtained from the Department of Agriculture, Forestry and Fisheries (DAFF) and were sourced locally (Phillipi) from a wholesale company.

3.2.2. Proximate analysis

Moisture content was measured using the AOAC method 934.01 where 2.5 g of the homogenised samples were dried at 100-105°C for 24 hours in a drying oven (Labcon drier, 334510) (AOAC, 2002a).

Total protein content was measured using the Dumas combustion method AOAC 992.15 (AOAC, 2002b) where 0.15 g of defatted, dried and ground fish sample was encapsulated in a Leco[TM] foil sheet and analysed in a Leco Nitrogen/Protein Analyser (FP-528, LECO Corporation, 3000 Lake View Avenue, St. Joseph, MI 49085-2396, USA). The Leco analyser was calibrated using ethylene-diamine-tetra-acetic acid (EDTA Part number

502-092) before each batch of samples was analysed. The accuracy and recovery rate of the method was ensured by running a calibration sample of EDTA after every 10 samples. The nitrogen content was multiplied by 6.25 to calculate the protein concentration in the sample.

The total lipid content was determined using the chloroform/methanol extraction gravimetric method as described by Lee *et al.* (1996). The present study used 2:1 (v/v) chloroform/methanol due to the high fat content (>5%) in the dried snoek (Lee *et al.*, 1996) as observed in a proximate composition pilot study. Five gram (± 0.02) of the homogenised snoek sample was mixed with 50 mL chloroform/methanol using a Bamix stick for 1minute where after the mixture was filtered through Whatman no. 1 filter paper into a separation funnel. Twenty mL of 0.5% NaCl was added to the filtrate and shaken gently, repeatedly. The mixture was allowed to stand for 30-60 minutes, until separation was clearly visible. The bottom layer was collected into an Erlenmeyer flask, from which 5 mL was pipetted into a weighed glass fat beaker. The fat beaker was placed onto a sand plate (setting 4) for 45 minutes to enable chloroform/methanol evaporation. The beaker was subsequently placed in a desiccator and allowed to cool for 30 minutes. The beaker was weighed after cooling.

The ash content was determined using the official AOAC, 942.05 (AOAC, 2002c) method where 2.5 g of the snoek samples were dried for 24 hours at 100°C, and subsequently ashed in a furnace at 500°C for 6 hours.

3.2.3. Sodium chloride analysis

Sodium chloride concentration was determined by titration using Mohr's method (Mol *et al.*, 2010). Five gram (± 0.02) of homogenised sample was diluted with 250 mL of distilled water. The mixture was heated in a water bath at 80°C for 1 hours, and filtered using Whatman no. 1 filter paper. Of the filtrate, 25 mL was mixed with 5% potassium chromate (K_2CrO_4) indicator (1 mL) and titrated with 0.1 N silver nitrate ($AgNO_3$) (Mol *et al.*, 2010).

3.2.4. Biochemical Analysis

3.2.4.1. Lipid oxidation

Lipid oxidation was determined using the thiobarbituric acid method (TBARS) (Gatellier *et al.*, 2001; Fernandez-Lopez *et al.*, 2008): Snoek fish sample of 1 g ± 0.02 was weighed using a chemical weighing balance (Radwag model AS 220/C/2 made in Poland) with ± 0.005 accuracy. To this, 10 mL of 0.15 M KCl buffer (1.12 g potassium chloride (KCl) + 0.002 g butylated hdroxytoluene (BHT) in 100 mL distilled water) was added. The mixtures were homogenized for 20 sec (Lasec homogeniser, Polytron PT 2500E), and 0.5 mL was pipetted into marked test tubes: To each sample, 0.25 mL of TBA (1% (w/v) TBA (2-thiobarbituric acid) and 50 mM NaOH (sodium hydroxide)) and 0.25 mL of TCA and (2.8%

(w/v) TCA (Trichloracetic acid)) was added. A standard solution range of 0 uM to 20 µM TMP (1,1,3,3-tetramethoxypropane) was made with distilled water. To 0.5 mL from each of the standard solutions, 0.25 mL TBA and 0.25 mL TCA were added. All test tubes were vortexed (standard and samples), and placed in a boiling water bath for 10 min. Two mL n-butanol was added to each standard and samples. The tubes were capped and vortexed and centrifuged for 30 min at 4ºC at 4 000 rpm. Then 1 mL of supernatant was pipetted into a cuvette and the absorbance was measured at 532 nm (CECIL, CE2021, 2000 series spectrophotometer supplied by LASEC SA). Concentration of TBARS was calculated using 1,1,3,3-tetramethoxypropane (0–8 µM) as standard (Gatellier *et al.*, 2001).

3.2.4.2. Histamine analysis

Histamine content was analysed using a RIDASCREEN® histamine competitive enzyme-linked immunosorbant assay (ELISA) kit (Art. No. R1601, supplied by AEC Amersham, Cape Town, South Africa) for the extraction and quantification of histamine in all snoek meat samples, in accordance with the instructions of the manufacturer. The ELISA kit has a detection limit of < 2.5 ppm and a range of quantification of 2.5-250.0 ppm for histamine in fresh and frozen fish products. According to the manufacturer the antibodies utilised in the kit are reported to exhibit 100% specificity to histamine.

3.2.4.3. Water activity

A Pawkit water activity meter (AquaLab Decagon devices Inc.) was used to measure the water activity of the snoek (Mol *et al.*, 2010). The meter has an accuracy of ± 0.02 a_w. Seven grams of the homogenised sample was used per reading.

3.2.4.4. Microbiological analysis

Microbiological quality of the fish samples was evaluated by quantifying the total aerobic mesophiles and screening for *Salmonella* spp. and *Escherichia coli*, respectively. One gram (± 0.02) of the homogenized dried snoek sample was aseptically weighed into 9 mL autoclaved physiological solution (PS) (9 g NaCl in 1 L distilled water) in test tubes and vortexed with sterile glass beads. Further serial dilutions (3 fold) were prepared using 1 mL of dilutions into 9 mL of PS. To enumerate the microbial load, 1 mL of aliquots of the dilutions were plated in triplicate onto appropriate media using the pour plate method.

Aerobic mesophilic bacteria were enumerated using the plate count method (Mahale *et al.*, 2008). Samples were plated on nutrient agar, and plates were incubated at 32°C for 24 hours. *E. coli* was detected using the ISO 4831:2006 method (Amado *et al.*, 2013). Isolation of *E. coli* was done using violet red bile lactose agar (VRBLA). The plates were allowed to set and incubated at 37°C for 24 hours. Confirmation of presence of *E. coli* was done using brilliant green bile broth (BGLB). *Salmonella* was enumerated using the

Horizontal method for the detection of *Salmonella* spp (ISO 6579:2003; Amado *et al*., 2013). Pre-enrichment in non-selective liquid medium was done using buffered peptone water (BPW).

Buffered peptone water was inoculated at ambient temperature with the test portion, and incubated at 37 ± 1°C for 18 ± 2 hours. A 0.1 mL sample of the culture obtained in pre-enriched non selective medium was transferred to a tube containing 10 mL of the Rappaport-Vassiliadis medium with soya (RVS) broth. Another 1 mL of the culture was also transferred to a tube containing 10 mL of Muller-Kauffman tetrathionate/novobiocin (MKTTn) broth. The RVS broth was incubated at 41.5 ± 1°C for 24 ± 3 hours and the inoculated MKTTn broth at 37 ± 1°C for 24 ± 3 hours. After incubation, using the culture obtained in the RVS broth, Xylose lysine deoxycholate (XLD) agar was inoculated by means of a loop, and this was repeated for the second selective agar: *Salmonella shigella* (SS) agar using a sterile loop as above. After incubation for 24 ± 3 hours, using the culture obtained in the MKTTn broth, the procedure was repeated with the two selective plating-out media. After incubation for 24 ± 3 hours, the plates were examined for the presence of typical colonies of *Salmonella.* Typical colonies of *Salmonella* grown on XLD agar have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator. They are colourless with a black centre on SS agar. Typical colonies of *Salmonella* and *Escherichia coli* were not detected.

3.2.5 Statistical analysis

All chemical and biochemical analyses were conducted in duplicate except microbial analysis which was carried out in triplicate. Statistical analysis of data was carried out using SAS software, Version 9. 3 (SAS System for Windows). General Linear Models (GLM) using one way analysis of variance (ANOVA) were employed to determine the level of significance in thiobarbituric acid reactive substances (TBARS), water activity, salt concentration, histamine and proximate composition of snoek sampled from four vendors in the Cape Peninsular region. Coefficient of variation was determined to evaluate the variability of the parameters tested. Where significant differences were found, a Bonferonni test was used to determine where the differences were.   Pearson correlation was used to determine relationships between the parameters tested. Boxplots were generated using Minitab 16 statistical software (O'Neill *et al*., 2011).

3.3. Results

3.3.1. Proximate composition

The proximate composition of fresh snoek sample (moisture, protein, lipid and ash) was 77.0%, 22.0%, 1.0% and 1.1%, respectively (Table 3.2). Lipid component of the fresh snoek

showed the greatest variation which is common in meat muscle. The proximate composition (protein, lipid, moisture and ash) of dried snoek varied considerably between the four vendors. The mean moisture, protein, lipid and ash content of all samples were 42.7%, 32.7%, 11.4% and 14.7%, respectively with a coefficient of variation (CV) of 6.28, 13.85, 27.36 and 12.86, respectively (Table 3.3). An inverse relationship (Pearson's correlation coefficient (r) of 0.795; $p < 0.01$) was observed between moisture and fat content of the dried snoek from the four vendors (Fig. 3.2, Table 3.4). This is particularly evident in fish sampled from Phillipi which had significantly lower ($p < 0.05$) percentage moisture and higher ($p < 0.05$) percentage lipid content compared to the other three vendors. Fish from both Strand vendors (A & B) had similar ($p \geq 0.05$) moisture and lipid contents.

The protein content of the dried snoek from Saldanha and Strand A were higher ($p < 0.05$) and lower ($p < 0.05$) respectively than fish from the other three vendors. It was evident that protein content between both Strand sites (A & B) differed ($p < 0.05$). Dried fish sampled from Saldanha and Strand A had the highest and lowest percentage ash respectively compared to the other vendors. However, this difference was not significant. From an observation of Figure 3.2 it is apparent that protein and ash followed similar trends for all four vendors analysed as high protein and ash content was found in Phillipi and Saldanha while protein and ash content was low in the Strand vendors. A summary of the proximate composition results for all vendors is presented in Figure 3.3.
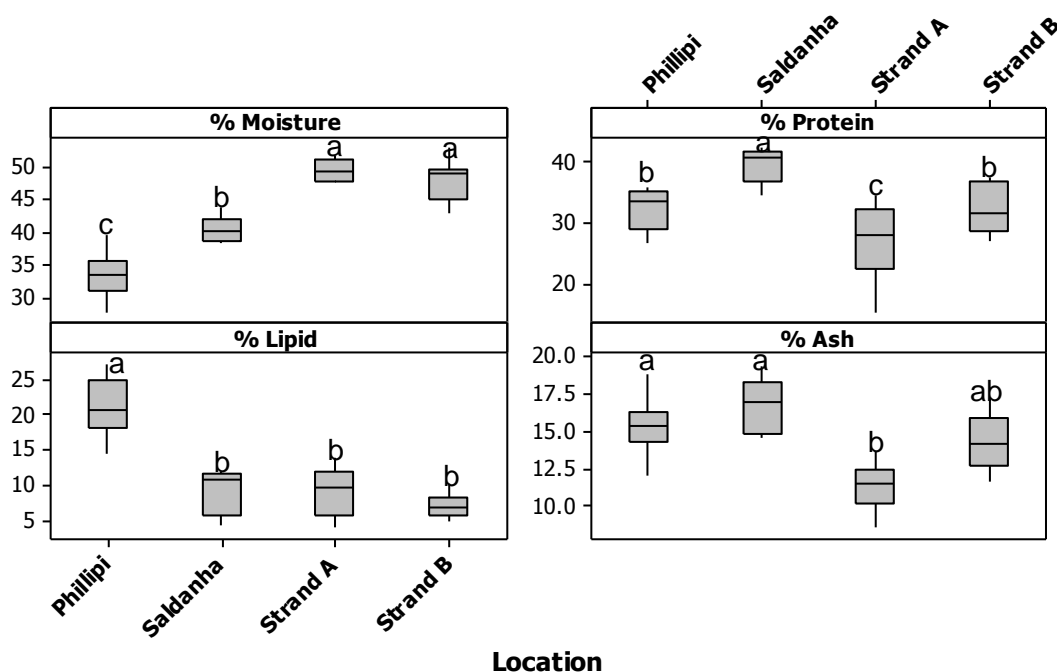
Figure 3.2 Variation in the proximate composition of dried snoek (*T. atun*) sampled from four different vendors within the Western Cape, South Africa. [a,b,c] Different letters indicate

significant differences ($p < 0.05$). The boxes represent the interquartile ranges; the whiskers (vertical lines) indicate values outside the interquartile range; the horizontal line denotes the median value.

Table 3.2 Summary (%) of the proximate composition of fresh snoek (*T. atun*)

Parameters	Mean ± Stdev	Minimum	Maximum	Coefficient of Variation
Moisture	77.0 ± 1.31	74.1	78.9	1.7
Protein	22 0 ± 1.60	19.9	24.7	7.2
Lipid	1.0 ± 0.18	0.7	1.3	18.8
Ash	1.1 ± 0.08	1.0	1.2	7.1

The results are the average value of ten replicates ± standard deviation

Table 3.3 Summary (%) of the proximate composition ± standard deviation of salted and dried snoek (*T. atun*) sampled from four vendors within the Western cape, South Africa (n=35).

Proximate composition	Strand (A)	Strand (B)	Phillipi	Saldanha	Mean	Min	Max	CV
Moisture	$49.3^a \pm 1.48$	$47.9^a \pm 3.07$	$33.4^c \pm 0.01$	$40.2^b \pm 2.09$	42.7 ± 7.38	27.6	51.7	6.28
Protein	$26.7^c \pm 6.01$	$32.3^b \pm 3.84$	$32.3^b \pm 3.41$	$39.4^a \pm 3.01$	32.7 ± 5.18	15.2	42.4	13.85
Lipid	$8.9^b \pm 3.53$	$7.0^b \pm 1.70$	$21.0^a \pm 3.39$	$9.0^b \pm 3.33$	11.4 ± 6.41	3.8	27.4	27.36
Ash	$11.3^b \pm 1.50$	$14.3^{ab} \pm 1.94$	$16.5^a \pm 3.95$	$16.6^a \pm 1.95$	14.7 ± 2.49	8.5	27.5	12.86

CV = Coefficient of variation, [a,b,c] Different letters within a row indicate significant differences ($p < 0.05$)

Min. = Minimum, Max. = Maximum

3.3.2. Biochemical composition

The salt, TBARS and water activity content ranged between 6.73-8.87%, 9.58-19.83 mg MDA.Kg^{-1}, and 0.75-0.85, respectively. A summary of the biochemical composition of the dried snoek sampled from the four vendors in Western Cape is outlined in Figure 3.3.

Overall the histamine content in the samples was lower than the detectable limit of 2.5 ppm of the ELISA kit used. Therefore histamine level was not statistically analysed. Significant variation in salt, TBARS and water activity was detected between the vendors sampled. An inverse relationship was also observed between salt and water activity of the dried fish at each of the four vendors ($r = - 0.566$, $p < 0.01$) (Table 3.4): the vendor which had low % salt tended to have high a water activity (Fig. 3.3). A negative and significant correlation was also observed between moisture content and salt content of the snoek sampled from the different vendors ($r = - 0.056$, p < 0.01) (Table 3.4).

Fish sampled from Phillipi had the highest salt concentration which was significantly higher ($p < 0.05$) than that of the fish sampled from Strand A; the latter had the lowest salt concentration of all the vendors. In addition, Phillipi and Strand A had significantly lower ($p < 0.05$) and higher ($p < 0.05$) water activity, respectively, when compared to the other sites (increased salt resulted in decreased water activity). Although both Strand vendors did not differ greatly ($p \geq 0.05$) in the % salt present, a difference ($p < 0.05$) in water activity was noted. No difference ($p > 0.05$) was observed between the water activity of fish from Saldanha and Strand B. Additionally, the TBARS content of dried fish from Saldanha was significantly (p ≤ 0.05) lower than the others. No other significant differences (p ≤ 0.05) in TBARS were evident.
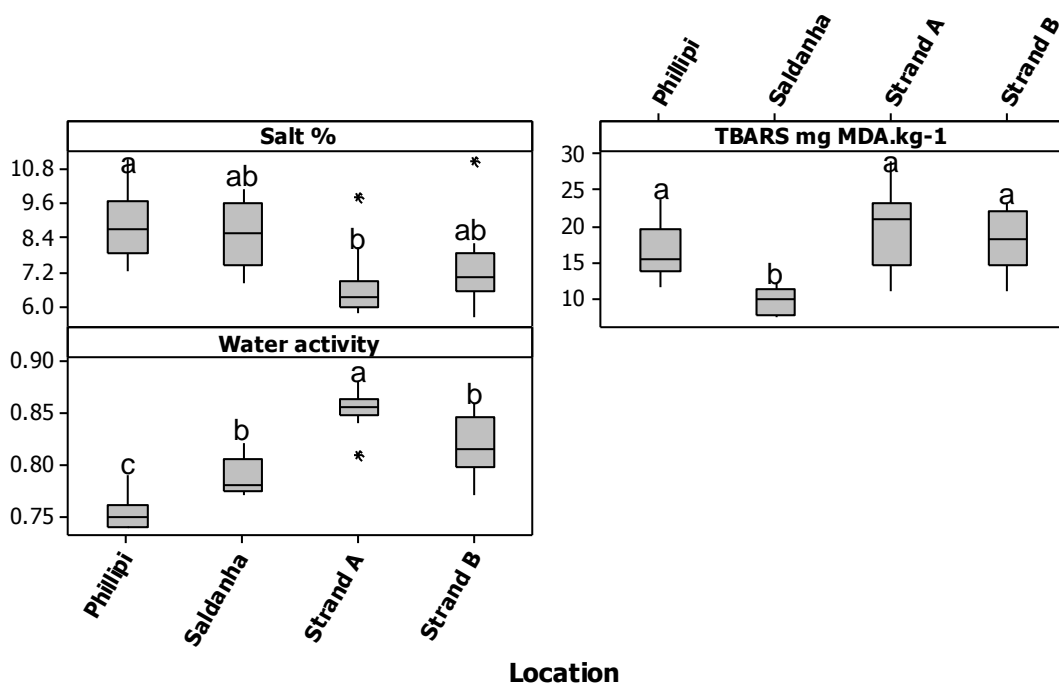
Figure 3.3 Variation in the biochemical composition (Salt (%), TBARS (mgMDA.kg^{-1}), water activity) of dried snoek (*T. atun*) sampled from different locations within the Western Cape, South Africa. [a,b,c] Different letters indicate significant differences ($p < 0.05$). The boxes represent the interquartile ranges; the whiskers (vertical lines) indicate values outside the interquartile range; the horizontal line denotes the median value.

A number of significant correlations ($p < 0.05$) were observed between the six measured variables (moisture, protein, lipid, ash, salt, TBARS and water activity) (Table 3.4).

Table 3.4 Summary of correlation analysis (proximate and biochemical) of salted dried snoek sampled from different vendors in the Western Cape (n=35)

Variable	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Salt (%)	TBARS (mg MDA.kg^{-1})	Water activity
Moisture (%)		**-0.426**	**-0.795**	**-0.568**	**-0.558**	0.272	**0.847**
Protein			-0.047	**0.358**	**0.451**	**-0.503**	**-0.350**
Lipid				0.210	0.257	0.051	**-0.623**
Ash					**0.575**	**-0.337**	**-0.688**
Salt (%)						-0.284	**-0.566**
TBARS(mg MDA/kg)							**0.387**
Water activity							

Bold numerals represent significant correlation coefficients at $p < 0.05$

A positive correlation was observed between moisture content and water activity, salt and ash content. However, negative correlation was observed between moisture and ash (r = -0.568), moisture and lipid (r = -0.795), moisture and salt (r = -0.558), protein and TBARS (r = -0.503), lipid and water activity (r = -0.623), ash and water activity (r = -0.688) and salt and water activity (r = -0.566)

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3.3.3. Total bacterial count and presence of Salmonella and Escherichia coli

No statistical differences ($p \geq 0.05$) were found in the microbial count of the snoek sampled from the four vendors (Figure 3.4). Dried fish from Phillipi had the highest microbial count at 3.39 ± 0.09 log CFU.g^{-1} sample while the dried fish from Strand A had the lowest count at 3.12 ± 0.11 log CFU.g^{-1} sample. *Salmonella* and *Escherichia coli* were not detected in any of the vendors.
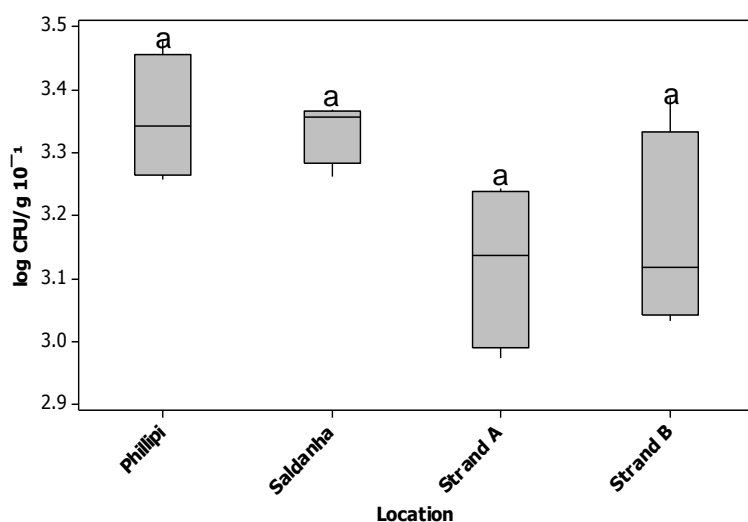
Figure 3.4. Total plate count in salted dried snoek from four vendors (n=4 samples per retail outlet). [a] No differences (p>0.05). The boxes represent the interquartile ranges; the whiskers (vertical lines) indicate values outside the interquartile range; the horizontal line denotes the median value.

3.4. Discussion

3.4.1. Proximate composition

Considerable variation was observed in the proximate composition of the salted dried snoek obtained from different vendors in the Western Cape. The variation could be as a result of different processing methods employed by the different producers whilst variability in processing techniques within producers was also identified (Strand A and Strand B). The moisture content of the dried snoek from all four vendors was lower (43.1%) compared to the fresh snoek analysed ($77.0 \pm 1.31\%$) in the current study. The reduced moisture content was due to the salting and drying process which resulted in dehydration and therefore moisture loss. The moisture content of the fish analysed in the current study was above the 25% recommend moisture content for salted dried fish (Nooralabettu, 2008) and therefore is considered too high to prevent microbiological and chemical degradation over prolonged periods of time (Immaculate *et al.*, 2013). Nooralabettu, (2008) reported that salted dried fish with moisture content < 25% can have an extended shelf life of up to several months. Therefore, moisture loss during salting and drying is crucial to storage stability of dried

products. Longer drying time or enhanced drying techniques is required if the vendors are to reduce the moisture content of their product to < 25%.

The greatest variation observed in the lipid content of the fresh snoek analysed could be due to feed intake, seasonal variation, sexual maturity, migratory behaviour and feeding cycles (Deka *et al.*, 2012). The protein (33.4-49.3%) and lipid (7.0-21.0%) content of the salted dried snoek was high when compared to the fresh equivalent (protein 22.0 ± 1.60% and lipid 1.0 ± 0.18%) This increase is due to the salting and drying process where moisture is removed and dehydration occurs, resulting in a concentration of other nutrients such as protein and lipid (Chukwu & Mohammed, 2009; Hwang *et al.*, 2012). This relationship between moisture, protein and lipid was reflected in the correlation analysis where both protein and lipid increased significantly with a decrease in moisture (Table 3.4). Similar negative relationships have been documented for a number of other fish species such as shark and catfish (Chukwu & Mohammed, 2009; Selmi *et al.*, 2010)**.**

The homogeneity of moisture and lipid content in fish sampled from Strand A and B may be due to similar processing strategies, as both A and B samples were sampled from the same vendor at different time points (three months apart). An informal discussion with the vendors indicated that they vary their processing techniques (salt concentration and drying time) depending on a number of factors (size of fish, quality of flesh, weather conditions etc) where these technique variations are based on experience.

Ash content, which gives an estimate of the mineral composition as well as the amount of residual salt of the fish products was considerably high (11.31-16.62%) in all four vendors and is generally found to range between 0.6-1.5% in fresh fish products (Opara *et al.*, 2004). The high ash content present in the current study could be attributed to the size of fish, the presence of salt and potentially bone fragments which may have not been removed from the snoek samples during initial lab processing (Selmi *et al.*, 2010).

The salt content of the dried salted snoek from the four vendors (6.73-8.88%) is considerably lower than that reported for numerous other dried fishes (15-25%) (Wang, 2011) and is likely due to the uncontrolled and unregulated salting and drying process in the artisanal South African snoek fishery as well as high moisture content observed in the snoek from the various vendors. When excess salt is added to fish products the salt can form a crust on the fish surface reducing the drying rate of the product (Mujaffar & Sankat, 2005; Guizani *et al.*, 2008); therefore, applying low salt concentration to fish products can facilitate faster drying. However, this was not apparent in this study and fish which contained low salt concentrations were dried to a suboptimal level, which may be due to consumer preference driven processing, where the consumers prefer a less dried product. The addition of high salt concentrations lowers moisture and thus extends storage stability by preventing microbial spoilage (Guizani *et al.*, 2008; Hwang *et al.*, 2012). However, the snoek sampled

within the current study had both low salt concentrations and high moisture content indicating the potential for early spoilage and therefore short shelf life. This is a major concern for consumers whom wish to store the snoek for prolonged periods of time.

3.4.2. Biochemical composition

The level of lipid oxidation (TBARS) of snoek from each of the four vendor sites (9.58-19.83 mg MDA.kg^{-1}) were higher than the 5-8 mg MDA.kg^{-1} limit characteristic of a good quality product (Kanatt *et al.*, 2006; Kilic, 2009; Halamícková & Malota, 2010; Hwang *et al.*, 2012). TBARS concentration is used as a quality marker in fish to determine the presence of secondary oxidation products such as malondialdehyde (MDA), the end product of lipid oxidation (Hwang *et al.*, 2012). The salting method, exposure to direct sunlight and high ambient temperature during salting, drying and storage can accelerate the oxidation processes in fish muscles and subsequently increasing the TBARS. When fish is exposed to high temperature and/or sunlight the polyunsaturated fatty acids in the muscle oxidize, which can result in changes in colour (brown), taste (off-flavour) and high TBARS leading to the development of rancidity (Pazos *et al.*, 2010; Maestre *et al.*, 2011; Utrera & Estévez, 2013). Kilic (2009) found that TBARS were high in fish dried at high temperatures and low at low temperatures. Salt can also aid the oxidation process (pro-oxidant) in fish where the level of salt related oxidation depends on the quantity present. High salt concentration and a dry salting method can accelerate lipid oxidation in fish (Yanar *et al.*, 2006; Gheisari & Motamedi, 2010).

The dried snoek sampled in this current study had relatively high water activities (0.75-0.85) which were above the recommended ranges (≤ 0.6) necessary for extending the shelf life of dried fish (Aberoumand, 2010). Therefore the snoek analysed cannot be classified as a 'dried' product but rather as semi-dried. Nonetheless, it is suggested that the major variation between vendors (including Strand samples) was predominantly due to the non-standardized processing procedures employed by the vendors where drying conditions and salt concentrations likely varied resulting in products of varying quality. In addition, consumer preference (saltiness and dryness of snoek) may vary from area to area which may have resulted in the high variability observed in the vendors examined.

A positive strong correlation was detected between % moisture and water activity of the vendors which is a common phenomenon in the drying process where water activity decreases as the moisture is removed (Toldrá, 2006; Goula *et al.*, 2008). Although dried fish can generally be stored at ambient temperatures for a prolonged period of time this does not apply to dried snoek products with high water activity (present study) as high water activity under ambient temperature conditions can facilitate the growth of spoilage organisms and subsequent spoilage and deterioration of the product (Quek *et al.*, 2007; Tsironi *et al.*, 2009).

Such deterioration can reduce the shelf life and the product can potentially be harmful to consumers. Therefore, fish containing high water activity should be further dried ($a_w \leq 0.6$) to reduce the water activity of the product (Abbas *et al.*, 2009; Aberoumand, 2010). When extended drying is not feasible, the product should be kept in cold storage, thereby, reducing spoilage and pathogen development. The overall high water activity of the products tested is a cause for concern as consumers may not be able to keep the fish products stable and safe to eat over prolonged storage periods without further processing.

The low histamine level (< detectable limit of 2.5 ppm) of the sampled snoek indicates the relative safety of the product and lack of spoilage at the time of product testing (Yesudhason *et al.*, 2013). Histamine is a biogenic amine formed in fish muscle as a result of amino acid (histidine) decarboxylation by the activities of some spoilage microorganisms such as *Vibrio*, *Photobacterium, Klebsiella, Morganella* and others at temperature > 0ºC (Auerswald *et al.*, 2006; Mateˇjkova *et al.,* 2013). The presence of histamine is therefore used as an indication of spoilage in fish. Consumption of fish with high histamine content can cause food poisoning which can result in headache, sweating, nausea, skin rash, stomach pain, vomiting, diarrhoea, dizziness, swelling of the tongue and face (Emborg *et al.*, 2005; Hungerford, 2010). The infection is usually mild and disappears between 8-12 hours except in patients with a history of atopy who are likely to suffer severe histamine poisoning which may be prolonged (Diaz & Hu, 2009; Wilson *et al.*, 2012). Severity of infection depends on the dose ingested and varies from individual to individual. Nonetheless, no death from histamine poison has been recorded (Diaz & Hu, 2009). Various fish species such as tuna, mackerel and marlin have been implicated in histamine poisoning (Tsai *et al.*, 2006) however, to date, no case of snoek related histamine poisoning has been reported (Auerswald *et al.*, 2006). Hwang *et al.* (2012) found that the addition of salt prior to drying inhibits the growth of histamine forming bacteria which may account for the low histamine level in the salted dried snoek samples. Although the salt content was low in the samples it is suggested that it was nonetheless sufficient to limit histamine levels at least at this early stage of storage. Therefore, salting fish and maintaining low temperature storage prior to drying can reduce histamine development as it can prevent microbial growth resulting in a safer product for human consumption.

3.4.3. Microbiological analysis

The microbial load observed in the dried fish products was lower than the recommended level of 5 log CFU.g^{-1} at 37°C for dried fish (Surendran *et al.*, 2006) while none of the two pathogens tested (*Escherichia coli* and *Salmonella spp.*) were detected. These results are in contrast to numerous studies which reported high microbial loads in salted dried fish (Immaculate *et al.*, 2013; Hwang *et al*, 2012; Saritha *et al.*, 2012). This variation in microbial

loads between the studies may be due to a number of factors such as; the salt concentration, the hygiene of the workers, the microbial population present at time of harvesting and/or variable harvesting and processing techniques. Although the salt content of the fish was low it may have been sufficient to limit microbial spoilage at least on a temporary basis as salting above 6% can inactivate some of the spoilage organisms (bacteria) and all vendors in this study were above 6% (Fig. 3.3) (Hwang *et al*., 2012).

The microbial population of fish represents the bacterial load present in the aquatic (marine environment) and processing environment (handling and storage) and can affect the post mortem bacterial load of salted dried fish. If the surrounding water or processing chain is contaminated with faecal material it will be reflected in the microbial load of the fish. Although the pathogens present in the environment cannot be controlled, those in the processing chain can be, where pathogens such as *Salmonella* and *Escherichia coli* can contaminate fish during handling and processing due to improper and unhygienic practices (Sifuna *et al*., 2009). The absence of *E. coli*, *Salmonella* and the low microbial count reported in this study, suggests absence of faecal contamination.

3.5. Conclusion

Substantial variation was observed in the proximate, biochemical composition and microbiological safety of the salted dried snoek between vendors in the Cape Peninsula area. This variation reflects inconsistencies in processing procedures between processors/vendors. The TBARS and water activity of fish from all four vendors was higher than the levels recommended to ensure minimal microbial growth and oxidation which could make the product unsafe. Given the adverse effects of consuming products with high TBARS and high potential for spoilage these results are worrying. Therefore, improved drying strategies, handling and packaging which is cost effective is suggested to increase the quality of the locally produced dried snoek.

The low histamine levels, low salt content, the proximate composition and absence of pathogens suggests that spoilage had not yet occurred and that the salted dried snoek sold in Western Cape province were of good quality and safe for consumption. Nonetheless, early spoilage is inevitable due to the low salt content present and high water activity of the samples. An experiment examining the rate of spoilage of salted dried snoek over time is recommended to identify the storage stability of these locally produced products. This would determine how long these products can be stored before they become spoiled. The results of the current study suggest that better standardisation of processing is required and that monitoring of these products is necessary in order to ensure the products are safe for consumption at all times.

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Chapter 4: Effects of salting and drying on water activity, moisture loss and salt content of Cape snoek (*Thyrsites atun*)

Abstract

The effect of various salt concentrations (saturated, 20% w/w and unsalted), temperature (30 & 40 °C) and relative humidity (40 & 50%) on water activity, moisture loss and weight loss of snoek portions during salting and drying were investigated. Moisture loss was high in snoek portions treated with saturated salt concentration compared to those treated with 20% (w/w) during the salting process. Progressive moisture loss, weight loss and reduced water activity were observed for all treatments examined. Salting, temperature and relative humidity of the drying environment had a significant effect on water activity and moisture loss of snoek during drying. The unsalted snoek portions had significantly higher moisture loss, lower weight and higher water activity than either of the two salted groups (20% w/w and saturated). Salting prior to drying considerably influenced the salt content, final water activity and weight of snoek.

4.1. Introduction

Salting is one of the oldest methods for preserving perishable meats such as marine and freshwater fish species (Sobukola & Olatunde, 2011). There are a number of different salting strategies (brining, dry salting and pickling) which are used depending on the specific product and the desired outcome. Although the main purpose of salting is preservation, salting is also practiced due to the desired organoleptic qualities it produces (Boudhrioua *et al.*, 2009). Salting has a bacteriostatic and dehydrating effect which helps to lower water activity and moisture content consequently, preventing microbial growth and other biochemical reactions which can lead to spoilage, thereby, improving the shelf life of fish (Goulas & Kontominas, 2005; Chaijan, 2011; Albarracín *et al.*, 2011; Agustinelli *et al.*, 2014). In addition, salt also helps to enhance flavour and improve textural characteristics of meat products by increasing the binding properties of protein (Desmond, 2006). During the salting process; water and soluble proteins diffuse out of the cell membranes as salt is absorbed (Gallart-Jornet *et al.*, 2007; Ibitwar *et al.*, 2008; Czerner & Yeannes, 2010). The rate of salt diffusion is influenced by many factors including muscle composition, salt concentration, temperature of the solute, salting method, contact time, size of the fish, weight, fillet thickness, level of agitation, fish to solute ratio, fat content, fish species, freshness, rigor mortis state and fish shape (Barat *et al.*, 2006; Gallart-Jornet *et al.,* 2007; Czerner & Yeannes, 2010).

Although the drying behaviour and final characteristics of fish is greatly influenced by salting (Sobukola & Olatunde, 2011), salting alone is inadequate for extending the shelf life

of salted fish, therefore, further processing methods such as drying are necessary (Albarracín *et al.*, 2011). Drying like salting also achieves preservation of fish by reducing the water content and water activity of the products and is achieved traditionally by exposing the fish to direct sunlight (Reza *et al.*, 2009; Darvishi *et al.*, 2013). This can take several days depending on the intensity of sunlight and the relative humidity of the environment. The limitation of this process is that non-uniform and poor quality products may be produced due to exposure to potential environmental contamination sources such as pests, insects, sand, microbial contamination and degradation (Wang *et al.*, 2013). Although insecticides can limit such infections, the insecticide itself can also cause contamination (Reza *et al.*, 2009). In addition, variable weather conditions can result in an extended drying period, uncontrollable processing condition and unstandardized processing procedures (Ali *et al.*, 2011). However, the use of different dying techniques such as vacuum freeze drying, solar and microwave drying have been developed to mitigate these problems and enable the drying process to be appropriately monitored and controlled resulting in consistently good quality fish products (Reza *et al.*, 2009).

Water activity is a useful indicator of the stability of dried food products and represents the energy state of water in a system (Abbas *et al.*, 2009). Water activity measures the amount of water available for microbial activities while moisture content describes only the overall quantity of water composition in the product (Quek *et al.*, 2007; Al-Harahsheh *et al.*, 2009). In addition, a non-linear relationship exists between water activity and moisture content of a food (Abbas *et al.*, 2009) where the relationship is dependent on the relative humidity and temperature of the material and is represented by sorption isotherms. Food products with water activity < 0.6 are generally referred to as microbiologically safe where spoilage only occurs as a result of chemical activities (Quek *et al.*, 2007; Aberoumand, 2010).

Salting and drying can significantly affect fish meat quality, product yield and shelf life of various marine and freshwater species (Goulas & Kontominas, 2005; Guizani *et al.*, 2008; Sobukola & Olatunde, 2011). However, only limited information is available on the effects of salting and drying on snoek meat quality. Given the socio-economic importance of the South African snoek fishery, this knowledge gap is considered substantial. Therefore, identifying and measuring parameters which indicate snoek quality such as spoilage is advantageous. This study aimed to evaluate the effect of salting and drying at different salt concentrations and drying conditions (°C and relative humidity), on moisture loss, water activity and the final salt content of snoek portions; which are valuable indicators of spoilage and storage stability.

4.2. Materials and methods

4.2.1. Raw material and salting pre-treatment

Whole fresh snoek (n = 54) were purchased from a snoek wholesale outlet at Phillipi, Western Cape province, South Africa. All fish were topped and tailed, the guts were removed whilst the skin was kept on the fish. The gutted fish were washed with clean water and cut into two equal parts along the line of symmetry. Each half was further divided into 3 portions and weighed individually (with an average weight of 130.76 ± 31.5 g). A group of 108 portions (n = 18 fish) were salted to saturation, 108 portions (n = 18 fish) were salted at 20% (w/w) and 108 portions (n = 18 fish) were unsalted (0%). Salting was carried out using the dry salting method. Saturated salting was done by burying the fish portions in excess coarse food grade salt. Salt was added excessively in between the fish to avoid fish to fish contact and the entire fish stack was covered with salt while the 20% salting was achieved by salting the snoek portions with salt equivalent to 20% of the weight. The brine produced by the salting was drained. The dry salting procedure was carried out at 4°C for 24 hours as suggested by Gallart-Jornet *et al.* (2007). All the fish portions were taken out of the salting medium for weight measurement every 3 hours (n = 8 sampling periods) which was used in the calculation of % weight loss. The fish portions were blotted dry using ply sheets in order to remove excess moisture and salt from the surface before weighing. Post salting, the fish portions were rinsed with clean fresh water to remove excess salt and were placed on racks at 4°C for 3 hours to allow excess water to drain before being weighed again. Weight loss (%) after salting was calculated using the following equation:

$$\frac{Initial\ weight - Final\ weight}{Initial\ weight} * 100$$

Fish water activity and moisture content of each fish portion were only examined at the beginning (time 0 hour) and end (time 24 hours) of the salting process.

4.2.2. Drying experiments

Fish drying was carried out for 72 hours using a convection dryer (AIRMASTER® 1200-25002, Reich, UK) which operates utilising the vertical air circulation principle. The air damper controls the inlet and outlet of air while relative humidity (RH) is measured psychrometrically. The air velocity in the drier was measured using a digital anemometer (Alnomar velometer AVM440 TSI incorporated, Shoreview MN 55126 USA) and averaged at 1.5 m s^{-1}. Each of the three groups (saturation, 20% and 0% salt) was each subdivided into three treatments where relative humidity (40 & 50% RH) and temperature (30 & 40°C) were altered. A summary of the various treatments is outlined in Table 4.1 below.

The fish portions were placed on the oven racks, skin down and sampling for each group (saturation, 20% and 0%) was done at random. Prior to placing on the oven racks, all

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the fish portions were individually weighed and marked and the individual weight change, moisture content and water activity were determined. Weight change is the actual weight of the fish portions sampled at each sampling point. Once sampled the fish portions were eliminated from the experiment. Moisture loss was determined from the moisture content data using the following equation:

$$\frac{Initial\ Moisture\ content - Final\ moisture\ content}{Initial\ moisture\ content} * 100$$

Table 4.1 Summary of the salting and drying treatments (n = 9 treatments) of Cape snoek

Treatment		Duration of drying (h)
Salt concentration (%)	**Temp (°C)-Relative humidity (%)**	
Saturated	30-40	72
Saturated	40-40	72
Saturated	40-50	72
20	30-40	72
20	40-40	72
20	40-50	72
0	30-40	72
0	40-40	72
0	40-50	72

Moisture content was measured using the AOAC method (934.01). For each group (% salt) and treatment (RH and °C), approximately 2.5 g of the homogenised fish samples were dried at 100°C for 24 hours in a drier (Labcon drier, 334510) (AOAC, 2002; Bosch *et al.,* 2013)

A Pawkit water activity meter (AquaLab Decagon devices Inc. Version: October 15, 2013 08:15:20), which has an accuracy of ± 0.02 a_w, was used to measure the water activity of the fish portions (Mol *et al.*, 2010). Approximately 7 g of the homogenised sample was used for water activity analysis.

Salt analysis was carried out using Mohr's method where 5 g (± 0.02) of each homogenised sample was diluted with 250 mL of distilled water. The mixture was heated in a water bath at 80°C for 1 hour, and filtered using Whatman no. 1 filter paper. Approximately 25 mL of filtrate was mixed with 5% potassium chromate (K_2CrO_4) indicator and titrated with 0.1 N silver nitrate ($AgNO_3$) (Mol *et al.*, 2010). All chemical analyses were conducted in duplicate.

4.2.3. Statistical analysis

Statistical analysis was conducted using SAS software (Version 9.3) for Windows unless otherwise stated. A General Linear Model procedure (GLM) was used for the factorial analysis of the data, incorporating salt concentration, temperature, relative humidity and their interactions in the model. The contribution of the interaction was calculated and where it is

high (> 30%) care is required when interpreting the main effects. Moisture loss, water activity, weight and salt concentration of salted dried snoek portions were dependent variables. Where significant differences were found, a Bonferonni test was used to determine pairwise comparisons. Time was not included as a main factor in the GLM as these samples were independent, i.e. no repeated measurement was taken for each fish portion at each sampling occasion, and portions were discarded post sampling. Therefore, statistical analysis was done separately for each sampling time. Although regression analysis would have been useful for estimating and predicting the effect of salting and drying conditions over time on moisture loss, water activity, weight and final salt content of dried snoek, it was not feasible in the current study as different portions were measured at the different sampling times.

Statistica 11 software was used to analyse weight loss between 20% salted and saturated salted snoek samples during salting only (one-way ANOVA); where differences were found a Bonferonni Post hoc test was used to identify where the differences were. The relationship between water activity and portion weight during drying was determined using a Pearson correlation using Statistica 11 software and the confidence interval was set at 95%.

4.3. Results

4.3.1. Moisture content, water activity and weight loss of snoek portions during the salting process

Moisture content and muscle weight loss varied ($p < 0.05$) between the 20% and saturated salted portions subsequent to the salting process. The saturated salted portions lost significantly ($p < 0.05$) more weight than the fish portions salted at 20% (Fig. 4.1). The average moisture content was 60% and 66%, while weight loss post salting was 17% and 8% for snoek portions salted to saturation and 20%, respectively. In addition, those portions salted to saturation had significantly lower ($p < 0.05$) water activity (average = 0.82) compared to the 20% salted portions (average = 0.88).
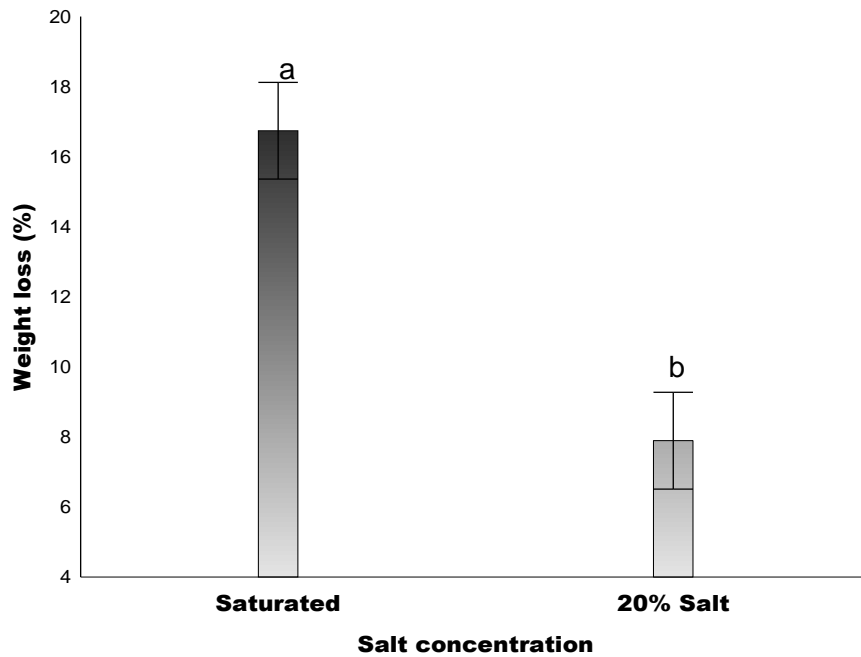
Figure 4.1 Variation in weight loss of salted snoek portions during the salting process. Different letters ([a,b]) denote where there are significant differences.

4.3.2. Moisture loss during drying

Moisture loss at time 0 for unsalted portions was not included in the statistical analysis as there was no moisture loss in that group at that time point. Moisture loss increased as the drying process proceeded for all the salt groups (0%, 20% and saturated) and treatments (30°C/40% RH, 40°C/40% RH and 40°C/50% RH) (Fig. 4.2). However, variation in moisture loss between treatments was observed at various sampling time points. At time 0 20% salted samples had 9.3. 9.2 and 5.6% moisture loss while the saturated salted samples had 15.5, 17.2 and 16.6%. Snoek portion salted at 20% and dried at 40°C/50% RH had moisture loss lower ($p < 0.05$) than the others in within the group and from the saturated salted group while there was no difference ($p \geq 0.05$) within the saturated group which were higher ($p < 0.05$) than the 20% salted. The difference in moisture loss at time 0 for the salted portions was as a result of the moisture loss that occurred due to the two brining treatments and probably due to different portions of the fish sampled.

A factorial ANOVA assessed the variation between all treatments analysed (n=9). Significant statistical differences ($p < 0.05$) were observed between 20% salted portions dried at 40°C/50% RH and other treatments within the same group and also between the 20% salted treatments dried at 40°C/50% RH and the saturated treatments. However, no significant difference ($p \geq 0.05$) was observed between the three treatments which were salted to saturation.

Although not consistently significant, the highest moisture loss was found in unsalted portions dried at 40°C/40% RH (time 12, 24, 36 and 60 hours) and 40°C/50% RH (48 and 72

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hours). Concurrently, the lowest moisture loss was observed in the saturated portions (except for time 0, 12 & 24 hours). The average moisture loss at the end of the experiment (Time 72) was 55.5, 42.0, and 41.5% for the unsalted, saturated and 20% salted groups, respectively. Due to the high inter-treatment (n=9 treatments) variability at each sampling point no additional trends for specific treatments were apparent. A summary of the ANOVA results is present in Table 4.2.

The influence of various treatments and their interaction on moisture loss at different stages of drying was examined and a summary of the result (including pairwise comparison) is presented in Table 4.3. The GLM identified similar trends as observed in the ANOVA analysis. Salt concentration had the most significant influence on moisture loss ($p < 0.001$) except at 12 hours. The GLM showed a specific trend in moisture loss where the unsalted portions were > 20% salted portions > saturated salted portions, throughout the drying experiment. The effect of temperature and relative humidity was significant ($p < 0.01$) over the dying period except at time 24 and 48 hours; where significant, the 40°C/40% RH treatment had the highest moisture loss. The effect of Salt*Temp-RH on moisture loss was significant at 36, 48 and 60 hours ($p < 0.001$). The interaction contribution was calculated for all significant interactions. The contribution was considered high for three of the sampling times and accounted for 34.7%, 31.5%, and 35.3% of the total variation (75%, 77% and 86%) at 36, 48 and 60 hours respectively (Table 4.3).

Table 4.2 Moisture loss, water activity and weight change (mean ± standard deviation) of snoek portions (salted to saturation, 20% salted and unsalted) during drying at different temperature and relative humidity over time (0-72 hours)

Time (h)	Treatment (Temperature/Relative humidity, salt concentration)								
	30°C/40% RH, 0% salt	40°C/40% RH, 0% salt	40°C/50% RH, 0% salt	30°C/40% RH, 20% salt	40°C/40% RH, 20% salt	40°C/50% RH, 20% salt	30°C/40% RH, Sat	40°C/40% RH, Sat	40°C/50% RH, Sat
Moisture loss (%)									
0				$9.3^b \pm 1.59$	$9.2^b \pm 1.23$	$5.6^c \pm 0.71$	$15.5^a \pm 2.05$	$17.2^a \pm 0.89$	$16.6^a \pm 0.96$
12	$13.3^b \pm 5.29$	$24.9^a \pm 7.85$	$19.1^{ab} \pm 2.53$	$16.6^{ab} \pm 1.59$	$22.5^a \pm 7.80$	$22.8^a \pm 2.71$	$20.8^{ab} \pm 3.34$	$18.4^{ab} \pm 3.65$	$19.0^{ab} \pm 2.35$
24	$30.3^{ab} \pm 2.56$	$39.1^a \pm 5.21$	$32.0^{ab} \pm 6.37$	$25.9^b \pm 3.26$	$26.6^{ab} \pm 0.98$	$34.1^{ab} \pm 9.36$	$25.3^b \pm 9.81$	$26.0^{ab} \pm 5.32$	$29.2^{ab} \pm 5.02$
36	$33.3^{bc} \pm 4.99$	$49.2^a \pm 6.47$	$45.1^{ab} \pm 9.40$	$30.8^{cd} \pm 5.64$	$32.9^{bc} \pm 3.74$	$35.8^{abc} \pm 4.75$	$38.5^{abc} \pm 9.47$	$30.1^{cd} \pm 5.53$	$18.2^d \pm 1.76$
48	$38.8^{bcd} \pm 7.54$	$50.1^{ab} \pm 9.05$	$51.4^a \pm 4.91$	$31.9^{cd} \pm 4.75$	$38.9^{bcd} \pm 1.15$	$39.2^{abcd} \pm 5.22$	$44.6^{abc} \pm 8.04$	$35.9^{cd} \pm 5.70$	$27.6^d \pm 2.71$
60	$44.2^{cb} \pm 4.78$	$57.7^a \pm 3.61$	$56.4^a \pm 7.81$	$36.9^{cd} \pm 5.81$	$41.9^{cd} \pm 1.92$	$43.1^{cb} \pm 3.77$	$48.2^{ab} \pm 4.51$	$40.5^{cb} \pm 5.79$	$28.8^d \pm 2.35$
72	$47.8^b \pm 1.98$	$59.2^a \pm 3.50$	$59.6^a \pm 6.30$	$38.3^{bc} \pm 5.07$	$40.1^{bc} \pm 6.00$	$46.3^b \pm 5.04$	$46.7^b \pm 5.55$	$47.0^b \pm 2.91$	$32.2^c \pm 1.97$
Water activity									
0				$0.9^b \pm 0.01$	$0.9^{ab} \pm 0.02$	$0.9^a \pm 0.00$	$0.8^c \pm 0.01$	$0.8^c \pm 0.01^c$	$0.8^c \pm 0.01$
12	$0.9^a \pm 0.01$	$0.9^a \pm 0.02$	$0.9^a \pm 0.01$	$0.8^{bc} \pm 0.02$	$0.8^{bc} \pm 0.06$	$0.8^b \pm 0.00$	$0.7^c \pm 0.00$	$0.7^c \pm 0.02^c$	$0.7^c \pm 0.02$
24	$0.9^a \pm 0.01$	$0.9^a \pm 0.03$	$0.9^a \pm 0.03$	$0.7^b \pm 0.03$	$0.8^b \pm 0.03$	$0.7^b \pm 0.06$	$0.7^b \pm 0.00$	$0.7^b \pm 0.01^b$	$0.7^b \pm 0.01$
36	$0.9^a \pm 0.01$	$0.8^b \pm 0.09$	$0.9^b \pm 0.07$	$0.7^b \pm 0.01$	$0.7^c \pm 0.03$	$0.7^c \pm 0.00$	$0.7^c \pm 0.02$	$0.7^c \pm 0.01^c$	$0.7^c \pm 0.00$
48	$0.9^a \pm 0.02$	$0.8^{ab} \pm 0.13$	$0.8^{ab} \pm 0.06$	$0.7^{bc} \pm 0.01$	$0.7^c \pm 0.05$	$0.7^{bc} \pm 0.02$	$0.7^c \pm 0.07$	$0.7^{bc} \pm 0.04^{bc}$	$0.7^{bc} \pm 0.01$
60	$0.9^a \pm 0.03$	$0.7^{bc} \pm 0.08$	$0.7^b \pm 0.13$	$0.7^{bc} \pm 0.01$	$0.7^{bc} \pm 0.01$	$0.7^{bc} \pm 0.02$	$0.6^c \pm 0.08$	$0.7^{bc} \pm 0.03^{bc}$	$0.7^{bc} \pm 0.02$
72	$0.8^a \pm 0.05$	$0.7^b \pm 0.07$	$0.7^b \pm 0.15$	$0.7^b \pm 0.01$	$0.7^b \pm 0.03$	$0.7^b \pm 0.04$	$0.6^b \pm 0.08$	$0.6^b \pm 0.04^b$	$0.7^b \pm 0.01$
Weight change (g)									
0	$154.2^{ab} \pm 47.31$	$135.2^{ab} \pm 4.32$	$156.7^{ab} \pm 1.70$	$121.6^b \pm 22.73$	$123.5^b \pm 8.34$	$118.3^b \pm 15.58$	$115.8^b \pm 20.94$	$168.1^{ab} \pm 27.60$	$198.2^a \pm 9.82$
12	$112.0^{ab} \pm 28.61$	$69.0^{ab} \pm 34.95$	$109.6^{ab} \pm 6.54$	$115.7^{ab} \pm 40.91$	$87.4^{ab} \pm 4.13$	$92.2^{ba} \pm 20.83$	$106.0^{ab} \pm 40.48$	$131.3^{ab} \pm 20.04$	$144.3^{ab} \pm 38.32$
24	$64.8^{ab} \pm 25.58$	$47.3^{ab} \pm 21.66$	$86.8^{ab} \pm 1.38$	$87.8^{ab} \pm 27.18$	$81.2^{ab} \pm 7.56$	$78.9^{ba} \pm 27.41$	$81.0^{ab} \pm 34.07$	$116.7^a \pm 40.83$	$123.1^a \pm 20.68$
36	$75.1^{bc} \pm 24.36$	$56.5^c \pm 17.16$	$57.6^c \pm 29.35$	$95.0^{abc} \pm 31.35$	$86.8^{abc} \pm 6.95$	$94.5^{bac} \pm 29.10$	$82.9^{abc} \pm 27.16$	$116.8^{ab} \pm 53.09$	$139.4^a \pm 32.16$
48	$77.5^b \pm 35.16$	$53.4^b \pm 21.63$	$56.3^b \pm 14.69$	$93.9^{ab} \pm 35.60$	$76.7^b \pm 15.51$	$74.3^b \pm 9.16$	$75.2^b \pm 18.35$	$109.3^{ab} \pm 50.75$	$138.7^a \pm 34.20$
60	$54.7^{bc} \pm 12.70$	$46.6^c \pm 17.29$	$62.2^{bc} \pm 2.46$	$91.6^{abc} \pm 20.19$	$108.3^{ab} \pm 3.60$	$72.8^{bc} \pm 20.64$	$80.7^{abc} \pm 32.74$	$99.0^{abc} \pm 5.27$	$134.9^a \pm 27.98$
72	$57.0^b \pm 16.46$	$46.7^b \pm 17.12$	$39.2^b \pm 13.31$	$87.6^{ab} \pm 20.74$	$87.2^{ab} \pm 0.12$	$75.0^b \pm 18.56$	$67.7^b \pm 28.08$	$74.7^b \pm 40.33$	$131.4^a \pm 26.34$

$P < 0.05$, [a,b,c,d] Different letters indicate where there are significant differences within each respective group (0%, 20% and Sat)

Sat = Saturated salt concentration

Table 4.3 Summary of GLM where the effect of salt, temperature and relative humidity and their interaction on moisture loss of snoek (salted to saturation, 20% salted and unsalted) was assessed during a 72 hour drying process

Time	Treatment	P-value	Pairwise comparison	Contribution of interaction (%)	Coefficient of determination (R^2)
0	Salt	<.001	S > 20%		
	Temp-RH	0.006	40°C/40 RH > 40°C/50 RH		
	Salt*temp-RH	0.002	S 40°C/50 RH, S 30°C/40 > 20% 30°C/40 RH, 20% 40°C/40 RH > 20% 40°C/50 RH	4.58	0.95
12	Salt	0.522	NS		
	Temp-RH	0.004	40°C/40 RH, > 30°C/40 RH		
	Salt*temp-RH	0.005	0% 40°C/40 RH, 20% 40°C/50, 20% 40°C/40 RH, > 0% 30°C/40	20.8	0.66
24	Salt	0.009	0% > 20%, S		
	Temp-RH	0.106	NS		
	Salt*temp-RH	0.101	NS	13.5	0.53
36	Salt	<.001	0% > 20% > S		
	Temp-RH	0.160	NS		
	Salt*temp-RH	<.001	0% 40°C/40 RH, 0% 40°C/50 RH> 20% 40°C/40 RH, > 0% 30°C/40 RH, 20% 40°C/40 RH > S 40°C/50 RH	34.72	0.75
48	Salt	<.001	0% > 20% > S		
	Temp-RH	0.263	NS		
	Salt*temp-RH	<.001	0% 40°C/50 RH > 20% 40°C/40 RH, 0% 30°C/40 RH > S 40°C/40, 20% 30°C/40RH, S 40°C/50	31.5	0.77
60	Salt	<.001	0% > 20%, S		
	Temp-RH	0.035	40°C/40 RH > 40°C/50 RH		
	Salt*temp-RH	<.001	0% 40°C/40 RH, 0% 40°C/50 > 0% 30°C/40 RH, 20% 40°C/50, 20% 40°C/40 RH, S 40°C/40 RH > S 40°C/50 RH	35.37	0.86
72	Salt	<.001	0% > 20%, S		
	Temp-RH	0.029	4°C/40 RH > 30°C/40 RH		
	Salt*temp-RH	<.001	0% 40°C/50 RH, 0% 40°C/40 > 0% 30°C/40 RH, S 40°C/40, S 30°C/40 RH, 20% 40°C/50 RH, 20% 40°C/40 RH, 20% 30°C/40 RH, > S 40 °C/50 RH	29.07	0.86

Temp-RH = Combination of temperature and relative humidity.
S = Saturated salted
0% = Unsalted
20% = 20% salted
NS = Not significant

Figure 4.2 Variation in cumulative moisture loss of all snoek portions (unsalted, 20% salted and salted to saturation) over the 72 hour drying period. Error bars represent standard deviation.

4.3.3. Water activity of snoek (unsalted, 20% salted and salted to saturation) during the drying process

The water activity decreased over the 72 hour drying period for all treatments (n = 9) at each of the seven sampling points (Table 4.2). A high, but not consistently significant water activity was observed in the unsalted portions compared to the salted. The result of the factorial ANOVAs showed that the water activity of the unsalted snoek portions dried at 30°C/40% RH was significantly ($p < 0.05$) higher than all the salted treatments at every time point except at time 0 and 12 and also varied significantly between treatments within the same salt group at time 36, 60 & 72 hour. Simultaneously, a low but inconsistently significant water activity was observed in saturated portions dried at 30°C/40% RH throughout the experiment except at time 0. At the end of the experiment the average water activity was 0.64, 0.68 and 0.72 for saturated, 20% salted and unsalted snoek portions, respectively (Table 4.2).

A GLM identified significant variability ($p < 0.001$) between the three salt groups at each sampling time (Table 4.4). The unsalted portions had higher ($p < 0.001$) water activity than the salted treatments except at time 0, where 20% salted portion had higher water activity than the saturated salted. A summary of the pairwise comparison of the treatments is presented in Table 4.4. The Temp-RH treatment also had a significant effect ($p < 0.01$) on water activity at 36, 60 and 72 hours where 30°C/40% RH had the highest ($p < 0.05$) water activity (0.71) and 40°C/40% RH the lowest (0.65). Additionally, Salt*Temp–RH was significant ($p < 0.001$) at 60 and 72 hours and the interaction contributed 31.4 and 29.3% of the total variation (76 and 77%) (Table 4.4). In addition, moisture loss and water activity displayed a negative linear relationship ($r^2 = -0.99$), where moisture loss increased as water activity decreased (Fig 4.3 & 4.4).

Table 4.4 Summary of GLM where the effect of salt, temperature and relative humidity and their interaction on water activity of snoek (salted to saturation, 20% salted and unsalted) was assessed during a 72 hour drying process

Time	Treatment	P-value	Pairwise comparison	Contribution of interaction (%)	Coefficient of determination (R^2)
0	Salt	<.001	20% > S		
	Temp-RH	0.075	NS		
	Salt*temp-RH	0.002	20% > 20% 30°C/40 > S group	7.67	0.94
12	Salt	<.001	0% > 20% > S		
	Temp-RH	0.064	NS		
	Salt*temp-RH	0.280	NS	0.7	0.96
24	Salt	<.001	0% > 20% > S		
	Temp-RH	0.619	NS		
	Salt*temp-RH	0.019	0% group > 20% group, S group	2.02	0.96
36	Salt	<.001	0% > 20%, S		
	Temp-RH	0.004	30°C/40 RH > 40°C/50 RH, 40°C/40 RH		
	Salt*temp-RH	0.002	0% 30°C/40 RH > 0% 40°C/50, 40°C/40 RH > the other treatments	7.94	0.89
48	Salt	<.001	0% > 20%, S		
	Temp-RH	0.252	NS		
	Salt*temp-RH	0.010	0% 30°C/40 RH > S group, 20% group	11.32	0.79
60	Salt	<.001	0% > 20%, S		
	Temp-RH	0.028	30°C/40 RH > 40°C/50 RH, 40°C/40 RH		
	Salt*temp-RH	<.001	0% 30°C/40 RH > the other treatments > S 30°C/40 RH	31.43	0.76
72	Salt	0.004	0% > S		
	Temp-RH	0.010	30°C/40 RH > 40°C/40 RH		
	Salt*temp-RH	<.001	0% 30°C/40 RH > the other treatments	29.25	0.77

Temp-RH = Combination of temperature and relative humidity.
S = Saturated salted
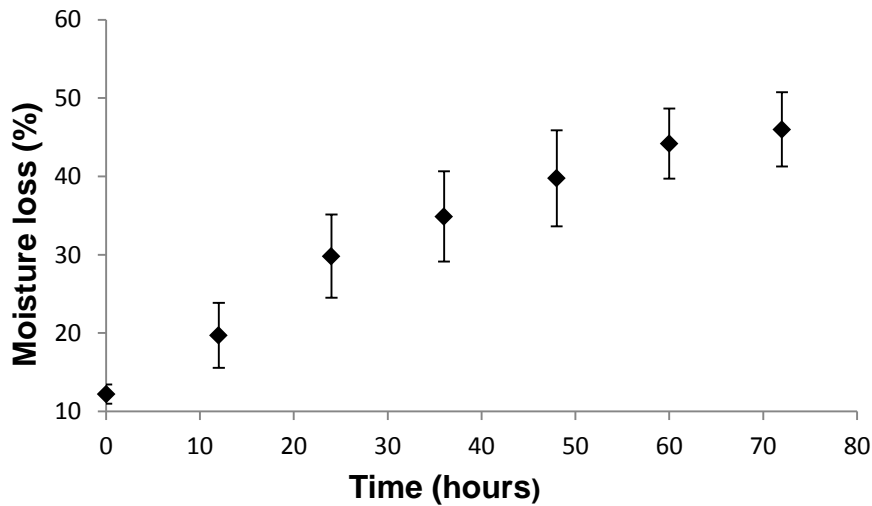0% = Unsalted
20% = 20% salted
NS = Not significant

Figure 4.3 Average water activity of snoek portions (salted to saturation, 20% salted and unsalted; n=63) over the experimental drying period (time 0-72 hours). The error bars represent standard deviation.
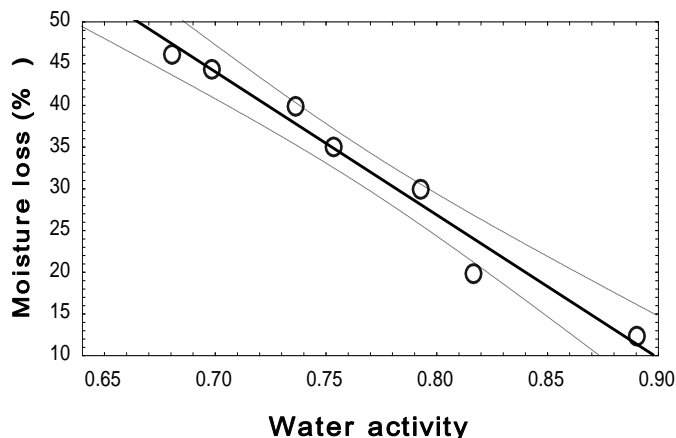
Figure 4.4 Relationship between water activity and moisture loss of snoek portions (salted to saturation, 20% salted and unsalted; n=63) over the experimental drying period (time 0-72 hours).

4.3.4. The effect of drying on weight of salted and unsalted snoek

A progressive decrease in portion weight was observed for all the groups and treatments as drying progressed from 0 to 72 hours. The result of the factorial ANOVA showed that the unsalted portions dried at 40°C/40% RH had the lowest weight (excluding time 0) and saturated salt portions dried at 40°C /50% RH had the highest weight compared to the other treatments; however these differences were not constantly significant. At times 36, 48, 60 and 72 hours, the saturated salted portions dried at 40°C/50% were significantly heavier than treatments within the unsalted treatments. The weight of the fish portions at 72 hours were on average 47.6, 83.3 and 91.3 g for unsalted, 20% salted and the saturated salted snoek portions.

The result of the GLM showed similar trend as indicated by the ANOVA results. Salt concentration had a significant ($p < 0.001$) effect on weight of snoek portions during drying (Table 4.5). The saturated salted portions had significantly higher weight than the 20% and unsalted throughout the experiment. Temperature and relative humidity had no significant effect on weight ($p \geq 0.05$) except at time 0 while Salt*temp-RH had a significant ($p < 0.01$) effect on weight at 0, 48 and 72 hours where the Salt*temp-RH contributed one third of the total variation (Table 4.5). A summary of the pairwise comparisons for all treatments is presented in Table 4.5.

Table 4.5 Summary of GLM where the effect of salt, temperature and relative humidity and their interaction on weight of snoek (salted to saturation, 20% salted and unsalted) was assessed during a 72 hour drying process

Time	Treatment	P-value	Pairwise comparison	Contribution of interaction (%)	Coefficient of determination (R^2)
0	Salt	0.001	S > 20%		
	Temp-RH	0.024	40°C/50% RH > 30°C/40% RH		
	Salt*temp-RH	0.003	S 40°C/50% > the other treatments	23.38	0.65
12	Salt	0.012	S > 20%, 0%		
	Temp-RH	0.167	NS		
	Salt*temp-RH	0.053	NS	13.88	0.61
24	Salt	0.001	S > 20%, 0%		
	Temp-RH	0.147	NS		
	Salt*temp-RH	0.079	NS	12.49	0.6
36	Salt	<.001	S, 20% > 0%		
	Temp-RH	0.354	NS		
	Salt*temp-RH	0.031	S 40°C/50% RH > 0%	12.79	0.69
48	Salt	0.003	S > 20%, 0%		
	Temp-RH	0.572	NS		
	Salt*temp-RH	0.008	S 40°C/50% RH > all treatments except S 40°C/40% RH, 20% 30°C/40% RH	20.21	0.64
60	Salt	<.001	S , 20% > 0%		
	Temp-RH	0.290	NS		
	Salt*temp-RH	0.011	S 40°C/50% RH > 0%, 20% 40°C/50% RH	17.6	0.67
72	Salt	<.001	S, 20% > 0%		
	Temp-RH	0.315	NS		
	Salt*temp-RH	0.002	S 40°C/50% RH >0%, S 40°C/40% RH, S 30°C/40% RH, 20% S 40°C/50% RH	23.95	0.66

Temp-RH = Combination of temperature and relative humidity.
S = Saturated salted
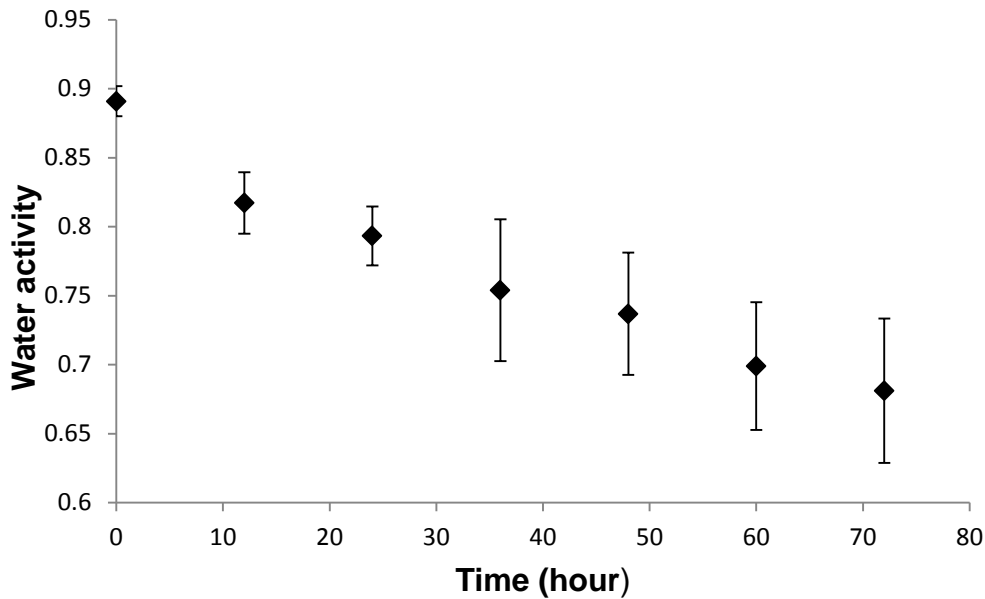0% = Unsalted
20% = 20% salted
NS = Not significant

4.3.5. Salt content of salted dried snoek

The salt content of the salted snoek was 23.6% for portions salted to saturation and 12.8% for those salted at 20%. The overall lowest salt content (11.8%) was observed in snoek portions salted at 20% and dried at 40°C/40% RH while the highest salt content (27.4%) was found in portions salted to saturation and dried at 40°C/40% RH (Table 4.6).

The one way ANOVA analysis found significant variation ($p < 0.05$) between the six salted variables at time 72 hours (Table 4.6). In addition, portions dried at 40°C/40% RH had higher ($p < 0.05$) salt contents than the portions dried at 40°C/50% RH within the saturated group. The salt concentration of portions salted at 20% did not differ significantly ($p \geq 0.05$).

Table 4.6 Variation in salt content of salted dried snoek portions (salted to saturation, 20% salted and unsalted) after a 72 h drying time (n = 6 per treatment)

	Treatments	
Salt concentration (%)	**Temperature/relative humidity**	**Mean ± standard deviation**
0	30/40	NA
	40/40	NA
	40/50	NA
20	30/40	$14.8^{cd} \pm 3.23$
	40/40	$11.8^{d} \pm 1.77$
	40/50	$12.8^{d} \pm 3.28$
Saturated	30/40	$23.5^{ab} \pm 4.81$
	40/40	$27.4^{a} \pm 8.03$
	40/50	$20.0^{cb} \pm 1.85$

$P < 0.05$, [a,b,c,d] Different letters indicate significant differences
NA = Not applicable

The GLM showed a similar trend as the ANOVA. The saturated salted portions had higher ($p < 0.001$) salt concentrations than the 20% salted group (Table 4.7). No differences ($p \geq 0.05$) were found for Temp-RH while a significant ($p \geq 0.01$) interaction effect (Salt*temp-RH) was identified; the calculated contribution of the interaction was 2.6%. The summary of the pairwise comparison analysis is presented in Table 4.7.

Table 4.7 Summary of GLM where the effect of salt, temperature and relative humidity and their interaction on salt content (salted to saturation and 20% salted) was assessed after a 72 hour drying process

Treatment	P-value	Pairwise comparison	Contribution of interaction (%)	Coefficient of determination (R^2)
Salt	<.001	S > 20%		
Temp-RH	0.0903	NS		
Salt*temp-RH	0.0119	S 40°C/40% RH > S 40°C/50% RH, 20% 30°C/40% RH > 20% 40°C/50% RH, 20% 40°C/40% RH	2.62	0.95

Temp-RH = Combination of temperature and relative humidity.
S = Saturated salted
0% = Unsalted
20% = 20% salted
NS = Not significant

4.4. Discussion

4.4.1. The salting process

The addition and concentration of salt can considerably affect protein stability/denaturation and subsequently the water retention capacity, moisture loss and the weight of fish meat (Nguyen *et al.*, 2011). Such an effect was observed in the current study where moisture loss increased and the water retention capacity and weight of fish decreased due to the addition of salt (Sahli *et al.*, 2009; Augustinelli *et al.*, 2014). Numerous studies found similar relationships between salt and weight loss (dehydration) in fish (Bellagha *et al.*, 2007; Gallart-Jornet *et al.*, 2007; Thorarinsdottir *et al.*, 2011). Low moisture content and water activity during salting are both desirable traits of salting process for extending shelf life of fish. Also salting is usually carried out as the first step in fish preservation. Salting is rarely done in isolation; since it is generally accompanied by drying (Wang *et al.*, 2011b).

4.4.2. The drying process

Drying is the traditional method mostly used in preservation of fish. It preserves fish by removing water and creating an environment which is unfavourable for microbial and chemical activities in fish products (Wang *et al.*, 2011a). Although moisture loss increased over the experimental drying period, which is desirable for extended shelf life of dried snoek, the rate of moisture loss for each treatment fluctuated substantially at each sampling point. The anatomical section analysed, size (volume to surface area ratio) of portion, contact with drying air, temperature, relative humidity and the salt concentration within the muscle of the snoek portions can all play a role in increased/reduced moisture loss in fish and could potentially explain the high variability observed (Guizani *et al.*, 2008).

Although reduced weight and high moisture loss was observed for the unsalted snoek portions, the final water activity was higher than those of the salted snoek. This is an indication that salted snoek will be able to resist microbial attack and other chemical reactions that can occur at high water activity. High moisture loss generally reflects low

water activity in both snoek, other fish species and shark fillets (Mujaffar & Sankat, 2006; Chaijan, 2011) dried under various conditions. Drying process should considerably remove moisture and subsequent decline in water activity for it to be effective as a preservative technique. This pattern between water activity and added salt content has been described for a number of other species such as dry salted tilapia (*Oreochromis niloticus*) (Chaijan, 2011), shark fillets (Mujaffar & Sankat, 2006), Tilapia (*Tilapia zilli*) (Ikrang *et al.*, 2014). The final water activity in the saturated treatment dried at 30°C/40% RH, and 40°C/40% RH (0.6) was adequate to prevent spoilage in the products as it was the same as the advised limit of 0.6 which is regarded as sufficient to prevent microbial growth (Quek *et al.*, 2007; Aberoumand, 2010). Further drying can be done especially for portions salted at 20% and portions salted to saturation and dried at 40°C/50% RH to further reduce the water activity to < 0.6.

Hinderance to moisture loss due to crust formation may have resulted in low weight loss observed in the saturated salted snoek especially the saturated salted dried at 40°C/50% RH during drying and the portions of the fish sampled at the different time point (Random sampling). The low weight of the unsalted fish portions may also be due to high moisture loss observed during the drying process. Low weight in dried fish is desirable for ease and reduced cost of transportation to the consumers. Although, salting can hinder drying process it is desirable for development of organoleptic characteristics and prolonged storability of fish products.

The salt content of snoek salted at 20% salt concentration was below the recommended level for salted dried fish (15-25%) while that of the saturated salted snoek was above the recommended level (Wang *et al.*, 2011a). The high salt content of saturated salted snoek portions could be as a result of salt molecules which replaced moisture during the salting process (Sobukola & Olatunde, 2011). The result of this investigation is similar to the high salt content reported for dry salted African catfish (*Clarias gariepinus*) Sobukola & Olatunde, 2011). Although, the Salt*Temp-RH on moisture loss, water activity and weight was significant at some time points during the experiment, its contribution can only be accounted for where it contributed considerably to the total variation. High salt content is desirable for extending shelf life of fish products but it must be done with caution as current trend in food processing requires the use of reduce salt content due to its health implication.

4.5. Conclusion

The result of this study demonstrated that variable drying and salting procedures significantly affected moisture loss, water activity and weight loss during salting and drying of snoek portions at different temperature and relative humidity combinations. Saturated salt concentration resulted in products with a high salt content above the recommended level while the use of 20% salt yielded products with a salt content within the recommended level for healthy salted dry fish. A higher weight yield was observed for 20% salted snoek portions

after salting which is desirable from an economic viewpoint. However, salting helped to achieve a final water activity lower than those of the unsalted snoek; which is ideal for the prolonged shelf life of dried fish products. This study suggests that snoek portions should be salted before drying in order to obtain effective water activity for extended shelf life of the products however, care should be taken not to salt above 20% as this will result in high salt content. Also, drying at 40°C/40% RH resulted in high moisture loss and low water activity. Therefore, salting of snoek at 20% and drying at 40°C/40% RH is suggested for a good quality product with low salt content, low water activity and high product yield. Further studies are required to determine the effect of salt concentration on the nutritional composition of snoek, lipid oxidation and the microbial load during and after drying and its effect on the storage stability of snoek portions.

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Chapter 5: Understanding the drying kinetics of salted and dried Snoek (*Thyrsites atun*)

Abstract

This study investigated the effects of oven drying conditions (temperature and relative humidity) on the drying characteristics (moisture content, drying rate and effective moisture diffisivity) of salted (20% w/w) and unsalted (control) snoek. Each group (salted and unsalted) was further subdivided into three drying treatments (30°C/40% RH; 40°C/40% RH; 40°C/50% RH). The moisture content, drying rate and effective moisture diffusivity was determined. A sharp initial decrease in moisture content was evident in both salted and unsalted snoek within 12 hours of experiment initiation which gradually tapered off. Drying rate and moisture content were significantly influenced by time, temperature, Relative humidity, salt concentration, and their interactions ($p \leq 0.05$), highlighting their individual and combined effect on snoek drying dynamics. Lower effective moisture diffusivity and drying rate were observed for salted snoek portions for all three treatments when compared to the unsalted portions emphasising the effect of salting on drying. A number of mathematical models [Lewis/Newton, Page, Henderson, Pabis and Asymptotic (Logarithmic)] were used to describe the drying characteristics of snoek where the optimal model was the Page due to its high coefficient of determination and low (0.9999) mean square error (6.6×10^{-6}).

5.1 Introduction

Fish drying is a popular method of fish preservation and is commonly used in tropical and sub-tropical countries where there is abundant solar heat necessary for reducing muscle moisture content (Jain & Pathare, 2007). Fresh fish contains approximately 80% water, 8-25% protein, 0.5-30% fat, and 0.6-1.5% minerals (Opara *et al*., 2004); where the high moisture content renders fish susceptible to microbial and chemical deterioration and spoilage, when not handled, stored and processed correctly (Bon *et al*., 2007; Jain & Pathare 2007). The reduction in moisture during the drying process can therefore enhance the shelf life of fish whilst simultaneously decreasing the fish muscle weight. Such a reduction in weight can lead to a considerable reduction in packaging and transportation cost (Vega-Gálvez *et al*., 2011). Prior to drying, fresh fish is routinely salted in order to enhance the stability of the product (Vega-Gálvez *et al.,* 2011). Fish salting also referred to as osmo-drying, reduces moisture content and can be done either by wet salting (immersion of fish in a hypertonic aqueous salt, sugar solutions or acids) or dry salting (sprinkling or burying the fish in salt) (Ibitwar *et al*., 2008; Albarracín *et al*., 2011).

The overall quality of salted and dried fish products depends on the processing standards employed such as the salting and drying techniques. Nooralabettu (2008) reported that sun dried fish sold at Tamilnadu and Maharashtra (India) were of low quality due to unhygienic handling such as dragging of catch along polluted beach, inadequate salting and improper drying of the products. Improper salting results in fungal and bacterial

contamination of fish which can render it unsafe for human consumption (Logesh *et al.*, 2012). In addition, the quality of traditional salted sun dried fish is dependent on the weather and can vary as a consequence (Jain & Pathare, 2005; Bellagha *et al.*, 2007; Wang *et al.*, 2011). Therefore, conventional fish and meat dryers have been developed and are extensively used in the food industry to improve production and standardise product quality (Reza *et al.*, 2009; Wang *et al.*, 2011). However, caution is required when oven drying fish where high temperatures can lead to the denaturation of various proteins, minerals and vitamins result in a lower nutritional quality (Wu & Mao, 2008).

During the drying process, hot air comes in contact with the surface of the fish, moisture diffuses out of the muscle to the surface and subsequently evaporates (Jain & Pathare, 2005; Bellagha *et al.*, 2007). Two basic phenomena take place during this drying process, the constant drying rate period and the falling drying rate period (Ghazanfari *et al.*, 2006; Bellagha *et al.*, 2007; Bon *et al.*, 2007). The constant drying rate period is the initial phase of the drying process where surface moisture evaporates at a high yet constant rate due to the initial high moisture content present (Bellagha *et al.*, 2007). The constant drying rate is mainly influenced by the external conditions (difference in temperature between the dry air and wet surface, external heat and mass transfer coefficients and surface area exposed to the dry air) while physiological and chemical nature of the product have negligible effects (Bon *et al.*, 2007; Srikiatden & Roberts, 2007). The falling drying rate follows the constant drying rate period and refers to the stage where slow moisture transfer occurs predominantly from the centre of the meat. The product is considered 'dried' in the falling drying rate period (Bellagha *et al.*, 2007). Moisture diffusivity measures the overall rate of movement/diffusion of moisture from the product to the external environment and is commonly used to measure the time taken for evaporation of moisture from the samples to occur (Zhao *et al.*, 2013).

As various drying conditions can result in variable product quality, understanding the efficiency of each drying strategy can be advantageous. Drying kinetics models is a useful mathematical tool used for assessing the efficiency of various drying method and can be utilised for method optimisation consequently resulting in superior product production and quality (Bon *et al.*, 2007; Lee & Kim, 2009). Drying kinetics is influenced by drying conditions such as temperature, relative humidity, air velocity and the nature of the material and moisture diffusivity (Wang *et al.*, 2011). Various mathematical models (Henderson-Pabis, Newton or Lewis, Page, Two-term model, Wang-Singh, Asymptotic (Logarithmic) model and others) have been developed and used to examine the drying kinetics of various fish species and agricultural products (Kaya *et al.*, 2007; Chong *et al.*, 2008; Boeri *et al.*, 2011; Wang *et al.*, 2011; Ngcobo *et al.*, 2013; Hubackova *et al.*, 2014) (Table 1). The choice of model depends on the variables (temperature, vapour pressure and moisture content) that may interfere with the drying process (Srikiatden *et al.,* 2011).

Table 5.1 Mathematical models that have been used to simulating the drying process of food products

Model name	Equation	Food product	Reference
Lewis/Newton	$M_R = exp(-kt)$	Quince (*Cydonia oblonga*) , Grape seed (*Vitis labrusca*)	Kaya *et al.* (2007); Roberts *et al.* (2008)
Page	$M_R = exp(-kt\ n)$	Sardine, cod (*Gadus morhua*), tilapia (*Oreochromis niloticus*), black grapes (*Vitis vinifera*), seaweed (*Himanthalia elongata*) , fermented sausage, red chili (*Capsicum annuum L.*), Cambodian fish species climbing (*Anabas testudineus*) perch, swamp eel (*Monopterus albus*), and walking catfish (*Clarias batrachus*)	Chong *et al.* (2008); Boeri *et al.* (2011); Yang *et al.* (2010); Djenboubi *et al.* (2009); Doymaz (2006); Fudholi *et al.* (2011); Ikonić, *et al.* (2012); Fudholi *et al.* (2013); Hubackova *et al.* (2014).
Henderson-Pabis	$M_R = aexp(-kt)$	Quince (*Cydonia oblonga*) , table grape (*cv. Regal Seedless*), big head carp (*Aristichthys nobilis*)	Kaya *et al.* (2007); Ngcobo *et al.* (2013); Duan *et al.* (2005)
Asymptotic (Logarithmic)	$M_R = aexp(-kt) + c$	Chelwa fish (*Cyprinus spp.*) and prawn (*Penaeus monodon*), chempedak (*Artocarpus integer*), onion (*Allium cepa L.*) , jumbo squid (*Dosidicus gigas*)	Jain & Pathare (2007); Chong *et al.* (2008); Djenboubi et al. (2009); Doymaz (2006); Mota *et al.* (2010); Vega-Gálvez *et al.* (2011)

M_R = Moisture ratio   *a, c* = Coefficient of drying models K = Drying coefficient
t = time   *n* = Exponential coefficient   *exp* = Exponential

Optimal and standardised salting and drying techniques of fish is necessary to ensure a constant high quality product which is safe for human consumption. However no current standard operating procedures are in place for salting and drying within the artisanal South African fishery and research on the drying characterises of snoek is limited. Therefore, the aim of this study was to determine the drying characteristics of snoek under various salting and drying conditions (temperature and relative humidity), assess the goodness of fit of four diverse mathematical models and evaluate the optimal drying scenario (salt %, temperature and relative humidity) of snoek.

5.2. Materials and methods

5.2.1. Raw material and sample preparation

Whole frozen snoek (n = 11) were purchased from a whole sale outlet at Phillipi, Western Cape province, South Africa. The whole fish samples were topped and tailed and the guts removed. The gutted fish were washed with clean water and cut into two equal parts along the line of symmetry. Each half was further divided into 3 portions with an average weight of 130.76 g (± 31.5), length 16.76 cm (± 2.9), width 10.2 cm (± 2.0) and thickness 1.68 cm (± 0.2). The thickness of the fish portions were measured using a Vernier calliper (list count - 0.01 mm). The fish portions were rectangular in shape. Dry salting method was used to salt 36 portions (n = 6 fish) at 20% of the total portion weight (Albarracín *et al.*, 2011; Oliveira *et*

al., 2012) and 30 portions (n = 5 fish) were unsalted (control). Where salting was done, salt was stacked extensively in between the fish to prevent fish to fish contact, and the entire fish pile was subsequently covered with salt. The brine produced by the salting was drained. The dry salting procedure was carried out at 4°C for 24 hours as was suggested by Gallart-Jornet *et al.* (2007). The fish portions were subsequently rinsed with clean fresh water to remove excess salt and were placed on racks at 4°C for 3 hours to allow excess water to drain.

5.2.2. Drying experiments

The fish portions were tagged and weighed prior to oven drying. The salted and unsalted fish portions were dried using a convection oven dryer (AIRMASTER® 1200-25002, Reich, UK). Three different drying treatments were employed for both the salted and unsalted portions; 30°C/40% RH, 40°C/40% RH and 40°C/50% RH, where 18 salted and 18 unsalted portions were used per treatment. The air velocity in the drying chamber was measured using digital anemometer (Alnomar velometer AVM440 TSI incorporated, Shoreview MN 55126 USA) and averaged 1.5 m s^{-1}. The fish portions were placed on the racks skin down. The inlet and outlet air was controlled by an air damper and RH was measured psychrometrically. The experiment was maintained until a constant weight for each treatment was reached (change in weight <1 g). Therefore, the duration of the experiment varied for each treatment. Sampling was carried out at 12 hours interval 0, 12, 24,.., and 168 hours and all fish portions were sampled and weighed on each sampling occasion. Subsequent to weighing the samples were returned to the oven. The drying rate (temporal loss in moisture) was determined by calculating the change in moisture content at consecutive time interval and is expressed as [(kg water (kg dry mass * h)$^{-1}$)]. Drying rate was measured in order to assess the quantity of moisture removed due to drying over the experimental duration.

Moisture content was measured using the AOAC method 934.01 (AOAC, International, 2002). For each drying condition approximately 2.5 g of the homogenised fish samples were dried at 100°C for 24 hours in a dry oven (Labcon drier, 334510). The AOAC moisture method was applied to all samples at time 0 and at 72 hours. The data collected from an experiment which ran concurrently with this experiment was used to determine the initial and moisture content at 72 hours as it was representative. The moisture content at each intermediate sampling time was assessed by calculating the change in portion weight at each sampling time.

Salt analysis was carried out using Mohr's method. Approximately 5 g (± 0.02) of homogenised sample was diluted with 250 mL of distilled water. The mixture was heated in a water bath at 80°C for 1 hours, and filtered using Whatman no. 1 filter paper. Approximately 25 mL of filtrate was mixed with 5% potassium chromate indicator and titrated with 0.1 N silver nitrate (Mol *et al.*, 2010).

5.2.3. Theoretical consideration

The change in moisture content was determined as the dimensionless moisture ratio expressed by equation (1)

$$M_R = \frac{M_t - M_e}{M_0 - M_e} \qquad (1)$$

M_R = Moisture ratio

M_t = Moisture content of sample at time t, (%)

M_0 = Initial moisture content, (%)

M_e = Equilibrium moisture content (%)

Moisture ratio can be simplified to $\frac{M_t}{M_0}$ rather than $\frac{M - M_e}{M_0 - M_e}$ because the dynamic equilibrium moisture content is very small compared to M_t and M_0 (Jain & Pathare, 2007; Wang *et al.*, 2011) and the RH was kept constant throughout the drying process. Moisture ratio represents the relationship between change in moisture of a sample at a particular time interval and the initial and final moisture content (Vega *et al.*, 2007). In order to determine the moisture content as a function of the drying time, various empirical models were investigated (Jain & Pathare, 2007). These included the Lewis/Newton, Page, Henderson and Pabis, and Asymptotic (Logarithmic) models. The acceptability of the models was determined by the coefficient of determination r^2 (the closer to one the better the fit) and mean standard errors (MSE).

5.2.4. Determination of the effective moisture diffusivity

When determining the effective moisture diffusivity, fish portions were assumed to be infinite slabs (rectangular in shape). The drying rate of the infinite slab was assessed using Fick's second law of diffusion (Crank, 1975).

$$M_R = \frac{M - M_e}{M_0 - M_e} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \, exp\left(\frac{-(2n+1)^2 D_{eff} \pi^2}{4L^2} . t\right) \qquad (2)$$

Where L = Half thickness of fish cuts (m)

D_{eff} = Effective moisture diffusivity (m^{2}s^{-1})

t = Drying time(s)

For extended drying time the first term in the series expansion of equation (2) could be used as in equation 3 (Kaymak-Ertekin, 2002):

$$M_R = \frac{8}{\pi^2} \, exp\left(\frac{\pi^2 . D_{eff}}{4L^2} . t\right) \qquad (3)$$

When converting equation 3 to straight line equation it becomes

$$\ln M_R = \ln\left(\frac{8}{\pi^2}\right) - \frac{\pi^2 . D_{eff}}{4L^2} . t \qquad (4)$$

D_{eff} values were calculated as the slope of a straight line graph obtained when $\ln M_R$ is plotted against time (Doymaz, 2006).

5.2.5. Statistical analysis

Mathematical models were analysed using Microsoft Excel's solver analytical tool (Microsoft Office 2010, WC, USA). A Pareto analysis was used to determine the effect of temperature, relative humidity, and salt concentration and their interactions on the drying rate and moisture content of the fish portions using Statistica software (Statistica v. 12.0, Statsoft, Tulsa, USA).

5.3. Results

5.3.1. Moisture content

The moisture and salt content of the fish portions was determined prior to the drying process where the moisture was on average 65.8% and 76.0% for salted and unsalted portions, respectively, where salt content ranged between 7.08-10.16% for the 20% salted portions. At 72 hours overall the unsalted treatments had lower moisture contents (mean = 19.5%) compared to the salted portions (mean = 24.1%), where the lowest moisture content was observed in the salted portions dried at 40°C and 40% RH (14.8%). In addition, the highest moisture content was found in the unsalted samples dried at 30°C and 40% RH (27.55%). The moisture content was measured at 72 hours because it was observed at that time point the drying rate was steady for all the treatments. A summary of the moisture content for the six treatments is outlined in Figure 5.1 a & b. High moisture loss was observed within the first 12 hours of the drying process which substantially reduced post 12 hours.

(a)

Control

Legend:
- 30°C/40% RH, 0% salt
- 40°C/40% RH, 0% salt
- 40°C/50% RH, 0% salt

(b)

Legend:
- 30°C/40% RH, 20% salt
- 40°C/40% RH, 20% salt
- 40°C/50% RH, 20% salt
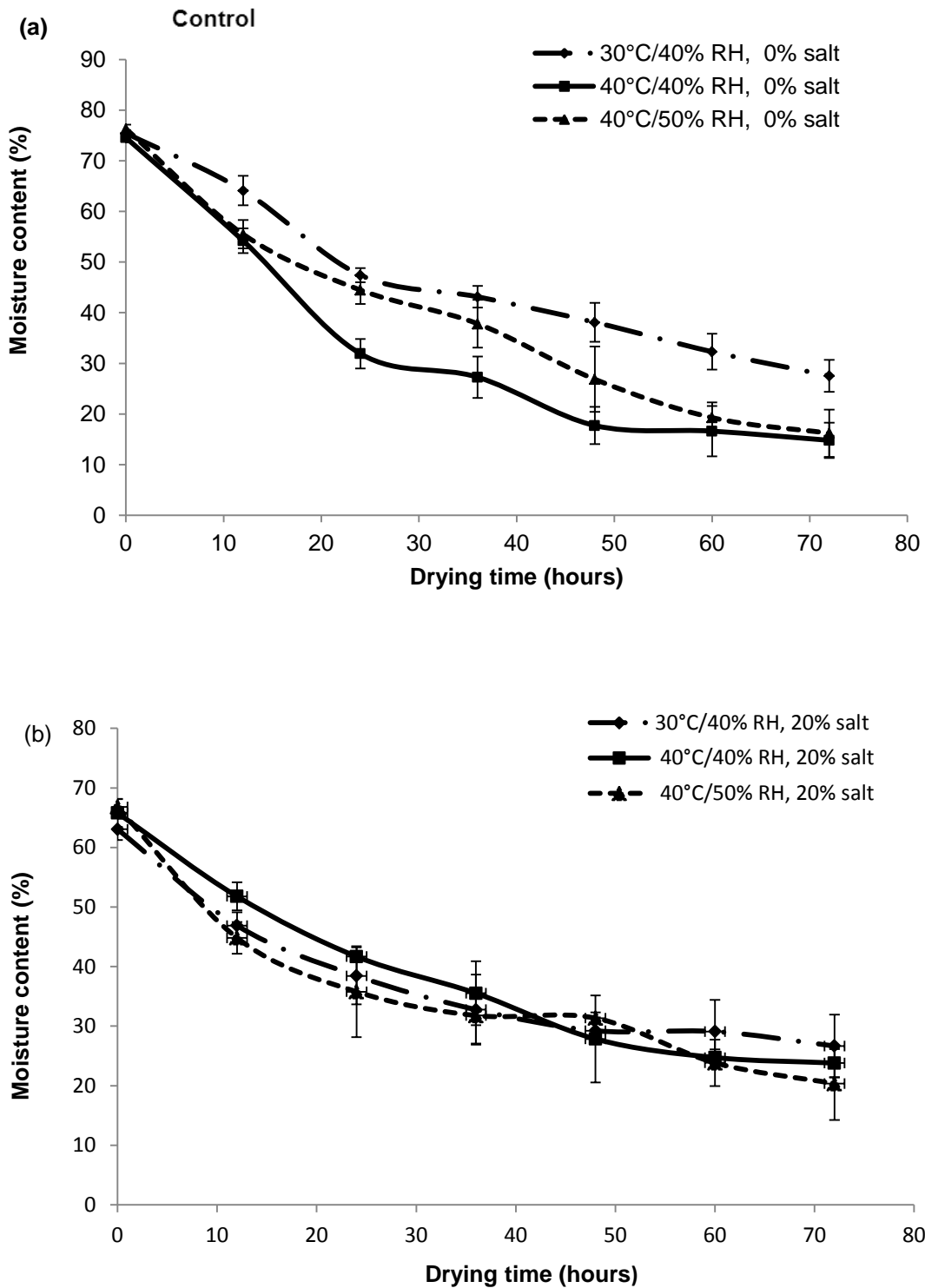
Figure 5.1 Influence of drying temperatures and relative humidity on moisture content (%) of unsalted (a) and salted (20% Bwt) (b) snoek portions over time (n=36). The error bar represents the standard deviation.

The moisture ratio ranged from between 0.7-0.8 and 0.4-0.6 for salted and unsalted snoek portions, respectively, over the entire experimental period. The Pareto analysis, which estimated the standardized effects of the various parameters and their interactions on the treatments (Fig. 5.2), found that drying temperature, RH, salt concentration, and time had a

significant effect on moisture content ($p < 0.05$). A significant ($p < 0.05$) influence of temperature*salt concentration, temperature*time, RH*salt concentration, salt*time on moisture content was also observed. No significant ($p \geq 0.05$) effect of RH*time on moisture content was found. The duration of the drying process varied for each treatment (Table 5.2) and was terminated when change in weight was < 1 g.

Figure 5.2 Pareto analysis examining the effects of treatments (temperature, salt concentration and relative humidity) and their interactions (*) on the moisture content of snoek portions. The Y-axis represents treatments and the X-axis represents the level of effect. Where the bars fall to the right of the vertical line a significant effect ($p < 0.05$) was observed while the numbers to the right of the bar represent the estimated standardized effect. On the y axis: 1 = Temperature; 2 = Relative humidity; 3 = Salt concentration; 4 = Time.

Table 5.2 Duration of the drying process for salted dried snoek (n=6 treatments)

Salt concentration (%)	Treatment	Duration of drying (h)
	Temp (°C)-Relative humidity (%)	
20	30-40	168
20	40-40	108
20	40-50	108
0	30-40	132
0	40-40	96
0	40-50	120

5.3.2. Drying rate

The drying rate decreased as moisture decreased and was slower for the salted snoek compared to the unsalted. At 72 hours of drying the drying rate was 0.04 kg water (kg dry matter * h)$^{-1}$ and 0.03 kg water (kg dry matter * h)$^{-1}$ for salted and unsalted snoek respectively. The unsalted fish portions dried at 40°C/40% RH displayed the fastest drying rate while those salted dried at 40°C/50% RH had an only moderately slower drying rate (Fig. 5.3a & b).
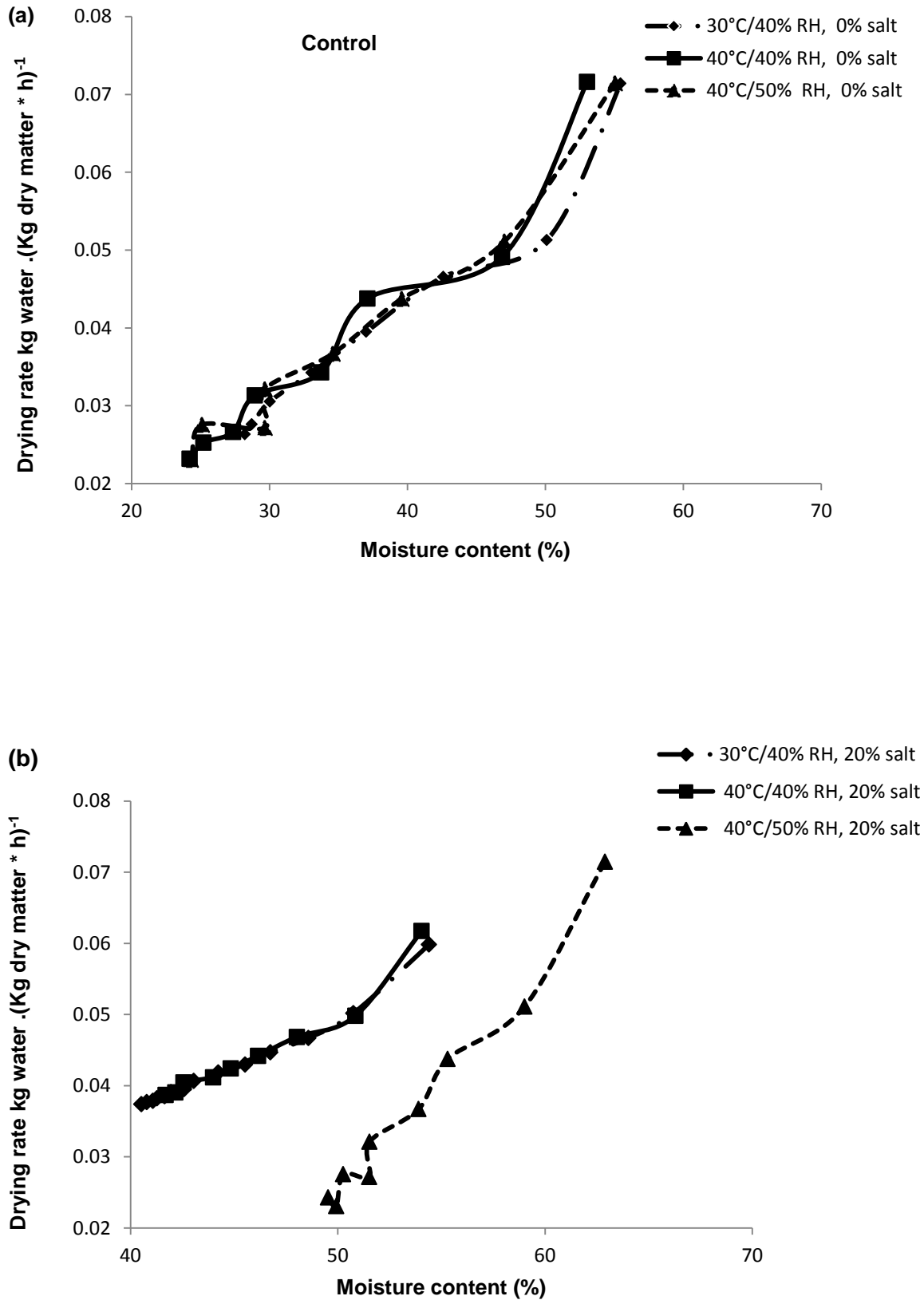
Figure 5.3 Variation in the drying rate (moisture content) of unsalted (a) and salted (b) snoek at three distinct temperature and relative humidity regimes.

Pareto analysis (Fig. 5.4.) showed that temperature, RH, salt concentration, time, temperature*salt concentration, temperature*time, RH*salt concentration, salt concentration*time had a significant ($p < 0.05$) effect on the drying rate of snoek while RH*time had no significant effect ($p > 0.05$).
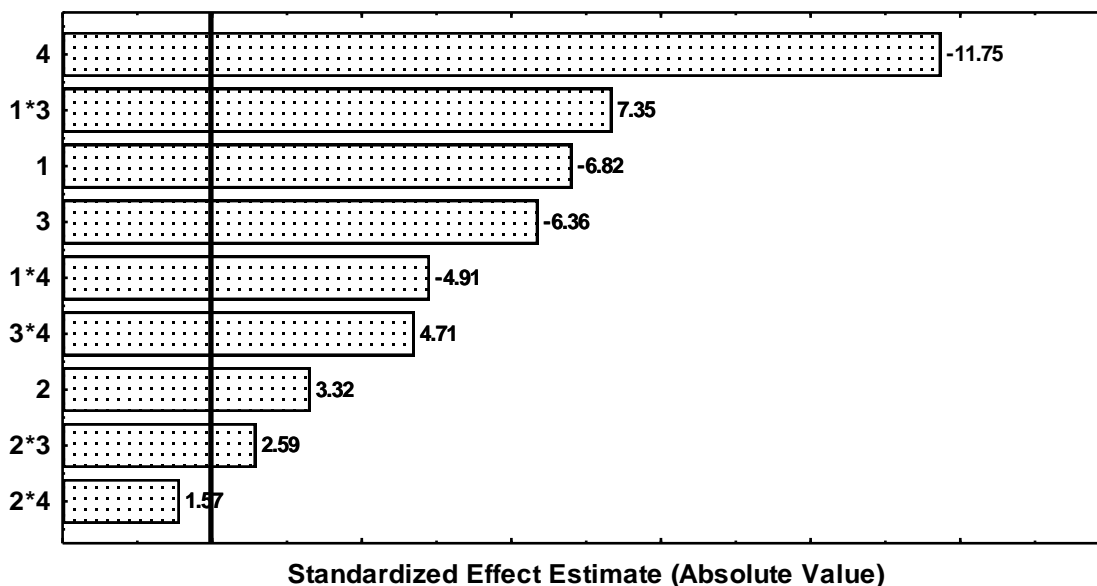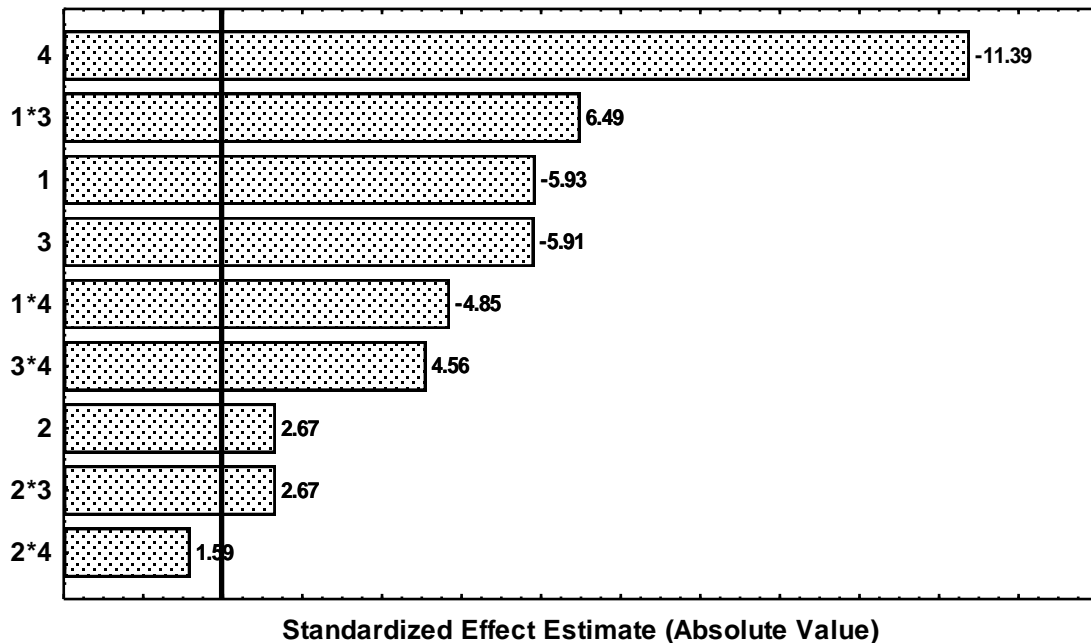

Figure 5.4 Pareto analysis examining the effects of treatments (temperature, salt concentration and relative humidity) and their interactions (*) on the drying rate of snoek portions. The Y-axis represents treatments and the X-axis represents the level of effect. Where the bars fall to the right of the vertical line a significant effect ($p < 0.05$) was observed, while the numbers to the right of the bar represent the estimated standardized effect. On the y axis: 1 = Temperature; 2 = Relative humidity; 3 = Salt concentration; 4 = Time.

5.3.3. Drying curve models

The Page model had the highest coefficient of determination ($r^2 = 0.99$) and lowest standard error (MSE = 6.6 x 10^{-6}) while the Lewis/Newton model gave the lowest ($r^2 = 0.84$) coefficient of determination and highest MSE (7.98 x 10^{-3}). A summary of the model outputs are presented in Table 5.3a and 5.3b.

Table 5.3 Summary of the four empirical models used for assessing the drying kinetics of salted (a) and unsalted (b) snoek portions. Three treatments were tested for both the salted and unsalted portions (30ºC/40% RH, 40ºC/40% RH, 40ºC/50% RH)

(a)

Salted (20%)	Model	Model constants	Value of model constants	Mean square error	Coefficient of determination (r^2)
Treatment (T^{o}C/RH)					
30/40	Lewis/Newton	k	3.80×10^{-4}	7.12×10^{-3}	0.8701
	Page	n	3.34×10^{-1}	1.02×10^{-4}	0.9981
		k	8.14×10^{-2}		
	Henderson & Pabis	a	8.63×10^{-1}	2.6×10^{-3}	0.9534
		k	2.30×10^{-3}		
	Asymptotic (logarithmic)	a_0	6.47×10^{-1}	1.72×10^{-4}	0.9971
		a	3.34×10^{-1}		
		k	3.20×10^{-2}		
40/40	Lewis/Newton	k	7.78×10^{-3}	4.46×10^{-3}	0.9773
	Page	n	4.27×10^{-1}	$6.86\ 10^{-6}$	0.9999
		k	7.87×10^{-2}		
	Henderson & Pabis	a	9.33×10^{-1}	2.90×10^{-3}	0.9846
		k	7.51×10^{-3}		
	Asymptotic (Logarithmic)	a_0	6.39×10^{-1}	3.14×10^{-4}	0.9988
		a	3.56×10^{-1}		
		k	5.90×10^{-2}		
40/40	Lewis/Newton	k	4.88×10^{-3}	6.0×10^{-5}	0.9999
	Page	n	9.40×10^{-1}	6.15×10^{-4}	0.9986
		k	5.28×10^{-2}		
	Henderson & Pabis	a	9.92×10^{-1}	7.09×10^{-4}	0.9984
		k	4.31×10^{-2}		
	Asymptotic (Logarithmic)	a_0	1.13×10^{-2}	1.05×10^{-3}	0.9977
		a	9.82×10^{-1}		
		k	4.22×10^{-2}		

$a_{o,}$ a – coefficient of drying model
k – Drying coefficient
n – exponential coefficient

(b)

Salted (20%) Treatment (T^oC/RH)	Model	Model constants	Value of model constants	Mean square error	Coefficient of determination (r^2)
40/40	Lewis/Newton	k	1.59×10^{-2}	6.28×10^{-3}	0.9146
	Page	n	5.87×10^{-1}	3.01×10^{-4}	0.9959
		k	8.27×10^{-2}		
	Henderson & Pabis	a	9.02×10^{-1}	4.08×10^{-3}	0.9446
		k	1.37×10^{-2}		
	Asymptotic (logarithmic)	a_0	3.06×10^{-1}	6.15×10^{-4}	0.9928
		a	6.77×10^{-1}		
		k	3.63×10^{-2}		
40/40	Lewis/Newton	k	1.40×10^{-2}	6.48×10^{-3}	0.88
	Page	n	5.90×10^{-1}	2.64×10^{-4}	0.9951
		k	7.78×10^{-2}		
	Henderson & Pabis	a	8.87×10^{-1}	3.96×10^{-3}	0.9266
		k	1.18×10^{-2}		
	Asymptotic (logarithmic)	a_0	2.86×10^{-1}	4.22×10^{-4}	0.993
		a	6.94×10^{-1}		
		k	3.11×10^{-2}		
30/40	Lewis/Newton	k	1.18×10^{-2}	7.98×10^{-3}	0.8415
	Page	n	0.55×10^{-1}	4.46×10^{-4}	0.9911
		k	8.33×10^{-2}		
	Henderson & Pabis	a	8.68×10^{-1}	4.60×10^{-3}	0.9086
		k	9.54×10^{-3}		
	Asymptotic (logarithmic)	a_0	3.28×10^{-1}	4.27×10^{-4}	0.9924
		a	6.51×10^{-1}		
		k	3.03×10^{-2}		

$a_0,$ a – coefficient of drying model
k – Drying coefficient
n – exponential coefficient

The relationship between the predicted (derived from the Page model) and expected moisture ratio was assessed and a strong relationship was observed (R^2=0.99). This indicates that the Page model sufficiently described the drying behaviour of the snoek portions.
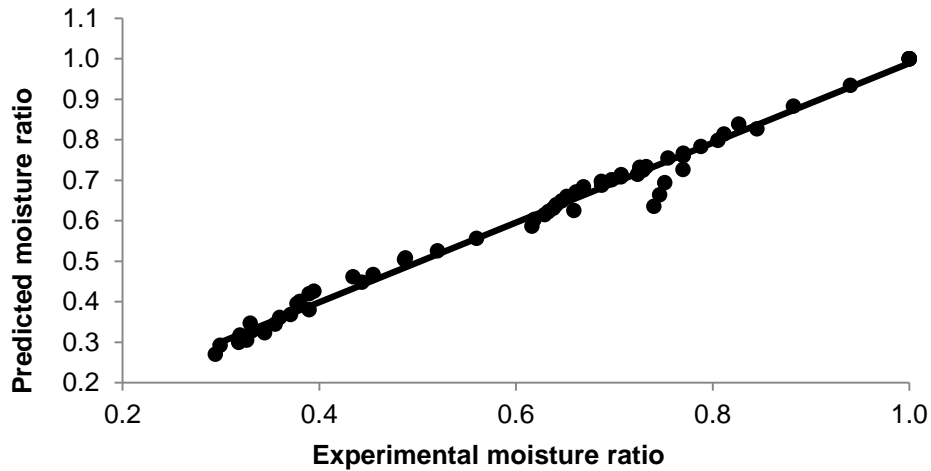

Figure 5.5. Relationship between the experimental and predicted moisture ratio of dried snoek using the Page model.

5.3.4. Effective moisture diffusivity

The effective moisture diffusivity ranged from 5.30×10^{-6} to 1.54×10^{-5} m^2 s^{-1} for salted portions and from 6.68×10^{-5} to 8.93×10^{-5} m^2 s^{-1} for the unsalted portions. Table 5.4 outlines the average effective moisture diffusivity for salted and unsalted snoek portions at the six different treatments. The effective moisture diffusivity was determined using equation 4 and calculated as the slope of a straight line which was obtained when $\ln M_R$ was plotted against time. The effective moisture diffusivity was considerably higher in unsalted snoek for all three treatments compared to the salted treatments. The highest moisture diffusivity (8.93×10^{-5} m^2 s^{-1}) was observed in unsalted portions dried at 40°C/40% RH while the salted portions dried at 40°C/50% RH had the lowest (5.3×10^{-6} m^2 s^{-1}). Within the unsalted the highest (8.93×10^{-5} m^2 s^{-1}) was found in the portions dried at 40°C/40% RH while the lowest was found in the portions dried at 30°C/40% RH. Within the salted treatments snoek portions dried at 40°C/40% RH had the highest moisture diffusivity (1.54×10^{-5} m^2 s^{-1}) while the lowest (5.3×10^{-6} m^2 s^{-1}) was observed in the samples dried at 40°C/50% RH.

Table 5.4 Effective moisture diffusivity of snoek at six different drying treatments

Salt (%)	Treatment (T $^{\circ}$C/RH)	Effective diffusivity (m^2 s^{-1})
20	30/40	1.43×10^{-5}
20	40/40	1.54×10^{-5}
20	40/50	5.3×10^{-6}
0	40/40	8.93×10^{-5}
0	40/50	7.94×10^{-5}
0	30/40	6.68×10^{-5}

5.4. Discussion

5.4.1. Effect of drying on moisture content and drying rate of snoek

The difference found in the initial moisture content between salted and unsalted snoek was a result of moisture loss during the salting process. Salt is hygroscopic in nature and therefore can dehydrate the fish muscle (Gravier *et al.*, 2006; kituu, 2009; Ikrang *et al.*, 2014). Similar results have been found in salted and unsalted shark (*Carcharhinus sorrah*) where the initial moisture content was 75.4% and 63.6% for unsalted and salted shark meat respectively (Guizani *et al.*, 2008). Additionally, the sharp decrease in moisture content observed within the first 12 hours of the drying process for all treatments is indicative of the constant drying rate and indicates that the snoek portions experienced most moisture loss within the first 12 hours of drying. Similar trends in high initial moisture loss have been found in salted and unsalted shark fillets (Mujaffar & Sankat, 2005). All the main factors (time, temperature, salt concentration and relative humidity) measured and most of their interactions (temperature*salt, temperature*time, salt*time and RH*time) affected the moisture content and drying rate of snoek.   This suggests that each factor needs to be taken into consideration when drying snoek. Mujaffar and Sankat (2005) also found similar results in shark meat where drying was considerably affected by drying time, temperature, salt and their various interactions.

A reduced drying rate of the salted snoek compared to the unsalted snoek was found and may be as a result of case hardening, where the added salt can form a crust on the surface of fish hindering the rate of evaporation during the drying process (Mujaffar & Sankat, 2005; Guizani *et al.*, 2008; Vega Galvez *et al.*, 2011). Similar results were found in tilapia (*Tilapia zilli*) and horse mackerel (*Trachurus japonicas*) where those salted tended to have a lower drying rate compared to unsalted fish (Shi *et al.*, 2008; Ikrang *et al.*, 2014). The drying rate is a significant factor in dried food processing where a slow drying rate results in an extended drying period and subsequently economic loss due to the high energy output required (Wu & Mao, 2008; Wang *et al.*, 2011).

Relative humidity played a significant role in snoek drying dynamics. A 10% increase in RH at the same temperature and air velocity significantly influenced the drying rate in the current study where salted snoek portions dried at 40°C and 40% RH had a faster drying rate than portions dried at 40°C and 50% RH. This slower drying rate observed at 40°C and 50%

RH is likely due to the effect of evaporation, which is reduced, when humidity is high (Kilic, 2009) therefore resulting in moisture retention. When RH is high and the drying temperature is low, the difference in the partial pressure of water vapour and pure water vapour pressure is low, therefore slowing the drying rate (Fudholi *et al*., 2013).

Kilic (2009) found that an increase in air drying temperature decreased the drying time of salted rainbow trout (*Oncorhynchus mykiss*). However, such a trend was not apparent in the current study where salted snoek portions dried at 30°C/40% RH dried faster than those dried at 40°C/50% RH. The absence of a negative association between moisture content and temperature may be due to the high relative humidity applied to the drying process of the snoek which likely dampened the effect of temperature. Within the current study it was observed that a combination of low temperature and high relative humidity had a negative influence on snoek drying rate. Increasing the drying temperature can therefore reduce the drying time. However, when temperature is increased heat labile nutrients such as vitamins and essential amino acids can be lost (Kilic, 2009; Wu & Mao, 2008). Therefore, a balance or trade-off is required when choosing drying temperature and careful monitoring is advised.

5.4.2. Evaluation of the drying curve mode

The Page model effectively described the drying process of snoek portions as it displayed the highest coefficient of determination and lowest mean square error of all models tested. On the contrary the Lewis/Newton model had the lowest and highest MSE of all models and therefore had the least suitable fit. Djendoubi *et al*. (2009) also found that the Page model was a best fit when evaluating the drying curve of sardine muscle (*S. pilchardus*). The Page model can therefore serve as a useful tool for optimising snoek drying process, predicting the equilibrium moisture content at any given time and can also aid in the design of hot-air facility for industrial dried snoek processing.

5.4.3. Effective moisture diffusivity

Considerable variation was observed in the effective moisture diffusivity coefficients (D_{eff}) of snoek portions exposed to the six diverse treatments. Overall lower D_{eff} were found in the salted snoek portions compared to the unsalted portions whilst a positive trend in D_{eff} values and drying temperature was apparent. Similar results describing the effect of salting on D_{eff} values have been found in shark meat dried at 30, 40, and 50°C where the fillets salted had lower D_{eff} values (1.09, 2.81 and 4.67 x 10^{-6}) compared to the unsalted fillets (2.47, 4.38 and 5.88 x 10^{-6}) (Mujaffar & Sankat, 2005). Temperature has also been found to play a vital role in the diffusion of moisture during drying where D_{eff} values decrease as temperature decreases (Vega-Galvez *et al*., 2011). This suggests that D_{eff} values can be a relative indicator of the salt content and drying rate of fish and shark meat.

The D_{eff} values of the salted snoek were higher than those obtained in previous studies which examined aquatic organisms, indicating a higher rate of moisture movement during the snoek drying process. Sun dried prawn and chelwa fish had an average effective moisture

diffusivity of 8.71 x 10^{-11} and 11.11x 10^{-11} m^2 s^{-1}, whilst sardine and silver carp had an effective moisture diffusivity range of 1.38 x 10^{-11}-2.21 x 10^{-11} $m^2 s^{-1}$ and 2.86 x 10^{-10} $m^2 s^{-1}$ and 4.94 x 10^{-10} m^2 respectively. The high variability in D_{eff} values between studies may be due to the experimental specific drying temperature, air velocity and RH (Wang *et al.*, 2013). In addition, the salt content of the fish and, muscle alignment, fat content, thickness, size of fillets and the presence or absence of skin can also affect the D_{effs} obtained (Vega-galvez *et al.*, 2011; Wang *et al.*, 2013). Monitoring of D_{effs} during the drying of snoek portions is symptomatic of the efficiency of the drying process (Mujaffar & Sankat, 2006) and can be useful to monitor the trend of moisture removal during fish drying process.

5.5. Conclusion

The salted snoek portions had a lower moisture content compared to the unsalted portion prior to the drying process. Once drying commenced a sharp initial (within 12 hours) decrease in moisture content was apparent for both the salted and unsalted portions which subsequently tapered off post 12 hours. Overall, it was the unsalted snoek portions for all three treatments which had lowest effective moisture diffusivity and high drying rate compared to the salted portions, which was likely due to the crusting effect of the added salt. Time, temperature, salt concentration and relative humidity and most of their interaction significantly affected the drying kinetics of snoek meat and therefore should be considered when drying fish and snoek products. Additionally, although high temperature generally results in a faster drying rate it was observed that a high relative humidity (40 and 50%) can dampen the drying effects of temperature in snoek and slow the drying rate. It is suggested, for improved effective moisture diffusivity and drying rate the RH should be maintained below 50%. The Page model was an excellent fit to the drying kinetics of snoek and can therefore be used to adequately predict the drying characteristics of snoek portions. This research can be used to optimize snoek drying process, improve dried snoek processing and in the design of dryer for dried snoek processing. Although this study describes the dynamics of salted and dried snoek, research on the effects of various salting and drying on snoek storage stability and the nutritional quality of the product is still required.

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Chapter 6. General discussion and recommendations

6.1. General discussion

Fish spoilage contributes to food insecurity and direct economic loss (kumolu-John & Ndimele, 2011); therefore, postharvest treatment of fish is essential to minimize losses and extend the shelf life of fish products. Various fish processing and preservation methods such as salting, drying, smoking, fermentation, cold storage, canning, vacuum packaging, irradiation and use of edible coatings have been developed to reduce postharvest loss and extend the shelf life (Thapa *et al.*, 2006; Jain & Pathare 2007; Reza *et al.*, 2009). Such processing and preservation techniques increase the availability and accessibility (year round) of fish for the communities which rely on them as their main protein source (Reza *et al.*, 2009; Tawari & Abowei, 2011).

Salting and drying are the most common and oldest methods of fish preservation and are currently widely used in developing countries such as South Africa due to ease of storage and low technology required (Bellagha *et al.*, 2007; Kilic, 2009). Snoek is one of the important species caught in South Africa by the small-scale fishermen. The common practise is that snoek is sold fresh but when it is surplus it is salted and dried in order to prevent spoilage. Snoek fisheries operate as an informal small scale sector with limited information on the regulations guiding the postharvest handling, storage and preservation of the fish. Hence, the aim of this study was to identify current market quality of salted and dried snoek and other processed products and identify ways of improving the overall quality. This was achieved with the following objectives:

- Investigate the quality of locally (Cape Peninsula) produced salted dried snoek products
- Determine the effect of salting at different salt concentrations and drying conditions on moisture loss, water activity and final salt content of snoek.
- Study the drying kinetics of snoek fish under different drying conditions in order to optimise salted dried snoek processing.

This study revealed different approaches in processing procedures between processors/vendors within the Western Cape province. The TBARS and water activity of fish sold locally were higher than suggested levels and are therefore a cause for concern due to potential adverse effects on consumer health. For instance consumption of lipid oxidation products has been linked to heart diseases and cancer while high TBARS and water activity can accelerate spoilage when stored for prolonged periods. Nonetheless the products were considered safe for consumption at time of sampling due to the low histamine levels and absence of pathogens. However, extended storage may compromise the safety and the shelf life of the products due to the high moisture content though the products were considered safe for consumption at time of sampling.

Water activity, moisture content and salt content are physical parameters which play important role on the stability of food products during storage and if properly controlled the shelf life of the products will be prolonged. Within the current study salting at 20% or to saturation resulted in water activity low enough to prevent microbial growth of most microorganisms whilst salting to saturation resulted in snoek containing salt above the recommended levels. Overall, this study identified that salting at 20% (20 g salt/100 g fish) and drying at 40°C/40% RH for 72 hours is ideal for the production of good quality salted dried snoek production where the salt content, water content, and water activity measured was considered adequate for inhibiting microbial spoilage thereby extending the shelf life.

The result of this study showed that moisture content and drying rate and effective moisture diffusivity of snoek was influenced by salting, temperature, relative humidity and their interactions. Low effective moisture diffusivity in salted snoek portions suggests that prolonged drying time due to crust formation during the drying process is a cause for concern in snoek processing. The Page model effectively described the drying kinetics of snoek portions and therefore, could help as a useful guide in predicting and optimising drying conditions of fresh snoek and in design of dryers for industrial dried snoek production.

Consumer awareness of the nutritional composition and safety of dried snoek products is important while the controlled drying conditions used in this study can help producers to achieve products with consistent product quality. Consequently, preserving nutritional constituents which can be lost when extreme processing conditions, are employed.

6.2. Recommendations

- Improved drying strategies such as oven drying at low temperature employed in this study, is suggested to increase the quality of the locally produced dried snoek.
- Although very difficult to enforce in informal sector, standardisation and monitoring of postharvest processing of snoek is required in order to ensure the products are safe for consumption. Measures such as random sampling of dried fish products should be put in place to investigate the histamine content, thiobarbituric reactive substances, nutritional quality and microbial safety of the products.
- Though high temperature generally results in a faster drying rate, high RH (50%) can also reduce the drying effects of temperature and slow the drying rate. Therefore, for improved effective moisture diffusivity and drying rate it is recommended that the RH should be maintained below 50%.
- The use of known salt concentration at 20% (20 g salt/ 100 g) fish is suggested for the processing of improved quality salted dry snoek.
- Further studies need is required to determine the effect of different air velocity on the snoek drying process.

- Due to the limited number of treatments examined in this thesis as result of time constraints, future studies should investigate the effects of various salting concentrations and drying conditions (not examined in this study) on snoek nutritional quality and microbial stability.

- A study investigating the rate of spoilage of locally salted dried snoek over time is recommended to identify the storage stability of these locally produced products.

- A study to investigate turnover rate of fresh and processed snoek from vendors to table is necessary for better understanding of the Western Cape province snoek industry.

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