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THE EFFECT OF BRINE ON THE QUALITY AND SAFETY OF SMOKED ATLANTIC MACKEREL (*Scomber scombrus*)

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ABSTRACT

The quality and safety of smoked pelagic fish products along the value chain is a complex event that required multiple analyses to define its stability. Consequently, changes in microbiological total viable count (TVC) and specific spoilage organisms (SSO) and physicochemical characteristics (total volatile base-nitrogen (TVB-N), total lipid, free fatty acids (FFA), peroxide value (PV) and thiobarbituric acid reactive substance (TBARS) of brined and unbrined smoked Atlantic mackerel (*Scomber scombrus*) were evaluated for 24 days period of storage. Total viable counts were significantly ($p=0.002$) higher in unbrined than in brined smoked *S. scombrus* stored at 20°C. Brined smoked *S. scombrus* stored at 0-4°C had lower TVC during the storage period. TVB-N levels increased with storage time, but the increase was evident in unbrined than brined samples stored at 20°C. TVB-N for brined smoked samples stored at 0-4°C below the acceptable limit for throughout the storage period. No significant differences ($p>0.05$) in lipid content were observed between the groups after smoking and during storage. Lipid hydrolysis and oxidation were higher in unbrined smoked samples stored at 20°C but was rather stable at 0-4°C. The shelf life of smoked *S. scombrus* was estimated to be 4 and 5 days for unbrined and brined samples stored at 20°C, while samples stored at 0-4°C had a shelf life of 20 days. The study shows that the use of brine in hot smoked mackerel creates the unavailability of ionically water molecules needed by micro-organisms. Low storage temperature decreases the level of hydrolysis by inhibiting enzyme activity responsible for spoilage. This has potential of achieving quality and safe value-added products along the pelagic fishery. Therefore, application of the technique in the Malawi fishery value chain cannot be over emphasized.

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1 INTRODUCTION

Inland fish and their fisheries are vital for nutritional, economic, and recreational roles and are necessary components of sustainable ecosystem function throughout the world (FAO, 2013). More than 60 million people in developing countries like Malawi are engaged in various aspects of fisheries (FAO, 2008). Malawi's fisheries sector plays a significant role to actors involved in fishing, processing and fish trading in the form of employment, income earnings and food security (Singini, Kaunda, Kasulo, & Jere, 2013). The sector directly employs around 178,000 people involved in fishing, fish processing, and marketing and over 600,000 people engaged in related fishing activities (DoF, 2018). The fisheries contribute over 70 % of the animal protein intake of Malawians and 40 % of the total protein supply, essential fatty acids, and micronutrients (Saliu, 2008). It is thus central to the food and nutrition security for millions of people.

However, fresh fish is a perishable commodity with a short shelf life due to high water activity, neutral pH and autolytic enzymes (Sallam, 2007). Hence, fish quality losses occur very rapidly after catch (McDonough et al., 2014). There is, therefore, a need to process fish after the catch to reduce physical and nutrient losses (FAO, 2008). The processing of fish refers to the processes associated with fish and fish products between the time fish is caught and the time the final product is delivered to the consumer (Antoine, Bohuon, Deumier, & Poligne, 2000). This is also an obvious means of improving supply of fish during the closed season, even without increased landings as it has the potential to prolong shelf life of products (Mostafa & Ayimba, 2014).

The magnitude to which losses are reduced depends on the type of processing method used. In Malawi, current fish processing methods such as open sun drying, smoking, and parboiling have a lot of limitations which range from contamination by dust, insect infestation, and non-uniform drying. This results in a high level of microbes and subsequent quality and safety loss of the products. Therefore, better methods must be used to improve the quality and safety of the products along the fishery value chain.

1.1 Fish production in Malawi

The catch and effort data collection system established and introduced to Malawi by the UN Food and Agriculture Organization shows a rather steady increase in production from 2000 to 2017 (FAO, 2018) (Figure 1). Although the annual production is increasing this seems to be done at the cost of the larger fish species such as *Rhamphochromis* species and *Oreochromis* species, which are decreasing (Mbalaka, Kanyerere, & Kawonga, 2018). Since 2000 to date, over 70 % of the total volume of the fish catch is dominated by small fish species such as *Engraulicypris sardella*, *Copadichromis* and *Diplotaxodon* species (DoF, 2018). The rise in production has also registered positive economic benefits. For instance in 2017, fish landings had a landed value of MWK 173.04 billion (USD 235.74 million) compared to MWK 129.74 billion (USD 172.74 million) in 2016. This is an increase of fish landing value by 26.8 percent. Among the small species, *Engraulicypris sardella* (Usipa) is the most significant cyprinid species in terms of production which varies by season. In 2011, about 46% of the estimated fish production of 82,414 tonnes was *E. sardella* while in 2017 the fishery contributed 67% to the national fish production estimated at 201,472,00 tonnes (Figure 2). Small pelagic fish species are nutrient rich food fish due to their high levels of micronutrients, unsaturated fat, and protein (Owaga et al., 2010; Kabahenda et al., 2011). Apparently, transition from big to small fish species means high perishability and high

perishability leads to high postharvest losses. Low value catches results in low financial returns and more volume to maintain profitability.

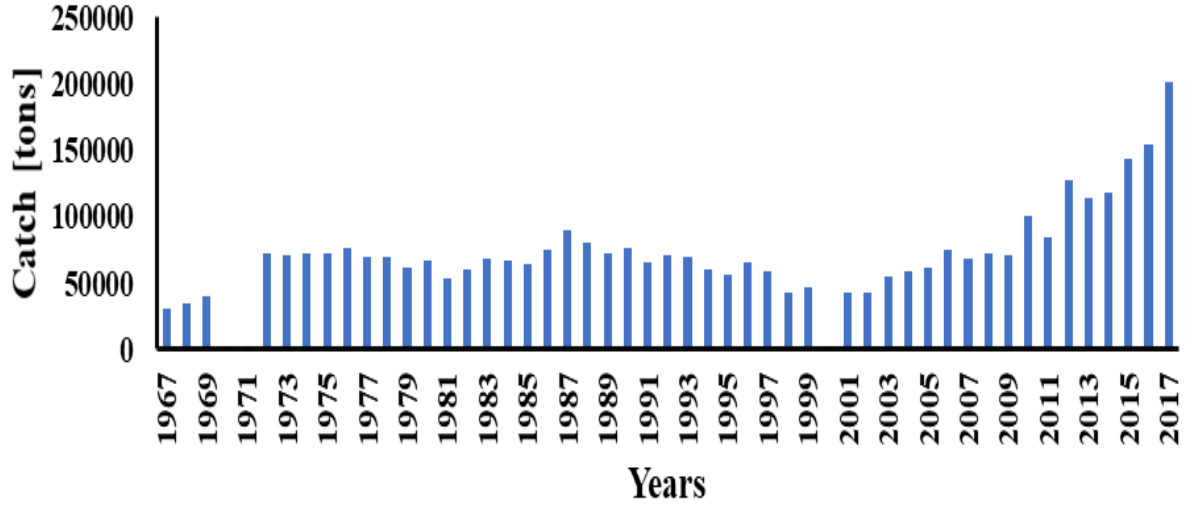

Figure 1 Annual fish production for Malawi capture fishery from 1967-2017. Source fao.org

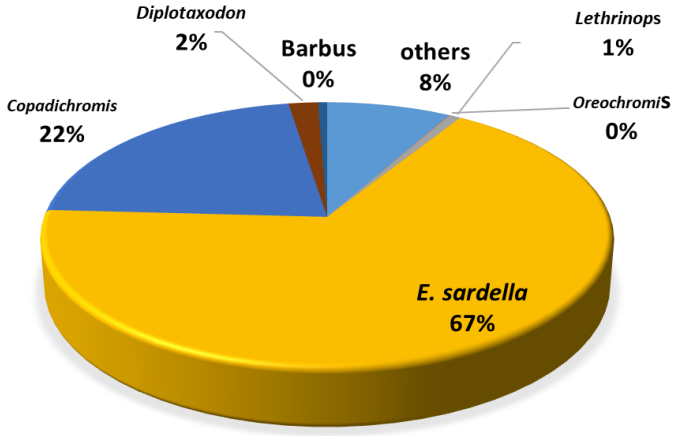

Figure 2 Current fish species composition of Malawi, 2017. Source DoF

1.2 Fish Consumption in Malawi

The per capita fish consumption in Malawi between 2007 and 2013 shows fluctuating trends due to unstable annual fish catches and post-harvest losses (DoF, 2018). Since 2016, there has been a fluctuation and significant increase in the per capita fish consumption, reaching 12.47kg/person/year in 2017, from 10.70kg/person/year (Table 1). Although the current average per capita consumption is a major achievement in nutrition security, the average per capita consumption of 12.47 kg/yr is far from the recommended 20.3 kg/yr set by the Food and Agriculture Organisation (FAO, 2018). This calls for approaches to reduce post-harvest losses. It is important to note that the revised National Fisheries and Aquaculture Policy, 2016 focuses on fish quality and value addition as a means of promoting the adoption of best practices (DoF, 2018). It is apparent that improved processing methods have the potential to

enhance the quality and safety of fish and fish products along the value chain. This will reduce the annual catch that is lost through post-harvest spoilage and ensure continued availability of fish in required amounts that will avert per capita consumption deficit (Banda, Chigwechokha, Msiska, & Simbeye, 2017). Equally, this demands actions towards reducing fish spoilage by improving current processing methods in order to feed the increasing population while sustaining the quality and safety of products along the value chain of Malawi.

Table 1 Per capita fish supply with estimated population growth for 2007-2017. Source DoF, 2018

Year	Population (million)	Total catch (ktonnes)	Fish supply/kg/person/year
2007	11,700,000	65,200,000	5.57
2008	13,100,000	71,266,000	5.44
2009	13,300,000	71,289,000	5.36
2010	13,500,000	95,724,000	7.09
2011	13,700,000	81,070,000	5.92
2012	13,900,000	81,070,000	8.66
2013	14,100,000	120,328,000	7.79
2014	14,300,000	117,094,878	8.19
2015	14,500,000	144,315,275	9.95
2016	14,700,000	157,267,660	10.7
2017	16,000,000	201,472,00	12.47

1.3 Problem statement

Fish supports livelihoods of over 800 million people in the world, most of which are in sub-Saharan Africa. However, on top of natural and anthropogenic effects on fish supply, fish dependent livelihoods are further threatened by high post-harvest losses. Fish post-harvest losses of pelagic fisheries are very high in Africa reaching as high as 40% (FAO, 2010). These losses are high in sub-Saharan Africa due to poor handling as a result of poor infrastructures such as roads and lack of ice plants and together result in a global annual economic loss of $2-5 billion (Béné 2011).

In Malawi, traditional processing methods such as smoking, parboiling, and open sun drying result in 34% post-harvest losses along the value chain (Chiwaula, Chirwa, Binauli, Banda, & Nagoli, 2018). Higher moisture content in smoked product creates a conducive environment for microbial multiplication leading to post-harvest losses (Chiwaula, Chirwa, Binauli, Banda, & Nagoli, 2018). These losses are manifested in physical damage, quality deterioration due to oxidation, and market value. Losses incurred in pelagic fisheries account for more than half of total fish production and translates into even big losses in food and nutrition security to millions of people in Malawi (Singini et al., 2017). Total economic losses due to post-harvest losses in Malawi is estimated at 42 million USD (DoF, 2018). This means that fish processors lose a substantial part of their profits. In addition, post-harvest losses cause a considerable loss to the population of nutritious food where fish provide 70% of animal protein (Chiwaula, Chirwa, Binauli, Banda, & Nagoli, 2018). Thus, the need for

suitable processing methods to ensure quality products that fit consumers along the value chain is important.

Smoking has been studied and applied in the fishery value chain for a long time (figure 3). However, most processors and consumers in Malawi are not aware of how to maintain quality and safe smoked products along the value chain. Spoilage of fish products is a result of lipid oxidation and microbiological activity, specifically specific spoilage organisms. This work is meant to increase the knowledge of how brine in smoking impacts the quality and shelf life of smoked fish. Using Atlantic Mackerel (*Scomber scombrus*) as a case study will help to understand the impact this method may have on other species in Malawi. This will eventually help to improve the value chain of Malawi fishery (figure 3).

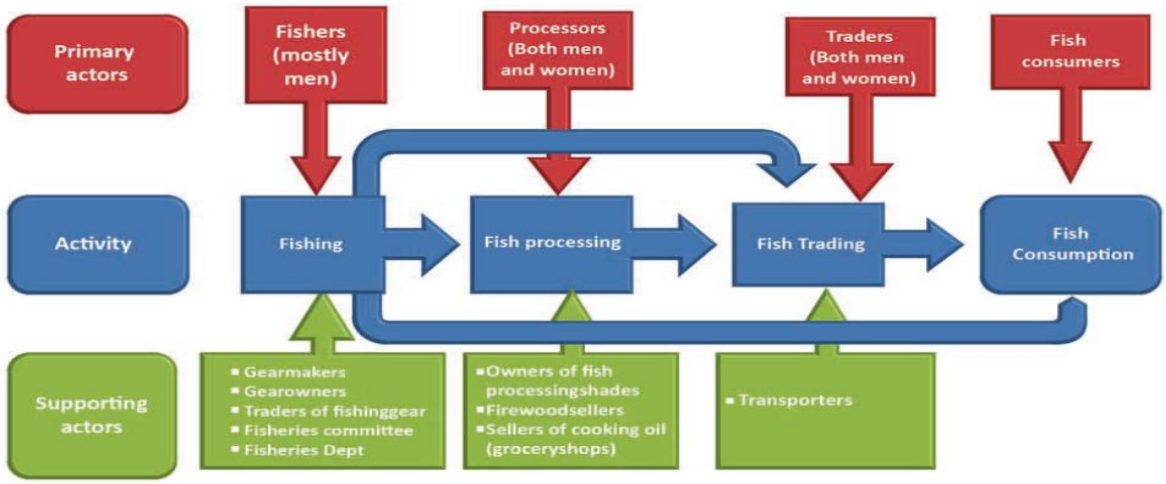

Figure 3 The value chain for Lake Malawi fishery

1.4 Research justification

The study will contribute to national and African development policies and goals in Malawi. For instance, reduction of losses and increase of quality and safety of pelagic fish products would substantially increase fish supply without increasing the pressure on the fishery resources. More importantly, the research supports the New Partnership for African Developments Plan of Action for the Development of African Fisheries and Aquaculture that specifically encourages investments in enhancing productivity through loss reduction and management along the value chain.

Furthermore, this will help to attain the goal in Malawi Growth Development Strategy which emphasizes creation of wealth and reduction of poverty through sustained and inclusive economic growth. From a fisheries perspective, the goal is to maintain fish product quality while contributing to economic growth. Similarly, the study will have a direct contribution to the Agriculture Sector Wide Approach (ASWAp-SP) which aims at creating avenues for consumers to access quality and nutritious food while increasing the contribution of agro-processing for the country's economic growth. This study will also have a positive impact through the improvement of the availability of quality fish products among consumers at a household level hence leading to the overall health improvement of consumers in Malawi.

1.5 Research Objectives

This project intends to optimize the brine smoking processing method with the aim of achieving a new quality and safe value-added product. Another objective of this research project is to learn about the fish brine smoking process and eventually apply the technique in Malawi. This will help in contributing to better use of raw materials through the introduction and out scaling of a new fish processing method in the Malawi pelagic fishery.

The specific objectives of this project are:

- To evaluate the effect of brining on the quality of smoked mackerel products, obtained from freshly thawed fish. For this, microbiological and physiochemical analysis will be carried out on the raw material and on the final smoked product.
- To combine different storage temperatures to determine and define the most appropriate storage temperature to produce a high-quality and safe brine smoked product. For this, a comparison between the raw material, practices, and components will be used during this study and existing conditions in Malawi will be used.

2 LITERATURE REVIEW

2.1 Atlantic mackerel *Scomber scombrus*

Atlantic mackerel (*Scomber scombrus*) is a pelagic species of mackerel found in the temperate waters of the Mediterranean Sea, Black Sea, and the northern Atlantic Ocean (Figure 4) (Saeed & Howell, 2002). The species occurs in the pelagic zone down to a depth of 200 meters. Studies have shown that Atlantic mackerel spends warmer periods close to shore and near the ocean surface (Stransky, Murta, Sclickeisen, & Zimmerman, 2008). It appears along the coastline in spring and leaves on arrival of the colder season in the fall and winter periods. It migrates out into deeper and moves to more southern in the cold times seeking out warmer temperate waters.

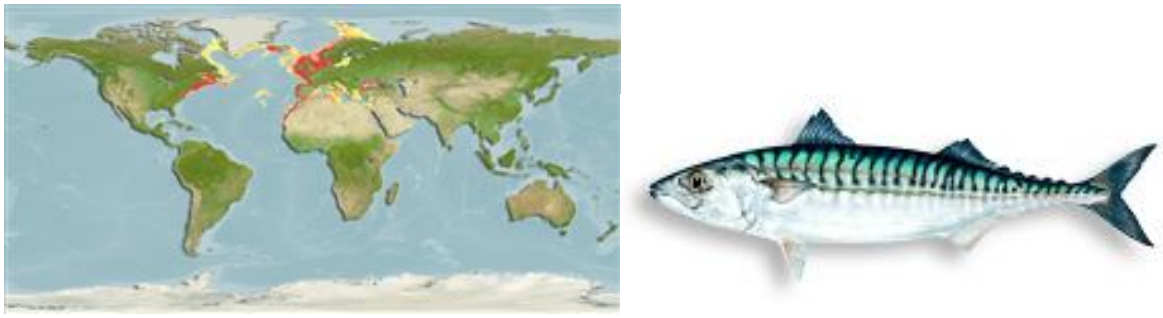

Figure 4 Distribution of Atlantic Mackerel (*Scomber scombrus*). Source: Fishbase.org

The Atlantic mackerel has an elongated body with steel-blue marked by wavy black lines in the dorsal fin and a long-pointed snout (Peterson & Ausubel., 1984). Its body tapers down its length and ends with a large tail fin. The normal size for matured Atlantic Mackerel is 30 cm, however, individuals have been measured at 60 cm with a maximum weight of 3.4 kg. The reproduction of the Atlantic Mackerel is oviparous and takes place near the shore during spring and summer periods (Stransky, Murta, Sclickeisen, & Zimmerman, 2008). It is reported that during reproduction time, female Atlantic mackerel produces over 450, 000 eggs. The Atlantic mackerel juveniles can reach sexual maturity after 2 years. Atlantic mackerel is primarily caught with purse seines, sometimes together with sardines. Surface catches are best when the summer thermocline is not deeper than 15 to 20 meters to prevent the mackerel from escaping into deeper water.

Studies have shown that Atlantic mackerel has good meat with a strong flavour (Saeed & Howell, 2002). It is an oily fish, with vitamin B_6, vitamin B_{12}, selenium, niacin, and omega 3 polyunsaturated fatty acids, containing nearly twice as much of the latter per unit weight as Salmon (Tenyang, Tiencheu, Dajikeng, Morfor, & Womeni, 2019). Approximately one million tonnes of Atlantic mackerel are caught each year globally, however, the bulk of which is sold fresh, frozen, smoked and canned.

2.2 Post-harvest losses in Malawi

The contribution of Malawi's inland fisheries to food and income security is being threatened by various factors including high post-harvest losses estimated at 34% (Banda, Chigwechokha, Msiska, & Simbeye, 2017). These losses have negative implications on fish

supply and income of actors in the fish value chain particularly women and youth, who play an important role in fish processing (Chiwaula, Chirwa, Binauli, Banda, & Nagoli, 2018). This is an essential thematic area for achieving the Sustainable Development Goals (SDGs). Additionally, the problem of post-harvest loss effects nutritional security of consumers in Malawi (Likongwe, Kasapila, Katundu, & Mpeketula, 2018). The current shift of the Malawi fisheries from large cichlids, catfishes and cyprinids to small pelagic fish presents a problem for management of fish post-harvest losses due to high perishability and low value of catch (Chiwaula et al., 2017). This is because all the small forage fishes are either sold sun-dried or smoked using poor processing methods with a lot of limitations (Likongwe, Kasapila, Katundu, & Mpeketula, 2018). Recognition of the important problem in which fish loss poses is reflected in the FAO Code of Conduct for Responsible Fisheries which promotes fish loss reduction (FAO, 2012). Hence, improved processing methods are required for enhancing quality fish products. This is also a means of increasing the supply of fish products in the value chain even without increased landings (FAO, 2010).

2.3 Microbial contamination

Live fish resist bacterial attacks except in the visceral organs and the respiratory area (Daramola, Oluwayemisi, & Fasakin, 2007). Fish and fish product contamination occurs immediately after the catch, during processing and storage (Macé, et al., 2014). Products such as fish have the potential to carry a lot of microorganisms (Evance, Kapute, Tembo, Phiri, & Holm, 2019). Over 600 million people are vulnerable to food borne diseases (WHO, 2015). The common pathogenic microbes responsible for fish contamination are *Shigella*, *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus* as well as *Vibrio* (Evance, Kapute, Tembo, Phiri, & Holm, 2019). These microbes can remain in fish products and further facilitate spoilage, eventually resulting in quality deterioration of the product. The acceptable threshold of microbial load in colony forming units/gram for fresh products is 5.0×10^{5}. However, the normal threshold for dried fish products is placed at $1.0\times10^{6\text{-}7}$ (Goulas & Kontominas, 2005). As an extremely perishable product, fish needs proper processing methods to prevent spoilage and microbial contamination that would greatly affect consumers who depend on it as a source of nutrients (Nguyen, Arason, & Eikevik, 2014).

2.3.1 Specific spoilage bacteria

Studies have shown that gram-negative psychrophilic or psychrotrophic organisms are key agents of fish spoilage (Arason, Thorarinsdottir, & Thorkelsson, 2013). However, accurate categorization of these organisms has been rather difficult and has varied significantly following new developments in methodology (Mostafa & Ayimba, 2014). Five spoilage bacteria species are known to affect different fish products including cooked and whole tropical fish stored in modified atmosphere packaging (Lone, et al., 2002). Common spoilage bacteria species include *Shewanella baltica*, *Carnobacterium maltaromaticum*, *Aeromonas salmonicida*, *Vibrio* species, *Gamma-Proteobacteria* containing one strain of *Pseudoalteromonas sp*. and one strain of *Psychrobacter* species. *C. maltaromaticum* and *S. baltica* are considered major spoilage bacteria for fish (Sabrina, et al., 2014). Therefore, analysis of specific spoilage bacteria in new processing methods such as the use of different brine solutions on the quality of small fish species remains crucial in ensuring safe fish products along the value chain of the pelagic fishery.

2.4 Biogenic amines

Biogenic amines are low molecular weight alkyl- or aryl-substituted derivatives of organic bases occurring in foods. These products are formed during the removal of carboxyl group and subsequent release of carbon dioxide in fish products (Guo *et al*., 2011). One of the common amines is tryptamine formed from tryptophane (Kim *et al*., 2011). Development of by-products and growth of microorganisms during post-harvest handling and during the storage of fish at ambient temperature are possible food safety risks (Huss, 1995). These products have potential to cause acute intoxications in the fish which can be harmful to consumers.

In certain fish species, including Atlantic mackerel, the development of biogenic amines is facilitated by bacteria that produce enzymes that decarboxylate amino acids to form toxic biogenic amines which are stable at a higher temperature (Shalaby, 1996). The limit value for histamine in fish products in European Union countries is 200 mg/kg (Saeed & Howell, 2002). Processing methods such as open sun drying pose high risks of contamination by both pathogenic and biogenic amine forming microbes. High moisture content after drying also facilitates the proliferation of pathogenic microorganisms as well as microorganisms responsible for biogenic amine formation in fish products (Macé, et al., 2014). In most rural areas, there is a significant potential for contamination of fresh products along the value chain (Taulo et al., 2008). This is due to a lack of cooling systems like refrigeration as few consumers processors cannot afford refrigeration due to poverty. Consequently, improved processing methods must reduce the likelihood of product contamination and eventually increase the shelf life of smoked fish products.

2.5 Water activity

Water activity is a term used to describe the freely available water for microorganisms to multiply; its measuring value is called water activity value (a_w) (Novasina, 2005). It measures the water in food products capable of facilitating reactions. It gives the ratio of partial pressures of water above food to the pure water under the same conditions (Arason, Thorarinsdottir, & Thorkelsson, 2013). Water activity is influenced by moisture content as a function of composition and temperature (Arason, 2003). A portion of the water content present in a product is strongly bound to specific sites on the chemicals that comprise the food product (Nguyen, Arason, & Eikevik, 2014). These site locations include the hydroxyl groups of polysaccharides, carbonyl and amino groups of proteins and polar sites like hydrogen bonds, ion-dipole bonds, and strong chemical bonds tightly bound water. Therefore, fish processing methods must lower the availability of water for specific spoilage microorganisms. Some groups of fungi which commonly contaminate processed fish products are also affected by water activity (Macé, et al., 2014). Moisture content can be used to predict the stability and safety of processed products with an emphasis on total viable counts, specific spoilage microorganisms and chemical deterioration reactions (Nguyen, Arason, & Eikevik, 2014). Water activity in food science is applied in product development, quality control and food safety along the value chain (Arason, Thorarinsdottir, & Thorkelsson, 2013). Consequently, processing methods must ensure that the final moisture content is below the point at which microorganisms grow. This is because moisture content serves an important role in determining the safety of the processed product when used as a control measure in quality control aspects of the Hazard Analysis Critical Control Point (HACCP – ISO 9001).

2.6 Lipid oxidation

Pelagic fish species such as Atlantic Mackerel (*Scomber scombrus*) and Atlantic Herring (*Clupea harengus*) tend to have high lipid contents which facilitate rancidity during storage (Connel, 1995). Oxidation limits storage time and thus also affect the marketing and distribution of food products along the value chain. Although this is a primary deteriorative reaction in many types of food, it is particularly critical in fish because of the high content of n-3 polyunsaturated fatty acids (Secci & Parisi, 2016). The free radical mechanism of lipid oxidation involves three major steps; initiation, propagation, and termination steps (Anna, Bjørkevoll, & Sigurjón, 2010). Lipid oxidation starts when oxygen reacts with unsaturated fatty acids between reactants and intermediates (Figure 5). The process results in the formation of hydroperoxide expressed as peroxide value (PV) which are primary oxidation products (Cyprian, et al., 2015). This is followed by the development of thiobarbituric acid-reactive substances (TBARS) which are secondary oxidation products as well as other tertiary products. In principle, peroxide value (PV) remains an indicator of the level of oxidation that has taken place and is used to determine primary oxidation of lipid in processed fish products (Anna, Bjørkevoll, & Sigurjón, 2010). The breakdown of hydroperoxide and further oxidation gives off substances which are responsible for rancid flavour in fish products (Cyprian, et al., 2015). Consequently, these effects must be assessed instantaneously to reflect a realistic model of the most likely pathway of lipid oxidation in smoked fish products to ascertain its quality along the value chain.

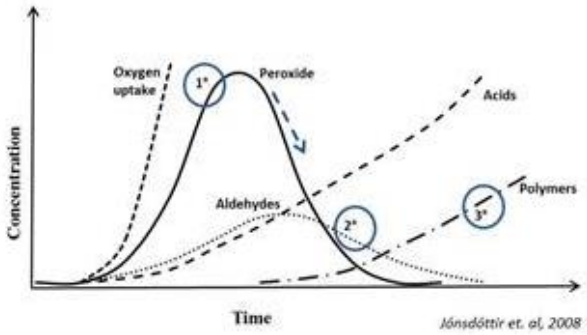

Figure 5 Process of lipid oxidation in fish products (Johnsdottir et al., 2008)

2.7 Fish processing methods in Malawi

2.7.1 Open sun drying

Open sun drying is used for all fish species. The process is facilitated by natural conditions with fresh fish products placed on racks raised above the ground (Banda, Chigwechokha, Msiska, & Simbeye, 2017). Drying is a complex process that involves instantaneous paired passing heat, mass and momentum transport where heat penetrates the fresh fish muscle. Water is then removed in the process resulting to decrease water activity in fish muscle. Water activity is of great importance for food preservation including fish. It is used to provide a dependable evaluation of microbial growth, lipid oxidation, non-enzymatic and enzymatic activities of food products (Likongwe, Kasapila, Katundu, & Mpeketula, 2018). Open sun drying has some limitations since the products are prone to fecal contamination and to cross-contamination (figure 6) (Banda, Chigwechokha, Msiska, & Simbeye, 2017). Also, case

hardening in which the surface of fish products dries and forms a thin layer while allowing the fish deeper underneath to remain soft (Arason, Thorarinsdottir, & Thorkelsson, 2013). This allows the multiplication of microorganisms such as spoilage and pathogenic microorganisms, hence facilitating degradation of the products (Mgwede, Msiska & Kapute, 2018).

Figure 6 Contamination by flies in open sun drying method

2.7.2 Smoking

Smoking processing method targets both small and big fish species including *Engraulicypris sardella*, *Lethrinops*, *Copadichromis* and *Diplotaxodon* species (Likongwe, Kasapila, Katundu, & Mpeketula, 2018). The importance of fish smoking is two-fold as it includes preservation and value addition through improved taste and product diversification (Arson, 2003). However, the final product can be compromised due to multiple handling steps (Mgwede, Msiska, & Kapute, 2018). Some smoking facilities use too much wood for a small amount of product thereby increasing deforestation - a drawback in the fight against climate change.

2.7.3 Deep frying

Deep frying is used for small pelagic fish species like *Copadichromis* and *Diplotaxodon* species. If the temperature of the oil is not controlled, it creates possibility of nutrients degradation. Furthermore, processors have the tendency of re-using the oil for process hence increasing the safety risk of the final product (figure 7).

Figure 7 Deep frying processing method in Malawi

2.7.4 Parboiling

Parboiling is a common processing method for *Engraulicypris sardella.* The process involves partial boiling of fresh *E. sardella* in hot water (Mgwede, Msiska, & Kapute, 2018). The partially boiled products are then subjected to open sun drying. Breaking the outer layer allows better drying of the inner parts but, similarly to the open sun drying method, the parboiled products are prone to contamination with fecal materials and flies during the final drying process on the open racks. This results in a high level of microbes that pose a health threat to consumers.

2.7.5 Solar tent drying

Solar tent fish drying in the fishery of Malawi is done with all small fish species. Due to its design, the driers are governed by the convection current process (Chiwaula et al., 2018). This involves providing sufficient heat that is more than ambient heat under certain relative humidity. This increases the vapor pressure of the moisture confined within fresh fish on racks and eventually decreases the relative humidity of the drying air, hence increasing the moisture carrying capacity of the air (Banda, Chigwechokha, Msiska, & Simbeye, 2017).

2.7.6 Salting and brining

Salting and brining are common in Iceland however, there is no traditional use of salt and brine in fish processing in Malawi. Iceland has placed a great emphasis on developing innovative products and processing methods to ensure the quality and freshness of fish products. The most important fish stock for salted production is Cod (Arason, Thorarinsdottir, & Thorkelsson, 2013). Salting methods achieve the right quality to meet the expectations of consumers in different countries (Nguyen, Arason, & Eikevik, 2014). The use of brine has potential to render the fish products an unsuitable environment for microbial proliferation. The increase in the concentration of soluble substances in the product help to remove water by causing soluble substances to disperse. This is because the ionically associated water molecules of brine processed products are unavailable for use by microorganisms (Arason, Thorarinsdottir, & Thorkelsson, 2013). This creates a tendency for the ionic forces to pull water molecules from the microbial cells hence dehydrating them to the point where they die and lie dormant at the lag phase. This helps to maintain the quality and safety of processed fish products. Consequently, it is important to determine the effect of brine on quality and safety of smoked pelagic fish species such as Atlantic Mackerel with the aim of applying the method in the pelagic fishery of Malawi.

3 MATERIALS AND METHODS

About 40 kg of frozen Atlantic Mackerel (*Scomber scombrus*) were used in this experiment. The samples were purchased from Sildarvinnslan (SVN) company (Iceland) and kept in frozen storage at -25°C. Before beginning the experiments, the samples were thawed at low temperature, 0 to 4°C, for about 24 hours.

3.1 Pre-trial

The aim of pre-trial was to set up the appropriate brining time and salt concentration in the final products. Two brine concentrations 8% and 15% were made and its concentration was measured using a refractometer (Figure 8). Fresh *S. scombrus* fish were taken from the freezer, thawed at 0-4°C overnight followed by filleting and washing with water at 4°C. The fillets were immersed in 8% and 15% salt solution for 45 minutes and 7 minutes respectively at 0-4°C. After brining, the fillets were placed on a tray to drain. The samples were then smoked using an electric smoker. Temperature degrees and time were set up accordingly, samples were smoked at an initial temperature of 30°C which was drying the samples for 30 minutes. After 30 minutes, the device temperature was set at 50°C for 30 minutes this was aimed at cooking the fish samples. In the second phase of smoking the device was set at 80°C for 60 minutes. So, in general, the time duration of the smoking process was 2 hours. The smoked samples were then taken to the chemical laboratory and separately ground in a waring blender for physicochemical (salt concentration, water activity and pH) analysis. The results from the pre-trials are shown in the appendix. The sample with 8% brine concentration and 45 minutes brining time was considered in designing the main experiments because it was less salty.

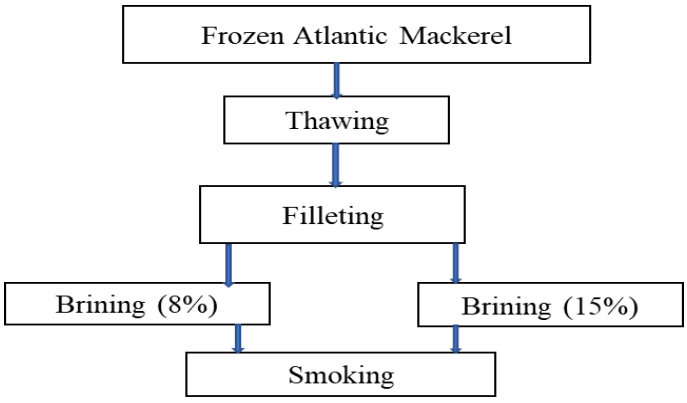

Figure 8 Flow diagram for pre-trial experiment

3.2 Sampling

The analysis of *Scomber scombrus* in the main experiments was carried out in 4 sampling points (Table 2). Samples were taken after thawing, smoking and storage temperature for analysis of TVC and SSO, physicochemical parameters (a_w, moisture content, salt content, lipid content, PV, TBARS, FFA, pH and TBARS) to determine quality, safety and best storage temperature (Figure 9).

Table 2 Analysis and sampling period for Atlantic Mackerel

Sample Type	Sampling Time						
	After filleting	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Raw material							
Un-brined smoked (20°C)							
Brined smoked (20°C)							
Brined smoked (0-4°C)							

3.3 Experimental design

To determine the quality and safety parameters on the brined smoked *Scomber scombrus* the samples, previously thawed, were divided into two sample groups brined (8%) and unbrined. The two groups of samples were smoked using an electric smoking kiln (Figure 10) at Matis. The microbiological and physicochemical analysis was carried out on the raw material (frozen *Scomber scombrus*), and on the final products during 24 days of storage as shown in the flowchart in Figure 9 below.

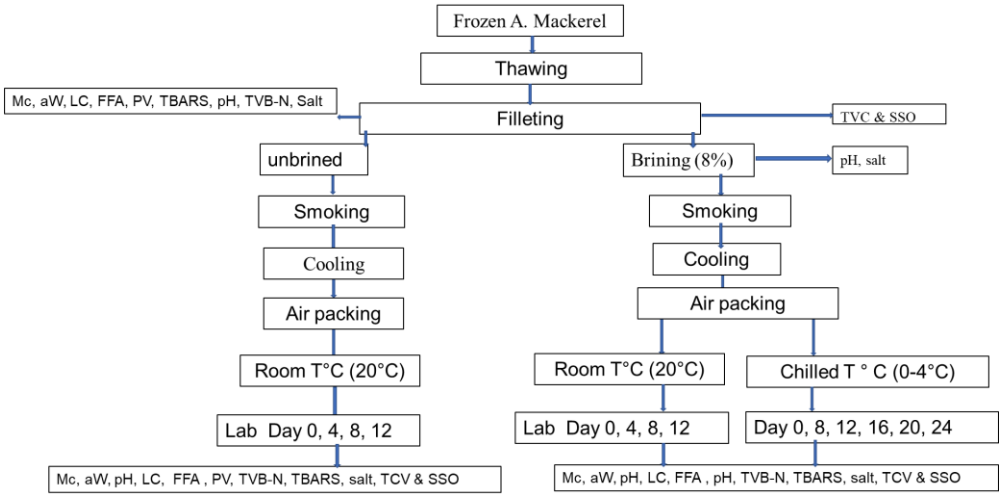

Figure 9 Process flow for experimental design and sample analysis

Figure 10 The electrical smoker equipment for sample (Atlantic Mackerel) processing

3.4 Microbiological analysis

To determine and quantify the microbial activity on the fish samples under study, two microbiological methods were assessed: Total Viable Counts (TVC) and Specific Spoilage Organisms (SSO). The analyses were done on thawed raw material and on final smoked products under storage. In this analysis, 1 ml of 1:10 dilutions were transferred using a pipette to Petri plates and melted Iron agar at 45°C poured on the plates and the content mixed to solidify. After solidification, the plates were covered with a thin layer of Iron agar then incubated at 22°C for 48 hours. All the microbiological analyses were conducted in duplicate; colonies recorded as colony forming units (cfu/g). Data was expressed as logarithm of the number of colony (white and black) forming units (log cfu/g).

3.5 Physicochemical Analysis

3.5.1 Total Volatile Base-Nitrogen (TVB-N)

The TVB-N was determined by dissolving 50 g of the *S. scombrus* samples extract with 100 ml 7.5% aqueous trichloroacetic acid in a metal beaker. This was followed by homogenizing the sample in a waring blender. The mixture was then filtered through a Whatman no 3-filter paper. 25 ml of the filtrate was pipetted into a distillation flask with 6 mL 10% NaOH. Thereafter, steam distillation was carried out using the Kjeldahl-type distillator (Struer TVN). The TVB-N was collected in 10 mL 4% boric acid (containing 0.04 mL of methyl red and bromocresol green) indicator which turned green when alkalinized by the TVB-N (Malle and Poumeyro, 1989). The solution was then titrated with 0.0407 N sulphuric acid until a complete neutralization of the base which was indicated by a colour change to pink. The TVB-N content was calculated by the following formula (equation 1):

$$\mathrm{TVB-N\ (mgN/100g)} = \frac{14\ \mathrm{mg/mol} \times \mathrm{a} \times \mathrm{b} \times 300}{25\ \mathrm{ml}} \qquad \textbf{Equation 1}$$

Where:

a: volume of sulphuric acid (ml)
b: normality of sulphuric acid

3.5.2 Salt content

The sodium chloride or salt content was determined according to (AOAC 17th ed 2000 no 976.18). This parameter was measured on thawed samples and both groups of smoked *Scomber scombrus* samples. In this analysis, 5 g of sample was weighed and put into an extraction bottle. 200 ml of deionized water was added to the sample and shaken for 50 minutes. 20 ml of nitric acid was then added to 20 ml of the supernatant and titrated with silver nitrate. The salt content in the water phase represented as Z-value for smoked mackerel was calculated following the equation below;

$$Z - \text{value} = [\%X(M_c + \%\ X)] \qquad \textbf{Equation 2}$$

Where:

%X = The percentage salt content
Mc = the percentage water content in the final product

3.5.3 pH analysis

The analysis of Hydrogen Ion Concentration (pH) was performed using a pH electrode (SE 104 – Mettler Toledo Knick, Berlin, Germany) connected to a portable pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was inserted directly in grounded fish sample. The pH meter was earlier calibrated with buffer solutions of pH 7.00 ± 0.01 and 4.00 ± 0.01 at 20 °C.

3.5.4 Moisture content (Mc)

A sample of ground fish (3g) was placed in a crucible and dried at 105ºC in an oven to a constant weight after the initial weighing. Moisture content of the fish was then calculated by subtracting the initial from the final weight of the fish sample as shown in the equation below.

$$\text{Mc (\%)} = \frac{W_2 - W_1}{W_2} \times 100 \qquad \textbf{Equation 3}$$

Where:

W_2= Weight of sample
W_1= Weight of dried sample

3.5.5 Water activity analysis (a_W)

An Aqua Lab water activity meter was used to measure the water activity. Both groups of smoked *Scomber scombrus* were evaluated in terms of water content.

$$aW = \frac{P}{P_0} \qquad \textbf{Equation 4}$$

Where:

a_W= water activity
P = vapor pressure in nutrients
P0 = vapor pressure of pure water at the same temperature and conditions

3.5.6 Total lipid content

Total lipid content was extracted from samples, according to the Bligh and Dyer (1959) method. 3 mL of the lower phase resulting from the lipid extraction was added in a screw cap culture tube which had its weight recorded before adding the lower phase. This was followed by removing the solvent present at 55°C using a nitrogen jet. After cooling down, the weight of screw cap was recorded again. Lipid content was calculated as the weight difference in the amount of lipids in the 3 mL. This was then multiplied with the total volume of the chloroform used (50 mL) and divided by the weight of the sample used for the lipid extraction.

$$\text{Lipid content} = \frac{X_2 - X_1}{E} \times V \qquad \textbf{Equation 5}$$

Where:

X_1 = weight of empty screw cap
X_2 = the weight of screw cap after nitrogen jetting process
E = sample weight
V = volume of chloroform used

3.7.7 Free fatty acids analysis (FFA) analysis

The free fatty acid (FFA) content was determined by Lowry & Tinsley (1976) method with modification by Bernárdez et al. (2005). This parameter was measured on both groups of smoked *Scomber scombrus* and raw material.

$$\text{FFA} = \frac{Y}{E} \times 282.46 \times 1 \times 10^{-6} \times 100 \qquad \textbf{Equation 6}$$

Where:

Y= Oleic acid is the oleic acid amount in µmol
282.46 = Molecular weight of oleic acid
E = Sample weight is the g lipid in the sample

3.5.7 PV analysis

Sample weighing 5 g was placed into 50 mL tubes with a red screw cap. 10 mL of ice-cold solvent was added into the tubes and homogenized at 6000 rpm for 10 seconds. 5 mL of the sodium chloride solution was added to the homogenized content. This was followed by centrifuging the content at 5100 rpm (2350 *g*) for 5 min at 4 °C. 3 mL of the bottom layer was collected and transferred using a pipette into a 15 mL tube (with red screw cap). 500 µL of the bottom layer was added to Eppendorf tubes followed by 5 µL of ammonium thiocyanate and ferrous chloride solution with a ratio of 1:1. The content was vortexed and

allowed to stand for 10 min at room temperature.100 µL was placed in the PP microplate and read at 500nm. The results were calculated using the following equation:

$$\text{PV (mmol/kg)} = \frac{W}{E} \times \frac{X_1}{X_2}$$ **Equation 7**

Where:

PV = Lipid hydroperoxide (mmol/kg)
W= The cumene peroxide amount (µ)
X_1= Amount of chloroform used (5 mL)
X_2= Chloroform collected chloroform (0.5)

3.5.8 Thiobarbituric Acid Reactive Substances (TBARS) analysis

To measure thiobarbituric acid-reactive substances (TBARS) Lemon (1975) method with modifications was used. This parameter was measured on both groups of brined and unbrined *Scomber scombrus* smoked samples.

$$\text{TBARS (µmol/kg)} = \frac{P}{E} \times \frac{S+M_c}{X} \times 1000$$ **Equation 8**

Where:

P= Malmoldedyde amount (µmol)
E = Sample weight (g)
S= Amount of TCA used (10 mL)
Mc= Moisture content in the sample (mL)
X= Supernatant amount collected after centrifugation (0.5 mL)

3.6 Data analysis

Microbiological data were log transformed to give a clear picture of the phase growth for microorganisms in smoked products under different storage conditions. Physicochemical data were analysed using Microsoft Excel 2013 to develop graphs for clear evolutions against the storage period.

4 RESULTS

This chapter reports the microbiological content for TVC and SSO and physiochemical changes in terms of Total Volatile Base-Nitrogen, salt content, free fatty acids, fat content, pH, moisture content and water activity of brined and unbrined smoked *Scomber scombrus*. These parameters represent the quality and safety of *S. scombrus* under storage.

4.1 Total Viable counts (TVC)

Changes in total viable counts in brined and unbrined smoked are shown in figure 11 below. Higher total viable counts were observed in smoked samples stored at 20°C for both brined and un brined smoked *S. scombrus* log 7.18 cfu/g and log 7.26 cfu/g respectively. Total viable counts at day 4 were above acceptable norms of log 6.0 cfu/g for smoked fish products. Visible moulds growth were observed on day 8 indicating complete spoilage of samples of smoked product stored at 20°C. Brined smoked *S. scombrus* stored at 0-4°C had low total bacteria counts along the storage period with higher population log 8.1cfu/g on day 20. It is apparent that smoked products stored at an ambient temperature of 20 °C pose high risk to consumers as it has the potential to cause infection and intoxication.

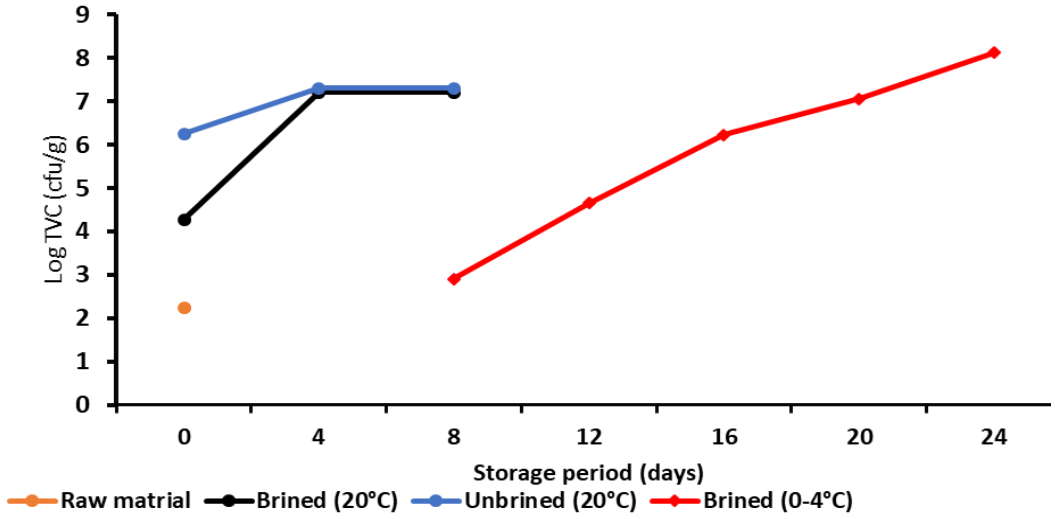

Figure 11 Total viable counts in smoked *S. scombrus* during storage

4.2 Specific spoilage organisms

The level of specific spoilage microorganisms in smoked *S. scombrus* during storage remained log 1.3 cfu/g in both groups of samples. This indicates that the samples were aseptically handled, and there no cross-contamination of the products during handling and smoking.

4.3 Total Volatile Base-Nitrogen (TVB-N)

Level of TVB-N mg/100g for brined and unbrined smoked *S. scombrus* stored at 20°C range were 16.90-137.20 mg/100g and 21.20-224.90 mg/100g respectively (Figure 12). The level of TVB-N for *S. scombrus* stored at 20°C was above the acceptable norms for smoked fish

products and was deemed unsafe for consumption at 8 days of storage. It was observed that TVB-N levels increased with storage time, but the increase was very noticeable in unbrined than brined samples, indicating brine has a positive effect on smoked *S. scombrus*. TVB-N for smoked samples stored at 0-4°C was lower and remained below the acceptable limit for throughout the storage period.

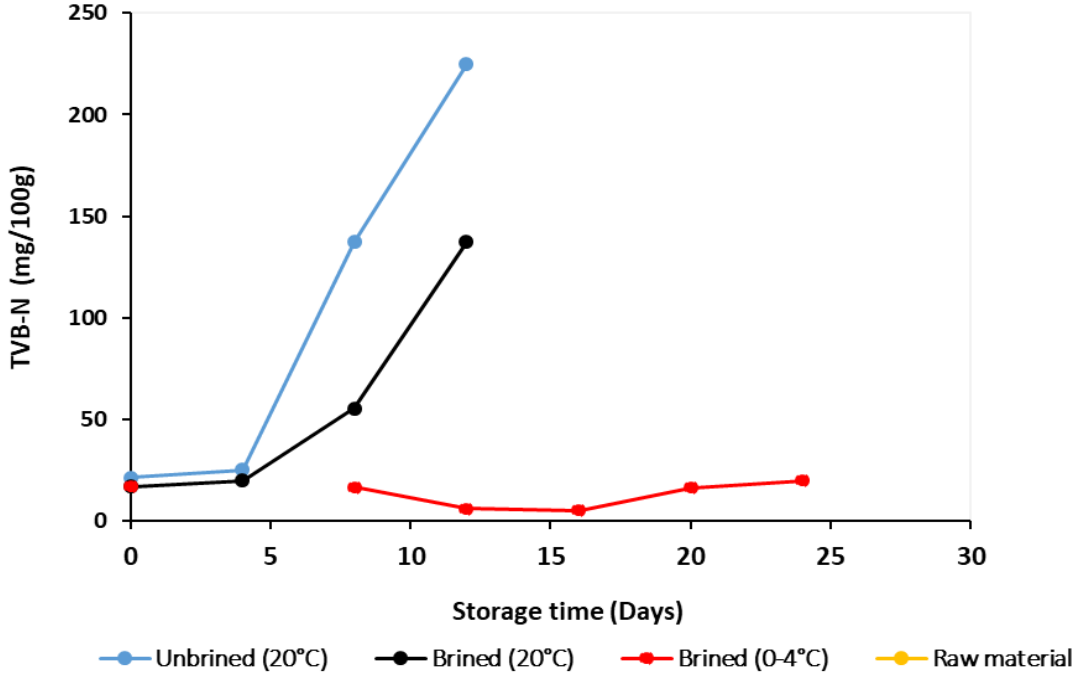

Figure 12 Level of TVB-N during storage period

4.4 Salt concentration

The pattern of salt concentration remained stable during the storage time (Figure 13). The salt content of brined smoked S. *scombrus* was higher than that of unbrined *S. scombrus*. However, no significant ($p>0.05$) differences in salt content were found between the groups throughout the storage period. The salt content in samples stored at 0-4°C was highest, followed by samples stored at 20°C and the least value was for unbrined samples stored at 20°C since the samples were not brined prior to the smoking process.

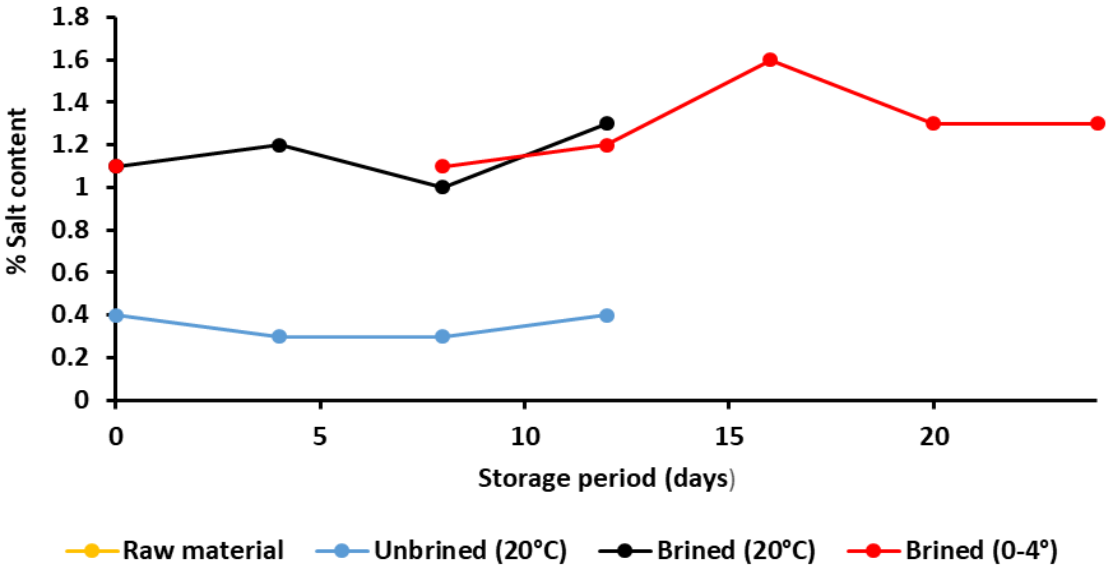

Figure 13 Percentage salt concentrations in smoked samples

4.5 Hydrogen Ion Concentration (pH)

Figure 14 shows the level of hydrogen ion concentration for brined and unbrined smoked *S. scombrus* during storage. The pH values were found to vary from 6.3-6.7 and 6.4-7.2 for brined and unbrined smoked *S. scombrus* stored at 20°C. The pH values of smoked products stored at 20°C showed a gradual increase with the storage period up to day 12. pH values for brined smoked products stored at 0-4°C varied from 6.2-6.1 on day 24 of storage.

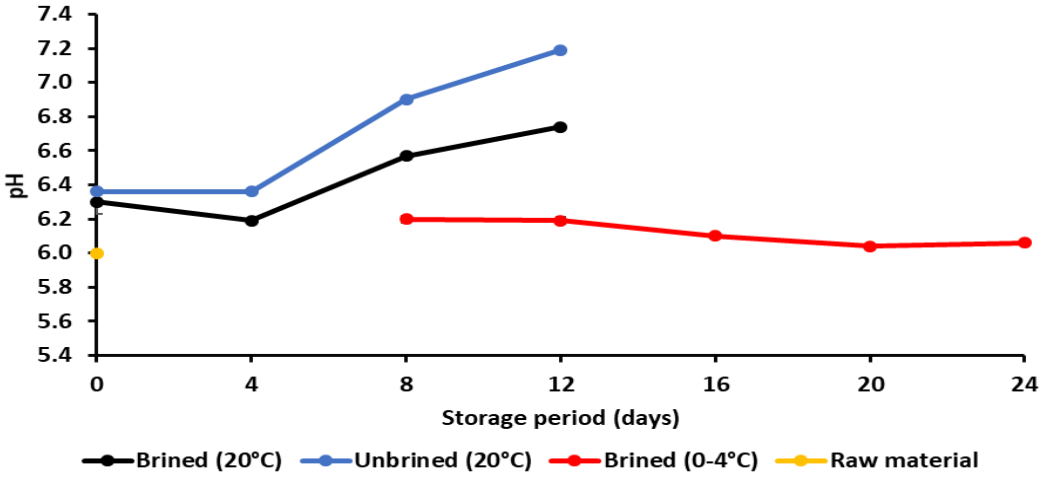

Figure 14 Changes in pH for samples during storage period

4.6 Moisture content (MC)

The moisture content of *S. scombrus* (brined and unbrined) decreased significantly ($p<0.05$) after the smoking process and subsequently remained stable during the storage period. The moisture content of *S. scombrus* (brined and unbrined) stored at 20°C was stable and there were no significant differences ($p>0.05$) (figure 15). Smoked samples stored at 0-4°C had lower moisture content than samples stored at 20°C and the level remained stable throughout the storage period.

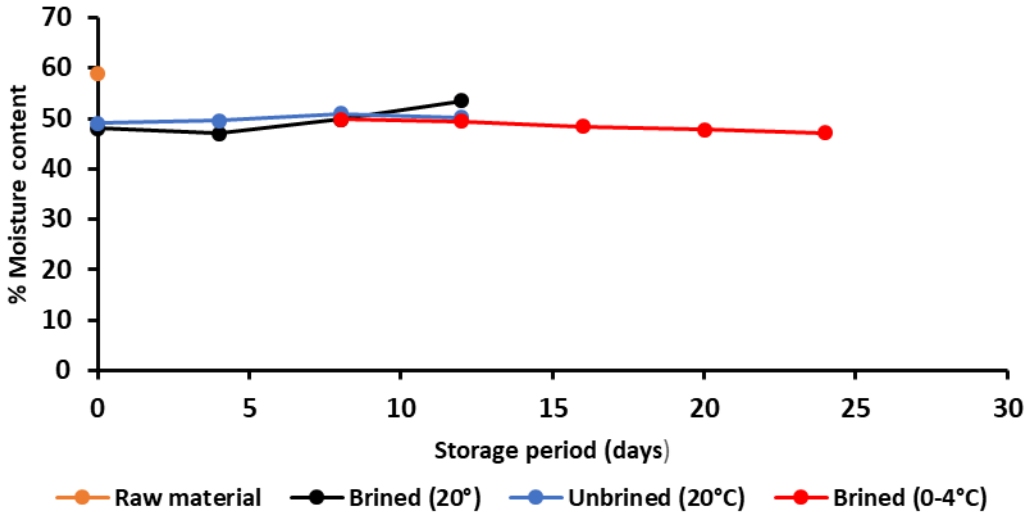

Figure 15 Level of moisture content in smoked fish samples during storage

4.7 Water activity (aw)

Level of water activity for raw material and smoked *S. scombrus* is presented in figure 16 below. The water activity after smoking brined and unbrined *S. scombrus* stored at 20 °C ranged from 0.98-0.99, 0.99-1.0 and 0.98-0.99 for samples stored at 0-4°C. The water activity was significantly different ($p<0.05$) for unbrined and brined smoked *S. scombrus*. This was marginally lower than that of the raw material, which was 0.999 however, it showed no significant loss ($p>0.05$). Water activity was stable during storage period for all groups.

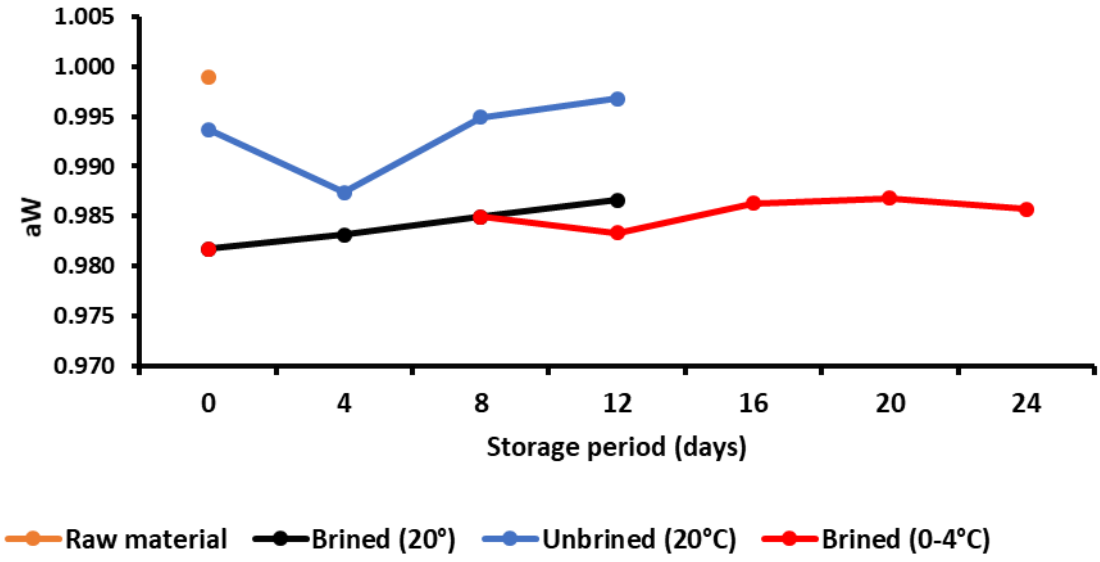

Figure 16 Level of water activity for smoked product during storage period

4.8 Lipid content

The lipid content of smoked *S. scombrus* smoked (brined and unbrined) samples was relatively stable after processing and storage (figure 17). There was an increase in lipid content of smoked samples and a decrease in day 4 and then an increase after 8 days of

storage. However, both groups had a stable evolution of lipid content during the storage period. The lipid content of raw material was lower than the smoked samples. The lowest lipid content was obtained in brined smoked *S. scombrus* but no significant ($p>0.05$) differences in lipid content were found between the groups.

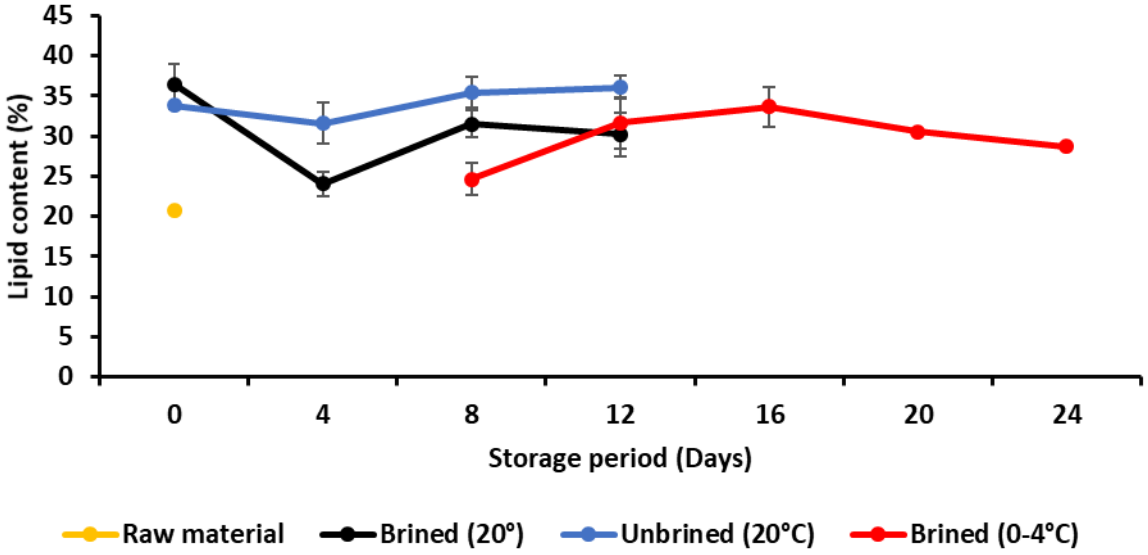

Figure 17 Lipid content of smoked *S. scombrus* under storage

4.9 Free fatty acids analysis (FFA)

Figure 18 shows the level of free fatty acid during the storage period. The level was not significant ($p>0.05$) differences between the groups. The free fatty acid in smoked *S. scombrus* ranged from 1.0-1.6 FFA/100 g lipids, 0.9-2.2 FFA/100g lipids, 1.0-0.8 FFA/g lipids for unbrined and brined stored at 20°C respectively. Brined smoked *S. scombrus* had stored at 0-4°C had a low level of FFA ranging from 1.4-0.8 FFA/g of lipids during the storage period. Raw material had 3.50 FFA/100g lipids of and was the highest.

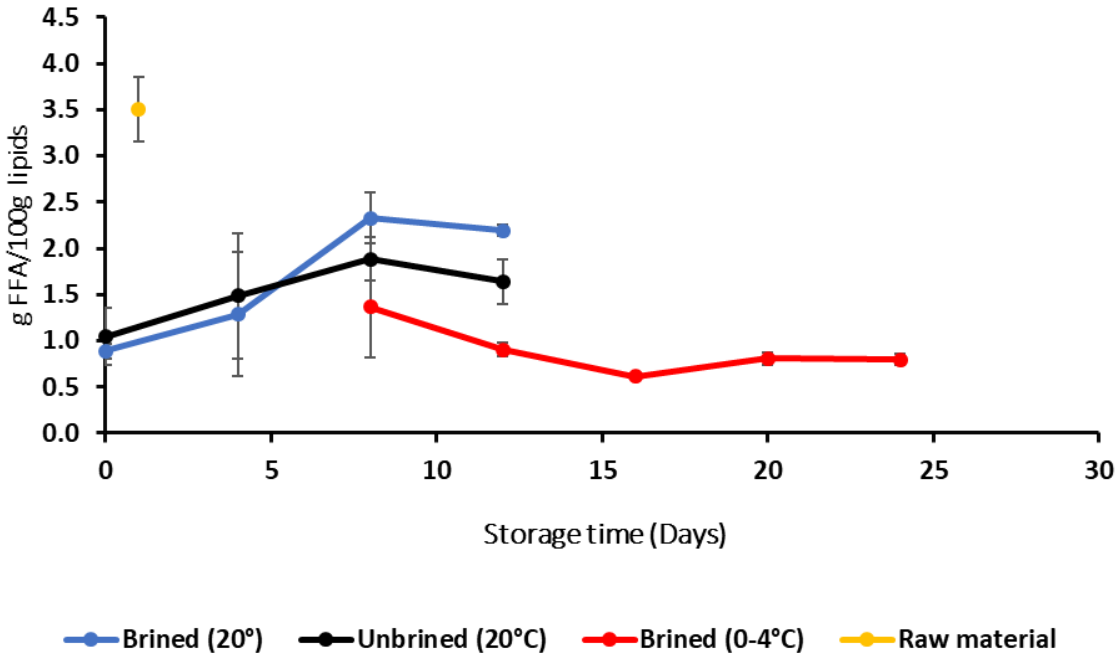

Figure 18 Level of free fatty acids during storage period

4.10 Lipid hydroperoxide value (PV)

Lipid hydroperoxide (PV) formation, a primary oxidation product, the analysis was done on 0 to day 4 in samples stored at 20°C (figure 19). Hydroperoxide value production appeared to be much higher and more rapid in unbrined than brined smoked *S. scombrus*.

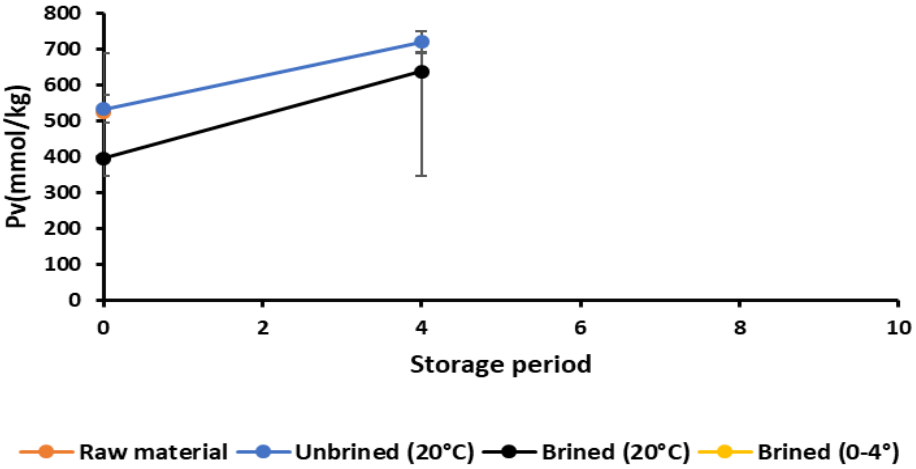

Figure 19 Level of hydroperoxide value (PV) during storage period

4.11 Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) shows the level of secondary oxidation in smoked *S. scombrus* during storage (figure 20). Result shows that between days 8 and 12 there are no significant differences ($p>0.05$) in samples stored at 20°C and 0-4 °C. From day

16 to day 24, there is an increase in secondary oxidation for samples stored at 0-4°C, but the process does not reach the peak level.

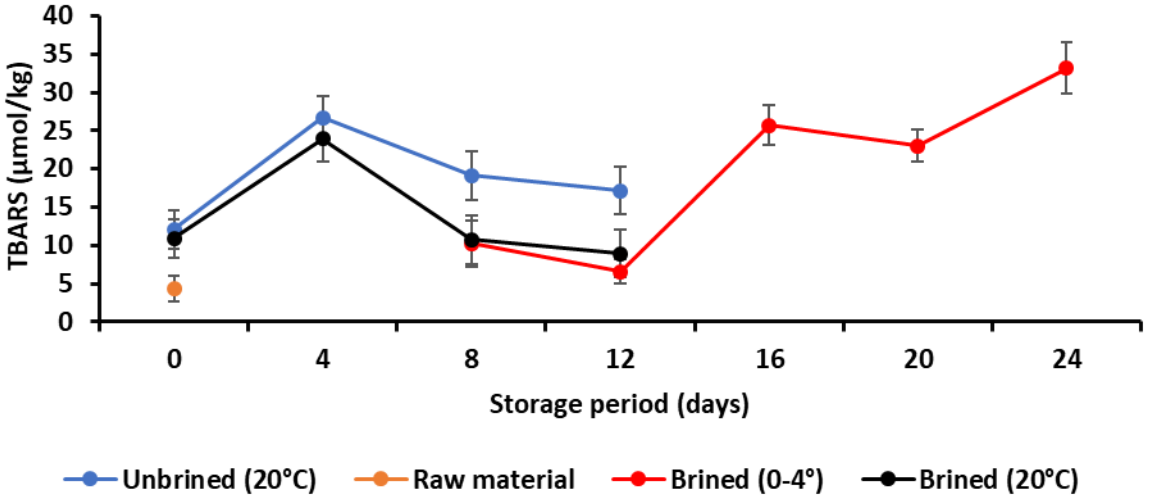

Figure 20 Level of thiobarbituric acid reactive substances (TBARS) during storage of smoked *S. scombrus*

5 DISCUSSION

This study focused on the quality and safety of brined and unbrined smoked *Scomber scombrus* under different storage conditions for 24 days. The analysis was conducted to determine microbiological and physiochemical changes on the treatments. This was because the various spoilage and quality changes in smoked fish products that occur during storage are known to lead to changes in these indices.

Low initial total viable count on day 0 of brined smoked *S. scombrus* indicates good fish quality. Microbial analyses showed an exponential phase at day 4 to 5 which was the day of complete spoilage for unbrined and brined smoked *S. scombrus* stored at 20°C. The values fall above the log 7.0 marginally accepted quality (Anna, Bjørkevoll, & Sigurjón, 2010). This means that TVC in brined and unbrined smoked *S. scombrus* under storage 20°C exceeded the proposed international limits for the evaluation of the shelf life of fish and fishery products. Studies have shown that enzymatic and microbiological activity is greatly influenced by temperature (Sabrina, et al., 2014; Antoine, Bohuon, Deumier, & Poligne, 2000). However, in the temperature range from 10 to 20°C, microbiological activity is relatively more important, and temperature fluctuations have a greater impact on microbiological growth than on enzymatic activity. This is evidenced by higher total viable counts in brined and unbribed smoked stored at 20°C. These bacteria populations are likely to prove difficult to control once smoked products are with consumers. This is a cause for worry in Malawi as product quality and safety awareness is not a significant concern as trade in fish and fish products increases, due to increased demand for fish among consumers (Chiwaula, Chirwa, Binauli, Banda, & Nagoli, 2018).

The brined smoked *S. scombrus* stored at 0-4°C had lower total viable counts during the whole storage period and remained safe for consumption to day 20 of storage. Total viable count levels for samples stored at 0-4°C were above the acceptable limit on day 24 and the level was determined as log 8.1 cfu/g. With these values, temperature storage conditions have an inhibitory effect on the total aerobic flora. The storage condition of 0-4°C is vital due to two factors governing the point at which microbiological growth stops. The reaction rates for the individual enzymes in the bacterium become much slower, low temperature reduces the fluidity and volatility of the cytoplasmic membrane. This leads to interfering with transport mechanisms and eventually controlling the microbial growth in the smoked products thereby preserving it over more days. It is apparent that storing brined smoked pelagic fish species at low temperatures has the potential to extend the shelf life of the products. Therefore, in view of improving the limitations of current fish processing methods in Malawi through smoking, brining prior to smoking must be adopted. Also, brined smoked products must be at a lower temperature to increase storage life.

Total volatile base nitrogen (TVB-N) is an important compound providing a measure of the progress of spoilage that is reliant on sensory assessment (Goulas & Kontominas, 2005). The development of TVB-N as spoilage indicators in smoked fish products is due to a combination of microbiological, chemical, enzymatic and physical events (Arason, Thorarinsdottir, & Thorkelsson, 2013). The concentration of TVB-N in freshly caught fish vary between 5 and 20 mg/100 g (Saeed, 2009). In this study, the concentration of TVB-N for fresh *Scomber scombrus* was 14.2 mg/100g. Quality fish products have TVB-N values of up to 25 mg/100 g, ‘‘good quality” has values of up to 30 mg/100 g and the ‘‘limit of acceptability” goes up to 35 mg/100g, and ‘‘spoilt fish” has above 35 mg/100 g (Goulas &

Kontominas, 2005). TVB-N contains the total amount of volatile nitrogen bases with nitrogen which is synthesized by the reaction from protein and includes ammonia, monomethyl amine, dimethylamine, sulphur compounds, and trimethylamine (Daramola, Oluwayemisi, & Fasakin, 2007). TVB-N reflects the extent of degradation of proteins and non-protein nitrogenous compounds hence producing ammonia (Cakli, Berna, Tolga, & Sebnem, 2006). The degradation of lipids in fatty fish generates rancid odours. Marine fish and some freshwater fish contain trimethylamine oxide (TMAO) that is degraded by several spoilage bacteria to trimethylamine (TMA) (Goulas & Kontominas, 2005). Trimethylamine compound responsible for fishy off odours in processed fish products under storage (Saeed, 2009). In this study, there were significant differences in the level of TVB-N among the groups. The unbrined smoked *S. scombrus* kept at 20°C group were above critical limits of consumption on day 4 while brined smoked *S. scombrus* had 8 days of storage life, that is an addition of 4 more days. This indicates that brine has an effect in extending the storage life of smoked *S. scombrus*. The level of TVB-N brined *S. scombrus* 0-4°C remained below the acceptable limit for the whole 24 days storage period. This is clear evidence that brine and lower temperature 0-4°C has a substantial effect in extending the storage life of smoked *S. scombrus*, due to a reduction in the rate of enzymes during the protein degradation process. Enzymes are susceptible to temperature, low temperature lead to loss of their activity in brined smoked *S. scombrus* stored at 0-4°C. Apparently, it can also be observed that the formation of TVB-N where primarily enthalpy and entropy driven.

Salting of fish can allow prolonged storage due to lowered water activity, among other factors. Brine smoked *S. scombrus* had its storage life extended for 1 day from 4 days, a clear indication of the effect of brine in smoked *S. scombrus*. The available salt content rendered the medium unsuitable environment for microbial proliferation (Antoine, Bohuon, Deumier, & Poligne, 2000). Increased concentration of soluble substances in brined smoked samples causes soluble substances to disperse. This eventually deprived water availability for microbes. This is because, the ionically associated water molecules of brine smoked product were unavailable for use by micro-organisms (Cakli, Berna, Tolga, & Sebnem, 2006). In this regard, there was a tendency for the ionic forces to pull water molecules from the microbial cells and dehydrating them to the point, where they die and lie inactive at the lag phase. Also, brining reduced the pH level of smoked fish products under storage, hence reducing the degradation of protein (Anna, Bjørkevoll, & Sigurjón, 2010). Lipid oxidation has been reported to increase proportionally to increase in brine concentrations and storage time in sardine and *S. scombrus*. Also, brining and storage temperature has shown to affect the lipases activity, hence affecting the degradation process of smoked *S. scombrus* under storage.

pH is an indicator of the extent of microbiological spoilage in products and other proteolytic microbes producing acid after decomposition of carbohydrates (Romotowska, et al., 2016). The pH value is a reliable indicator of the degree of freshness and spoilage of fish products. Decomposition of nitrogenous compounds leads to an increase in pH for fish products under storage (Goulas & Kontominas, 2005). Apparently, the increase in pH indicates the loss of quality for smoked fish products. In this study, the pH value of the raw material was 6.0. Smoked fish products are acceptable up to a pH of 6.8 but are spoiled above pH 7.0. Samples stored at 0-4°C had stable pH during the storage period. Although the initial pH values of smoked *S. scombrus* were like the findings of other researchers, it showed a gradual increase with the storage period at room temperature (Anna, Bjørkevoll, & Sigurjón, 2010) (Hans, 995). The probable reason for these differences is differences in fish species. The decline was

due to the accumulation of end products of spoilage of both alkaline and acidic nature which tend to neutralize each other (Arason, Thorarinsdottir, & Thorkelsson, 2013). The increase in pH for samples stored at 20°C coincided with an increase in Total Viable Counts in this study. This explains the rapid spoilage and reduced shelf life in unbrined smoked and brined smoked *S. scombrus* stored at 20°C. A critical linkage between increased pH and spoilage of smoked *S. scombrus* at 20°C is that it favoured more microbial activity, hence high total viable counts.

Water activity remains a critical standard for the assessment and control of fish product quality and safety (Saeed, 2009). It describes the range of energy states of the water in fish products and the relationship of water activity to the moisture content in a non-linear known as a moisture sorption isotherm curve (Romotowska, et al., 2016). Processing such as smoking decreases the water activity in the final fish product and becomes crucial for storage (Odoi, 2014). The water activity determines the storage life of fish and fish products along the value chain. It is apparent that the microbial and chemical stability of fish and fish products depends on the (aw) of the product. Water activity for freshly fish was 0.999 and was decreased through brining and smoking. This resulted in a reduced rate of microbial population growth. Nguyen, Arason, & Eikevik (2014), reported that microbiological and physicochemical stability of fish products after smoking and storage is extremely dependent on water activity contents. The fish products with a water activity of less than 0.7 are microbiologically stable. The free or available water in food supports microbial growth and participates in and supports chemical and enzymatic reactions and spoilage processes (Saeed & Howell, 2002; Arason, Thorarinsdottir, & Thorkelsson, 2013). Furthermore, chemical and biochemical reactions are affected by the amount of available water. Consequently, one of the effects of reducing the water activity in smoked *S. scombrus* is to reduce the rate of these reactions.

In this study, there was different lipid content among the different in smoked samples. This might be due to the influence of brine concentration and on lipid oxidation to its components after processing and during storage (Arason, Thorarinsdottir, & Thorkelsson, 2013) (Romotowska, et al., 2016). Fish oils are rich in highly unsaturated fatty acids, which are susceptible to oxidation resulting in the formation of free radicals and hydroperoxides (Romotowska, Karldottir, Gudjonsdottir, Kristinsson, & Arason, 2016). The brined smoked *S. scombrus* had a lower percentage of total lipid than unbrined *S. scombrus*, this can be attributed to the effect brine which increased the lipid loss during smoking. The result of total lipid in *S. scombrus* shows a significant difference among the treatment at different sampling times. Total lipids dropped sharply on day 4 in samples stored at 20 °C, this might be due to more lipids oxidizing into primary and secondary products as indicated by higher free fatty acids (Nguyen, Arason, & Eikevik, 2014).

Lipid hydroperoxides are primary products of lipid oxidation in smoked *S. scombrus* (Nguyen, Arason, & Eikevik, 2014). The breakdown of hydroperoxides generates several secondary products like organic acids, aldehydes, ketones, alcohols and hydrocarbons (Cyprian, et al., 2015). Products of hydroperoxide breakdown are recognized as secondary oxidation products. In the sampled days for this study, there was an increase in hydroperoxide value observed in unbrined than brined smoked *S. scombrus*. The increase in hydroperoxide was due to the generation of primary products caused by the presence of oxidation initiators such as enzymes (Romotowska, Karldottir, Gudjonsdottir, Kristinsson, & Arason, 2016). It is apparent that the storage of untreated samples at higher temperatures drives oxidation during

the early stages of oxidation process. This, therefore, calls for storing smoked samples at 0-4°C as a measure of reducing enzymatic activity responsible for the oxidation of smoked *S. scombrus*. This will enhance the quality and safety of smoked products along the value chain.

Free fatty acid is a tertiary product of rancidity in fish products and its level increases through storage (Cakli, Berna, Tolga, & Sebnem, 2006). The free fatty acid is a measure of hydrolytic rancidity - the extent of lipid hydrolysis by lipase action (Romotowska, et al., 2016). The smoked *S. scombrus* showed lower levels of free fatty acids than raw material, this shows that the smoking process had an effect in reducing the oil leading to loss of free oil resulted hence loss of some free fatty acids in the process. Increased accumulation of the free fatty acids during the storage of smoked *S. scombrus* at 20°C is a result of enzyme activity particulary lipase and phospholipase enzymatic (Romotowska, et al., 2016). Free fatty acid content shows that there was an extensive breakdown of lipid of smoked *S. scombrus* under 20 °C unlike brined smoked samples stored at 0-4°C. Total lipid content was decreasing with storage time whereas free fatty acid was increasing with storage time for sample stored at 20°C than samples stored at 0-4°C. This is might be because of the temperature of the activity of enzymes. This relationship of total lipid to free fatty acid echoed the previous study for *S. scombrus* stored at chilling temperature. This study has also shown that enzymatic action in smoked *S. scombrus* was greatly influenced by brine and temperature. Brine and low temperature reduced the formation of free fatty acids as it reduced the enzymatic activity in smoked *S. scombrus* products under storage.

Thiobarbituric acid reactive substances (TBARS) values are usually used to measure the level of rancidity (Goulas & Kontominas, 2005). These products are mainly related to the development of secondary oxidation products (Romotowska, et al., 2016). In this study, thiobarbituric acid reactive substances increased from day 0 to day 4 and decreased at day 8 in samples stored at room temperature. The presence of brine in smoked fish products acted as a barrier against oxygen during brining, hence the oxidative process did not proceed as rapidly as expected. Although salting effectively prevents the growth of both total viable counts and specific spoilage bacteria, it is reported that salt concentration in fish muscle enhances the oxidation of the highly unsaturated lipids (Nguyen, Arason, & Eikevik, 2014). The thiobarbituric acid reactive substances level fluctuations in this study for brine smoked *S. scombrus* stored at 0-4°C. This indicates that lower temperatures reduce secondary oxidation in smoked *S. scombrus*.

6 CONCLUSIONS AND RECOMMENDATIONS

The study suggests that brining prior to smoking process and storage temperature has a significant effect on the quality and safety of smoked *S. scombrus*. This increases the quality of smoked *S. scombrus* by reducing microbiological multiplication, protein degradation and lipid oxidation. The use of brine in smoked *S. scombrus* stored at 20°C increases quality and shelf life to 5 days. However, brining and lowering storage temperature increases the storage life by 15 days. In this regard, it is recommended for adoption and out-scaling among fish processors to produce quality and safe smoked fish products in the Malawi fishery. Also, this will be vital in meeting the matching issues of harmonizing between the quality and safety of smoked fish products for the country's population while reducing post-harvest losses. Furthermore, it is important to continue this work; there is a need to conduct a consumer preference study for brined smoked fish products in the Malawi fishery value chain.

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APPENDIX

Physiochemical results for smoked samples

[Brine]%	Brine time	[Salt]%		pH		Water activity	
		A	B	A	B	Wa	°C
8	45	2.0±3	2.3±3	5.91	5.9	0.97	24.78
15	7	2.7±3	3.0±3	5.88	5.93	0.96	24.81

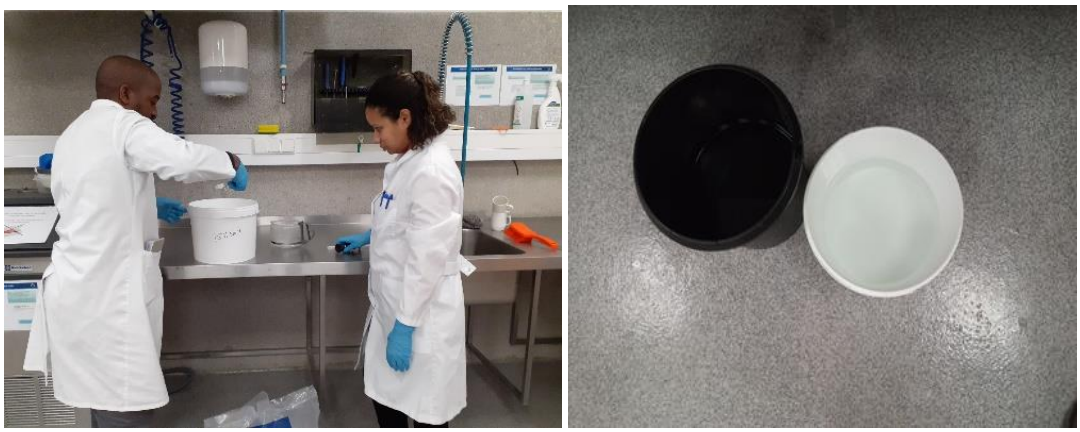

Figure 21. Process of brine formation for pre-trial experiment

Figure 22. Filleting process of Atlantic Mackerel (*S. scombrus*)

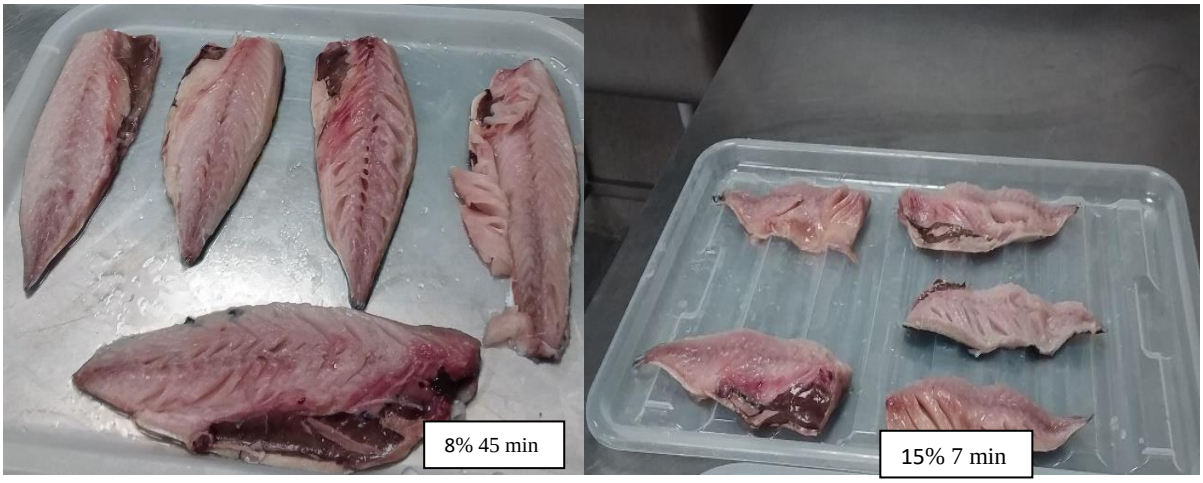

Figure 23. Two brined filleted *S. scombrus* samples

Figure 24. Smoking process of brined samples

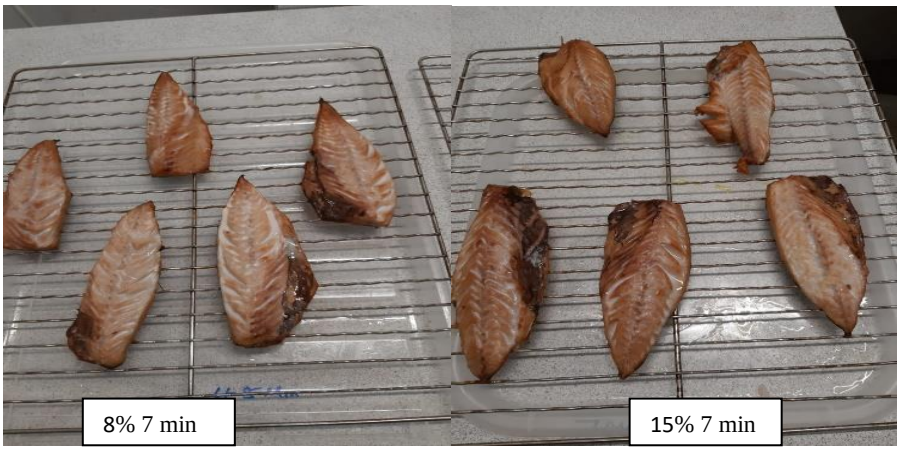

Figure 25. Smoked samples of *S. scombrus*

Figure 26. Physiochemical analysis of smoked samples

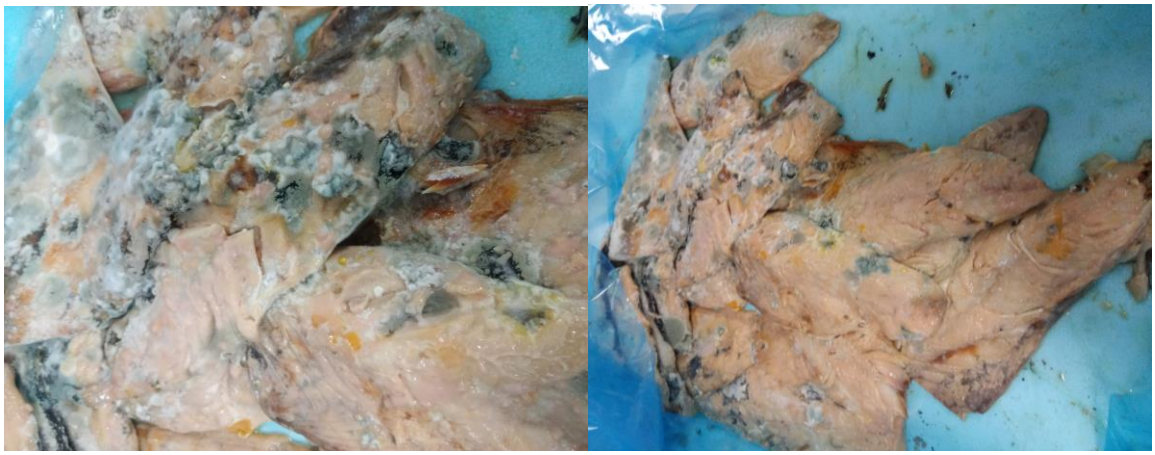

Figure 27. Moulds on brined and brined smoked *S. scombrus* stored at 20°C

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